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Keçi Sütünde Isıl İşlem Sonrası Melatonin Seviyeleri: Toplam Protein İlişkisi

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ÖZET

Bu çalışmada, pastörizasyon (72 °C'de 15 saniye) ve kaynatma işlemlerinin (100 °C'de 5 dakika) keçi sütü melatonin ve toplam protein seviyeleri üzerindeki etkilerinin araştırılması amaçlanmıştır. Çalışmada Hatay ilinde özel bir çiftliğe ait 20 adet sağlıklı keçiden alınan sütler kullanılmıştır. Her bir süt örneği çiğ süt, pastörizasyon işlemi (72 °C'de 15 saniye) ve kaynatma işlemi (100 °C'de 5 dakika) için eşit şekilde bölünmüş, 3 grup oluşturulmuştur. Sütlerin melatonin düzeyleri ticari ELISA kiti ile, toplam protein düzeyleri spektrofotometrik olarak manuel yöntemlerle belirlenmiştir. Kaynatılmış süt örneklerinde (4.20 ± 0.39 pg ml^{-1}) melatonin seviyelerinin, çiğ süt (3.19 ± 0.25 pg ml^{-1}) örneklerine kıyasla yüksek olduğu belirlenmiştir (P<0.05). Isıl işlem görmüş gruplar arasında Bradford yöntemi ile belirlenen toplam protein düzeyleri açısından fark olmamakla birlikte, Lowry yöntemi ile belirlenen kaynatılmış süt grubu toplam protein düzeylerinin diğer gruplara göre önemli ölçüde düşük olduğu belirlenmiştir (P<0.001). Bradford ve Lowry yöntemleri ile belirlenen toplam protein düzeyleri açısından çiğ süt grubu (r=0.723, P<0.01), pastörize süt grubu (r=0.838, P<0.01) ve kaynatılmış süt grubu (r=0.449, P<0.05) için pozitif korelasyonlar tespit edilmiştir. Keçi sütüne pastörizasyon işlemi uygulamasının süt melatonin ve toplam protein düzeylerini etkilemediği, ancak kaynatma işlemi uygulanması ile süt toplam protein düzeylerinin düştüğü, melatonin düzeylerinin ise yükseldiği belirlenmiştir. Bu bağlamda, sütte ELISA yöntemi ile melatonin analizi yapılmadan önce, melatoninin bağlı olduğu proteinden ayırmak amacı ile, süte ön işlem yapılmasının yararlı olabileceği öngörülmüştür.

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Melatonin Levels After Heat Treatment in Goat Milk: Relation of Total Protein

ABSTRACT

Objective of this study was to investigate the effects of pasteurization (15 seconds at 72 °C) and boiling processes (5 minutes at 100 °C) on goat milk melatonin and total protein levels. The milk of 20 healthy goats obtained from a private goat farm were used as materials. Each milk sample was divided into 3 for raw milk, for pasteurization and for boiling process. Melatonin levels in raw, pasteurized and boiled milk were determined with commercial ELISA kit while total protein levels were determined spectrophotometricly by manual methods. Melatonin levels in boiled milk samples (4.20±0.39 pg ml^{-1}) increased (P<0.05) compared to raw milk (3.19±0.25 pg ml^{-1}) samples. While there was no difference between the heat-treated groups in terms of total protein levels determined by Bradford method, the total protein levels determined by Lowry method decreased significantly in boiling process group compared to the other groups (P<0.001). Positive correlations were determined between total protein levels determined by Bradford and Lowry methods in raw (r=0.723, P<0.01), pasteurized (r=0.838, P<0.01) and boiled (r=0.449, P<0.05) milk samples. Pasteurization process applied to goat milk did not change milk

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melatonin and total protein levels, and boiling process decreased total protein levels, while increased melatonin levels. Before melatonin analysis was performed in milk by ELISA method, it was predicted that a pre-treatment of milk may be useful in order to separate melatonin from the bound protein.

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GİRİŞ

Keçi sütü üretimi sadece az gelişmiş ülkelerde, temel beslenme ve geçim kaynağı sağladığı için değil, aynı zamanda gelişmiş ülkelerdeki insanların sofistike tüketici zevklerine çeşitlilik sağlaması ve inek sütü ürünleri ile ilgili bir takım sorunlar yaşayan kişiler (alerji, mide-bağırsak problemleri vb.) için bir alternatif sunması gibi nedenlerle günümüzde önem taşımaktadır (Haenlein,1986; Park 2010; Lad ve ark., 2017; Yılmaz, 2017). Türkiye'de gerek doğal, gerekse ticari olarak en yaygın olarak kullanılan süt inek sütüdür. 2010-2011 verilerine göre, Türkiye toplam süt üretiminin % 91.7'sini inek sütü, % 6'sını koyun sütü, % 2'sini keçi sütü ve % 0.3'nü manda sütü oluşturmaktadır (Atasever ve Gülaç, 2012). Ortalama bileşim bakımından inek sütüne benzerlik göstermekle birlikte, fizikokimyasal niteliklerindeki bazı farklılıklar nedeniyle, keçi sütü inek sütünden daha değerli olarak kabul edilmektedir. İnek sütüne göre daha yüksek oranda küçük çaplı yağ globülleri bulundurması ve süt proteinlerinin özellikle de α-kazeinin kalitatif ve kantitatif açıdan farklılıklar göstermesi, keçi sütünün daha kolay sindirilebilmesini sağlamaktadır. Bu özelliğiyle de bebekler, yaşlılar ve süt veren kadınların beslenmesinde üstünlükleri bulunmaktadır (Yılmaz, 2017). Ayrıca keçi sütü, sığır sütündeki önemli bir alerjen olan αs-1 kazeini önemli ölçüde daha düşük seviyede içerir (Lara-Villoslada ve ark., 2004). Kısacası, keçi sütü, inek sütü ile kıyaslandığında, daha düşük kolesterol içermesi (Haenlein, 2004), yağ sindirilebilirliğinin daha yüksek olması (Park ve ark., 2007), bazı vitamin ve mineral düzeyleri ile (Belewu ve Aiyegbusi, 2002; Park ve ark., 2007), non-protein nitrojen içeriğinin daha yüksek olması (Park ve ark., 2007) gibi nedenlerle, insan beslenmesinde inek sütüne tercih sebebi olabilmektedir (Lad ve ark., 2017).

Pineal bez tarafından, triptofan amino asitinden sentezlenen bir indol bileşiği olan melatonin, sentez ve salınımı ışık tarafından kontrol edilen bir hormondur. Melatonin sentez ve salınımı karanlıkta artmakta, aydınlıkta ışık ile birlikte baskılanmaktadır (Özgüner ve ark., 1995). Karanlıkta ve uykuda iken sentezlenen melatoninin hücre yenilenmesi, anti-aging, anti-kanserojen, bağışıklık sisteminin güçlenmesi, uyku düzeninin sağlanması gibi bir takım fonksiyonları bulunmaktadır. Melatoninin, inek ve keçi sütünde 5-25 pg ml^{-1} düzeylerinde bulunduğu rapor edilmiştir (Eriksson ve ark., 1998; Baskett ve ark., 2001; Valtonen ve ark., 2005). Melatoninin sütteki konsantrasyonu, serum konsantrasyonu ile paralel olarak diürnal bir seyir izlemektedir ve gece sağılan sütlerde melatonin düzeyleri gündüz sağılan sütlere göre yüksektir (Milagres ve ark., 2014). Yüksek melatonin seviyeli gece sütü tüketen yaşlı kişilerin uyku düzeninin iyileştiği rapor edilmiştir (Valtonen ve ark., 2005).

İnsan ve rat kan plazmasında melatoninin özellikle albümine bağlanarak taşındığı (Cardinali ve ark., 1972), toplam melatoninin (bağlı ve serbest) % 23 kadarının ise serbest olduğu bildirilmiştir (Kennaway ve Voultsios, 1998). Keçilerde beyin omurilik sıvısında, protein düzeyleri düşük olduğundan, melatoninin büyük oranda serbest olarak bulunduğu tahmin edilmektedir (Cardinali ve ark., 1972). Sütlerde ise melatoninin proteinlerle ilişkisine dair bir çalışmaya rastlanılmamıştır.

Sütler, fabrikasyon işlemleri sırasında besinsel düzeylerinde değişime maruz kalmaktadır. Ticari olarak satılan uzun ömürlü (UHT) ve pastörize inek sütünün yağsız kuru madde, yağ, protein, laktoz ve kazein düzeylerinin, çiğ inek sütüne kıyasla düşük olduğu bildirilirken, ticari olarak satılan UHT keçi sütü ile çiğ keçi sütü kıyaslandığında bahsedilen parametrelerin önemli düzeyde değişmediği bildirilmiştir (Yılmaz, 2017). Diğer yandan keçi sütünün fizikokimyasal kompozisyonunun inek sütü ile kıyaslandığında ısıl işleme daha duyarlı olduğunu bildiren çalışmalar da bulunmaktadır (Raynal-Ljutovac ve ark., 2007; Chen ve ark., 2019). İşlenmiş inek sütündeki (UHT, pastörize) melatonin düzeylerinin, çiğ süttekine göre daha düşük olduğu bildirilmiştir (Schaper ve ark., 2015). Ancak, literatür taramasında, keçi sütlerinde ısıl işlem sonrasında, melatonin düzeylerinin araştırıldığı herhangi bir çalışmaya rastlanılmamıştır.

Besinsel yönden değerli bir süt olan keçi sütünün ısıl işlem sonrasında melatonin yönünden bir kayba uğrayıp uğramadığının ortaya konulması, ısıl işlemin keçi süt proteinleri üzerine etkisinin ve melatoninin bu süreçte süt proteinleri ile ilişkisinin belirlenmesi amaçlarıyla; sunulan çalışmada keçi sütüne

uygulanan pastörizasyon (72 °C'de 15 sn) ve geleneksel olarak evlerde uygulanan kaynatma işleminin (100 °C'de 5 dk) süt melatonin ve toplam protein düzeylerine etkileri araştırılmıştır.

MATERYAL ve METOD

Hatay ilinde bulunan yarı entansif özel bir keçi çiftliğinden, nisan ayında (Bağıl nem % 60-70, sıcaklık en yüksek 22 °C, en düşük 12 °C), aynı bakım beslemeye tabi tutulan, 1-4 yaşlı, sağlıklı, 20 adet keçiden (10 Halep, 10 Kilis keçisi), 20 adet süt örneği toplanmıştır. Sütler, hayvan sahibinin sabah (10:30-11:00) meradan dönen keçilerden süt sağımı yaptığı sırada temin edilmiştir. Sütler klinik olarak sağlıklı görünen memelerin sağ lobundan alınmıştır. Meme başları % 70'lik etil alkollü pamuk ile sterile edilmiştir. İlk 2-3 sıkım süt atılmış ve 50 ml'lik steril falkon tüplere 20'şer ml süt örnekleri alınmıştır. Toplanan süt örnekleri soğuk zincirde biyokimya laboratuvarına getirilmiş ve uygulamalara kadar -20 °C'de saklanılmıştır.

Yirmi adet keçiden elde edilen süt örnekleri, deney sırasında çözündürülerek, 5'er ml'lik 3 eşit parçaya bölünmüş ve gruplar oluşturulmuştur: Grup 1, çiğ süt grubu olup, her bir numuneden 5 ml çiğ süt alınarak oluşturulmuştur. Grup 2, pastörize süt grubu olup, süt örneklerine (5 ml) laboratuvarda pastörizasyon işlemi (72 °C'de 15 sn) uygulanmıştır (Grant ve ark., 1999). Bu amaçla sütler, ısıya dayanıklı cam tüpler içerisinde (20 adet cam tüp ile), bir tüp sporu üzerinde, ben-mari'ye yerleştirilmiştir. Sütlerin iç ısısı Dijital termometre (Testo SE&Co.KGaA, Türkiye) ile ölçülmüştür. Tüp sporu üzerinde bulunan bir süt örneği içerisine daldırılan termometre ile, sütün iç ısısının 72 °C'ye yükseldiği gözlemlendiğinde, sütler 15 sn bekletildikten sonra hemen kırık buz bulunan bir küvete alınarak 6 °C'ye soğutulmuştur. Grup 3 kaynatma süt grubu olup, süt örneklerine (5 ml) kaynatma işlemi (100 °C'de 5 dk) uygulanmıştır (Metwally ve ark., 2011). Bu amaçla ısıya dayanıklı cam tüplerdeki süt örnekleri (20 adet cam tüp ile), su ısısı 100 °C olan ben-mari içerisinde (termometre ile süt iç ısısı 90 °C ölçülen) 5 dk bekletildikten sonra hemen kırık buz bulunan bir küvet içerisinde 6 °C'ye soğutulmuştur.

Süt melatonin düzeylerinin analizi Milagres ve ark. (2014) ile Schaper ve ark. (2015)'nın inek sütü için modifiye ettiği insan direkt tükürük melatonin ELISA kiti (RE54041, IBL International, Almanya) ile gerçekleştirilmiştir. Bu amaçla sütler 5400 rpm x 15 dk x +4 °C'de santrifüj edilmiş ve üstteki yağlı tabaka uzaklaştırılmıştır. Geriye kalan süt serumu kit prosedürüne göre analizlenmiştir. Örnekleme öncesinde süt serumu örnekleri kit içerisindeki örnek sulandırıcı ile dört kat sulandırılmıştır (Milagres ve ark., 2014, Schaper ve ark. 2015).

Prosedür: Melatonin antijeni ile kaplanmış mikropleyt kuyucuklarının kullanıldığı yöntemde, kuyucuklara standart çözeltileri ve süt örnekleri eklendikten sonra tracer olarak biotinle işaretlenmiş melatonin antikoru ve HRP (Horse radish peroxydase) birlikte verilerek inkube edilmiştir. İnkubasyon sonrası plaklar yıkanarak tracer'a bağlanmamış HRP'ler uzaklaştırılmış, yıkanan plaklara subsrat eklenerek inkube edilmiş ve inkubasyon sona erince stop solusyonu ile enzim reaksiyonu durdurulmuş ve 10 dakika içinde 450 nm'de absorbanslar okunmuştur. Standart eğri oluşturularak sonuçlar hesaplanmıştır. Sonuçlar pg ml^{-1} olarak verilmiştir.

Süt toplam protein düzeylerinin analizleri

Süt toplam protein tayini amacıyla, yaygın olarak, sütteki nitrojen seviyelerinin belirlendiği Kjeldahl metodu (Barbano ve ark., 1990) kullanılmasına rağmen, sunulan çalışmada ısıl işlemin süt proteinleri ve melatonin üzerine etkilerinin, ayrıca bu süreçte melatonin ile protein ilişkilerinin araştırılması amacıyla, peptid bağlarının varlığında protein tayini gerçekleştirilen Lowry yöntemi (Lowry ve ark., 1951) ile amino asitlerin de reaksiyon verdiği Bradford yöntemi (Bradford, 1976) süt proteinlerinin tayini amacıyla kullanılmıştır. Sığır serum albümini (Sigma-A2153) standart olarak kullanılmış ve protein düzeyleri, standart ile çizilen absorbans-konsantrasyon grafiği üzerinden hesaplanmıştır. Sonuçlar g dl^{-1} olarak verilmiştir. Süt toplam proteinlerinin analizi 5400 rpm x 15 dk x +4 °C'de santrifüj edilmiş ve yağı alınmış süt serumlarında gerçekleştirilmiştir.

Lowry yöntemi

Prensip: Bu yöntemde, alkali ortamda Cu^{+2}, proteinin yapısını oluşturan peptit bağlarıyla birleşerek Cu^{+1}'e indirgenir ve biüret kompleksini oluşturur. Daha sonra, Folin ve Ciocolteu'nun fenol reaktifi ilave edilir ve fosfotungstik ve fosfomolibdik asitlerin indirgenmesiyle molibdenyum mavisi ve tungsten mavisi renkleri meydana gelir. Oluşan renkli kompleksin 700 nm'deki optik dansitesi, spektrofotometrede (UV 2100 UV–VIS Recording Spectrophotometer Shimadzu, Japonya) ölçülerek protein düzeyleri belirlenir (Lowry ve ark., 1951). Prosedür: Çalışma reaktifi hazırlamak için; 0.1 N NaOH'te (Merck 1,06462) % 2'lik Na_2CO_3 (Merck 1,06392) çözeltisi hazırlanmış, bu çözelti daha sonra % 1'lik $CuSO_4$ çözeltisi (Carlo Erba UN3077) ve % 2'lik Na-K tartarat çözeltisi (Carlo Erba 474117) ile 98/1/1(v) oranında karıştırılmıştır. Numune ve standart tüplerine 10 µl süt serumu ve standart çözeltisi eklenmiştir. Kör tüpüne 10 µl bidistile su eklenmiştir. Tüplere çalışma reaktifinden 2,5 ml eklenmiştir. Tüpler vortekslenmiş ve 25 °C'de 10 dk bekletilmiştir. Tüplere, hacimsel olarak 1/1 oranda distile su ile hazırlanan Folin-Ciocalteu's reaktifinden

(Carla Erba UN3264) 0,25 ml eklenmiştir. Tüpler karıştırılmış ve 25 °C'de 30 dk bekletildikten sonra, absorbanslar spektrofotometrede 700 nm'de köre karşı okunmuştur.

Bradford yöntemi

Prensip: Bu yöntemde, Coomassie brillant blue G250 boyası (Sigma 27815), negatif bir yüke sahiptir ve protein üzerindeki amino asitlerin pozitif yüküne bağlanır (Bradford, 1976). Oluşan renkli kompleksin 595 nm'deki optik dansitesi spektrofotmetrede ölçülerek, protein düzeyleri belirlenir. Prosedür: Bradford reaktifi hazırlamak amacıyla 40 mg Coomassie Brillant blue G250, 50 ml % 95'lik etanolde çözüldükten sonra, üzerine 55 ml % 85'lik fosforik asit (Sigma 79617) eklenmiştir. Son hacim bidistile su ile 1 L'ye tamamlanmıştır. Hazırlanan reaktiften her bir örnek, standart ve kör tüpüne 1 ml eklenmiştir. Reaktifin üzerine süt serumu örnekleri ve standartlardan 20'şer µl eklenmiştir. Kör tüpü için 20 µl bidistile su kullanılmıştır. Tüpler karıştırılıp 10 dk oda ısısında bekletildikten sonra 595 nm'de spektrofotometre ile absorbans ölçümleri yapılmıştır.

İstatistik Analizler

Elde edilen değerler SPSS 15.0 programında ANOVA ile değerlendirilerek, gruplar arası farklılıklar post-hoc Duncan testi ile ortaya konulmuştur. Bradford ve Lowry metodları ile belirlenen toplam protein düzeyleri arasındaki ilişkiler student's t-testi ile belirlenmiştir. Korelasyon analizleri, veriler normal dağılım gösterdiğinden, Pierson testi ile yapılmıştır. $P<0.05$ önem düzeyinde önemli kabul edilmiştir.

BULGULAR ve TARTIŞMA

Çiğ sütte, pastörize edilen ve kaynatılan keçi sütlerinde melatonin ve toplam protein düzeyleri Çizelge 1'de sunulmuştur. Kaynatma işlemi uygulanan süt örneklerinde melatonin düzeyleri (4.20±0.39 pg ml^{-1}), çiğ süt örneklerine (3.19±0.25 pg ml^{-1}) kıyasla yüksek bulundu ($P<0.05$). Toplam protein düzeyleri değerlendirildiğinde, Bradford yöntemi belirlenen toplam protein düzeyleri açısından gruplar arasında bir farklılık bulunmaz iken, Lowry yöntemi ile belirlenen toplam protein düzeylerinin kaynatma uygulanan grupta diğer gruplara göre önemli düzeyde düşük olduğu belirlenmiştir ($P<0.001$). Çiğ süt ve pastörize sütteki toplam protein düzeyleri Bradford yöntemi ve Lowry yöntemi ile belirlenen düzeyler açısından birbiri ile yakın bulundu ($P>0.05$). Kaynatma uygulanan gruplarda ise Bradford yöntemi ile belirlenen toplam protein düzeyleri (2.26±0.13 g dl^{-1}), Lowry metodu ile belirlenen toplam protein düzeylerinden (1.92±0.09 g dl^{-1}) yüksek olarak belirlenmiştir ($P<0.05$).

Table 1 Melatonin and total protein levels in raw, pasteurized and boiled milk (Mean±SE) (n=20)
Çizelge 1. Çiğ sütte, pastörize edilen ve kaynatılan sütlerde melatonin ve toplam protein düzeyleri (Ort±SH) (n=20)

Süt Numuneleri	Melatonin (pg ml^{-1})	Toplam Protein Bradford (g dl^{-1})	Toplam Protein Lowry (g dl^{-1})
Çiğ süt	3.19±0.25 ^{b}	2.33±0.07	2.32±0.07 ^{a}
Pastörize süt	3.42±0.24 ^{ab}	2.27±0.10	2.28±0.05 ^{a}
Kaynatılan süt	4.20±0.39 ^{a}	2.26±0.13	1.92±0.09 ^{b}
P	<0.05	>0.05	<0.001

^{a,b,c} Aynı sütundaki farklı harfler istatistiksel olarak önemli düzeyde farklıdır. $P<0.05$ and $P<0.001$.

Çiğ süt, pastörizasyon ve kaynatma ısıl işlemi uygulanan sütlerde ilgili biyokimyasal parametreler arasındaki korelasyonlar Çizelge 2, 3 ve 4'de sunulmuştur. Çiğ süt örneklerinde Bradford metodu ile Lowry metodu kullanılarak belirlenen toplam protein düzeyleri arasında yüksek pozitif korelasyon belirlenmiştir ($r=0.723$, $P<0.01$). Pastörizasyon uygulanan süt örneklerinde Bradford metodu ve Lowry metodu kullanılarak belirlenen toplam protein düzeyleri arasında yüksek pozitif korelasyon belirlenmiştir ($r=0.838$, $P<0.01$). Kaynatma işlemi uygulanan sütlerde Bradford metodu ile Lowry metodu kullanılarak belirlenen toplam protein düzeyleri arasında, diğer uygulamalara göre kıyaslandığında, zayıf pozitif korelasyon belirlenmiştir ($r=0.449$, $P<0.05$).

Çiğ sütte insan sağlığı açısından zararlı mikroorganizmalar bulunduğundan, insan tüketimine sunulmadan önce çiğ sütler bir takım ısıl işlemlere tabi tutulmaktadır. Ancak ısıl işlem uygulamalarının süt bileşenlerinde bir takım değişikliklere neden olduğu bilinmektedir (Metwally, 2011; Yılmaz, 2017). Süte uygulanan ısıl işlemler sonrasında sütte bulunan proteinlerin konformasyonlarının değiştiği ve böylece proteinlerin denatürasyona ve/veya agregasyona uğradıkları bildirilmektedir (Jimenez-Saiz ve ark., 2015, Lamberti ve ark., 2018). Keçi sütünde temel olarak 5 protein tipi bulunur; bunlar αs2-kazein, β-kazein, κ-kazein, β-laktoglobülin ve α-laktalbümindir. Majör protein fraksiyonu inek sütünde αs1-kazein iken, keçi sütünde β-kazeindir (Chandan ve ark., 1992). Keçi sütünün kazein miselleri inek sütününkinden daha az çözünür formdadır ve ısı karşısındaki dayanılırlığı daha azdır, dolayısı ile ısı artışı ile β-kazein kaybı daha fazladır (Juarez ve Ramos, 1986). Günümüzde geleneksel kaynatma yönteminin süt proteinleri içeriği ve konformasyonel değişimi hakkında sınırlı veri bulunmaktadır:

proteinlerin 55-70 °C'de ikincil yapılarının bozulduğu, 70-80 °C'de disülfit bağlarının koptuğu, 80-90 °C'de yeni intra- ve inter-moleküler bağların kurulduğu ve 90-100 °C'de agregatların oluştuğu bildirilmektedir (Wal, 2003).

Table 2 Correlations between melatonin and total protein in raw milk
Çizelge 2. Çiğ sütlerde melatonin ile toplam protein arasındaki korelasyonlar

Çiğ Süt	Toplam Protein Bradford (g dl^{-1})	Toplam Protein Lowry (g dl^{-1})
Melatonin (pg ml^{-1})	0.270	0.264
Toplam Protein Bradford (g dl^{-1})		0.723*

*P<0.01 (Pierson testi, n=20)

Table 3 Correlations between melatonin and total protein in pasteurized milk
Çizelge 3. Pastörizasyon işlemi uygulanan sütlerde melatonin ile toplam protein arasındaki korelasyonlar

Pastörize Süt	Toplam Protein Bradford (g dl^{-1})	Toplam Protein Lowry (g dl^{-1})
Melatonin (pg ml^{-1})	0.321	0.218
Toplam Protein Bradford (g dl^{-1})		0.838*

*P<0.01 (Pierson testi, n=20)

Table 4 Correlations between melatonin and total protein in boiled milk
Çizelge 4. Kaynatma işlemi uygulanan sütlerde melatonin ile toplam protein arasındaki korelasyonlar

Kaynatılmış Süt	Toplam Protein Bradford (g dl^{-1})	Toplam Protein Lowry (g dl^{-1})
Melatonin (pg ml^{-1})	-0.077	-0.026
Toplam Protein Bradford (g dl^{-1})		0.449*

*P<0.05 (Pierson testi, n=20)

İnek sütüne kaynatma (98 °C'de 16 dk) ve mikrodalga yöntemi ile ısıl işlem uygulaması sonucunda, tek yönlü elektroforezde peynir altı suyu protein (α-laktalbümin) bantlarının zayıfladığı, ß-laktoglobülin bantlarının ise neredeyse kaybolduğu belirlenmiştir (Lamberti ve ark., 2018). Bloom ve ark. (2014) da 20 dk'dan uzun kaynatma işlemi uyguladıkları inek sütünde α-laktalbümin ve ß-laktoglobülinin yüksek molekül ağırlıklı agregatlarının oluştuğunu bildirmişlerdir. Yılmaz (2017), taze keçi sütü ile marketlerden toplanan UHT keçi sütlerini karşılaştırdığı çalışmasında keçi sütü toplam protein düzeylerinin (FOSS MilkoScanTM FT-120, Foss electric, Denmark) taze süt (% 3.04± 0.17) ve ticari sütlerde (% 2.94 ±0.02) istatistiksel olarak farklı olmadığını bildirmiştir. Taze inek sütünde ise toplam protein düzeylerinin (% 3.66±0.16) ticari inek sütü toplam protein düzeylerinden(% 2.71±0.07) önemli düzeyde yüksek olduğunu gözlemlemiştir. Formol titrasyon testi ile belirlenen toplam protein düzeyleri, taze ve pastörize keçi sütünde 2.90 g dl^{-1}, 63 °C'de 30 dk ısıl işlem uygulanmış, yağı ve hücreleri alınmış (9800 rpm x 15 dk x 4 °C) sütte 2.63 g dl^{-1} olarak bildirilmiştir (Paz ve ark., 2014). Diğer yandan Moreno-Montoro ve ark. (2015), Kjeldahl metodu ile belirledikleri keçi sütü toplam protein düzeylerini çiğ sütte 3.62 ± 0.19 g $100g^{-1}$, santrifüj edilmiş çiğ sütte 4.10 ± 0.33 g $100g^{-1}$olarak bildirmişlerdir. Rukke ve ark. (2010) da santrifüj edilmiş ve yağı alınmış çiğ keçi sütünde Kjeldahl metoduna göre belirledikleri toplam protein düzeylerini 1.72-3.31g $100g^{-1}$ olarak bildirmişlerdir. Sunulan çalışmada 5400 rpm x 15 dk x +4 °C'de santrifüj edilerek hem yağı hem de süt hücrelerinden ayrımlanan çiğ süt örneklerinde Bradford yöntemi ile belirlenen toplam protein düzeyleri 2.33±0.07 g dl^{-1}, Lowry metodu ile belirlenen toplam protein düzeyleri 2.32±0.07 g dl^{-1} olarak belirlenmiştir. Düzeylerin, Rukke ve ark. (2010)'nın bildirdiği düzeyler arasında olduğu, diğer araştırmacıların bildirdiği düzeylerden ise biraz düşük olduğu görüldü. Bu durum, protein analiz metodlarının farklılığı, keçi ırklarının farklılığı, bölgesel farklılık, süt alımının yılın farklı zamanlarında gerçekleştirilmesi gibi nedenlerden kaynaklanabilir.

Sunulan çalışmada Bradford yöntemi ile belirlenen toplam protein düzeyleri ısıl işlem uygulamaları ile değişim göstermezken, Lowry yöntemi ile belirlenen toplam protein düzeyleri 5 dk süreyle kaynatma uygulanan sütlerde, diğer gruplara göre önemli düzeyde düşük olarak belirlenmiştir. Bradford yönteminde Commassie blue G250 anyonunun proteinler üzerinde bulunan NH_3^+ katyonik grubuna bağlandığı, polilizin, poliarjinin, politirozin, polihistinin peptidleri, arjinin, histidin gibi bazı amino asitler ve bazı di ve tri-peptidlerle bağlanarak renk verdiği bildirilmiştir (De Moreno ve ark., 1986). Diğer yandan, Lowry metodunda ise Biüret ve Folin reaksiyonu sonucu, Cu^+ iyonları proteinlerde bulunan peptid bağlarına bağlanmaktadır (Lowry ve ark., 1951). Amino asit düzeyi arttıkça Folin reaksiyonunda meydana gelen rengin, proteinlere göre azaldığı da bildirilmiştir (Andersch ve Gibson, 1933). Dolayısı ile sunulan çalışmada, keçi sütü proteinlerinin 5 dk kaynatma işlemi sonucunda peptid bağlarının sayısının azaldığı ve böylece Lowry yöntemi ile belirlenen toplam protein düzeylerinin düşük

bulunduğu sonucuna varılabilir. Bu nedenle sütlerde, özellikle ısıl işlem uygulaması sonrasında protein düzeylerinin belirlenmesi amacıyla 'toplam nitrojen düzeylerinin belirlenmesinin' daha hassas ve net sonuçlar vereceği öngörülmektedir.

Melatonin, sirkadyen ritim üzerine etkili, aynı zamanda antioksidan (Maksimovich, 2002), ve antikanser (Dullo ve Chaudhary, 2009) özellikleri bulunan bir hormondur. Melatonin sentez ve salınımı karanlıkta artmaktadır (Özgüner ve ark., 1995). Özellikle gece saat 23:00-05:00 sıralarında melatonin salgılanması en yüksek seviyeye ulaşır; hücre içinde ve kandaki konsantrasyonu gündüze nazaran 3-10 kat artar (Claustrat ve ark., 2005). Melatonin hormonunun insan, inek ve keçi sütünde varlığı ve düzeyleri (5-25 pg ml^{-1}) ortaya konulmuştur (Eriksson ve ark., 1998; Valtonen ve ark., 2005). Süt melatonin düzeylerinin, gece sağılan (gece 02:00-04:00), sütlerde gündüz sağılan sütlere oranla daha yüksek olduğu bildirilmiştir (Milagres ve ark., 2014). Sunulan çalışmada gündüz saatlerinde mera dönüşünde alınan keçi sütü melatonin düzeyleri, ineklerde gündüz sütünde bildirilen değerlere yakın çıkmıştır. Schaper ve ark. (2015), farklı marketlerden elde edilen işlenmiş sütler ile bir çiftlikten elde edilen 10 ineğe ait çiğ sütlerdeki melatonin düzeylerini karşılaştırdıkları çalışmada, işlenmiş inek sütündeki (UHT sütte 4.5 pg ml^{-1}, pastörize sütte 7 pg ml^{-1}) melatonin düzeylerinin, çiğ süttekine (13.6 pg ml^{-1}) göre daha düşük olduğunu bildirmişlerdir. Sunulan çalışmada ise aynı süt üzerine pastörizasyon uygulamasının, çiğ sütlere göre melatonin düzeylerinde bir değişime neden olmadığı belirlenmiştir. İnek sütü üzerine ısıl işlemin etkileri ile ilgili çalışmada (Shaper ve ark., 2015) aynı süt örneği üzerine ısıl işlem uygulamasının etkileri değil, marketlerden temin edilen sütler (UHT, pastörize) ile çiftliklerden elde edilen çiğ sütler karşılaştırılmıştır. Sunulan çalışma ise, hem keçi sütünde olması hem de aynı süt örneği üzerine uygulanan ısıl işlemin melatonin düzeylerine etkilerinin araştırılması yönlerinden özgündür.

Yapılan literatür taramalarında melatoninin sütte taşınması ile ilgili bir çalışmaya rastlanılmamıştır. İnsan ve rat kan plazmasında melatoninin özellikle albümine bağlanarak taşındığı (Cardinali ve ark., 1972), toplam melatoninin (bağlı ve serbest) % 23 kadarının ise serbest olduğu bildirilmiştir (Kennaway ve Voultsios, 1998). Sunulan çalışmada, serbest melatonin düzeylerinin ölçüldüğü yöntemde, kaynatma işlemi sonrasında süt melatonin düzeylerinin, pastörize ve çiğ süt örneklerine göre yüksek olduğu gözlemlenmiştir. Keçi sütü yapısında bulunan α-laktalbüminin sütte melatonin taşınmasında rolünün olabileceği düşünülebilir. İnek sütünde, ısıl işlem uygulaması sonucunda, elektroforetik incelemede, α-laktalbümin bantlarının zayıfladığı, ß-laktoglobülin bantlarının ise neredeyse kaybolduğu belirlenmiştir (Lamberti ve ark., 2018). Yine 20 dk'dan uzun kaynatma işlemi uygulanan inek sütünde α-laktalbümin ve ß-laktoglobülinin yüksek molekül ağırlıklı agregatlarının oluştuğu bildirilmiştir (Bloom ve ark., 2014). Bu bilgiler ışığında, keçi sütünde bulunan laktalbumine bağlı melatoninin, ısıl işlem uygulaması sonrasında laktalbuminin denatürasyonu ve/veya agregasyonu neticesinde serbest hale geçerek, ELISA analizinde çiğ süt ve pastörize süte göre daha yüksek düzeyde belirlenmiş olması olasıdır.

SONUÇ ve ÖNERİLER

Keçi sütüne uygulanan pastörizasyon işleminin, süt melatonin ve toplam protein düzeylerini çiğ süte göre değiştirmediği, 100 °C'de 5 dk kaynatma uygulamasının ise proteinlerin denaturasyonu ve/veya agregasyonuna neden olabildiği belirlenmiştir. Bu bilgiler ışığında keçi sütünün biyoyararlanımında pastörizasyon işleminin, geleneksel kaynatma işlemine göre daha üstün olduğu düşünülmüştür. Sütlerde ELİSA metodu ile melatonin analizi yapılmadan önce, melatoninin sütte bağlı bulunduğu proteinden ayrıştırılması amacıyla, sütün, bir ön işlemden geçirilmesinin, melatonin düzeylerinin daha doğru olarak belirlenmesinde faydalı olabileceği öngörülebilir.

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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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Evaluation of Antioxidant and Antimicrobial Activities of *Potentilla* recta L.

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ABSTRACT
The objective of this study was to determine the effectiveness and the antioxidant activity of *Potentilla recta* L. (Rosaceae), which is traditionally used in the treatment of many diseases, against pathogenic microorganisms in the skin. The antioxidant activity was determined using 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) and 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS·+) radical scavenging activities, inhibition of ß-carotene bleaching, protection of 2-deoxyribose and bovine brain-derived phospholipids against hydroxyl radical-mediated degradation assay, at different concentrations ranging from 0.001 to 2 mg mL^{-1}. Antimicrobial activity was evaluated against *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 3699), *Pseudomonas aeruginosa* (ATCC 27853) *and Candida albicans* (ATCC 90028) by microdilution method. Besides, viability enhancing effects on murine fibroblast cells (L929) were determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The key parameters for the extract included the following: DPPH· IC_{50}: 0.19 mg mL^{-1}, TEAC: 0.76 mmol L^{-1} Trolox, reduction: 0.87 mmol g^{-1} AsscE, and protection of lipid peroxidation IC_{50}: 0.07 mg mL^{-1}. A strong effect on *S. epidermidis* was observed with 79% inhibition at a concentration of 125 mg mL^{-1} but did not show toxicity to L929 cells below 250 mg mL^{-1} concentration. The present findings highlight the need for research of plants for traditional medicinal uses and the importance of scientific evaluations.

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Potentilla recta L.'nin Antioksidan ve Antimikrobiyal Aktivitelerinin Değerlendirilmesi

ÖZET
Bu çalışmada geleneksel olarak birçok hastalığın tedavisinde kullanılan *Potentilla recta* L. (Rosaceae) 'nin ciltteki patojen mikroorganizmalara karşı etkinliği ve antioksidan aktivitesi araştırılmıştır. Antioksidan aktivite 1,1-Difenil-2-pikrilhidrazil (DPPH·) ve 2,2-Azinobis (3-etilbenzotiyazolin-6-sülfonat) (ABTS·+) radikal temizleme, ß-karoten ağartmanın inhibisyonu, 2-deoksiriboz ve sığır beyninden türetilmiş fosfolipidlerin hidroksil radikal aracılı degradasyonuna karşı koruma etkileri deneyleri ile 0.001 - 2 mg mL^{-1} arasında değişen farklı konsantrasyonlarda belirlenmiştir. Antimikrobiyal aktivite, mikrodilüsyon yöntemi ile *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 3699), *Pseudomonas aeruginosa* (ATCC 27853) *Candida albicans* (ATCC 90028) üzerinde değerlendirilmiştir. Ayrıca fare fibroblast hücreleri (L929) üzerindeki canlılığı artırıcı etkiler MTT (3- (4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromür) yöntemi ile değerlendirilmiştir. Ekstreler için temel parametreler: DPPH • IC_{50}: 0.19 mg mL^{-1}, TEAC: 0.76 mmol L^{-1} Trolox, redüksiyon: 0.87 mmol g^{-1} AsscE ve lipid peroksidasyonun korunması IC_{50}: 0.07 mg mL^{-1} olarak belirlenmiştir. 125 mg mL^{-1} konsantrasyonda % 79 inhibisyonla *S. epidermidis*

Biyoloji

Araştırma Makalesi

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üzerinde güçlü bir etki gözlenirken 250 mg mL^{-1} konsantrasyonunun altında L929 hücrelerine toksisite gözlenmemiştir. Mevcut bulgular, geleneksel tıbbi kullanımı olan bitkilerin araştırılması gerektiğine ve bilimsel değerlendirmenin önemli olduğuna ışık tutmaktadır.

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INTRODUCTION

The Rosaceae family, which includes the *Potentilla* genus, is the 19th largest family in the plant kingdom. It has approximately 100 genera and 2830-3100 species (Folta and Gardiner 2009). Sixty *Potentilla* species are widely found in Turkish Flora (Güner and Aslan 2012). *Potentilla* species are known as "Beşparmak otu, İncibar kökü, Sarı kabusluk" in Turkey and are popularly used as antipyretic and tonic agents and also for constipation since ancient times (Baytop 1999). Underground and aerial parts of species have traditionally been used to treat inflammations, some types of cancer, wounds, infections, diarrhea, diabetes, and some other diseases. Greek physician Dioscorides recommended the use of decoction prepared from underground parts of *Potentilla erecta* in inflammatory facial eczema and ulcerations of oral cavities. In the Middle Ages, physicians and botanists defined the *Potentilla* species in their botanical books for Europe. Extracts prepared with different solvents such as water, milk, honey, and ethanol have been used as an ailment for toothache, sore throats, wound healing, jaundice, mouth ulcers, dysentery, and homeostatic (Tomczyk and Latté 2009). The herb parts of species have been studied in many phytochemical studies. *P. erecta* is the first species with the highest number of identified components among the *Potentilla* species, (a total of 68 compounds in the aerial and underground parts), followed by *P. anserina* (37 compounds in the aerial and underground parts). The main groups of these compounds are flavonoids, condensed tannins, organic and phenolic carboxylic acids, coumarins, and sterols (Tomczyk and Latté 2009). In a study for investigating the polyphenols *of P. recta;* 10 components; 1 neolignan glycoside and 9 flavonoids, were identified and their structures were elucidated (Şöhretoğlu and Kırmızıbekmez 2011).

Due to the known toxic effects of synthetic drugs, people's tendency to herbal medicines has increased, especially in the treatment of simple diseases. For this reason, it is important to determine the biological effects and possible toxic effects of plants traditionally used in treatment. One of the current examples of these herbal medicines for Anatolia is *Potentilla recta.* This plant, which grows naturally in Turkey, is traditionally used in the treatment of respiratory diseases, various skin diseases, gastrointestinal and neurological disorders, as well as for its wound healing properties (Tuzlacı, 2006). To better understand the traditional usage of *Potentilla recta*, the antioxidant activity of the plant has been investigated in detail, and its effectiveness on the microorganisms that cause infection in the skin has been evaluated. The toxicity of the plant extract was evaluated using the mouse fibroblast (L929) cell line, which is frequently used in toxicity studies.

MATERIAL and METHOD

Plant material, reagents, and extraction

Potentilla recta was collected from Pınarbaşı-Kayseri/Turkey, in June 2013. The herbarium material from the plant was kept in the Herbarium (ERCH) of Erciyes University Faculty of Science, Department of Biology. All the chemicals are analytical grade and purchased from Sigma Chemical Company (St. Louis, MO).

Aerial parts of the 150 g dried plant material were macerated with an appropriate volume of 70% methanol (MeOH) in 24 hrs in the shaking water bath (3 times repeats). Methanol phase concentrated under vacuum (37° C). The obtained concentrated extract was lyophilized and maintained at -18 C until analysis.

Reduction of iron (III) to iron (II)

The reduction of iron (III) to iron (II) was assessed by (Oyaizu 1986) method. The solutions, 0.2 M phosphate buffer (pH 6.6), 1% (w/v) potassium hexacyanoferrate solution, 10% w/v trichloroacetic acid (TCA,), and 0.1% (w/v) $FeCl_3$, were used. Extract dissolved in 70% MeOH and then phosphate buffer and potassium hexacyanoferrate solutions (2.5 mL each) were added. TCA (2.5 mL) was added after incubation at 50°C for 30 min. The reaction mixture solution was centrifuged (1000 g, 10 min). Separated upper layer (2.5 mL) was added in H_2O (2.5 mL) and mixed with 0.5 mL of $FeCl_3$. The absorbance of all samples was read at 700 nm. The results were evaluated as ascorbic acid equivalents (AscAE, mmol ascorbic acid/g sample). Triplicate analyses were done and then mean values are given.

DPPH● Scavenging Assay

DPPH• radical scavenging effects of the extract were evaluated by Gyamfi, Yonamine et al. (1999) method.

1 mL of DPPH• methanolic solution (0.1 mM) was mixed with 50 µL of extract solution and 450 µL of Tris-HCl buffer. The absorbance at 517 nm against the blind, which is composed of MeOH, was recorded after incubation of the reaction solutions at room temperature for 30 min. The inhibition of samples was calculated with Eq. 1 and the mean IC_{50} values are given after triplicate analyses.

$$\%\ inhibition = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \quad \text{(Eq. 1)}$$

Inhibition of ß-carotene bleaching

In this assay, the antioxidant capacity of the extract and standards were–evaluated using the method of Velioglu et al. (1998). After mixing, 1.2 mL of *ß*-carotene solution (1 mg mL^{-1} in chloroform), Tween 20, and linoleic acid, chloroform removed from media via rotary evaporator. The emulsion was prepared by adding distilled water to this mixture and mixing gently. For the blanks of the control and samples, the same procedure was performed without using ß-carotene. After the samples were put into tubes together with the emulsion, these samples were kept in a water bath for 2 hours at 50 °C for autoxidation and the fading level was measured at 470 nm every 15 minutes. Inhibition of ß-carotene bleaching was calculated using Eq. 2.

$$AA\% = [1-(Abs0\ sample - Abs120\ sample)/(Abs0\ control - Abs120\ control)] \times 100 \quad \text{(Eq. 2)}$$

$ABTS^{+\bullet}$ Radical Scavenging Activity

The concentrate $ABTS^{+\bullet}$ solution was prepared using 2.45 mM $K_2S_2O_8$ and after 12-16 hours' incubation in dark. This prepared $ABTS^{+\bullet}$ radical solution was measured at 734 nm and diluted with ethyl alcohol until an absorbance of 0.700 (± 0.030) was reached. For the measurement, standard/sample (10 µL) and diluted $ABTS^{+\bullet}$ solution (990 µL) were mixed and then the kinetic profiles were recorded at 734 nm for 30 minutes (1 min interval). Inhibitions (%) were calculated using Trolox (TEAC) calibration equations. Results are given in mean values (n=3) (Re et al. 1999).

Ascorbate-Iron (III)-Catalyzed Phospholipid Peroxidation

Folch VII type bovine brain extract, 10 mM phosphate buffer saline (PBS) (pH 7.4), $FeCl_3$ (1 mM), 2% (w/v) butylated hydroxytoluene (BHT), TCA (2.8%, w/v), and 2-thiobarbituric acid (TBA) (1%, w/v) were used in the experiment. Liposome was prepared with bovine brain extract and PBS. Liposomes (0.2 mL), PBS buffer (0.5 mL), $FeCl_3$ (0.1 mL) and extract/standard (0.1 mL) were mixed. Ascorbate solution (0.1 mL) was mixed to speed up the peroxidation reaction. After incubation (37 °C, 60 min), BHT (50 µL) of, TCA (1 mL) of and TBA (1 mL) were added. After the heating process (100 °C) was done in a water bath for 20 minutes, chromogens formed by malondialdehyde and thiobarbituric acid (MDA- (TBA) 2) were extracted with 2 mL of n-butanol. The absorbance was measured at 532 nm and Equation 1 was used for calculations. After triplicate analyses, IC_{50} values are given as mean values (Aruoma et al. 1997).

Iron (II) Chelate Activity

200 µL extract, 100 µL aqueous $FeCl_2$ (2.0 mM) and 900 µL methanol were added in a tube. After 5 minutes of incubation, the reaction was accelerated by ferrozine (400 µL, 5.0 mM) addition. The absorbance was recorded at 562 nm after 10 minutes. Na_2EDTA equivalent iron-chelating activity ($mg_{Na2EDTA}/g_{sample}$) is given as the average of triplicate analyses (Carter 1971).

Non-site Specific Hydroxyl Radical (●OH) Mediated 2-deoxy-D-ribose Degradation

2-deoxyribose (5.6 mM, pH 7.4), $FeCl_3$/ EDTA (1: 1, v /v), H_2O_2 (1.0 mM), aqueous ascorbic acid (1.0 mM), 2.8% TCA, and 1.0% TBA were used in the experiment. 100 µL of extract, 500 µL of 2-deoxyribose, 200 µL of premixed $FeCl_3$/ EDTA, H_2O_2 (100 µL), and ascorbic acid (100 µL) were mixed. After incubation at 50 °C for 30 minutes, TCA (1 mL) and TBA (1 mL) were added and incubated again at 50 ° C for 30 minutes. The oxidation was calculated as % inhibition after measuring the absorbance at 532 nm. Equation 1 was used for IC_{50} (n=3) (Halliwell, Gutteridge et al.1987).

Antimicrobial activity

The study to determine the minimum inhibition concentration was performed according to the Clinical and Laboratory Standards Institute with microdilution method (Wayne 2009). Gram (+) strains *Staphylococcus aureus* ATCC29213, *Staphylococcus epidermidis* ATCC 3699, Gr-(-) strain *Pseudomonas aeruginosa* ATCC27853 and fungal culture *Candida albicans* ATCC 90028) were used in the study. Mueller Hinton Agar and Mueller Hinton Broth were used for bacterial strains and Sabouraud Dextrose Broth and Sabouraud Dextrose Agar were used for the culture of *C. albicans*

Bacterial cultures were transferred to agar medium to obtain isolated colonies, after 24 hours' incubation at 37 ° C, 4-5 well-isolated colonies were taken into 0.9% saline. The turbidity of the prepared bacterial suspension was set to 0.5 (1×10^8 CFU/mL) McFarland for bacteria and 5×10^6 CFU/mL for yeast in the turbidimeter. In a 96-well plate, 5% of bacteria were cultivated on 0.2 mL nutrient broth containing different concentrations of plant extract and left for 24 hours at 37 ° incubation. At the end of incubation, the concentration without turbidity was determined as Minimum inhibitory concentration (MIC). All analyzes

were carried out in three parallel.

Cell viability assay on L929 cells

The L929 (mouse fibroblast) cells were cultivated in EMEM medium containing 1% penicillin/streptomycin mixture and horse serum (10%, 37 °C, in 5% CO2 and 95% air). The cell line was purchased from American Type Culture Collection (CCL-1 Manassas, VA, USA).

Using MTT colorimetric method, the toxicity, and viability enhancing effect of the extract on the L929 cell line was determined. The cultured cells were counted 24 hours before the experiment and distributed on a 96-well microplate at a number of 1 x 10^4 cell/well. After 24 hours, the supernatant of cells adhering to the plate base was discarded and in 100 µL medium, 15.6; 31.25; 62.5; 125; 250; 500; 1000 and 2000 µg mL^{-1} concentrated extract was added. After 24 hours' incubation, the wells were drained and MTT solution (100 µL, in PBS (5 mg/mL)) was mixed. After 2 hours' in the incubator, the wells were emptied and dimethyl sulfoxide (100 µL, DMSO) was dispensed into the wells. The absorbance was recorded at 570 nm wavelength using ELISA (Biotek Synergy HT). Results are given as mean ± standard error after triplicate analyses.

Statistical analyses

Variance homogeneity was tested with the Levene test. One-way analysis of variance was used for comparisons between more than two groups. Dunnett's test and Tukey test were used for multiple comparisons. The data were evaluated with SPSS Version 11.0 statistic software package. The significance level was accepted as $p < 0.05$.

RESULTS

Iron (III) to iron (II) reduction activity

The capabilities of the samples on the reduction of iron were evaluated and given in Table 1 as ascorbic acid equivalent. The reducing capacity of the extract was 0.87 mmol g^{-1} AscAE. The extract was not found to be as active as ascorbic acid (AA), BHT, butylated hydroxyanisole (BHA), rosmarinic acid (RA) and gallic acid (GA) used as positive controls for reducing iron (III). The activity of the extract was statistically significant ($P < 0.001$) than the activity of the standards.

DPPH• Radical Scavenging Activity

The IC_{50} value is defined as the necessary amount to scavenge 50% of the radical. The decrease in absorbance is indicative of high free radical scavenging ability. The IC_{50} value of the extract and the standards are given in Table 1. *P. recta* extract scavenged the DPPH· radical depending on the concentration at physiological pH and the IC_{50} value was found 0.19±0.00 mg mL^{-1}. However, it has been determined that the scavenging effect of the AA, BHT, BHA, GA, and RA were more active than the extract ($P < 0.001$).

Inhibition of β-carotene bleaching

Free radicals formed by the emergence of hydrogen from linoleic acid attacks β-carotene and the resultant color changes. The degree of inhibition of oxidation with *P. recta* extract was given in Table 1. AAC was compared to the different groups and statistically significant variances were identified in the intergroup comparisons. According to the results, the activity of the extract was significantly higher ($P < 0.001$) than the GA and RA groups and lower than the BHT and BHA groups ($P < 0.001$).

ABTS+●Radical Scavenging Activity

This radical scavenging test measures the amount of antiradical spectroscopically using the $ABTS^{+\bullet}$ radical. In this method, hydrophilic and lipophilic antioxidants such as flavonoids, hydroxycinnamic acids, and carotenoids can be measured. $ABTS^{+\bullet}$ scavenging ability of the *P. recta* was evaluated and given in Table 1. The extract and all standards were analyzed at 2 different concentrations. After comparison of the TEAC values, TEAC variance was found statistically significant in the intergroup comparisons. The activity of the extract was significantly found lower than GA, RA, AA, BHT, BHA groups at 0.2 mg mL^{-1} concentration ($P<0.001$) and significantly lower than the BHT, BHA, AA, GA groups at 0.1 mg mL^{-1} concentration ($P<0.001$) whereas significantly higher than RA group ($P<0.001$). The TEAC value of the extract and the most active GA standard were as follows respectively: 0.76±0.04 mmol L^{-1} Trolox and 2.49±0.01 mmol L^{-1} Trolox.

Ascorbate-Iron (III)-Catalyzed Phospholipid Peroxidation

Biologically important phospholipids in which contain a high amount of polyunsaturated fatty acids susceptible to degradation due to hydroxyl radicals (·OH). In the experiment, the phospholipid liposomes obtained from the bovine brain were rapidly subjected to ·OH induced peroxidation, resulting in the formation of MDA and similar aldehydes. These reactive aldehydes formed were reacted with TBA and the resulting pink color was measured. Antioxidants, which act by removing ·OH and preventing the formation of TBARS, show increasing color intensity with increasing concentration. As seen in Table 1, the extract showed higher activity than AA used as standard. The IC50 value of the extract was 0.07±0.00 mg mL^{-1} and the IC50 value of the AA was 0.90±0.13 mg mL^{-1}. IC_{50} (mg/mL) values were compared between the groups and found statistically significant in the intergroup comparisons. As a result, the activity of the

extract was significantly lower than the AA group ($P < 0.001$), and there were no significant differences from BHT, BHA, GA, and RA.

Iron (II) Chelate Activity

The metal chelating activity is based on the principle that ferrozine quantitatively complexes with Fe^{+2}. To bind Fe^{+2} ions, ferrozine reagent, a strong iron chelator, the metal-binding compounds present in the medium compete with the reactive. If the chelating power is high, the production of Fe^{+2}/ferrozine complex is prevented. The effect of the extract on the chelation of iron (II) ions was investigated and the activity of the extract was found to be greater than 10 mg mL^{-1} and the IC_{50} value was not calculated.

Non-site Specific Hydroxyl Radical (•OH) Mediated 2-deoxy-D-ribose Degradation

The experiment was based on the principle that the ·OH produced by the Fenton reaction causes deoxyribose attack and degradation products resulting from fragmentation react with TBA with low pH and temperature and constitute pink color. Only AA was used as a standard in this experiment. The IC_{50} value of the ascorbic acid was 0.81±0.12 mg mL^{-1} and of the extract was 0.62±0.03 mg mL^{-1}. IC50 values were compared between the groups. The extract was not significantly different from the AA group ($P>0.05$).

Table 1 The antioxidant activity results of P. recta extract and standards
Çizelge 1. P. recta ekstresinin ve standartların antioksidan aktivitesi

Sample[A]	AscAe[B](mmol g^{-1})	DPPH[C] IC50 (mg mL^{-1})	TBAD IC50 (mg mL^{-1})	AAC[E]	TEAC[F] (mmol L^{-1} Trolox)	
					0,1 mg mL^{-1}	0.2 mg mL^{-1}
BHT	2.26±0.005*	0.12±0.01*	0.09±0.00	933.23±1.25*	0.55±0.01*	1.45±0.01*
BHA	1.93 ±0.006*	0.07±0.00*	0.02±0.00	987.55±1.33*	0.86±0.01*	1.87±0.01*
RA	3.10±0.03*	0.04±0.0*	0.05±0.01	661.42±1.10*	0.37±0.01*	1.32±0.00*
GA	4.08±0.01*	0.02±0.00*	0.16±0.06	639.13±0.88*	2.09±0.02*	2.49±0.01*
AscAs	5.72±0.1*	0.13±0.00*	0.90±0.13*	-	1.18±0.01*	1.91±0.00*
P. recta	0.87±0.00	0.19±0.00	0.07±0.00	857.62±1.14	0.41±0.02	0.76±0.04

[A] BHT: butylated hydroxytoluene; BHA: butylated hydroxyanisole; RA: rosmarinic acid; GA: gallic acid; AA: ascorbic acid; Pr 70: 70% methanol extract. [B] Iron (III) reduction. CDPPH radical scavenging. [D] Inhibiton of malondialdehyde formation. [E] Determination of Inhibition of ß-Carotene/Linoleic acid Co-oxidation. [F] TEAC is defined as the concentration of Trolox (mmol/L) having the $ABTS^{+\bullet}$ radical scavenging activity. * expresses difference with Pr70 extract ($P<0.05$). Data are expressed as mean ± standard error.

Antimicrobial activity

The extracts were studied at a concentration of 0.0625-8 mg mL^{-1} in the antimicrobial activity test. Inhibition of viability at a concentration of 0.0625 mg mL^{-1} was found as follows: 44.18% for *S. aureus* (ATCC 29213), 78.29% for *S. epidermidis* (ATCC3699), 24.05% for *P. aeruginosa* (ATCC 27853), and 32.05%, for *C. albicans* (ATCC 90028). The extract had the strongest effect *on S. epidermidis* and the viability could not exceed 22%. The weakest effect was on *P. aeruginosa*, with inhibition of 71.78% at 8 mg mL^{-1}. Results are presented in Figures 1-4.

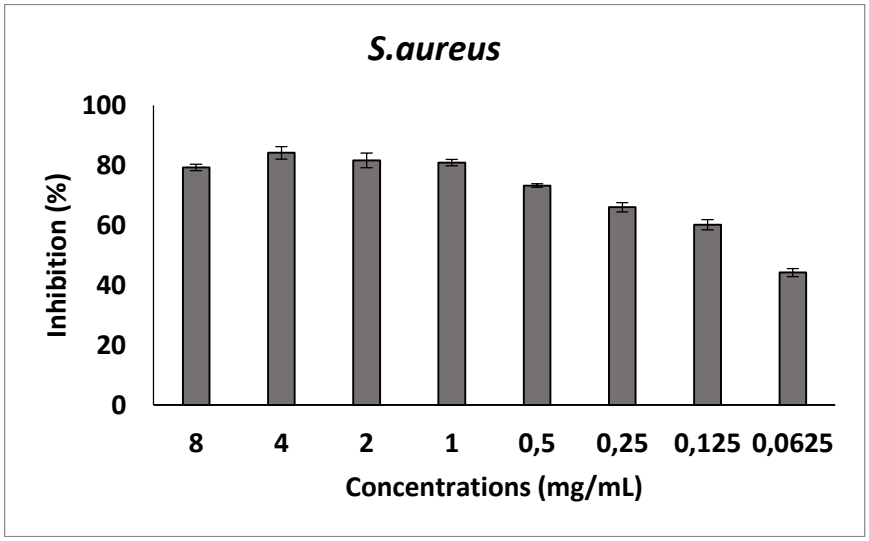

Figure 1. Inhibitory effects of P. recta extract on S. aureus. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

Şekil 1. P. recta ekstresinin S. aureus üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi

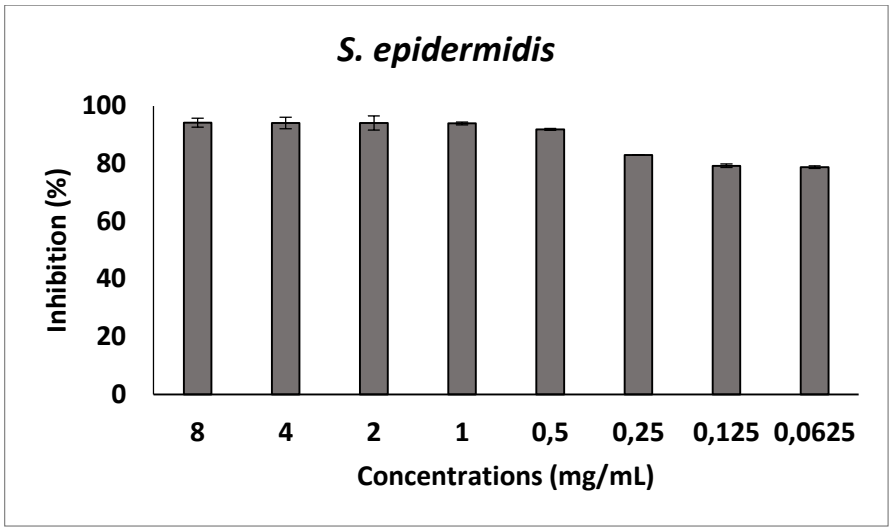

Figure 2. Inhibitory effects of P. recta extract on S. epidermidis. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

Şekil 2. P. recta ekstresinin S. epidermidis üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi

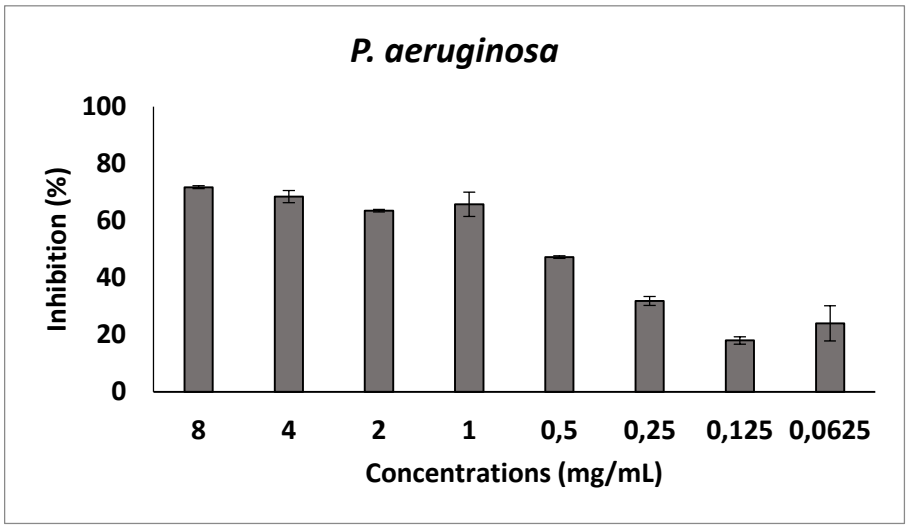

Figure 3. Inhibitory effects of P. recta extract on P. aeruginosa. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

Şekil 3. P. recta ekstresinin P. aeruginosa üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi

Cell viability assay on L929 cells

The effects of the extract studied in the 7.8-2000 µg mL^{-1} concentration range on cell viability were evaluated by the MTT method. Experimental data was presented in Figure 5. The extract has decreased the viability below 50% between the concentrations of 500-2000 µg/mL at $P<0.001$ significant comparisons with the control. At 250 µg mL^{-1}, the viability was found to be 73% ($P<0.05$), while at 7.8 -62.5 µg mL^{-1} concentrations, the viability exceeded 100%.

DISCUSSION

In this study, antioxidant capacity, antimicrobial potential, and vitality enhancing effects on L929 cells were evaluated to better understand the ethnomedical applications of *P. recta*. In our previous study, *P. recta* was found to have a high amount of total phenolics (185.85 ± 6,51 mg_{GAE} $g^{-1}_{extract}$). In phytochemical analyzes, kinic acid, ellagic acid, caffeic acid derivative, kaempferol glycoside, quercetin glucuronide, quercetin derivative, Bis-HHDP glucose, and potentillin was found (Ökdem et al. 2018). The fact that the content is rich in phenolic compounds has enabled *P. recta* to have high antioxidant capacity. Phenolic acids and flavonoids exhibit antioxidant activity by donating electrons (Kang et al. 1996), (Leopoldini et al. 2004). Antioxidant activity increased in proportion to the tannin content and the increase in the tannin concentration has been reported to increase the iron (III) reducing power in the literature (Zhang and Lin 2009). From recently published articles

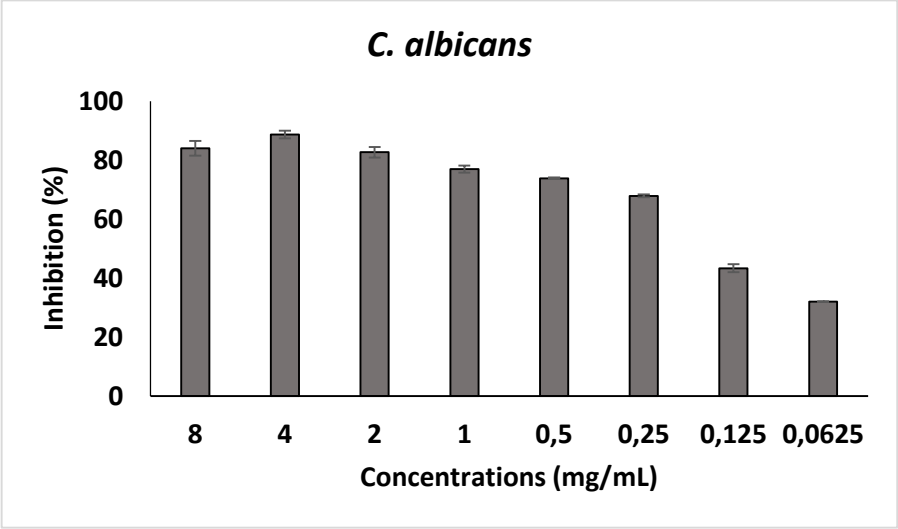

Figure 4. Inhibitory effects of P. recta extract on C. albicans. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

Şekil 4. P. recta ekstresinin C. albicans üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi

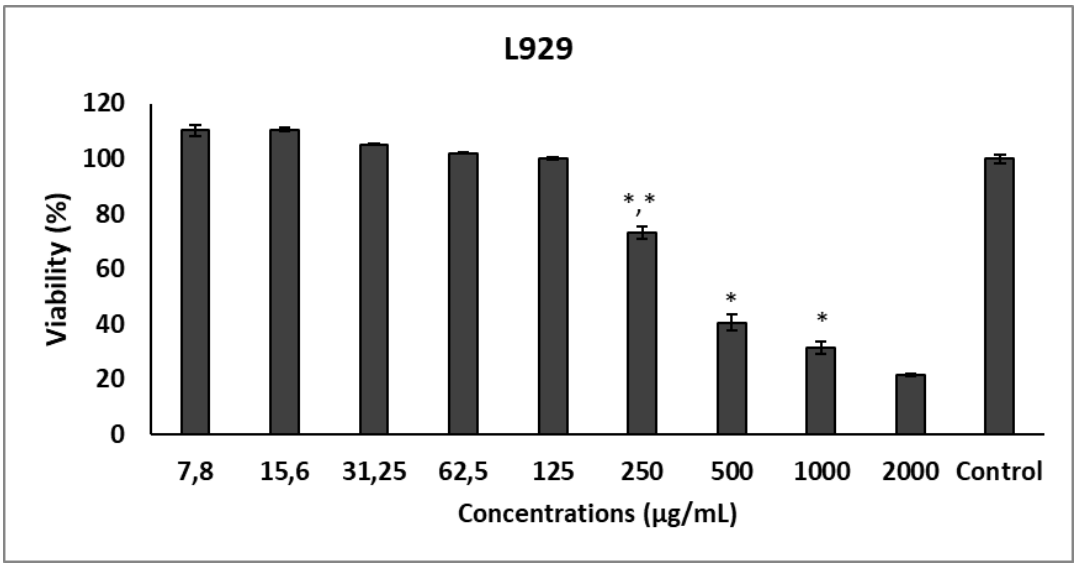

Figure 5. Effect of P. recta extract on L929 cell line viability. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3) * P < 0.01; ** P < 0.05.

*Şekil 5. P. recta ekstresinin L929 hücre hattı canlılığı üzerindeki etkisi. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi, * P < 0.01; ** P < 0.05.*

(Sut et al. 2019), *P. recta* methanol extract has been reported to exhibit 175.60 mg TE g^{-1} extract iron (III) reducing activity as measured by Trolox equivalent. In a different study conducted with *Potentilla reptans* and *Potentilla speciosa*, the iron reduction activities of extracts were found to be 81.59 to 219.97 mg TE g^{-1} extract for *P. reptans* and 56.97-214.49 mg TE g^{-1} extract for *P. speciosa* (Uysal et al. 2017).

The DPPH• radical scavenging effect of *P. recta* was not as high as AA, BHT, BHA, GA, and RA used as positive controls, but comparing with the literature we set a lower IC_{50} (0.19±0.00 mg mL^{-1}) value from the results found by Şöhretoğlu et al (2015).

In the DPPH radical scavenging activity with *P. reptans*, the IC_{50} value of the aerial part extract was found to be 12.11 µg mL^{-1} and the IC_{50} value of root was found to be 2.57 µg mL^{-1}. It has been found that the radical scavenging effect of the root was higher than that of the standard BHT (Tomovic et al. 2015). The biological activities of aqueous extracts of *Potentilla fruticosa*, *P. norvegica*, *P. pensylvanica*, *P. thuringiaca*, *P. crantzii,* and *P. nepalensis* were analyzed and their antiradical activities were found to be dose-dependently (Tomczyk et al. 2013).

While antioxidant activity is being investigated, it is essential to use lipid oxidation assay media. The *β-*

carotene/linoleic acid method, which is one of the most widely used methods for this purpose, is based on the principle that the degradation products resulting from linoleic acid oxidation will turn on the color of the *β*-carotene molecules (Liyana-Pathirana and Shahidi 2006). It has been observed that the *P. recta* extract has higher activity than GA and RA standards. Although there is no carotene bleach test of *P. recta* in the literature, the activity of *P. fruticosa* was evaluated and it was reported to exhibit strong antioxidant activity (Miliauskas et al. 2004).

$ABTS^{\cdot+}$ radical removal effect of extract and standards were evaluated at two different concentrations of 0.1 and 0.2 mg mL^{-1}. It has been determined that the scavenging effect of the extract is not as high as AA, BHT, BHA, GA, and RA used as positive controls at a concentration of 0.2 mg mL^{-1}. The scavenging effect of the extract was found to be higher than RA at the concentration of 0.1 mg mL^{-1}. It has been observed that extracts and all standards were more active in the high concentration in terms of $ABTS^{\cdot+}$ radical scavenging effect. In Sut et al.'s (2019) study, it was reported that *P. recta* water extract was more active than methanol and ethyl acetate extracts with a value of 3.85 mmol TE $g^{-1}_{extract}$ in $ABTS^{\cdot+}$ test system (Sut et al. 2019). Similar results were reported in the study conducted with *P. fruticosa*, *Potentilla glabra,* and *Potentilla parvifolia*, with activity results of 2140.22-2763.48 µmol Trolox g^{-1} at a concentration of 0.2 mg mL^{-1} (Wang et al. 2013).

The most important effects of free oxygen radicals are on the lipid systems. This phenomenon is known as lipid peroxidation and is briefly defined as the conversion of membrane phospholipids in cells into oxidized peroxide derivatives (Yarsan 2014). In this experiment, phospholipidic liposomes were rapidly produced MDA with iron (III) and ascorbic acid after $^{\cdot}OH$- induced peroxidation. Strong antioxidant effect was obtained from *P. recta* extract by preventing the formation of TBARs. The extract had higher activity than the ascorbic acid standard and did not show a significant difference in activity according to BHA, BHT, RA, and GA standards. Leaf, flower, and stem extracts of *P. fruticosa,* in the experiment using egg yolk, have been declared to demonstrate strong peroxidation inhibition (Yu et al. 2016). In alloxan-induced diabetic rats *P. fulgens* at 250 mg kg^{-1} bw dose caused a reduction (75%) in TBARS levels in liver tissue (Saio et al. 2012). Strong antioxidant properties of *Potentilla* species are also prominent with their lipid peroxidation inhibitory activities, and in our study, strong inhibition of *P. recta* on lipid peroxidation was reported by us for the first time.

Ferrozine test is frequently used in determining the chelating activity of iron ions. In the test, ferrozine complexes with Fe^{+2} ions, and these complexes are evaluated quantitatively. Chelating agents in the environment inhibit the formation of Fe^{+2} complexes with ferrozine (Sarikurkcu et al. 2015). The iron (II) chelating activity of *P. recta* extract was found to be greater than 10 mg/mL. When we compare the iron (II) chelating activity of extract with free radical scavenging activity results, any correlation is not observed. This can be attributed to the fact that the radical scavenging activity experimentation mechanisms are different. Contrary to our results, (Grochowski et al. 2017) state that *P. thuringiaca* showed strong metal chelating activity and they associated this with the compound 1-*o*-beta-D glucopyranosyl synapate. In a different study evaluating the metal chelating activity of methanol, water, and ethyl acetate extracts of *Potentilla anatolica,* the results are given between the range of 27.44- 32 mgEDTA g^{-1} (Uysal and Aktumsek 2015).

The $^{\cdot}OH$ is one of the most active radical that damages endogen components within the living organisms. In *in vitro* Fenton reaction, the $^{\cdot}OH$ bind to deoxyribose and converts to reactive TBA products by directing non-specific (Fe^{+2} + H_2O_2 + EDTA) and specific (Fe^{+2} + H_2O_2) $^{\cdot}OH$ radicals. Phenolics in the extracellular composition are liable for the scavenging of the $^{\cdot}OH$ in the $^{\cdot}OH$ -induced 2-deoxyribose degradation assay (Halliwell et al. 1987). The ability to inhibit 2-deoxyribose degradation directed by the non-specific $^{\cdot}OH$ of the extract was observed to be no significant ($P>0.05$) difference when compared to the AA used as a positive control.

The role of microorganisms in chronic wound pathology is quite important, and the combination of topical antimicrobial agents is used in the treatment. Wounds provide the microorganisms from the skin surface that make up the skin microbiota, a combined opportunity to gain access to the underlying tissues and to find optimal conditions for colonization and growth. *S. aureus, S. epidermidis, P. aeruginosa and C. albicans* are microorganisms found in wounds (Tomic-Canic et al. 2020; Wolcott et al. 2016). In a study evaluating the antimicrobial activity of extracts of *P. recta* against streptococci mutants, the visibility of inhibition zones was evaluated by measuring the zone diameters. The results presented that 50% methanol extract and subfractions (chloroform, diethyl ether, ethyl acetate, and n-butanol) inhibited the growth of all the tested oral *Streptococci* (Tomczyk et al. 2011). In another study, the MIC value of *P. recta* for *S. aureus* was stated as 25 mg mL^{-1}, and for *P. aeruginosa* as >100 mg mL^{-1} (Tomczyk et al. 2008). The low effect of *P. recta* against *P. aeruginosa* in our study is similar to the literature. Our result that *P. recta* is effective against *C. albicans* is also supported by the research of Tosun et al. (2006). According to their results, *P. recta* showed 21 mm inhibition zone diameter in the disk diffusion method against *C. albicans*.

L929 mouse fibroblast cells are a reference cell often used in many studies to determine the cytotoxicity of samples (Karatoprak et al. 2020; Pitz et al. 2016; Talekar et al. 2017). The extract does not decrease the viability below 250 µg mL^{-1} concentration compared to the control and even increases the viability in the concentration range of 7.8-62.5 µg mL^{-1} compared to the control. These results prove that the extract showed both antioxidant and antimicrobial activity in the concentration range where it is not toxic to fibroblast cells and increases proliferation.

CONCLUSION

In this study, antioxidant, antimicrobial and toxic effects of *P. recta* on fibroblast cell line were evaluated. *P. recta* has been found to have potent antioxidant capacity and moderate antimicrobial activity. At effective concentrations evaluated in both antioxidant and antimicrobial activity tests, the extract did not show toxicity to fibroblast cell lines and even increased viability. The results of the study provided preliminary information to understand the use of *P. recta* as a traditional herbal remedy, but *in vivo* studies are needed to fully prove efficacy.

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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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Removal of Heavy Metals in Water by Biosorption Method Using Three Different Bacillus sp-derived Biosorbents

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ABSTRACT
Water is an important part of the ecosystem for life. With industrialization, pollution in water resources has reached a worrying level. Water pollution due to heavy metals and their increasing concentrations have caused researchers to increase their interest in the subject due to the damage they cause to water ecosystems. It requires serious cost and time to eliminate the pollution caused by heavy metals in water. In recent years, the use of biosorption method using bacteria to remove heavy metals in water has become widespread. The main reason why this method is preferred is that gram-positive bacteria have a thick peptidoglycan layer on the cell wall and increases the adsorption capacity. In this study, in drinking, waste, river water and artificially prepared samples, batch method of heavy metal biosorption and biosorption competition in multiple prepared heavy metal solutions were investigated. For these processes, *Bacillus licheniformis sp. Bacillus subtilis sp.* and *Bacillus subtilis (ATCC 6051)* strains were used as a biosorbent. Biosorbtion of Cd (II), Cu (II), Pb (II), Fe (II), Ni (II) and Zn (II) metals from waters with these biosorbents at different pHs at 25 ° C with 0.25 mg L^{-1} It was carried out using. Surface morphological structures of biosorbents were evaluated using SEM images and element compositions were evaluated using EDAX profile. Element content was determined using ICP-OES. It was determined that heavy metal ions were removed up to 98% with maximum biosorption at pH 6.0.

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Farklı Biyosorbentler Kullanarak Su Ortamında Ağır Metallerin Biyosorbsiyon Metodu ile Giderilmesi

ÖZET
Su canlılar için ekosistemin önemli parçasıdır. Endüstrileşme ile birlikte su kaynaklarındaki kirlenme endişe edilir boyutlara ulaşmıştır. Ağır metallere bağlı su kirliliği ve artan konsantrasyonları su ekosistemlerine verdiği zarar nedeniyle araştırmacıların konuya olan ilgisinin artmasına sebep olmuştur. Ağır metallerin sularda oluşturduğu kirliliği gidermek ciddi maliyet ve zaman gerektirmektedir. Son yıllarda suda bulunan ağır metallerin uzaklaştırılması için bakteriler kullanılarak biyosorbsiyon yönteminin kullanılması yaygınlaşmıştır. Bu yöntemin tercih edilmesinin temel sebebi gram pozitif bakterilerin hücre duvarında kalın bir peptidoglikan tabakasına sahip olması ile adsorbsiyon kapasitesini artırmasıdır. Bu çalışmada içme, atık, nehir suları ve suni olarak hazırlanan numunelerde çalkalamalı metod kullanarak ağır metal biyosorbsiyonu ile birlikte çoklu hazırlanmış ağır metal çözeltilerinde biyosorbsiyon rekabeti incelenmiştir. Bu işlemler için Dicle nehri bölgesine ait topraklardan izole edilen *Bacillus licheniformis sp. Bacillus subtilis sp.* ve *Bacillus subtilis (ATCC 6051)* suşları ile sulu çözeltiden Cd (II), Cu (II), Pb (II), Fe (II), Ni (II) ve Zn

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 22.05.2021
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Anahtar Kelimeler
Bacillus subtilis
Bacillus licheniformis sp.
Biyosorpsiyon
ICP-OES
Atık su

(II) metallerinin biyosorbsiyonu değerlendirildi. *B. subtilis* suşları ve *B.licheniformis sp.* organizmalarının yüzey morfoljik yapıları SEM görüntüleri, element kompozisyonları EDAX verileri ile incelendi. ICP-OES kullanılarak element içeriği tespit edildi. Sulu çözeltideki Cd (II), Cu (II), Pb (II), Fe (II), Ni (II) ve Zn (II) metal iyonları farklı pH'larda 25 °C de 0,25 mg L^{-1} biomass ile biyosorbsiyon gerçekleştirildi. pH 6.0 da maksimum biyosorbsiyon ile metal iyonlarının % 98 varan oranda giderildiği belirlendi.

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INTRODUCTION

Heavy metal pollution is a worrying global problem as a result of rapid industrialization (Yahiaoui et al., 2020). High concentrations of the heavy metals pose a toxic threat to all life forms (Wu et al., 2020) (Morcali and Baysal, 2019). Heavy metal-containing wastewater from metal coating facilities, mining activities, fertilizer industry, battery and paper industries are directly or indirectly discharged into water sources. Heavy metals are not biodegradable in water, soil or atmosphere. Biologically accumulating heavy metals cause toxic and carcinogenic effects for many organisms (Kucukcongar et al., 2020; Lieswito et al., 2019).

Various methods are used to remove heavy metals from water and wastewater (Qin et al., 2020). Ion exchange (Zendehdel et al., 2019), chemical precipitation (Eltarahony et al., 2020), coplexing (Eggermont et al., 2020), membrane filtration (El-yazeed et al., 2020), adsorption (Subramani et al., 2019), biyosorbsiyon (Sabri et al., 2018) methods are some of them. Biosorption is a favorite technique for heavy metals removal. Being environmentally friendly and not requiring high energy increases the interest in this field by making the method economical. In biosorption applications, heavy metal adsorbing sources are considered as biosorbents (Kouli et al., 2020; Halimahtussaddiyah, 2017; Nasab et al., 2020). Biosorbent sources such as bacteria (Abedinzadeh et al., 2020), fungi (Qin et al., 2020; Verma et al., 2013) yeasts (Yasmin et al., 2020), algae (Liu et al., 2018), shellfish ((Keshvardoostchokami et al., 2017), plants (Parthasarathy and Narayanan, 2014) are used in biosorption applications. Biosorbtion studies performed with biologically sourced adsorbents are in a very interesting position in terms of easy application stages, low cost, high biosorption efficiency, reuse of the adsorbent and not containing toxic chemicals that will harm health (Qin et al., 2020; Shokoohi et al., 2020; Khameneh and Moharreri, 2020).

In studies using bacteria as adsorbents, it has been observed that gram positive bacteria exhibit better character than gram negative bacteria in terms of biosorption efficiency. Gram-positive bacteria have a thick cell wall, which increases biosorption (Baran and Duz, 2019; Biswas et al., 2020).

In this study, the removal of Cd (II), Cu (II), Pb (II), Fe (II), Ni (II), Zn (II) heavy metals prepared at appropriate pH values with different biomasses using the batch biosorption technique, and the removal of these heavy metals It was also aimed to examine the biosorption competition in the solution.

MATERIAL and METHOD

Isolation of Bacterial Strains

1 g soil sample was taken for each strain to be used in the isolation of the strains from the soils of the Tigris river coastal region. The samples were mixed with 4.5 mL of sterile distilled water. The homogenized samples were kept at 80 °C for 10 minutes (Lennete et al., 1985). Samples were diluted using sterile water to obtain colonies. It was incubated at 37 °C by inoculation on nutrient agar medium containing 10 % NaCl. At the end of the period, the colonies grown in the medium were evaluated according to their morphology. Colonies with Bacillus morphology were seeded on nutrient agar medium and incubated at 37°C for 1 night (Sneath et al., 1986). Taxonomic descriptions of *Bacillus licheniformis sp. (B. licheniformis sp.*) and *Bacillus subtilis sp.* (*B. subtilis sp.*) by Dicle University, Dr. It was carried out by Hüsamettin Aygün.

Equipment and Chemicals

Metal salts of analytical purity $Cd(NO_3)_2.4H_2O$, $Pb(NO_3)_2$, Cu $(NO_3)_2.3H_2O$, $Zn(NO_3)_2.4H_2O$, $Fe(SO_4).7H_2O$ and $Ni(NO_3)_2.7H_2O$ were used. Stock solutions and pH adjustment (NaOH, HCl and HNO3) were used as acid and base in experimental studies. Stock solutions of iron, lead, copper, nickel, zinc and cadmium were prepared as 1000 mg/L from these metal salts.

Perkin-Elmer OPTİMA 5300 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES),

HANNA HI-2211 7000 seven multi brand pH meters, ALC-4235 A brand model centrifuge devices, as well as EVO 40 LEQ model SEM-EDAX devices for biosorbent characterization was used.

Biosorbent Preparation Process

Isolated from the soils on the banks of the Tigris river *B.licheniformis sp.* and *B. subtilis* wild strains as well as the *Bacillus subtilis ATCC 6051* (*B. Subtilis*) strain were used for biosorption as a biosorbent. Nutrient agar, nutrient broth solid media forms were used to the cultivate bacteria.

The microorganisms used are gram positive bacilli and have a thick peptidoglycan layer. The structure of this layer is a large polymer formed by cross-linking peptide chains with N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) pentapeptide chains (Reith and Mayer, 2011; Tocheva et al., 2013).

Inoculation was performed on nutrient agar medium. It was left to incubate at 37 °C for 24 hours. Then the microorganisms grown were transferred from solid media to 1.0 liter nutrient broth media. The bacteria were left in a shaker at 37 °C for 24 hours to grow. The media content obtained after growth was subjected to centrifugation at 7,000 rpm for 15 minutes. The bottom pellet was washed several times with sterile distilled water. Then it was left to dry. The dried pellet was passed through a 180 µm sieve. *B. licheniformis sp.* (Fl), *B. subtilis sp.* (Fs) and *B. subtilis* (B1) biosorbent naming was done. Biosorbents have been made ready for the biosorption process.

Heavy Metal Biosorption with Batch Method

Biosorbtion processes with biosorbents were performed using the physical adsorption batch method (Ali et al., 2020). This method is based on the removal of heavy metals from the aqueous solution by stirring (Mwandira et al., 2020; Nazmara et al., 2020). Different pH biosorption trials were carried out and the pH values in which the metals showed the best activity among these studies were selected for the biosorption application. In the studies conducted with the batch method, the removal of heavy metals at different pH's and low concentrations in water was investigated. (Shokoohi et al., 2020; Tengxia et al., 2020).

Biosorption at Low Concentration

From the stock solutions of 1000 ppm containing metal salts, dilutions were made for each metal (Cd, Cu, Fe, Ni, Pb and Zn) and the solutions were prepared with an initial concentration of 1.5 ppm. The pH of the solutions of Cd, Cu, Fe, Ni, Pb and Zn was adjusted as 5, 4.5, 6, 6, 5.5 and 6.5, respectively. Then, B1, Fl and Fs biosorbents were weighed 0.25 mg and left to the 1000 mL flasks. Metal solutions were added and agitation was carried out at 25 °C. After the samples were taken and centrifuged, readings were made with ICP-OES. After the equilibrium concentrations were determined, the percentage metal removal capacities of B1, Fs and F1 biosorbents for each metal were evaluated. Percent metal removal was calculated using the equation stated below (Baran and Duz, 2019).

$$\%A=((Co-Cd))/Co \times 100 \quad (1)$$

Co = Initial metal concentration,

Cd = Refers to the concentration of metal at equilibrium.

Low Concentration Biosorption Competition

A mixture of metal ions Ni (II), Cd (II), Pb (II), Cu (II), Fe (II) and Zn (II) with an initial concentration of 1.5 ppm was prepared from the stock solutions. The proper pH was adjusted. The biosorbents (F1, Fs and B1) were weighed 0.25 mg and each was placed in a separate 1000 mL flask. The prepared metal mixture solution was added on them. It was left to stir during equilibrium at 25 ° C. Samples were centrifuged and readings for metal contents were made with the ICP-OES device. Then percent recovery was calculated with the equation shown in formula (1).

Biosorption Competition in Drinking, River and Wastewater

B1 biosorbent with the best biosorbent ability was selected in the applications. This stage, which is the last stage of biosorption applications, was evaluated for its applicability to drinking, waste and river waters. Samples were taken from different stations (Table 5-6). The initial concentrations of the water samples taken were determined ~~with~~ via the ICP-OES device. 0.25 mg L-1 of B1 biosorbent was weighed and put into the flasks and water samples taken from different points were added on them. ~~It~~ Samples ~~was~~ were left in a shaking water bath for one hour. The samples taken at different times were centrifuged and the Equilibrium concentration was determined. Percent metal removal was calculated by the equation defined in formula (1).

FINDINGS and DISCUSSION

Biosorbent Characterization

SEM-EDAX micrographs

Morphological appearances and element compositions of Fs, F1 and B1 biosorbents before and after biosorption were evaluated by the SEM analysis. The bacterial cell is morphologically rod-shaped and has a smooth surface. However, its morphology changed after metal biosorption (Figure 1-3) (Pugazhendhi et al., 2018; Dahaghin et al., 2017).

In the EDAX profile, a decrease was observed in B1 biosorbent after biosorbtion in Potassium (K) ions. In

the study conducted with the *Bacillus gibsonii s-2* strain, it is reported that the biosorbent absorbs lead ions as a result of the biosorption of the lead metal. In the EDAX analysis, it was stated that the effective decrease in Na^+ ions, one of the ions in the bacterial profile after biosorption, was based on the ion exchange between Pb-Na (Zhang et al., 2013). The decrease in K^+ in the EDAX profile indicates that biosorption occurs by ion exchange in similarly between K-Pb, K-Fe and K-Cd. In addition, heavy metals adsorbed after biosorption are also reflected in the EDAX profile (Figure 4). Similar findings of SEM-EDX micrographs were presented in recent biosorption studies (Ayucitra et al., 2017; Zendehdel et al., 2019; Kucukcongar et al., 2020; Su et al., 2020).

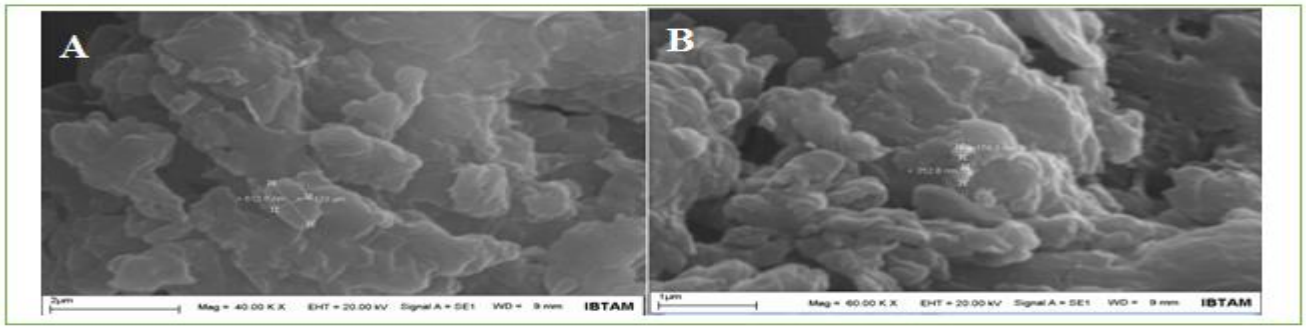

Figure 1. SEM images of the B1 biosorbent (A) before and (B) after metal interactions.
Şekil 1. B1 Biyosorbentinin metal etkileşiminden önceki (A) ve metal ile etkileştikten sonraki SEM görüntüleri. (B)

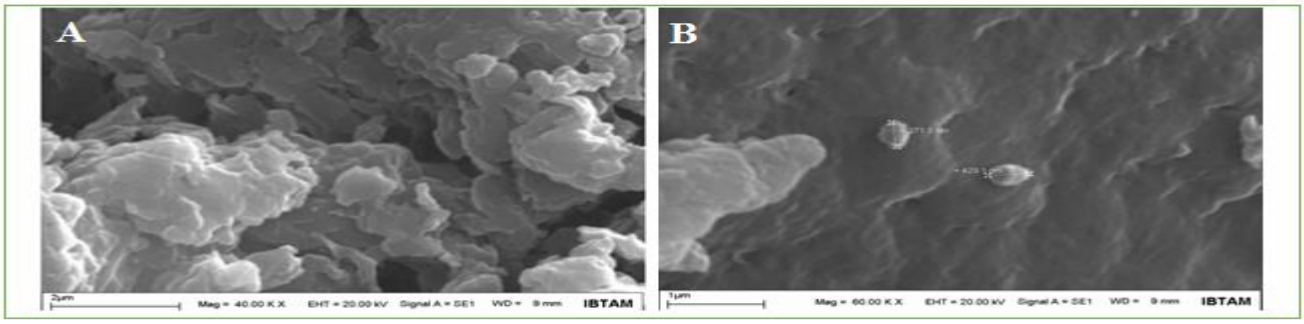

Figure 2. SEM images of the F1 biosorbent (A) before and (B) after metal interactions.
Şekil 2. F1 Biyosorbentinin (A) metal etkileşiminden önceki ve (B) metal ile etkileştikten sonraki SEM görüntüleri.

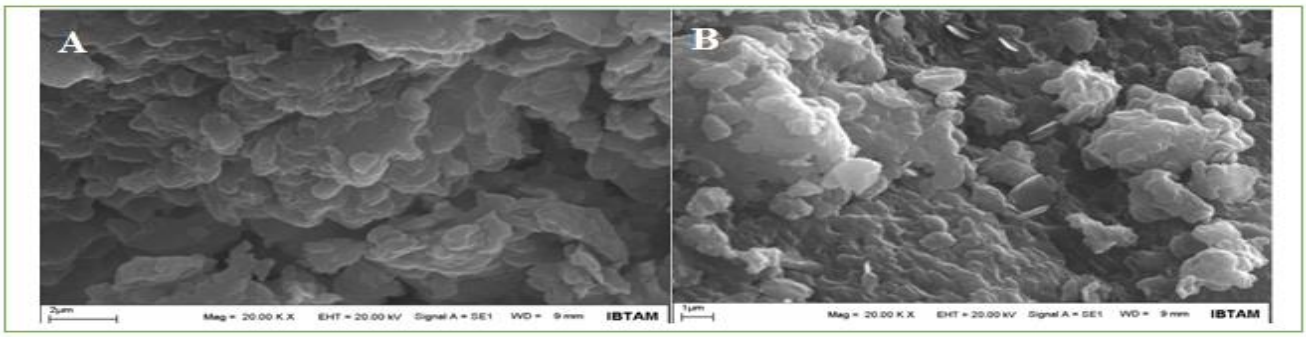

Figure 3. SEM images of the Fs biosorbent (A) before and (B) after metal interactions.
Şekil 3. Fs Biyosorbentinin (A) metal etkileşiminden önceki ve (B) metal ile etkileştikten sonraki SEM görüntüleri.

Biosorption Studies

Cd, Cu, Fe, Ni, Pb and Zn 1.5 ppm solutions were evaluated by biosorption at pH 5, 4.5, 6, 6, 5.5 and 6.5, respectively. Equilibrium concentrations and metal removal percentages were calculated using the equation in formula (1).

When table 1 and figure 5 data were examined and compared for biosorbtion at low concentrations, it is seen that the metals that F1, Fs and B1 biosorbents adsorb best were Cd and Pb. It is seen that the biosorbent that provides the best Cd and Pb removal in biosorbents is B1 with % 98.13 and % 97.53 removal rates, respectively. It has been reported in the biosorption studies that Cd and Pb ions are largely adsorbed (Abedinzadeh et al., 2020; Mwandira et al., 2020). It has been stated in studies that bacteria are more resistant to Pb and Cd stress (Su et al., 2020; Borralho et al., 2020).

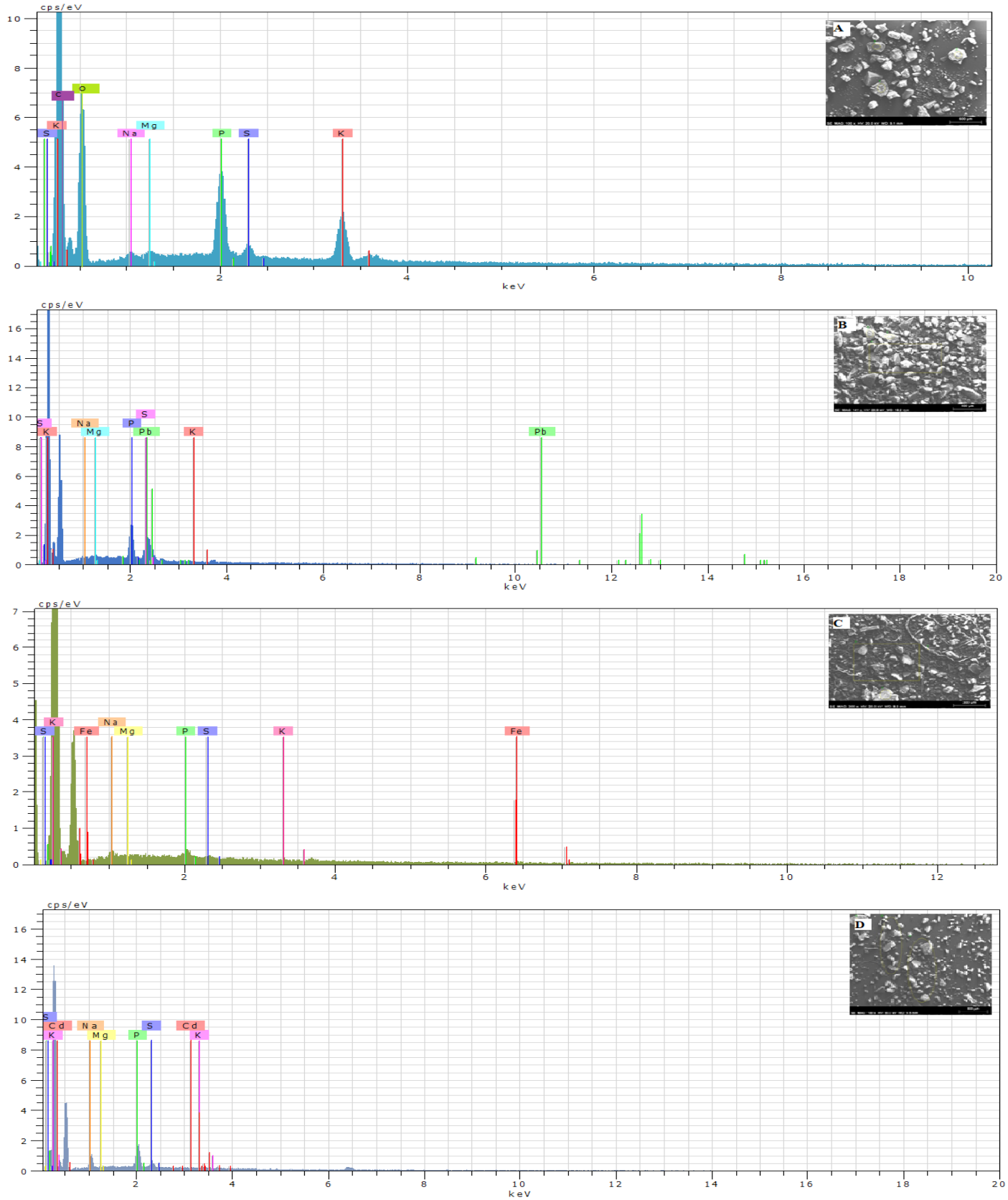

Figure 4. EDAX data of B1 biosorbent (A), after interaction with B1-Pb (B), after interaction with B1-Fe (C), after interaction with B1-Cd (D).

Şekil 4. B1 biyosorbentine ait EDAX verileri (A), B1-Pb ile etkileşme sonrası (B), B1-Fe ile etkileşme sonrası (C), B1-Cd ile etkileşme sonrası (D)

Table 1. Low concentration metal ion equilibrium concentrations and % recovery data of biosorbents (F1, B1 and Fs)

Çizelge 1. Biyosorbentlerin (F1, B1 ve Fs) düşük konsantrasyonda metal iyonu denge konsantrasyonları ve % kazanım verileri

			B1		FI		Fs	
Metal Ions	**pH**	**$C_{o(ppm)}$**	**$C_{e(ppm)}$**	**Recovery%**	**$C_{e\ (ppm)}$**	**Recovery%**	**$C_{e\ (ppm)}$**	**Recovery%**
Cd	5	1.5	0.028±0.0008	98.13	0.054±0.0007	96.13	0.083±0.0008	94.46
Cu	4.5	1.5	0.178±0.0043	88.13	0.312±0.0032	79.20	0.472±0.0066	68.53
Fe	6	1.5	0.116±0.0038	92.26	0.161±0.0020	89.25	0.375±0.0015	75.00
Ni	6	1.5	0.311±0.0034	79.26	0.339±0.0127	77.40	0.635±0.0052	57.66
Pb	5.5	1.5	0.037±0.0016	97.53	0.059±0.0021	96.06	0.114±0.0017	92.40
Zn	6.5	1.5	0.099±0.0023	93.40	0.135±0.0038	91.00	0.236±0.0055	84.26

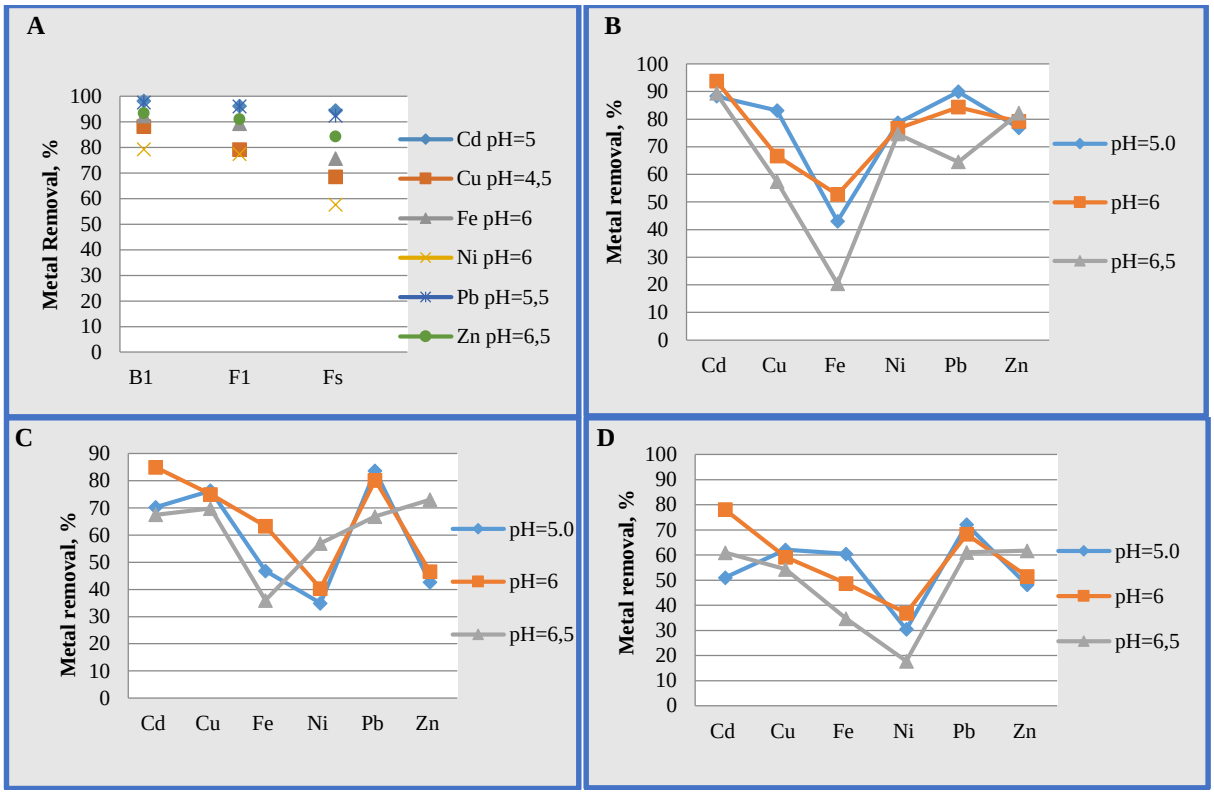

Figure 5. A. Low concentration metal ion biosorption Table, B. The graph of the biosorbent competition with the pH effect of the B1 biosorbent in the medium containing all metals, C. The biosorbent competition graph with the pH effect of the F1 biosorbent in the medium containing all metals, D. Graph of biosorbtion competition with pH effect of Fs biosorbent in media containing all metals.

Şekil 5. A. Düşük konsantrasyonda metal iyonu biyosorbsiyon grafiği, B. biyosorbentinin metallerin tümünü içeren ortamda pH etkisi ile biyosorbsiyon rekabeti grafiği, C. F1 biyosorbentinin metallerin tümünü içeren ortamda pH etkisi ile biyosorbsiyon rekabeti grafiği, D. Fs biyosorbentinin metallerin tümünü içeren ortamda pH etkisi ile biyosorbsiyon rekabeti grafiği.

Biosorbtion competitions of B1, F1 and Fs biosorbents at different pH values in a mixture solution of Cd (II), Cu (II), Fe (II), Ni (II), Pb (II) and Zn (II) metal ions were investigated. When Figure 5 and Table 2-4 are examined, it is observed that the biosorption capacity of some metals increases and some decreases with the increase of pH. This reveals the importance of pH in biosorbent metal adsorption. Studies have indicated that pH changes affect the biosorption capacity (Pratush, 2018; Zhao et al., 2019).

When we evaluated the results of biosorption application in drinking, river and wastewater, values were found below the biosorption capacity efficiency obtained in experimental studies. The presence of different metal contents in this type of water and their participation in biosorption may have affected this situation. The highest biosorption efficiency was obtained with % 81.09 removal of Pb and % 75 of Cd in the sample taken before the Dicle river facility (Table 5-6).

Biosorbtion studies performed with different biosorbents are shown in Table 7.

Table 2. Results of biosorbent competition of B1 biosorbent in different pH solutions containing a mixture of metals

Çizelge 2. B1 biyosorbentinin metallerin tümünü içeren farklı pH da ortamlarında biyosorbsiyon rekabetinin incelenmesi

	pH=5		pH=6		pH=6.5	
Metal Ions	$C_{e\ (ppm)}$	Recovery %	$C_{e\ (ppm)}$	Recovery %	$C_{e\ (ppm)}$	Recovery %
Cd	0.176±0.0012	88.26	0.093±0.0009	93.80	0.160±0.0003	89.33
Cu	0.253±0.0039	83.13	0.500±0.0028	66.66	0.639±0.0060	57.40
Fe	0.854±0.0066	43.00	0.510±0.0038	52.66	1.194±0.0030	20.40
Ni	0.320±0.0111	78.66	0.350±0.0040	76.67	0.381±0.0096	74.60
Pb	0.151±0.0011	89.93	0.235±0.0027	84.33	0.532±0.0014	64.53
Zn	0.348±0.0061	76.80	0.313±0.0039	79.13	0.268±0.0063	82.13

Table 3. Results of biosorbent competition of F1 biosorbent in different pH solutions containing a mixture of metals

Çizelge 3. F1 biyosorbentinin metallerin tümünü içeren farklı pH da ortamlarında biyosorbsiyon rekabetinin incelenmesi

	pH=5		pH=6		pH=6.5	
Metal Ions	$C_{e\ (ppm)}$	Recovery %	$C_{e\ (ppm)}$	Recovery %	$C_{e\ (ppm)}$	Recovery %
Cd	0.446±0.0856	70.26	0.229±0.0036	84.93	0.487±0.0028	67.53
Cu	0.355±0.0054	76.33	0.375±0.0052	75.00	0.453±0.0040	69.8
Fe	0.798±0.0028	46.80	0.550±0.0088	63.33	0.960±0.047	36.00
Ni	0.976±0.0297	34.93	0.895±0.0185	40.33	0.646±0.0090	56.93
Pb	0.246±0.0021	83.66	0.297±0.0015	80.20	0.497±0.0057	66.86
Zn	0.857±0.0059	42.86	0.802±0.0057	46.53	0.404±0.0008	73.06

Table 4. Results of biosorbent competition of Fs biosorbent in different pH solutions containing a mixture of metals

Çizelge 4. Fs biyosorbentinin metallerin tümünü içeren farklı pH da ortamlarında biyosorbsiyon rekabetinin incelenmesi

	pH=5		pH=6		pH=6.5	
Metal Ions	$C_{e\ (ppm)}$	Recovery %	$C_{e\ (ppm)}$	Recovery %	$C_{e\ (ppm)}$	Recovery %
Cd	0.735±0.0458	51.00	0.328±0.0033	78.13	0.586±0.0145	60.93
Cu	0.568±0.0066	62.13	0.612±0.0050	59.2	0.685±0.0012	54.33
Fe	0.983±0.0104	60.46	0.769±0.0021	48.73	0.979±0.0021	34.72
Ni	1.043±0.0729	30.46	0.947±0.0229	36.86	1.234±0.0069	17.73
Pb	0.418±0.0043	72.13	0.476±0.0015	68.26	0.586±0.0074	61.06
Zn	0.775±0.0062	48.33	0.727±0.0096	51.53	0.574±0.0055	61.70

Table 5. Biosorption efficiency values of Cu, Cd and Pb metals in drinking water and wastewater samples were taken from different stations.

Çizelge 5. Farklı bölgelerden alınan içme ve atık su örneklerinde Cu, Cd ve Pb metlallerine ait biyosorbsiyon verimi değerleri

Sampling Point	Cu			Cd			Pb		
	Co ppb	Ceppb	% recovery	Co ppb	Ce ppb	% recovery	Co ppb	Ce ppb	% recovery
DI	15.62±1.8	8.128±0.6	48.07	0.97±0,001	0.44±0.02	54.63	0.66±0.008	0.13±0.005	71.73
DNO	106.6±0.35	37.6±0.02	66.03	9.48±0.09	2.37±0.03	75.05	2.75±0.3	0.52±0.8	81.09
DAG	24.04±0.81	11.04±1.34	54.16	<LOD	<LOD	-	1.13±0.30	0.29±0.004	74.36
DAC	14.25±0.5	7.03±0.11	51.84	<LOD	<LOD	-	0.96±0.005	<LOD	-
DNS	113.7±0.25	113.7±0.25	60.17	13.37±0.06	4.71±0.04	63.16	5.16±0.98	1.29±0.007	75.00
MI	99.74±2.95	99.74±2.75	57.98	2.26±0.08	1.03±0.06	54.42	2.34±0.36	0.67±0.009	68.34

Table 6. Biosorption efficiency values of Ni, Zn and Fe metals in drinking water and wastewater samples taken from different stations

Çizelge 6. Farklı bölgelerden alınan içme ve atık su örneklerinde Ni, Zn ve Fe metlallerine ait biyosorbsiyon verimi değerleri.

	Ni			Zn			Fe		
Sampling Point	**Co ppb**	**Ce ppb**	**Recovey %**	**Co ppb**	**Ce ppb**	**Recovey %**	**Co ppb**	**Ce ppb**	**Recovey %**
DI	15.80±08	0.08±0.6	61.56	101.0±9.1	42.57±3.02	57.90	8.1±0.21	2.26±0.06	72.09
DNO	11.54±0.75	3.57±0.21	68.12	52.28±1.08	17.3±1.48	67.01	162.1±3.4	58.32±1.55	67.09
DAG	30.16±2.2	9.6±1.22	69.00	110.34±9.6	48.6±1.43	56.48	10.8±1.12	3.91±0.07	68.76
DAC	14.9±0.86	4.46±0.07	71.04	51.48±1.28	19.5±0.04	61.64	7.17±0.05	2.63±0.04	63.00
DNS	22.3±1.45	8.17±0.42	63.33	79.13±2.9	34.3±1.48	56.96	213±1.95	79.38±0.38	62.91
MI	9.76±0.95	3.51±0.61	64.98	257.7±4.8	90.73±1.6	62.96	71.5±1.36	27.16±1.09	62.31

DI: Diyarbakir Drinking Water, **DNO:** Tigris River Before Installation, **DAG:** Introduction to Dicle Treatment Plant, **DAC:** Leaving Dicle Treatment Plant, **DNS:** Tigris River After Facility and **MI:** MI: named as Mardin Drinking Water samples. ***Dİ:****Diyarbakır İçme suyu,* ***DNO:*** *Dicle Nehr i Tesis Öncesi,* ***DAG:*** *Dicle Arıtma Tesisine Giriş,* ***DAÇ:*** *Dicle Arıtma Tesisinden Çıkış,* ***DNS:*** *Dicle Nehri Tesis Sonrası,* ***MI:*** *Mardin İçme Su numuneleri olarak adlandırılmıştır.*

Table 7. Results of biosorption studies for heavy metal removal

Çizelge 7. Ağır metal adsorbsiyonu için yapılan çalışmalar

Biosorbent	Metals	pH	Temperature °C	Removal %	References
Chitosan / oxide nanocomposite	Ni(II), Cd(II), Pb (II)	3	22	98	(Keshvardoostchokami, et al., 2017)
Algae	Cu(II)	4	25-45	96.4	(N A Lieswito, 2019)
Pseudomonas sp	Cd(II)	7	-	92	(Xu et al., 2020)
Bacillus subtilis	Zn(II)	7.6-8.4	30	89.5	(Sun et al., 2020)
Bacillus gibsonii S-2	Pb (II)	4	40	-	(Zhang et al., 2013)
Talaromyces islandicus	Pb (II)	5	30	90	(Sharma et al., 2020)
Eucalyptus camaldulensis	Pb (II)	7	25	85	(Sabri et al., 2018)
Algae (Mixed culture)	Pb (II)	6	30	95.43	(Mousavi et al., 2019)
Ralstonia solanacearum	Pb (II)	6	35	90	(Pugazhendhi et al., 2018)
Sargassum muticum	Pb (II)	5	20	96	(Hannachi and Hafidh, 2020)
B. licheniformis	Pb (II)	6	30	98	(Wen et al., 2018)

CONCLUSION

Depletion of water resources and exposure to toxic pollution with heavy metals is a major problem. Finding ways to deal with them is a serious issue that every segment should focus on. Various methods are used to remove toxic contaminated water resources from heavy metals or to protect them from such situations. Using biosorbent, heavy metal removal with biosorption has advantages over physical and chemical applications. Some of these advantages are the ease of the application process, the absence of toxic chemicals in the process stages, and the economical nature of the biosorbent after the process. When choosing a biosorbent for biosorbent, qualities such as its applicability to drinking and wastewater at low concentrations, high biosorption capacity, cheap cost and no risk of being a pathogen for human health should be considered. Considering these criteria, soil bacilli resistant to extreme conditions, which do not carry pathogenicity risk, were used as biosorbents. As a result of the experiments carried out at different pH values at 25 °C, the maximum adsorption capacity at pH 6.0 was determined. The morphological change of biosorption in biomass and its element composition were also revealed ~~by~~ via the SEM and EDAX micrographs. It was determined that the highest adsorption capacities were about % 98 of Pb (II) and Cd (II) metals. As a result, heavy metal removal up to 98 % was achieved with the high metal binding capacity of bacteria. Considering the widespread presence of many biosorbents in nature, the biosorption method will provide significant benefits for heavy metal removal in water.

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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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Reyhan Bitkisinin (*Ocimum basilicum* L.) Adventif Kök Kültürlerinde Rosmarinik Asit Üretim Olanaklarının ve Antioksidan Kapasitenin Araştırılması

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ÖZET

Bu çalışmada, reyhan bitkisinin (*Ocimum basilicum* L.) adventif kök süspansiyon ve katı kültürlerinde rosmarinik asit üretim olanakları, toplan fenolik ve flavonoid içerikleri ve antioksidan kapasitenin belirlenmesi amaçlanmıştır. Adventif kök katı kültürlerin oluşturulmasında *in vitro* koşullarda yetiştirilen 30 günlük bitkilerin hipokotil kısımları eksplant kaynağı olarak kullanılmıştır. Eksplantlar 3.3 g L^{-1} MS (Murashige ve Skoog), 30 g L^{-1} sukroz ve 2 g L^{-1} phytagel ve 2 mg L^{-1} indol-3-bütirik asit içeren besin ortamında karanlık koşullarda kültüre alınmıştır. Bu ortamda gelişen adventif kökler süspansiyon kültürlerinin oluşturulmasında kullanılmıştır. Süspansiyon kültürünün 10, 20 ve 30. günlerinde adventif kökler hasat edilerek analizler yapılmıştır. Adventif köklerin rosmarinik asit içeriği HPLC cihazıyla analiz edilmiştir. Antioksidan kapasiteleri katyon radikali giderme (ABTS), indirgeme gücü (FRAP) ve serbest radikal giderme (DPPH) metotları ile belirlenmiştir. Rosmarinik asit içeriği en yüksek adventif kök süspansiyon kültürünün 30. gününde 32.38 mg g^{-1} olarak belirlenmiştir. En yüksek toplam fenolik bileşik içeriği süspansiyon kültürünün 20. gününde 32.94 mg GAE g^{-1} olarak belirlenmiştir. DPPH, ABTS ve FRAP aktivitesi en yüksek süspansiyon kültürünün 30. gününde belirlenmiştir. Sonuç olarak reyhan bitkisinin süspansiyon kültüründen elde edilen adventif köklerin rosmarinik asit üretimi için uygun materyaller olduğu düşünülmektedir.

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Investigation of Rosmarinic Acid Production Possibilities and Antioxidant Capacities in Adventitious Root Cultures of Basil (*Ocimum basilicum* L.)

ABSTRACT

In this study, It was aimed to determine the rosmarinic acid production possibilities, total phenolic and flavonoid contents and antioxidant activities of adventitious root suspension and solid cultures of basil (*Ocimum basilicum* L.). The hypocotyl parts of 30-day-old plants grown under *in vitro* conditions were used as the source of explants for the establishment of adventitious root solid culture. The explants were cultured in dark conditions in nutrient medium containing 3.3 g L^{-1} MS (Murashige and Skoog), 30 g L^{-1} sucrose, 2 g L^{-1} phytagel and 2 mg L^{-1} indole-3-butyric acid (IBA). Adventitious roots obtained under these conditions were used in the establishment of suspension cultures. Adventitious roots were harvested and analyzed at 10, 20 and 30 days of suspension culture.

The rosmarinic acid content of adventitious roots was analyzed by HPLC. Antioxidant capacities were determined by reducing power (FRAP), free radical scavenging (DPPH) and cation radical scavenging (ABTS) methods. The highest rosmarinic acid content was determined as 32.38 mg g^{-1} on the 30th day of the adventitious root suspension culture. The highest total phenolic compound content was determined as 32.94 mg GAE g^{-1} on the 20th day of suspension culture. The highest DPPH, ABTS and FRAP activities were determined on the

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30th day of the suspension culture. Overall, it can be concluded that adventitious roots obtained from suspension cultures of basil plant are suitable materials to produce rosmarinic acid.

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GİRİŞ

Lamiaceae familyasının Ocimum cinsine ait türleri Türkiye'de yaygın olarak reyhan veya fesleğen olarak adlandırılmaktadır (Günay ve Telci, 2017). Reyhan bitkisi (*Ocimum basilicum* L.) tıbbi ve aromatik bir bitki olup aromaterapi, kozmetik, parfüm ve gıda ürünlerinde yaygın olarak kullanılmaktadır (Ekmekci ve Aasim, 2014). Ayrıca reyhan bitkisinin antibakteriyel, antifungal, antikanser, antiinflamatuar, antioksidan, antiülser, antiviral, insektisidal, solucan düşürücü, hipoglisemik, hipolipidemik, kardiyak uyarıcı ve yara iyileştirici gibi farmakolojik aktivitelere sahip olduğu da bildirilmiştir (Marwat ve ark., 2011). Çok sayıda genotipi bulunan reyhan bitkisinin kalitesinin belirlenmesinde kimyasal bileşimi çok önemli bir yer tutmakta ve başlıca fenilpropanoid bileşiğini rosmarinik asit oluşturmaktadır (Zeljkovi´c ve ark., 2020). Bu bitkiye ait genotiplerin yüksek miktarda fenolik bileşik içerdiği ve en fazla bulunan fenolik bileşiklerin sırası ile rosmarinik asit, rutin, sisorik asit, kaftarik asit, 4-hidroksibezoik asit ve kafeik asit olduğu belirlenmiştir (Genç, 2016). Rosmarinik asit; kafeik asit ve 3,4-dihidroksifenillaktik asit esteri olan fenolik bir bileşik olup biyosentezi fenilpropanoid yolu ile tirozin türevli yol aracılığıyla ile gerçekleşmektedir (Zhang ve ark., 2014; Jiang ve ark., 2016). Rosmarinik asit ve türevlerinin antienflamatuar, antioksidan, antialerjik, antianjiyojenik, antitümör, antimikrobiyal, antiviral ve nöroprotektif gibi biyolojik aktivitelerin belirlenmesi nedeniyle son zamanlarda yoğun ilgi görmektedir (Kim ve ark, 2015). Ancak doğal kaynakların sınırlı olması ve kimyasal sentezinin kompleksliği nedeni ile bu bileşiği olan talep karşılanamamaktadır (Jiang ve ark., 2016). Rosmarinik aside olan talebi karşılamak için bitki hücre kültürü ve saçak kök kültürleri gibi biyoteknolojik yöntemlerle üretim olanakları yoğun bir şekilde araştırılmıştır (Khojasteh ve ark., 2014). Bu bağlamda *Salvia miltiorrhiza, Agastache rugosa, Coleus forskohlii ve Coleus blumei* gibi Lamiaceae familyasına ait türlerden elde edilen saçak köklerde rosmarinik asit üretimi, elistasyonu, biyosentezi ve biyoaktivitesi kapsamlı bir şekilde incelenmiştir (Srivastava ve ark., 2016). Ayrıca rosmarinik asit üretimi *Salvia officinalis* L. bitkisinin *in vitro* sürgün kültürlerinde (Krac˘un-Kolarevic´ ve ark., 2015), *Satureja khuzistanica* Jamzad bitkisinin hücre süspansiyon kültürlerinde (Sahraroo ve ark., 2016) ve *Coleus blumei* bitkisinin kallus ve hücre süspansiyon kültürlerinde (Qian ve ark., 2009) araştırılmıştır.

Reyhan bitkisinin (*Ocimum basilicum*) saçak kök kültüründe (Srivastava ve ark., 2016), kallus kültüründe (Nazir ve ark., 2019; Duran ve ark., 2019) ve hücre süspansiyon kültürlerinde (Kintzios ve ark., 2004) rosmarinik asit üretimi önemli ölçüde incelenmiştir. Ancak adventif kök kültürlerinde üretim olanakları üzerine çalışmalar oldukça sınırlı kalmıştır. Adventif kökler bitkilerin kök dışındaki yaprak ve gövde gibi kısımlarından kök gelişimi yönünde uyarılmasıyla elde edilen kökler olarak tanımlanmaktadır (Steffens ve Rasmussen, 2016; Rahmat ve Kang, 2019). Bu kökler; hızlı büyümeleri ve biyoaktif bileşiklerin istikrarlı üretkenliği nedeniyle ticari boyutta metabolit üretimine olanak sağlayan uygun materyallerdir (Cui ve ark., 2013; Le ve ark., 2018; Rahmat ve Kang, 2019). Bu çalışmada reyhan (*Ocimum basilicum*) bitkisinden elde edilen adventif köklerin katı ve sıvı süspansiyon kültür koşullarında rosmarinik asit üretim olanakları, toplam fenolik ve flavonoid içerikleri ve antioksidan kapasitelerinin (DPPH, ABTS ve FRAP yöntemlerine göre) belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Adventif Kök Katı Kültürlerin Oluşturulması

Araştırmada bitkisel materyal olarak *in vitro* koşullarda yetiştirilen reyhan (*Ocimum basillicum* L.) bitkisi kullanılmıştır. Adventif kök kültürlerinin oluşturulmasında steril şartlarda yetiştirilen bir aylık bitkilerin hipokotil kısımları eksplant kaynağı olarak kullanılmıştır. Eksplantlar 3.3 g L^{-1} MS (Murashige ve Skoog, 1962), 30 g L^{-1} sukroz ve 2 g L^{-1} phytagel ve 2 mg L^{-1} indol-3-bütirik asit (IBA) içeren besin ortamında karanlık ortam koşullarda inkübatörde (25 °C) kültüre alınmıştır. Eksplantlar 15. günde aynı içerikli besin ortamına alt kültüre alınmıştır. Kültürün 30. gününde adventif kökler eksplant kaynaklarından ayrılarak süspansiyon kültürünün oluşturulmasında kullanılmıştır. Ayrıca bir kısım adventif kök kurutularak metabolit içeriği ve antioksidan aktiviteleri belirlenmiştir.

Adventif Kök Süspansiyon Kültürlerinin Oluşturulması

Katı besin ortamında gelişen adventif kökler kültürün 30. gününde eksplant kaynaklarından ayrılarak süspansiyon kültürlerinin oluşturulmasında başlangıç materyali olarak kullanılmıştır. Sıvı kültürlerin oluşturulmasında kullanılan besi ortamı 3.3 g L^{-1} MS (Murashige ve Skoog, 1962), 2 mg L^{-1} IBA ve 30 g L^{-1} sukrozdan oluşmaktadır. Besiyerinin pH'sı 1 M NaOH veya 1 M HCl kullanılarak 5.8'e ayarlandıktan sonra 121°C, 1.2 atmosfer basınçta 20 dakika otoklav edilmiştir. Adventif kökler (3 g) 100 mL besin ortamı içeren 250 mL'lik erlanmayerlerde kültüre alınmıştır. Erlenmayerler 120 devir dak^{-1} hızla çalışan çalkayıcı üzerinde, 25 ± 2 °C sıcaklıkta ve karanlık ortam koşullarında inkübe edilmiştir. Bu kökler süspansiyon kültürünün 10, 20 ve 30. günlerinde hasat edilerek 35 °C'de inkübatör koşullarında kurutulmuştur.

Ekstraktların Hazırlanması

Sekonder metabolit içeriği (rosmarinik asit, toplam fenolik bileşik, toplam flavonoid) ve antioksidan aktivitelerinin belirlenmesinde 0.2 g kurutulmuş adventif kök numuneleri kullanılmıştır. Bu numuneler metanol-diklorometan (4:1) çözeltisinde ekstrakte edildikten sonra filtre (0.22 µm) edilerek analizlerde kullanılmıştır.

Rosmarinik Asit Miktarının HPLC ile Belirlenmesi

Adventif köklerin rosmarinik asit içeriği yüksek performanslı sıvı kromatografi (HPLC) (Shimadzu Nexera-i LC-2040C 3D Plus, Shimadzu, Japan) cihazı ile analiz edilmiştir. HPLC uygulamalarında reverse phase C18 Phenylhexyl 4.6 x 150 mm kolon (GL Sciences InterSustain, Japan) kullanılmıştır. Analiz aşamasında cihaz ve kolon koşulları; akış hızı 1 mL dak^{-1}, kolon sıcaklığı 30 °C, enjeksiyon hacmi 10 µL, analiz süresi 25 dakika ve dalga boyu 330 nm olarak ayarlanmıştır. Mobil fazlar çözücü A (deiyonize suda % 0.1 formik asit) ve çözücü B (asetonitril)'den oluşmuştur. Çözücüler 0 dak, % 100 A ve % 0 B; 5 dak, % 80 A ve % 20 B; 10 dak,% 50 A ve % 50; 15 dak, % 30 A ve % 70 B ve 20 dak, % 100 A ve % 0 B ayarlı gradient programına göre yürütülmüştür. Adventif köklerin rosmarinik asit içerikleri standart bileşik kullanılarak hazırlanan kalibrasyon grafiğinin denklemi ile belirlenmiştir.

Total Fenolik Bileşik Miktarının Belirlenmesi

Adventif köklerin toplam fenolik bileşik içeriği spektrofotometrik olarak belirlenmiştir. Adventif kök ekstraktlarından 100 µL alınarak üzerine sırasıyla 4,5 mL saf su, 100 µL Folin-Cioceltaus reaktifi ve 300 µL Na_2CO_3 (%2'lik) ilave edilmiştir. Elde edilen karışımlar oda sıcaklığında iki saat inkübe edilmiştir. Bu sürenin sonunda numunelerin absorbanları spektrofotometrede (Shimadzu UVmini-1240) 720 nm dalga boyunda ölçülmüştür. Numunelerin toplam fenolik bileşik içerikleri gallik asit (mg GAE g^{-1}) kullanılarak hazırlanan standart grafik ile belirlenmiştir (Slinkard ve Singleton, 1977).

Flavonoid Miktarının Belirlenmesi

Adventif köklerin toplam flavonoid içerikleri spektrofotometrik olarak belirlenmiştir. Numune ekstraktlarından 200 µL içeren deney tüplerinin üzerine sırasıyla 1.5 mL etanol, 100 µL $AlCl_3$ (%10'luk), 100 µL $NaCH_3COO$ (1 M) ve 3.1 mL saf su ilave edilmiştir. Elde edilen karışımlar oda sıcaklığında 30 dakika inkübe edilmiştir. Bu sürenin sonunda numunelerin absorbanları spektrofotometrede (Shimadzu UVmini-1240) 427 nm dalga boyunda ölçülmüştür. Adventif köklerin toplam flavonoid içerikleri kuersetin (mg KUE g^{-1}) kullanılarak hazırlanan standart grafik ile belirlenmiştir (Pekal ve Pyrzynska, 2014).

Antioksidan Aktivite Analizleri

Serbest Radikal Giderme Aktivitesi (DPPH)

Adventif köklerin serbest radikal giderme aktivitesi, ekstraktların DPPH• radikalini (2,2-difenil-1-pikril hidrazil) temizleme kapasitelerinin ölçülmesiyle belirlenmiştir. Farklı miktarlarda (50 µg mL^{-1}, 100 µg mL^{-1}, 150 µg mL^{-1}) numune ekstraktları bulunan deney tüplerinin hacimleri etanol ile 3 mL'ye tamamlanmıştır. Bu karışımın üzerine 1 mL DPPH• (0,26 mM, etanolde hazırlanmış) radikali ilave edilerek güçlü bir şekilde vortekslenmiştir. Reaksiyon karışımı oda sıcaklığında 30 dakika (karanlıkta) bekletildikten sonra 517 nm'deki absorbansları spektrofotometrede (Shimadzu UVmini-1240) ölçülmüştür. Adventif köklerin serbest radikal giderme kapasiteleri radikalin başlangıç konsantrasyonunu %50 oranında azaltan örnek konsantrasyonu (IC_{50}) olarak verilmiştir. Numunelerin IC_{50} değerleri derişim/aktivite (% aktivite) grafiğinin denklemi kullanılarak hesaplanmıştır (Blois,1958).

DPPH aktivitesi (%) = $[(A_k - A_n)/A_k * 100]$

A_k: Kontrol absorbansı

A_n: Numune absorbansı

İndirgeme Gücü Aktivitesi (FRAP)

Adventif köklerin indirgeme gücü aktiviteleri Oyaizu metoduna göre belirlenmiştir (Oyaizu, 1986). Numune ekstraktlarından farklı derişimlerde (100 µg mL^{-1}, 200 µg mL^{-1}, 400 µg mL^{-1}) alınarak son hacimleri 0.2 M fosfat tamponu (pH 6.6) ile 1.25 mL'ye tamamlanmıştır. Bu karışımın üzerine 1.25 mL $K_3Fe(CN)_6$ (%1) ilave edilerek 50 °C'de etüvde 20 dakika inkübe edilmiştir. İnkübasyon süresinin sonunda karışımın üzerine sırasıyla 1.25 mL TCA

(%10) ve 0.25 mL $FeCl_3$ (% 0.1) ilave edilerek absorbansları (700 nm) spektrofotometrede (Shimadzu UVmini-1240) ölçülmüştür. Adventif köklerin indirgeme gücü kapasiteleri troloks standart antioksidan bileşiğinden hazırlanan standart grafiğin denklemi ile (µmol TE g^{-1} doku) belirlenmiştir.

Katyon Radikali Giderme Aktivitesi (ABTS)

Adventif köklerin katyon radikali giderme aktivitesi, ekstraktların $ABTS^{\cdot+}$ (2,2'-azino-bis 3-ethylbenzothiazoline-6-sülfonik asit) katyon radikalini temizleme kapasitelerinin ölçülmesiyle belirlenmiştir. Numune ekstraktlarından farklı miktarlarda (20 µg mL^{-1}, 40 µg mL^{-1}, 80 µg mL^{-1}) alınarak son hacimleri 0.1 M fosfat tamponu (pH 7.4) ile 3 mL'ye tamamlanmıştır. Bu karışımların üzerine 1 ml ABTS-$K_2S_2O_8$ çözeltisi ilave edilerek vortekslenmiştir. ABTS-$K_2S_2O_8$ çözeltisi 2 mM ABTS çözeltisi ile 2.45 mM $K_2S_2O_8$ çözeltisinin 1:2 oranında karıştırılarak karanlık koşullarda altı saat inkübe edilmesiyle elde edilmiştir. Reaksiyon karışımları karanlıkta oda sıcaklığında 30 dakika inkübe edildikten sonra absorbansları (734 nm) spektrofotometrede (Shimadzu UVmini-1240) ölçülmüştür. Adventif köklerin katyon radikali giderme kapasiteleri radikalin başlangıç konsantrasyonunu %50 oranında azaltan örnek konsantrasyonu (IC_{50}) olarak verilmiştir. Numunelerin IC_{50} değerleri derişim/aktivite (% aktivite) grafiğinin denklemi kullanılarak hesaplanmıştır (Re ve ark., 1999).

ABTS aktivitesi (%) = $[(A_k - A_n)/A_k * 100]$
A_k: Kontrol absorbansı
A_n: Numune absorbansı

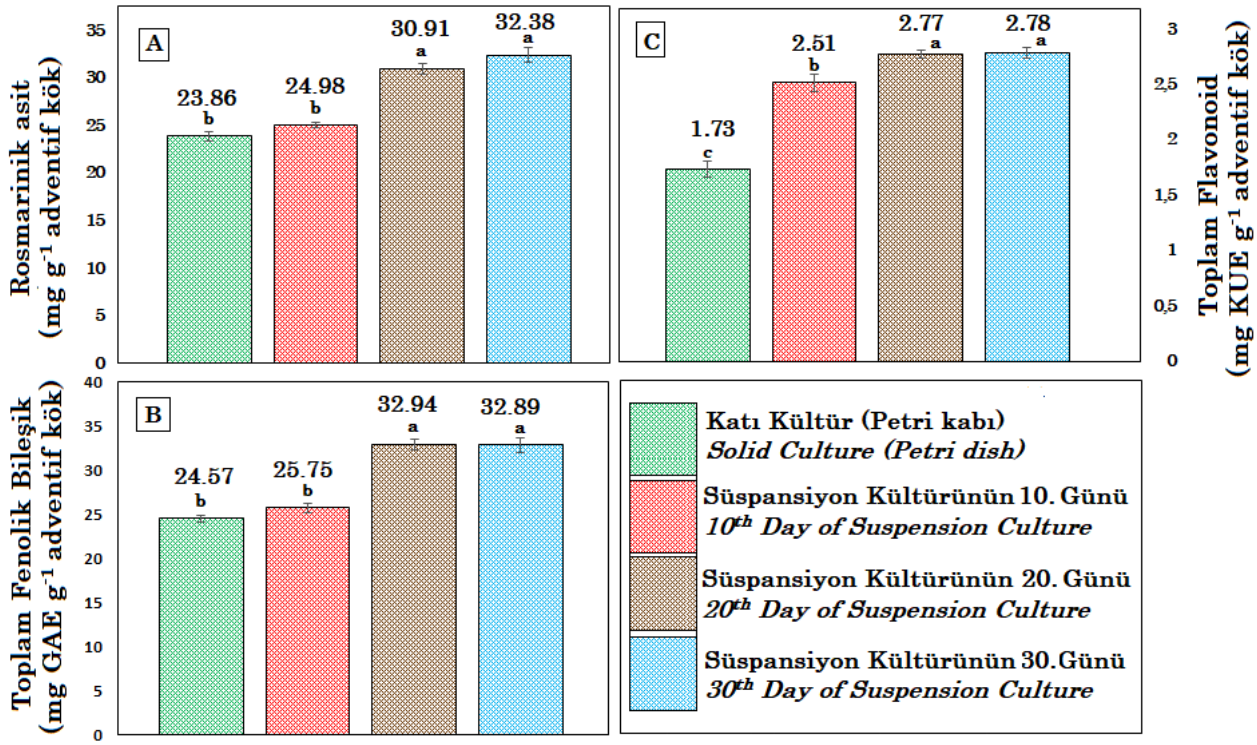
İstatistik Analizler

Bu çalışmadaki her uygulama ve analiz üç tekrarlı olarak yapılmış olup elde edilen verilerin istatistiki analizleri SPSS 20 (Armonk, NY: IBM Corp.) programı kullanılarak gerçekleştirilmiştir. Deney grupları arasındaki farklılıklar Duncan çoklu aralık testine göre belirlenmiştir (Duncan, 1955).

BULGULAR ve TARTIŞMA

Sekonder Metabolit İçeriği

Katı ve süspansiyon kültüründen elde edilen adventif köklerin rosmarinik asit, toplam fenolik bileşik ve flavonoid içeriği Şekil 1'de verilmiştir.



Şekil 1. Farklı kültür koşullarından elde edilen adventif köklerin rosmarinik asit (A), toplam fenolik bileşik (B) ve flavonoid (C) içerikleri (Şekil üzerinde verilen farklı harfler uygulamalar arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir)

Figure 1. Rosmarinic acid (A), total phenolic compound (B) and flavonoid (C) content of adventitious roots obtained from different culture conditions (The different letters given on the figure show that the difference between the applications is statistically significant)

Katı kültüründen elde edilen adventif köklerin metabolit içeriği kültürün 30. günde belirlenirken süspansiyon kültürünün metabolit içeriği 10, 20 ve 30. günlerde belirlenmiştir. Rosmarinik asit miktarı HPLC cihazı ile belirlenirken toplam fenolik bileşik ve flavonoid içeriği spektrofotometrik olarak

belirlenmiştir. Adventif köklerin toplam fenolik bileşik içeriği gallik aside (mg GAE g^{-1}) eşdeğer olarak verilirken flavonoid içeriği kuersetine (mg KUE g^{-1}) eşdeğer olarak ifade edilmiştir.

Adventif köklerin rosmarinik asit içeriği katı kültürde 23.86 mg g^{-1} olarak belirlenirken süspansiyon kültürlerinde 24.98 ile 32.38 mg g^{-1} arasında değiştiği belirlenmiştir. En yüksek rosmarinik asit içeriği süspansiyon kültürün 30. gününde 32.38 mg g^{-1} olarak belirlenmiştir. Süspansiyon kültürünün 30. günündeki rosmarinik asit içeriği katı kültürden ve süspansiyon kültürünün 10. gününden önemli ölçüde yüksektir ($P<0.01$). Ancak süspansiyon kültürünün 30. ve 20. günleri arasındaki farkın istatistiki olarak önemli olmadığı belirlenmiştir.

Srivastava ve ark. (2016), *Ocimum basilicum* bitkisinden elde edilen yedi saçak kök hattının rosmarinik asit içeriğinin 3.13 ile 28.83 mg g^{-1} kuru ağılık (20. gün) arasında değiştiğini ifade etmişlerdir. Genç ve ark. (2020) reyhan bitkisinin tarla koşullarında yetiştirilen dört çeşidinin rosmarinik asit içeriğinin 2610.15 ile 18460.56 mg kg^{-1} kuru ağırlık (2.61015- 18.46056 mg g^{-1} kuru ağılık) arasında değiştiğini belirlemişlerdir. Reyhan (*Ocimum basilicum* L) bitkisinde yapılan bir diğer çalışmada ise hücre süspansiyon ve kallus kültüründe rosmarinik asit üretim olanakları incelenmiştir. Bu çalışmada hücre süspansiyon kültürlerinin rosmarinik asit içeriğinin en yüksek 10 mg g^{-1} kuru ağırlık olarak belirlendiği ve bu değerin kallus kültüründen ve eksplant kaynağı olarak kullanılan bitkinin yapraklarından 11 kat daha yüksek olduğu ifade edilmiştir (Kintzios ve ark., 2003). Verma ve ark., (2016) farklı kültür koşullarında (*in vitro, ex vitro*) yetiştirilen reyhan (*Ocimum basilicum* L.) bitkilerinde en yüksek rosmarinik asit içeriğinin çiçeklenme dönemindeki yapraklarda (13.0 mg g^{-1}) olduğunu belirtmişlerdir. Bu çalışmada süspansiyon kültüründen elde edilen rosmarinik asit miktarının reyhan bitkisinde daha önce yapılan kallus, hücre süspansiyon, saçak kök, *in vitro* koşullarda yetiştirme ve tarla koşullarında yetiştiricilikten elde edilen sonuçlardan daha yüksek olduğu görülmektedir. Bu bağlamda reyhan bitkisinin adventif kök süspansiyon kültürleri rosmarinik asit üretimi için uygun biyoteknolojik yöntemler olduğu anlaşılmaktadır.

Adventif köklerin toplam fenolik ve flavonoid içeriği kültür süresi ve tipine göre önemli ölçüde değişmiştir. En yüksek toplam fenolik bileşik içeriği süspansiyon kültürünün 20. gününde 32.94 mg GAE g^{-1} olarak belirlenirken en yüksek flavonoid içeriği süspansiyon kültürünün 30. gününde 2.78 mg KUE g^{-1} olarak belirlenmiştir. Süspansiyon kültürünün 20. ve 30. günlerinde elde edilen sonuçlar katı kültürden ve süspansiyon kültürünün 10. gününden önemli ölçüde yüksektir ($P<0.01$). Ancak süspansiyon kültürünün 20. ve 30. günleri arasında önemli farklılıklar meydana gelmemiştir.

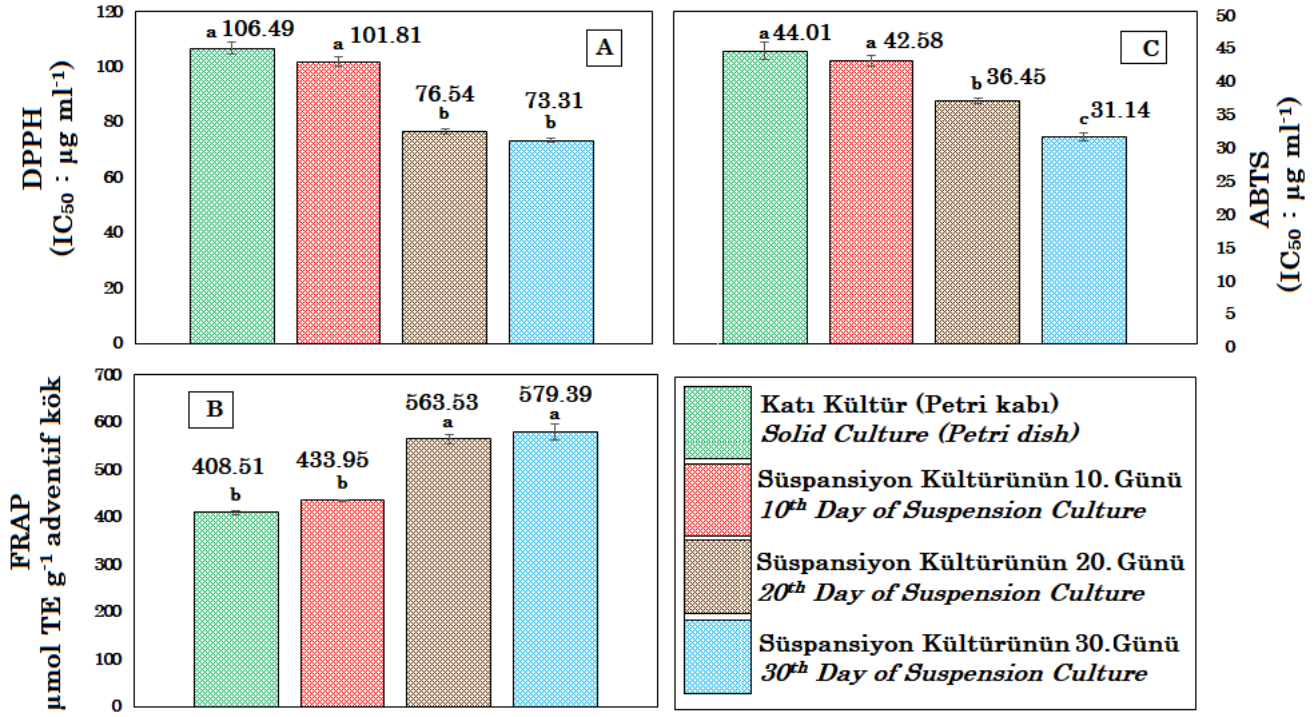
Srivastava ve ark., (2016) reyhan bitkisinden elde edilen saçak kök hatlarının toplam fenolik bileşik içeriği 55.75 ile 161.47 mg g^{-1} kuru ağılık (20. gün) arasında değiştiğini ifade etmişlerdir.

Bu bitkide yapılan benzer bir diğer araştırmada *in vitro* koşullarda yetiştirilen bitkilerin fenolik bileşik içeriğinin 23 ile 71 mg GAE g^{-1} kuru ağılık arasında değiştiği ifade edilmiştir (Verma ve ark., 2016). Belirtilen sonuçların yürütülen bu çalışmadan elde edilen sonuçlardan oldukça yüksek olduğu anlaşılmıştır. Ancak yapılan önceki çalışmalar incelendiğinde saçak kök kültüründe ve *in vitro* koşullarda yetiştirilen bitkilerin rosmarinik asit içeriğinin toplam fenolik bileşik içeriğine oranının oldukça düşük kaldığı görülmüştür. Yürütülen bu çalışmada adventif kök süspansiyon kültürlerinin rosmarinik asit içeriğinin oldukça yüksek olduğu ve miktarının toplam fenolik bileşik içeriğine çok yakın değerlerde olduğu belirlenmiştir. Bu durumun adventif kök süspansiyon kültürüyle üretilen rosmarinik asidin saflaştırma aşamasında önemli bir avantaj sağlayacağı düşünülmektedir.

Antioksidan Aktivite

Adventif köklerin antioksidan kapasiteleri DPPH, FRAP ve ABTS yöntemlerini kullanılarak belirlenmiştir. FRAP aktivitesinin sonuçları troloks'a eşdeğer olarak verilirken DPPH ve ABTS sonuçları ise radikalin % 50'sini gideren doku miktarı (IC_{50}) olarak belirlenmiş ve Şekil 2'de verilmiştir.

Adventif köklerin antioksidan aktiviteleri kültür tipi ve süresine bağlı olarak önemli ölçüde değişmiştir ($P<0.05$). Analizi yapılan üç antioksidan metoduna göre en yüksek antioksidan aktivite süspansiyon kültürünün 30. gününde belirlenmiştir. Bu köklerin DPPH, ABTS ve FRAP aktivitesi sırasıyla IC_{50} : 73,31 µg mL^{-1}, IC_{50} : 31.14 µg mL^{-1} ve 579.39 µmol TE g^{-1} olarak belirlenmiştir. DPPH ve FRAP aktiviteleri kültürün 20 ve 30. günleri arasında önemli ölçüde değişmemiştir. En düşük antioksidan aktivite katı kültürden elde edilen adventif köklerde belirlenmiştir. Antioksidan aktivitesi yüksek olan adventif köklerin rosmarinik asit, fenolik ve flavonoid içeriğinin de önemli ölçüde yüksek olduğu belirlenmiştir. Genç ve ark. (2020) tarla şartlarında yetiştirilen dört reyhan (*Ocimum basilicum* L.) çeşidinin FRAP aktivitesinin 150.8 ile 650.2 µmol TE g^{-1} arasında değiştiğini bildirmişlerdir. Gülçin ve ark. (2007) reyhan bitkisinin su ve etanol ekstraktlarında DPPH serbest radikal giderme, hidrojen peroksit giderme, süperoksit anyon radikal giderme, ferrik tiyosiyanat yöntemi, toplam indirgeme gücü ve metal şelatlama yöntemlerine göre yaptıkları antioksidan aktivite analiz sonuçlarına göre bitki ekstraktlarının güçlü antioksidan aktiviteye sahip olduğunu ifade etmişlerdir.



Şekil 2. Farklı kültür koşullarından elde edilen adventif köklerin DPPH (A), FRAP (B) ve ABTS (C) yöntemlerine göre antioksidan aktiviteleri (Şekil üzerinde verilen farklı harfler uygulamalar arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir)

Figure 2. Antioxidant activities of adventitious roots obtained from different culture conditions according to DPPH (A), FRAP (B) and ABTS (C) methods (The different letters given on the figure show that the difference between the applications is statistically significant)

SONUÇ ve ÖNERİLER

Bu çalışmada reyhan bitkisinde süspansiyon ve katı kültür koşullarında elde edilen adventif köklerin rosmarinik asit, toplam fenolik ve flavonoid içeriği ile DPPH, ABTS ve FRAP yöntemlerine göre antioksidan aktiviteleri incelenmiştir. Adventif kök süspansiyon kültürlerinin rosmarinik asit, toplam fenolik ve flavonoid içeriği katı kültürden oldukça yüksek olduğu belirlenmiştir. En yüksek rosmarinik asit içeriği süspansiyon kültürünün 30. gününde belirlenmiştir. Bu çalışmadan elde edilen verilerin ışığında adventif kök süspansiyon kültürlerinin rosmarinik asit üretimi için uygun biyoteknolojik yöntem olduğu anlaşılmaktadır. Ayrıca reyhan bitkisinden elde edilen adventif köklerin rosmarinik asit içeriğinin toplam fenolik bileşik içeriğine çok yakın değerlerde olması üretilen rosmarinik asidin saflaştırma aşamasında önemli bir avantaj sağlayacağı düşünülmektedir. Süspansiyon kültüründen elde edilen adventif köklerin DPPH, ABTS ve FRAP yöntemlerine göre oldukça yüksek antioksidan aktiviteye sahip olduğu görülmüştür. Kültür süresinin artmasıyla antioksidan aktivitenin de arttığı ve en yüksek antioksidan aktivite süspansiyon kültürünün 30. gününde belirlenmiştir. Daha sonra yapılacak olan çalışmalarda hormon, MS ve sukroz gibi temel besin ortam koşullarının optimizasyonun sağlanması yüksek verimde metabolit ve biyomas elde etmek için avantaj sağlayacaktır. Ayrıca rosmarinik asidin biyosentez öncüllerinin besin ortamına ilave edilmesi ve elisitasyon çalışmalarının yapılması büyük önem arz etmektedir.

TEŞEKKÜR

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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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Methylation Modelling and Epigenetic Analysis of Sunflower (*Helianthus annuus* L.) Seedlings Exposed to Cadmium Heavy Metal Stress

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ABSTRACT
Environmental pollution, especially heavy metal pollution, is an important environmental problem all over the world. Heavy metals that accumulate in high concentrations in soil and water ecosystems are known to damage most functional biomolecules such as DNA, RNA and protein in living organisms and cause genotoxicity. For example, cadmium heavy metal is one of the heavy metals that negatively affect plant growth and development. The purpose of this study was to determine the methylcytosine level in the sunflower plant genome and the changes in the methylation pattern under cadmium stress. Sunflower seeds were grown with different concentrations of cadmium heavy metal solution (Control, 20, 40, 80, 160, 320, 640 and 1280 ppm) for 3 weeks. According to the data obtained in the study, as the cadmium concentration increased, the growth and development of sunflower seedlings decreased. After detecting DNA band variations by RAPD analysis, methylcytosine levels in the sample genome were determined by CRED-RA technique. As a result of RAPD analysis, the highest GTS rate was 87.83% at 20 ppm cadmium concentration and the lowest rate was 81.75% at 320 ppm. Four different methylation patterns (Type I-IV) were determined according to the CRED-RA analysis. As a result of the study, significant changes in the DNA methylation pattern were observed by CRED-RA analysis in the sunflower genome exposed to cadmium heavy metal stress.

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Kadmiyum Ağır Metal Stresine Maruz Kalmış Ayçiçeği (*Helianthus annuus* L.) Fidelerinin Metilasyon Modellemesi ve Epigenetik Analizi

ÖZET
Çevre kirliliği, özellikle ağır metal kirliliği, tüm dünyada önemli bir çevre sorunudur. Toprak ve su ekosistemlerinde yüksek konsantrasyonlarda biriken ağır metallerin canlı organizmalardaki DNA, RNA ve protein gibi çoğu fonksiyonel biyomoleküle zarar verdiği ve genotoksisiteye neden olduğu bilinmektedir. Örneğin kadmiyum ağır metal, bitki büyümesini ve gelişmesini olumsuz etkileyen ağır metallerden biridir. Bu çalışmanın amacı, ayçiçeği bitki genomundaki metilsitozin düzeyini ve kadmiyum stresi altında metilasyon modelindeki değişiklikleri belirlemektir. Ayçiçeği tohumları, 3 hafta boyunca farklı konsantrasyonlarda kadmiyum ağır metal çözeltisi (Kontrol, 20, 40, 80, 160, 320, 640 ile 1280 ppm) ile büyütüldü. Çalışmada elde edilen verilere göre kadmiyum konsantrasyonu arttıkça ayçiçeği fidelerinin büyüme ve gelişmesi azalmıştır. RAPD analizi ile DNA bandı varyasyonları tespit edildikten sonra, numune genomundaki metilsitozin seviyeleri CRED-RA tekniği ile belirlendi. RAPD analizi sonucunda, en yüksek GTS oranı 20 ppm kadmiyum konsantrasyonunda % 87.83 ve en düşük oran 320 ppm'de % 81.75 olmuştur. CRED-RA analizine göre dört farklı metilasyon modeli (Tip I-IV) belirlendi. Çalışma sonucunda kadmiyum ağır metal stresine maruz kalan ayçiçeği genomunda CRED-RA analizi ile DNA metilasyon modelinde önemli değişiklikler gözlemlendi.

Tarımsal Biyoteknoloji

Araştırma Makalesi

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Geliş Tarihi : 20.02.2021
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Anahtar Kelimeler
Metilasyon
CRED-RA
Ağır Metal
Kadmiyum
Ayçiçeği

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INTRODUCTION

The sunflower plant is an oil plant that is very high commercial importance (belongs to the Helianthus annuus L. Asteraceae family) and has economic value for agriculture worldwide (Davis 1985). Sunflower (*H. annuus*) is a mostly annually grown economically valuable crop. Its seeds contain oil and are very important for nutrition. The high fatty acid rate (~70%) shows the importance of the sunflower plant. The economic value of sunflower was realized after the second world war. Subsequently, studies with physical and chemical content were carried out for quality oil with a very high oil content, good edible, refinery quality and high nutritional value. Also, Sunflower seeds are rich in potassium and vitamin-E as well as being an important food source in terms of linoleic acid (Lentz et. al., 2008; Blackmana et, al., 2011).

Important commercial oil crops such as sunflower, soybean, rapeseed, safflower, poppy, peanut and sesame are often subjected to various abiotic stresses such as drought, low temperature, salinity, excessive water, ultraviolet radiation and heavy metal contamination (Khurana and Chatterjee, 2001). Environmental pollution, especially metal pollution, is one of the global environmental and health problems affecting many organisms, from microorganisms to plants, animals and humans. Heavy metals, which can accumulate intensively in the air, water and soil have become a dangerous environmental problem that requires quick action (Yarsan et., al, 2000). Heavy metal excess in the soil causes damage to the morphological, cytological, metabolic and genomic integrity of the plants (Meyerowitz and Somerville, 1994; Hu, 2005; Kosnett, 2007; Kumar et., al, 2009; Bolukbasi and Aras, 2016; Jia et., al, 2020; Dash et., al, 2020). Heavy metals are taken into the cell by various carriers specific to their structure. They cause the formation of reactive oxygen species in organelles by affecting metabolism with various heavy metal redox reactions (Yıldız et., al, 2011; Yalcin et., al, 2020).

Some metals such as zinc (Zn), copper (Cu), manganese (Mn) and nickel (Ni) are required in low concentrations for the growth and development of plants (Kachenko and Singh, 2004). However, heavy metals such as lead (Pb), mercury (Hg) and cadmium (Cd) have severe **toxicity. Cadmium, which is not an essential element** especially for plants, is generally found in low amounts in the soil and adversely affects plant growth and development. It is not an essential nutrient for plants, but it quickly enters the cells. This metal has some serious effects on plants such as growth inhibition, decrease in enzyme activities, photosynthesis and nutrient intake (Hart et., al, 1998; Toppi and Gabrielli, 1999; Schutzendubel et., al, 2001; Kumar et., al, 2009; Bolukbasi and Aras, 2016; Jia et., al, 2020; Dash et., al, 2020).

Some plants that are called hyperaccumulators can accumulate 50 to 500 times more metal in their above and underground parts than the metal concentration in the soil (Memon et., al, 2001; Clemens, 2006). Sunflower (*Helianthus annuus* L.) which is among the plant specimens (as *Nicotiana tabacum* L., *Brassica juncea* L. and *Zea mays* L.) that can accumulate moderate heavy metals but generate high amounts of biomass, is also defined as a hyperalumulatory plant (Ozay and Mammadov, 2013; Bolukbasi and Aras, 2016; Kayakoku and Dodru, 2020).

Epigenetics is the branch of molecular biology that studies gene expression changes that are not caused by changes in DNA sequences, but are also inherited and can be passed down from generation to generation. In other words, it examines the inherited phenotypic variations that occur with non-genetic environmental effects. Such changes in DNA sequences can directly affect the cell or the organism, but there is no change in the DNA sequence (Martin and Zhang, 2007; Niu et., al, 2020).

DNA methylation is one of the best known and applied DNA modification models, which is formed by enzymatic attachment of a methyl group to the 5th carbon of cytosine (the transfer of a methyl group from S-adenosyl methionine to the 5th position of the DNA cytosine residue catalyze by DNA Methyltransferase enzymes) and plays an important role in controlling gene expression in plants. And also DNA methylation is thought to also contribute to biological defense in plants (Boyko and Kovalchuk, 2008; Pontvianne et., al, 2010; Taspinar et., al, 2017; Arslan, 2019; Shams et., al, 2020).

Many plants adapt to different stresses, such as heavy metal stress that occurs by changing their own DNA through the DNA methylation process. DNA methylation is an inherited modification that is passed down from generation to generation. The ability to remove the methyl group to return to the original DNA structure is a reversible process. Therefore, DNA methylation is an inherited mechanism and an important and common treatment method in determining the methylation pattern (Suzuki and

Bird, 2008; Chinnusamy and Zhu, 2009; Mirouze and Paszkowski, 2011; Yagci et., al, 2019; Aydin et., al, 2021).

There are many molecular analyzes used in determining methylation patterns in the genome (Cai et., al, 1996; Leljak et., al, 2004). The technique of coupled/combined restriction enzyme digestion-random amplification (CRED-RA) is an old however an effective and valid technique for detecting methylation patterns in plants. There are many studies in which the CRED-RA technique has been used successfully to determine DNA methylation patterns (Grigg and Clark, 1994; Rein et., al, 1998; Tani et., al, 2005; Karan et., al, 2012; Bolukbasi and Aras, 2016; Taspinar et., al, 2017; Arslan, 2019; Shams et., al, 2020; Aydin et., al, 2021).

In this current study, sunflowers (*Helianthus annuus* L.) groups exposed to stress with various cadmium solutions compared with control group plants for possible methylation differences were evaluated. Thus, it was aimed PCR-based CRED-RA technique was used to detect changes in DNA methylation pattern originating from cadmium heavy metal.

MATERIALS and METHODS

Growth of plant samples and cadmium stress treatments

Before planting sunflower seeds, their surfaces were sterilized with 70% alcohol and 30% sodium hypochlorite solution. The seeds were then washed three or four times with distilled water. For the germination and growth of sunflower seeds, viols prepared using sterile perlite were arranged. The viols prepared were divided into eight groups. Seven groups for different cadmium solutions and one group for the control group. Control group seedlings were irrigated only with 15 ml distilled water. The other batches were treated at a reaction volume of 15ml each for concentrations of 20, 40, 80, 160, 320, 640, 1280 ppm cadmium solution, respectively. The cultivation process adjusted in this way was continued for 21 days. At the end of 21 days, control group and plant samples treated with cadmium solution were harvested and stored at -20 degrees until DNA isolation.

DNA isolation from samples

Root fragments (200 mg) taken from samples which exposed to cadmium stress were powdered using liquid nitrogen. Subsequently, DNA was isolated from these samples. For DNA extracting, Lefort's (Lefort et., al, 1998) DNA isolation protocol was followed. Quantity and quality measurement of isolated genomic DNAs were determined by Nanodrop (NanoDrop ND-1000 Spectrophotometer, Thermo Scientific). And then it was confirmed by 1.5% agarose (containing 0.05µl/ml EtBr) gel electrophoresis.

PCR (RAPD) Procedure

The RAPD-PCR prosedure was carried out with a total reaction volume of 25 µl for each DNA samples. Amplification conditions were optimized with 200 ng of genomic DNA, 1 × reaction buffer, 3.5 mM $MgCl_2$, 20 µM dNTPs, 0.2 mM primer and 0.7U Taq DNA polymerase (Promega) and these amounts were used for PCR mix. Fourteen primers were used for RAPD-PCR reactions (Table-1). The PCR programme performed an initial denaturation step of 7.5 minutes at 95 °C, followed by 94 °C for 90 seconds denaturation, 36 °C for 60 seconds annealing and 72 °C for extension at 120 seconds. And then, the prosedure followed by at 72 ° C a final extension period of 5 minutes. The negative control was run on each of the samples to test for other types of contamination without any DNA template.

Table 1. Nucleotide sequences of primers were used for RAPD-PCR reactions
Çizelge1.RAPD-PCR reaksiyonda kullanılan primerlerin sekansları

Primers	*Nucleotide sequence (5´→ 3´)*
OPC-01*	TTCGAGCCAG
OPC-02*	GTGAGGCGTC
OPC-04*	CCGCATCTAC
OPC-06*	GAACGGACTC
OPC-07	GTCCCGACGA
OPC-08*	TGGACCGGTG
OPC-09	CTCACCGTCC
OPC-10	TGTCTGGGTG
OPC-11*	AAAGCTGCGG
OPA-08	GTGACGTAGG
OPB-07	GGTGACGCAG
OPF-05	CCGAATTCCC

*** refer to primers which used for CRED-RA analysis**

Technique of CRED-RA

DNA digestion with the restriction enzymes

MspI and HpaII enzymes were used to detect variation in methylation models of genom of samples between control and experimental groups. The CRED-RA run was carried out with a total volume of 20 µl for all samples. Approximately 1 µg of genomic DNA, 2 µl of 10X reaction buffer and 10U enzyme for restriction were used in a reaction volume of 20 µl. Microcentrifuge tubes containing the above components were kept in a 37 ° C water bath for 3 hours. Following a 3 hour incubation, samples were kept in a 95 ° C heat block for 15 minutes to inactivate the reaction.

PCR components and conditions

Approximately 200 ng digestion product, 2.5 µl 10 X of reaction preservative, 20 mM dNTPs, 2.5 µl MgCl2, 0.2 mM, 0.7 U Taq polymerase for each primer were used

in 25 µl reaction volume. Six of 14 RAPD primers (indicated in Table 1, as *) were showed monomorphic band profiles in RAPD-PCR. And so these primers were used in CRED-RA assay. For optimized reactions, an initial denaturation step of 96 °C was performed for 90 seconds. Then 45 cycles of 95 °C (denaturation) for 30 seconds, 36 ° C for 60 seconds (binding), 72 °C for 120 seconds (extension) followed by a final extension period of 72 °C for 10 minutes complete with. And then samples were confirmed by 1.6 % agarose gel electrophoresis. A negative control was used for each group to determine if there was any contamination.

Analysis of CRED-RA data

The digestion reactions were carried out via enzymes of *HpaII* and *MspI* separately. The data obtained as a result of the CRED-RA technique were evaluated with reference to Table 2. The enzymes of HpaII and MspI have different digestive abilities depending on the status of cytosine in the methylation model. The methylation models were evaluated and the band profiles were scored as yes/presence (1) and no/absence (0). While performing CRED-RA analysis, the scores obtained from the bands were evaluated according to previous studies (Liu et., al, 2005; Pan et., al, 2011; Wang et., al, 2011) and 4 different methylation patterns were determined.

Table 2. Methylation types of *HpaII* and *MspI* restriction enzymes according to their digestion
Çizelge 2. HpaII ve MspI restriksiyon enzimlerinin kesim kabliyetlerine göre metilasyon türleri

Type	Methylation Patterns	*HpaII*	*MspI*	Score of Band Profile			
				x	y	z	
Type I	CCGG GGCC	digestion	digestion	-/1	+/0	+/0	Non-methylation
Type II	**C**CGG GGCC **C**CGG GGCC	digestion	undigestion	-/1	+/0	-/1	Semi-methylation
Type III	C**C**GG GG**C**C	undigestion	digestion	-/1	-/1	+/0	Full-methylation
Type IV	**CC**GG GG**CC**	undigestion	undigestion	-/1	-/1	-/1	Full-methylation

x: PCR product is not digested by either enzyme "+" refer to digestion and "-" refer to undigestion
y: PCR product is digested by the *HpaII* enzyme
z: PCR product is digested by the MspI enzyme "1" refer to band presence and "0" refer to band absence

RESULTS

RAPD data analysis

In this study, a significant degree of polymorphism was observed in sunflower samples exposed to cadmium stress according to the results of RAPD analyzes. In 12 of the 18 RAPD-PCR primers performed in this current study, different polymorphic DNA bands were detected from the control group. It showed significant polymorphic band patterns in primers OPC 09 (57.2%), OPC 08 (55.50%), OPC 07 (50.00%) and OPC 11 (50.00%) (Table 3).

According to the GTS rates adapted with RAPD profiles, the highest rate was 87.83% at 20 ppm Cd concentration. The lowest rate was 81.75% at 320 ppm Cd stress (Table 4).

CRED-RA analysis

As a result of the analysis; it has been observed that heavy metals are effective in epigenetic mechanisms, especially in DNA methylation differences, and this situation provides resistance to cadmium heavy metal by forming different types of methylation in sunflower plants. By CRED-RA analysis, 4 different methylation types were obtained from sunflower seedlings exposed to heavy metal stress at different concentrations of cadmium. In detecting these differences, 6 different primers giving monomorphic and clear bands were used to detect methylation differences as a result of the PCR band profiles. The

Table 3. Results of polymorphism rate of primers were used for RAPD-PCR reactions
Çizelge 3. RAPD-PCR reaksiyonunda kullanılan primerlerin polimorfizm oranınları

Primers	Polymorphism rate (%)
OPC-01*	15.4
OPC-02*	12.5
OPC-04*	33.3
OPC-06*	36.4
OPC-07	50.0
OPC-08*	55.5
OPC-09	57.2
OPC-10	33.3
OPC-11*	50.0
OPA-08	37.5
OPB-07	26.3
OPF-05	25.3

*** refer to primers which used for CRED-RA analysis**

Table 4. % change of GTS rates

Çizelge 4. GKS oranlarındaki değişim yüzdesi

Samples	GTS rate (%)
20 ppm	87.83
40 ppm	87.16
80 ppm	86.48
160 ppm	85.13
320 ppm	81.75
640 ppm	83.78
1280 ppm	82.43

sequences of the primers used for this analysis are given in Table 1. And then, Table 2 is taken as a reference for determining the methylation patterns and determining their differences. While evaluating the methylation pattern, scoring was made as yes/presence (1) and no/absence (0) in line with the information in table 2. As a result of these analysis; the percentages of different methylation types have been calculated and the highest and lowest concentration percentages of the methylation types are specifically indicated (Liu et., al 2007; 2009; Karan et., al, 2012; Bolukbasi and Aras, 2016). The formulation used in the calculation is given in Table 5 in detail. The most striking point here is; Type-IV methylation has the highest value at all cadmium concentrations.

Table 5. The average (%) rates of methylation types based on data obtained from CRED-RA analysis
Çizelge 5. CRED-RA analizinden elde edilen verilere göre metilasyon türlerinin ortalama (%) oranları

	Control	20	40	80	160	320	640	1280
Type-I (%)	3.20	***2.30***	3.40	***5.60***	3.60	3.50	3.20	4.10
Type-II (%)	2.30	2.40	2.50	5.20	***5.50***	2.80	1.60	***0.80***
Type-III (%)	6.90	***8.00***	6.20	6.20	4.40	***3.80***	7.70	7.80
Type-IV (%)	87.60	87.30	87.90	***83.00***	86.50	***89.90***	87.50	87.30
Total methylated bands ratio (%)[a]	96.80	96.70	96.60	94.40	96.40	96.50	96.80	***97.50***
Full-methylated bands ratio (%)[b]	94.50	95.30	94.10	89.20	90.90	93.70	95.20	***97.40***
Semi-methylated bands ratio (%)[c]	2.30	2.40	2.50	5.20	***5.50***	2.80	1.60	0.80

aTotal methylated bands ratio (%) = [(II+III+IV)/(I+II+III+IV)]x100
bFull-methylated bands ratio (%) = [(III+IV)/(I+II+III+IV)]x100
cSemi-methylated bands ratio (%) = [(II)/(I+II+III+IV)]x100

The (%) ratio of methylation types obtained from CRED-RA analysis in sunflower samples given in Figure 1 comparatively.

And also, the (%) ratio of methylation pattern types obtained from CRED-RA analysis in sunflower samples given in Figure 2.

Additionally, the significant correlation was observed between the total methylation pattern and non-methylation pattern in sunflower seedlings subjected to cadmium stress. The R^2 value was 0.9873 (Figure 3).

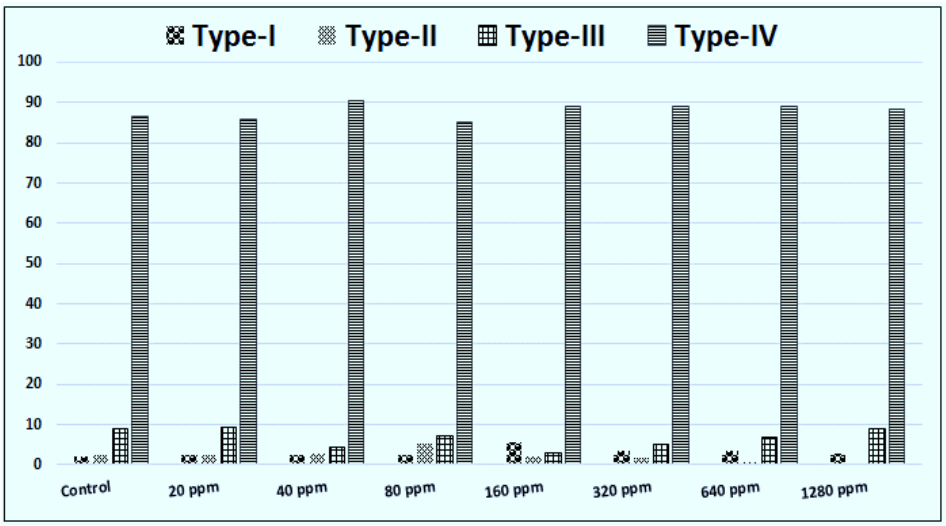

Figure 1. The average (%) rates of methylation types obtained from CRED-RA analysis in sunflower samples
Şekil 1. Ayçiçeği örneklerinde CRED-RA analizinden elde edilen metilasyon türlerinin ortalama (%) oranları

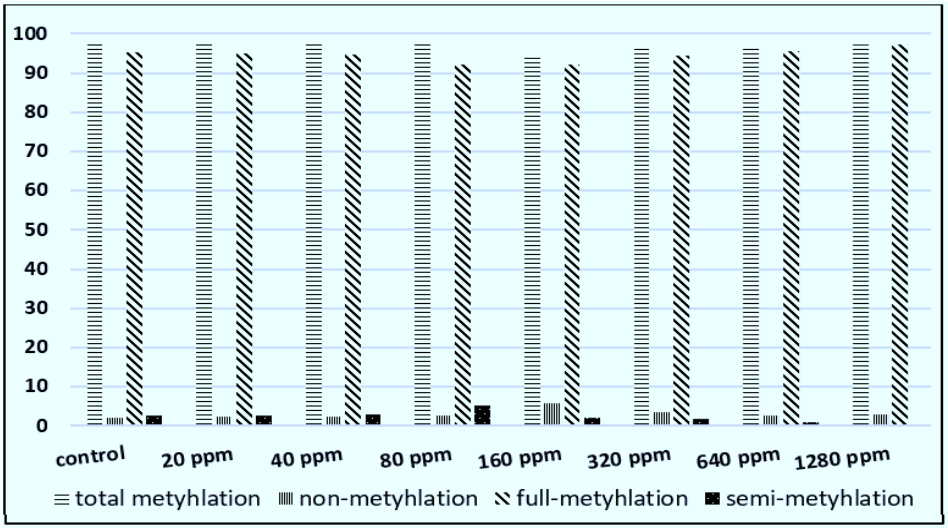

Figure 2. The (%) ratio of methylation pattern types obtained from CRED-RA analysis in sunflower samples
Şekil 2. Ayçiçeği örneklerinde CRED-RA analizinden elde edilen metilasyon modellerinin (%) oranı

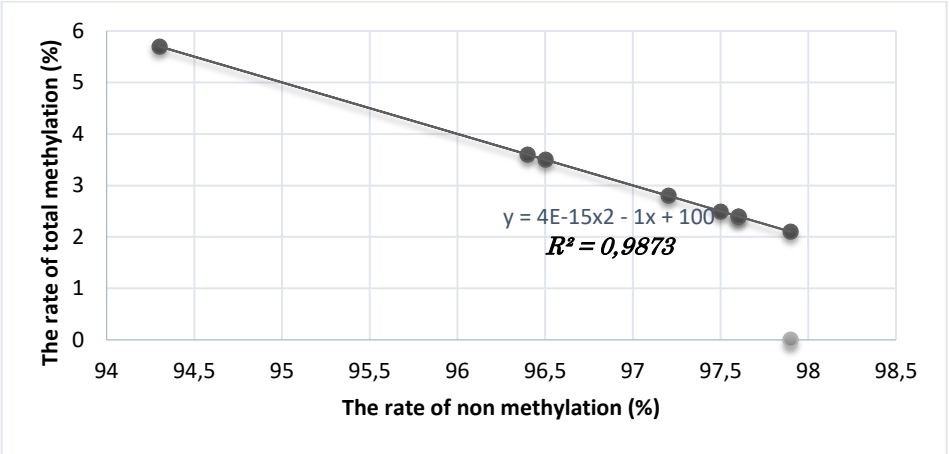

Figure 3. Influence of total methylation pattern to non-methylation pattern
Şekil 3. Toplam metilasyon modelinin (%) metillenmemiş modele (%) etkisi

DISCUSSION and CONCLUSION

One of the most important issues in environmental research is the toxic effects of heavy metals. The most important effects of heavy metals is to inhibit plant growth (Liu et., al, 2005; Pan et., al, 2011; Wang et., al, 2011; Karan et., al, 2012; Taspinar et., al, 2017; Arslan, 2019). The accumulation of heavy metals in plant tissues adversely affects the germination of seeds and the growth of roots and stems. In many studies, it has been stated that the toxicity created by heavy metals as catalysts in the oxidative degradation of biological macromolecules damages the DNA structure by causing oxidative damage (Liu et., al, 2005; 2007). It is possible to detect the toxic effects of heavy metals with molecular parameters related to DNA mutation. DNA fingerprinting techniques such as RAPD-PCR are widely used to identify DNA changes in plants induced by contaminants such as heavy metals (Theodorakis et., al, 2001; Pan et., al, 2011; Bolukbasi and Aras, 2016; Taspinar et., al, 2017; Gallo-Franco et., al, 2020; Harshitha et., al, 2020).

In this study, RAPD-PCR technique was used to detect changes in DNA band profiles. When DNA band profiles of sunflower samples exposed to cadmium heavy metal stress at different concentrations for 21 days were examined, it was seen that there were significant changes compared to the control group. RAPD-PCR profiles were analyzed by agarose gel electrophoresis containing ethidium bromide. The strips were counted one by one from the top of the strips. All RAPD-PCR profiles amplified by the primers were scored by comparison with the control group. The amplification profiles of the twelve primers were compared to the control group and the bands of the DNA fragments were scored as yes/presence (1) and no/absence (0). All primers showed significant polymorphic band patterns, particularly at OPC09 (57.2%), OPC08 (55.50%), OPC07 (50.00%) and OPC11 (50.00%). In addition, when the obtained RAPD-PCR band profiles were evaluated, the highest change in GTS ratios was 87.83% at 20 ppm Cd concentration. When RAPD-PCR data were evaluated, it was

observed that there was a significant amount of polymorphism in samples exposed to different concentrations of cadmium heavy metal stress. It was also stated in previous studies that this difference occurred as a result of mutations in the places where the primers were linked in the genome (Savva, 2000; Liu et., al, 2007; Bolukbasi and Aras 2016; Taspinar et., al, 2017; Arslan, 2019; Hosseinpour et., al, 2020). These results showed that the primers used are a strong marker or indicator for detecting mutagenic effects of cadmium heavy metal in sunflower plants. In addition, changing in GTS rates clearly highlight the importance of different cadmium stress concentrations (Conte et., al, 1998; Theodorakis et., al, 2001; Atienzar et., al, 2002; Harshitha et., al, 2020; Aydin et., al, 2021). It indicates that RAPD-PCR markers can be used successfully to detect various DNA damage in plants such as sunflower exposed to environmental pollution (Gupta and Sarin, 2009; Gallo-Franco et., al, 2020; Jia et., al, 2020).

Epigenetic mechanism like DNA methylation is an important biological defense mechanism in plants. Through DNA methylation, many plants resist various abiotic stresses such as drought, salinity, and heavy metal contamination. DNA methylation is an applied DNA modification models, which is formed by enzymatic attachment of a methyl group to the 5th carbon of cytosine (the transfer of a methyl group from S-adenosyl methionine to the 5th position of the DNA cytosine residue catalyze by DNA Methyltransferase enzymes) and plays an important role in controlling gene expression in plants (Cai et., al,1996; Leljak et., al, 2004; Suzuki and Bird, 2008; Chinnusamy and Zhu, 2009; Mirouze and Paszkowski, 2011; Bolukbasi and Aras, 2016; Arslan, 2019; Aydin et., al, 2021). Many techniques are used to detect changes in methylation patterns in the genome due to heavy metal pollution such as cadmium (Liu et., al, 2005; 2007; 2009). The CRED-RA technique used in this study is one of them (Tani et., al, 2005; Karan et., al, 2012; Bolukbasi and Aras, 2016; Taspinar et., al, 2017; Arslan, 2019; Aydin et., al, 2021).

In the study via PCR-based CRED-RA technique, based on the rates of average methylation types, the highest rate for Type-I methylation was 5.60 at 80ppm. Type-I methylation model represents non-methylated models. This indicates that methylated cytosine is not on double stranded DNA or internal methylated cytosine in a single strand. It has been regarded as unmethylated cytosine in previous studies (Liu et., al, 2007; Mirouze and Paszkowski, 2011; Karan et., al, 2012; Bolukbasi and Aras 2016; Arslan, 2019; Aydin et., al, 2021). In this regard, methylation appeared to be absent at all cadmium stress concentrations.

The highest rate at which the Type-II methylation pattern detected was 5.50% at 160ppm. Type-II methylation represents the externally methylated semi-methylation pattern of cytosine nucleotide on the DNA that is a single strand.

As stated in previous studies, the presence of inner methylated cytosine in both strands of DNA indicates Type-III or full methylation model and the presence of outer methylated cytosine indicates Type-IV methylation model. When the data were examined, it was seen that the Type-IV methylation was at the highest level in all different concentration between 20 to 1280 ppm compare to other methylation models. Additionally, the Type-III and Type-IV methylation patterns, which are full methylation types have appeared at all cadmium stress concentrations.

In conclusion, DNA polymorphisms investigated in sunflower plants in response to abiotic stress conditions were analyzed by CRED-RA technique at different concentrations of cadmium heavy metals. Significiant polymorphisms and methylation changes has been observed that changes in the level of methylation patterns have an effect on the biological defense mechanism in sunflower plants. In addition, the results of current study indicate that cadmium is a serious genotoxic agent for sunflower plants. Also this research have clearly shown that such studies will be effective in cleaning and restoring areas contaminated with various heavy metals. In addition, the hyperacumulator feature of the sunflower plant was once again determined.

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Conflicts of Interest Statement

Author had no any financial or personal relationships with other individuals or organizations that might inappropriately influence this work during the submission process.

Statement Contribution of the Author

This study's experimentation, analysis and writing, etc. all steps were made by the author.

Statement of Ethics

There is no need for an ethics committee decision for the studies in the article.

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UV-C'nin, *Deinonoccus radiodurans* ve *Vitreoscilla* Hemoglobin (vgb) Geni Aktarılmış Rekombinantlarında; SOD, KAT ve Karoten Miktarı Üzerine Etkisi

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ÖZET

Ultraviyole radyasyon (UV); biyolojik dokularda reaktif oksijen türlerinin meydana gelmesine neden olarak oksidatif stres oluşturmaktadır. UV' nin indüklediği reaktif oksijen türleri, bunların etkileri ve bunlara karşı hücresel savunma mekanizmaları ve reaktif oksijen türlerinin temizlenmesinden sorumlu antioksidan sistemleri günümüzde üzerinde oldukça fazla araştırma yapılan konulardır. Bu çalışmada, yüksek seviyede iyonize radyasyon ve UV radyasyon, kuraklık ve DNA' ya zarar veren kimyasallar gibi birçok ajan ve koşula olan direnciyle iyi bilinen bir ekstremofil olan *Deinococcus radiodrans* ile *Vitreoscilla* hemoglobin (vgb) geni klonlanmış rekombinantı ve kontrol olarak da vgb¯ rekombinant suşu kullanılmıştır. UV-C' nin *D. radiodurans*' ın antioksidan savunma sistemleri (süperoksit dismutaz, katalaz ve karoten) üzerine etkisi araştırılıp, buna ek olarak organizmaya daha fazla oksijenli ortam sağlayarak daha fazla büyümesini sağlayan vgb geninin, bakterinin UV direncine yapacağı katkısı araştırılmıştır. Buna göre, *D. radiodurans* (vgb¯) in UV-C uygulanan örnekleri kontrol gruplarıyla kıyaslandığında süperoksit dismutaz ve katalaz enzim aktivitesinin yabanıl ve vgb genini taşıyan rekombinantına oranla daha düşük olduğu tespit edilmiştir. Yine yüksek karoten içeren yabanıl tipi bakterilerde, UV-C uygulamasına bağlı olarak karoten miktar artışı net bir şekilde gözlenmiştir.

Bahçe Bitkileri

Araştırma Makalesi

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Geliş Tarihi : 15.04.2021
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Anahtar Kelimeler
UV-C radyasyon
Antioksidan enzim
Süperoksit dismutaz
Katalaz
Karoten

In UV-C, *Deinonoccus radiodurans* and *Vitreoscilla* Hemoglobin (vgb) Gene Transferred Recombinants; Effect on SOD, KAT and Carotene Amount

ABSTRACT

Ultraviolet radiation (UV); creates oxidative stress by causing the formation of reactive oxygen species in biological tissues. Reactive oxygen species induced by UV, their effects and cellular defense mechanisms against them, and antioxidant systems responsible for cleaning reactive oxygen species are the subjects of much research today. In this study, Deinococcus radiodrans which is well known an extremophile for its resistance to many agents and conditions such as high levels of ionizing radiation and UV radiation, drought and chemicals that damage DNA and Vitreoscilla hemoglobin (vgb) gene cloned recombinant with, and vgb¯ recombinant strain as a control were used. The effect of UV-C on the antioxidant defense systems of D. radiodurans (superoxide dismutase, catalase and carotene) was investigated, and in addition, the contribution of the vgb gene, which provides more oxygenated environment to the organism, to the UV resistance of the bacteria, was investigated. Accordingly, when UV-C treated samples of D. radiodurans (vgb¯) were compared with the control groups, it was determined that the superoxide dismutase and catalase enzyme activities were lower than the wild and the recombinant carrying the vgb gene. Again, in wild-type bacteria with high carotene, an increase in the amount of carotene was clearly observed due to UV-C application.

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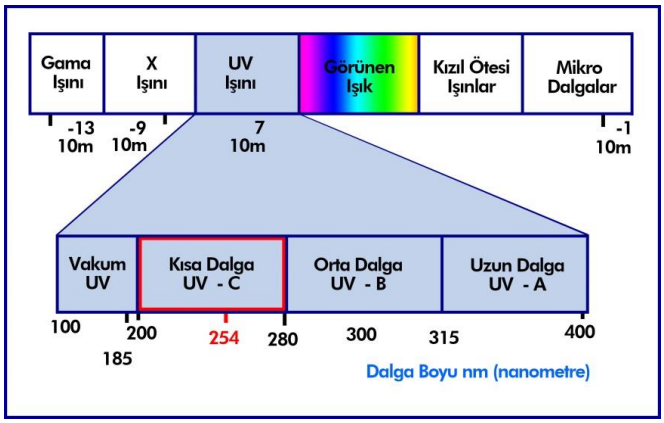
GİRİŞ

Elektromanyetik Dalgalar

Işık veya elektromanyetik dalga foton olarak isimlendirilen kütlesiz bir parçacık akışı olmak üzere birbirini tamamlayan iki biçiminde tanımlanmaktadır. (http://astom.omu.edu.tr, 2006). X-ışınları, ultraviyole (mor ötesi) ışınları, mikro dalgalar ve radyo dalgaları başlıca elektromanyetik dalgalar arasında sayılmaktadır (Henden, 2000). Elektromanyetik dalgalar, dalga boylarına veya enerjilerine göre çok geniş bir alana yayılırlar. Elektromanyetik spektrum bölgeleri Şekil 1'deki gibi gösterilebilir.



Şekil.1 Elektromanyetik spektrum bölgeleri (Perincek, 2006)

Figure.1 *Electromagnetic spectrum regions (Perincek, 2006)*

Ultraviyole Radyasyon

UV, güneş tarafından yayılan ve göz tarafından algılanmayan radyasyon tipidir. Güneş, elektromanyetik spektrum olarak bilinen bir dizi enerji yaymaktadır ve bu enerjinin değişik şekilleri dalga boylarına göre sınıflandırılır. Buna göre UV radyasyon, farklı dalga boylarına göre üç tipte incelenebilmektedir;

UV-A: 315-380 nm

UV-B: 280-315 nm

UV-C: 190-280 nm dalga boyları arasındadır.

Dalga boyları ile radyasyonun enerjisi arasında ters bir ilişki bulunmaktadır. Bu nedenle dalga boyu en az ve dolayısıyla enerjisi en yüksek olan UV-C' nin daha ağır hücresel hasarlara neden olabileceği söylenebilmekte (Caspari, 2000) ve yine dalga boyu en yuksek ve enerjisi en az olan UV-A' nın da hücresel zararlarının tolere edilebilecek duzeyde olduğundan bahsedilmektedir (Leena ve Marikki, 2005).

UV ışınları aynı zamanda serbest radikaller meydana getirerek hücrelerde oksidatif strese neden olurlar (Latonen ve Laiho, 2005).Oluşan bu serbest radikaller, hücrede biyomoleküllere hasar vererek, hücrenin bütünlüğünün bozulmasına, yaşlanmasına ve hücre ölümüne neden olabilmektedirler (Özalpan, 2001).

Deinococcus radiodurans

Deinococcus radiodurans, yüksek seviyede iyonize radyasyon ve UV radyasyon, kuraklık ve DNA' ya zarar veren kimyasallar gibi birçok ajan ve koşula olan direnciyle iyi bilinen bir ekstremofildir (Battista, 1997). Aynı zamanda çeşitli ağır metalleri ve radyoaktif metalleri yıkabilme özelliğine sahip bir bakteridir (Battista ve Raney, 1997).

D. radiodurans, UV-C radyasyona (190- 280 nm) son derece dirençli bir organizmadır ve UV-C' nin tetiklediği siklobütan, primidin dimerleri (CPDs) ve primidin-(6-4) primidon (6-4 PPs) gibi biprimidin ışık ürünlerini (BPPs) etkili bir şekilde tamir edebilmektedir (Setlow ve Duggan, 1964). UV-C' e maruz kalmış *D. radiodurans'* ın en önemli primidin ürünleri primidin dimerleridir (Moeller, 2010). UVC' nin tetiklediği DNA zararı iki nükleotit eksizyon tamir mekanizması (Moseley ve Evans, 1983;Minton, 1994) ve bir genetik rekombinasyon mekanizması ile tamir edilmektedir (Moseley ve ark.,1972; Moseley ve Copland, 1975).

D. radiodurans' da Oksidatif Stres

Oksidatif stres, prooksidanlarla, reaktif oksijen türlerinin (ROT) ortamda artmasına engel olan antioksidanların savunma yeteneği arasındaki dengesizliğin bir sonucu olarak ortaya çıkmaktadır (John ve Gutterdige, 2010).

Yüksek oranda çeşitlilik gösteren antioksidan savunma sistemleri, protein oksidasyonuna engel olur ve Reaktif Oksijen Türlerini temizleyerek *D. radiodurans'* daki oksidatif stresi hafifletmektedir. *D. radiodurans'* daki antioksidan savunma mekanizması, genel olarak hidroksil radikali (·OH) (Mello ve Meneghini, 1984; Dunford, 1987), süperoksit radikali (O_2 ·–), hidrojen peroksit (H_2O_2) (Imlay, 2006) gibi 3 reaktif oksijen türüne karşı etkili olmaktadır.

D. radiodurans' ın H_2O_2, ·OH, O_2 ·– gibi reaktif oksijen türlerini temizleme kapasitesi, *E.coli'* den sırasıyla 30 kat, 17 kat ve 6 kat daha yüksektir (Tian ve ark.,

2004). *D. radiodurans* oldukça yüksek katalaz ve SOD aktivitesine sahiptir (Lipton, 2002). Katalazlar ve peroksidazlar H_2O_2' i uzaklaştırırken SOD' lar hücrelerden süperoksit radikallerini elimine etmektedirler (Makarova, 2001;Markillie ve ark., 1999).

Karoten

Enzimatik olmayan antioksidanlar arasında yer alan karoten, yağda çözünebilen doğal antioksidanlardır. Başta singlet oksijen ve peroksi radikalleri ($ROO^{\cdot}$) olmak üzere ROT türlerine karşı etkili bir savunma mekanizması oluşturmaktadır (Tatsuzawa ve ark., 2000;Stahl ve Sies, 2003). Karotenoidler, DNA' yı oksidatif zarardan, proteinleri karbonilasyondan ve membran lipitlerini lipit peroksidasyonundan korumaktadır (Stahl ve ark., 1998; Zhang ve Omave, 2000). *D. radiodurans* 'ın hücre içi karetenoidleri, ($^{\cdot}OH$, $O_2^{\cdot -}$, H_2O_2, ve $^1O_2$) gibi ROT (Zhang ve ark., 2007; Tian ve ark., 2007) ve 2,2-difenil-1-pikrilhidrazil gibi RNT (Reaktif Nitrojen Türleri)'ni ortamdan temizleyebilmektedir. Karotenoidlerin antioksidan aktivitesi, yapısındaki konjuge çifte bağlardan ileri gelmektedir. *D. radiodurans* bakteriye turuncu rengini veren çok miktarda karoten sentezlemektedir. *D. radiodurans*' ın renksiz mutant soyunun indüklenmiş oksidatif stres sonucu oluşan reaktif oksijen türlerine yabanıla oranla daha duyarlı olduğunu tespit edilmiştir (Carbonneau ve ark., 1989).

Vitreoscilla Hemoglobin

Hemoglobinler, 1986 yılına kadar ökaryotik orjinli proteinler olarak bilinmekteydi. Ancak Dr. Webster ve arkadaşları Gram (-) bir bakteri olan *Vitreoscilla stercoraria'* nın doğal olarak hemoglobin içerdiğini tespit etmişlerdir (Wakabayashi ve ark.,1986). *Beggiatoaceae* familyasında bulunan *Vitreoscilla* zorunlu aerob, Gram (-) ve kemoorganotrof flamentli bir bakteridir. Zorunlu aerob olmasına rağmen doğal yaşam alanı oksijeni düşük ortamlardır ve bu koşullarda yaşamını sürdürebilmek için hemoglogin (vgb) genini sentezlemektedir (Woose, 1987). *Vitreoscilla* hemoglobininin (VHb) ökaryotik hemoglobinlerle yüksek homoloji göstermekte olup, farklı yapısal organizasyonu ve konformasyonu stres durumlarında kalma yeteneği gösterebilmesi onun birden fazla işlevi gerçekleştirebilmesine imkan sağlamaktadır (Khosla ve Bailey, 1988;Liu ve ark., 2008).

Bu çalışmada, yabanıl *D. radiodrans* ile *Vitreoscilla* hemoglobin (*vgb*) geni klonlanmış rekombinantı ve kontrol olarak da *vgb*$^-$ rekombinant suşu kullanılmıştır. Bakterilerin UV-C radyasyon direnci araştırılıp, bunlara ek olarak organizmaya daha fazla oksijenli ortam sağlayarak daha fazla büyümesini sağlayan *vgb* geninin, bakterinin UV-C direncine yapacağı katkının araştırılması planlanmıştır. Aynı zamanda UV-C radyasyon uygulamalarının yabanıl ve rekombinant *D. radiodurans'* ın antioksidan savunma sistemleri (süperoksit dismutaz, katalaz ve karoten) üzerine etkisi araştırılıp, *vgb*' nin bu savunma sistemine etkileri saptanacaktır. Çünkü UV' nin hücresel zararına karşı antioksidan savunma mekanizmalarında, oksijen gerektiren çeşitli oksijenaz ve deoksijenazlarla katalizlenen, etkin bir oksijen alımı ve kullanımını sağlayan *vgb* geni aktarılmış rekombinant bakterinin daha etkili olacağı düşünülmektedir. Bu bağlamda radyasyona dirençliliğiyle bilinen *D. radiodurans'* ın vgb/VHb sisteminin UV-C direnç kapasitesine etkisi ve organizmanın bundan nasıl etkileneceği araştırılmıştır.

MATERYAL ve METOD

Bakteri Soyları

Deinococcus radiodurans R1 (ATCC BAA-816), bu bakterinin vgb geni klonlanmış rekombinantı ve kontrol olarak da vgb$^-$ rekombinant suşu kullanıldı. *D. radiodurans*' in iki rekombinantından puc8 plazmitini taşıyan Dr[pUC8] olarak, aynı plazmitin vgb geni taşıyan formu ise Dr[pUC8:15] olarak adlandırılmaktadır (Şekil 2).

Şekil 2. D. radiodurans ve rekombinantları
Figure 2. *D. radiodurans and its recombinants*

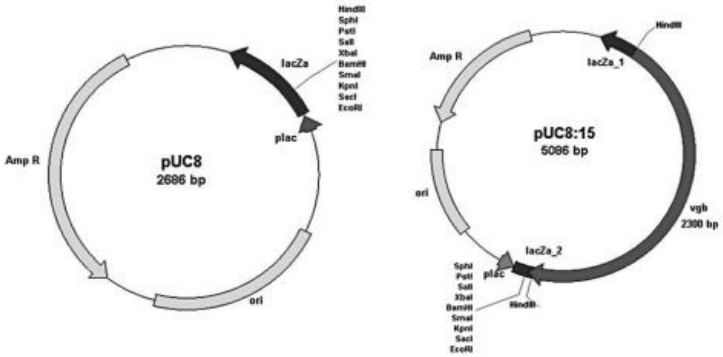
vgb Klonları

D. radiodurans'ın Dr[pUC8] ve Dr[pUC8:15] rekombinantları İnönü Üniversitesi Moleküler Biyoloji Bölümünde Hikmet Geçkil' in laboratuvarından temin edilmiştir. pUC8 plazmiti 2.7 kb büyüklüğünde olup işlevsel bir lacZ geni taşımaktadır. pUC8:15 plazmiti ise, 5 kb büyüklüğündedir ve 2.3 kb uzunluğundaki multi klonlama bölgesine vgb geni yerleştirilmiştir (Şekil 3).

Bakterilerin UV-C Radyasyona Maruz Bırakılması

UV-C uygulaması için Philips TUV 15 Watt/G15 T8 254 nm dalga boyuna sahip 45 cm boyunda UV-C

lambası kullanılmıştır. UV-C radyasyon kaynağı tavana 6 cm mesafe ile yerleştirilmiştir. Işınlama için 90 x 60 x 70 cm boyutlarındaki alüminyum ile kaplı bir kabin kullanılmıştır. Lamba ile örnekler arası ışınlama mesafesi 11 cm'dir. Lambanın yüzeyinden 1 cm mesafede 15 w'lık bir güç var olduğundan dolayı 11 cm'deki uygulanan gücün değeri 0.124 wattır. 11 cm'lik mesafeden yapılan bu ışınlama 46 x 13 cm'lik bir yüzey alanını kapsamaktadır. Enzim aktivite tayini için UV-C uygulama süresine bağlı olarak uygulanan doz miktarları aşağıdaki çizelge 2' de verilmiştir.



Şekil 3. pUC8 ve pUC8:15 plazmitlerinin fiziki haritası.
Figure 3. *Physical map of the pUC8 and pUC8: 15 plasmids.*

Çizelge 1. UV-C uygulama süresine bağlı olarak uygulanan doz miktarları
Table 1. *Doses applied depending on the application time of UV-C.*

UV-C Uygulama süresi UV-C Application time	Doz Miktarı (j cm -2) Dosage Amount (j cm -2)
6 saat	4.47
12 saat	8.94
24 saat	17.88
48 saat	35.76

Enzim Aktivite ve Karotenoid Miktar Tayini

Enzim aktivitesi tayin işlemlerinde, spektrofotometre (SHİMADZU UV-visible Spectrophotometer UV-1601) kullanıldı. Bütün enzimlerin aktiviteleri her bakteri için üç tekrarlı olarak ölçüldü. Katalaz enziminin aktivite tayini Luck (1963) yöntemine göre, SOD enziminin aktivitesi Mc Cord ve Fridovich (1969) yöntemine göre yapıldı. .Absorbans değerleri belirlendikten sonra ml' deki enzim ünite sayısı spektrofotometrik olarak hesaplandı. Elde edilen değerler süpernatanın mililitresindeki miligram proteine bölünerek spesifik aktivite tespit edildi. 6, 12, 24 ve 48 saat UV-C radyasyon uygulanan örneklerin karoten tayini, spektrofotometrik olarak yapıldı (Bhosole ve Gadre, 2001). Litredeki miktarı bulmak için elde edilen sonuçlar 1000 ile çarpılarak toplam karotenoid miktarı mg L^{-1} olarak hesaplanmıştır.

İstatistiki Analizler

Elde edilen sonuçların istatistiksel olarak değerlendirilmesi amacıyla istatiksel paket program (SPSS 10.0 for Windows İnc., USA) kullanıldı. Bu programda önem kontrolü için Duncan testi uygulandı (Duncan, 1995).

BULGULAR

UV-C Uygulaması Sonrası SOD Aktivitesi

D. radiodurans (yabanıl) ve rekombinant bakterilerinde UV-C radyasyona maruz bırakılan ve bırakılmayan gruplarda süperoksit dismutaz (SOD) aktiviteleri saptanmış ve sonuçlar Çizelge 2'de verilmiştir. Buna göre *D. radiodurans* (yabanıl)' da 12 ve 24 saatlik uygulamalarda kontrol grubuna göre bir artış saptanmıştır. *D. radiodurans (vgb)'* de UV-C uygulamasına bağlı olarak kontrol gruplarıyla karşılaştırıldıklarında SOD enzim aktivitesinde genel olarak bir artış gözlenmiştir. *D. radiodurans (vgb) ve D. radiodurans (pUC8)'* in UV-C uygulamasına bağlı olarak kontrol gruplarıyla karşılaştırıldıklarında SOD enzim aktivitesinde genel olarak bir artış gözlenmiştir.

UV-C Uygulaması Sonrası Katalaz Aktivitesi

D. radiodurans ve rekombinant suşlarında UV-C radyasyona maruz bırakılan ve bırakılmayan gruplarda katalaz (KAT) aktiviteleri saptanmış ve sonuçlar Çizelge 2'de verilmiştir. Buna göre *D. radiodurans* (yabanıl)' da en yüksek enzim aktivitesi de 24 saat UV-C uygulanan örneklerde tespit edilmiştir. *D. radiodurans* (vgb)' de UV-C uygulamasına bağlı olarak kontrol gruplarıyla kıyaslandığında 24 saatlik UV-C uygulanan örneklerdeki KAT enzim aktivitesi kontrol grubundan yüksek olmasına rağmen 6 saatlik ışın uygulanan örneklerden daha düşük tespit edilmiştir. Bunun dışında diğer örneklerde doza bağlı olarak bir artış gözlenmiştir. *D. radiodurans (pUC8)'* in UV-C uygulanan örnekleri kontrol gruplarıyla kıyaslandığında 12 saatlik UV-C uygulanan örneklerdeki KAT enzim aktivitesi kontrol grubundan yüksek olmasına rağmen 6 saatlik ışın uygulanan örneklerden daha düşük tespit edilmiştir.

Çizelge 2. Farklı dozlarda UV-C radyasyon uygulanan D. radiodurans ve rekombinant suşlarda süperoksit dismutaz (SOD) ve katalaz (KAT) aktivitesi

Table 2. *Superoxide dismutase (SOD) and catalase (KAT) activity in D. radiodurans and recombinant strains treated with different doses of UV-C radiation.*

Bakteri Bacterium	UV-C Uygulama Süresi UV-C Application Time	SOD Aktivitesi (µmol ml -1mg -1 protein) SOD Activity (µmol ml -1mg -1 protein)	KAT Aktivitesi (µmol ml -1mg -1 protein) CAT Activity (µmol ml -1mg -1 protein)
D. radiodurans (yabanıl) *(wild)*	6 saat (kontrol)	124.3 ± 10.1	158.66 ± 8.5
	6 saat (UV-C)	79.06 ± 7.5	53.34 ± 6.4
	12 saat (kontrol)	229.4 ± 8.6	189.21 ± 10.4
	12 saat (UV-C)	235.06 ± 9.3	197.15 ± 8.6
	24 saat (kontrol)	252.93 ± 15.5	178.36 ± 9.4
	24 saat UV-C	939.04 ± 14.2	278.66 ± 11.2
	48 saat (kontrol)	216.07 ± 7.6	166.34 ± 9.3
	48 saat (UV-C)	19.2 ± 2.3	1.77 ± 0.9
D. radiodurans (vgb)	6 saat (kontrol)	139.27 ± 4.7	52.94 ± 4.2
	6 saat (UV-C)	534.8 ± 26.4	102.24 ± 5.3
	12 saat (kontrol)	265.1 ± 8.7	52.27 ± 4.5
	12 saat (UV-C)	372.5 ± 9.6	155.66 ± 8.7
	24 saat (kontrol)	201.5 ± 6.3	57.18 ± 5.6
	24 saat UV-C	1002.2 ± 18.5	118.06 ± 9.2
	48 saat (kontrol)	330.2 ± 12.4	60.81 ± 6.3
	48 saat (UV-C)	1427.07 ± 19.6	237.4 ± 12.3
D. radiodurans (pUC8)	6 saat (kontrol)	223.3 ± 11.3	38.58 ± 11.2
	6 saat (UV-C)	563.27 ± 10.4	165.27 ± 9.7
	12 saat (kontrol)	310.3 ± 9.6	41.74 ± 6.7
	12 saat (UV-C)	370.13 ± 11.2	149.74 ± 8.9
	24 saat (kontrol)	252.5 ± 8.9	60.57 ± 7.2
	24 saat UV-C	569.32 ± 11.2	172.11 ± 9.7
	48 saat (kontrol)	210.6 ± 7.8	65.90 ± 4.5
	48 saat (UV-C)	579.19 ± 13.2	178.10 ± 6.6

UV-C Uygulaması Sonrası Karoten Miktarındaki Değişimler

6, 12, 24 ve 48 saatlik UV-C uygulaması sonrası toplam kareten miktarındaki değişimler, *D. radiodurans* (yabanıl) ve rekombinantları için tespit edilmiştir. Buna göre 6 saatlik UV-C uygulanan örneklerle kontrol grupları karşılaştırıldığında *D. radiodurans* (yabanıl)'a turuncu rengini veren karotenin kontrol gruplarında, rekombinant bakterilerden yaklaşık olarak 1,5 kat daha fazla olduğu görülmüştür. Aynı zamanda UV-C uygulaması sonrası bu fark yaklaşık 4,5 kata çıkmıştır. 6 saatlik UV-C uygulaması sonrası bakterilerin karoten miktarındaki değişimler Şekil 4' de verilmiştir.

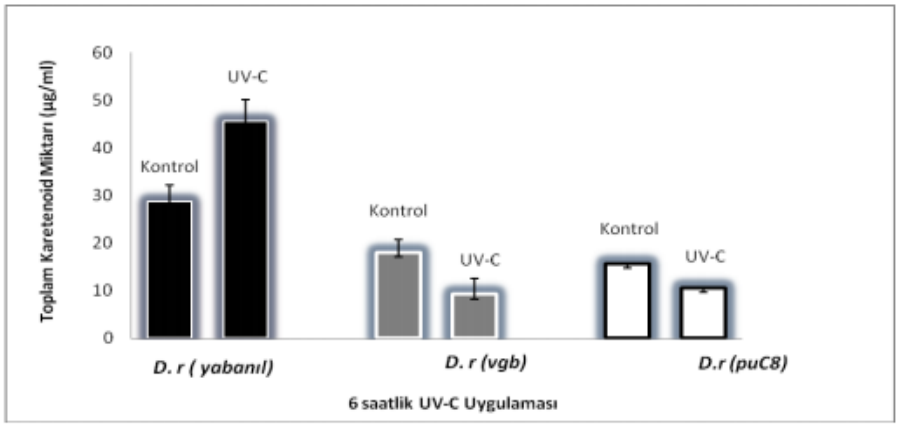
12 saatlik UV-C uygulanan örneklerle kontrol gruplarını kıyaslandığında *D. radiodurans* (yabanıl)' ın kontrol gruplarında, rekombinant bakterilerden yaklaşık olarak 3,5 kat daha fazla olduğu görülmüştür. Aynı zamanda UV-C uygulaması sonrası bu fark yaklaşık 6 kata çıkmıştır. 12 saatlik UV-C uygulaması sonrası bakterilerin karoten miktarındaki değişimler Şekil 5' de verilmiştir.

24 saatlik UV-C uygulanan örneklerle konrol gruplarını kıyaslandığında *D. radiodurans* (yabanıl)'ın kontrol gruplarında, rekombinant bakterilerden yaklaşık olarak 3,5 kat daha fazla olduğu tespit edilmiştir. Aynı zamanda UV-C uygulaması sonrası bu fark yaklaşık 8 kata çıkmıştır. 24 saatlik UV-C uygulaması sonrası bakterilerin karoten miktarındaki değişimler Şekil 6' da verilmiştir.

48 saatlik UV-C uygulanan gruplarla kontrol grupları kıyaslandığında *D. radiodurans* (yabanıl)' ın kontrol gruplarındaki karoten miktarının rekombinant bakterilerden yaklaşık 3,5 kat daha fazla olduğu ortaya çıkmıştır. UV-C uygulamasına bağlı olarak bu fark 4,2 kata çıkmıştır. 48 saatlik UV-C uygulaması sonrası bakterilerin karoten miktarındaki değişimler Şekil 7' de verilmiştir.

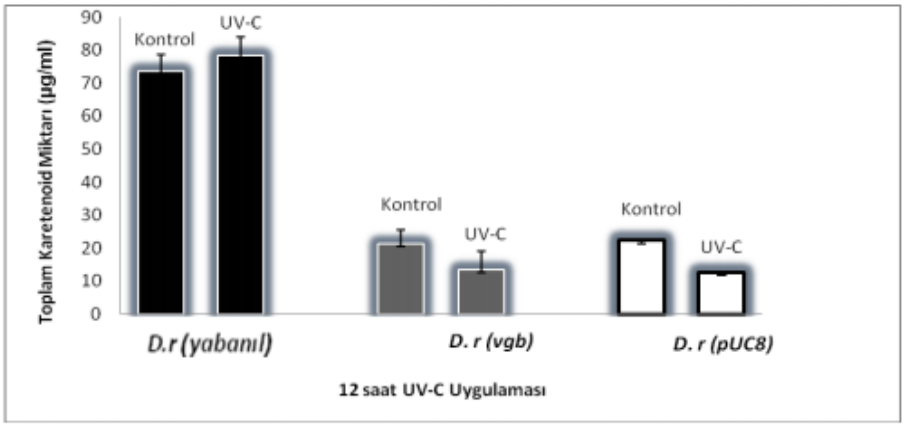
Bakterilerin UV-C Uygulaması Öncesi ve Sonrası SEM Resimleri

D. radiodurans (yabanıl) ve rekombinantlarının, UV-C uygulaması öncesi ve sonrası bakterilerin hücresel yapılarındaki değişimler Scaning Elektron Mikroskobunda tespit edilmiştir (Şekil 8). *D. radiodurans* (yabanıl), kok şeklinde ve genel olarak ikili veya dörtlü hücre kümeleri şeklinde bulunurlar.



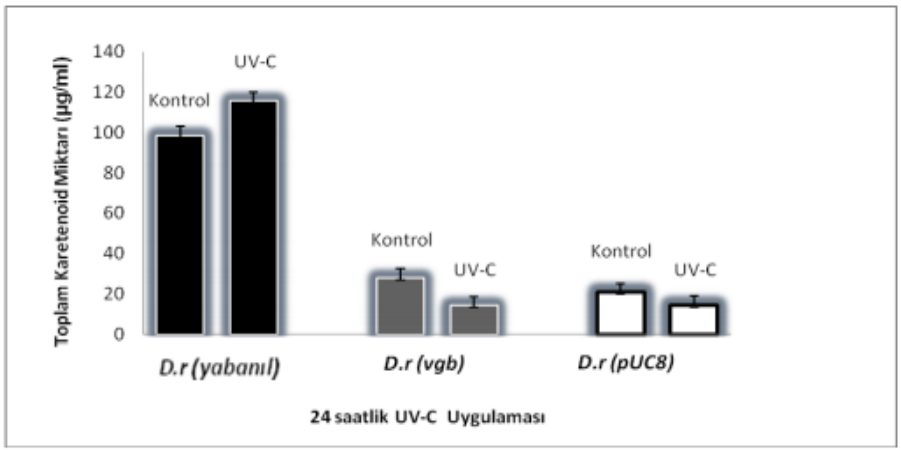
Şekil 4. D. radiodurans (yabanıl) ve rekombinantlarının 6 saatlik UV-C uygulaması sonrası toplam karoten miktarındaki değişimler

Figure 4. *Changes in total carotene amount after 6 hours UV-C application of D. radiodurans (wild) and recombinants*



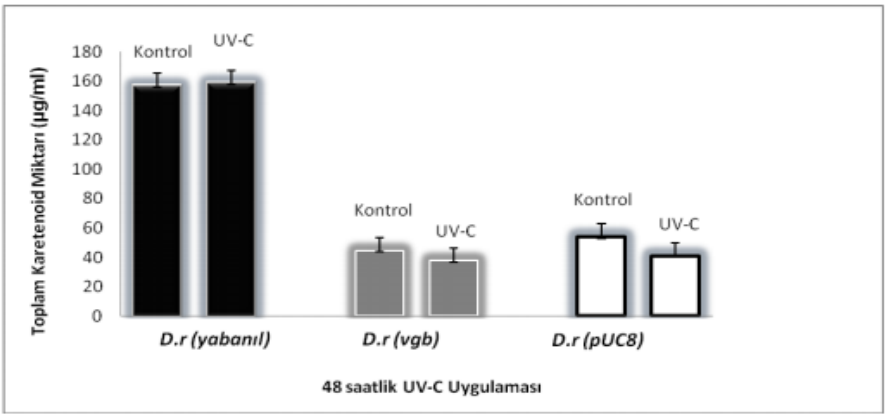
Şekil 5. D. radiodurans (yabanıl) ve rekombinantlarının 12 saatlik UV-C uygulaması sonrası toplam karoten miktarındaki değişimler

Figure 5. *Changes in the total carotene amount after 12 hours UV-C application of D. radiodurans (wild) and recombinants*



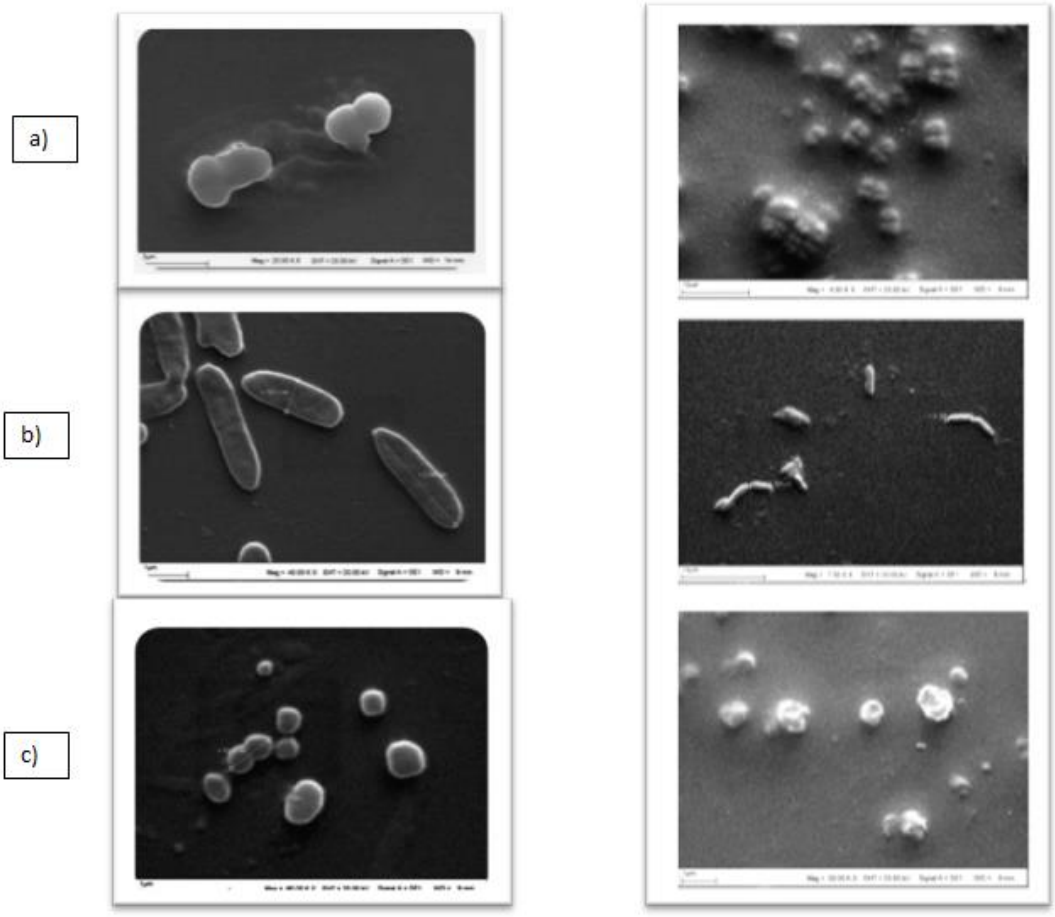
Şekil 6. D. radiodurans (yabanıl) ve rekombinantlarının 24 saatlik UV-C uygulaması sonrası toplam karoten miktarındaki değişimler

Figure 6. *Changes in the total carotene amount after 24 hours UV-C application of D. radiodurans (wild) and recombinants*



Şekil 7. D. radiodurans (yabanıl) ve rekombinantlarının 48 saatlik UV-C uygulaması sonrası toplam karoten miktarındaki değişimler.

Figure 7. *Changes in the total carotene amount after 48 hours UV-C application of D. radiodurans (wild) and recombinants*



Şekil 8. D. radiodurans (yabanıl) ve rekombinantlarının 48 saatlik UV-C uygulaması sonrası SEM görüntüleri. a) D. radiodurans (yabanıl), b) D. radiodurans (vgb), c) D. radiodurans (pUC8).

Figure 8. *SEM images of D. radiodurans (wild) and its recombinants after 48 hours UV-C application. a) D. radiodurans (wild), b) D. radiodurans (vgb), c) D. radiodurans (pUC8).*

48 saatlik UV-C uygulaması sonrası SEM resimleri karşılaştırıldığında, bakteri UV-C uygulamasından sonra boyut olarak büyümüş ve daha çok dörtlü hücre kümeleri halinde görülmüştür. Aynı zamanda hücre örtülerinin pürüzsüz yapısı bozularak deformasyonlar gözlenmiştir. *D. radiodurans* (vgb) rekombinantının yabanılından farklı olarak uzun basil yapısında olan şekli, 48 saatlik UV-C uygulaması sonrası boyut olarak oldukça küçülmüş ve hücre yapısında bozulmalar gözlenmiştir. *D. radiodurans* (pUC8),

yabanıl tipteki bakteri gibi kok şeklindedir. 48 saatlik UV-C uygulaması sonrası bakteri boyut olarak büyümüş ve hücre örtülerinin pürüzsüz yapısı bozularak deformasyonlar gözlenmiştir.

TARTIŞMA ve SONUÇ

UV-C uygulamasının antioksidan sistem üzerine etkisinin karoten pigmenti ile ilişkilendirildiği çalışmada, aynı zamanda vgb geninin de antioksidan savunmaya katacağı katkı araştırılmak istenmiştir. UV-C'nin hücresel zararına karşı antioksidan savunma mekanizmalarında, oksijen gerektiren çeşitli oksijenaz ve deoksijenazlarla katalizlenen, etkin bir oksijen alım ve kullanımını sağlayan sistemle (vgb) geninin etkinliği tespit edilmiştir (Liu ve ark., 1995).

VHb' nin hem endojen hem de eksojen hidrojen peroksite karşı savunmada rol aldığı aynı zamanda stres altında SOD ve KAT gibi enzimlerin aktive olmasına neden olduğu tespit edilmiştir. VHb' nin oksidatif strese karşı bu koruyucu etkisinin heterelog hücrelerdeki üretkenliği arttırma yeteneğinden kaynaklandığı düşünülmektedir (Akbas ve ark., 2011)

D. radiodurans (pUC8) in UV-C uygulanan örneklerini kontrol gruplarıyla kıyaslandığında SOD ve KAT enzim aktivitesinin yabanıl ve vgb genini taşıyan rekombinantına oranla daha düşük olduğu tespit edilmiştir. Enzim aktivitelerinin yüksek olduğu yabanıl ve vgb genini taşıyan bakterilerde, radyasyonun zararlı etkisi yabanıl için 48. saatte kendini gösterirken, vgb rekombinantı VHb/vgb gen sisteminin vermiş olduğu avantajlar sayesinde 48. saatte en yüksek enzim aktivitesine sahiptir.

Karotenoid pigmentinin iyonize ve iyonize olmayan radyasyona karşı canlıları koruduğu bilinmektedir. (Jagannatham ve ark., 2000). Bu çalışmada özellikle yüksek karoten içeren yabanıl tipi bakterilerde, UV-C uygulamasına bağlı olarak miktar artışı net bir şekilde gözlenmiştir (Tian ve ark., 2007). 6, 12, 24 ve 48 saatlik UV-C uygulanan *D. radiodurans (yabanıl)* ve rekombinant bakteriler için toplam karoten miktarı *D. radiodurans (yabanıl)*'a turuncu rengini veren karotenin kontrol gruplarında, rekombinant bakterilerden yaklaşık olarak 1,5 kat daha fazla olduğu görülmüştür. Aynı zamanda UV-C uyglaması sonrası bu fark yaklaşık 4,5 kata çıkmıştır. Bu sonuçlar *D. radiodurans* karotenoidlerinin çevresel stresle mücadelede bakteriye katkısının olduğunu düşündürmektedir.

TEŞEKKÜR

D. radiodurans' ın Dr[pUC8] ve Dr[pUC8:15] rekombinantları İnönü Üniversitesi Moleküler Biyoloji Bölümünde Prof. Dr. Hikmet Geçkil' in laboratuvarından temin edilmiştir. Ayrıca bu çalışma 2012-192 no'lu proje ile İnönü Üniversitesi Bilimsel Araştırma Projeleri tarafından desteklenmiştir.

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Araştırma, yazma, orijinal taslak hazırlama, inceleme ve düzenleme, görselleştirme ve biçimsel analiz ile ilgili olarak yazarlar makaleye eşit oranda katkıda bulundu ve son halini okuyarak onayladı.

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Makale yazarları arasında herhangi bir çıkar çatışması bulunmamaktadır.

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Effects of Callus Cell Suspension Cultures and Elicitor Applications on Bioactive Components in Globe Artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori]

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ABSTRACT

Globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] has many health-protecting properties due to its valuable bioactive components which are not stable and produced in high amounts in the raw plant material in nature. These bioactive components that gaining interest can be increased thanks to the contribution of valuable *in vitro* techniques, such as callus and cell suspension cultures, and various new applications such as elicitor treatments. The present study aimed to determined bioactive components in three globe artichoke cultivars by using callus cell suspension cultures in various media combinations and by applying two elicitor treatments, namely methyl jasmonate and chitosan, at 3 different concentrations (methyl jasmonate 50 µM, 100 µM, and 200 µM; chitosan 200 mg L^{-1}, 400 mg L^{-1}, and 800 mg L^{-1}) with 3 different application durations (24h, 48h, and 72h). The bioactive compounds profile of cultivars was determined by HPLC-DAD. Obtained results revealed that using well-balanced concentrations of auxin: cytokinin (1:1 or 10:1) in a media composition is a must for triggering the callus formation process for globe artichoke. Results also showed that accumulated bioactive components and their amounts varied based on cultivars. Experiment results revealed that different types of elicitors other than methyl jasmonate and chitosan, or different doses of elicitors and application durations should be used/tested to get desired levels of bioactive components. The findings of the present study may play a supportively and complementarily mission in several important fields such as agriculture, and pharmaceutical engineering.

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Flavonoids
Chitosan
Methyl jasmonate

Kallus Hücre Süspansiyon Kültürleri ve Elisitör Uygulamalarının Enginarda [*Cynara cardunculus* var. *scolymus* (L.) Fiori] Biyoaktif Bileşenler Üzerine Etkisi

ÖZET

Enginar [*Cynara cardunculus* var. *scolymus* (L.) Fiori] doğada ham bitki materyalinde yüksek miktarlarda ve stabil olarak üretilmeyen değerli biyoaktif bileşenleri nedeniyle sağlık üzerine koruyucu birçok özelliğe sahiptir. Bu biyoaktif bileşenler, kallus ve hücre süspansiyon kültürleri gibi değerli *in vitro* tekniklerin ve elisitör gibi çeşitli yeni uygulamaların katkısıyla artırılabilmektedir. Bu çalışmada, çeşitli besi ortam kombinasyonlarında kallus hücre süspansiyon kültürleri kullanılarak ve 3 farklı konsantrasyonda (metil jasmonat 50 µM, 100 µM ve 200 µM; kitosan 200 mg L^{-1}, 400 mg L^{-1} ve 800 mg L^{-1}) 3 farklı uygulama süresiyle (24 saat, 48 saat ve 72 saat) uygulamaların biyoaktif içerikler üzerine olan etkisi araştırılmıştır. Çeşitlerin biyoaktif bileşik profili HPLC-DAD ile belirlenmiştir. Elde edilen sonuçlar, besi ortamı bileşiminde iyi dengelenmiş oksin: sitokinin (1:1 veya 10:1) konsantrasyonlarının kullanılmasının, enginarda kallus oluşum sürecini tetiklemek için bir zorunluluk olduğunu açıkça ortaya koymuştur. Sonuçlar ayrıca biriken biyoaktif bileşenlerin ve miktarlarının çeşitlere göre değiştiğini ortaya koymuştur. Araştırma sonuçları, istenen biyoaktif bileşen seviyelerini elde etmek için metil

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
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Kitosan
Metil jasmonat

jasmonat ve kitosan dışında farklı tipte elisitörlerin veya aynı elisitörlerin farklı dozlarının ve/veya uygulama sürelerinin kullanılması/test edilmesi gerektiğini açık bir şekilde ortaya koymuştur. Mevcut çalışmanın bulgularının, tarım ve ilaç mühendisliği gibi birçok önemli alanda destekleyici ve tamamlayıcı bir etkisi olacağı düşünülmektedir.

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INTRODUCTION

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] with special therapeutic features for human is native to Mediterranean Basin. Globe artichoke has gained its reputation because it has valuable bioactive components in its edible parts as well as its non-edible parts. Although its non-edible parts are treated as waste, their value has recently been more understood and has become important (Pandino et al., 2011; Pandino et al., 2013).

Plants incorporate precious bioactive compounds with healing properties on human are produced and used in many fields, incuding pharmaceuticals, agrochemicals, food flavor, texture, and color. But it is difficult to attain the desired level of bioactive compounds in plants and since it is dependent on the variation of environmental conditions, stresses, genotypes, and plant parts. To overcome such uncontrollable circumstances, biotechnological practices like callus and cell suspension culture techniques are used (Pandino et al., 2017; El-Bahr et al., 2018). However, plant parts from which explants are taken to initiate callus and cell suspension cultures, which also allow the production of bioactive compounds, are also important. Starting *in vitro* callus and cell suspension cultures using plant parts where the target bioactive compound is concentrated will be advantageous for the potential bioactive compound obtained during the culture (Sökmen and Gürel, 2001). In addition to using these morphologically unorganized and undifferentiated biotechnological techniques, other treatments namely precursors, elicitors, immobilizations, can also be used for cell growth and production of targeted bioactive compounds in large volumes. Considering the above stated potential of *in vitro* techniques, they serve as reliable, sustainable, continuous and standardized production processes of precious bioactive compounds.

The use of elicitors provides an opportunity for effective and practical work called elicitation. Using various types of elicitors (biotic and abiotic) at different doses and/or applying durations, changes, or enhancements in the amount of the desired bioactive component can be achieved (Naik and Al–Khayri, 2015). Two different elicitors were preferred in this study; methyl jasmonate and chitosan. Methyl jasmonate is a phytohormone which is a volatile methyl ester form of jasmonic acid, has many roles in the plant, and is known to play a critical role in a number of important physiological and developmental processes that take place in the plant. Chitin is a component formed in the wall structure of various plant fungal pathogens. Chitosan, on the other hand, is a component derived from chitin and is often used as an elicitor (Ahmed and Baig, 2014; Alsoufi et al., 2019).

Using these techniques has been practiced in many medicinal and aromatic plants from past to present and their use is getting increased day by day in basic fields such as agriculture, food, and pharmacology. Based on the literature search, it is found out that there are limited studies using callus cell suspension culture techniques and elicitor treatments in globe artichoke. The present study aimed to determine bioactive components in three globe artichoke cultivars by using callus cell suspension cultures in various media combinations and by applying two elicitor treatments.

MATERIAL and METHOD

Plant materials and surface sterilization

Two open-pollinated (OP) globe artichoke cultivars, namely 'Bayrampaşa' and 'Sakız', and one F_1 hybrid globe artichoke cultivar 'Olympus' were used as plant materials. For callus induction and formation, especially newly formed leaves were separately collected and carried to the tissue culture laboratory for surface sterilization. To serve the purpose: (1) leaf explants were kept under running tap water for 15 minutes, (2) in an antibacterial soap solution (5 mL antibacterial soap + 95 mL water) for 15 minutes. After rinsing, the leaf explants were taken to laminar airflow workbench for further surface sterilization process which was carried out by treating with 20% (v/v) of a commercial bleach solution (40 g/L active chlorine) for 10 minutes, followed by 3 times rinsing with sterilized distilled water (López-Pérez and Martínez JA 2015; Ozsan and Onus, 2020a; b).

Media compositions, induction and conditions of callus culture, biomass yield

For establishing the callus cultures from newly formed leaf explants, several media compositions were assessed according to Ozsan and Onus, (2020b). The medium compositions consisted of Gamborg B5 basic media (Gamborg et al., 1968) supplemented with plant growth regulators (BAP, NAA, Kin, 2,4-D) at various concentrations (0.1, 0.5, 1.0, 2.0 mg L^{-1}), and all medium combinations contained 30.0 g L^{-1} sucrose and 6 g L^{-1} plant agar, pH was adjusted to 5.8 before autoclaved. After the necessary surface sterilization processes, initial plant leaf explants were cut at the size of 0.5-1.0 cm (Abbas et al., 2018; Sarmadi et al., 2018) and placed on these medium combinations. Plant growth regulator-free Gamborg B5 basal medium (Gamborg B5-0) was used as a control medium. All callus cultures were incubated at a growth chamber having 24±2 °C temperature, 16 hours light and 8 hours dark photoperiod under 3000 µ $E.m^{-2}.s^{-1}$ light intensity.

Differences between callus morphology were recorded weekly intervals based on cultivars and media combinations. In about 3-4 weeks, among all media combinations, well-responded calli growths were recorded on various media combinations for each cultivar. Five sub-cultures were performed in the same media combinations for each cultivar. Formed calli were maintained and utilized for further steps of experiments.

For fresh weights biomass yield of calli belonging to cultivars, the harvested calli from each media composition were measured and it was recorded as fresh weights.

Establishment of cell suspension cultures

Calli developed from various media combinations as stated above were used for initiating the cell suspension cultures. Suspension cultures were established for each cultivar by transferring 1.0 g fresh weight of friable calli onto Gamborg B5 liquid medium; (50 mL) within 250 mL capacity of Erlenmeyer flasks. They were strengthened with several concentrations of plant growth regulators depending on each cultivar's response as down stated and based on findings of Ozsan and Onus, (2020b).

For 'Bayrampaşa' OP cultivar, (1) 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 18), (2) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (3) 0.1 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 23), (4) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (5) 1.0 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 28).

For 'Sakız' OP cultivar, (1) 0.1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 13), (2) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19), (3) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (4) 0.5 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 25), (5) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (6) 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium no 6).

For 'Olympus' F_1 hybrid cultivar, (1) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19).

These media combinations were chosen for present study because they were found to be highly effective on callus formation with good enough amounts (Ozsan and Onus, 2020b).

All Erlenmeyer flasks were kept on an orbital rotary shaker, at 130 rpm, in the growth chamber until full suspension. Afterwards suspended cells were filtered individually through 0.45 µm filters, washed out with sterile distilled water, bare down gently on sterilized filter paper to remove excess water, then weighed, and subsequently, cells were sub-cultured in a fresh medium. The stated process was repeated at the end of each sub-culture with 12-days intervals. For determining dry weights, cell suspension cultures were subjected to the oven-dry process at 60 °C until reaching the constant weights.

Preparation of elicitors' concentrations and elicitation

Stock solution of methyl jasmonate (MeJa, Sigma) was prepared by dissolving in 70% (v/v) ethanol. Distilled pure water was used for further dilutions. Filter sterilization with 0.22 µm syringe filter (Millipore) was conducted for final solution. Afterwards MeJa at the concentrations of 50 µM, 100 µM, and 200 µM with 3 different application durations (24h, 48h, and 72h) were supplied to cell suspension cultures (Krzyzanowska et al., 2012; Tanoori et al., 2015; Liu et al., 2018).

Regarding chitosan application; preparation of stock solution of chitosan from crab shells (Sigma) was prepared by dissolving in 0.1 HCl by heating gently and stirring continuously. The sterilization of the stock solution was provided by autoclaving. After this process, chitosan at the concentrations of 200 mg L^{-1}, 400 mg L^{-1} and 800 mg L^{-1} with 3 different application durations (24h, 48h, and 72h) were supplied to cell suspension cultures (Lim et al., 2013; Ahmed and Baig, 2014; Jiao et al., 2018). It was prepared 50% (v/v) ethanol solution for both elicitor treatments' control media.

To see the effects of elicitors on bioactive compounds first step is weighting up formed calli. To serve the purpose calli weight was scaled at the end of each application duration.

Reagents and solvents

For polyphenol analysis, evaluated chemicals were high purity. Methanol, acetonitrile, acetic acid (≥99.5%) were purchased from Isolab; mono- and di-caffeoylquinic acids were purchased as powder form namely were 3-*O*-caffeoylquinic acid (>98%) and 1,5-*O*-

dicaffeoylquinic acid (99%) (Toronto Research Chemicals); 4-*O*-caffeoylquinic acid (99%), 5-*O*-caffeoylquinic acid (99%), 1,3-*O*-dicaffeoylquinic acid (99%) (Cayman Chemical). Other phenolics were purchased by several firms, such as narirutin (99%), luteolin (99%), apigenin (99%) and apigenin 7-*O*-glucuronide (99%) (Cayman Chemical); *p*-coumaric acid (≥99%) and caffeic acid (≥98%) (Sigma-Aldrich); ferulic acid (99%) (J&K Scientific); luteolin 7-*O*-glucronide (87%) (Harbison Walker International). To conduct HPLC analysis, the Millipore Mill-Q Direct Q-3 ultrapure water system was used for ultrapure water.

Extraction of bioactive compounds and HPLC analysis

The extraction procedure and HPLC analysis of each bioactive compound were carried out as described in Pandino et al., (2010) with a few modifications. Each sample was grounded, weighed as 0.5±0.01 g, and then extracted with 80% ethanol solution (5.0 mL). These samples were vortex for 30 seconds and then kept overnight in a shaker with 250 rpm. At the end of this process, the samples were filtered with 0.45 µm PTFE filter, centrifuged at 10000 rpm for 10 minutes, then the supernatant was collected, 1 mL sample extract transferred to 2 mL vials and injected to HPLC-DAD instrument for bioactive compound analysis, the same procedure was repeated twice.

The Agilent 1100 HPLC instrument with a quaternary HPLC pump (G1311A), column oven (G1316A), auto sampler (G1313A), degasser (G1379A) and diode array detector (DAD) (G1315A) was used to conduct the bioactive compound analysis. To achieve the chromatographic separation of bioactive compounds, the Agilent Hypersil ODS 250 mm x 4.6 mm I.D., 5 µm particle size C18 column, operated at 28 ºC, was used.

For the determination of bioactive compounds quantitatively, the HPLC analysis method was adapted from Pandino et al., (2010); mobile phases were 5% acetic acid in water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate 1.0 mL/min, the column oven temperature was 28 °C and the injection volume was 20 µL. The gradient started with 10% mobile phase B to reach 20% percent at 5 minutes, 40% mobile phase B at 45-minute, 100% mobile phase B at 55 minutes. HPLC-DAD chromatograms were determined by the limits of detection (LOD) and quantification (LOQ) values and the spectrum data were collected at 310 nm, 330 nm, and 280 nm. Each bioactive compound was identified based on the retention time (RT) and wavelength (λmax).

Statistical analysis

The experiment was carried out as a completely randomized factorial design with 3 replications. The data were analyzed with the statistical program JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA). It was performed ANOVA to determine the effects of cultivars and sub-cultures on certain bioactive components. Comparisons that obtained $P \leq 0.05$ were considered statistically significant. Additionally, correlation among all the obtained results was carried out through multivariate methods with the statistical program JMP version 5.0.1, with $P \leq 0.05$ as the threshold.

RESULTS and DISCUSSIONS

Callus and suspension culture

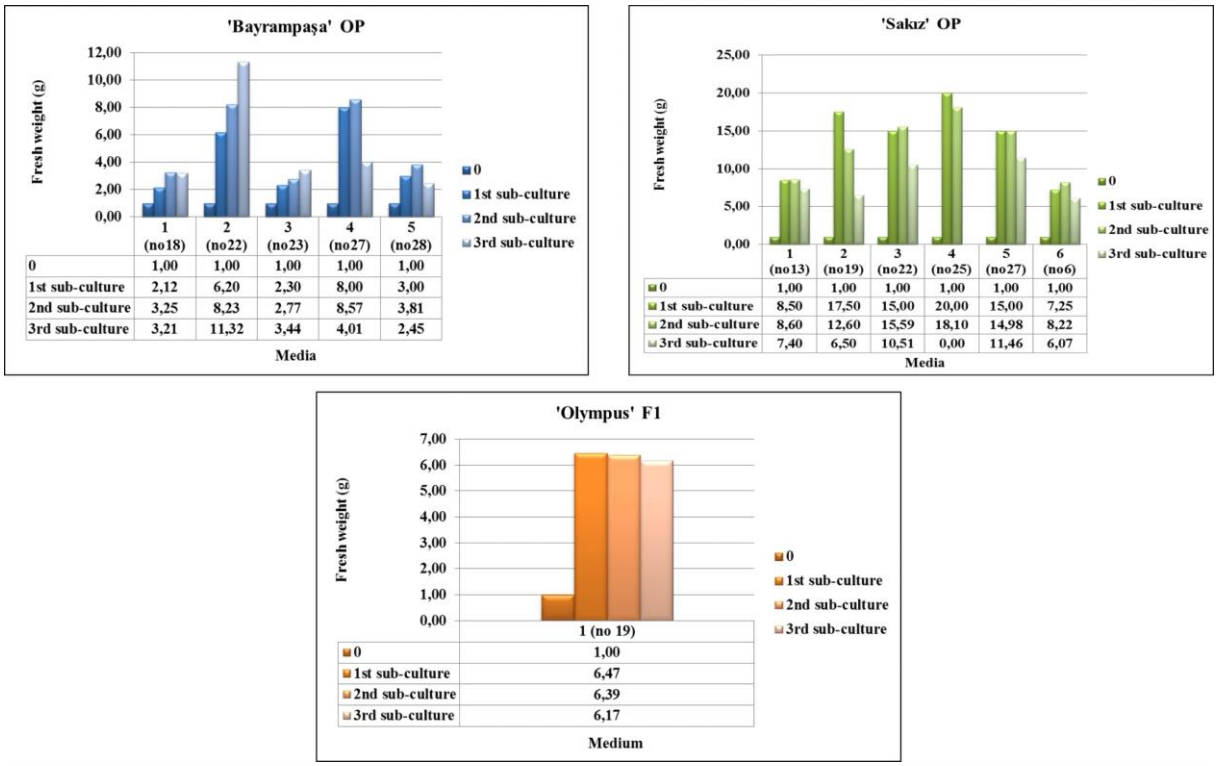
When experimental results were analyzed for callus and suspension culture, it was observed that each cultivar responded differently to all media combinations tested. Among the 29 callus induction media combinations, the most suitable media combinations for each cultivar were as down stated:

Five media combinations for Bayrampaşa OP cultivar: (1) 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 18), (2) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (3) 0.1 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 23), (4) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (5) 1.0 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 28). Two media combinations (media no 22 and 23) among 5 stated media also increased biomass yield while others caused to a decrease (Fig. 1).

Six media combinations for Sakız OP cultivar: (1) 0.1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 13), (2) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19), (3) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (4) 0.5 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 25), (5) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (6) 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium no 6). Biomass yield got decreased in all media combinations (Fig. 1).

One medium combination for Olympus F_1 hybrid cultivar: (1) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19). Unfortunately stated medium resulted with a decrease for biomass yield during each sub-culture for Olympus F_1 hybrid cultivar (Fig.1). Therefore Olympus F_1 hybrid cultivar was omitted for rest of the study.

Within the scope of callus cell suspension culture studies conducted under aseptic and controlled conditions, it is known that callus cultures are affected by many factors such as genotype, explant source, and composition of media. Compared to callus culture, cell suspension cultures require longer procedures but are considered a good source of uniform cells that enable scale production. Cell suspension cultures continue to differentiate with a short growth cycle under controlled conditions, thereby increasing the chances of repetition within and between experiments (Ngara et al., 2008; Abbas et al., 2018). Therefore, callus and cell suspension cultures can be conducted quickly using related *in vitro* culture techniques, and therefore accumulation of valuable bioactive components is enabled (Verpoorte et al., 2002; Abbas et al., 2018).



	1 (no18)	2 (no22)	3 (no23)	4 (no27)	5 (no28)
0	1,00	1,00	1,00	1,00	1,00
1st sub-culture	2,12	6,20	2,30	8,00	3,00
2nd sub-culture	3,25	8,23	2,77	8,57	3,81
3rd sub-culture	3,21	11,32	3,44	4,01	2,45

	1 (no13)	2 (no19)	3 (no22)	4 (no25)	5 (no27)	6 (no6)
0	1,00	1,00	1,00	1,00	1,00	1,00
1st sub-culture	8,50	17,50	15,00	20,00	15,00	7,25
2nd sub-culture	8,60	12,60	15,59	18,10	14,98	8,22
3rd sub-culture	7,40	6,50	10,51	0,00	11,46	6,07

	1 (no 19)
0	1,00
1st sub-culture	6,47
2nd sub-culture	6,39
3rd sub-culture	6,17

Figure 1. Biomass yield belongs to globe artichoke cultivars based on media combinations and number of sub-cultures

Şekil 1. Besi ortamı kombinasyonlarına ve alt kültür sayılarına göre enginar çeşitlerine ait biyokütle verimi

In many previous studies conducted on callus and suspension culture in different crops, it has been revealed that PGRs are so important factor for callogenesis. It has been also reported that another essential factor is that these PGRs should be added at appropriate concentrations and combinations to culture media (Ozsan and Onus, 2020b). It is possible to say that the PGRs combination of kinetin + 2,4-D is more effective than the BAP + NAA combination. Besides PGRs combination, the results revealed that concentrations of PGRs are also an essential factor for triggering calli formation. For obtaining calli, balance of auxin:cytokinin concentration based on cultivars was found to be important. It was observed that the most inducing concentrations of auxin:cytokinin 1:1 or 10:1. Therefore, obtained results from the current study were in accordance with previous studies (Ruta et al., 2013; Joshaghani et al., 2014; Abbas et al., 2018; Hesami and Daneshvar, 2018; Wani et al., 2018). If a general evaluation is made based on the findings of the present study, the accumulated bioactive compounds and their amounts varied according to cultivars and media combinations.

Elicitor treatments

Methyl jasmonate treatments belonging to Bayrampaşa OP cultivar ended with reducing on callus weights and the maximum change was determined when 200 µM MeJa was applied for 24 hours. However, the least loss of callus weight was detected in the application of 200 µM MeJa for 48 hours. When MeJa applications were evaluated in the Sakız OP cultivar, there was not as much loss in callus weights as in Bayrampaşa. However considering that most callus fresh weight losses took place in control media, it might be assumed that MeJa application prevented heavy callus weight loss for both cultivars.

Considering the results obtained in the observations made after chitosan application, decreases in callus fresh weights were noticeable in both cultivars.

Tanoori et al., (2015), who applied 200 µM MeJa to callus cultures, reported that with this application dose maximum phenolic acid accumulation took place and the increase rate was 3.96 times higher than the control group. The same researchers also reported that they achieved the highest flavonoid accumulation with a 100 µM MeJa application. Regarding chitosan application Ahmad et al., (2019) reported that different doses of chitosan applied to cell cultures in *Linum usitatissimum* L. (flax) had a positive effect on total phenolic accumulation, and the application increased their antioxidant activity by 1.3 times more than the control group. Since there was no increase on calli weight for both cultivars after elicitor applications, it was not possible to get enough amount of calli to see the effect of elicitor treatments on bioactive component contents. It is considered that cultivars may respond

differently to each elicitor type, and it is thought that the quality of elicitors, application doses and durations important factors.

In a study conducted on strawberries, it was shown that the production of anthocyanins and other metabolites caused pectinase consumption in cell cultures. Researchers concluded that there was a link between the formation of cell clusters and bioactive compounds (Edahiro and Seki, 2006). Therefore, the view arises that cell size can regulate bioactive compounds accumulation. Although no research was conducted on cell sizes in the present study, it could be speculated that MeJa and chitosan elicitor applications did not have any effect on the formation of cell clusters in both artichoke cultivars.

Evaluating of bioactive metabolites

Results of HPLC analysis regarding bioactive components revealed that there were statistically significant differences among cultivars and media combinations. Based on the results of the present study it is possible to say that cultivars respond differently to the content of media composition and the content of each media composition have different effects on different bioactive compound accumulation.

Regarding evaluated bioactive components, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, and 1,3-*O*-dicaffeoylquinic were determined in both OP cultivars, while caffeic acid, ferulic acid and luteolin 7-*O*-glucuronide were detected in only Sakız OP cultivar. Other bioactive components were detected in neither Bayrampaşa OP nor Sakız OP cultivars.

Accordingly, when suspension cultures of the Bayrampaşa OP cultivar were evaluated, two of the important bioactive components namely, 3-*O*-caffeoylquinic acid (227.43 mg kg^{-1}), and 5-*O*-caffeoylquinic acid (106.42 mg kg^{-1}), were detected in the medium 27 (1.0 mg L^{-1} kinetin + 1.0 mg L^{-1} 2,4-D). The other important bioactive components namely, 4-*O*-caffeoylquinic acid (108.10 mg kg^{-1}) and 1,3-*O*-dicaffeoylquinic acid (5.92 mg kg^{-1}), were the highest in the medium 22 (0.1 mg L^{-1} kinetin + 1.0 mg L^{-1} 2,4-D) (Table 1).

Table 1. Bioactive compounds and their values for Bayrampaşa OP cultivar based on media combination
Çizelge 1. Besi ortamı bazında Bayrampaşa çeşidi için biyoaktif bileşenler ve değerleri

	Media numbers				
Bioactive compounds	**1 (no 18)**	**2 (no 22)**	**3 (no 23)**	**4 (no 27)**	**5 (no 28)**
3-*O*-CQ	8.19^{d}	117.56^{b}	3.20^{e}	227.43^{a}	20.40^{c}
LSD value	1.82				
4-*O*-CQ	0.0^{b}	108.10^{a}	0.0^{b}	0.0^{b}	0.0^{b}
LSD value	0.59				
5-*O*-CQ	0.0^{d}	0.0^{b}	24.42^{c}	106.42^{a}	38.44^{b}
LSD value	1.64				
1,3-*O*-diCQ	0.0^{b}	5.92^{a}	0.0^{b}	0.0^{b}	0.0^{b}
LSD value	0.82				

Different letters in the same column and row show that the mean difference is a statistically significant difference at P≤0.05 level.
3-*O*-CQ: 3-*O*-caffeoylquinic acid, 4-*O*-CQ: 4-*O*-caffeoylquinic acid, 5-*O*-CQ: 5-*O*-caffeoylquinic acid, 1,3-*O*-diCQ: 1,3-*O*-dicaffeoylquinic acid.

When the Sakız OP cultivar was evaluated regarding suspension cultures, the highest values of 3-*O*-caffeoylquinic acid (171.67 mg kg^{-1}), 4-*O*-caffeoylquinic acid (138.21 mg kg^{-1}), 5-*O*-caffeoylquinic acid (12.99 mg kg^{-1}), and caffeic acid (38.50 mg kg^{-1}) were obtained in the medium 13 (0.1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA). In addition, the other important bioactive component 1,3-*O*-dicaffeoylquinic acid (78.27 mg kg^{-1}) was detected in the medium 6 (5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA). The other important bioactive components namely, ferulic acid (36.14 mg kg^{-1}) and luteolin 7-*O*-glucuronide (56.30 mg kg^{-1}), were obtained in the medium 27 (1.0 mg L^{-1} kinetin + 1.0 mg L^{-1} 2,4-D) (Table 2).

Regarding the media combinations media no 22 and 27 were common both for Bayrampaşa and Sakız OP cultivars. Results revealed that both cultivars responded differently to each media combination as 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid came into prominence in Bayrampaşa, while 1,3-*O*-dicaffeoylquinic acid, luteolin 7-*O*-glucuronide, caffeic acid, ferulic acid were determined at high amounts in Sakız (Table 3).

The study conducted by Pandino et al., (2017) is accepted as one of the first studies on the accumulation of phytochemicals through cell suspension cultures in artichoke, and therefore comparison in the literature is limited. Pandino et al., (2017) reported that they detected the highest accumulation of phenolic substances on the 5th day of suspension culture in their study in artichoke, and this amount decreased by about 26% on the 10th day of culture. As a possible cause of this situation, they showed that the cells were

in the rapid growth phase in this process. Since there was also a decrease on the level of bioactive compounds at the end of the suspension culture studies in the present study, the cells of callus suspension cultures could be also at rapid growth phase, too.

Table 2. Bioactive compounds and their values for Sakız OP cultivar based on media combination
Çizelge 2. Besi ortamı bazında Sakız çeşidi için biyoaktif bileşenler ve değerleri

	Media numbers					
Bioactive compounds	**1 (no 13)**	**2 (no 19)**	**3 (no 22)**	**4 (no 25)**	**5 (no 27)**	**6 (no 6)**
3-*O*-CQ	171.67^{a}	89.45^{b}	78.17^{c}	57.83^{de}	56.06^{e}	60.31^{d}
LSD value	2.88					
4-*O*-CQ	138.21^{a}	54.20^{d}	67.34^{b}	60.61^{c}	29.35^{f}	40.48^{e}
LSD value	2.73					
5-*O*-CQ	12.99^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
LSD value	0.31					
1,3-*O*-diCQ	30.13^{c}	0^{d}	0^{d}	0^{d}	52.22^{b}	78.27^{a}
LSD value	1.05					
Caffeic acid	38.50^{a}	0^{e}	13.26^{c}	0^{e}	8.85^{d}	21.76^{b}
LSD value	0.84					
Ferulic acid	12.41^{c}	19.23^{b}	0^{e}	6.22^{d}	36.14^{a}	0^{e}
LSD value	0.75					
Lut. 7-*O*-gluc.	0^{c}	18.58^{b}	0^{c}	0^{c}	56.30^{a}	0^{c}
LSD value	0.97					

Different letters in the same column and row show that the mean difference is a statistically significant difference at P≤0.05 level.
3-*O*-CQ: 3-*O*-caffeoylquinic acid, 4-*O*-CQ: 4-*O*-caffeoylquinic acid, 5-*O*-CQ: 5-*O*-caffeoylquinic acid, 1,3-*O*-diCQ: 1,3-*O*-dicaffeoylquinic acid, Lut. 7-*O*-gluc.: luteolin 7-*O*-glucuronide.

Results from the current study revealed that extractions originating from cell suspension cultures had different bioactive compounds and the amount of each compounds are found to be at a different level. The reason for obtaining different bioactive compounds at different amounts can be attributed to several factors. For example Negrel and Javelle, (1995), reported that this could be caused by defense responses produced by enzymes acting on the cell wall, initiating the biosynthesis of various metabolites even in the same cell lines. It is argued that the production of certain compounds in cell cultures may not occur because their origin is undifferentiated cells, and as a consequence these cells lack specific metabolites and therefore require tissue-specific biosynthesis (Reis et al., 2018).

CONCLUSION

The present study aimed to increase bioactive components in three globe artichoke cultivars by using callus cell suspension cultures in various media combinations and by applying two elicitor treatments, namely methyl jasmonate and chitosan, at 3 different concentrations. The experimental results revealed that the Bayrampaşa OP and Sakız OP cultivars reacted differently from Olympus F_1 hybrid cultivar, and they were found to be more responsive to callus cell suspension culture based on the results of HPLC-DAD analysis. Regarding the media combinations media no 22 and 27 were common both for Bayrampaşa and Sakız OP cultivars. Results revealed that both cultivars responded differently to each media combination for different bioactive compounds. Regarding elicitor treatments, after two elicitors (MeJa and chitosan) application at different concentrations and durations decreases in callus fresh weights were noticeable in both cultivars. Although there was no increase on levels of bioactive compounds, it could not be obtained, it should be kept in mind that genotype, type of elicitor applied, concentration and duration are very important factors for elicitor application. Therefore different kinds of elicitors, application times, and concentrations should be used and tested for different artichoke cultivars, assuming that it is essential to pay attention to these factors for of the studies and ensuring their optimization for further artichoke callus cell suspension studies.

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Conflict of interest statement

The authors declared no conflict of interest.

Table 3. Comparison of bioactive compounds and their values for Bayrampaşa and Sakız OP cultivars based on media combination

Çizelge 3. Besi ortamı bazında Bayrampaşa ve Sakız çeşitlerinin biyoaktif bileşenler ve değerlerinin karşılaştırılması

3-*O*-CQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	117.56^{b}	227.43^{a}	78.17^{c}	56.06^{d}	**Medium 1 =** 97.86^{b}
Mean values of cultivars	172.50^{a}		67.11^{b}		**Medium 2 =** 141.75^{a}
LSD values	**LSD_{media} =** 1.53	**$LSD_{cultivars}$ =** 1.53	**$LSD_{cultivars\ x\ media}$ =** 2.17		
4-*O*-CQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	106.42^{a}	0^{b}	0^{b}	**Medium 1 =** 0^{b}
Mean values of cultivars	53.21^{a}		0^{b}		**Medium 2 =** 53.21^{a}
LSD values	**LSD_{media} =** 1.13	**$LSD_{cultivars}$ =** 1.13	**$LSD_{cultivars\ x\ media}$ =** 1.60		
5-*O*-CQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	106.42^{a}	0^{b}	0^{b}	**Medium 1 =** 0^{b}
Mean values of cultivars	53.21^{a}		0^{b}		**Medium 2 =** 53.21^{a}
LSD values	**LSD_{media} =** 1.06	**$LSD_{cultivars}$ =** 1.06	**$LSD_{cultivars\ x\ media}$ =** 1.50		
1,3-*O*-diCQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	5.92^{b}	0^{c}	0^{c}	52.22^{a}	**Medium 1 =** 2.96^{b}
Mean values of cultivars	2.96^{b}		26.11^{a}		**Medium 2 =** 26.11^{a}
LSD values	**LSD_{media} =** 0.95	**$LSD_{cultivars}$ =** 0.95	**$LSD_{cultivars\ x\ media}$ =** 1.35		
Caffeic acid	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{c}	0^{c}	13.26^{a}	8.85^{b}	**Medium 1 =** 6.63^{a}
Mean values of cultivars	0^{b}		11.05^{a}		**Medium 2 =** 4.42^{b}
LSD values	**LSD_{media} =** 0.30	**$LSD_{cultivars}$ =** 0.30	**$LSD_{cultivars\ x\ media}$ =** 0.43		
Ferulic acid	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	0^{b}	0^{b}	36.14^{a}	**Medium 1 =** 0^{b}
Mean values of cultivars	0^{b}		18.07^{a}		**Medium 2 =** 18.07^{a}
LSD values	**LSD_{media} =** 0.49	**$LSD_{cultivars}$ =** 0.49	**$LSD_{cultivars\ x\ media}$ =** 0.70		
Lut. 7-*O*-gluc.	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	0^{b}	0^{b}	56.30^{a}	**Medium 1 =** 0^{b}
Mean values of cultivars	0^{b}		28.15^{a}		**Medium 2 =** 28.15^{a}
LSD values	**LSD_{media} =** 0.76	**$LSD_{cultivars}$ =** 0.76	**$LSD_{cultivars\ x\ media}$ =** 1.07		

Different letters in the same column and row show that the mean difference is a statistically significant difference at P≤0.05 level.

3-*O*-CQ: 3-*O*-caffeoylquinic acid, 4-*O*-CQ: 4-*O*-caffeoylquinic acid, 5-*O*-CQ: 5-*O*-caffeoylquinic acid, 1,3-*O*-diCQ: 1,3-*O*-dicaffeoylquinic acid, Lut. 7-*O*-gluc.: luteolin 7-*O*-glucuronide.

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Yozgat İli Beyaz Baş Lahana Üretim Alanlarında Bakteriyel Yumuşak Çürüklük Hastalığına Neden Olan *Pectobacterium* İzolatlarının Tanılanması

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ÖZET

Yozgat ili lahana üretim alanlarında 2018-2019 vejetasyon döneminde yumuşak çürüklük hastalık etmenlerinin tanılanmasına yönelik survey çalışmaları yapılmıştır. Yaprak, gövde ve kök kısımlarında sulu-ıslak lezyonlar ve çürüme belirtisi sergileyen dokulardan bakteriyel etmeninin izolasyonu için Crystal Violet Pectate (CVP) besi yeri kullanılmıştır. Farklı tarlalardan toplanan 24 şüpheli bitki örneğinden CVP besi yerinde çukur oluşturan 16 adet pektolitik izolat elde edilmiştir. Şeffaf, konveks ve kenarları krater formda olduğu tespit edilen bakteriyel izolatların gram negatif, fakültative anaerob, oksidaz negatif, katalaz pozitif, UV-ışık altında King B besi yerindeki kolonilerinin floresans parlama göstermediği belirlenmiştir. Patates dilimlerinde ve lahana bitkilerinde yumuşak çürüklük belirtilerine neden olan izolatların 37 ºC'de gelişebildiği, %5'lik NaCl içeren sıvı besi yerinde türbidite oluşturduğu ve tütün yapraklarında aşırı duyarlılık reaksiyonuna neden olduğu belirlenmiştir. Elde edilen 16 izolat ile yapılan PCR analizlerde, izolatların tamamı *Pectobacterium* spp. için spesifik Y1/Y2 primerleri ile 434 bç, *P. carotovorum* subsp. *carotovorum* için spesifik EXPCCF/EXCPCCR primerleri ile ise 550 bç büyüklüğünde PCR ürünü oluşturmuştur. Yapılan biyokimyasal, fizyolojik, patojenite ve moleküler analizler sonucu test edilen 16 izolatın *P. carotovorum* subsp. *carotovorum* olduğunu göstermiştir. Yapay inokulasyon yapılan lahana bitkilerinde patojenik izolatların sulu-ıslak lezyonlar şeklinde yumuşak çürüklük belirtilerine neden olduğu gözlemlenmiştir. *P. carotovorum* subsp. *carotovorum*'un Yozgat ili beyaz baş lahana üretim alanlarında yumuşak çürüklük hastalığının enfeksiyon kaynağı olduğu ilk kez bu çalışma ile tespit edilmiştir.

Bitki Koruma

Araştırma Makalesi

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Anahtar Kelimeler
Bakteriyel hastalık
Yumuşak çürüklük
Pectobacterium
Beyaz baş lahana
Yozgat ili

Identification of *Pectobacterium* Isolates Causing Bacterial Soft Rot Disease in White Head Cabbage Production Areas of Yozgat Province

ABSTRACT

During the 2018-2019 vegetation period, survey was conducted to identify soft rot disease agent in the white head cabbage production areas of Yozgat. Crystal Violet Pectate (CVP) medium was used for the isolation of bacterial agent from tissues showing watery-wet lesions and rotting signs on leaves, stems and roots. A total of 16 pectolytic isolates that formed cavity in the CVP medium were obtained from 24 suspicious plant samples collected from different fields. The colonies of bacterial isolates detected as transparent, convex and crater with edges were gram negative, facultative anaerobe, oxidase negative, catalase positive, and did not show fluorescence on the King B-medium under UV-light. It has been determined that the isolates caused soft rot symptoms in potato slices and cabbage plants could grow at 37 ºC, had turbidity in liquid medium containing 5% NaCl and cause hypersensitivity reaction in tobacco leaves. In PCR analyses with obtained 16 isolates revealed that all of the isolates generated 434 bp and 550 bp PCR product using

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Y1/Y2 and EXPCCF/EXCPCCR primers which were recommended for the identification of *Pectobacterium* spp, and *P. carotovorum* subsp. *carotovorum*, respectively. Based on biochemical, physiological, pathogenicity and molecular analysis, 16 isolates were identified as *P. carotovorum* subsp. *carotovorum*. It has been also observed that all pathogenic isolates caused signs of soft rot in the form of watery-wet lesions in artificially inoculated cabbage plants. It was determined for the first time with this study that *P. carotovorum* subsp. *carotovorum* is the source of soft rot disease infection in the white head cabbage production areas of Yozgat province.

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GİRİŞ

Brassica oleracea grubu içerisinde 37 adet alt tür bulunmaktadır. Bunlar arasından beyaz baş lahana (var. *capitata* L. f. *alba*) bitkisi günümüzde en çok tüketilen lahana tipidir ve 90'dan fazla ülkede kültürü yapılan en önemli sebze türlerinden birisidir (Nieuwhof, 1969). Dünya lahana üretiminde Türkiye 778 bin ton lahana üretimi ile 15. sırada yer almaktadır (Anoymous, 2018). Türkiye genelinde lahana bitkisi üretilen kışlık sebze türleri arasında 10. sırada yer almaktadır (Balkaya ve ark., 2017). Türkiye'de 2020 yılında en fazla lahana üretimi 129.310 ton ile Niğde ilinde yapılmakta olup bu ili sırasıyla, 111.181 ton ile Samsun, 37.549 ton ile Bursa, 24.106 ton ile Mersin, 22.504 ton ile İzmir illeri takip etmektedir (Anonim, 2020). Lahana üretimini ve uzun dönem muhafazasını kısıtlayan birçok hastalık mevcuttur (Maskell ve ark. 1999). Lahana bitkilerinde hastalıklara neden olan başlıca bakteriyel hastalık etmenlerinin *Pectobacterium* sp., *Pseudomonas syringae* pv. *maculicola*, *P. marginalis*, *Xanthomonas campestris* pv. *campestris* olduğu bildirilmiştir (Rimmer ve ark., 2007).

Pectobacterium spp.'nin neden olduğu yumuşak çürüklük hastalığı lahana üretiminde verim ve kalite kayıplarına yol açan en önemli bakteriyel hastalıklar arasındadır (Hauben ark., 1998; Gardan ve ark., 2003; Popovic ve ark., 2019). Yumuşak çürüklük hastalığının küresel olarak sebze, meyve ve süs bitkilerinde toplamda % 30 ürün kaybına neden olduğu bildirilmektedir (Toth ve ark., 2011; Charkowski, 2015; Charkowski, 2018). *Pectobacterium* spp., oldukça geniş bir coğrafyada monokotiledon ve dikotiledon bitkilerde enfeksiyona neden olmaktadır. Dünyanın birçok yerinde başta patates olmak üzere farklı konukçu bitkilerde yaygın olarak rapor edilen patojenik izolatlara sahiptir (Duarte ve ark., 2004; Ma ve ark., 2007; Marquez-Villavicencio ve ark., 2011; Mansfield ve ark., 2012;). *Pectobacterium* izolatlarının en önemli virülenslik faktörü, bitki hücre duvarı eriten pektolitik enzimlere sahip olmasıdır (Barras ve ark., 1994). Bitki hücrelerinde hücre duvarı ve orta lamel bileşeni olan pektini parçalayarak substrat olarak kullanan bakteriler konukçu doku entegrasyonunun bozulması ve hücre içeriklerinin dışarı salınmasına neden olmaktadır. Sızıntı daha sonra birçok saprofitik mikroorganizmanın saldırısıyla ağır bir çürük koku oluşumuna davetiye çıkarmaktadır (Barras ve ark., 1994; Perombelon, 2002).

Çevresel faktörlerden sıcaklık, *Pectobacterium* türlerinin dağılımı ve epidemiyolojisinde en belirleyici faktördür (Perombelon ve Kelman, 1980; Perembelon, 2002). Avrupa kıtası gibi ılıman bölgelerde *P. atrosepticum* (Syn. *E. carotovora* subsp. *atroseptica*) patojeni 20-22 °C sıcaklık değerlerindeki daha fazla oranda enfeksiyon oluşturur ve konukçu olarak en fazla patates bitkisinde olmakla birlikte sınırlı sayıda da olsa biber (Stommel ve ark., 1996), ayçiçeği (Baştaş ve ark., 2009) ve kala (Popovic ve ark., 2017) bitkilerinde de hastalığa neden olduğu bildirilmektedir (Marquez-Villavicencio ve ark., 2011). Daha sıcak bölgelerde (ılıman-subtropik) ise *P. carotovorum* subsp. *carotovorum* (Syn. *Erwinia carotovora* subsp. *carotovora*) enfeksiyonlarının fazla oranda olduğu bilinmektedir (Czajkowski ve ark., 2011; Czajkowski ve ark., 2015). *P. carotovorum* subsp. *carotovorum* etmeni dünya genelinde en patojenik tür olarak gösterilmektedir. 25 °C civarındaki sıcaklıklarda ılıman ve yarı - tropik bölgelerde marul, sarımsak, tatlı biber, patlıcan, bamya, lahana, hindiba, kabak, şeker pancarı, domates, biber, şalgam, havuç, hıyar, kereviz, soğan, tütün ve dut gibi konukçularda ekonomik kayıplara neden olur (Peltzer ve Sivasithamparam, 1985; Waleron ve ark., 2002; Perombelon, 2002; Gardan ve ark., 2003; Fiori ve Schiaffino, 2004; Mahmoudi ve ark., 2007; Xia ve Mo, 2007; Golkhandan ve ark., 2013; Cariddi ve Sanzani, 2013; Dees ve ark., 2017; Zaczek-Moczydłowska ve ark., 2019). *P. carotovorum* subsp. *carotovorum* etmeninin daha geniş coğrafi dağılıma ve konukçu aralığına sahip olması izolatlar arasında genetik çeşitliliğinin fazla olmasından dolayı tahmin

edilmektedir. (Toth ve ark., 2003).

Türkiye genelinde *Pectobacterium* türleri bir dizi konukçu kültür bitkisi üzerinde rapor edilmiştir. En erken tanı çalışmaları Benlioğlu (1991) tarafından patates örneklerinde *P. carotovorum* subsp. *carotovorum* ve *P. atrosepticum* patojenlerinin varlığının bildirilmesi ile başlamıştır (Benlioğlu ve ark., 1991). Çetinkaya-Yıldız ve ark. (2004) Doğu Akdeniz Bölgesi'nde *Dieffeanbachia amoena* bitkilerinde *P. carotovorum* etmenini, Aysan ve ark. (2004) Mersin ve Hatay illeri domates seralarında *P. carotovorum* ve *Dickeya* spp. (*E. chrsanthemi*) izolatlarının yumuşak çürüklüğe neden olduğunu bildirmişlerdir. Boyraz ve ark. (2006) Konya ili lale üretim alanlarında *P. carotovorum*'u, Baştaş ve ark. (2009) Konya ili ayçiçeği üretim alanlarında *P. atrosepticum*'u, Ozturk (2016) Yozgat ili patates üretim alanlarında *P. parmentieri*'yi, Ozturk (2017) Orta Karadeniz bölgesi patates üretim alanlarında *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliense*, *P. parmentieri* ve *P. atrosepticum*'u karabacak ve yumuşak çürüklük hastalığı etmenleri olarak belirlemiştir. Dadaşoğlu ve Kotan (2017), *P. carotovorum* subsp. *atrosepticum*'un çilek, dut, maydonoz, lahana, patates, biber ve patlıcanda, *D. chrysanthemi*'nin soğan, biber ve maydonozda yumuşak çürüklüğe neden olduğunu belirlemişlerdir. *P. carotovorum* subsp. *carotovorum* etmeni ayrıca Aydın ili enginar üretim alanlarında (Üstün ve Arslan, 2016), Samsun ili beyaz baş lahana üretim alanlarında (Aksoy ve ark., 2017a), Mersin ili muz üretim alanlarında (Basım ve ark., 2019) hastalığa neden olan patojen olarak bildirilmiştir. Ozturk ve ark. (2019), Yozgat ili şeker pancarı üretim alanlarında *P. betavasculorum*'un vasküler nekroz ve yumru çürüklük enfeksiyonlarına neden olduğunu rapor etmişlerdir. Son yıllarda toplam 540 dekar alanda 1.280 ton beyaz baş lahana üretiminin gerçekleştiği Yozgat ilinde (Anonim, 2020), artan ekim alanları ile birlikte lahanalarda bakteriyel ve fungal hastalıklarla ilişikli şikâyetlerde artışlar kayıt edilmeye başlanmıştır.

Yozgat iline ait ilçe ve köylerde yürütülen bu çalışmada, beyaz baş lahana üretiminin gerçekleştiği tarlalarda yumuşak çürüklük belirtileri gösteren bitkilerden bakteriyel hastalık etmeninin izolasyonu, biyokimyasal, fizyolojik, patojenisite ve moleküler analizlerle tanılanması amaçlanmıştır.

MATERYAL ve METOT

Bitki Örnekleri ve Bakteriyel İzolasyon

2018-2019 yılları Eylül-Kasım aylarında Yozgat iline bağlı Sorgun, Sarıkaya ve Merkez ilçelerinde yapılan sörveylerde, yapraklarda kloroz, sulu-ıslak nekrotik leke, yaprak ana damar, gövde, kök dokularında kahverengileşerek ilerleyen zamanla sulu-ıslak hale gelen ve yumuşama belirtileri gösteren lahana bitkileri toplanmıştır. Simptomatik dokular % 1'lik sodyum hipoklorit (NaOCl) ile 2-3 dakika süreyle yüzeysel dezenfeksiyonu yapıldıktan sonra izolasyon için bitki dokuları 1-3 cm büyüklüğünde kesitlere ayrılmış, %70 etil alkolde 1 dakika süreyle bekletilen örnekler üç kez steril suda durulandıktan sonra fizyolojik serum (% 0.85'lik NaCl çözeltisi) içeren steril ekstraksiyon torbalarına konulup süspansiyonlar elde edilmiştir (Kara ve ark., 2020). Elde edilen süspansiyonlardan steril öze yardımıyla alınan örnekler, Crystal Violet Pectate Medium (CVP) besi yerine ekimi yapılmıştır (Helias ve ark., 2012). *Pectobacterium* spp. ait izolatlar 24-72 saat 26 ºC'de inkübasyon sonucunda besi yerinde çukur oluşumu şeklinde morfolojik görünümlü kolonilerinden Nutrient Agar (NA) besi yerine saflaştırmaları yapılmıştır.

Patateste Pektolitik Aktivite Testi

NA besi yerine saflaştırılan pektolitik aktiviteye sahip bakteriyel izolatların belirlenmesi amacıyla patates dilimlerinde (cv. Marabel) pektolitik aktivite testi uygulanmıştır. Sağlıklı patates yumruları % 5'lik sodyum hipokloritte 10 dakika bekletilerek yüzeyleri dezenfekte edilmiştir. Daha sonra kabukları soyularak dezenfekte edilen yumrular yaklaşık 20 mm dilimlenerek içerisinde steril nemli kağıtlar içeren steril petri kaplarına yerleştirilmiştir. Patates dilimlerinin yüzeylerine 24 saatlik bakteri kültürlerinden steril kürdan ile alınan bakteri hücreleri bulaştırılmış, petriler 26±2°C'de inkübasyona bırakılmıştır. İnkübasyondan 24-72 saat sonra inokulasyon noktalarında maserasyon şeklinde görülen yumuşak çürüklük oluşumu pozitif olarak değerlendirilmiştir (Bozkurt ve Soylu, 2019). Pozitif kontrol olarak referans kültür *P. carotovorum* subsp. *carotovorum* A8G4, negatif kontrol olarak steril saf su kullanılmıştır. Patates dilimlerinde pektolitik aktivite gösteren şeffaf renkte ve kenarları krater koloni morfolojisine sahip 16 farklı izolat morfolojik, biyokimyasal, patojenisite ve moleküler analizler için % 40'lık steril glyserol içerisinde -20 ºC'de muhafaza edilmiştir (Lojkowska ve ark., 1995).

Lahana Bitkilerinde Patojenisite Testleri

NA besi yerinde geliştirilen 24-48 saatlik kültürlerin kolonileri steril kürdan yardımıyla alınarak 4 haftalık lahana bitkilerinin gövdesine bulaştırılmıştır. İnokulasyon bölgesi parafilm ile kaplanmıştır. Bitkiler 16:8 saat (aydınlık:karanlık) fotoperiyoda sahip yetiştirme odasında sırasıyla 25ºC (16 saat) ve 18ºC (8 saat) inkübasyona bırakılmış, inokulasyon bölgesinde 5-7 gün sonra oluşan ıslak-sulu doku lezyonlarının oluşumu incelenmiştir (Caruso ve ark., 2016).

Biyokimyasal ve Fizyolojik Testler

Potasyum hidroksit (KOH), oksidaz, katalaz,

oksidatif/fermantatif (O/F), arjinin dehidrolaz, floresan parlama, 37 °C de gelişebilme, % 5 NaCl içeren LB (Luria broth) besi yerinde türbidite oluşturma ve tütünde aşırı duyarlılık testleri açısından 16 farklı izolat değerlendirilmiştir (Lelliot ve Stead, 1987; Schaad ve ark., 2001).

Tütünde Aşırı Duyarlılık Testi

NA besi yerinde geliştirilen 24 s'lik kültürlerin konsantrasyonları 10^8 CFU ml^{-1}'ye ayarlandıktan sonra elde edilen süspansiyonlardan enjektör yardımıyla tütün (*Nicotina tabacum* kv. *bentamiana*) yapraklarının damarları arasına enjekte edilmiştir. Aşırı duyarlılık oluşturan izolatlar HR pozitif (+), oluşturmayanlar ise HR negatif (–) olarak değerlendirilmiştir. Kontrol bitkilerine Pozitif kontrol olarak *Pseudomonas syringae* pv. *phaseolicola* Psp12, negatif kontrol olarak steril saf su uygulanmıştır (Soylu ve ark., 2020).

Pectobacterium İzolatlarının PCR Tekniği ile Moleküler Tanısı

NA besi ortamında 24-48 saatlik süreyle geliştirilen bakteriyel kolonilerin PCR ile tanısında Çizelge 1.'de belirtilen primer oligonükleotidleri kullanılmıştır. Bakteriyel koloniler LB (Luria broth) besi yerinde 26°C'de 48 saat orbital çalkalayıcıda geliştirilmiştir. Genomik DNA izolasyonu Genomik DNA prüfikasyon kiti (Katalog numarası: K0721, ThermoFisher firması GeneJET) kullanılarak yapılmıştır. Nanodrop cihazından elde edilen DNA miktar tayinine göre örneklerin DNA'ları 10 ng μl^{-1} final konsantrasyon olacak şekilde hazırlanarak DNA örnekleri -20 °C'de saklanmıştır. PCR karışımı, 10 µl 2x master mix (Bioline my Taq, İngiltere), 1 µl forward primer (10 pmol), 1 µl reverse primer (10 pmol), 7 µl steril distile saf su ve 1 µl DNA olacak şekilde hazırlanmıştır. Thermocycler cihazı PCR döngü işlemleri, Touchdown (kademeli sıcaklık düşürme) programına göre yapılmıştır. Buna göre, 95 °C'de 4 (dak.), ilk 10 döngü; 94 °C'de 30 (s), 65-56 °C'de 30 (s) (her döngüde 1°C azalır), 72°C'de 1 (dak.), 72°C'de 5 (dak.) şeklinde ayarlanarak ilaveten 24 döngü sabit 56°C'de aynı parametreleri kullanarak uygulanmıştır (Aksoy ve ark., 2017b). PCR ürünleri agaroz jele (% 1) yüklenerek 100 voltluk elektrik akımında 30 dakika süreyle koşturulmuş ve UV ışın altında görüntülenerek çoğaltılan DNA fragmentlerinin boyutları referans markör (G-BIOSCIENCE DNAmark 100 bp ladder) boyutlarına göre belirlenmiştir (Sambrook ve ark., 1989). Referans kültür olarak *P. carotovorum* subsp. *carotovorum* A8G4, *P. carotovorum* subsp. *brasiliense* VK22G8 ve *P. atrosepticum* ÇA2G8 izolatları kullanılmıştır (Ozturk ve ark., 2018).

Çizelge 1. Enfekteli lahana örneklerinden izole edilen *Pectobacterium* izolatların moleküler tanısında kullanılan primerler ve oligonükleotid dizinleri

Table 1. Primers and sequences of oligonucleotides used for molecular identifications of bacterial strains obtained from infected samples

Tür/Alt tür	Primer	Sekans 5'→3'	Ürün Boyutu (bç)	Kaynakça
Pectobacterium spp.	Y_1	TTACCGGACGCCGAGCTGTGGCGT	434	Darrasse ve ark. (1994)
	Y_2	CAGGAAGATGTCGTTATCGCGAGT		
P. atrosepticum	Y_{45}	TCACCGGACGCCGAACTGTGGCGT	439	Frechon ve ark. (1998)
	Y_{46}	TCGCCAACGTTCAGCAGAACAAGT		
P. carotovorum subsp. *carotovorum*	EXPCCF	GAACTTCGCACCGCCGACCTTCTA	550	Kang ve ark. (2003)
	EXPCCR	GCCGTAATTGCCTACCTGCTTAAG		
P.carotovorum subsp. *brasiliense*	BR1f	GCGTGCCGGGTTTATGACCT	322	Duarte ve ark. (2004)
	L1r	CA(A/G)GGCATCCACCGT		

BULGULAR ve TARTIŞMA

Hastalık Etmeninin Izolasyonu, Biyokimyasal ve Fizyolojik Testler

Yozgat ilinin lahana yetiştiriciliğinin yapıldığı Sorgun'da 12, Sarıkaya'da 6 ve Merkez ilçesinde 8 olmak üzere toplam 26 farklı lahana tarlasındaki bitkiler güdümlü olarak hastalık açısından incelenmiş olup, Sorgun'da 10, Sarıkaya'da 6 ve Merkez ilçesinde 8 olmak üzere toplam 24 tarladan her tarlayı temsilen şüpheli örnekler alınmıştır. Hastalık belirtilerinin tespit edildiği arazilerde bulunan lahana bitkilerinde; yaprak ve yaprak saplarında sulu lezyonlar, lezyonların daha fazla ilerlediği dokularda sulu-ıslak lezyonların bulunduğu dokuların kahverengi-siyah renk alarak yumuşama gösterdiği, doku entegrasyonunun zayıfladığı bitkilerde ağır çürük koku oluşumu gözlemlenmiştir (Şekil 1A,B). Popovic ve ark. (2019)'da belirtildiği gibi, farklı tarlalardan toplanan yumuşak çürüklük belirtisi gösteren 24 şüpheli lahana (Şekil 1C) dokularından CVP besi

yerine yapılan izolasyonlarda çok sayıda bakterinin geliştiği gözlemlenmiştir. Daha önceden bildirildiği gibi (Helias ve ark., 2012) lahana dokularında izole edilen pektolitik koloniler CVP besi yerinde pektat kaynağını parçalayarak bulundukları bölgelerde çukur oluşumuna neden olmuş, sonuçta pektolitik izolatların diğer bakteriyel kolonilere göre CVP besi yerinde daha dominant olduğu (Popovic ve ark., 2019) gözlemlenmiştir (Şekil 1D). Tüm izolatların patates dilimlerinde 24 saat sonra yapılan gözlemlerde pektolitik aktivite gösterdiği ve dokularda yumuşamalara (maserasyona) neden olduğu belirlenmiştir (Aksoy ve ark., 2017a). Oskiera ve ark. (2017)'de bildirildiği üzere, pektolitik aktivite gösteren 24 izolat arasında 16 pektolitik izolatın NA besi yerinde gelişen kolonilerinin şeffaf, konveks ve kenarları krater formda olduğu belirlenmiştir (Şekil 1E.) (Benlioğlu, 1991; Ozturk ve ark., 2018).

Şekil 1. **A.** Gözlemlerin yapıldığı alanlardaki lahana bitkilerinde tespit edilen yumuşak çürüklük (ok) belirtileri, **B.** Enfekteli bitkide kök bölgesine kadar ilerlemiş şiddetli yumuşak çürüklük (ok). **C.** *Pectobacterium* spp. kaynaklı enfekteli kök dokusu (ok), **D.** Pektat besin kaynağını kullanan pektolitik izolatlarının CVP besi yerinde çukur oluşturması (ok), **E**. NA besi yerinde *Pectobacterium* izolatının 5 günlük kolonileri

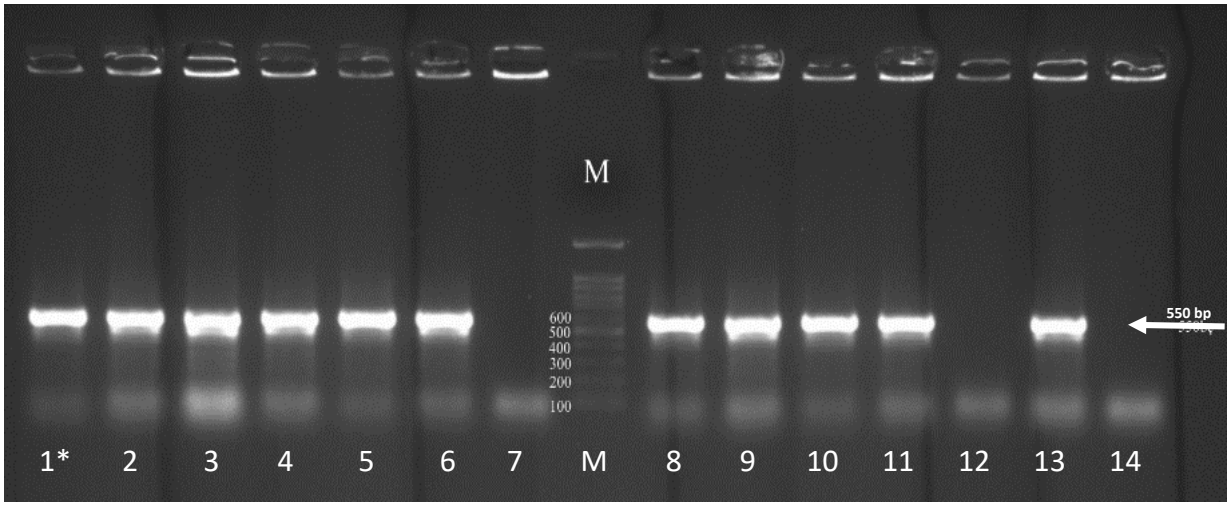
Figure 1. (A) Typical soft rot symptoms (arrow) observed on cabbage plants in inspected fields. (B) Severe soft rot that has advanced to the root area of the infected plant (arrow). (C) Pectobacterium spp. originated infected root tissue (arrow). (D) Cavitiy formation (arrow) formed by pectolytic bacterial isolates using pectate nutrient source on CVP nutrient media . (E) Growth of Pectobacterium isolates on NA medium after 5 days.

CVP besi yerinde ve patates dilimlerinde pektolitik aktiviteye sahip olduğu belirlenen izolatların, potasyum hidroksit (KOH) testine göre gram negatif olduğu belirlenmiştir. Oksidaz ve arjinin dehidrolaz testlerinde negatif, katalaz testinde pozitif reaksiyon gösteren fakültatif anaerob karakterdeki izolatların King B besi yerindeki kültürlerinin UV ışık altında floresan parlama göstermediği belirlenmiştir. İzolatların 37 °C de gelişebildiği, % 5 NaCl içeren LB (Luria broth) besi yerinde türbidite oluşturduğu ve tütün yapraklarında aşırı duyarlılık reaksiyonuna neden olduğu belirlenmiştir (Lelliot ve Stead, 1987; Schaad ve ark., 2001). Lee ve ark. (2019) belirtildiği üzere, yumuşak çürüklüğe neden olan bakteriyel etmenler *Bacillus*, *Pseudomonas*, *Pectobacterium* ve *Xanthomonas* spp. olarak bildirilmektedir (Aksoy ve ark., 2017a; Charkowski, 2018; Lee ve ark., 2019). *Pectobacterium* izolatlarının patates yumru dilimlerinde daha başarılı maserasyona neden olduğu

ve pektolitik aktiviteye neden olan enzimlerin daha fazla oranda üretildiği bildirilmektedir (Marín-Rodríguezve ark., 2002).

Bakteri İzolatlarının PCR Tekniği ile Moleküler Tanılaması

İzolatların moleküler tanısında *Pectobacterium* spp. izolatlarının cins düzeyinde tanılanmasını sağlayan Y1/Y2 primer çifti kullanılarak 434 bç büyüklüğünde PCR amplikonu çoğaltılmıştır (Darrasse ve ark., 1994). Brassicaceae bitkileri üzerinde *P. carotovorum* subsp. *carotovorum* patojeninin, *P. carotovorum* subsp. *brasiliense* ve P. *carotovorum* subsp. *oderiferum* patojenlerinden daha yaygın enfeksiyon kaynağı olarak rapor edildiği bilinmektedir (Arsenuevic ve Obradovic, 1996; Bhat ve ark., 2010; Nazerian ve ark., 2011; Waleron ve ark., 2015). *Pectobacterium* izolatlarının tür/alt tür tanısı için kullanılan primer oligonüklotidleri ile yapılan PCR analizlerinde (De Boer ve ark., 2012) referans *P. carotovorum* subsp. *carotovorum* A8G4 izolatı ile 16 lahana *Pectobacterium* izolatının EXPCCF/EXPCCR primerleri (Kang ve ark., 2003) ile 550 bç büyüklüğünde PCR ürünü oluşturduğu belirlenmiştir (Şekil 2). Diğer yandan, *P. carotovorum* subsp. *brasiliense* VK22G8 ve *P. atrosepticum* ÇA2G8 izolatlarının aynı primerle yapılan PCR çalışmalarında söz konusu PCR ürününü oluşturmadığı tespit edilmiştir (Şekil 2). Türkiye genelinde yetiştiriciliği yapılan başta patates olmak üzere birçok yumrulu ve yaprağı tüketilen (etli yapraklı) sebzelerde yumuşak çürüklük hastalığına neden olan bakteri izolatları arasından en yaygın bakteriyel türün *P. carotovorum* subsp. *carotovorum* olduğu bildirilmiştir (Benlioğlu, 1991; Öztürk, 2017).



Şekil 2. Lahana yumuşak çürüklük izolatlarının EXPCCF/EXPCCR primerleri ile 550 bç PCR ürünü (ok) oluşturması (***1=*Pcc* A8G4**, 2=BRY1, 3=BRY2, 4=BRY3, 5=BRY4, 6=BRY5, **7=*Pba* ÇA2G8**, **M=Moleküler DNA marker**, 8=BRY6, 9=BRY7, 10=BRY8, 11= BRY9, 12=*Pcbr* VK22G8, 13= BRY9,14=Kontrol, su)

*Figure 2. Typical 550 bp PCR product (arrow) generated by cabbage soft rot bacterial isolates using EXPCCF/EXPCCR primer pairs. (***1=Pcc A8G4**, 2=BRY1, 3=BRY2, 4=BRY3, 5=BRY4, 6=BRY5, **7=Pba ÇA2G8**, **M=Molecular DNA marker**, 8=BRY6, 9=BRY7, 10=BRY8, 11= BRY9, **12=Pcbr VK22G8**, 13= BRY9, **14=Control, water**)*

P. carotovorum subsp. *brasiliense* VK22G8 ve *P. atrosepticum* ÇA2G8 izolatlarının *P. carotovorum* subsp. *brasiliense* izolatlarına spesifik Br1f/L1r (Duarte ve ark., 2004) ve *P. atrosepticum* izolatlarına spesifik Y45/Y46 primerleri ile beklenen PCR ürünlerini (322 ve 439 bç, sırasıyla) oluşturduğu, EXPCCF/EXPCCR primerlerine göre pozitif olduğu belirlenen lahana izolatlarının ise Br1f/L1r ve Y45/Y46 primerleriyle negatif reaksiyon gösterdiği belirlenmiştir (Dees ve ark., 2017; Öztürk, 2017; Ozturk ve ark., 2018).

Lahana Bitkilerinde Patojenisite Testi

Moleküler tanısı yapılan izolatların konukçu bitki üzerinde yapılan patojenisite testinden 5-7 gün sonra gövdede sulu ıslak dokuların oluştuğu ayrıca inokulasyon bölgesine en yakın noktada yer alan yaprakların kenarlarında haşlanma ile başlayıp daha sonrasında kurumaların meydana geldiği Alvarado ve ark. (2011)'de belirtildiği gibi gözlemlenmiştir. Lahana bitkilerinde patojenitesi yapılan izolatlar inokulasyon noktalarından tekrar izole edilmiş ve yapılan biyokimyasal, fizyolojik ve moleküler testlerde re-izolatların orijinal izolatları ile aynı reaksiyonları verdiği tespit edilmiştir.

SONUÇ ve ÖNERİLER

Sonuç olarak, Yozgat ili lahana üretim alanlarında *Pectobacterium* spp.'de yer alan pektolitik izolatlardan kaynaklı yumuşak çürüklük hastalığı tespit edilmiştir. Hastalıklı dokulardan elde edilen *Pectobacterium* izolatları ile yapılan biyokimyasal, fizyolojik, patojenisite ve PCR ile moleküler tanı çalışmaları sonucunda 16 izolatın *P. carotovorum* subsp.

carotovorum olduğu belirlenmiştir. *P. carotovorum* subsp*. carotovorum* izolatlarının tamamı cins ve tür düzeyinde tanılanmalarına olanak sağlayan Y1/Y2 ve EXPCCF/R primeri ile sırasıyla 434 ve 550 bç PCR ürünü oluşturmuştur. Elde edilen izolatların lahana bitkilerinde patojenik olduğu konukçu bitkiler üzerinde yapılan patojenite testleri ve inokulasyon noktalarından tekrar yapılan geri izolasyonları neticesinde anlaşılmıştır. Hastalığın mücadelesinde etkin bir kimyasal bulunmamaktadır. Etmen geniş konukçu aralığında enfeksiyona neden olduğu için patojenin izole edildiği alanlarda konukçusu olmayan bitkiler ile üretiminin yapılması önem arz etmektedir. Hastalıktan ari, sertifikalı üretim materyali kullanımı ve patojen ile enfekteli bitkilerde etmenin erken dönemlerde bitki dokusunda daha fazla çoğalmasını önlemek amacıyla patojenin erken teşhis edilerek baskılanmasına yönelik uygulamaların yapılması sayesinde hastalık şiddetinin daha fazla görülmemesine önem verilmelidir.

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Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Biberiye [*Rosmarinus officinalis* L.] Uçucu Yağının *Callosobruchus maculatus* (F.)'un Yumurta Bırakma Davranışına Etkisi ve Erginlere Karşı Toksisitesi

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ÖZET

Depolanmış baklagillerde kayıplara yol açan zararlılara karşı mücadelede sentetik organik insektisitlere alternatif kimyasallar belirlemek amacıyla, biberiye (*Rosmarinus officinalis* L.) uçucu yağının *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) erginlerine toksik etkisi ve dişilerin yumurta bırakmasını engelleme etkisi laboratuvar koşullarında test edilmiştir. Ayrıca dişilerin yumurta bırakma tercihine etkisi de Free-choice (serbest-seçenekli) testi ile belirlenmiştir. Tüm denemelerde biberiye uçucu yağının % 0.0125, 0.025, 0.05, 0.1 (w/w) olmak üzere 4 farklı konsantrasyonu kullanmış olup, püskürtme şeklinde uygulanmıştır. Toksisite denemesinin sonucunda erginlerde LC_{50} değeri 24 saat sonunda % 0.368; 48 saat sonunda ise % 0.148 olarak belirlenmiştir. Biberiye uçucu yağının konsantrasyonu arttıkça dişilerin daha az yumurta bıraktığı gözlenmiş ve en yüksek konsantrasyonda yumurta bırakmayı engelleme oranı %19.11 olarak belirlenmiştir. Yumurta bırakma tercihi denemelerinde ise biberiye uçucu yağının konsantrasyonu arttıkça bırakılan yumurta sayısının azaldığı belirlenmiştir. Elde edilen bu veriler, *C. maculatus*'un mücadelesinde biberiye uçucu yağının kimyasal mücadeleye alternatif olabilecek bileşiklerin geliştirmesinde veri kaynağı oluşturabileceğini göstermiştir.

Bitki Koruma

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Anahtar Kelimeler
Callosobruchus maculatus
Rosmarinus officinalis
Toksisite
Uçucu yağ
Yumurta bırakma davranışı

Toxicity of Rosemary (*Rosmarinus officinalis* L.) Essential Oil on *Callosobruchus maculatus* (F.) Adults and Its Effect on Oviposition Behavior

ABSTRACT

Rosemary essential oil was tested the toxicity and oviposition deterrent activity against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) under laboratory conditions, in order to determine alternative chemicals to synthetic organic insecticides in the protection of stored grains against insect pests. The effect on egg laying preference of females was also determined by Free-choice test. Four different concentrations of rosemary essential oil as 0.0125, 0.025, 0.05, 0.1 (w/w) were used in all toxicity experiments in which spraying technique was applied. As a result of toxicity experiment, LC_{50} value for adults was determined as 0.368% and 0.148% after 24 and 48 hours of exposure, respectively. It was observed that as the concentration of rosemary essential oil was increased, females laid less eggs and at the highest concentration of 0.1%, oviposition deterrent rate was determined as 19.11%. As a result of egg laying preference experiment, it was found that as the concentrations of rosemary essential oil were increased, the number of laid eggs decreased. Data obtained from this study showed that rosemary essential oil can serve as a potential source of compounds that can be developed and used as an alternative to chemical control of *C. maculatus*.

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GİRİŞ

Baklagiller tüm dünyada insan beslenmesinde önemli bir protein kaynağıdır. Karbonhidrat oranı yüksek, yağ oranı düşük ve oldukça besleyicidir. Dünya ve Türkiye'de tarla bitkileri üretimi yapılan alanlarda ilk sırayı tahıllar alırken bunu yemeklik dane baklagiller izlemektedir (Gülümser, 2016). Bu açıdan bakıldığında yemeklik dane baklagiller Türkiye ve dünya için oldukça önemli bir yere sahiptir.

Baklagil tohum böceklerinden olan *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) hem tarlada baklaları hem de depodaki tohumları istila eden önemli bir baklagil zararlısıdır (Baidoo ve ark., 2010). Larvaların zarar verdiği tohumlarda çimlenme görülmez ve insan tüketimi için uygun değildir (Rahman ve Talukder, 2006). Depodaki bulaşmalarda 3-4 ay içinde zarar oranı %50' nin üzerine çıkabilir (Baidoo ve ark., 2010).

Depo zararlılarından dolayı meydana gelen zararı en aza indirmek için yaygın olarak kimyasal uygulamalar yapılmaktadır. Kimyasal mücadelede kullanılan kimyasal maddeler bıraktığı kalıntılar nedeniyle, insanlar ve çevre üzerinde çeşitli tehlikeleri beraberinde getirmiştir. Ayrıca bu kimyasalların sürekli kullanımı nedeniyle zararlıda dayanıklılık problemi ortaya çıkarmakta ve mücadele zorlaşmaktadır. Tüm bu olumsuzluklardan dolayı *C. maculatus*' un mücadelesinde sentetik organik böcek ilaçlarının yerine bitkilerden elde edilen uçucu yağların ve sekonder bileşiklerin kullanımı alternatif bir yol olarak ortaya çıkmıştır. Uçucu yağ içeren bitkiler, içeriğinde bulunan alkoloid, terpenoid ve flavanoid gibi aktif bileşikler bakımından zengin olup, bu bileşikler zararlılara karşı kullanılabilecek potansiyeldedir (Topuz ve Madanlar, 2012). Bugüne kadar yapılan çalışmalarda birçok bitkinin uçucu yağı ve bileşenlerinin zararlılara karşı insektisit, repellent, ovisit, cezbedici, beslenmeyi engelleyici, gelişme ve üremeyi engelleyici gibi etkileri belirlenmiştir (Prajapati ve ark., 2005; Moravvej ve ark., 2010; Nerio ve Olivera-Verbel, 2010; Wagan ve ark., 2018)

Biberiye (*Rosmarinus officinalis* L.) (Lamiaceae) önemli bir tıbbi ve aromatik bitki (Begum ve ark., 2013) olup, çalı görünümünde, herdem yeşil, çiçekleri soluk mavimsi renkte ve çok yıllıktır.(Baytop, 1984). Dünyada süs ve tıbbi- aromatik bitki olarak geniş bir kullanım alanına sahip olan biberiye, Akdeniz iklimi görülen yerlerde yabani olarak ta yetişebilmektedir (Ceylan, 1987).

Biberiye uçucu yağının çeşitli böcek ve akar türlerine karşı kaçırıcı (repellent) (Hori, 1998; Koschier ve Sedy, 2003) ve kontakt etkiye sahip olduğu ve özellikle bazı depolanmış ürün zararlılarına karşı fumigant etkileri olduğu ortaya konulmuştur (Papachristos ve Stamopoulos, 2004; Işıkber ve ark., 2006; Theou ve ark., 2013; Güdek ve Çetin, 2017). Amerika Birleşik Devletleri'nde, biberiye uçucu yağının böcek öldürücü özelliğinden yola çıkarak aktif bileşen olarak biberiye yağı içeren ticari böcek öldürücü geliştirilmiştir (Isman ve ark., 2008).

Daha önce biberiye uçucu yağıyla yapılan çalışmalar, *Callosobruchus maculatus*'a fumigant etkisi üzerinde yürütülmüştür. Bu çalışmada biberiye bitkisinden elde edilen uçucu yağın, önemli depo zararlısı *Callosobruchus maculatus* dişilerinin yumurta bırakma davranışı üzerine etkileri ve erginlere toksisitesi araştırılmıştır.

MATERYAL ve METOD

Callosobruchus maculatus (F.)'un Yetiştirilmesi

Denemede kullanılan *C. maculatus* erginleri Selçuk Üniversitesi Ziraat Fakültesinde Bitki Koruma bölümüne ait 28±2 ºC sıcaklık ve %55± 5 nispi neme sahip iklim odasında bulunan stok kültürden temin edilmiştir. Stok kültürden alınan erginler, içerisinde yem olarak nohut (*Cicer arietinum* L.) bulunan 1 l'lik cam kavanozlara aktarılmıştır. Yumurta bırakmaları için 5 gün süreyle kavanozlarda tutulan erginler tekrar elenerek alınıp stok kültür kavanozlarına bırakılmıştır. Bundan sonra kavanozlardaki çıkışlar gözlemlenmiş ve ilk ergin çıkışından 3-4 gün sonra erginler alınarak stok kültür kavanozuna aktarılmıştır. Ertesi gün daha fazla sayıda çıkan erginler (1 günlük) denemelerde kullanılmıştır.

Biberiye Uçucu Yağının Elde Edilmesi

Denemelerde kullanılan biberiye bitkisi Antalya' nın Geyikbayırı köyünden (755 m rakım 36.876 enlem ve 30.457 boylam) toplanarak gölge ve havadar bir ortamda kurutulmuştur. Daha sonra dallarından ayrılan yapraklar öğütülmüş ve hassas terazide 100 g tartılıp Clevenger düzeneğinde 1:10 oranında su ile karıştırılmıştır. Denemelerde kullanılan uçucu yağ 2-3 saat sulu destilasyonu sonucu elde edilmiştir.

Elde edilen biberiye uçucu yağının %0.0125, 0.025, 0.05, 0.1' lik (w/w) konsantrasyonları %0.2 oranında Tween 20 (yayıcı yapıştırıcı olarak) içeren saf su kullanılarak hazırlanmıştır. Kontrollerde %0.2 oranında Tween 20 (yayıcı yapıştırıcı olarak) içeren saf su kullanılmıştır.

Toksisite Denemeleri

Denemelerde kullanılan 1 günlük erginler 20' şer tane sayılarak petri kaplarına (90 mm çapında ve 17 mm yüksekliğinde) alınmış ve 5-6 dk soğutma kabininde (+2°C) bekletilerek uyuşmaları sağlandıktan sonra denemeye başlanmıştır. Petri kabında bulunan uyuşuk haldeki erginlere, püskürtme kulesi (Manual Potter Spray Tower-Burkard Scientific Limited, Uxbridge, UK) kullanılarak her bir konsantrasyondan 2 ml biberiye uçucu yağı 0.8 bar basınçla püskürtülmüştür (Gökçe ve ark., 2007). Petri kaplarının ağızlarına ince şerit kağıt koyulduktan sonra kapakları kapatılarak hava alması sağlanmış ve 28±2ºC sıcaklık ve %55±5 nispi neme sahip iklim kabinine yerleştirilmiştir. Ölü erginler, uygulamadan 24 ve 48 saat sonra dikkatlice sayılmıştır. İnce uçlu bir fırçayla dokunulduğunda az da olsa hareket belirtisi gösteren erginler canlı, hiç hareket etmeyenler ise ölü olarak sayılmıştır. Deneme 3 tekerrürlü olarak yürütülmüştür.

Yumurta Bırakmayı Engelleyici Etkisini Belirleme Denemeleri

Denemede mümkün olduğunca eşit büyüklükte seçilen nohutlardan her bir petriye 10'ar tane bırakılmış ve üzerlerine püskürtme kulesi yardımıyla biberiye uçucu yağının hazırlanan 4 konsantrasyonundan püskürtülmüştür. Kontrol olarak ise %0.2 oranında Tween 20 içeren saf su püskürtülmüştür. Nohutların bir süre kuruması beklendikten sonra herbir petri kabına bir günlük 2 dişi 1 erkek birey konulmuştur. Deneme tüm böcekler ölünceye kadar 28±2 ºC sıcaklık ve %55±5 orantılı neme sahip iklim kabininde sürdürülmüştür. Sonuçta her bir nohut stereo zoom mikroskopta incelenmiş ve bırakılan yumurtalar tek tek sayılarak kayıt altına alınmıştır. Deneme 4 tekerrürlü olarak yürütülmüştür.

Yumurta Bırakma Tercihi Testi (Free-choice Testi)

Tercih denemesi için daire şeklinde bir kabın içerisine eşit aralıklı 5 bölme olacak şekilde özel bir düzenek oluşturulmuştur (Şekil 1). Her bir bölmeye biberiye uçucu yağının farklı bir konsantrasyonu (%0.0125, 0.025, 0.05, 0.1'lik konsantrasyonları) püskürtülmüş ve kurutulmuş 10 nohut bırakılmıştır. Düzeneğin tam orta kısmına bir günlük 5 dişi ve 10 erkek olmak üzere böcekler yerleştirilmiştir (Vanmathi ve ark., 2010). Düzeneğin üzeri tamamen tül ile kapatılmıştır.

Şekil 1. Yumurta koyma tercihinin belirlenmesinde kullanılan düzenek
Figure1. The mechanism used to determine egg laying preference

Denemede kullanılan düzenekler 28±2ºC sıcaklık ve %55±5 orantılı neme sahip iklim kabininde tutulmuş ve deneme tüm böcekler ölünceye kadar sürdürülmüştür. Sonuçta her bir bölmedeki nohutların üzerindeki yumurtalar stereo zoom mikroskop altında sayılıp kaydedilmiştir. Deneme 3 tekerrürlü olarak yürütülmüştür.

İstatistik Analizler

Toksisite testi sonucunda farklı süre ve uygulama konsantrasyonlarından elde edilen ölüm oranı verilerine SPSS 22 versiyon (Statistical Package for Social Sciences) yazılım paketi kullanılarak varyans analizleri (ANOVA) yapılmış, farkın önemli olduğu tespit edilen değerlere %5 önem seviyesinde DUNCAN testi yapılarak ortalamalar arasındaki farklar tespit edilmiştir.

Ayrıca ölüm oranı verileri "Poloplus" (Le Ora Software, 1994) programı ile probit analizine tabi tutulmuştur. Probit analiz metoduna göre de birebir regresyon ile letal konsantrasyon$_{50}$ (LC_{50}) ve letal konsantrasyon$_{90}$ (LC_{90}) değerleri hesaplanmıştır.

Yumurta bırakmayı engelleme etkisi testlerinden elde edilen verilerin değerlendirilmesinde Lundgren (1975) tarafından tanımlanan ovipozisyonu engelleme indeksi (1),

$$O.E.İ = [(X-Y)/(X+Y)] \times 100 \quad (1)$$

(X: Kontrol kabındaki nohutlara bırakılan toplam yumurta sayısı, Y: Muamele kabındaki nohutlara bırakılan toplam yumurta sayısı), kullanılmıştır.

Free-choice testi sonucunda elde edilen verilerin istatistik analizleri SPSS 22 versiyon yazılım paketi kullanılarak varyans analizleri (ANOVA) yapılmış, farkın önemli olduğu tespit edilen değerlere %5 önem seviyesinde DUNCAN testi yapılarak ortalamalar arasındaki farklar tespit edilmiştir.

BULGULAR ve TARTIŞMA

Callosobruchus maculatus erginleri üzerine biberiye uçucu yağının 4 farklı konsantrasyonunun püskürtülmesiyle 24 ve 48 saat sonunda elde edilen sonuçlar Çizelge 1'de verilmiştir. Buna göre, ölüm oranlarında istatistiksel olarak önemli farklılıkların ($P<0.05$) olduğu, uygulanan konsantrasyonlar ve maruz bırakma sürelerinin artışına bağlı olarak ölümlerin arttığı tespit edilmiştir.

Çizelge 1. Biberiye uçucu yağının farklı konsantrasyonlarının 24 ve 48 saat sonunda *Callosobruchus maculatus*'un erginlerindeki ölüm oranları (%)

Table 1. Mortality rates (%) after 24 and 48 hours of different concentrations of rosemary essential oil against adults of Callosobruchus maculatus

Ergin ölüm oranı (%)±Standart hata *(Adult mortality rate (%)±Standart error)*		
Konsantrasyonlar (% w/w) *(Concentrations) (% w/w)*	24 saat *(24 hours)*	48 saat *(48 hours)*
Kontrol (%0.2 Tween 20)	0.00±0.00 a*	0.00±0.00 a
0.0125	6.67±2.35 ab	18.33±7.21 b
0.025	11.67±1.66 bc	23.33±3.33 b
0.05	18.33±3.33 c	30.00±5.00 b
0.1	28.33±4.40 d	46.67±1.66 c

*Aynı sütunda bulunan harfler aynı ise istatistiksel olarak (*P<0.05)* bir farklılık yoktur .

Biberiye uçucu yağının 24 ve 48 saat sonunda LC50 ve LC90 değerleri Çizelge 2'de verilmiştir. Uygulamadan 24 saat sonra *C. maculatus* erginlerinde LC_{50} ve LC_{90} değerleri sırasıyla % 0.368 ve %6.565 (w/w) olarak belirlenmiştir. Uygulamadan 48 saat sonra ise LC_{50} ve LC_{90} değerleri sırasıyla %0.148 ve %3.891 olarak tespit edilmiştir. Bu değerler biberiye uçucu yağının *C. maculatus* erginleri üzerinde yüksek toksik etkiye sahip olduğunu göstermiştir.

Çizelge 2. Biberiye uçucu yağının *Callosobruchus maculatus*'un erginlerine toksisitesi

Table 2. Toxicity of rosemary essential oil against adults of Callosobruchus maculatus

Uygulama Süresi (Saat) *Application time (hour)*	N (*Total numbers of individulas tested)*	Eğim±SH *(Slope±SE)*	LC_{50} (Güven Aralığı) *(Confidence interval)*	LC_{90} (Güven Aralığı) *(Confidence interval)*	SD *(Degree of freedom)*	Heterojenite
24	300	1.024±0.308	0.368(0.15-8.49)	6.565(0.98-9.89)	10	0.290
48	300	0.902±0.261	0.148(0.08-0.96)	3.891(0.70-5.69)	10	0.641

n=Test edilen toplam birey sayısı
SH=Standart hata
Güven aralığı (%95 önem seviyesinde)
SD=Serbestlik derecesi

Bu sonuç birçok araştırıcı tarafından yapılan çalışmalarla da desteklenmektedir. Güdek ve Çetin (2017), *R. officinalis* L. uçucu yağının *C. maculatus*'un erginlerine karşı fumigant etkisini araştırdığı çalışmada uygulama konsantrasyonu ve süresine göre değişmekle beraber yüksek fumigant etki gösterdiğini rapor etmişlerdir. En yüksek konsantrasyon (60 µl l^{-1}-1air) ve 96 saat uygulama süresinde %100 ölüm görüldüğünü bildirmişlerdir. Bouchikhi Tani ve ark. (2008), *R. officinalis* uçucu yağının *Acanthoscelides obtectus* (Say)'un erginlerine insektisidal etkisini araştırdığı çalışmada, uçucu yağın oldukça etkili olduğunu ve 48 saatlik uygulamadan sonra LC_{50} değerinin 0,59 µl/30g tohum olduğunu bildirmişlerdir. Çetin ve ark. (2014), 18 adet tıbbi ve aromatik bitkinin uçucu yağlarının *A. obtectus* erginlerine karşı fumigant etkilerini test etmişler ve *R. officinalis* ve *Salvia fruticosa* Mill. uçucu yağlarının en etkili yağlar olduğunu tespit etmişlerdir. Hannour ve ark. (2018)'da, biberiye uçucu yağının *Bruchus rufimanus* (Coleoptera: Chrysomelidae) erginlerine yüksek fümigant etki gösterdiğini bildirmişlerdir.

Çizelge 3'de verilen ovipozisyonu engelleme indekslerine bakıldığında, konsantrasyon artışına bağlı olarak bu değerin arttığı belirlenmiştir. Biberiye uçucu yağının en yüksek konsantrasyonu, %19.11 ovipozisyonu engelleme indeksi değeriyle en yüksek etkiyi göstermiştir. En düşük konsantrasyon olan %0.0125'in yumurta verimini çok az etkilediği ve ovipozisyonu engelleme indeksi değerinin %6'nin altında kaldığı görülmektedir.

Çalışmada, biberiye uçucu yağının, *C. maculatus'* un dişilerinin kontrole göre daha az yumurta bırakmasına neden olduğu belirlenmiştir. Douiri ve ark. (2014), biberiye uçucu yağının farklı konsantrasyonları ile fumige edilen nohut tohumları üzerindeki *C. maculatus*'un doğurganlığının kuvvetli bir şekilde etkilendiğini ve bu tohumlara bırakılan yumurta sayısının kontrole bırakılandan oldukça düşük olduğunu belirlemişlerdir. Pandey ve ark. (2011) 4 bitkiden elde edilen uçucu yağların hepsinin (*Chenopodium ambrosioides* L., *Ocimum sanctum* L, *Clausena pentaphylla* (Roxb.) ve *Mentha arvensis* L.) *C. maculatus* üzerinde ovipozisyonu engelleyici etkiye

sahip olduğunu bildirmişlerdir. Nyamador ve ark. (2017), *Bidens borianiana, Cymbopogon nardus, Cymbopogon giganteus* ve *Chromolaena odorata* bitkilerinden elde ettikleri uçucu yağların, *C. maculatus* üzerindeki toksik ve ovipozisyonu engelleme etkisini araştırdıkları çalışmada *C. giganteus* bitkisinden elde edilen uçucu yağın erginlere karşı en fazla fumigant etki (LC_{50} = 20.06 µl L^{-1}) gösterdiğini bildirmişlerdir. Ayrıca aynı çalışmada ovipozisyonu engelleme etkisine en fazla *C. nardus* uçucu yağının neden olduğunu belirlemişlerdir.

Free-choice çalışması sonucunda biberiye uçucu yağının farklı konsantrasyonları ile kontrol arasında önemli farklılıklar görülmüştür (Çizelge 4). Konsantrasyon azaldıkça bırakılan yumurta sayısı artmış ve ergin dişiler en çok kontroldeki danelere (40.6 adet) yumurta koymayı tercih etmiştir. En az tercih edilen ise en yüksek konsantrasyon olan %0.1 konsantrasyonu (28.6 adet) olmuştur.

Çizelge 3. Biberiye uçucu yağının farklı konsantrasyonlarının *Callosobruchus maculatus*'un yumurta bırakmayı engellemeye etkisi

Table 3. The effect of different concentrations of rosemary essential oil on egg laying of Callosobruchus maculatus

Konsantrasyonlar (%) (Concentrations)	Bırakılan ortalama yumurta sayısı (Adet) *(Mean number of eggs laid)*	*OEİ (%)* (Oviposition Deterrent index)*
0.0125	35.75±0.34	5.92
0.025	32.75±0.26	10.27
0.05	29±0.35	16.24
0.1	27.75±0.38	19.11
Kontrol	40.25±0.98	

*OEİ=Ovipozisyonu engelleme indeksi

Çizelge 4. Biberiye uçucu yağının farklı konsantrasyonlarının yumurta bırakma tercihi üzerine etkisi

Table 4. The effect of different concentrations of rosemary essential oil on egg laying preference

Konsantrasyonlar (%) *(Concentrations)*	En az *(Minimum)*	En Çok *(Maximum)*	Ortalama yumurta sayısı (adet) *(Mean egg number)*
0.0125	25	45	38.3±0.31d*
0.025	33	44	37.0±0.25bc
0.05	25	43	34.6±0.30bc
0.1	22	38	28.6±0.34b
Kontrol	37	47	40.6±0.24a

*Aynı sütunda bulunan harfler aynı ise istatistiksel olarak (P>0.05) bir farklılık yoktur.

Yapılan yumurta bırakma tercihi denemelerinde, konsantrasyon arttıkça bırakılan yumurta sayısı azalmıştır. Stamopoulos (1991), *A. obtectus*' un yumurta bırakmasına 4 farklı uçucu yağın (Sardunya, okaliptüs, selvi ve acı badem) etkisini ikili seçenek testi şeklinde (Kontrol ve uçucu yağ) test ettiği denemesinin sonucunda, bütün test edilen uçucu yağlara bırakılan yumurta sayısının kontrolden az olduğunu ve kontrole göre en az yumurta sayısının okaliptüs uçucu yağının uygulandığı tohumlarda olduğunu tespit etmiştir. Ayrıca bu tercih durumunu, uçucu yağın gaz haline dönüşmesi nedeniyle dişilerin çoğunluğunun yumurtalarını bu tohumların üzerine koymamalarından kaynaklanmış olabileceğini bildirmiştir.

SONUÇ ve ÖNERİLER

Çalışma sonucunda biberiye uçucu yağının erginlere karşı toksik etki gösterdiği ve aynı zamanda dişilerde özellikle yüksek konsantrasyonda yumurta bırakmayı engelleyici etki gösterdiği tespit edilmiştir. Buna ilaveten konsantrasyona bağlı olarak yumurta bırakma tercihi testinde ise konsantrasyon arttıkça bırakılan yumurta sayısının azaldığı belirlenmiştir.

Bu çalışma ile elde edilen sonuçların bu konuda yapılacak yeni çalışmalara ışık tutacağı düşünülmektedir. Uçucu yağların ana bileşenlerinin saf olarak elde edilmesi ve bunların depolanmış ürün zararlılarına karşı formülasyon haline getirilerek kullanımına yönelik çalışmalar uçucu yağların potansiyellerinin anlaşılmasında faydalı olacaktır.

Bu konuda yapılacak yeni araştırmalarda özellikle bu bileşenlerin yapılarının tespit edilerek sentetik olarak üretilebilmesi ve hatta yapısal olarak değişikliğe uğratılarak zararlılara karşı etkinliğinin arttırılması çabaları konuyu daha ileriye taşıyacak adımlar olacaktır. Bunun yanında bitkisel kökenli pestisitlerin çevre ve insan sağlığı açısından yan etkilerinin olup-olmadığı tespit edilerek güvenilirliğinin yapılacak olan bilimsel çalışmalarla ortaya konulması gerekmektedir.

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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Use of Extreme Low Temperatures Against *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) in Storage Management

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ABSTRACT
This study was carried out to determine the effect of extreme low temperatures on different biological stages of *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae). Different extreme low temperatures were tested for egg, larva, pupa and adult stages in all experiments with different exposure times. For larval, pupal and adult stages, 100% mortality was obtained after 45 minutes at -16 °C, 30 minutes at -20 °C and 20 minutes at -26 °C. LT95 value was determined as 38.3, 39.0 and 36.8 minutes at -16 °C for larva, pupa and adult, respectively. LT95 value was determined as 27.4, 27.5 and 26.0 minutes at -20 °C for larva, pupa and adult, respectively. At the end of 120 minutes at -20 °C, 100% mortality was obtained in the egg stage and it was determined that the adults did not hatch from the eggs kept at -20 °C for 60 minutes. From these results, it is understood that extreme low temperatures are effective in all biological stages of *C. chinensis* that cause damage in storage.

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Depolama Yönetiminde *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae)'e Karşı Aşırı Düşük Sıcaklıkların Kullanılması

ÖZET
Bu çalışma, aşırı düşük sıcaklıkların *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae)'nin farklı biyolojik dönemleri üzerindeki etkisini belirlemek için yapılmıştır. Farklı maruz kalma sürelerine sahip tüm deneylerde yumurta, larva, pupa ve ergin dönemleri için farklı aşırı düşük sıcaklıklar test edilmiştir. Larva, pupa ve ergin dönemleri için, -16 °C'de 45 dakika, -20 °C'de 30 dakika ve -26 °C'de 20 dakika sonunda %100 ölüm oranı elde edilmiştir. LT95 değeri larva, pupa ve ergin için -16 °C'de sırasıyla 38.3, 39.0 ve 36.8 dakika olarak belirlenmiştir. LT95 değeri larva, pupa ve ergin için -20 °C'de sırasıyla 27.4, 27.5 ve 26.0 dakika olarak belirlenmiştir. -20 °C'de 120 dakika sonunda yumurta döneminde %100 ölüm sağlanmış ve -20 °C'de 60 dakika bekletilen yumurtalardan erginlerin çıkmadığı tespit edilmiştir. Bu sonuçlardan, aşırı düşük sıcaklıkların depolamada zarara neden olan *C. chinensis*'in tüm biyolojik dönemleri üzerinde etkili olduğu anlaşılmaktadır.

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 01.02.2021
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INTRODUCTION

Legumes are an essential part of the human diet, as they are very rich in protein, carbohydrates and dietary fiber (Chibbar, 2009). Chickpea (*Cicer arietinum* L., Fabales: Fabaceae) is one of the most important legumes in many parts of the world and it originated in Turkey's southeast (Ali et al., 2009; Baloch and Zubair, 2010). According to FAO-2019 data, chickpeas are grown on an area of approximately 13.7 million hectares with 14.2 million tons of production in the world. India, Turkey, Russia, Myanmar and Pakistan are the biggest chickpea

producing countries in the world (Faostat, 2020). Chickpeas are highly nutritious and rich in energy in terms of high protein content and balanced fat, carbohydrate and fiber values (Ravi and Harte, 2009).

Chickpeas are exposed to the damage of many pest species both in the field and in storage conditions (Clement et al., 2000; Hill, 2003). Stored product pests are very dangerous because of the extent and prevalence of the irreversible losses they cause in legumes (Kumar et al., 2009; Jat et al., 2013). *Callosobruchus chinensis* L. is one of the most destructive pests of stored legumes, including chickpeas (Kutbay et al., 2011; Tessema et al., 2015). This pest not only causes loss of quantity and quality of chickpeas, but also reduces the economic value and nutritional content of the product (Righi-Assia et al., 2010; Homan and Yubak Dhoj, 2011).

Chemical management methods using fumigation with methyl bromide, phosphine or dusting with pirimiphos methyl are very successful against this pest in stored chickpeas (Athanassiou et al., 2004; Shaheen and Khaliq, 2005), however there are some disadvantages such as shipping hazards, pesticide residues, the possibility of developing resistance, health risks and environmental pollution, so there are a few limitations on chemicals (Boateng and Kusi, 2008; Boyer et al., 2012).

In recent years, due to the negative effects caused by chemical control methods, it has focused on alternative reliable control methods against stored product pests. One of these methods is to prevent the development of stored product pests by keeping the product at low temperature for a certain period of time (Fields and White, 2002; Zhong et al., 2013). Studies have shown that low temperatures can have negative effects on the growth of many stored product pests (Fields and Muir, 1996; Loganathan et al., 2011). During storage, pests are highly susceptible to dropping the storage temperature below 10 °C, and this sensitivity level can vary with biological stages (Zakladnoi and Ratanova, 1987; Maharjan et al., 2017). Also, susceptibility to deadly low temperatures; depends on pest species, exposure temperature, exposure time, sex of insects and ambient humidity (Mason and Strait, 1998; Zhong et al., 2013).

The aim of this research was to determine the adverse effects of extreme low temperatures on the different biological stages of *C. chinensis*, thereby minimizing the damage this pest can cause to stored products. In this way, an alternative method will be developed for stored product pests.

MATERIAL and METHODS

Insect Rearing

C. chinensis adults were obtained from stock cultures grown on chickpea seeds at the Ondokuz Mayıs University, Faculty of Agriculture, Entomology Laboratory. *C. chinensis* were reared in glass jars (1 L) covered with muslin cloth in climate cabins at 28±1 °C, 70±5% RH and a 16:8 h (L:D) photoperiod.

The Extreme Low Temperature Experiments

The healthy chickpea seeds to be used in the experiments were sterilized in the oven at 50 °C for 24 hours, then the chickpea seeds were cooled. The moisture content of the sterile seeds was adjusted to 14% moisture and the seeds were made ready for experiments (Wright et al., 1987). The moisture content of chickpea seeds was monitored regularly throughout the experiments with a digital moisture meter (WILE Grain Moisture Meter). Low temperature experiments were performed in a temperature-controlled refrigerator (SIEMENS KG57NP01NE) and temperatures were monitored hourly using a digital data logger.

Five male-5 female *C. chinensis* adults were placed in sterile glass jars each containing 40 sterile chickpea seeds. *C. chinensis* adults were allowed to lay eggs on sterile seeds, and these egged seeds were transferred to sterile petri dishes. There were 20 chickpea seeds in each petri dish. In this way, more than 100 sterile petri dishes were prepared. The adults emerged from these eggs were collected daily. Some of the adults emerged from these eggs were used to determine the effect of low temperatures on adults. In addition, 2 male-2 female pairs from newly emerged adults were transferred to new sterile petri dishes each containing 10 sterile chickpea seeds. In this way, more than 300 new sterile petri dishes were prepared. Eggs laid on chickpea seeds in each petri dish by the pest pairs within 24 hours were used in all experiments.

Low-temperature experiments were performed on eggs, larvae, pupae and adults of 24-48 hours ages. Since larval and pupal stages occur in chickpea seeds, eggs between 0-24 hours at 28±1 °C - 70±5% RH were used to determine the biology of the *C. chinensis*. Accordingly, the larval stages started to form on the 6th day on average, and the pupal stages on the 22nd day on average. For 24-48 hour larval and pupal studies, 6-day-old chickpea seeds were used in the larval trials and 22-day-old chickpea seeds were used in the pupal trials. Different temperatures and different exposure times were used to determine the effects of extreme low temperatures on different biological stages of *C. chinensis*. The effects of low temperature on *C. chinensis'* eggs were studied at two constant temperatures (4 and -20 °C) and eight exposure times (5, 10, 15, 30, 45, 60, 120 and 180 minutes). The effects of low temperature on *C. chinensis'* larvae and pupae were studied at three constant temperatures (-16, -20 and -26 °C) and seven exposure times (10, 20, 30, 45, 60, 120 and 180 minutes). The effects of low temperature on *C.*

chinensis' adults were studied at six constant temperatures (8, 4, 2, -16, -20 and -26 ° C) and seven exposure times (10, 20, 30, 45, 60, 120 and 180 minutes). 480 eggs, 120 larvae, 120 pupae, and 60 adult pests were used in 4 replicates for each exposure time of each temperature. In addition, control groups with 4 replicates were formed under normal growth conditions for each biological stage in the experiments (28±1 °C, 70±5% RH, 16:8 h (L:D) photoperiod).

It was observed that adults started to emerge from chickpeas 28-36 days after the first egg was laid under normal growth conditions. It has been determined that the lifespan of *C. chinensis* is about 36-42 days. Egg, larva, pupa and adult stages exposed to different low temperatures in the experiments were taken to normal growth conditions after different exposure times. Eggs that have not completed their development or have not reached the larval stage are considered dead as a result of the controls made for 30 days after being placed in normal growing conditions. Larvae that did not complete their development or did not reach the pupal stage as a result of the controls made for 60 days after being placed in normal growth conditions were considered dead. Pupae that did not complete their development or did not mature as a result of the controls made for 60 days after being placed under normal growing conditions were considered dead. In order to determine whether the adults died as a result of the experiment, adults were touched with a fine-tipped brush and adults who did not respond were considered dead. Adults were checked for 30 days after being placed under normal growing conditions, and dead adults were counted again for control after 30 days.

Statistical Analysis

The data obtained from low temperature applications and exposure times were tried to be analyzed using a One-Way Anova program (SPSS 21). Mortality rates was considered significantly different at $P<0.05$. Statistical means are separated by Duncan's mean separation test. Statistical significance was established by comparing the p value in the "t" test table. The data obtained were corrected using Abbott's control formula (Abbott, 1925) and subjected to probit analysis (Finney, 1971) to estimate the LT50 and LT95 values (Polo Plus, LeOra Software, Robertson et al., 2003).

RESULTS and DISCUSSION

Effect of Low Temperatures on Eggs

Mortality and adult emergence rates of *C. chinensis'* eggs were studied at different temperatures and different exposure times (Fig. 1), and significant differences were found between exposure times at 4 °C and -20 °C ($P<0.001$). After the eggs were kept at 4 °C for 180 minutes, it was determined that 44% of the eggs died and 78% of the remaining eggs were emerged as adults. After the eggs were kept at -20 °C for 120

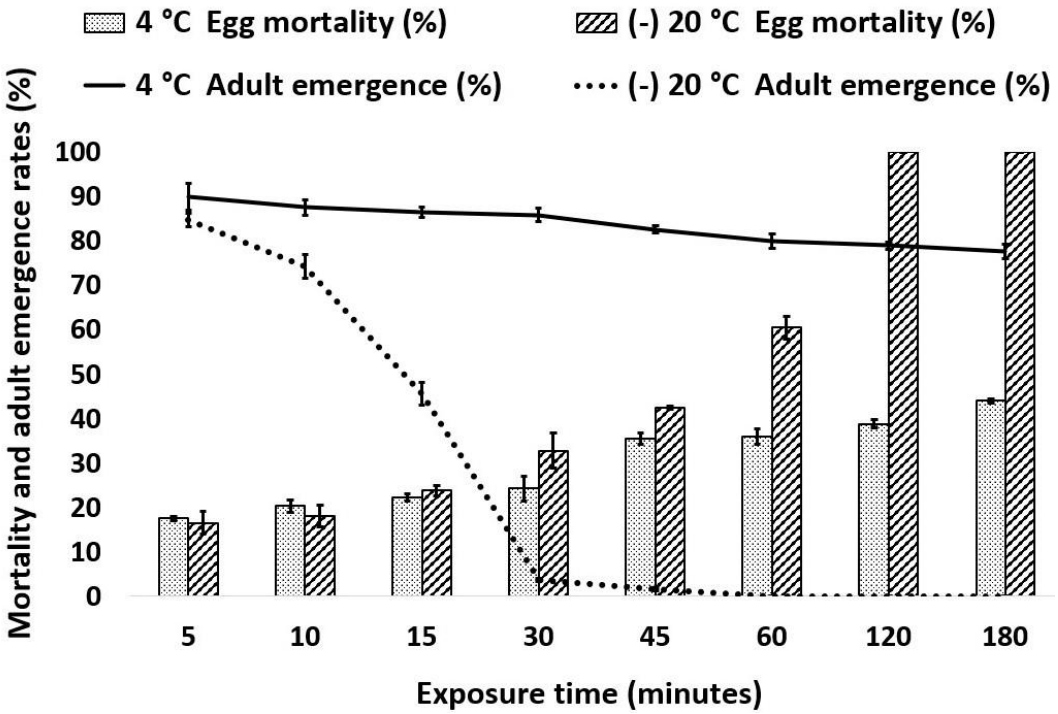

Figure 1. Mortality and adult emergence rates of *Callosobruchus chinensis'* eggs at different exposure times at 4 °C and -20 °C (P<0.001).

Şekil 1. 4 °C ve -20 °C'de farklı maruz kalma sürelerinde Callosobruchus chinensis yumurtalarının ölüm oranı ve ergin ortaya çıkma oranları (P<0.001).

minutes, it was determined that all the eggs died, and the eggs were kept at -20 °C for 60 minutes did not reach adulthood (Fig. 1). It was observed that egg mortality increased due to the increase in exposure time to -20 °C and at the same time the probability of reaching adulthood from egg was significantly reduced.

Effect of Low Temperatures on Larvae

Mortality rates of *C. chinensis'* larvae were studied, and significant differences were found between temperatures and exposure times (*P<0.001*). When the exposure time of the larvae to low temperatures was examined, it was observed that deaths occurred within the first 10 minutes. After the larvae were exposed to extreme low temperatures at -16 °C, -20 °C and -26 °C at the end of the first 10 minutes, mortality rates were determined as 7.36 %, 16.65 %, 54.24 %, respectively (*P<0.001*). As a result of the experiments, 100% larval mortality was achieved after 20 minutes, 30 minutes and 45 minutes at temperatures of -26 °C, -20 °C, -16 °C, respectively (Fig. 2).

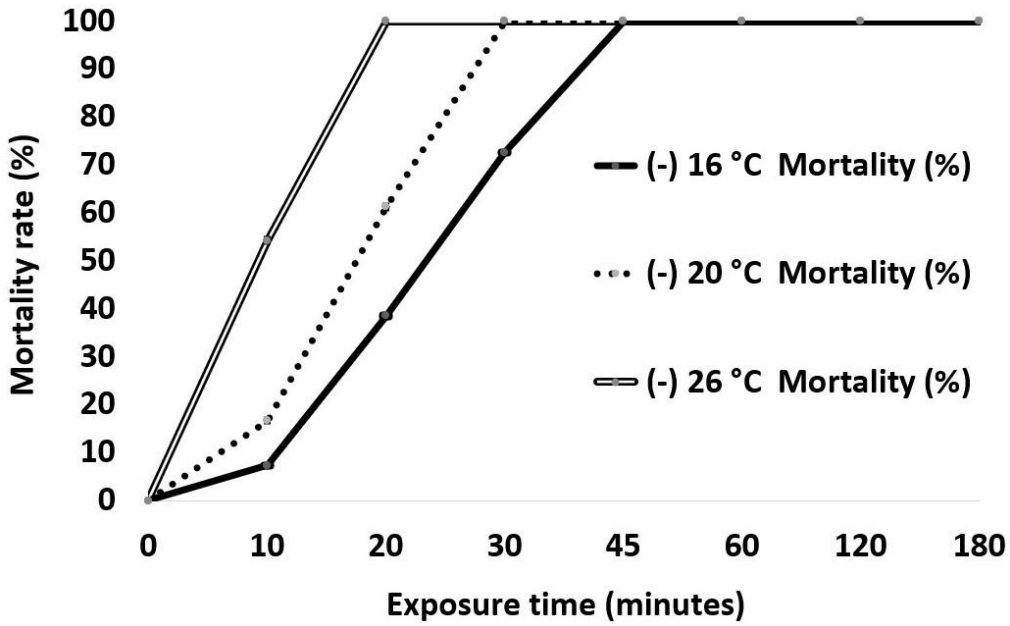

Figure 2. Mortality rates of *Callosobruchus chinensis'* larvae at different exposure times at -16 °C, -20 °C and -26 °C (P<0.001).

Şekil 2. Callosobruchus chinensis larvalarının -16 °C, -20 °C ve -26 °C'de farklı maruz kalma sürelerinde ölüm oranları (P<0.001).

Effect of Low Temperatures on Pupae

The differences between pupae death rates according to exposure times for each low temperature were found to be statistically significant (*P<0.001*). Mortality rates have been observed to increase as the exposure time to low temperatures increases. Deaths started in the first 10 minutes of the experiment, and this mortality rate progressed very rapidly. After the pupae were exposed to extreme low temperatures at -16 °C, -20 °C and -26 °C at the end of the first 10 minutes, mortality rates were determined as 8.42 %, 17.12 %, 57.64 %, respectively (*P<0.001*). As a result of the experiments, 100% pupal death was achieved at temperatures of -16 °C, -20 °C, -26 °C after 45 minutes, 30 minutes and 20 minutes, respectively (Fig. 3).

Effect of Low Temperatures on Adults

Comparing the 3 low temperatures applied to adults in 7 different exposure times, no deaths occurred in adults exposed to 8 °C after 180 minutes. 8.08 % and 3.31 % deaths were observed after 180 minutes at 2 °C and 4 °C, respectively (*P>0.05*) (Fig. 4). Comparing the 3 extreme low temperatures applied to adults in 7 different exposure times, the adults died after 10 minutes. 9.24 %, 18.64 % and 60.04 % deaths were observed after 10 minutes at -16 °C, -20 °C and -26 °C, respectively (*P<0.001*). Mortality increased with increases exposure to extreme low temperatures and 100% adult deaths were reached at -16 °C, -20 °C, -26 °C after 45 minutes, 30 minutes and 20 minutes, respectively (Fig. 4). It was determined that the control groups at each biological stage were between 95-100% successful.

Pests cause large amounts of damage in stored products, which can reach 5-10% in temperate climatic regions and 20-30% in tropical regions (Caswell, 1981; Nakakita, 1998). Temperature application is one of the most promising methods to control stored product pests (Fields, 1992). The lethal low temperature is highly effective in controlling the pests of stored seeds

(Loganathan et al., 2011). At low temperatures, the progeny of stored product pests decreases, and their development slows down to a certain point (Flinn and Hagstrum, 1990; Abdelghany et al., 2015). Stored product pests usually stop growing at temperatures below 20 °C, and stored product pests usually begin to die at temperatures below 5 °C (Fields and Muir, 1996). The results of this study have shown that extreme low temperatures have a lethal effect on all developmental stages of *C. chinensis*, and the egg stage is relatively more tolerant of cold than the other three stages (Fig. 5, 6, 7). Many extreme low temperature applications on *Callosobruchus* species also support these results (Mullen and Arbogast, 1979; Dohino et al., 1999; Johnson and Valero, 2000; Johnson and Valero, 2003).

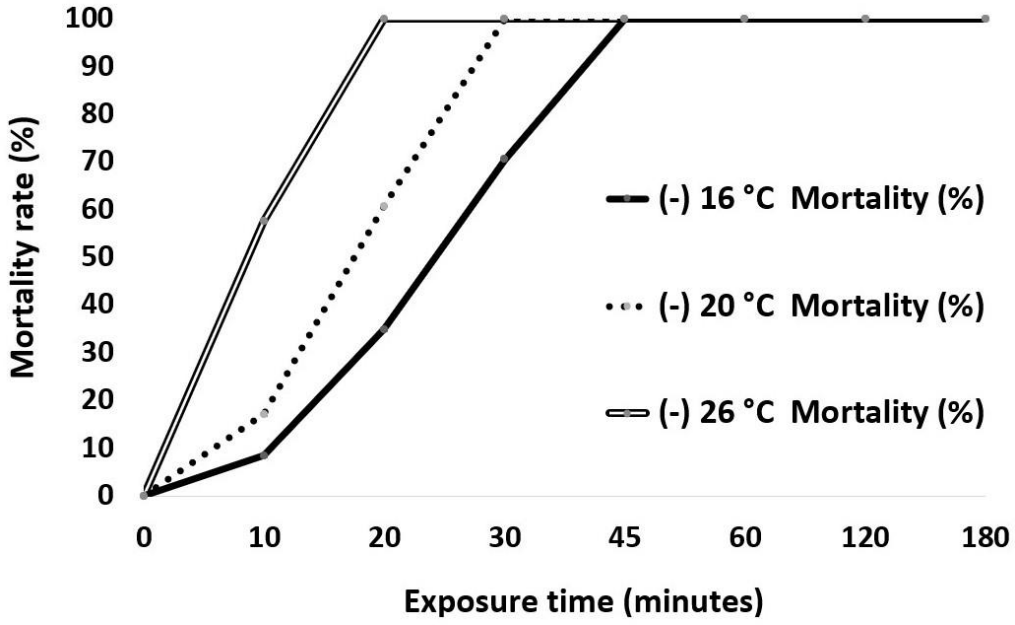

Figure 3. Mortality rates of *Callosobruchus chinensis'* pupae at different exposure times at -16 °C, -20 °C and -26 °C (P<0.001).

Şekil 3. Callosobruchus chinensis pupalarının -16 °C, -20 °C ve -26 °C'de farklı maruz kalma sürelerinde ölüm oranları (P<0.001).

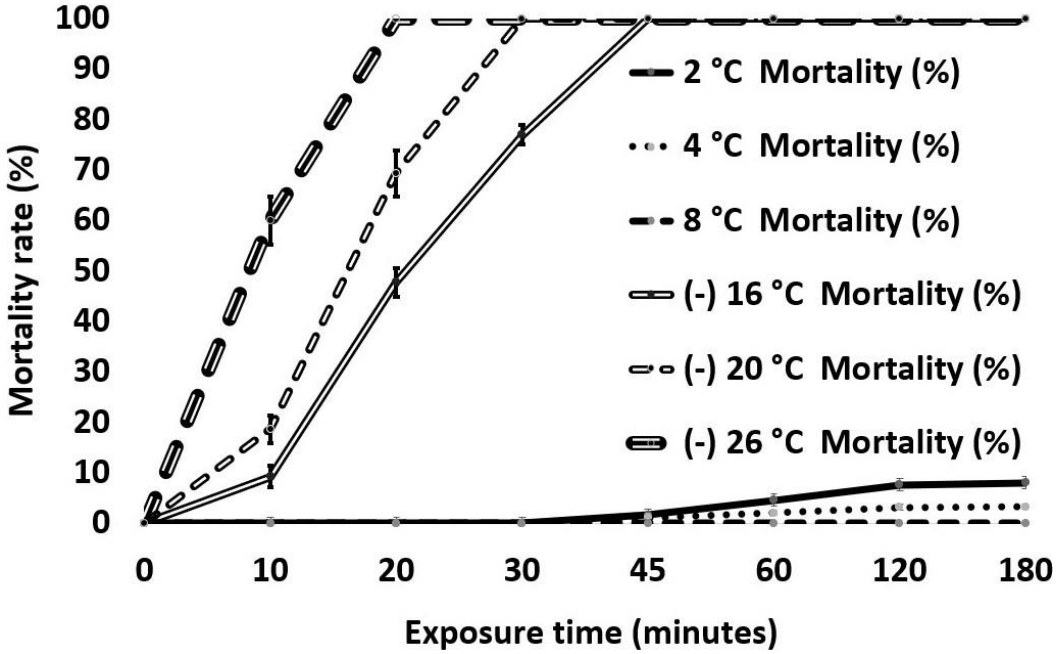

Figure 4. Mortality rates of *Callosobruchus chinensis'* adults at different exposure times at 2 °C, 4 °C, 8 °C and at -16 °C, -20 °C, -26 °C (P<0.001).

Şekil 4. Callosobruchus chinensis erginlerinin 2 °C, 4 °C, 8 °C ve -16 °C, -20 °C, -26 °C'de farklı maruz kalma sürelerinde ölüm oranları (P<0.001).

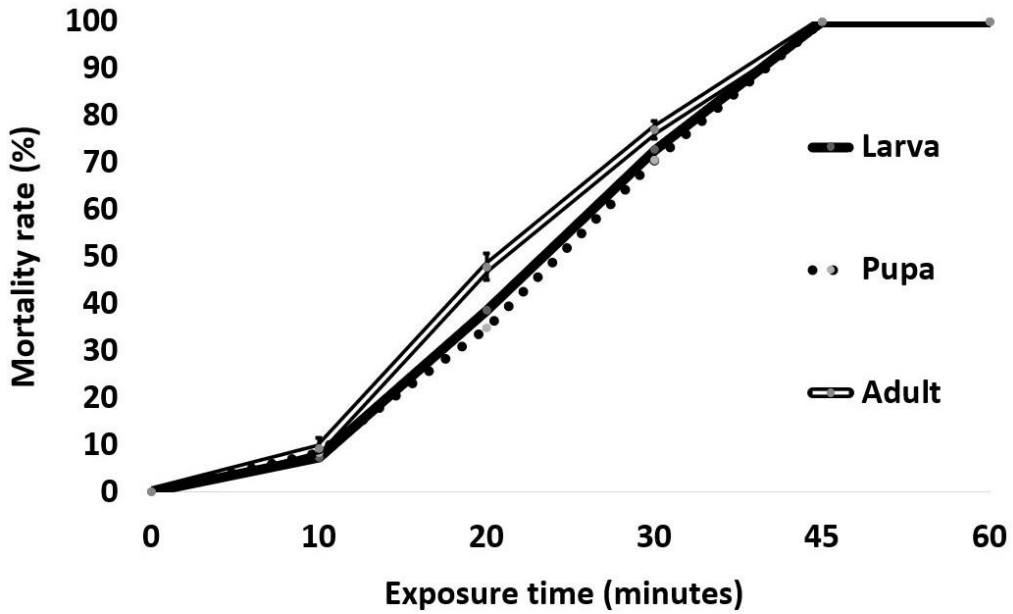

Figure 5. Mortality rates of *Callosobruchus chinensis'* larvae, pupae and adults exposed to -16 °C (P<0.001).
Şekil 5. -16 °C'ye maruz kalan Callosobruchus chinensis larva, pupa ve erginlerinin ölüm oranları (P<0.001).

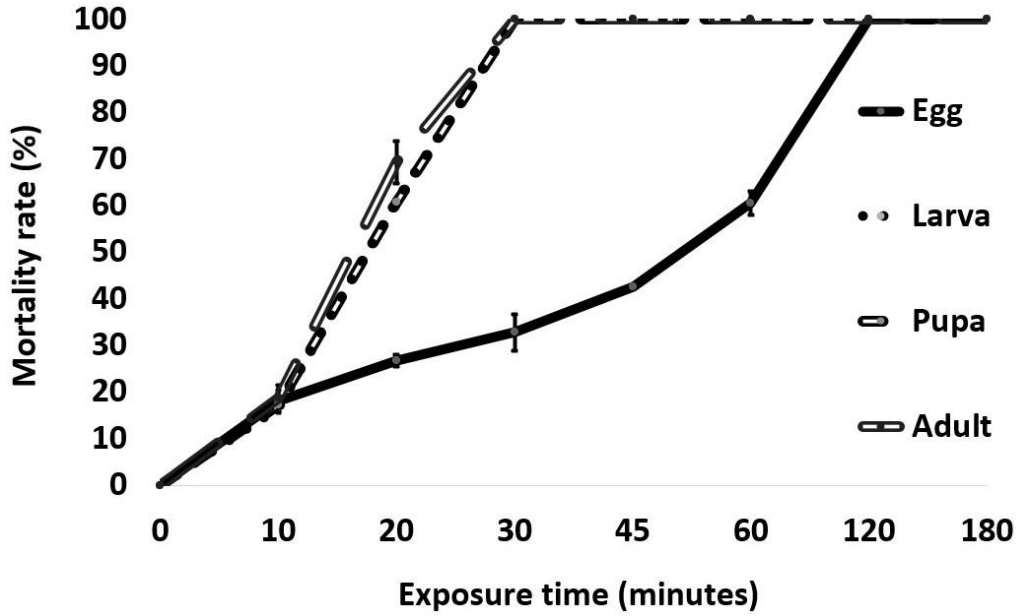

Figure 6. Mortality rates of *Callosobruchus chinensis'* eggs, larvae, pupae and adults exposed to -20 °C (P<0.001).
Şekil 6. -20 °C'ye maruz kalan Callosobruchus chinensis yumurta, larva, pupa ve erginlerinin ölüm oranları (P<0.001).

In this study, 100% death was achieved in the eggs after 120 minutes at -20 °C and it was observed that these eggs did not reach maturity after the eggs were kept at -20 °C for 60 minutes (Figure 1, 6). In other low temperature studies performed on *C. chinensis* eggs, LT50 and LT99 values at -5 °C were found to be 12.57 and 77.03 hours, respectively (Zhong et al., 2013). In another study, it was said that the time required for *C. chinensis* eggs to die completely at -4 °C should be about 20 days (Maharjan et al., 2017). In the study of Dohino et al (1999) on *Callosobruchus rhodesians* (Col: Chrysomelidae), they found a value very close to the results we found and recorded the LT99 value of the eggs as approximately 124 minutes at -18 °C. Johnson and Valero (2000) stated that after exposure of *Callosobruchus maculatus* (F) (Col: Chrysomelidae) eggs to an extreme low temperature such as -18 °C for 180 minutes, no egg reached maturity. As noted in another study by these authors, the complete mortality rate of *C. maculatus* eggs on *Vigna unguiculata* (L.) occurred after 14 days of storage in a commercial cold storage at -18 °C, and no adults emerged from these eggs kept at -18 °C for 24 hours (Johnson and Valero, 2003). Similarly, Loganathan et al. (2011) found that

the LT50 and LT95 values of *C. maculatus* eggs were 1.0 hour and 1.3 hours at -15 °C, respectively. A study on *Tribolium castaneum* (Col: Tenebrionidae), an important stored product pest, reported that more than 8 hours of exposure at -18 °C was required to kill all eggs (Arthur et al., 2015).

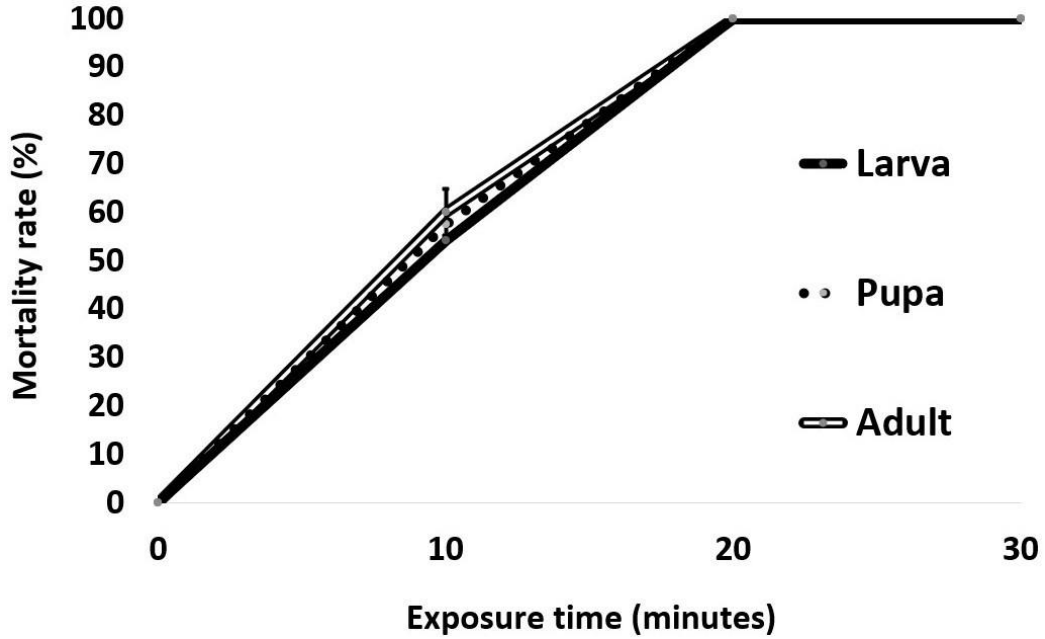

Figure 7. Mortality rates of *Callosobruchus chinensis'* larvae, pupae and adults exposed to -26 °C (P<0.001).
Şekil 7. -26 °C'ye maruz kalan Callosobruchus chinensis larva, pupa ve erginlerinin ölüm oranları (P<0.001).

This study showed that 100% mortality was obtained in larvae, pupae and adults after 20 minutes, 30 minutes and 45 minutes at -26 °C, -20 °C, -16 °C, respectively (Fig. 5, 6, 7). As a result of the findings, lethal time of the various life stages of *C. chinensis* exposed to -16 °C and -20 °C, it is understood that the lethal times of larvae and pupae are very similar. In addition, the adult mortality rate is very close to the lethal time of larvae and pupae. In this study, that LT95 value at -16 °C was 38.3, 39.0 and 36.8 minutes for larvae, pupae and adults, respectively. LT95 value at -20 °C for larvae, pupae and adults were 27.4, 27.5 and 26.0 minutes, respectively (Table 1, 2).

Table 1. LT50 and LT95 values (minutes) of *Callosobruchus chinensis'* larvae, pupae and adults exposed to -16 °C.
Çizelge 1. -16 °C'ye maruz kalan Callosobruchus chinensis larva, pupa ve erginlerinin LT50 ve LT95 değerleri (dakika)

Stage	LT50 (95% CL)	LT95 (95% CL)	Slope ± SE	Intercept ± SE	X^2 (df)
Larva	23.4 (22.1 - 24.8)	38.3 (35.9 - 41.3)	0.111 ± 0.008	-2.594 ± 0.196	3.36 (4)
Pupa	23.8 (22.5 - 25.2)	39.0 (36.6 - 42.1)	0.108 ± 0.008	-2.584 ± 0.193	3.51 (4)
Adult	21.8 (19.9 - 23.7)	36.8 (33.6 - 41.4)	0.109 ± 0.011	-2.375 ± 0.257	2.71 (4)

Table 2. LT50 and LT95 values (minutes) of *Callosobruchus chinensis'* larvae, pupae and adults exposed to -20 °C.
Çizelge 2. -20 °C'ye maruz kalan Callosobruchus chinensis larva, pupa ve erginlerinin LT50 ve LT95 değerleri (dakika)

Stage	LT50 (95% CL)	LT95 (95% CL)	Slope ± SE	Intercept ± SE	X^2 (df)
Larva	17.1 (15.9 - 18.1)	27.4 (25.6 - 29.6)	0.160 ± 0.013	-2.720 ± 0.232	6.461 (4)
Pupa	17.0 (14.9 - 19.2)	27.5 (24.5 - 32.8)	0.157 ± 0.013	-2.671 ± 0.226	7.411 (4)
Adult	16.1 (14.6 - 17.6)	26.0 (23.8 - 29.4)	0.166 ± 0.019	-2.672 ± 0.329	1.741 (4)

In another extreme low temperature study on *C. chinensis*, LT50 values at -5 °C were 24.93, 30.54 and 15.76 hours for larvae, pupae and adults, respectively. In the same study, LT99 values for *C. chinensis* at -5 °C were 171.36, 189.70 and 126.11 hours for larvae, pupae and adults, respectively. In addition, *C. chinensis* pupal LT99 values were found as 189.70, 33.81 and 2.90 hours at -5, -10 and -20 °C, respectively (Zhong et al., 2013). When two studies were compared in terms of extreme low temperature application applied to the pupal stage at -20 °C, the LT95 value in this study was found to be 27.5 minutes, and the LT99 value was found to be 175 minutes in the study conducted in 2013. In another similar study, all the

larvae and pupae of *C. chinensis* used in the studies died at -4 °C after 25 and 10 days, respectively (Maharjan et al., 2017).

Looking at similar studies on *C. maculatus*, Johnson and Valero (2000) noted that the newly emerged adults died completely after 50 minutes of exposure to -18 °C. In another study by the same authors, it was stated that adults living on seeds died completely within 40 minutes at -18 °C (Johnson and Valero, 2003). In a study on mites, all nymphs and adults of *Tyrophagus putrescentiae* (Acaridae) died within 30 minutes after cooling to -18 °C (Eaton and Kells, 2011). It took about 30-60 minutes for all life forms of *Plodia interpunctella* (Hübner) (Lep: Pyralidae) to die at -18 °C (Gvozdenac et al., 2019). The results of these four studies are very similar to the values found at -16 °C for this study. However, Loganathan et al. (2011) showed that the LT50 and LT95 values of *C. maculatus* pupae were 1.8 hours and 2.5 hours at -15 °C, respectively.

Generally, -18 °C was used against stored product pests and generally the same results were obtained. Donahaye et al. (1995) determined that approximately 180 minutes of exposure at -18 °C was required to kill all life stages of *T. castaneum, Oryzaephilus surinamensis* (Col: Silvanidae) and *Ephestia cautella* (Lep: Pyralidae). In a similar study, Ferizli et al. (2004) found that eggs, larvae, pupae, and adults of *C. maculatus* can be fully controlled by keeping them at -18 °C for 180 minutes. However, Gvozdenac et al. (2019) stated differently that the LT50 and LT99 values of *P. interpunctella* larvae died at -18 °C in a very short time such as 1.9 minutes and 12.8 minutes, respectively.

When extreme low temperature studies were examined, it was seen that there were differences in the same extreme low temperature results applied to the same stored product pest. Eliopoulos et al. (2011) found that the LT50 value of larvae of *T. granarium* at -16 °C was less than four hours. However, Abdelghany et al. (2015) found that the LT50 value of larvae of *T. granarium* at -16 °C was more than one day. In another extreme cold application study on *P.interpunctella, P.interpunctella* adults died after 120 minutes at -15 °C (Athanassiou et al., 2018), and *P.interpunctella* larvae died after 180 minutes at -15 °C. (Gvozdenac et al., 2019).

Low temperature studies have been conducted on *C. chinensis*, but not extreme low temperature studies as in this study. However, very low temperature studies have been done on similar stored product pests. While some researchers came to different conclusions that eggs are the most sensitive stage in low temperature applications (Maharjan et al., 2017), some researchers have reached different conclusions that eggs are the most resistant stage in low temperature applications (Johnson and Valero, 2003). In this low temperature study, the egg stage was found to be the most durable stage and the adult stage to be the most susceptible stage. The larval and pupal stages in this study have almost similar results. Adult stage results are close to larval and pupal stage results. There may be many reasons why different biological stages of *C. chinensis* and other stored product pests show different sensitivity and different responses to extreme low temperatures. These; There may be different factors such as the species of pests, the geographies where the pests are located, climates and temperature requirements, the species of stored product that the pests live on, the storage conditions of the product where the pests live. All these different factors can cause similar or different results in low temperature studies on stored product pests. Therefore, it is important to carry out low temperature studies carefully for each region. It would be in the interest of valuable scientists working in this field to come together in comprehensive regional and global studies on stored product pests.

CONCLUSION

Today, it is known that healthy protection of agricultural products is as important as the safe cultivation of agricultural products. It is known that the use of chemical pesticides in the storage of agricultural products has some side effects. As can be understood from previous studies, low temperature applications as a reliable way to control of stored product pests have gained importance in recent years and have been defined as an alternative management strategy against pests. As a result of this study, it was found that *C. chinensis* was susceptible to extreme low temperatures and deaths increased rapidly due to the increase in exposure time. This study will set an example for future low temperature studies, thus shedding light on the development of new strategies for a healthier and more reliable storage of stored agricultural products for a long time.

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Author's Contributions

The contribution of the authors is equal.

Conflicts of interest

The authors declare no conflict of interest.

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The Effects of Root Lesion Nematodes (*Pratylenchus thornei*) on Rhizobium Bacteria of Chickpea Plant

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ABSTRACT

Chickpea (*Cicer arietinum*) is one of the most significant legume crops and supply high-quality protein for human nutrition.Legume crops such as chickpea are important agriculturally because of their symbiotic ability to nitrogen fixation with specific soil bacteria. Legumes like chickpea depend on nitrogen provided by the activity to grow, but these rhizobium bacteria are affected by plant-parasitic nematodes that cause less activity and decrease the number of nodules in chickpea plant. The root lesion nematodes (*Pratylenchus thornei*) are common and economically important pests described as one of the limiting factors in agriculture and the growing chickpea field in the world. In this study, the effects of this nematode on the number of rhizobia (nodules) and rhizobium bacteria activity were assessed in both wild and domesticated accession of *Cicer* species under laboratory conditions. We inoculated all *Cicer* accession with the *Mesorhizobium* bacteria and with one species of the genus *Pratylenchus* (*P. thornei*). The result showed that *P. thornei* has a negative impact on the number of nodules and the activity of rhizobium bacteria. Nematode infection on chickpea caused decreased nodulation. Overall, nematode infected plant formed 4-8 nodules/root and less nodule number than an uninfected plant.

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Kök Lezyon Nematodlarının (*Pratylenchus thornei*) Nohut Bitkisi ve Rhizobium Bakterileri Üzerindeki Etkisi

ÖZET

Nohut (*Cicer arietinum*) en önemli baklagil bitkilerinden biridir ve insan beslenmesi için yüksek kaliteli protein sağlar. nohut gibi baklagil bitkileri, spesifik toprak bakterileriyle azot fiksasyonuna yönelik simbiyotik yetenekleri nedeniyle tarımsal açıdan önemlidir. Nohut gibi baklagiller büyüme aktivitesi sağladığı nitrojene bağlıdır, ancak bu rizobium bakterileri nodül bitkilerinde daha az aktiviteye neden olan ve nodül sayısını azaltan bitki paraziti nematodlardan etkilenir. Kök lezyon nematodları (*Pratylenchus thornei*), dünya tarımında büyüyen nohut tarlalarında en önemli sınırlayıcı faktörlerden biri olarak tanımlanan yaygın ve ekonomik açıdan önemli zararlılardır. Bu çalışmada, bu nematodun rizobium (nodül) sayısı ve rhizobium bakteri aktivitesi üzerindeki etkileri, laboratuvar koşullarında *Cicer* türlerinin hem yabani hem de yerli genotiplerinde değerlendirilmiştir. Tüm nohut genotipleri Mesorhizobium bakterileri ve *Pratylenchus* cinsinin bir türü (*Pratylenchus thornei*) ile bulaştırılmıştır. Sonuç olarak *P. thornei*'nin nodül sayısı ve rhizobium bakterilerinin aktivitesi üzerinde olumsuz bir etkisi olduğunu göstermiştir. Nematod enfeksiyonu, nohutta nodülasyon sayısının azalmasına neden olmuştur. Nerdeyse, nematod ile enfekte olmuş bitki, enfekte olmayan bitkiye göre 4-8 arası az nodül sayısı oluşturmuştur.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important plants between legumes crops in the world. The most important chickpea production areas in the world are India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, USA, and Canada (FAO, 2017).

Almost all species want a symbiotic relationship with other species to assess easily carry out the crucial biological activity (Shapira, 2016). Legumes crop can make a symbiotic relationship with nitrogen-fixing soil bacteria that is called rhizobia bacteria. This symbiosis relationship causes to form nodules on the plant root by this bacteria that convert atmospheric nitrogen into ammonia that can be used by the plant. Also, the advantage result of symbiotic nitrogen fixation by chickpea nodules and rhizobium bacteria can make the facility of growing the crop in many nitrogen-poor soils (Carter et al., 1994). Most of the plants depend on symbiotic bacteria or fungi to grow, and on their activity for reproduction (Friesen, 2013; Busby et al., 2017). The fixation of nitrogen by legumes plays an important role in agricultural production. Legumes crops such as chickpea are partners with rhizobium bacteria for the fixation of nitrogen.

The average chickpea yield in the world is changing because of many biotic plant-parasitic nematodes and abiotic soil factors that can cause an important reduction in grain quantity and quality of chickpea (Singh et al., 1994; Sudupak et al., 2002). Castillo and Vovlas (2007) indicated that the plant-parasitic nematodes are one of the most economically and important pests affecting chickpea as the biotic factors. Parasites can cause important changes in the symbiotic relationship, usually in two ways (Strauss and Irwin, 2004). Sasser and Freckman (1987) indicated that annual yield losses of the total global production of chickpea was 14% by the effect of plant-parasitic nematodes. Different species of nematode cause important economic damage in chickpea. Among these nematodes, root-lesion nematodes (*P. thornei*) is one of the most important species. Ballhorn et al. (2014) showed that the activity of symbiotic partnerships and formed rhizobium associations was decreased because of this parasite in the infected host plants. The symbiotic relationship between rhizobia and plant genotype together can affect the yield potential of chickpea and the symbiosis response to biotic and abiotic stresses (Correa and Barneix, 1997). Hussey et al. (1976) observed that plant-parasitic nematodes can affect symbiotic nitrogen fixation on legumes roots by Rhizobium bacteria. Also, the study by Vovlas et al. (1998) showed that plant-parasitic nematodes (*Meloidogyne incognita*) affected more than 25% of *Mesorhizobium* bacteria activity in the chickpea nodule.

The aim of study was to know about the interaction activity between legumes and nitrogen-fixing bacteria (rhizobia), and how do parasitic nematodes can affect the rhizobium bacteria activity. For this purpose, we inoculated all *Cicer* genotypes with one of the root-lesion nematodes (*Pratylenchus thornei*). Using the number of nematodes was to estimate the damage caused by *P. thornei* in chickpea, we observed that chickpea genotypes differed in susceptibility to nematode infection, and between the number of rhizobia (nodules) and nematode number differed in infectivity.

The number of nodules plays an important in the fixation of nitrogen and agricultural production by legumes. Many legume crops are infected by plant-parasitic nematodes causing photosynthates damage (Dhandaydham et al., 2008; Goverse and Smant, 2014).

This research provides scientific information to understand how root-lesion nematodes (*P. thornei*) can impact the number of nodules and activity of the rhizobium bacteria in the infected chickpea plant. Because nodule number is correlated with rhizobium fitness in legumes and also it can provide the benefit as a proxy for plant partner quality in the rhizobium symbiotic (Heath and Tiffin, 2009).

MATERIALS and METHODS

In this study, 9 accessions of Turkish domesticated and wild *Cicer* species 3 *Cicer arietinum* (Azkan, Çagatay, and Gökçe), 3 *Cicer echinospermum* (Karabahçe, Ortace, and Destek), and *3 Cicer reticulatum* (Şırnak, Kallen and Eğil) were used to test how root-lesion nematodes (*P. thornei*) impact the number of nodules (rhizobium) under laboratory conditions. For this purpose, we compared the nodule number and total nodules between nematode-infected and uninfected plants.

Cicer genotypes using in this study were collected from 3 collection sites of Şanliurfa, Şirnak, and Diyarbakir province of Turkey. The collection seeds were sacrificed by making a small cut in the seed coat before germinating to improve water absorption and germination in the wild *Cicer* spp. The individual chickpea seeds were disinfected with hypochlorite (4%) and alcohol (30%). Moreover, to enhance seed germination, about 30 seeds of each accession were

placed on the surface of wet filter paper at 4°C for 3 days in sterile Petri dishes (Garcia et al. 2006). We incubated seeds at room temperature for 16 hours before planting.

Germinated seeds were then planted in the open-ended standard small tube (16 cm in high 2.5 cm in diameter) that contained 60 gr field soil, (73% clay, 16.5% silt, and 10% river sand) and supported by a box frame. The soil inside the tube was sterilized by an autoclave machine for 5 minutes at 121°C before sowing the seeds and keep in the laboratory at the University of Cukurova at 25°C, on a 16:8 light: dark cycle.

Isolation and culture of nematode

Soil and root samples were collected from chickpea fields between June and July of 2019. Nematodes were extracted by using the Baermann funnel technique in the laboratory (Hooper, 1986). Each root and soil sample consisted of 5- 10 samples (taken at depth of 5-10 cm), with the final weight of 1-2 kg soil per sample. Root-lesion nematodes (*Pratylenchus thornei*) used in this study were cultured on carrot cultures by using the method described by Nicol and Vanstone (1993). This nematode was collected originally from growing chickpea regions in Şanliurfa province (Harran district) located in the Southeastern Anatolia Region of Turkey and cultured in the nematology laboratory at Çukurova University.

Isolation and culture of bacteria

Rhizobia were isolated from nodules of chickpea from field-grown chickpea from regions in Şanliurfa province (Harran district) located in the Southeastern Anatolia Region of Turkey. For rhizobia isolation (*Mesorhizobium*), 2–11 nodules from chickpea and a maximum of five nodules/plant from field-grown chickpea were selected. The rhizobia from the selected nodules were isolated and cultured following standard protocols using CRYEMA (yeast extract mannitol agar medium with congo red) described in Somasegaran and Hoben (1994).

Two weeks after germination, each chickpea plant was inoculated with 1 ml of Mesorhysobium culture and 1 ml of nematode consisting of 225 *P. thornei* (Behmand et al., 2019). Nematodes and rhizobia were inoculated onto the plant at the same time to prevent effects associated with different arrival times. The plants were harvested 16 weeks after planting and the number of nodules and on each root system were counted under a dissecting microscope.

Statistical analyses

The data were analyzed using a randomized block design (One-way ANOVA) in Genstat (V13). These analyses included treatment (nematode presence or absence) and random effects of genotype, block, treatment × genotype, and treatment × block. Significant differences between treatment and replication of data were calculated at P< 0 .001. Outliers and variance distribution was assessed using residual plots. Data were transformed as necessary.

RESULTS AND DISCUSSION

The result showed that *P. thornei* has a negative impact on the number of nodules and the activity of rhizobium bacteria. Seed inoculation with *Mesorhyzobium* bacteria and *P. thornei* had a significant effect on the number of formed nodules (rhizobium). The average nodule number in the inoculation seed with *Mesorhyzobium* bacteria was more than inoculation seed with *Mesorhyzobium* bacteria and *P. thornei* (Figures 1 and 2).

There was a significant difference observed between root-lesion nematodes (*P. thornei*) and the number of nodules produced in the absence of nematodes P<.001 (Table 1). Nematode-infected plants produced fewer nodules than uninfected plants (Figures 1 and 2). Where, nematode infected plant formed 4-8 nodules/root and less nodule number than the uninfected plant. The frequency distribution of nodules on the infected and uninfected plant with nematode is shown in figure 1.

Also, the results of the study indicated that the number of nodules (rhizobium) not only was different between legume species even cultivars within a species were different. There was a significant effect of *Cicer* cultivars for total nodule and the ability of them to attract rhizobia was changed between *Cicer* species P<0.001 (Table 1). The number of nodules among the cultivars studied, 'Çagatay', 'Gökçe', and Menemen was higher than other cultivars. These effects showed that plant genotypes differed in how nodule traits were impacted by nematode infection. A similar study by Bhuiyan et al. (2008) and Sattar et al. (1998) showed that genotypic diversity affected the number of nodules in both wild and domesticated chickpea and other legumes crops.

In addition, results demonstrated that the maximum of nodule numbers among the cultivars of *C. arietinum* was higher than other *Cicer* species and there was a significant difference observed between *Cicer* species for the nodulation potential P<.001 (Table 1 and Figure 3), but the negative effect of nematode on rhizobium (nodules) in the wild *Cicer* species (*C. echinospermum* and *C. reticulatum*) was less than *C. arietinum* (Figures 2 and 3).

Both wild *Cicer* sp. have a similarly responsive to *P. thornei* and the performance of them for the nodulation was better in nematode infected plant than any *Cicer arietinum* cultivars (Figure 2). However, among the *C. echinospermum* and *C. reticulatum* cultivars, Karabahce, Ortace, and Destek have given a good

performance to *P. thornei* than any *C. reticulatum* cultivars (Figures 2 and 3). Both bacterial and plant genes can affect the development of root nodules (Buttery et al., 1997 and Danso et al., 1987). Therefore, any changes in these organisms cause to decrease the development of the nodules and therefore affect nitrogen fixation (Rupela and Saxena, 1987).

Table 1. Analysis of variance
Çizelge 1. Varyans analizi

Source of variation	Degree of Freedom (df)	Sum Square (SS)	Mean square (MS)	F Ratio	P Value
treatment	1	387.347	387.347	47.51	<.001
species	2	895.194	447.597	54.90	<.001
Treatment xspecies	2	48.694	24.347	2.99	0.057
Residual	66	538.083	8.153		
Total	71	1869.319			

* df: contains degree of freedom which are measure of how much information is contained in each variance;
s.s: Means squares, which are calculated by multiplying the mean square and degree of freedom in the same row;
ms (Means squares): The variance between treatment;
v.r: The ratio of the between treatment variance to the within treatment variance;
F pr or P value: Significance value P < 0.001.

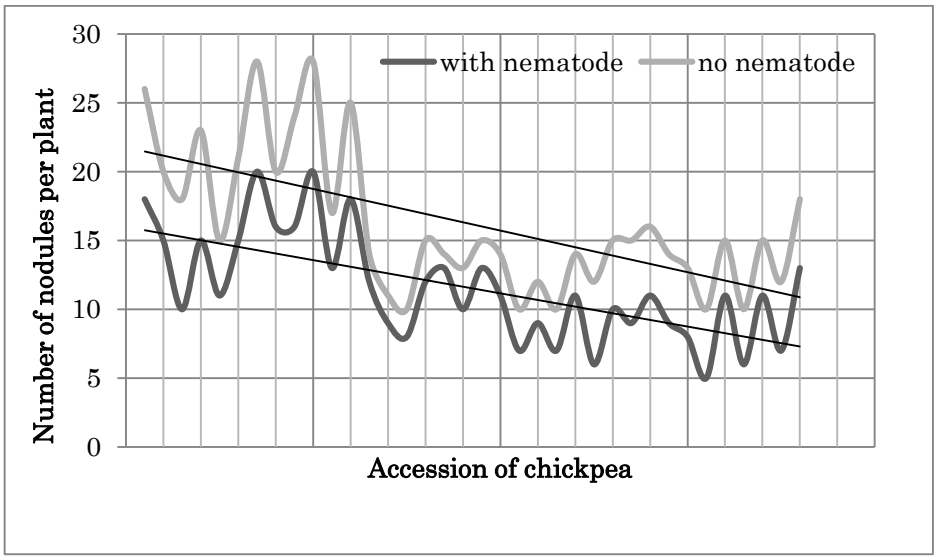

Figure 1. Frequency distribution of nodules on the infected and uninfected plant with nematode
Şekil 1. Nematod ile enfekte ve enfekte olmayan bitki üzerindeki nodüllerin frekans dağılımı

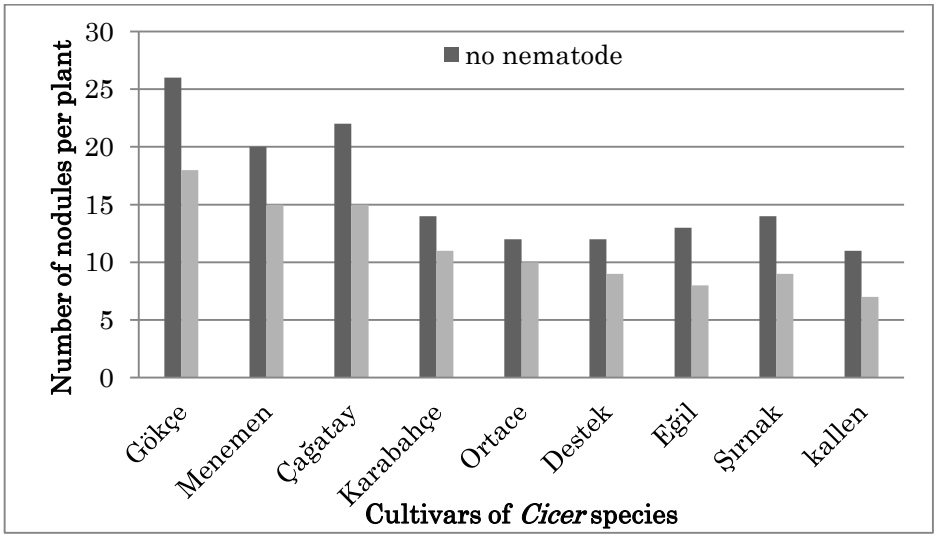

Figure 2. The effects *of Pratylenchus thornei* on the number of nodules between *Cicer* cultivars.
Şekil 2. *Pratylenchus thornei*'nin nohut çeşitleri arasındaki nodül sayısı üzerine etkileri.

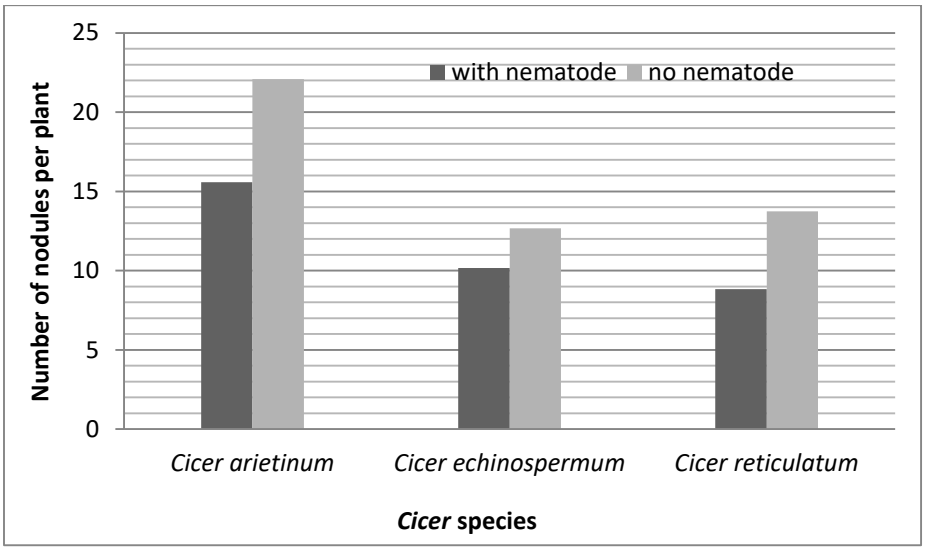

Fig 3. The effects of *Pratylenchus thornei* on the number of nodules between *Cicer* species.
Şekil 3. *Pratylenchus thornei*'nin nohut türleri arasındaki nodül sayısı üzerine etkileri.

According to the study showed that biotic factors such as plant-parasitic nematodes disrupt the symbiotic relationship between leguminous plants and nitrogen-fixing rhizobia bacteria. In this study chickpea that were infected by parasitic nematodes (*P. thornei*) formed fewer total nodules than uninfected plants. The number of nodules in the accession of domesticated *Cicer* with rhizobium bacteria had a more nodules number than wild genotype species. However, plants that formed more nodules with rhizobia were more attack and heavily infected by nematodes. *Mesorhyzobium ciceri* that infects the chickpea is specific and rarely present in the soil in which the chickpea is not recently grown (Elhadi and Elsheikh, 1999). Also, Abdalla et al. (2011) indicated that the number nodulation of the chickpea and the success of bacterial inoculation increased in the presence of the native bacterial population.

The study showed that the symbiotic relationship between rhizobium and plants is negatively affected by parasite infection. Also, in the presence of nematodes, plants formed fewer or prevent associations with symbiotic rhizobia. We found that nematode-infected plants formed 29% fewer total nodules on root than uninfected plants. A similar study by Wood et al. (2018) showed that *Meloidogyne hapla* nematode has a negative impact on the number of nodules and formed 23% fewer nodules and 19% less total nodule biomass per gram in Medicago plant roots.

The effect of nematode on nodule number is different between *Cicer* species. Moreover, while some *Cicer* genotypes formed fewer nodules when infected by nematodes (*C. arietinum*), others including *C. echinospermum* and *C. reticulatum* genotypes were not more affected. In this study, the relationships between wild and domesticated *Cicer* sp. indicated that negatively affected of *P. thornei* on rhizobium in both wild *Cicer* sp. (*C. reticulatum* and *C. echinospermum*) were less than domesticated varieties (*C. arietinum*).

The useful effect of rhizobia symbiosis on legume crops nutrition and growth and the association of rhizobia with plant-parasitic nematodes in the rhizosphere cause investigations into the potential role of nematode parasitism on nodulation, and accordingly on symbiotic nitrogen fixation. Future studies in the effect of root-lesion nematodes on rhizobium should explore the genetic capacity is the influences the degree of these relationships or not. Wood et al. (2018) demonstrated that the effect of nematode on crop plants is different because of genotype variation in the plant. Burghardt et al. (2017) also found significant genotype variation between plant genotypes in the definition of defense genes in nodules. The information provided by these results will be useful for the clarification of nematodes, root-nodule bacteria, and host plant relations, but more studies are needed to test the effect of root-lesion nematodes on rhizobium for developing chickpea breeding programs to keep the economic damage below the threshold level.

CONCLUSION

It is concluded that root-lesion nematodes (*P. thornei*) infection can impact the number of nodules in the chickpea plant. Disease management by using resistance cultivars is suitable to be used for control of root lesion nematodes and lessen the negative impact on the number of nodules and the activity of rhizobium bacteria.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

TB designed the study, conducted the experiments, and prepared the manuscript. IHE provided technical guidance, and critically revised the manuscript for intellectual content. Two authors read and approved the final manuscript.

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Molecular Identification of Root-knot Nematode Species (*Meloidogyne* spp.) on Lavender of Isparta and Burdur Provinces in Turkey

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ABSTRACT

This study was conducted to identify and to determine the distribution of root-knot nematode species in total of 625 ha of Laventer (*Lavandula × intermedia* Emeric ex Loisel. var. Super) cultivated area of Isparta and Burdur Provinces of Turkey. A total of 60 samples were collected in autumn of 2020. Root knot nematode species molecular identification was determined by species-specific primers from egg masses. The 17 samples taken from cultivated lavender fields were found to be infected with Root knot nematode. As a result of molecular identification, 12 of samples were found to be *Meloidogyne incognita*, while 5 of them were found to be *M. arenaria*. This was the first report of infestation of lavender by *M. incognita* in Turkey. Of studied areas, Keçiborlu district of Isparta Province with most cultivated lavender area sustained 7 samples of *M. incognita* and 3 of samples *M. arenaria*.

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Meloidogyne arenaria
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Türkiye'nin Isparta ve Burdur İllerinin Lavantalarında Kök-ur Nematod Türlerinin (*Meloidogyne* spp.) Moleküler Tanımlanması

ÖZET

Bu çalışma, Türkiye'nin Isparta ve Burdur illerinde Lavanta (*Lavandula × intermedia* Emeric ex Loisel. var. Super) yetiştirilen yaklaşık 625 ha'lık bir alanda Kök-ur nematodu türlerinin belirlenmesi ve yayılışlarının saptanması amacıyla yürütülmüştür. 2020 yılının sonbaharında toplam 60 örnek toplanmıştır. Kök-ur nematod türlerinin moleküler tanımlaması, yumurta paketinden türe özgü primerler ile belirlenmiştir. Kültürü yapılan lavanta tarlalarından alınan 17 örneğin Kök ur nematodları ile enfekte olduğu bulunmuştur. Moleküler tanılama sonucunda bu örneklerin 12 tanesinde *Meloidogyne incognita* bulunurken, 5 tanesinde *M. arenaria* saptanmıştır. Bu, Türkiye'de lavantada *M. incognita* enfeksiyonuna ilişkin ilk rapordur. Lavantanın en çok yetiştirildiği Isparta İli Keçiborlu İlçesi'nin Kök-ur nematodu ile enfekte olduğu belirlenmiştir. Keçiborlu İlçesi'nde 7 örnek *M. incognita* tespit edilirken, 3 örnek *M. arenaria* tespit edilmiştir.

Bitki Koruma

Araştırma Makalesi

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Anahtar Kelimeler Lavanta
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INTRODUCTION

Lavender (*Lavandula* spp.) is an aromatic herb of Lamiaceae. The essential oil obtained from the spikes of the lavender is in great demand around the world which is mostly used in cosmetics, perfumery, flavoring and pharmaceutical industries (Tarhan et al., 2019). Lavender (*L. angustifolia* = *L. officinalis* = *L. vera*), Lavandin (*L. angustifolia* × *L. latifolia* = *Lavandula × intermedia* = *L. hybrida*), and Spike lavender (*L. spica* = *L. latifolia*) are important lavender species (Erbaş et al., 2017). Lavender is densely cultivated in France, Bulgaria, Spain, Italy, Greece, England, Russia, the USA, Austria, and the North African countries worldwide (Tucker, 1985; Erbaş et al., 2017). Lavender is cultivated approximately 1200 ha area in Turkey and in Burdur and Isparta Provinces, 167.8 and 456.8 ha of cultivated area are

reported respectively (TÜİK, 2021). The most lavender cultivation is in Kuyucak village in Keçiborlu district of Isparta Province which consisted of 93% lavender production of Turkey (Başaran, 2017). The largest lavender field in Turkey is in Akçaköy in Yeşilova district in Burdur Province where 37 ha of cultivation are done in a single plot (Anonim, 2021). The cultivated lavender cultivar in Isparta and Burdur provinces is *Lavandula × intermedia* var. Super which has very well adapted particularly to the non-irrigated, arid, and sloping lands of this locality (Erbaş et al., 2017).

Lavender is infected by many pests and pathogens and that might cause significant damage and effect negatively in terms of their quality and essence yields (Gorustovich et al., 1997). Root-knot nematodes (*Meloidogyne* spp.) are the most economically important nematodes in agriculture due to damage to vascular tissues, wide host range, forming disease complexes with soil pathogens (Udo et al., 2008; Moens et al., 2009; Lobna et al., 2016; Siddiqui and Zaki, 2017). *Meloidogyne incognita* (Kofoid and White, 1912) Chitwood, 1949*, M. javanica* (Treub, 1885) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949 and *M. hapla* Chitwood, 1949 are the most dominant species in agricultural and horticultural crops (Hussey and Janssen, 2002; Brito et al., 2008; Sarkar, 2020) and are common in the Mediterranean area (Ornat and Sorribas, 2008; Devran and Söğüt, 2009; Uysal et al., 2017; Gonçalves et al., 2020). *Meloidoyne incognita* was reported in lavender in Argentina (Gorustovich et al., 1997) and Egypt (Ibrahim and Mokbel, 2009) in cultivars *L. hybrida* and *L. officinalis*, respectively. Carneiro et al. (2014) found *M. luci* on *L. spica. Meloidogyne hapla* from Greece (Gonçalves et al., 2020) and *M. arenaria* (Özalp et al., 2020) from Turkey are reported in *L. angustifolia.*

The aim of this study was to identify root knot nematode species in lavender cultivation areas of Isparta and Burdur provinces of Turkey by molecular methods and consequently determine their distribution.

MATERIAL and METHOD

Root-knot nematode sampling locations

Lavender fields in Isparta and Burdur provinces of Turkey were surveyed in autumn of 2020. A total of 60 samples were collected in the study (Table 1). In each field, root and soil samples were taken from lavender localities indicating symptoms of stunting plants. Root samples were placed in a separate bag and brought to the laboratory in a cold chain and stored at 4ºC for further use.

Nematode extraction

Each lavender root samples were gently washed with tap water and examined under a stereomicroscope for

Table 1. Locatians of Root-knot nematode samples
Çizelge 1. Kök-ur nematodu örneklerinin lokasyon bilgileri

Sample no *Örnek no*	Code Kod	Village or smalltown/ District/ Province (*Köy yada Kasaba/ İlçe/ İl)*
1	E1	Sorkuncak/Eğirdir/Isparta
2	E2	Sorkuncak/Eğirdir/Isparta
3	E3	Sorkuncak Eğirdir/Isparta
4	E4	Sarıidris/ Eğirdir/Isparta
5	E5	Sarıidris/ Eğirdir/Isparta
6	E6	Sarıidris/ Eğirdir/Isparta
7	E7	Eğirdir/Isparta
8	ISP1	Centre/Isparta
9	ISP2	Centre/Isparta
10	KL1	Kılıç/Keçiborlu/Isparta
11	KL2	Kılıç/Keçiborlu/Isparta
12	KL3	Kılıç/Keçiborlu/Isparta
13	KL4	Kılıç/Keçiborlu/Isparta
14	A1	Aydoğmuş/Keçiborlu/Isparta
15	A2	Aydoğmuş/Keçiborlu/Isparta
16	A3	Ardıçlı/Keçiborlu/Isparta
17	A4	Ardıçlı/Keçiborlu/Isparta
18	C1	Çukurören/Keçiborlu/Isparta
19	C2	Çukurören/Keçiborlu/Isparta
20	C3	Çukurören/Keçiborlu/Isparta
21	C4	Çukurören/Keçiborlu/Isparta
22	C5	Çukurören/Keçiborlu/Isparta
23	C6	Çukurören/Keçiborlu/Isparta
24	C7	Çukurören/Keçiborlu/Isparta
25	C8	Çukurören/Keçiborlu/Isparta
26	C9	Çukurören/Keçiborlu/Isparta
27	K1	Kuyucak//Keçiborlu/Isparta
28	K2	Kuyucak//Keçiborlu/Isparta
29	K3	Kuyucak//Keçiborlu/Isparta
30	K4	Kuyucak//Keçiborlu/Isparta
31	K5	Kuyucak//Keçiborlu/Isparta
32	K6	Kuyucak//Keçiborlu/Isparta
33	K7	Kuyucak//Keçiborlu/Isparta
34	K8	Kuyucak//Keçiborlu/Isparta
35	K9	Kuyucak//Keçiborlu/Isparta
36	K10	Kuyucak//Keçiborlu/Isparta
37	K11	Kuyucak//Keçiborlu/Isparta
38	K12	Kuyucak//Keçiborlu/Isparta
39	K13	Kuyucak//Keçiborlu/Isparta
40	K14	Kuyucak//Keçiborlu/Isparta
41	K15	Kuyucak//Keçiborlu/Isparta
42	K16	Kuyucak//Keçiborlu/Isparta
43	K17	Kuyucak//Keçiborlu/Isparta
44	K18	Kuyucak//Keçiborlu/Isparta
45	K19	Kuyucak//Keçiborlu/Isparta
46	K20	Kuyucak//Keçiborlu/Isparta
47	S1	Boğazköy/Sütçüler/Isparta
48	S2	Boğazköy/Sütçüler/Isparta
49	B1	Centre/Burdur
50	B2	Centre/Burdur
51	B3	Akçaköy/Yeşilova/Burdur
52	B4	Akçaköy/Yeşilova/Burdur
53	B5	Akçaköy/Yeşilova/Burdur
54	B6	Akçaköy/Yeşilova/Burdur
55	B7	Akçaköy/Yeşilova/Burdur
56	B8	İlyas/Yeşilova/Burdur
57	B9	İlyas/Yeşilova/Burdur
58	B10	İlyas/Yeşilova/Burdur
59	B11	Salda/ Yeşilova/Burdur
60	B12	Salda/ Yeşilova/Burdur

evidence of galls. Then, egg masses and mature females were collected from infested roots using needle and placed in Eppendorf tubes under a stereomicroscope.

Molecular identification

DNA extraction from nematode isolates was performed following cetyl trimethyl ammonium bromide (CTAB) method with slight modifications (El-Qurashi et al., 2017; Mondino et al., 2015). Two species-specific primers were used in the PCR amplifications, which was conducted by thermocycler (Veriti Thermal cycler, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) in a total volume of 25 µL (Table 2). Reaction mixture consisted of 10 ng DNA (5 µl), PCR buffer (2.5 µl), 2 mM MgCI2 (1 µl), 0.2 mM dNTP (1 µl), 10 mM Primer F (1 µl), 10 mM Primer R (1 µl), 1 unit Taq DNA polymerase (GenEon, San Antonio, TX, USA) (0.25 µl) and ddH2O (13.25 µl). PCR cycles; initial denaturation at 94°C for 3 min, followed by 35 cycles each consisting of 30 sec at 94°C, 30 sec at 56°C and 60 sec at 72°C for Far/Rar and 94°C for 3 min for INCK14F/INCK14R primers, followed by 30 sec at 94°C, 30 sec at 60°C and 60 sec at 72°C with a final extension at 72°C for 7min.

PCR products were separated using agarose electrophoresis in 2% gel (Agarose Type I, Sigma-Aldrich, St. Louis, MO, USA) with ethidium bromide. The gel was run for 2 hours using constant voltage of around 90 V and then visualized and photographed under UV light using a gel documentation system. The specific band was detected for each SCAR marker separately.

Table 2. Species specific primers of root-knot nematodes for molecular identification
Çizelge 2. Moleküler tanımlama için kök-ur nematodlarının türe özgü primerleri

Nematode species *Nematod türü*	*Primers* *Primer*	*Primer sequences (5-3)* *Primer sekansları*	*Fragments (bp)* *Uzunlukları*	*Reference* *Kaynak*
M. arenaria	FAR	TCGGCGATAGAGGTAAATGAC	420	Zijlstra vd., 2000
	RAR	TCGGCGATAGACACTACAACT		
M. incognita	INCK14R	CCCGCTACACCCTCAACTTC	399	Randing vd., 2002
	INCK14F	GGGATGTGTAAATGCTCCTG		

RESULT and DISCUSSION

Root knot nematode infested plant roots were found in 17 (28.3%) of 60 samples collected from lavender fields in Isparta and Burdur provinces. Root knot nematodes were found in 3 of 12 samples in Burdur Province and 14 of 48 samples in Isparta Province (Table 3).

As a result of the molecular analysis, while 12 of the 17 samples with Root knot nematode were identified as *Meloidogyne incognita* (Figure 1), 5 of them were identified as *M. arenaria* (Figure 2). *Meloidogyne arenaria* were detected in one sample (B12) in Burdur Province and four samples (E1, A3, K5, K17) from Isparta Province. Only two samples of *M. incognita* (B3 and B7) were found in Yeşilova district in Burdur Province whereas in Isparta Province, 10 samples of *M. incognita* (C5, C9, K4, K11, K16, K20, E4, ISP1, KL3, S1) were identified. Seven samples of *M. incognita* and 3 samples of *M. arenaria* were determined in intensive lavender cultivated Keçiborlu district (Table 3).

Table 3. Number of infested samples with Root knot nematode in lavender fields
Çizelge 3. Lavanta tarlalarında Kök ur nematodu ile enfekte olmuş örnek sayısı

Province *İl*	*District* *İlçe*	*Number of samples* *Örnek sayısı*	*Number of samples with nematodes* *Nematod ile bulaşık örnek sayısı*	*Meloidogyne incognita*	*Meloidogyne arenaria*
Isparta	Eğirdir	7	2	1	1
	Centre	2	1	1	x
	Keçiborlu	37	10	7	3
	Sütçüler	2	1	1	x
Burdur	Centre	2	x	x	x
	Yeşilova	10	3	2	1
Total		60	17	12	5

In Turkey, Lavender is most cultivated in Keçiborlu district of Isparta province and no study has been found on the detection of root knot nematode in this region. In the study, it was determined that Keçiborlu district was significant locality infected with root-knot nematode. It was confirmed that tomatoes were grown before lavender in the C5 and K20 sampling areas of Keçiborlu district where *M. incognita* was detected. On the other hand, it was found that potatoes were grown before lavender in B3 and B7 sampling areas in

Yeşilova district where *M. incognita* was detected. Previously, *M. arenaria* was detected in lavender fields of Edirne and Kırklareli in Turkey (Özalp et al., 2020). However, there is no report of *M. incognita* infecting lavender in Turkey. The present study is the first report of *M. incognita* in lavender in Turkey by using molecular markers. Moreno et al. (1990) reported that lavender species were a suitable host for *M. arenaria*. *Meloidoyne incogita* was found infecting *L. hybrida* Rev. from Argentina (Gorustovich et al., 1997) and *L. officinalis* Chaix et Vill. from Egypt (Ibrahim and Mokbel, 2009).

Figure1. PCR products amplified using primers INCK14R/INCK14F
Şekil 1. INCK14R/INCK14F primerleri kullanılarak çoğaltılmış PCR ürünleri

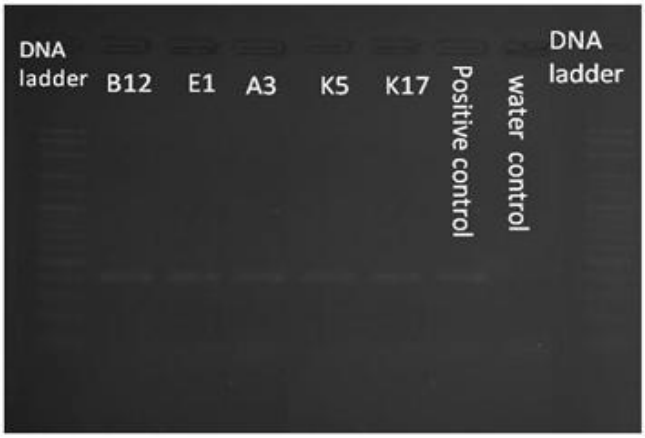

Figure2. PCR products amplified using primers FAR/RAR
Şekil 2. FAR/RAR primerleri kullanılarak çoğaltılmış PCR ürünleri

CONCLUSION

In conclusion, this study showed the detection of root knot nematode infestation in the lavender fields of Isparta and Burdur provinces. *M. incognita* was more common nematode species in studied area. It is necessary to pay attention to lavender seedlings transportion to prevent the dispersal of nematodes from this region to other regions of the country. In addition, weeds that are known to be host to root knot nematodes should be controlled in the fields. Newly to be established lavender field should have soil analyses before starting production with a nematode resistant variety.

Conflict of Interest

The author declare that does not have any competition and any conflicts of interest.

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Yapraklı Barajı (Burdur/Gölhisar) *Alburnus carianorum* (Teleostei: Cyprinidae) Populasyonuna ait Yaş, Büyüme ve Ölüm Parametreleri

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ÖZET

Bu çalışmada, *Alburnus carianorum*'un Yapraklı Barajı popülasyonuna ait bazı popülasyon dinamiği parametreleri incelenmiştir. Uzatma ağları ile 2014-2016 yılları arasında baraj gölünden toplanan 280 birey ile çalışma gerçekleştirilmiştir. İncelenen bireylerin 0-IV'üncü yaş grupları arasında değiştiği ve II. yaş grubunun en baskın yaş grubu olduğu belirlenmiştir. Örneklenen bireylerin total boy değerinin 3.6-14.4 cm ve ağırlık değerinin ise 2.2-35.46 gr arasında değişim gösterdiği belirlenmiş olup ortalama boy ve ağırlık değerleri sırasıyla 11.06±1.56 cm ve 13.95±6.10 g olarak hesaplanmıştır. Boy-ağırlık ilişkisi ise W= 0.0260*$L^{2.5747}$ olarak belirlenmiştir. Popülasyon parametreleri L_∞: 19.73 cm, k: 0.189, to: -0.743, Φ': 1.86 ve K: 0.95±0.13 olarak hesaplanmıştır. Toplam, doğal ve balıkçılık ölüm oranları sırasıyla Z: 0.42, M: 0.32, F: 0.09 olarak tahmin edilmiş olup stoktan yararlanma düzeyi ise E: 0.22 olarak hesaplanmıştır.

Zooloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 13.02.2021
Kabul Tarihi : 03.07.2021

Anahtar Kelimeler
Doğu Akdeniz Havzası
Dalaman Çayı
Ölüm Oranları
Avcılık Baskısı
İzometrik Büyüme

Age, Growth and Mortality of *Alburnus carianorum* (Teleostei: Cyprinidae) from Yapraklı Dam Lake (Burdur/Gölhisar)

ABSTRACT

In this study, some population dynamical parameters were determined for *Alburnus carianorum* which is distributed in Yapraklı dam lake. A total of 280 specimens were collected from 2014 to 2016 using gill nets. Age of the examined specimens varied between 0 and IV age groups and the II. age group was the most dominant. It was determined that the total length varied from 3.6 to14.4 cm and the body weight ranged from 2.2 to 35.46 g, and the average length and weight values were 11.06±1.56 cm and 13.95±6,10 g, respectively. The length-weight relationship was determined as W = 0.0260* $L^{2.5747}$. The population parameters were calculated as $L\infty$: 19.73 cm, *k*: 0.189, *to*: -0.743, *Φ'*: 1.86 and K: 0.95±0.13. Instantaneous rate of total, natural and fishing mortalities were 0.42, 0.32 and 0.0 9 $year^{-1}$, respectively and the exploitation rate was calculated as 0.22.

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GİRİŞ

Leuciscidae familyası üyesi olan *Alburnus* Rafinesque, 1820 cinsi Avrupa ve Ortadoğu ülkelerinin büyük bir kısmında dağılım göstermekte olup Türkiye'nin tüm havzalarından bulunmaktadır. Türkiye'de *Alburnus* cinsi 25 türle temsil edilmekte olup bu türlerin 18'i endemiktir (Çiçek ve ark., 2015; 2018; 2020). Endemik türlerden biri olan *Alburnus carianorum* Freyhof, Kaya, Bayçelebi, Geiger & Turan, 2018 Dalaman Çayı ile Büyük Menderes havzasında dağılım göstermektedir (Mangıt ve Yerli, 2018; Freyhof ve ark., 2018). Göllerin ve hızlı akan akarsuların su filmine yakın zonlarında gruplar halinde yaşarlar ve maksimum total boy 95 mm olarak kaydedilmiştir

(Freyhof ve ark., 2018). IUCN tehlike kategorisi ise EN (Endangered=Tehlikede) olarak belirlenmiştir (IUCN, 2020). Yeni bildirilen bir tür olduğu için söz konusu tür ile ilgili herhangi bir popülasyon dinamiği çalışmasına rastlanmamıştır.

Balık popülasyonlarının büyüme, üreme, ölüm parametreleri gibi bazı popülasyon dinamiği parametrelerinin belirlenmesi ilgili popülasyonun hakkında bilgi sağlamaktadır. Bu nedenle balık popülasyonları ile ilgili kapsamlı ve sürekli araştırmaların yapılması, popülasyonlarda meydana gelen değişikliklerin izlenmesi, türlerin korunması, sürdürülebilir stok yönetimi için gereklidir (Sarıhan ve ark., 2007). Özellikle endemik ve dağılım alanı sınırlı olan türler için bu verilerin sağlanması türlerin korunması bakımından ayrıca önem arz etmektedir. Yapraklı baraj gölünde yapılan bu çalışma ile *A. carianorum* türünün bazı popülasyon dinamiği parametreleri ve ölüm oranlarının belirlenmiştir.

MATERYAL ve METOD

Bu çalışma Dalaman Çayı üzerinde kurulu olan Yapraklı barajında Mayıs 2014-Eylül 2016 tarihleri arasında toplanmış olan numuneler kullanılarak yapılmıştır. Örneklemede TS EN 14757 standartları dâhilindeki yöntemler esas alınmış olup avcılıkta farklı göz açıklığına sahip (5, 6,25, 8, 10, 12,5, 15,5, 19,5, 24, 29, 35, 43 ve 55 mm) uzatma ağları kullanılmıştır. Yakalanan örnekler %10'luk formaldehit solüsyonu kullanılarak tespit edildikten sonra içerisinde %4'lük formaldehit olan bidonlara konularak Nevşehir Hacı Bektaş Veli Üniversitesi İhtiyoloji Laboratuvarına getirilmiştir.

Formaldehit içinde korunan örnekler çeşme suyu altında bekletilerek formaldehitten arındırılması sağlandıktan sonra standart (SB), çatal (ÇB) ve total (TB) boy 1 mm hassasiyetle boy ölçüm tahtası ile ölçülmüş olup total ağırlık (TA) ise hassas elektronik terazi ile 0,01 g hassasiyette tartılmıştır. Pektoral yüzgecin gerisinden alınan pul örnekleri ışık mikroskobu kullanılarak iki farklı okuyucu tarafından bireysel yaş tayini yapılmıştır.

Boy-boy (L-LR) ve boy-ağırlık (L-WR) ilişkileri sırasıyla doğrusal ve üssel regresyon yöntemi kullanılarak belirlenmiştir (Sparre ve Venema, 1998).

L-LR: $L_x=(a*L_y)-b$ (1)

(L_x ve L_y: boy ölçümleri, a ve b: regresyon sabitleri)

L-WR: $W = aL^b$ (2)

(W: bireysel ağırlığı (g), L: total boyu (cm), a ve b: regresyon sabitleri)

von Bertalanffy büyüme eşitliği yardımıyla büyüme özellikleri ortaya konmuştur (Sparre ve Venema, 1998).

$$Lt = L_\infty * \left[1 - e^{-k\,(t-to)}\right] \text{ ve } Wt = W_\infty * \left[1 - e^{-k\,(t-to)}\right] \quad (3)$$

(Lt: t anındaki boy (cm), L_∞: sonsuz boy (cm), k: Brody büyüme katsayısı ($yıl^{-1}$), ve t: yaşı, t_o: yumurtadan çıktığı andaki kuramsal yaş (yıl) ve W_∞: sonsuz ağırlığı (g))

Popülasyonun besililik durumunun değerlendirilmesi için Fulton'un Kondisyon Faktörü (K) ve Büyüme Performans İndeksi (Φ'): eşitliği kullanılarak belirlenmiştir (Pauly ve Munro, 1984; Sparre ve Venema, 1998).

$$K = 100 * \frac{W}{L^b} \quad (4)$$

$$\Phi' = \log k + 2\log L_\infty \quad (5)$$

Popülasyona ait Toplam (Z), Doğal nedenlerle (M) ve Balıkçılık (F) nedeniyle olan ölüm oranları ve stoktan yararlanma düzeyi aşağıdaki formüller kullanılarak hesaplanmıştır (Beverton ve Holt, 1957; Pauly, 1980).

$$Z = k\frac{\left(L_\infty - \bar{L}\right)}{\left(\bar{L} - L'\right)} \quad (6)$$

(Z: toplam ölümler, L_∞: sonsuz boy (cm), $\bar{L}$: ortalama boy (cm), L': örneklenen bireyler içerisindeki en küçük boydaki balığın dahil olduğu sınıf aralığı (cm))

$$Log10M = -0{,}0152 - 0{,}279 * log10L? + 0{,}6543 * log10k + 0{,}463 * log10T \quad (7)$$

(T: örnekleme alanının ortalama su sıcaklık (°C) değeri. Ortalama yıllık sıcaklık değeri olarak Gölhisar ilçesinin yıllık ortalama sıcaklığı olan 12,7°C kullanılmıştır (Anonim, 2012))

$F = Z - M$ (8) $\quad E = \frac{F}{Z}$ (9)

BULGULAR ve TARTIŞMA

Yapraklı Barajında *A. carianorum*'un yanısıra *Atherina boyeri* Risso, 1810; *Cyprinus carpio* Linnaeus, 1758; *Carassius gibelio* (Bloch, 1782); *Barbus xanthos* Güçlü, Kalaycı, Küçük ve Turan 2020; *Pseudorasbora parva* (Temminck & Schlegel, 1846); *Squalius fellowesii* (Günther, 1868); *Oncorhynchus mykiss* (Walbaum, 1792) ve *Scardinius elmaliensis* Bogutskaya, 1997 türleri de örneklenmiştir.

Üzerinde çalışılan bireylerin 0 ile IV. yaş grupları arasında dağılım gösterdiği en baskın yaş grubunun % 50'lik oran ile II. yaş grubu olduğu bunu % 27.14 ile I. ve % 10'luk bir değer ile III. yaş grubu takip etmektedir (Tablo 1). Örneklerin total boy değerlerinin 3.6-14.4 cm ve total ağırlık değerinin ise 2.2-35.46 g arasında değişim gösterdiği belirlenmiş olup ortalama boy ve ağırlık değerleri ise sırasıyla11.06±1.56 cm ve 13.84±5.75 g olarak hesaplanmıştır. Yıllık büyüme oranı dikkate alındığında en fazla büyüme oranının birinci yılda olduğu görülmüştür ve takip eden yaş gruplarında boyca ve ağırlıkça büyüme oranı azalmaktadır.

Çizelge 1. *Alburnus carianorum* popülasyonunda yaş-boy ve yaş-ağırlık frekans dağılımları, her yaş grubuna ait ortalama boy ve ortalama ağırlık

Table 1. Age-lenght and age-weight frequency distributions in Alburnus carianorum population, average height for each age group

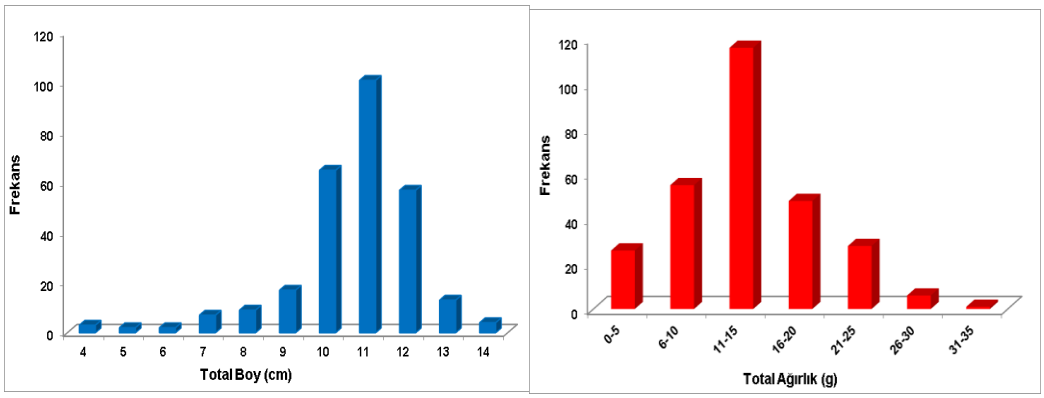
Yaş (Age)	N	%	Total Boy (OB±SD) Total Lenght (mean± SD)	Total Ağırlık (OA±SD) Total Weight (mean± SD)
0	7	2,50	3.6-6.90 (5.36±1.18)	2.2-4.2 (3.16±0.84)
I	100	27.14	7.10-11.2 (9.93±0.36)	5.32-14.80 (9.01±2.73)
II	140	50	10.7-12.7 (11.67±0.48)	9.38-23.07 (15.51±3.04)
III	28	10	11.1-13.5 (12.94±0.26)	19.2-30.11 (22.52±2.51)
IV	5	1.79	13.6-14.4 (14.18±0.33)	22.92-35.46 (30.03±4.51)
Σ	280		3.6-14.4 (13.84±5.75)	2.2-35.46 (13.84±5.75)

Örneklerin boy-frekans değişimi incelendiğinde normal bir dağılım sergilediği ve en baskın boy grubunun 11 cm boy grubu olduğu bunu 10 ve 12 cm boy gruplarının takip ettiği belirlenmiştir (Şekil 1).

Total boy, çatal boy ve standart boy arasındaki ilişkiler ÇB=(0.8621*TB)-0.4617, SB=(0.877*TB)-0.5605 ve TB=(0.9958*SB)-0.8174 olarak formülize edilmiştir (Şekil 2).

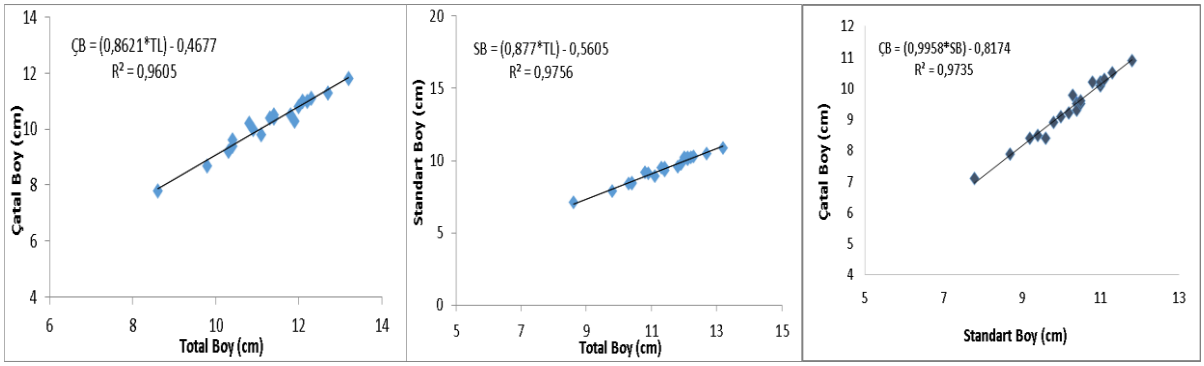
Boy-ağırlık ilişkisi ise $W = 0.0260*L^{2.5747}$ olarak belirlenmiştir (Şekil 3). Bu çalışmada elde edilen bireylerden hesaplanan *b* değerinin %95'lik güven aralığı ile 2.5747-3.6291 olarak hesaplanmış olup büyümenin izometrik olduğu belirlenmiştir.

von Bertalanffy büyüme parametreleri, büyüme performans indeksi ve Fulton'un kondisyon faktörü değerleri Tablo 3'te verilmiştir.



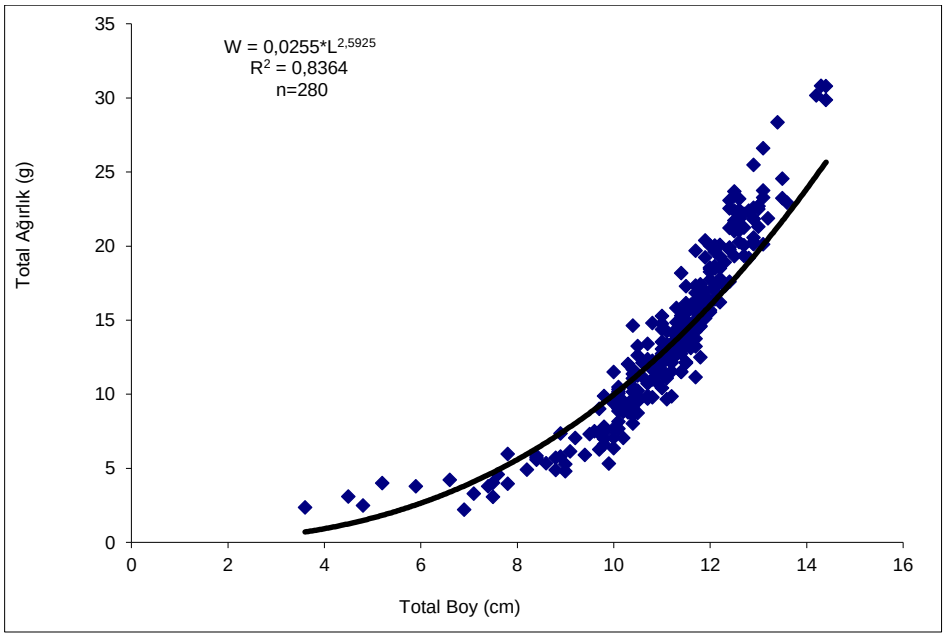
Şekil 1. Yapraklı Barajı *Alburnus carianorum* popülasyonuna ait total boy ve total ağırlık frekans dağılımları

Figure 1. Total length and frequency distributions in Alburnus caeruleus population from Yapraklı Dam



Şekil 2. Yapraklı Barajı *Alburnus carianorum* popülasyonuna ait total boy, çatal boy ve standart boy arasındaki ilişkiler

Figure 2. Relationships between the total length, fork length and standard length of Yapraklı Dam Alburnus carianorum population



Şekil 3. *Alburnus carianorum* popülasyonuna ait boy-ağırlık ilişkisi grafiği
Figure 2. Lenght and weight relationships in Alburnus carianorum population

Tablo 3. Yapraklı Barajı *Alburnus carianorum* popülasyonuna ait von Bertalanffy büyüme parametreleri
Table 3. Growth parameters of von Bertalanffy belonging to the Yapraklı Dam Al*burnus carianorum* population

a	*b*	*95% CI of b*	r^2	L_∞ (cm)	k (yıl^{-1})	t_0 (yıl)	W_∞ (g)	Φ'	*K*
0.026	2.5747	2.5747-3.6259	0.8319	19.73	0.189	-0.743	57.36	1.86	0.95

İncelenen popülasyon için doğal sebeplerle meydana gelen ölüm oranı (M) 0.32 ve balıkçılık sebebiyle oluşan ölüm oranı ise (F) çok daha düşük 0.09 olarak hesaplanmıştır. Bu değerler kullanılarak sömürülme oranının ise (E) 0.22 olduğu görülmüştür.

von Bertalanffy büyüme parametrelerinden yararlanılarak boyca ve ağırlıkça büyüme denklemi kullanılarak yaş gruplarına göre boy ve ağırlık değerleri hesaplanmıştır. Ölçülen ve eşitlik yardımıyla hesaplanan boyca ve ağırlıkça büyüme grafikleri oluşturulmuştur (Şekil 3). Ölçülen ve hesaplanan boy ve ağırlık değerleri bakımından istatistiksel anlamda bir farklılık olmadığı tespit edilmiştir ($p>0.05$).

SONUÇ ve ÖNERİLER

Boy-ağırlık ilişki sabitlerinden b değerinin 3 olması balığın fusiform yapıda olduğunu, 3'ün altında olması ince uzun 3'ün üstünde olması ise vücudun daha küt bir yapıda olduğunu gösterir (Avşar, 2005). Yapraklı Barajı *A. carianorum* popülasyonunda b değeri 2.5747 ve değişim aralığı ise 2.5747-3.6259 (%95) olarak belirlenmiştir. Bu durum ilgili popülasyonda büyümenin izometrik bir yapı sergilediğini göstermektedir.

Balıklarda besililik düzeyini belirlemek için Fulton'un Kondisyon Faktörü (K) değeri kullanılmaktadır. *Alburnus carianorum* Yapraklı barajı popülasyonu için K değeri 0.95±0.13 olarak hesaplanmıştır. Bu değerin 1'in altında olması nedeniyle bu türde kondisyonun düşük olduğu ileri sürülebilir. Büyüme özelliğini ortaya koymada kullanılan b değerinin de 2.57 olarak bulunduğu göz önüne alındığında türün nispeten ince uzun zayıf bir yapıya sahip olduğu söylenebilir.

Büyümenin yorumlanmasında kullanılan büyüme indeksi (Φ') 1.86 olarak hesaplanmıştır. Bu değerin 2'nin altında olması nedeniyle bu türde büyümenin düşük performans sergilediği iddia edilebilir.

Yapraklı barajı, vejetasyonun olmadığı, dip yapısı akarsuyun taşıdığı erozyon materyali ile kaplı olduğu, suyun oldukça berrak olduğu ve plankton yoğunluğunun düşük olduğu görülmüştür. Bu nedenle sucul makrofitlerin ve planktonun sağladığı besinden yoksun olması nedeniyle baraj gölünün besin açısından oldukça fakir olduğu gözlemlenmiştir.

LWR sabitlerinden a, b, Φ' ve K değerleri göz önüne alındığında *A. carianorum*'un büyüme performansının düşük olduğu görülmektedir. Yapraklı Barajında *A. carianorum*'un yanı sıra 8 farklı türün bulunduğu ve bunlar içerisinde *A. boyeri*, *C. gibelio* ve *P. parva* egzotik istilacı türlerin bulunduğu görülmüştür. Besin açısından zaten fakir olan barajda aynı nişi paylaşan türler bulunması, istilacılık potansiyelinin yüksek olduğu türlerin varlığı ve barajda ticari balıkçılığın yapılmıyor olması *A. carianorum* üzerinde ciddi baskı yaratmaktadır. Özetle A. *carianorum*'un düşük büyüme performansı sergilemesinin sebebi yukarıda bahsedildiği üzere yüksek popülasyon yoğunluğu, besin azlığı ve niş çakışmasına bağlı rekabete bağlanabilir.

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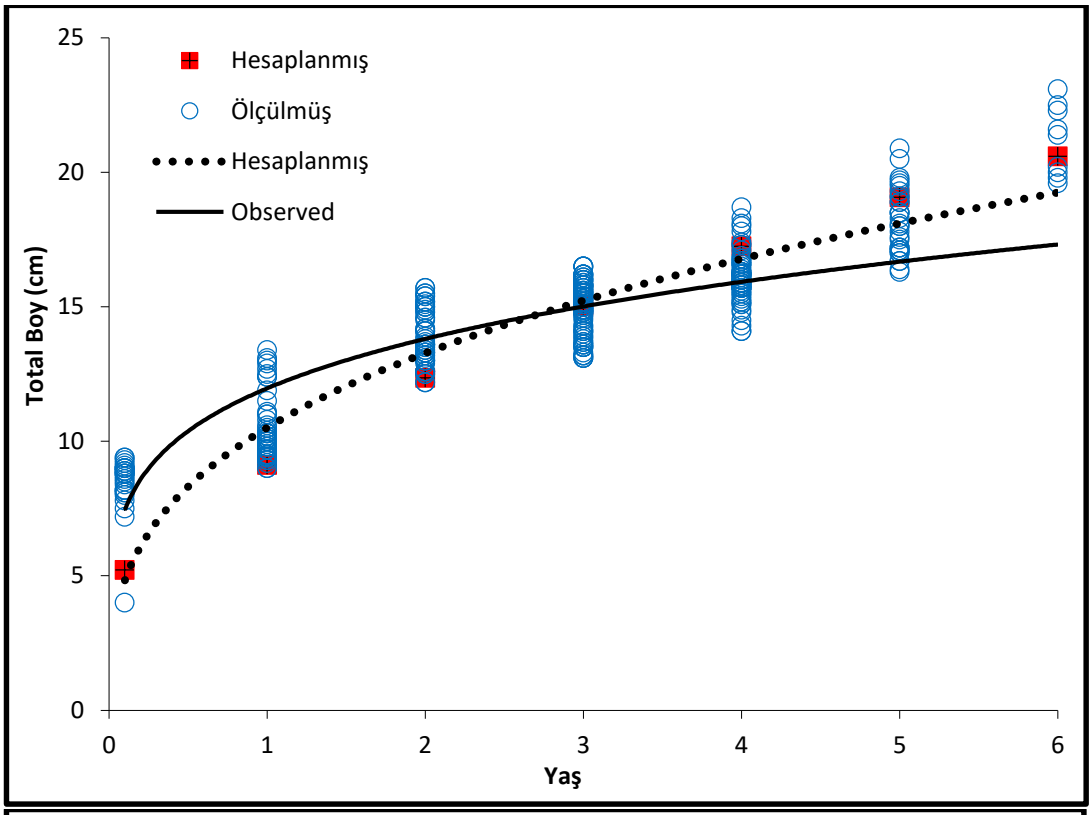

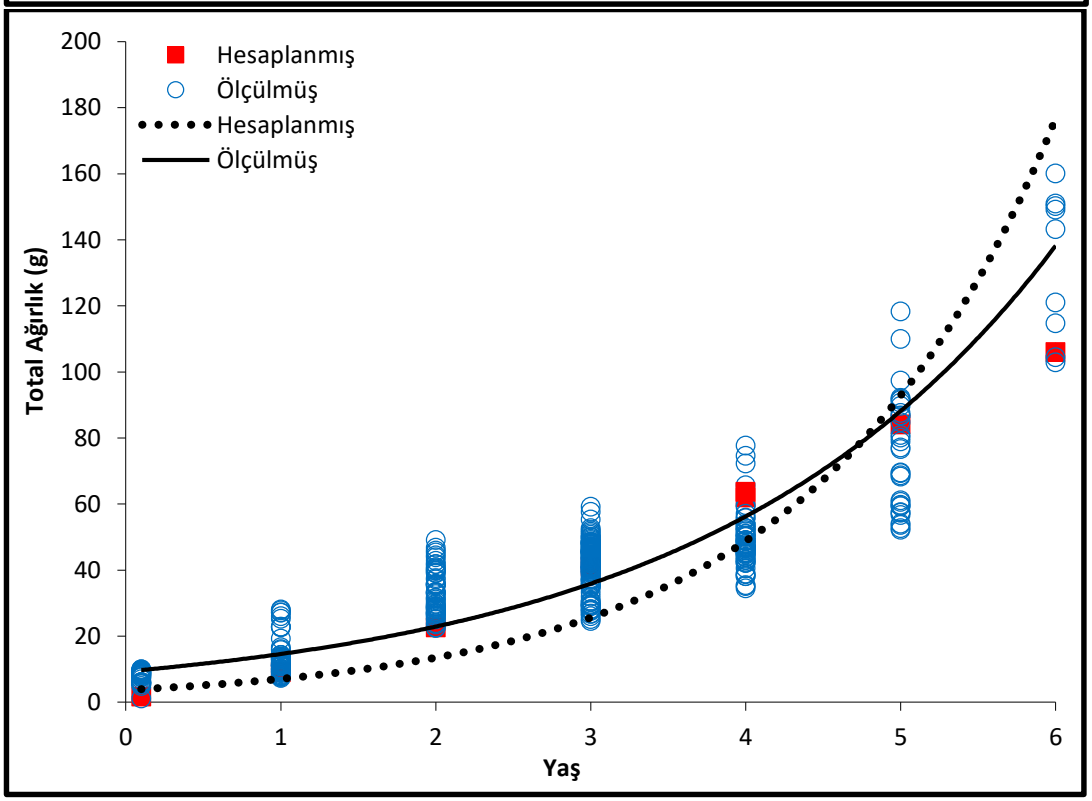

Şekil 4. Yapraklı Barajı *Alburnus carianorum* popülasyonu için ölçülen ve hesaplanan boy ve ağırlık değerlerine ait büyüme grafiği

Figure 4. Growth chart of measured and calculated height and weight values for Yapraklı Dam Alburnus carianorum population

Yapraklı barajı *A. carianorum* popülasyonunda doğal nedenlerle olan ölüm oranının (M=0.32) ve balıkçılık nedeniyle olan ölüm oranından (F=0.09) çok daha yüksek olduğu hesaplanmış olup sömürülme oranı (E)

0.22 olarak tahmin edilmiştir. Gerçekten de barajda ticari balık avcılığı yürütülmediği belirlenmiştir. Bu durumun önüne geçilmesi için yoğun popülasyonu azaltmak adına barajda balıkçılık faaliyetlerinin yürütülmesinin balıkların büyümesini pozitif yönde etkileyeceği düşünülmektedir.

Bu çalışmada *Alburnus carianorum* türünün popülasyon dinamiği parametrelerinin belirlendiği herhangi bir çalışmaya rastlanmamış olup bu çalışma ilk niteliğindedir. Bu nedenle bu çalışmada hesaplanmış olan popülasyon dinamiği parametreleri başka çalışmalarla kıyaslanamamıştır.

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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Notes on the Seasonal Dynamics of *Polyphylla turkmenoglui* Petrovitz (Coleoptera: Scarabaeidae: Melolonthinae) in the vineyards of Manisa, western Anatolia

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ABSTRACT

Polyphylla turkmenoglui Petrovitz, 1965 is one of the the most important pests of vineyards in the Aegean Region of Turkey. Larvae of this species feed on the roots of vineyards and in general, the larvaes are found under the soil. For this reason, controlling this pest is very difficult. The adults mostly feed on pines foliage. In the study, the seasonal activity of the adult beetles of *P. turkmenoglui* was studied via two light traps between May and August in 2016 and 2017 in two vineyard locations in Alaşehir and Sarıgöl districts, Manisa province of Turkey. At the end of the study, 1.552 specimens of *P. turkmenoglui* were evaluated. Total 362 and 1.190 specimens were collected from the localities in Alaşehir and Sarıgöl, respectively. In spite of the small differences in trapping localities, the number of the specimens belonging to this species increased as of June, reached the highest level in mid-June and early July, and started to decrease after that date. No specimens were collected after the second half of July. In addition, the morphological features of this species, both male and female, were defined and illustrated.

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Manisa yöresi bağlarındaki *Polyphylla turkmenoglui* Petrovitz (Coleoptera: Scarabaeidae: Melolonthinae) türünün mevsimsel aktivitesi üzerine notlar

ÖZET

Polyphylla turkmenoglui Petrovitz, 1965 türü Ege Bölgesi bağlarında bulunan en önemli zararlılardan biridir. Zararlının toprakta yaşayan larvası bitki kökleri ile beslenir. Bu nedenle kontrolü çok zordur. Erginleri çoğunlukla çam yaprakları ile beslenmektedir. Bu çalışmada, 2016-2017 yıllarında Mayıs-Ağustos tarihleri arasında Manisa'nın Alaşehir ve Sarıgöl ilçelerinde bulunan iki bağ alanına kurulan ikişer tane ışık tuzağı aracılığı ile ergin *P. turkmenoglui* türünün mevsimsel aktivitesi incelenmiştir. Çalışma sonucunda, toplam 1.552 örnek değerlendirilmiş olup bu örneklerin 362'si Alaşehir, 1.190'ı ise Sarıgöl bağ alanlarından toplanmıştır. Tuzak lokaliteleri arasında küçük farklılıklara rağmen, bu türe ait örnek sayısı Haziran ayından itibaren artmaya başlamış, Haziran ayının ortası ve Temmuz ayının başında en yüksek populasyon yoğunluğuna ulaşmış ve bu tarihten sonra düşmeye başlamıştır.Temmuz ayının ikinci yarısından sonra ise hiç örnek toplanamamıştır. Ek olarak, bu türün dişi ve erkeğine ait morfolojik bilgiler ve şekillere de çalışmada yer verilmiştir.

Zooloji

Araştırma Makalesi

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INTRODUCTION

Turkey hosts a large number and variety of agricultural products thanks to its climatic and geographical characteristics. Viticulture has an important place among these products. Turkey ranks the sixth in grape production in the world and

viticulture provides the livelihood of many farmers (Bashimov, 2017). In 2018, 3.9 million tons of grapes were produced on a total area of 4.8 million decares in Turkey (Anonymous, 2021a). At the same time, table grapes and raisins have a very important place in Turkey's exports of agricultural products. Especially in the production of seedless raisins, Manisa in western Turkey, and Alaşehir and Sarıgöl districts of Manisa stand out. The most important economic activity in both districts is vine growing. According to 2015 data, 495 thousand tons of grapes were produced in an area of 200 thousand decares in Alaşehir. In Sarıgöl district, approximately 238,000 tons of grapes were obtained from an area of 90,000 decares (Anonymous, 2021a).

The genus *Polyphylla* Harris, 1841 is divided into 7 subgenera and it contains 33 species in the Palaearctic region (Löbl and Smetana, 2006). A total of five species of the genus are known from Turkey, which are *Polyphylla fullo* (Linnaeus, 1758), *P. boryi* Brullé, 1832, *P. olivieri* (Laporte, 1840), *P. adspersa* Motschulsky, 1854 and *P. turkmenoglui* Petrovitz, 1965 (Löbl and Smetana, 2006). Among these species, *P. fullo* has a wide distribution in whole Europe and Turkey. *P. boryi* is known from Bulgaria, Greece, Croatia and Turkey; *P. olivieri* is known from Greece, Turkey, the Caucasus, Iran, Syria, and Israel. *P. adspersa* is dispersed over Turkey, Iran, the Caucasus and the Middle East. Lastly, *P. turkmenoglui* is only known from Turkey. The larvae of *Polyphylla* species may damage almost all types of fruit saplings and vineyards. The main harm of this species is that they gnaw and pierce plant roots.

P. turkmenoglui is described by Petrovitz in 1965 as *Polyphylla fullo turkmenoglui* from the Menemen district of Izmir (Petrovitz, 1965) and was later raised to the species level (Löbl and Smetana, 2006). Very few studies have been done so far regarding the phenology, biology and the damage caused by this species. Among these, Türkmenoğlu (1967) has included important information about this species in his publication. In summary, it has been reported in this study that this species damages different cultivars in the Aegean Region but specifically vineyards. In addition, he gave information about the ways of damage and economic importance, morphological features, phenologies and struggle against *P. turkmenoglui* species. Accordingly, the biggest damage of the larvae of this species is observed in the roots of vine. Especially the third, i.e. last-stage larvae cause significant damage by gnawing vines erected on sandy soils. The damage is very high especially in newly established vineyards. It may sometimes rise to an extent of 50-80%. In the same publication, it was also reported that this species preferred sandy, fine sandy and alluvial soils and that it caused economic damage at the roots of the cultivated plants grown there. In addition, Türkmenoğlu (1967) reported that this species is found in Nazilli (Aydın), Denizli Central District, Edremit (Balıkesir), Manisa Central District, Akhisar, Alaşehir, Sarıgöl, Saruhanlı, Turgutlu (Manisa) and Köyceğiz (Muğla) in the Aegean Region. In another study, according to Önuçar and Ulu (1987), this species is one of the most important pests for cherries, peach and potatos and some other fruits and crops.

There are many pests in the vine plant, as in every agricultural product. One of the important pests of the Aegean Region vineyards is *P. turkmenoglui.* This insect, known as "Halkalı Şeker" among the people in Alaşehir and Sarıgöl region, tends to grow in sandy and loosely structured soils in the region. It is also more common in fertilized and organic soils and in vineyards where weeds are abundant. This species, commonly found in Alaşehir and Sarıgöl vineyards, causes significant damage, damaging the root of the vine and causing the plant to weaken and to completely dry out . However, integrated combat methods are often not successful or at least the desired result is not fully achieved. The presence of the larvae of the species in the depths of the soil reduces the effectiveness of chemical control.

It is possible to collect and examine *Polyphylla* species by means of light traps because they exhibit light-directed behavior. No specific studies regarding the seasonal activities of *P. turkmenoglui*'s mature individuals located in Turkey vineyards have been conducted until now. In this study, *P. turkmenoglui* found in Alaşehir and Sarıgöl vineyards were collected by light traps for the first time in Turkey. It has been sought to demonstrate seasonal activities of the species by determining its seasonal densities. Thus, it was aimed to obtain the information which may be useful for the struggle against this species.

MATERIAL and METHOD

Light trap method was used to collect samples of *P. turkmenoglui* species in this study. In this method, two light trap assemblies were set up in each vineyard area selected in Alaşehir and Sarıgöl districts of Manisa, covering May-August periods of 2016 and 2017. The study in 2016 was made within the scope of the master's thesis of the second author, and the study in 2017 was an independent study. In the master's thesis, this species was identified as *P. fullo*, but later on it was found out that the species was in fact *P. turkmenoglui.*

Some preliminary studies were carried out in the selected areas before the study was established. In this framework, the presence of this insect's activity was determined through observations on visits to selected areas in advance. In addition, a short-term light trap was established in 2015 in the same

vineyards for study purposes. It was investigated whether there were larvae in the soil on visits to both study areas. The vineyard with a light trap in Sarıgöl has a total of 9 decares of land and a 3-year-old "Thompson seedless" grape variety. The planting frequncy is 3.00 X 1.8 m and the vineyard is established with an open pergola trellis system. The vineyard with a light trap in Alaşehir has a total of 5 decares of land and a 13-year-old "Red Globe" grape variety. The planting frequncy is 3.00 X 2 m and the vineyard is established with an open pergola trellis system. All cultural practices in the vineyard have been carried out under farmer conditions.

Soil analyzes of the studied localities were also conducted. For this purpose, soil samples were taken from the depths of 0-30 cm and 30-60 cm in the test plots. In soil samples, grain size distribution was determined by using hydrometer method, soil reaction (pH) was determined in sature soil paste with glass electrode pH-meter, salinity was determined in sature soil paste with EC-meter, organic matter was determined through wet burning with potassium dichromate ($K_2Cr_2O_7$), lime ($CaCO_3$ %) was determined with Scheibler calcimeter by volumetric method, Total-N was determined with modified macro kjeldahl method, extractable K, Na, Ca, Mg 1, and N were determined through NH_4OAc (pH 7) extraction, extractable phosphor was determined through NaHCO3 extraction, extractable Fe, Cu, Zn, Mn were determined by extraction method with DTPA+$CaCl_2$+TEA solution (Kacar, 2016). The results of the analysis were evaluated according to Müftüoğlu et al. (2014).

Light traps were established in the coordinates of 38°19'50.35"N 28°36'31.36"E and 38°19'50.36"N 28°36'30.95"E in Alaşehir and 38°14'18.93"N, 28°42'25.35"E and 38°14'19.57"N 28°42'29.38"E in Sarıgöl (Figure 1). A 125 watt Philips energy saver white day light bulb was used at each trap and traps were cleared at weekly intervals.

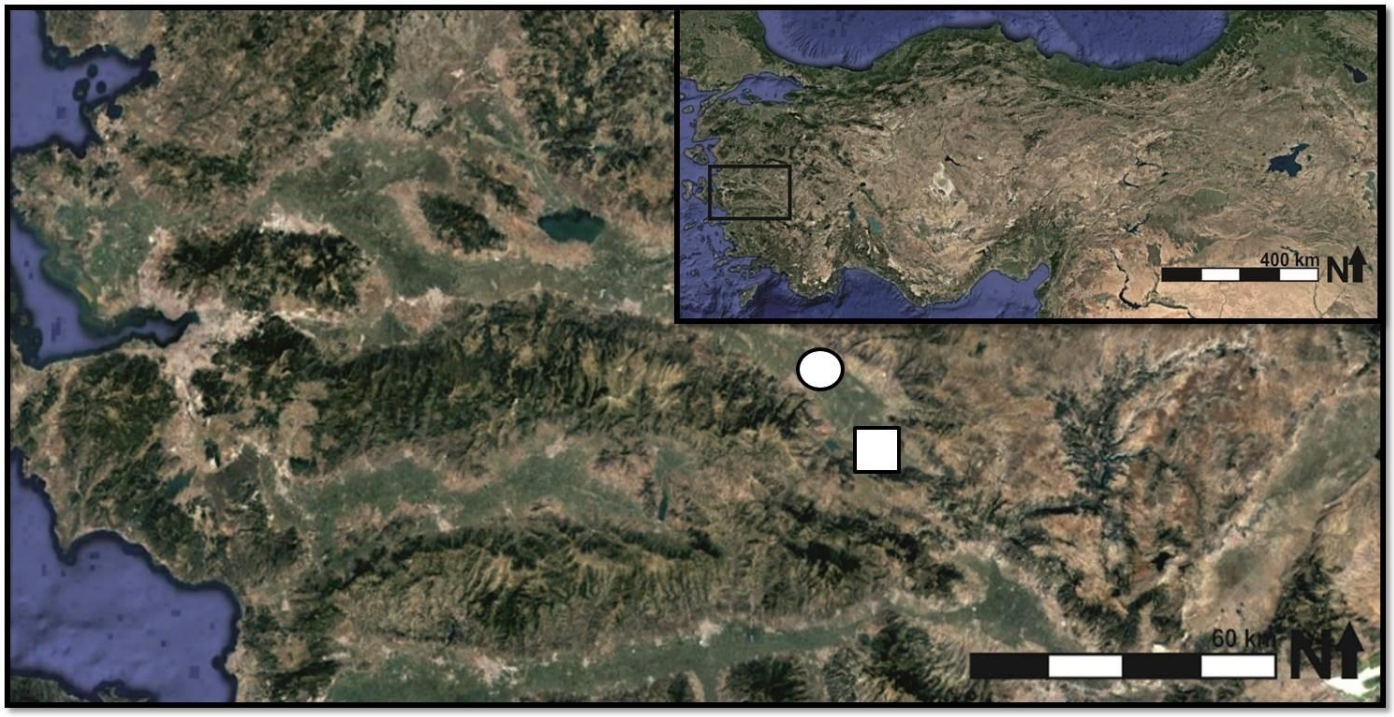

Figure 1. The locality of light trapping study area in Alaşehir (circle) and Sarıgöl (square), Manisa, western Anatolia, Turkey.

Şekil 1. Batı Anadolu'da (Türkiye) bulunan Manisa'daki çalışma yapılan Alaşehir (daire) ve Sarıgöl (kare) ışık tuzağı lokaliteleri.

All specimens falling into the chamber of the light trap was brought to the laboratory by taking them in the field into jars containing 70 % ethanol. Labels containing the name and date of the vineyard where the study was set up were placed in the jars in which the specimens collected in the field were put. The jars which were brought to the laboratory and which contained insect specimens were first poured into a large white area in the form of a tray. Since many specimens belonging to other groups were collected with the light trap, the samples of the target species, namely *Polyphylla turkmenoglui,* were separated and then counted, and labeled in different jars. The separated specimens were cleaned from various dust and substances adhering to them with a soft painting brush. After that, all samples were examined morphologically under a microscope. The identified specimens were dried and pinned. Locality and identified labels were also added to these specimens.

The morphological studies were conducted using a Stemi 508 microscope (Zeiss, Germany). Photographs of the studied specimens were taken with a digital camera (Zeiss Axiocam ERC5s). All photographs were edited with the Helicon Focus v. 6, and Coreldraw X5 software. The map (Figure 1) was made using the software Google Earth Pro (2019). The materials were identified by the third author and were deposited in the Alaşehir Zoological Museum, Manisa, Turkey (AZMM).

RESULTS

Morphology of P. *turkmenoglui* Petrovitz, 1965 (Figures 2-4)

Diagnosis. Female body 3.0-3.6 cm in length. General coloration: head dark brown; pronotum, elitra, antenna and legs reddish brown in dorsal view (Figure 2A); the whole body brown in ventral view (Figure 2B).

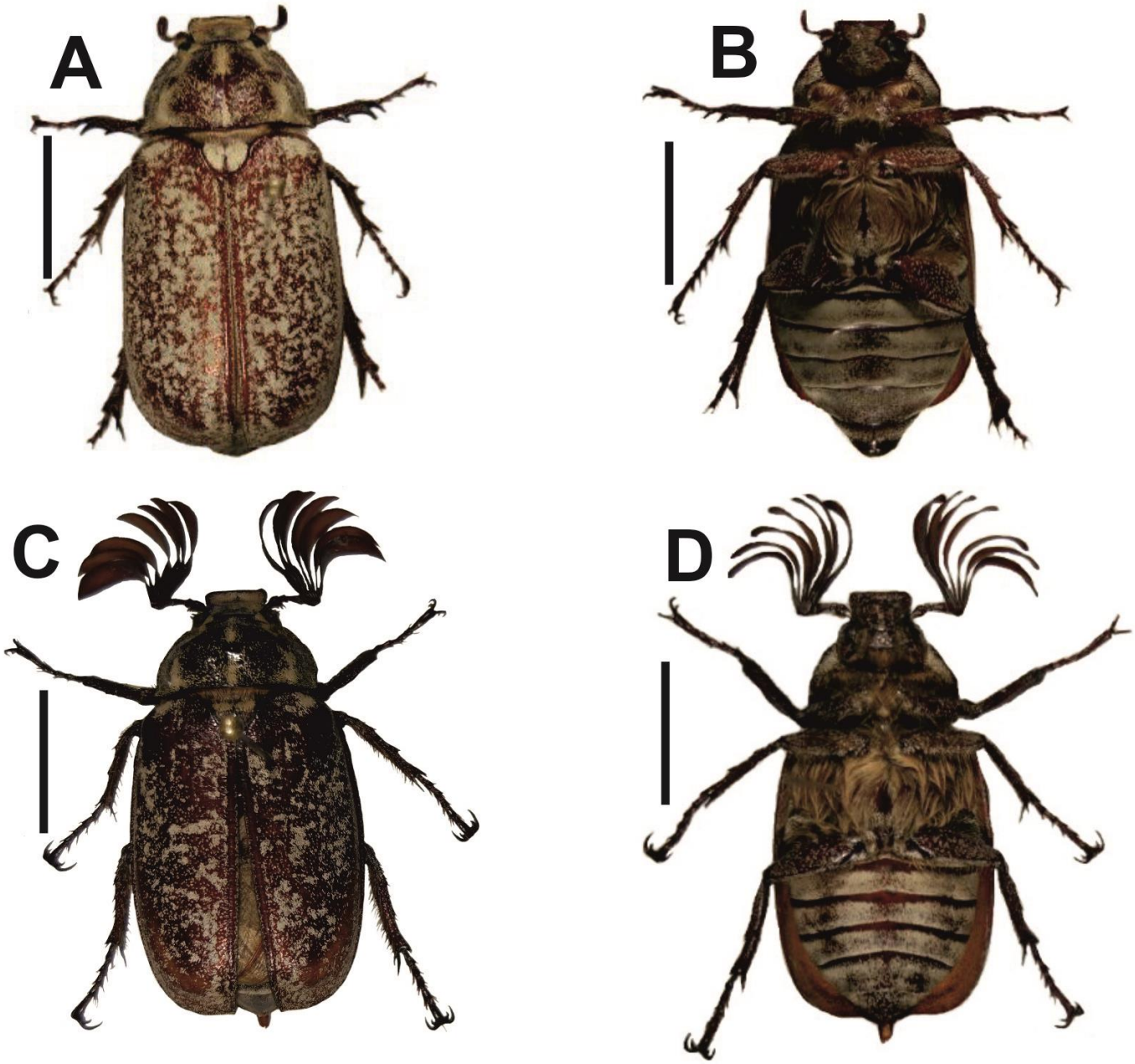

Figure 2. Habitus of *P. turkmenoglui* Petrovitz, 1965. A) female in dorsal view; B) female in ventral view; C) male in dorsal view; D) male in ventral view. Scale bars: 1 cm (A-D).

Şekil 2. P. turkmenoglui Petrovitz, 1965 türünün vücut şekli. A) dişi, dorsal görünüş; B) dişi, ventral görünüş; C) erkek, dorsal görünüş; D) erkek, ventral görünüş. Ölçek çubukları: 1 cm (A-D).

Male body 2.9-3.3 cm in length.

General coloration: All body darker than the female in dorsal and ventral view (Figures 2C-D).

In female; dorsal surface of head covered with flakes of color ranging from white to golden yellow; flakes more frequent on the front and back of the head and less frequent at the lateral, these structures in the front resembling thick hair growth rather than flakes (Figure 3A). Clypeus elongated forward with sharp corners, light brown hairs on the indented structures on the sides of the head. The antenna 10 segments, the tip knobed with five lamellas, the colour varying from dark brown to reddish; frequently whitish yellowish hairs on the sides of the first segment of the antenna, these structures gradually changing as they go towards the ends of the antenna. Eyes small, yellowish structures on the upper part of the eyes in the form of eyelashes.

In male; head brown to blackish and covered with yellowish-whitish flakes, these flakes more intense on the lateral of the head and the margins of the eyes (Figure 3B). Clypeus elongating anterior and its corners more roundish than the female. Antennae strikingly seven-leaf / lamellar fan-shaped, with sparse pubesences on these fans, antennomere I with dense and light brown pubesences. Eyes small and lash-shaped golden yellow flakes present on the upper part, more frequent and evident towards the antennae.

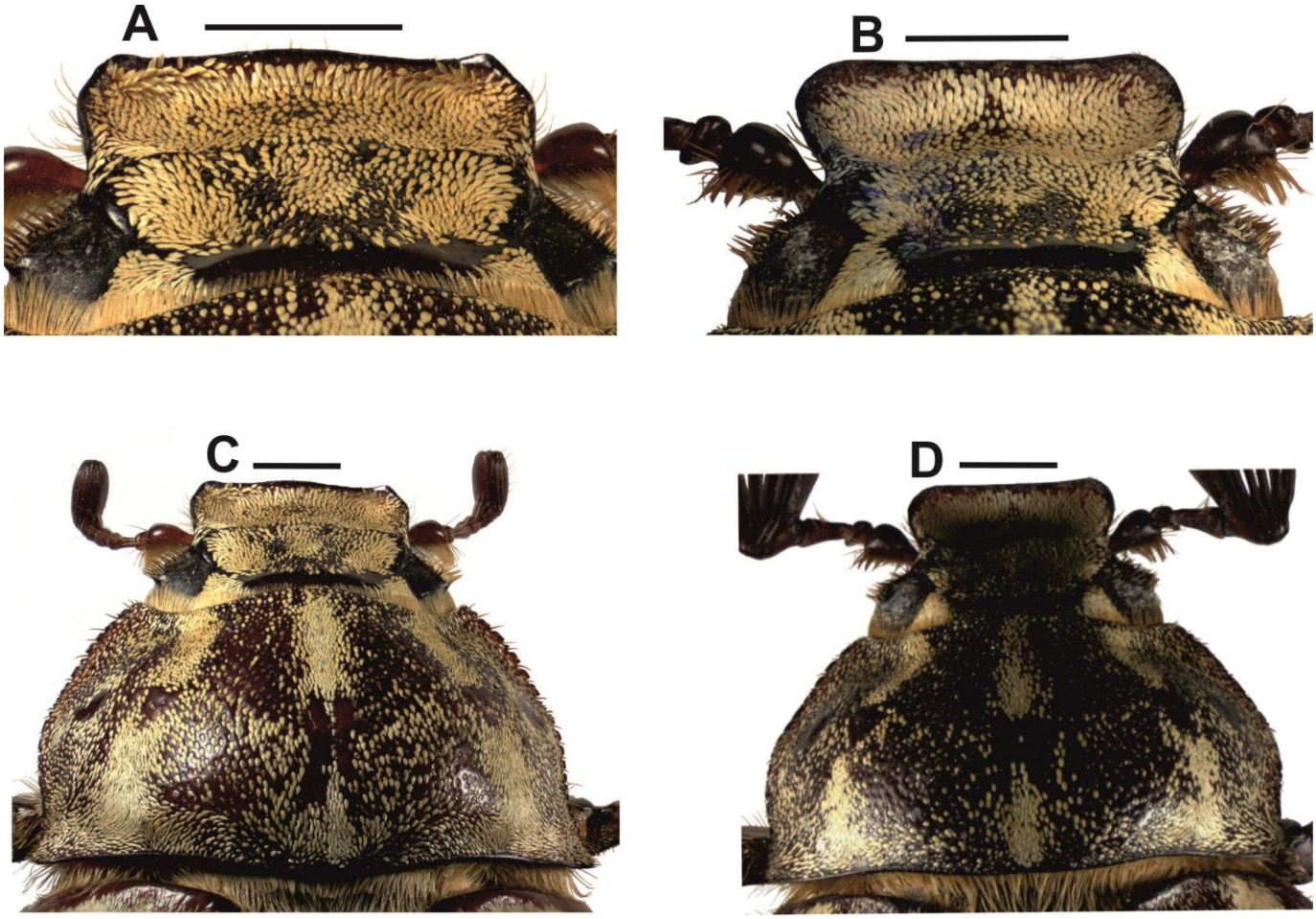

Figure 3. In *P. turkmenoglui* Petrovitz. A) female head; B) male head; C) female pronotum; D) male pronotum. Scale bars: 2 mm (a-d).

Şekil *3. P. turkmenoglui Petrovitz türünde, A) dişi, baş; B) erkek, baş; C) dişi, pronotum; D) erkek, pronotum. Ölçek çubukları: 2 mm (A-D).*

In female; pronotum with single sclerits, the lateral margins indented similar to tooth; flakes less denser than that of head (Figure 3C).

In male; pronotum with single sclerits, and smaller number of indented structures on the margins compared to the female; flakes less denser than that of head (Figure 3D).

In female; elitra with colored flakes ranging from white to golden yellow on the reddish brown as small cubicles, flakes more frequent at the median area and less frequent along the sides. Legs long, the coxa with sparse yellowish pubesences, the femur of prothoracic

legs (the first pair of legs) with thick and rake-shaped toothed ridges, the last part of the tarsus with strong nails in the form of hooks (Figure 4A), these structures very small in the femoral part of mesothoracic legs (the second pair of legs) and metathoracic legs (the third pair of legs) compared to the prothoracic legs (Figures 4B-C).

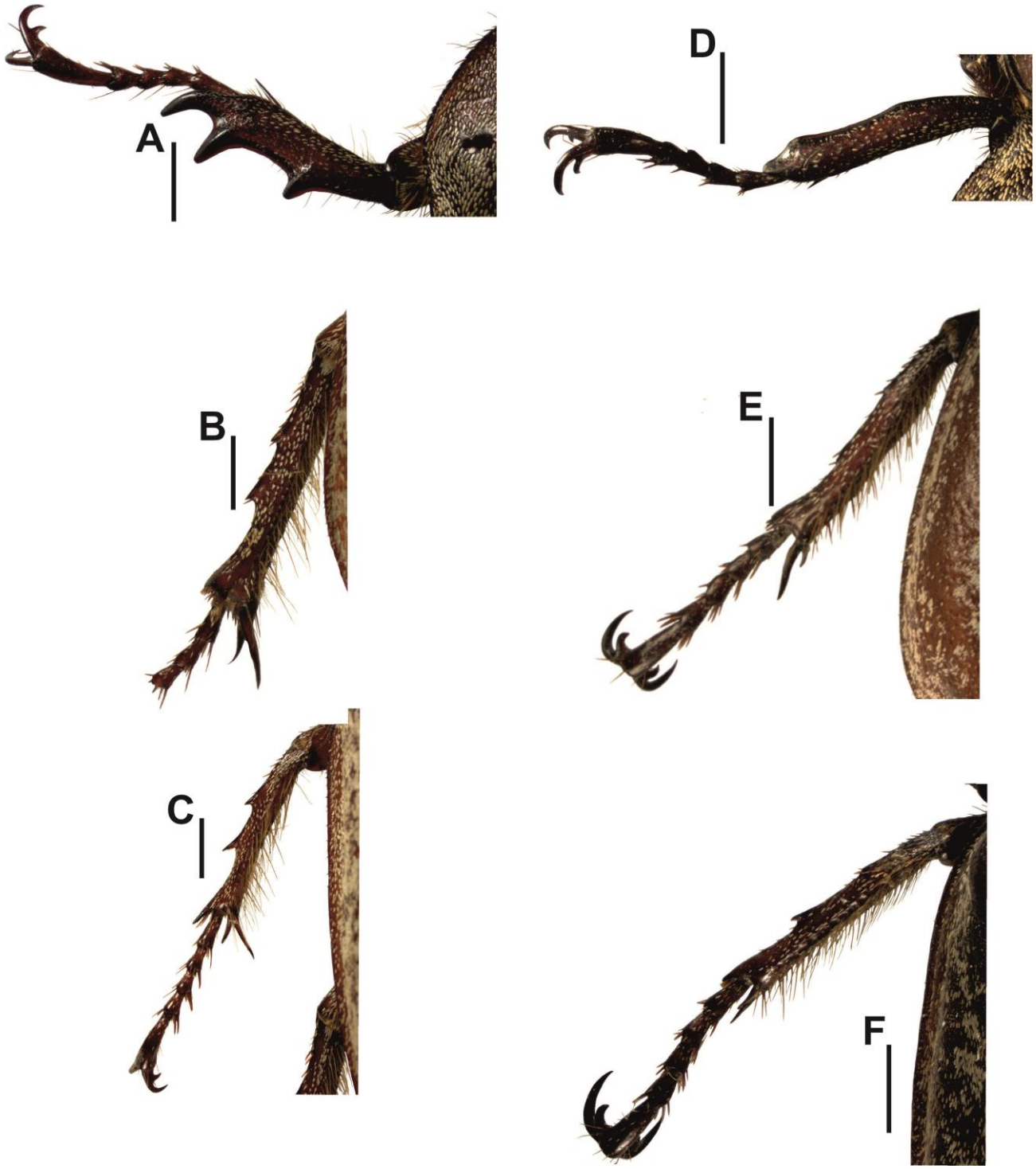

Figure 4. In *P. turkmenoglui* Petrovitz A) female prothoracic legs; B) female mesothoracic legs; C) female metathoracic legs; D) male prothoracic legs; E) male mesothoracic legs; F) male metathoracic legs; Scale bars: 1 mm (A-F).

Şekil *4. P. turkmenoglui Petrovitz türünde, A) dişi prothorasik bacaklar; B) dişi mesothorasik bacaklar; C) dişi metathorasik bacaklar; D) erkek prothorasik bacaklar; E) erkek mesothorasik bacaklar; F) erkek metathorasik bacaklar; Ölçek çubukları: 1 mm (A-F).*

In male; elitra with dense flakes ranging from white to golden yellow on a dark brown background. Legs less developed than the female, dark brown coloration and gradually darkening and turning into black towards tarsus; femur yellowish and dense pubesences; a single spine at the end of the femur of the prothoracic legs (Figure 4D), and curved structures in the form of hooks at the end of the tarsus of the legs (Figures 4D-F).

In female and male; abdomen with visible six segments in ventral view, with white pubesences, and very dense between mesothoracic and metathoracic legs.

Characteristics of the Vineyard Soils in the Study Areas

Some physicochemical properties of the soils in the two study areas are shown in Table 1.

The soil of the vineyard, which is located in Sarıgöl locality, is classified as sandy-loam at a depth of 0-30 cm, and as loamy-sand at a depth of 30-60 cm. While the sand amount increases at 30-60 cm depth compared to 0-30 cm depth, silt rate decreases. The soil reaction is alkaline. The soil is classified as slightly lime in terms of lime and there is no salinity. The organic matter is very low, total nitrogen is low, extractable P is high, and extractable Ca K, Mg, Fe, Zn, Mn and Cu are sufficient (Table 1).

Table 1. Some physicochemical properties of the study area soils in Sarıgöl and Alaşehir.
Çizelge 1. Sarıgöl ve Alaşehirdeki araştırma alanı toprağının bazı fizikokimyasal özelikleri.

Region	Sarıgöl		Alaşehir	
Soil Properties/ Soil depth	0-30cm	30-60cm	0-30cm	30-60cm
pH	8,09	8,13	7,94	7,98
Electrical Conductivity (µS cm^{-1})	274	170	201	204
Lime ($CaCO_3$) %	3,28	2,96	0,80	1,08
Soil Texture	Sandy Loam	Loamy sand	Sandy Loam	Sandy Loam
Sand (%)	68,56	79,84	59,56	58,56
Silt (%)	23,28	14,00	31,28	29,28
Clay (%)	8,16	6,16	12,16	12,16
Soil Organic matter (%)	0,84	0,54	1,32	1,79
Total-N (%)	0,056	0,045	0,101	0,090
Available-P (mg kg^{-1})	40.82	30.53	35.29	27.48
Extractable-K (mg kg^{-1})	272,2	135,8	261,9	271,6
Extractable -Ca (mg kg^{-1})	2860	2761	2761	2663
Extractable -Mg (mg kg^{-1})	361	232	439	422
Available -Fe (mg kg^{-1})	9,01	9,93	7,83	6,29
Available –Zn (mg kg^{-1})	2,29	0,59	19,97	2,68
Available -Mn (mg kg^{-1})	3,96	4,67	13,92	12,50
Available -Cu (mg kg^{-1})	8,65	1,64	5,28	3,15

The soil of the vineyard, which is located in Alaşehir locality, is classified as sandy-loam at both depths. Soil reaction is alkaline. The soil is classified as less lime in terms of lime and there is no salinity. Organic matter is very low, total nitrogen is sufficient, extractable P is high, and extractable Ca K, Mg, Fe, Zn, Mn and Cu are sufficient (Table 1).

Seasonal Dynamics

In this study, four light traps were established in the vineyard areas determined in Sarıgöl and Alaşehir districts of Manisa between May and August in 2016 and 2017, and a total of 1.552 specimens belonging to *P. turkmenoglui* were collected. Accordingly, 362 of the collected specimens fell on the light traps which were set up in Alaşehir and 1.190 on the traps in Sarıgöl. When the distribution of sample numbers per year was examined, it was seen that a total of 792 samples were collected in 2016 and 760 samples were collected in 2017 (Figure 5, Table 2).

When the seasonal activities of the *P. turkmenoglui* in the study area in 2016 and 2017 were examined, it the data of both years were found to be similar. According to the study, the date when the adults of the species first appeared was between May 22 and May 29. However, only 4 examples fell into the traps in this period. Later, the number of specimens of the species started to increase and the number of the specimens was 30 between May 29 and June 5, and 240 between June 5 and June 12. After this date, that is between June 12 and June 19, the number of the collected specimens reached the highest with 543. Afterwards, the number of specimens decreased to 281 bewteen June 19 and June 26, and then increased again to 408 between June 26 and July 3. After this date, the number of specimens of the species

decreased rapidly to 41 between July 3 and July 10 and then to 5 between July 10 and July 17. After this date, no specimens could be collected with traps (Figure 5, Table 2).

Accordingly, when the seasonal activity of the *P. turkmenoglui* species was evaluated in general, it was found that the adults of this species first had begun to appear at the end of May. Later, it was observed that their numbers had increased since the beginning of June and reached the highest level in the middle of June and at the beginning of July. It was determined that the number of adults of this species had decreased since the beginning of July. Adult activity ended after the second half of July (Figure 5, Table 2).

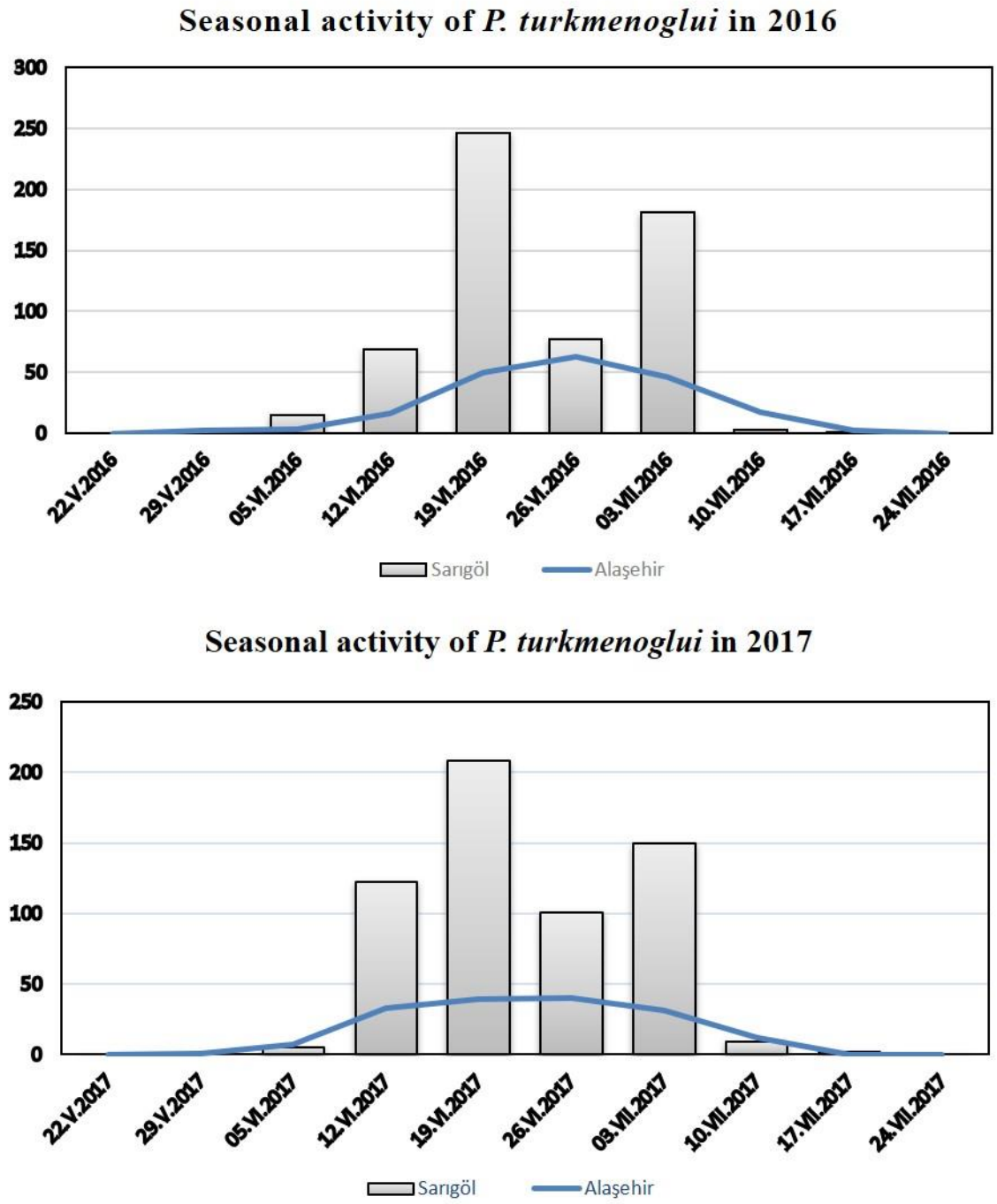

Figure 5. Seasonal activity of *P. turkmenoglui* in 2016 and 2107 in Sarıgöl and Alaşehir localities.
Şekil 5. P. turkmenoglui türünün Sarıgöl ve Alaşehir lokalitelerindeki 2016-2017 yıllarındaki mevsimsel aktivitesi.

Ecological Observations

Observations were made in the localities where light traps were established in order to obtain additional ecological data. According to these observations, *P.*

turkmenoglui individuals were observed to fly mostly between 20:30 and 21:30 with their heads toward the light. It was also observed that the specified time range and flight density varied depending on the weather's being windy, rainy and cool. According to this, rainy weather prevented the flights completely, while cool and windy weather reduced the density of the flying population of the species. It was determined that individuals belonging to the species entered the soil again after mating. After that, it was observed that the mating female individuals laid their eggs 10-20 cm below the soil in groups of 20-28, and that their ovulation periods reached a week. It was found that their eggs hatched in the last week of July.

Table 2. Numbers of specimens by date and localities.
Çizelge 2. Lokalite ve tarihlere göre örnek sayıları.

Collecting date	Year	Alaşehir	Sarıgöl	Total
22 May	2016	-	-	-
	2017	-	-	-
29 May	2016	2	1	3
	2017	1		1
5 June	2016	3	15	18
	2017	7	5	12
12 June	2016	16	69	85
	2017	33	122	155
19 June	2016	50	246	296
	2017	39	208	247
26 June	2016	63	77	140
	2017	40	101	141
3 July	2016	46	181	227
	2017	31	150	181
10 July	2016	17	3	20
	2017	12	9	21
17 July	2016	2	1	3
	2017	-	2	2
24 July	2016	-	-	-
	2017	-	-	-
22 May-24 July	2016	199	593	792
	2017	163	597	760
Total	**2016-2017**	**362**	**1.190**	**1.552**

Besides, some adult beetles were observed to hide among the surrounding weeds after flight and mating. When the adults were disturbed in their areas, they moved away from their environment by making a characteristic sound. In addition, due to the fact that the study established in Alaşehir was close to the residential area, this insect was also observed to hover around the lamps which lit up in front of various houses in the evening. Since the stations in Sarıgöl were further from the residential area than those in Alaşehir, the night flights of the insect took place only around the traps.

According to the observations made during the research, it was determined that *Corvus cornix* Linnaeus, 1758 and *C. monedula* Linnaeus, 1758 were predators of *P. turkmenoglui*. In addition, *Megascolia maculata maculata* (Drury, 1773) and *Scolia hirta* (Schrank, 1781) species of the family Scoliidae (Hymenoptera), which are thought to be parasitoids of *P. turkmenoglui*, were also observed to be flying in vineyards.

DISCUSSION

This study on the seasonal activity of the adults of *P. turkmenoglui species*, which damaged the vineyards identified in Alaşehir and Sarıgöl districts between 2016 and 2017, serves as the first study conducted in this respect.

However, according to the literature research conducted, there are some observational studies on the activity of this species. According to one of them, Türkmenoğlu (1967), adult appearance of the species continues from mid-June to the end of the first week of July, and the number of adults reached the highest level on June 20. According to our findings, however, adult appearance of the species occurs as of the end of May. Nevertheless, the highest number of samples that fell in our traps was 543 in the period between June 12 and 19. The number of samples decreased in the following week. Then, again, an increase was observed between June 26 and July 03. In the same study, according to Türkmenoğlu (1967), it was stated that the adults of the species appeared from the soil 40 minutes after sunset on average, and flew and

mated for 40 minutes on average again, and then they got into the soil again. According to our observations in the vineyards where the study was carried out, the adults of the species started to fly maximum half an hour after sunset. It was also observed that adults of the species flew, though rarely, until late at night.

Another study on *P. turkmenoglui* was carried out by Önuçar and Ulu (1987) in a peach orchard in the province of Manisa between the end of May and the beginning of July between 1984 and 1987. According to this study, this species was observed every five minutes between 20.40 and 21.00 between the same two peach trees in the orchard. In the same study, it was stated that the flight of the species started 10-20 minutes after sunset and varied depending on the climatic conditions. At the same time, predation of this species by *Caprimulgus europaeus* Linnaeus, 1758 was observed. In the observations made in this study, it was found that *C. cornix* and *C. monedula* species were predators of *P. turkmenoglui*.

Compared to the traps set up in Alaşehir, the traps set up in the Sarıgöl vineyard area had three times more samples. Among the reasons for this may be that the vineyard area in Sarıgöl consists of younger vines compared to the vineyard area in Alaşehir. *Polyphylla* spp. are especially harmful in young vineyards (Lodos, 1995). As a matter of fact, the larvae of this insect have better feeding opportunities with the roots of the young vine seedlings in Sarıgöl. According to our observations, in the vineyard areas of Sarıgöl, withederedness in the green parts and seedlings of the vine was detected. In addition, the fact that the study area in Sarıgöl district was farther from the residential area was interpreted as a reason for the higher density of the species. Besides, soil type is thought to have an effect. Among soil properties, soil structure is the most important feature which will affect the population of *Polyphylla* spp. Sand content of 0-30 cm depth of the test area soil in Sarıgöl region was determined as 68.56 % and sand content of 30-60 cm depth was determined as 79.84 %. Sand content of 0-30 cm and 30-60 cm depth of the soil of the test area in Alaşehir region was determined the same for both depths, as 58.56 %. As a result, one of the reasons why *P. turkmenoglui* was more abundant in Sarıgöl locality may be the higher sand content of the soil in Sarıgöl locality. In fact, Türkmenoğlu (1967) stated in his study that this species preferred sandy, fine sandy and alluvial soils. Besides, it was evaluated that other soil characteristics in the study areas were not different enough to affect the activity of the species.

In their studies, Vereecken and Carriere (2003) and Vuts et al. (2012) stated that Scoliidae species, which is a parasitic wasp family, is the ectoparasite of some Scarabaeidae species, and also mentioned *P. fullo* among these species. From this perspective, scoliid species are also thought to be ectoparasites of *P. turkmenoglui*, as well. It is known that species belonging to the family Scoliida are abudantly present in Turkey and in Manisa province (Anlaş and Çevik, 2004; Özbek and Anlaş, 2012). As a matter of fact, in the observations made during the study, some scoliid species were identified in the localities where light traps were established.

In conclusion, it will be useful to take the following measures in the fight against *P. turkmenoglui* species:

1) to clean vineyards off weeds especially in June and July since *P. turkmenoglui* species usually lay their eggs on weedy areas,

2) to reduce the population of larvae by making deep soil cultivation in the autumn period, when the larvae are active and by leaving the larvae to sunlight and the effect of predators,

3) to set up light traps in areas where the pest is densely found by taking the seasonal activity of *P. turkmenoglui* species into consideration, and, thereby to take the population of adults under control,

4) to plant flowering plants (e. g. *Vitex agnus-castus* L., *Rubus canescens* DC.) in the sides of the vineyards in order to enable scoliid wasps to combat the pest effectively.

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Statement of Conflict of Interest

Author has declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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Evaluation of the Seed Flow Uniformity of the Fluted Feed Roller Designed for Coarse-Grained Seeds

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ABSTRACT

In this study, the nine fluted rollers designed for coarse-grained seeds and with the flute diameters of 18, 20, and 22 mm and the flute depths of 5, 8, and 11 mm were evaluated for dry bean seed. Each fluted roller were operated at the ground speeds of 1.0, 1.5, and 2.0 m s^{-1} for at the seed rates of 80, 120, and 160 kg ha^{-1}. Performance for the flow accuracy of the seeds is taken into account in the evaluations and for this; the values of coefficient of variation were used. The optimum dimensions were determined by applying statistical analyses for the CV values obtained from each replication in the study conducted in three replications. According to the analysis results, it was determined that the prescribed flute diameters do not have a stable effect on the flow accuracy, the flow accuracy partially increased as flute depth was increased, and better as the seed rate and the speed of the feed shaft was increased. In this regard, the optimum dimensions for the best seed flow accuracy were obtained from the flute depth of 8 mm, seed rate of 160 kg ha^{-1}, and at the ground speed of 2.0 m s^{-1}.

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İri Daneli Tohumlar için Tasarlanan Oluklu Makaranın Tohum Akış Düzgünlüğünün Değerlendirilmesi

ÖZET

Bu çalışmada, iri taneli tohumlar için 18, 20 ve 22 mm oluk çaplarında ve 5, 8 ve 11 mm oluk derinliklerinde tasarlanan dokuz adet oluklu makara kuru fasulye tohumunun akış düzgünlüğü için değerlendirilmiştir. Her bir oluklu makara, 80, 120 ve 160 kg ha^{-1} ekim normları için 1.0, 1.5 ve 2.0 m s^{-1} ilerleme hızlarında çalıştırılmıştır. Tohum akış düzgünlüğünün belirlenmesinde varyasyon katsayısı değerleri kullanılmıştır. Üç tekerrürlü olarak yürütülen çalışmada, her tekerrürde elde edilen CV değerleri için istatistiksel analizler yapılarak, optimum boyutlar belirlenmiştir. Analiz sonuçlarına göre, öngörülen oluk çaplarının akış oranı düzgünlüğü üzerinde kararlı bir etkisinin olmadığı, oluk derinliği arttıkça akış oranı düzgünlüğünün kısmen arttığı, ekim normu ve ilerleme hızı arttıkça akışın daha iyi olduğu belirlenmiştir. Bu bağlamda, en iyi tohum akış düzgünlüğü için optimum boyutlar, 8 mm'lik oluk derinliği, 160 kg ha^{-1} ekim normu ve 2,0 m s^{-1} ilerleme hızından elde edilmiştir.

Biyosistem Mühendisliği

Araştırma makalesi

Makale Tarihçesi
Geliş Tarihi : 19.02.2021
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Anahtar Kelimeler
Oluk çapı
Oluk derinliği
Ekim normu
İlerleme hızı
Fasülye

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INTRODUCTION

One reason for reduced yield is uneven seed germination (Nafziger, 1996). The uniform development of the plant depends on the even of the plant distribution in the field, that is, the precision and accuracy of the application rate of the seed metering mechanism. The uneven flow of seeds during sowing, which depends on the metering mechanism of the seeder, causes uneven seed distribution in the field and affects competition among plants in terms of existing moisture, light, and nutrients (Griepentrog, 1998; Karayel and Özmerzi, 2002).

Dry bean seeds can be planted with both cereal seeders and precision seeders. However, there are restrictive reasons in choosing a seeder, such as purchasing power of the enterprise, the farmer's lack of technical knowledge about precision seeders (Ess et al., 2005), precision seeders cannot technically perform the sowing operation in narrow row spacing (Parish et al., 1999), and sowing performance deteriorates in spacing less than 5 cm intra-row (Önal, 2011).

Fluted roller seeders can be used as an alternative to these seeders for the sowing of small and coarse seeds (Halley and Soffe, 1988). Griepentrog (1994) stated that the fluted roller seeders, which are cheaper for narrow row spacing, were relatively preferred precision seeders. Fluted roller metering devices have been in use for over 300 years and are the most widely metering devices used in today's mechanical cereal seeders (Brown, 2003; Maleki et al., 2006; Li et al., 2016; Yang et al., 2018). Despite widely preferred due to its design, simplicity, ease to manufacture, lightweight, easy flow rate adjustment, and suitable for high-speed sowing, when the appropriate roller structural and operating parameters are not selected for seeds with different physical properties, the metering device may give an uneven seed distribution pattern (Ryu and Kim, 1998; Brown, 2003; Ess et al., 2005; Jin et al., 2018).

Current developments in seeders have been allowing higher sowing speeds. However, it is not easy to achieve a successful distribution uniformity pattern at these speeds for seeds of different physical properties. Therefore, improving the flow rate uniformity in fluted roller seeders is still an important research focus. Important studies have been carried out on the structural features of fluted roller metering devices. Nukeshev et al. (2016) proposed a new design of roller pin, where the pins are in the form of a truncated pyramid to prevent passive zone on the roller during sowing; they reported this configuration allowed the metering devices to operate properly in slow roller rotational speed. And, Sugirbay et al. (2020) showed that the discharge uniformity of the pin and the discharge amount increase by optimizing the design parameters of the new pin-roller for variable-rate and variable-speed applications. Yıldırım and Kuş (2014) designed fluted rollers with different flute diameters (18, 20, and 22 mm) and flute helical angles (0, 10, and 20^{o}) to examine the flow rate uniformity of metering device for coarse seeds. They reported that the best flow rate values were obtained from the flute diameter of 18 mm and the helical angle of 20^{o}.

One of the most important parameters affecting sowing performance in metering devices of the fluted roller is the physical properties of seeds such as shape, size, and mass. Fluted feed rollers, especially in the case of using the suitable flute diameters for the seed sizes, have a universal structure that can sow various seeds from small seeds to coarse seeds. In this context, in this study, the flow accuracy of the dry bean was investigated for different seed rates and ground speeds using fluted feed rollers designed in different flute diameters and depths.

MATERIALS and METHODS

This research was carried out at workshop of department the farm machinery and technologies engineering of the agricultural faculty, Ataturk University, Turkey. Dry bean (*Phaseolus vulgaris L.*) seeds cleaned of foreign materials such as stones, crust, garbage, and so on were used in tests. Thousand-grain weight, angle of repose, sphericity, bulk density, and moisture content values of dry bean seeds were 309 g, 20 °, 64%, 770 kg m^{-3} and 10%, respectively. In addition, seed sizes (length, width, and thickness) ranged from 9.5-14.1 mm, 6.7-9.0 mm, and 3.2-6.1 mm, respectively. The test setup consisted of a fluted roller seed metering mechanism coupled to a DC motor via a chain drive (Fig. 1). A speed control unit was used to synchronize the DC speed corresponding to the ground speed of operation. The active area of the rollers was adjusted by a screw mechanism.

Nine fluted rollers made of Delrin was used in the tests. The fluted rollers with bottom delivery used in the study were manufactured in the flute diameters of 18, 20, and 22 mm according to dry bean sizes and the flute depths of 5, 8, and 11 mm. The rollers were used in the feed unit at the bottom of the hopper of the seeder. The width of the slot between the roller and the bottom plate of the feed unit was 13.5 mm.

In the study, which was carried out according to the completely randomized factorial design, the flow uniformity of the dry bean seeds from the fluted roller metering device was examined. The experiments were arranged in three replications including three flute diameters, three flute depths, three roller rotation speeds (i.e. three ground speeds) for three seed rates of dry bean. Dry bean is sown in inter-row distances of 40-60 cm and the rates of 60-180 kg ha^{-1} based on the various big of seed. The seed rates of 80, 120, and 160 kg ha^{-1} for dry beans were used in the study. The inter-row distance of dry beans was selected 45 cm according to the values used in practice. The individual flow rates of the fluted rollers were calculated to obtain the seed rates of 80, 120, and 160 kg ha^{-1} at the roller rotation speeds of 8, 13, and 18 min^{-1} corresponding to the ground speeds of 1.0, 1.5, and 2.0 m s^{-1} in a seeder for the inter-row distance of 45 cm (Table 1).

A precision balance was employed at the bottom end of the seed delivery mechanism to detect the flow rates of seeds. The seed flowed from the fluted roller metering device for each replication was weighed at intervals of 0.1 second cumulatively with an accuracy of 0.01 g and the data were transmitted simultaneously to a PC with a continuous stream by RS 232 C interface circuit of

the balance (Yıldırım and Kuş 2016). The coefficient of variation (CV) values defined as the standard deviation divided by the mean value were used to evaluate the seed flow uniformity of the fluted roller metering device. For this, by taking the differences between successive cumulative weighing values of each repetition, absolute weighing values were obtained and the CV values were computed from these values. However, Duncan multiple range tests were conducted to show whether there was a difference between parameter levels. The CV values were evaluated according to the criteria reported by Turgut et al. (1995). According to this the CV values of 0-5%, 5-10%, and 10-20% were "very good", "good", and "acceptable", respectively. For this, the 200 (20 s), 133 (13.3 s), and 100 (10 s) data were evaluated for the ground speed of 1.0, 1.5, and 2.0 m s^{-1} for the row of 20 m, respectively. The study, in which 27 trials were carried out for each roller, was completed with a total of 243 experiments.

Table 1. The individual flow rates and effective flute length values of fluted rollers
Çizelge 1. Oluklu makaraların akış oranları ve etkin makara uzunlukları

Ground speed, m s^{-1} (min^{-1})	Seed rate (kg ha^{-1})					
	80		120		160	
	Flow rate (g s^{-1})	ERL (mm)	Flow rate (g s^{-1})	ERL (mm)	Flow rate (g s^{-1})	ERL (mm)
1.0 (8)[a]	3.6	24	5.4	34	7.2	42
1.5 (13)	5.4	21	8.1	31	10.8	39
2.0 (18)	7.2	18	10.8	28	14.4	36

[a]: Values in brackets is feed shaft speeds, ERL: effective flute length

RESULTS and DISCUSSION

The results of variance analysis and multiple range test applied to the CV values obtained from each repeat are shown in Table 2. According to Table 2, the effect of the ground speed (or feed shaft revolution), flute diameter, and flute depth on the flow accuracy of dry bean seeds was found to be highly significant ($P<0.001$). As seen in multiple range tests, increased ground speed and seeding rate decreased CV values, thus increasing seed flow accuracy. Although the results of the variance analysis were significant and there was a general difference between the levels in the results of the multiple comparison test, the CV values of the flute diameter did not show a significant decrease or increase. This shows that the projected roller flute diameters do not provide a uniform flow uniformity for dry bean seeds. It is also possible to understand the instability in seed flow accuracy occurring due to the flute diameter, from the change in CV values, showed in Figures 2, 3, and 4.

The results of the multiple range test show that all levels of ground speed differ significantly from each other in each seeding rate. As the seed rate increased, the CV values decreased for all ground speeds. For this reason, it can be said that the higher seed rate and the ground speed increased the flow accuracy in bean sowing with fluted roller metering devices. With the increase of seed rate from 80 kg ha^{-1} to 160 kg ha^{-1}, the decrease rate in the CV values occurring at 1.0, 1.5, and 2.0 m s^{-1} ground speeds, were 28%, 32%, and 35%, respectively. CV values, which are higher at the same ground speeds and lower seeding rates, increased the flute fill rate with the increase of the seeding rate and accordingly decreased the CV values. In addition, the rate of decrease in the CV values that occurred at 80, 120, and 160 kg ha^{-1} seed rates with the increase of the ground speed were 47%, 50%, and 52%, respectively.

Since sufficient time cannot be provided while working at low roller rotation speeds with lower seeding norms, the flutes are not filled with seeds and as a result, pulsed flow occurs. Seed flow mass increased, as effective roller length was increased to achieve seed rates of 80, 120, and 160 kg ha^{-1} at the same ground speed. It is thought that it was provided a more stable flow as the flutes will fill better by seeds as the seed flow mass increases. However, it is thought that the decrease in the CV values with the increase in the ground speed is not due to the better filling of the flutes, but by the faster rotating roller providing a more stable flow.

When the results in flute depth were examined, there was a more stable situation compared to the flute diameter. While the CV values obtained depending on the flute depth were lower at lower seeding rates, these values also increased with the increase of the seeding rate. In fact, with the greatest seeding rate and ground speed, CV values decreased with increasing flute depth and seed flow became more stable. Increasing the seeding rate improved seed flow uniformity by decreasing CV values at all levels of both flute diameter and flute depth. While CV values in all flute depths were statistically different from each other in the lowest seeding rate, there was no statistical difference between 5 and 8 mm flute depths in the remaining seeding rates. The lowest CV values in flute depth are generally obtained from 8 mm. With the increase of seeding rate from 80 kg ha^{-1} to 160 kg ha^{-1}, the decrease rate in the CV values occurring at 5, 8, and 11 mm flute depths, were 27%, 34%, and 33%, respectively. It is obvious that the greatest decrease in

CV values occurred with the increase of the ground speed in each seeding rate. In this context, the parameter that most affects the flow accuracy of the dry bean seed was the ground speed (i.e. the rotational speed of the roller).

The CV values obtained in all combinations of fluted metering roller's ground speed, flute diameter, and flute depth for 80, 120, and 160 kg ha^{-1} seeding rates are respectively; it is shown in Figures 2, 3, and 4. The data point given for each flute depth in the figures is the average of three repeats. In all seed rates, for each flute diameter and depth, the highest mean CV values were obtained at 1.0 m s^{-1} ground speed and the lowest values at 2.0 m s^{-1} ground speed. The mean values of CV given in Figures 2, 3, and 4 vary between 4-20%. All CV values obtained from the current study, it was below the "acceptable" (CV <20%) limit value reported by Turgut et al. (1995).

Table 2. The results of variance analysis and Duncan's Multiple Range Test (DMRT)
Çizelge 2. Varyans analizi ve Duncan Çoklu Karşılaştırma Testi sonuçları

Variance sources	Seeding rate (kg ha^{-1})								
	80			120			160		
	df	SS	P	df	SS	P	df	SS	P
Ground speed (GS)	2	822.82	0.000	2	636.72	0.000	2	530.77	0.000
Flute diameter (FD)	2	14.69	0.000	2	20.16	0.000	2	20.58	0.000
Flute depth (FDH)	2	47.94	0.000	2	17.40	0.000	2	34.28	0.000
GS x FD	4	10.58	0.001	4	9.03	0.000	4	9.91	0.000
GS x FDH	4	5.26	0.037	4	2.71	0.133	4	4.93	0.000
FD x FDH	4	6.64	0.013	4	0.384	0.901	4	13.20	0.000
GS x FD x FDH	8	12.48	0.004	8	11.25	0.001	8	13.57	0.000
Error	54	25.79		54	19.82		54	5.44	
Total	80			80			80		
DMRT									
Seed rate^{+}	Ground speed (m s^{-1})			Flute diameter (mm)			Flute Depth (mm)		
	1.0	1.5	2.0	18	20	22	5	8	11
80 kg ha^{-1}	16.35**a**	11.19**b**	8.69**c**	12.33**a**	12.43**a**	11.48**b**	12.00**b**	11.18**c**	13.06**a**
120 kg ha^{-1}	13.64**a**	9.35**b**	6.85**c**	9.34**c**	10.56**a**	9.92**b**	10.18**a**	9.30**b**	10.35**a**
160 kg ha^{-1}	11.77**a**	7.56**b**	5.64**c**	8.72**a**	7.61**b**	8.64**a**	8.79**a**	7.40**b**	8.78**a**

$^{+}$: Means followed by the same letter in each group are not significantly different at the 0.95 level.

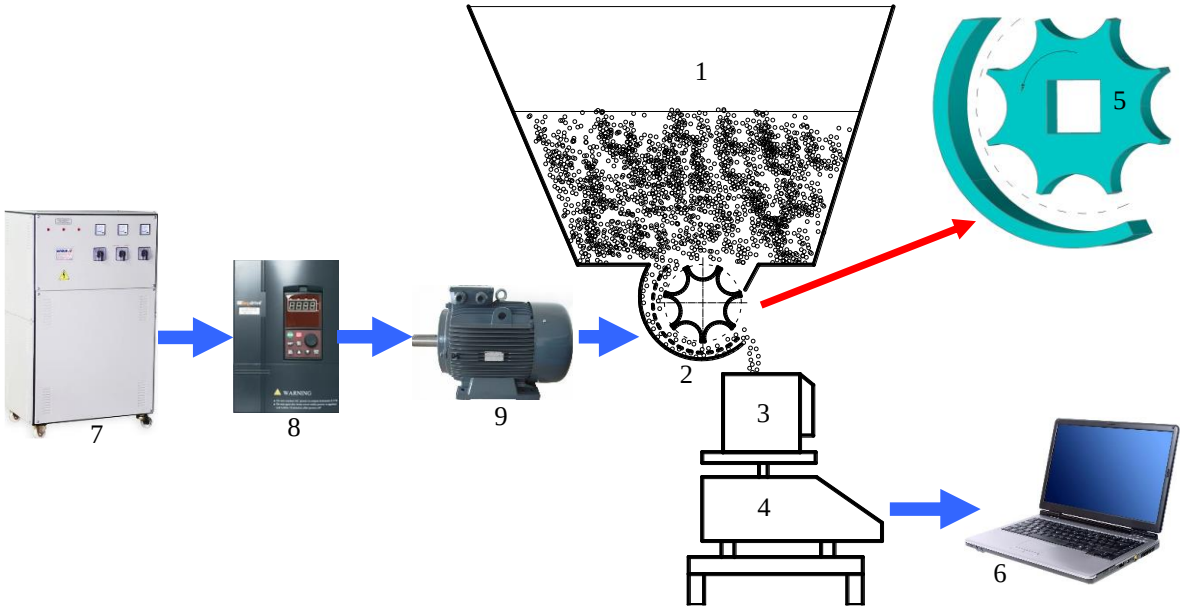

Figure 1. The instruments and equipment used in experimental test rig: hopper (1), seed metering device (2), container (3), electronic scale (4), and fluted roller (5), personal computer (6), regulator (7), speed control unit (8), and AC motor. (9)

Şekil 1. Deneme düzeneği: tohum deposu (1), ekici düzen (2), toplama kabı (3), hassas terazi (4), oluklu makara (5), bilgisayar (6), regülatör (7), hız kontrol ünitesi (8) ve elektrik motoru (9)

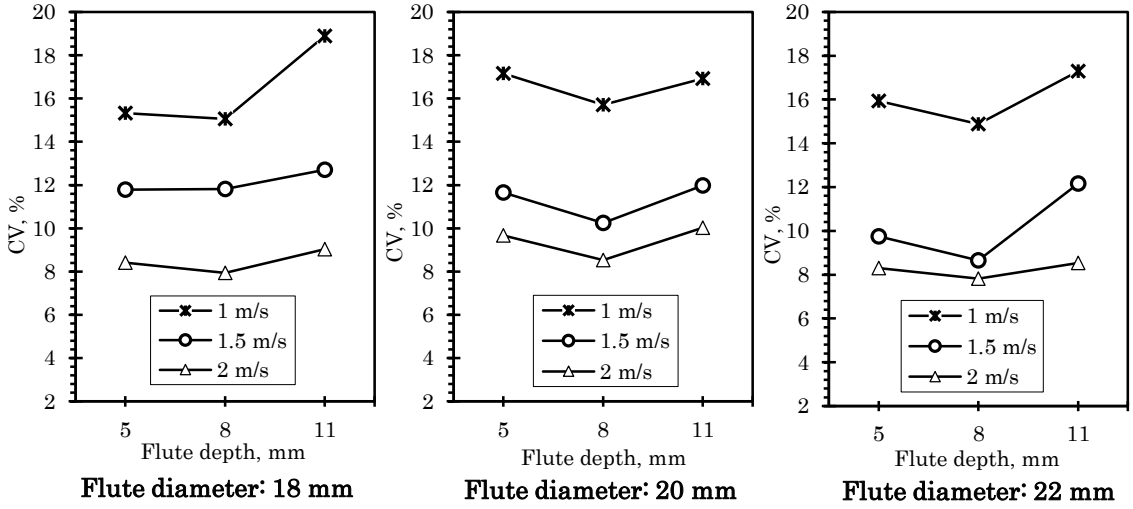

Figure 2. The line graphs of CV values for seeding rate of 80 kg ha^{-1}
Şekil 2. 80 kg ha^{-1} ekim normu için CV değerlerinin değişimi

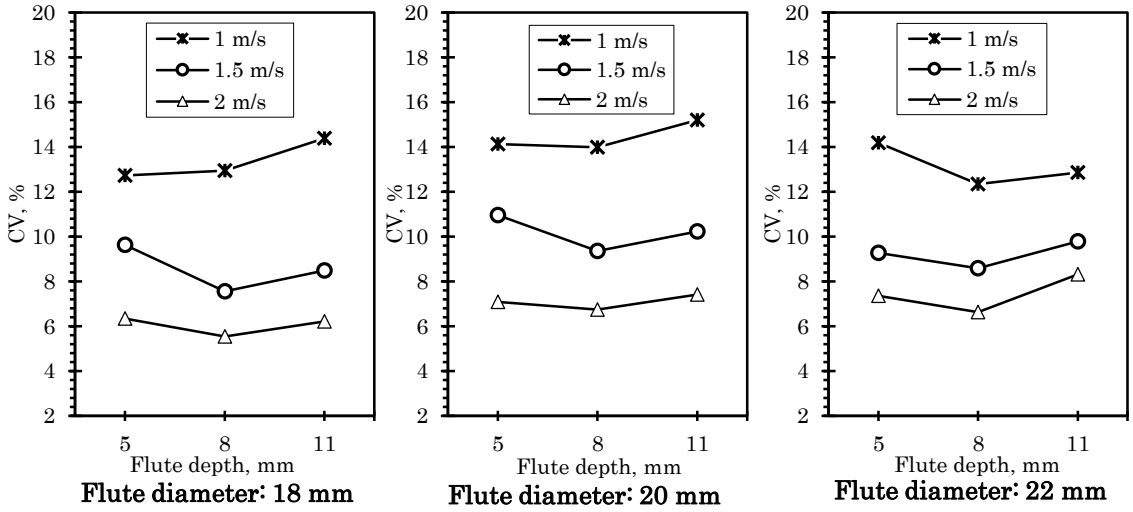

Figure 3. The line graphs of CV values for seeding rate of 120 kg ha^{-1}
Şekil 3. 120 kg ha^{-1} ekim normu için CV değerlerinin değişimi

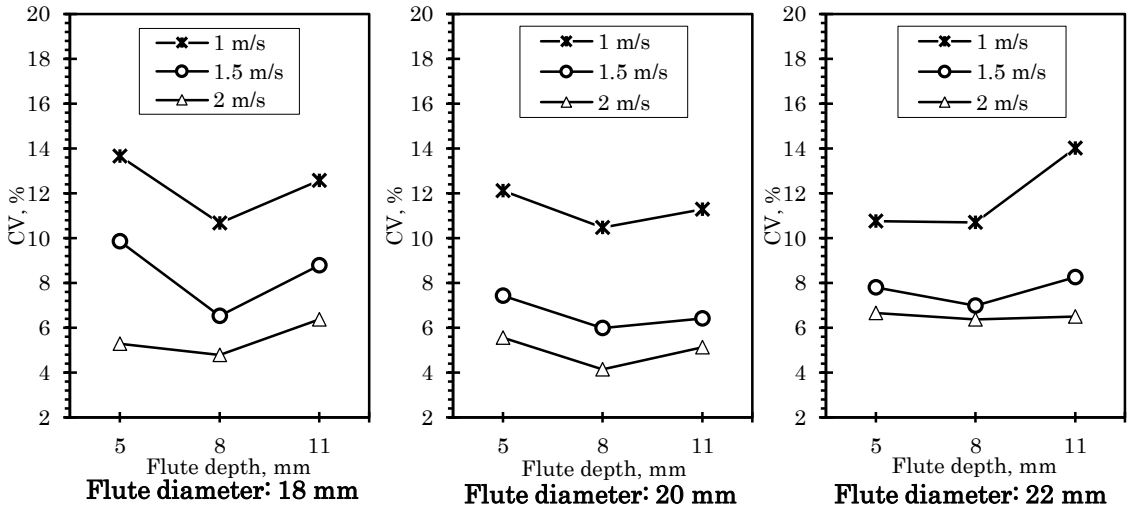

Figure 4. The line graphs of CV values for seeding rate of 160 kg ha^{-1}
Şekil 4. 160 kg ha^{-1} ekim normu için CV değerlerinin değişimi

CONCLUSIONS

In this study, the possibilities of sowing dry bean seed with a fluted roller metering device were investigated and optimum feed roller structural properties and operating parameters were determined for this seed. In this context, the results of current study are as showed:

1) The parameter that had the greatest effect on seed flow accuracy was the ground speed. With the increase in the ground speed, CV values decreased approximately by half.
2) It defected that, in the sowing of dry bean seeds with a fluted roller metering device, the increase in the seeding rate and ground speed improves the seed flow accuracy, the prescribed flute diameters do not have a stable effect on the flow accuracy, and the flow accuracy in the smallest and largest flute depths impaired.
3) It was determined that the CV values obtained for all parameters were within the acceptable limits specified in the literature.

As a result, it is recommended to use an 8 mm flute depth, and 2.0 m s^{-1} ground speed, where the best flow accuracy is achieved in all seeding rates, when sowing dry beans with a fluted roller metering device.

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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Improving Functional Properties of Kefir Produced with Cow and Goat Milk

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ABSTRACT
The purpose of this study was to research some properties of kefir that was obtained from the 1% (w/v) and 2% (w/v) inulin addition to cow-goat milk mixture. In this present study, changes of titrable acidity, pH value, total mesophilic aerobic bacteria, *Lactobacillus* spp., *Lactococcus* spp. and yeast counts of samples in storage were determined. Additionally, samples' total fat content, total solid and viscosity values were reported and taste, consistency, and total acceptance of samples were evaluated. Control group, 1% (w/v) and 2% (w/v) inulin added samples' total solid and fat content, viscosity, pH and titrable acidity (equivalent to lactic acid %) values were investigated and found at the range of; 11.84 – 13.53, 4.4 – 4.8, 365.8 – 488.7, 4.45 – 4.53, 0.80 – 0.84, respectively. On the 40th day of the storage total mesophilic aerobic bacteria, *Lactobacillus* spp *Lactococcus* spp. and yeasts were determined as 10.50-10.55, 10.24-10.58, 10.25-10.58 and 7.60-7.93 log cfu ml^{-1}, respectively.

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İnek ve Keçi Sütleriyle Üretilen Kefirin Fonksiyonel Özelliklerinin Geliştirilmesi

ÖZET
Bu araştırmada % 1 (w/v) ve % 2 (w/v) inülin ilavesiyle üretilmiş inek-keçi sütü kefirlerindeki fiziksel, kimyasal ve mikrobiyal özellikleri araştırılmıştır. Bu çalışmada tüm örneklerde titrasyon asitliği, pH değeri, toplam mezofilik aerobik bakteri sayısı, *Lactobacillus* spp., *Lactococcus* spp. ve maya sayıları depolama süresi boyunca tespit edilmiştir. Aynı zamanda örneklerin toplam yağ, kurumadde ve vizkozite değerleri raporlanmıştır. Tat, yoğunluk ve toplam kabul edilebilirlikdeğerlendirilmiştir. Kontrol grup, %1 (w/v) ve % 2 (w/v) inülin ilaveli gruplar için toplam kurumadde ve yağ miktarı, vizkozite, pH ve titrasyon asitliği (%1 laktik asit eşdeğeri) miktarları sırasıyla 11.84 – 13.53, 4.4 – 4.8, 365.8 – 488.7, 4.45 – 4.53, 0.80 – 0.84 olarak belirlenmiştir. Depolamanın 40. gününde, toplam mezofilik aerobik bakteri sayısı 10.50-10.55, *Lactobacillus* spp. 10.24-10.58, *Lactococcus* spp. 10.25-10.58 ve mayalar 7.60-7.93 log kob ml^{-1} olarak tespit edilmiştir.

Gıda Bilimi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 10.03.2021
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Anahtar Kelimeler
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Keçi sütü
İnülin
Kefir
Lactobacillus spp.

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INTRODUCTION

Fermented dairy products are highly consumed all around the world (Gaware et al., 2011; Rotar et al., 2015). The dairy industry is globally expanding, and some functional milk products are particularly preferred by consumers for their positive health effects. Among these dairy products, kefir is known to be an acidic fermented milk product, originated in the Caucasus area and mostly popular in Russia, North-Eastern Europe and Southwest Asia locations (Leite et al., 2013; Oliveira et al., 2017; Lima et al., 2018). Kefir grains are composed of kefiran which is a kind of polysaccharide containing D-glycose and D-galactose (Guzel-Seydim et al., 2005; Turan and Ilter, 2007). Kefir's chemical composition depends not only on the starter-kefir grains but also on its geographical origin, the temperature, and time-related conditions of

fermentation, and especially on the type and volume of the milk used. Traditional Kefir is obtained from starter culture called 'kefir grains' which is a semi-hard granule that consists of several lactic acid bacteria and probiotics (Wang et al., 2017). Kefir grain microflora comprises *Lactobacillus* spp. (dominantly; *Lactobacillus acidophilus*, *Lb. lactis, Lb. casei*, *Lb. kefir* and *Lb. delbrueckii* subsp. *bulgaricus*) *Streptococcus lactis*, *S. cremoris*, *Leuconostoc* spp. acetic acid microorganisms (*Acetobacter aceti*, *A. rasens*) and mainly some yeasts (*Candida kefir*, *Saccharomyces cerevisiae*, *Kluyveromyces fragilis*. Thus, kefir is known to be a good source of probiotic microorganism with potential health benefits (Santos et al., 2003; Kok-Tas et al., 2010).

Kefir is made from various types of milk (cow, goat, camel, buffalo, or mare), and is usually produced by mixing two types of milk to enhance its benefits, flavour, and texture, and subjected to secondary fermentation or the addition of additives such as inulin to improve the final product properties (Farag et al., 2020). Goat milk has higher nutrient contents than that of cow's milk (Vitamin A, Vitamin B1 and B2) and it can be digested more easily with the 3.49 µm size fat globules and higher amounts fatty acids (short chain). In addition to that, goat's milk contains caproic, caprilic and capric fatty acids that reduce serum cholesterol content in metabolism. Goat milk has less allergenic properties than cow's milk and its proteins are more easily degraded and absorbed in gastrointestinal system (Ahmed et al., 2015). It is notable that goat's milk is widely consumed for health purposes such as its antiallergenic effect (Haenlein, 2004). Technologically, goat's milk has also some good properties as compared with cow's milk; such as small size fat particles which provide a smoother texture in products, containing low quantity of αs1-casein results soft gel products, as well as higher water binding potential (Gomes et al., 2013).

Probiotic microorganisms and lactic acid bacteria in fermented products show beneficial effects on health if they are consumed adequately. Basically, prebiotics are food ingredients that increase the viability of useful microorganisms in host's metabolism. Inulin, commercially produced from chicory's roots in Belgium and Netherland in the early 1990's, is one of the prebiotics that can be used for this purpose (Yabancı, 2010). Inulin is a non-digestible oligosaccharide with prebiotic property, and it has been successfully applied to well-known dairy products. It is a storage material present in many plants such as wheat, onion and bananas; however, chicory is one of the main raw materials used for industrial production. One of the most important advantages of inulin and certain non-digestible oligosaccharides is their ability for selective stimulation of the bifidobacteria growth in the colon (Glibowski and Zielinska, 2015). Consumers are demanding for foods with increasingly properties, such as pleasant flavor, low calorie value or low-fat content and beneficial health effects (Goncu et al., 2017). In order to improve nutraceutical benefits of kefir, an appropriate approach could involve the enrichment with suitable components able to confer to the drink specific and valuable properties (Aiello et al., 2020). However, there has been limited research conducted on the products fermented with goat's milk. Inulin is generally used to modify the texture, viscosity and sensorial properties of dairy products (Tratnik et al., 2006; Moatsou and Park, 2017).

It is remarked that inulin can increase *Lactobacillus* and *Bifidobacterium* spp. in yoghurts (Oliveira et al., 2012). It is proved that inulin supplementation not only has conservation effect on activity and viability of some *Lactobacillus* strains (casei and acidophilus) but also it decreases the generation time of *Streptococcus* and *Lactobacillus*, significantly (Moghadam et al., 2019). As shown in Birkett and Francis' (2010) study, fructo-oligosaccharides (FOS) can support the growth of *Lactobacillus* and *Bifidobacterium* species, but other microorganisms such as *Escherichia coli* and *Clostridium difficile* do not metabolize the FOS. In inulin-added dairy products, there has been an increase in rheological properties especially water binding capacity and dry matter content.

The objectives of this research were to:

1) Produce functional traditional fermented product kefir and determine the effects of addition 1% (w/v) and 2% (w/v) inulin to cow and goat milk mixture on the survival of total mesophilic aerobic bacteria, *Lactobacillus* spp., *Lactococcus* spp. and yeast counts.
2) Examine some quality parameters such as pH, viscosity values and sensory properties of inulin added kefir and control samples over the course of 40 days of cold storage.

MATERIALS and METHOD

Kefir production

Goat's milk has solitary sensorial characteristics as standard and definite 'goaty' aroma. As some buyers do not like the taste of goat's milk, cow and goat milks were mixed (1:1 v/v) in kefir production. Cow and goat raw milks were obtained from a farm and pasteurized at 85 °C for 10 min. Kefir granules were purchased from market and inulin (Orafti, HPX) was provided from company Artisan Food (Istanbul). Kefir production steps can be seen in Figure 1. Trial groups' names are coded as A, B and C for 1% w/v, 2% w/v inulin added groups and control samples, respectively.

Chemical and physical analyses

An acidity indicator pH was determined using a pH meter (Sartorious PT-15). The dry matter, titrable acidity and fat amounts of samples were measured

according to A. O. A.C procedures (Anonymous, 2006). Viscosities were tested with Brookfield DV viscosimeter (11, Pro Extra Model).

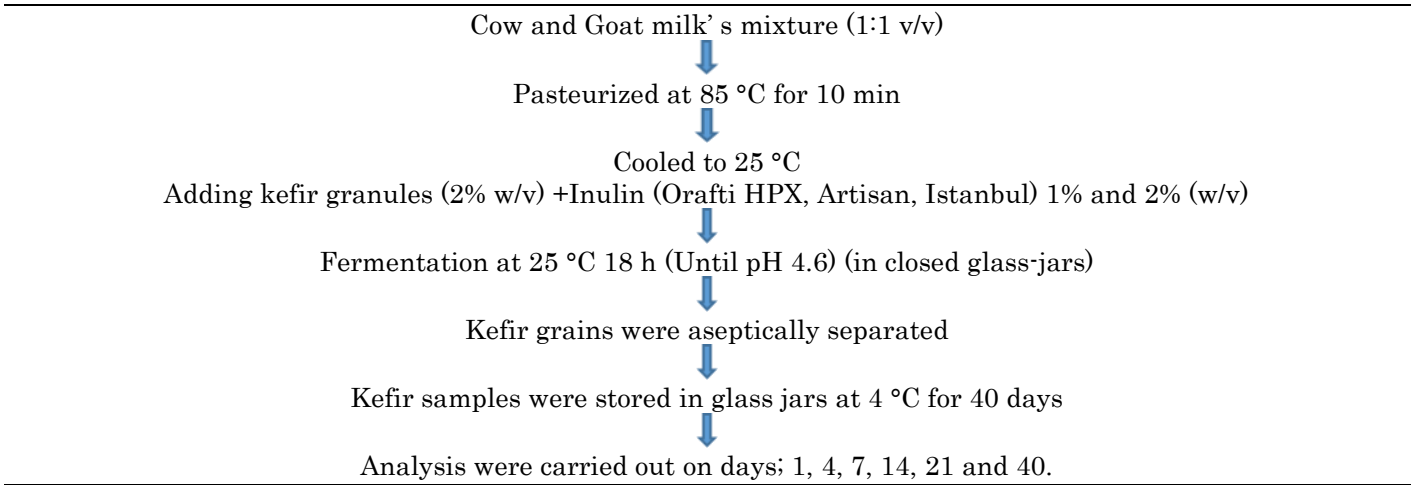


Figure 1. Production of cow and goat milk kefir with inulin addition
Şekil 1. İnülin ilaveli inek-keçi sütü kefirlerinin üretimi

Microbiological analyses

Ten ml of kefir samples were diluted with 90 ml of 0.1% (w/v, pepton) sterile water and decimal dilutions were prepared in 9 ml of 0.1% (w/v, pepton) sterile water. Lactic acid bacteria numbers were determined by pour plate technique and counted on de Man Rogosa Sharpe agar (MRS Merck 1.10660.0500) under anaerobic conditions at 37°C/72 h. Total mesophilic aerobic microorganisms were detected on plate count medium (PCA, Merck 1.05463.0500) and incubated at 28-30°C/48 h. *Lactococcus* spp. were counted on M17 plates (Merck 1.15108.0500) using pour plate technique after the incubation at 37°C/48 h in anaerobic conditions. Then, yeasts were enumerated on yeast extract glucose chloramphenicol plates (YGC, Merck 1.0375.0500) and plates were incubated at 25°C/5 days (Halkman and Kayhan, 2000).

Sensorial analyses

Sensory evaluation was conducted by using 5 trained panellists (age 18-40) in Balikesir University. The samples were served in 100 ml portions at about 8 °C. The kefir samples were examined and tested by the panellists who were asked to rate the samples sensorially by using marks on a full-score levels in terms of the flavour, odour, colour and texture quality parameters (0-1; it is not consumed as a human food, 2: unpleasant, 3: mildly 4: good, 5: very good).

Statistical Analyses

SPSS 19.0 software for windows (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. A one-way analysis of variance (ANOVA) test was performed to determine mean differences between the A, B and C sample groups. The level of significance between the means was obtained by the Tukey HSD and LSD tests.

RESULTS and DISCUSSION

The average percent dry matter % ±S.D. without fat for cow's milk and goat's milk was 7.93 %±0.18, 8.53 ±0.21, and the fat content % ±S.D. was 3.5± % 0.15, 4.5± % 0.10, respectively.

Average % dry matter contents ± S. D. of A, B and C samples were 13.53 ± 0.04, 13.22 ± 0.04, 11.84 ± 0.1; % fat contents ± S. D. of A, B and C samples were 4.6± 0.12, 4.8± 0.18 and 4.4± 0.14, respectively.

Raw cow and goat milks analyses results were compatible with Turkish Food Codex Raw Milk standards. Guneser and Karagul-Yuceer (2010) also determined 3.25% ± 0.05 fat content averagely and between 10.49%± 0.01 - 15.49% ± 0.19 dry matter contents for goat's milk samples collected from Canakkale region. In the study differences between the dry matter contents % and fat contents % of the samples were of importance when compared with the control groups. Dry matter contents% and fat contents of the samples were not changed during the storage.

Viscosity is a parameter that is directly related with the texture of product and a factor for consumer's preference (Gomes et al., 2013). In the research, during the storage viscosity average values were determined as 488.7± 0.50, 365.8±0.43, 380.1±0.50 cP±S.D. for A, B and C samples, respectively. In the present research, it was found that 1% (w/v) inulin added kefir samples have higher viscosity values than the others. The incorporation of inulin caused an increase in the viscosity of the synbiotic yoghurt drink samples in Soh et al. (2021) study. Also, it was stated that inulin has unique ability to form a discrete highly stable particle gels and contribute to the rheological and textural properties of foods. In a similar research, inulin

demonstrated the highest rheological and sensory performance as well as the best viability of probiotics in synbiotic fermented milk (Ozturkoglu-Budak et al., 2019). Helal et al. (2018) found yogurt apparent viscosity increased with inulin addition till 2% and was comparable to full-fat yogurt, the addition of inulin has significantly affected the yogurt viscosity resulted in increasing the viscosity value with the inulin addition. Guven (2005) and Tratnik et al. (2006) put forth that inulin addition (2% w/v) in kefir samples have higher viscosities than the control groups. Also, Iriyogen et al. (2005) found 179-501 cP viscosity values for kefir samples in their research. It was stated that increasing the kefir granules ratio in kefir leads to higher viscosity in kefir samples. It can be explained that total dry matter, protein, fat contents (casein and serum protein ratio), heat process, serum protein denaturation, homogenisation, salt stability of milk, starter culture activities, storage temperature may have an impact on the viscosity of the product (Uslu, 2010).

In the study, the titrable acidity values showed an increasing trend. And the pH values of kefir samples were on a decrasing. In the literature there are many research that describe the effect of pH on viability of probiotic viability. Changes in lactic acid values in inulin added samples were found significantly important ($p<0.05$). Nevertheless, differences for control samples were not found significantly important during the storage ($p>0.05$). Guneser and Karagul-Yuceer (2010) found 0.73-0.79 lactic acid contents in kefir samples produced from different ratio of cow and goat milks mixtures.

In the present research, pH values of samples were determined as between 4.45-4.62. Changes in pH values were not found significant ($p>0.05$). It can be seen in Table 1.

Table 1- Lactic acid% ±S.D. and pHvalues of kefir samples during the +4°C storage
Çizelge 1- Kefir örneklerinin +4°C'de depolamada % laktik asit ±S.D. ve pH değerleri

	A		B		C	
Storage days	**L. a.%**	**pH**	**L. a.%**	**pH**	**L. a. %**	**pH**
1.	$0.63 \pm 0.13^{a*}$	4.62 ± 0.23^{a}	0.65 ± 0.14^{a}	4.54 ± 0.13^{a}	0.62 ± 0.09^{a}	4.61 ± 0.28^{a}
4.	0.70 ± 0.14^{a}	4.47 ± 0.29^{a}	0.70 ± 0.26^{a}	4.48 ± 0.13^{a}	0.69 ± 0.23^{a}	4.49 ± 0.34^{a}
7.	0.67 ± 0.14^{ab}	4.59 ± 0.14^{a}	0.70 ± 0.12^{ab}	4.61 ± 0.13^{a}	0.68 ± 0.20^{a}	4.61 ± 0.13^{a}
14.	0.71 ± 0.14^{bc}	4.53 ± 0.10^{a}	0.72 ± 0.10^{b}	4.52 ± 0.13^{a}	0.71 ± 0.09^{a}	4.54 ± 0.13^{a}
21.	0.78 ± 0.20^{c}	4.46 ± 0.01^{a}	0.75 ± 0.06^{bc}	4.48 ± 0.13^{a}	0.75 ± 0.18^{a}	4.51 ± 0.04^{a}
40.	0.84 ± 0.05^{c}	4.45 ± 0.13^{a}	0.80 ± 0.14^{c}	4.48 ± 0.13^{a}	0.80 ± 0.23^{a}	4.53 ± 0.15^{a}

*Means ±SD within each row not sharing the same lowercase letters are statistically different ($p < 0.05$).

In another research, the pH values of inulin and kefir culture added yoghurt samples were determined as between 4.40-4.70 (Okur et al., 2008). Likewise, Glibowski and Kowalska (2012) determined pH values between 4.47-4.53 after the 24 hours' fermentation in inulin added kefir samples. Agata and Jan (2012) produced fermented goat milk beverage with *Lactococcus lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, *Saccharomyces fragilis* culture and they observed 4.57-4.63 pH values changes in samples.

Total mesophilic aerobic bacteria count increased during the storage days but in the control samples there was a drop in bacteria numbers on the 14th day. Increase in bacteria numbers was not found significantly important ($p>0.05$). In other words, for all the sample groups, bacteria numbers were found very close to each other on the 40th days of storage (Fig. 2a)

In Uslu (2010) study, mesophilic aerobic bacteria numbers were found 6.41 log cfu/ml in commercially sold kefirs in Ankara markets. Similarly, Karabiyikli and Dastan (2016) determined 7.91-8.50 log cfu/ml and 6.12-7.24 log cfu/ml total mesophilic bacteria in produced kefir samples and commercially sold kefir, respectively.

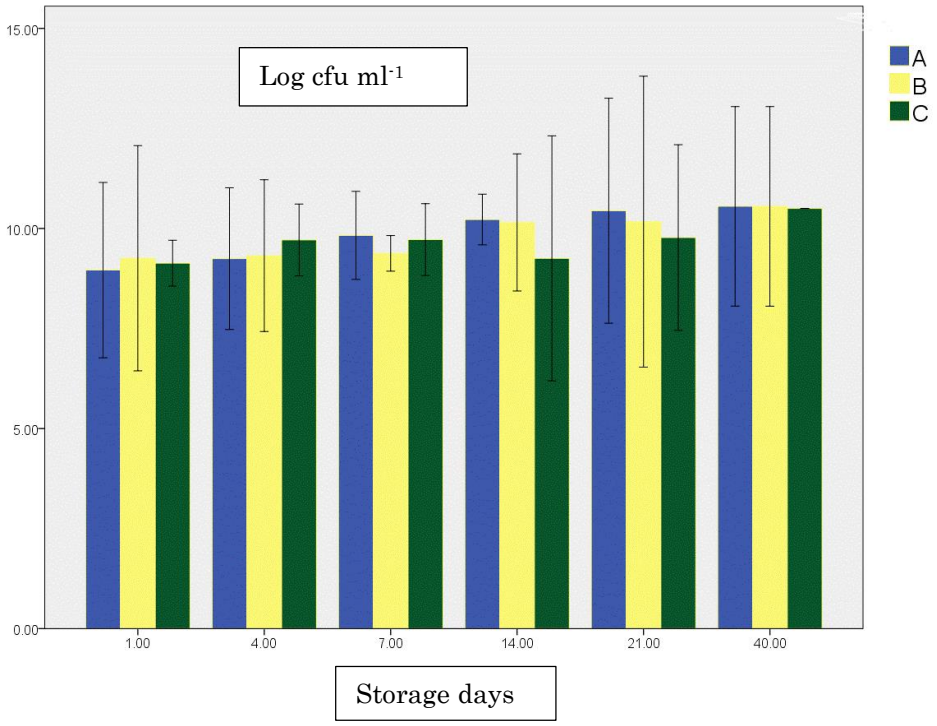
The highest *Lactobacillus* spp. count were determined in 2% inulin added samples with 11.17 log cfu/ ml on the 21st day. It was observed minimum of 8.55 log cfu/ ml of *Lactobacillus* spp. in control groups on the 1st day of storage. In the study, changes in bacteria counts on the 4 th and 14 th days of storage for 1% inulin added samples, 4 th, 14 th and 40th days of storage for 2% inulin added samples and 4 th, 7 th and 21st days of storage for control samples were found significantly important ($p<0.05$) (Fig. 2b). Similarly in an onoter study, the viability of *L. delbrueckii* ssp. *bulgaricus* was increased by the addition of 1 and 2% of inulin, while the addition of 3% had negative effect. However, no effect was reported in case of *Streptococcus thermophiles* viability in low fat yoghurt samples during the 14 days of storage (Helal et al., 2018). In another study, inulin, added as a prebiotic, increased acidity, as well as enhanced survival of LAB in yogurt-like plant milk fortified with inulin (at 6 °C for 21 days storage) (Łopusiewicz et al., 2020). In a study it was investigated the effects of inulin on some properties of cow milk kefir and goat milk kefir. *Lactobacilli* and *Streptococci* count in goat milk kefir were almost similar to the cow milk kefir.

The cow milk kefir with 2% inulin exhibited the highest *Streptococci* and *Lactobacilli* counts at the end

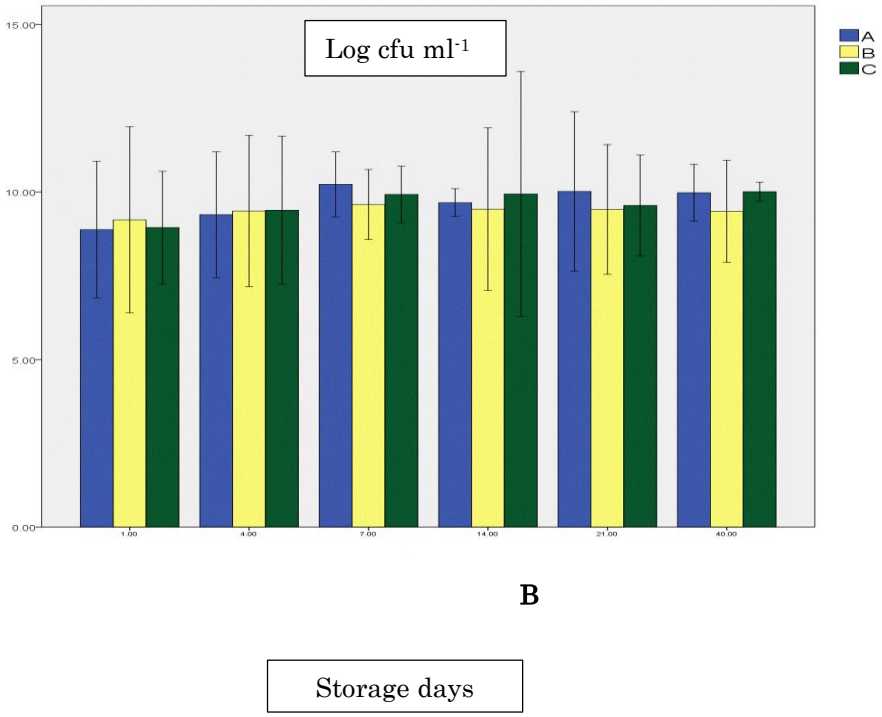
of the storage (14 days). It was explained as inulin-type fructans can promote the development of *Bifidobacteria* and *Lactobacilli* (Kef and Arslan, 2021).

The results obtained in the study was consistent with the previous reports. Witthuhn et al. (2005) observed 6.88-8.30 log cfu/ml in kefir samples, Kok-Tas et al. (2010) found 8 log cfu/ml in inulin added probiotic ayran samples. Moreover, Cetinkaya and Elal-Mus (2012) determined 4.68-8.26 log cfu/ml in 50 kefir samples from Bursa. In another study, *Lactobacillus* spp. numbers were found 9.96 log cfu/ml in kefir samples which were produced with the addition of 4% oligosaccharides (Oh et al., 2013).

Viability of *Lactococcus* spp. of kefir samples are presented in Fig 2c. The viable cell counts of *Lactococcus* spp. were 8.08-11.17 log cfu /ml during the storage. Changes in 1% inulin added kefir samples were found significant on the 4th, 7th and 14th days of storage ($p<0.05$). *Lactococcus* spp. numbers for 2% inulin added kefir samples ranged from 11.17 to 10.25 log cfu /ml on the 40th day of storage.



a



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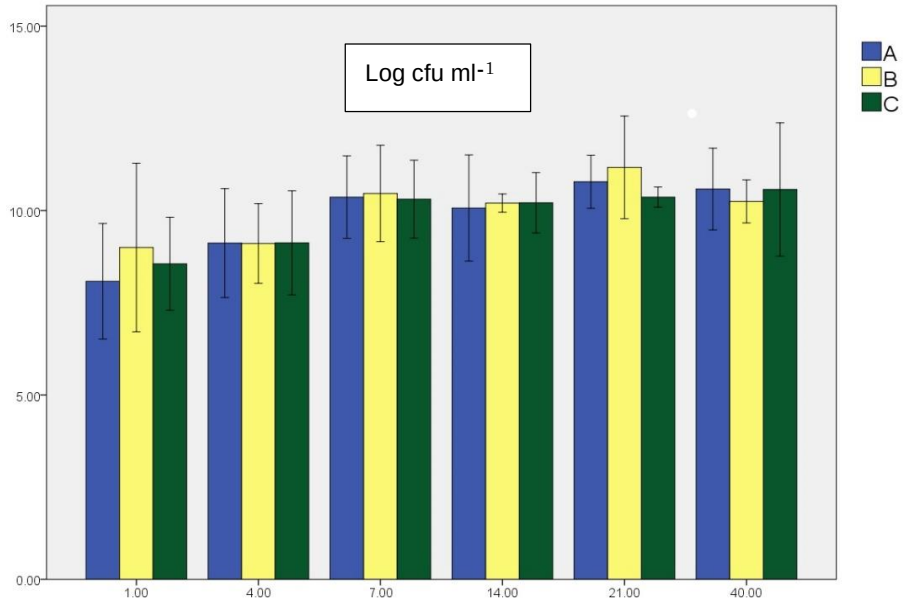
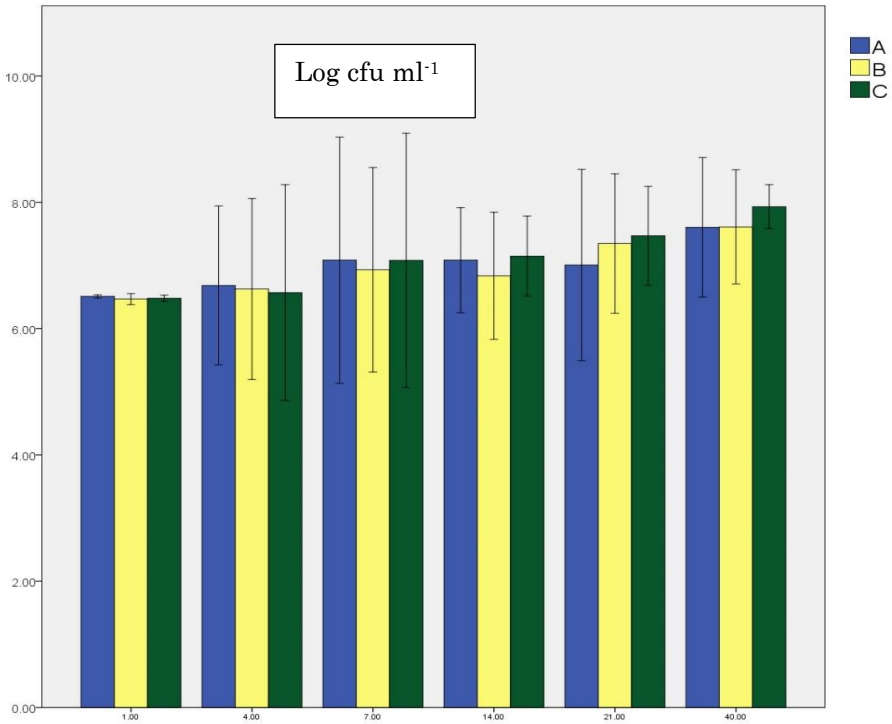


C

Storage days



D

Storage days

Figure 2. a) Total aerobic mesophilic bacteria numbers b) Lactobacillus spp. numbers c) Lactococcus spp. numbers d) yeast numbers (log cfu/ml) of kefir samples with the standard deviation bars. (A: 1% w/v inulin added samples; B: 2% w/v inulin added samples; C: Control groups).

Şekil 2. Kefir örneklerinin standart sapmaları ile birlikte a)Toplam aerobic mezofilik bakteri sayıları b) Lactobacillus spp. sayıları c) Lactococcus sayıları d) maya sayıları (log kob /ml) (A: % 1 inülin ilaveli örnekler; B:% 2 inülin ilaveli örnekler C: Kontrol grupları).

Garcia-Fontan et. al. (2006) found 8 log cfu/ml *Lactococcus* spp. in cows' milk kefir samples and Karatepe and Yalcin (2014) determined *Lactococcus* numbers as 7.26- 8.17 log cfu/ml in kefir samples. They also observed an increase in the viable bacteria to 8.23 log cfu/ml after 15 days of storage in their research. Kim et al. (2014) determined 8.84 log cfu /ml *Lactococcus* spp. as a dominant flora in kefir samples. The data were found similar to prior research results, but it was indicated that all kefir samples had higher bacteria numbers than the other research findings. It may be said that *Lactococcus* numbers can be affected by variables, namely inulin addition, milk type and milk's nutrient compounds, acidity of samples, and so forth.

No mould growth in all the kefir samples during the storage time was observed. However, yeast growth was significantly important in control kefir samples ($p<0.05$), but changes were not found important for inulin added samples. On the 40th day yeast numbers were higher (7.93 log cfu/ml) in control samples than the others (Fig. 2d). Other researchers determined lower numbers in yeasts counts regarded as 5.29-5.63 log cfu/ml in goat's milk kefir samples (Satir et al., 2015), 6 log cfu/ml yeasts in kefir samples after 28 day of storage (Leite et al., 2013) and 5.47, 5.44, 5.00 log cfu/ml yeasts numbers in cow's, ewe's and goat's milk kefir samples (Yaman et al., 2010).

Since the flavour of goat's milk has been found more intense in comparison to cow's milk, the production of dairy products using mixtures of goat and cow milks may be an interesting approach for the dairy market in order to add value to products, supporting some sensory and texture properties and acceptance by the consumers (Gomes et al., 2013). The sensory properties of the samples were applied by the scaling procedure. The kefir samples were evaluated for colour, texture, taste and overall acceptability (yeasty taste, fermented taste, sour taste, sour odour, viscosity, serum separation). Samples were coded with randomly chosen three numbers and served as 8°C. In the general sense, changes in acidity was found to affect the organoleptic characteristics of the products. It was found out that 1% inulin added samples preferred by the panellists took higher marks in total (4.1 points) than the others on the 40th day (Fig. 3). Ertekin and Guzel-Seydim (2009) added inulin in kefir samples in their research and they did not determine any negative effect on product quality. Tratnik et al. (2006) reported that sensorial differences were not significant in kefir samples produced with or without inulin addition until 5th or 10th days of storage, but marks given to taste of inulin added kefir samples were lower than the control samples.

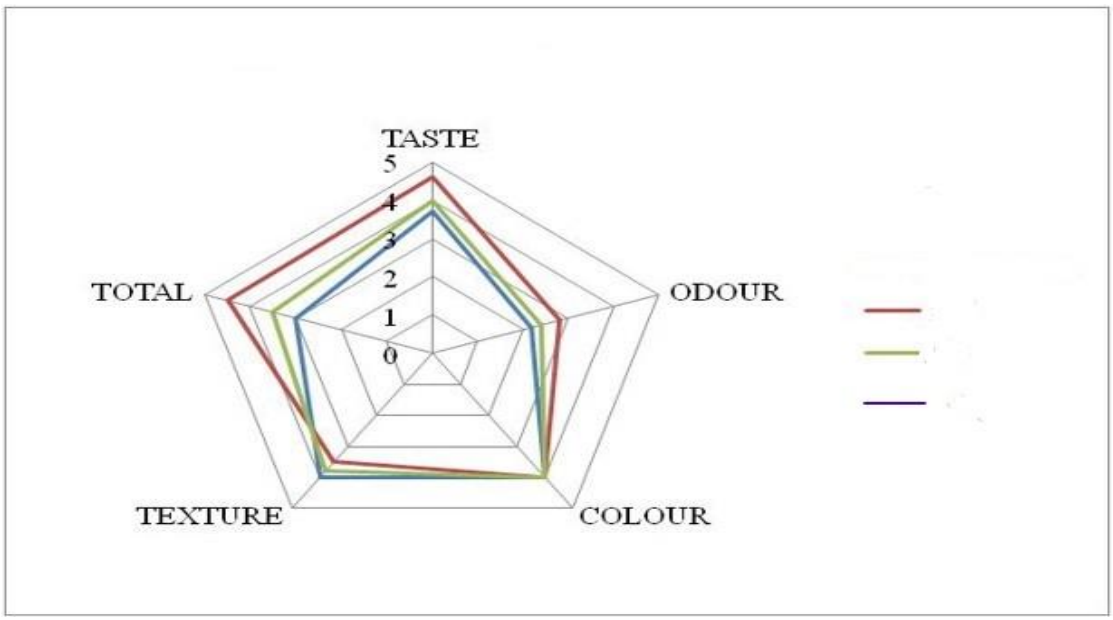


Figure 3. Sensory analyses result of kefir samples (A: 1% w/v inulin added samples; B: 2% w/v inulin added samples; C: Control groups)

Şekil 3. Kefir numunelerinin duyusal analiz sonuçları (A: %1 w/v inülin ilaveli örnekler; B: % 2 w/v inülin ilaveli örnekler; C: Kontrol grupları).

It can be concluded that inulin addition had no effect on the pH values of the product, but the lactic acid changes were found significant for inulin added samples. Inulin addition was also found out to improve viscosity, viability of *Lactococcus* spp., and *Lactobacillus* spp. and sensory properties of kefir. To this end, control kefir samples can be evaluated as a functional probiotic product because of containing >10

log cfu/ ml *Lactobacillus* spp. and *Lactococcus* spp. bacteria numbers. Fortification of goat's milk kefir with inulin can be regarded as an alternative to develop a functional beverage having health and nutritional benefits. As a prebiotic, inulin can provide viability of the probiotic bacteria in kefir for a long storage time. The sensorial properties of kefir can be enhanced with inulin addition as 1% (w/v) concentration.

Contribution of Authors

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

Conflict of Interest

Artcle authors declare that there are no conflicts of interest among them.

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The Effects of Two-Step Tempering Treatment on the Rheological Characteristics of Flour in Bread Wheat (*Triticum aestivum* L.)

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ABSTRACT
In flour milling, tempering is the last process applied to the wheat before grinding and it has a unique and vital role in wheat processing technology. In wheat milling, tempering is conventionally done in one step. In this study, two bread wheat (*Triticum aestivum* L.) varieties (Adana-99 and Russian) with different hardness degree were subjected two-step tempering process in addition to single-step (classical) tempering process. The potential of two-step tempering treatment a) to improve the wheat and flour qualities and b) on the rheological characteristics of wheat flour was the explored. Each wheat variety was subjected to four different tempering treatments. These were; a. no tempering (control), b. single-step tempering for 24 h, c. single-step tempering for 48 h and d. two-step tempering for 48 h. The tempering treatment resulted in a significant improvement in the rheological properties. This positive effect was more evident in the extensograph measurements compared to farinograph measurements. Two-step tempering treatment significantly increased the resistance, ratio and energy values of the dough. This showed that two-step tempering significantly increased strength, force, resistance, and the ability to retain gas properties of dough, which are the most important quality criteria for bread dough. Tempering treatment for 48 h among single step tempering treatments resulted in improved flour quality. The findings conclude that, water when added to the wheat in the tempering process in two-steps results in an improved flour quality in terms of rheological properties.

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Farinograph
Extensograph
Rheological features
Tempering process

İki Aşamalı Tavlama Uygulamasının Ekmeklik Buğdayda (*Triticum aestivum* L.) Unun Reolojik Özelliklerine Etkileri

ÖZET
Un değirmenciliğinde tavlama, öğütmeden önce buğdaya uygulanan son işlemdir ve buğday işleme teknolojisinde benzersiz ve hayati bir role sahiptir. Buğday öğütmede tavlama geleneksel olarak tek aşamada yapılır. Bu çalışmada, farklı sertlik derecelerine sahip iki ekmeklik buğday (*Triticum aestivum* L.) çeşidi (Adana-99 ve Rus), tek aşamalı (klasik) tavlama işlemine ek olarak iki aşamalı tavlama işlemine tabi tutulmuştur. İki aşamalı tavlamanın a) buğday ve un kalitesini iyileştirme ve b) buğday ununun reolojik özelliklerini ıslah etme potansiyeli araştırılmıştır. Her bir buğday çeşidi, dört farklı tavlama işlemine tabi tutulmuştur. Bunlar; a. tavsız (kontrol), b. 24 saat süreyle tek aşamalı tavlı, c. 48 saat süreyle tek aşamalı tavlı ve d. 48 saat süreyle iki aşamalı tavlı. Tavlama işlemi, reolojik özelliklerde önemli iyileşme sağlamıştır. Bu olumlu etki, ekstensograf ölçümlerinde, farinograf ölçümlerine kıyasla daha belirgindir. İki aşamalı tavlama işlemi hamurun direnç, oran ve enerji değerlerini önemli ölçüde artırmıştır. Bu, iki aşamalı tavlamanın ekmek hamuru için en önemli kalite kriterleri olan mukavemet, kuvvet, direnç ve gaz tutma yeteneği özelliklerini önemli ölçüde artırdığını göstermiştir. Tek aşamalı tavlanmış örnekler arasında 48 saat tavlama un

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kalitesini geliştirmiştir. Bulgular, tavlama prosesinde suyun buğdaya iki aşamada eklenmesinin reolojik özellikler açısından un kalitesini geliştirdiğini ortaya koymuştur.

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INTRODUCTION

Wheat is consumed by processed to semi-finished cereal products like flour, semolina, starch, bulgur, and/or to final products like bread, pasta, noodle, cakes, biscuits, crackers, wafers, cookies. Among these products, wheat flour has a particular importance as it constitutes the basic structure of many bakery products both in terms of quality and quantity (Dizlek and Ozer, 2016). As in the factors that determine the quality of all other intermediate and finished products, the quality of wheat flour is also; it depends on the quality of the wheat(s) used in its production and the processing steps applied in the production of the wheat flour.

The processes involved in the processing of wheat into flour and semolina in milling can be grouped under three major groups: 1) Preparation processes (buying and storing of wheat, cleaning, and separating it from foreign substances, blending, washing, and tempering), 2) Milling operation and 3) Flour storage and blending operations. All these process steps affect milling products' qualitative and quantitative properties to be obtained (Delcour and Hoseney, 2010). Additionally, the labor and the rigor shown in the preparation processes help obtain high-quality flour and semolina. Also, there is overall reduction in energy consumption of the milling factory and these results in roller-sieve systems to be used efficiently and an increased lifespan. Therefore, as shown tempering has a special and important place in cereal industry.

In flour milling, tempering, which is the last process applied to the wheat in the preparation stage for grinding before feeding to the rolls to break the wheat and reduce the size to produce the desired material; it is the process of adding cold or hot water to the wheat and resting the it for a while to absorb this water. As it can be understood from the definition, in the tempering process; first the moisture content of the wheat mass is determined and then the appropriate amount of water (target moisture in soft wheat 15-16.5%, in hard wheat 16-18%) is added. After this process, wheat is left to rest in the silos to equilibrate the moisture content and allow evenly distribution, which will result in optimum properties for grinding. The tempering process's main objectives can be specified as follows: To provide easier crushing of wheat, to reduce the energy consumption of the factory, to obtain more flour from a unit amount of wheat, to obtain flour with low ash content (Keskinoğlu et al., 2001; Yoo et al., 2009). Tempering time, tempering water temperature and different tempering methods (warm, hot, steamy, microwave, ultrasound etc.) have been emphasized in the studies (Butcher and Stenvert, 1973; Stenvert and Kingswood, 1976; Moss, 1977; Finney and Bolte, 1985; Ibanoglu, 2001; Keskinoğlu et al., 2002; Sünter, 2003; Kweon et al., 2009; Warechowska et al., 2016).

The rheological properties are significant in grain and milling technology and give an essential idea about bread-making (Diraman et al., 2013; Dizlek and Ozer, 2017a). The rheological properties of the dough prepared from wheat flour can be evaluated by various methods such as farinograph, extensograph, mixograph and alveograph (Diósi et al., 2015; Aslan and Gul, 2016; Gul et al., 2017; Dizlek and Ozer, 2017a). The farinograph test is the essential in terms of being a basic analysis that reveals the rheological properties and bread qualities of flour during kneading (Basar et al., 2016). The most critical rheological properties of dough are often described as resistance (elasticity), strength and extensibility (EX) are determined by Extensograph (Dizlek and Ozer, 2017a).

The amount of water to be supplied to the wheat mass during tempering is usually given at one (single) step. However, in this study, two bread wheat (*Triticum aestivum* L.) varieties with different hardness degree were subjected to a two-step tempering process in addition to single-step (classical) tempering process. The potential of this treatment to improve the wheat and flour rheological and bread properties was then investigated.

MATERIAL and METHODS

Material

Wheat: In the study, two different bread wheat (*Triticum aestivum* L.) varieties namely a low-protein, soft 'Russian' variety imported from Russia and a domestic, medium hard 'Adana-99' wheat variety were used. Both wheat samples were 2017 crop season. The Adana-99 variety was supplied from İslamoğlu Trade in Osmaniye (Turkey) and the Russian variety was supplied from Sunar Özlem Flour Factory (Osmaniye, Turkey). Approximately 100 kg of each wheat variety was supplied. The samples taken from the wheat

masses in accordance with the sampling method were stored in 50 kg jute bags and stored in cold storage (+4 °C) until they were analyzed and ready for milling.

Flour: After applying different tempering processes to the two wheat varieties, the wheat varieties were milled according to method (Kurt and Dizlek, 2020) described in method section to obtain subsequent flours.

Water: Potable water supplied within the campus of University of Osmaniye Korkut Ata (Osmaniye, Turkey) was used in tempering of the wheat.

Laboratory Type Mill: Wheat samples were milled into flour by using; the first three pieces of breaking, the last two reducing rolls, 'Chopin' brand 'CD1' model tempered wheat grinding mill (Chopin Technologies, Paris, France).

Methods

Formation of Experimental Wheat Groups

With the tempering, the target moisture content of the Adana-99 wheat used in the study was 17% and the Russian wheat was 16.5%. The amount of tempering water for given a wheat masses was calculated according to American Association of Cereal Chemists International (AACCI) Method 26-95.01 (AACCI, 2010). Wheat samples were tempered by cold tap water. Within the scope of this research, the experimental materials and the pattern were formed by applying the following different tempering treatments in each of the two bread wheat varieties whose qualities are different (Table 1):

a) no tempering (= control)
b) single-step tempering for 24 h (The amount of water calculated according to the target moisture is given to the wheat at one time and tempered for 24 h)
c) single-step tempering for 48 h (The amount of water calculated according to the target moisture was given to the wheat in one time and tempered for 48 h)
d) two-step tempering for 48 h (Adana-99 and Russian wheat were initially treated with 15% and 15.5% moisture content, respectively, with the first tempering water and resting for 24 h. At the end of the 24 h period, second tempering water added to Adana-99 and Russian wheat to a target moisture of 17% and 16.5%, respectively (In summary, the wheat samples were tempered two times and the final moisture content of the wheat was adjusted to the percentage in b) and c)).

Taking into account commercial conditions, in the study, also the flour obtained from wheat, (tempered according to item d)), was rested in cool and dry room conditions for 30 days. The rheological properties of this sample were also studied.

Table 1. Experimental design of the research.
Çizelge 1. Araştırmanın deneysel tasarımı.

Tempering Time (*Tavlama Süresi*) (h) (*saat*)	Tempering Number (*Tavlama Sayısı*)	Wheat Variety (*Buğday Çeşidi*)	
		Adana-99	Russian Rus
0 (non-tempered=control) (*tavsız=kontrol*)	0	x	x
24	1	x	x
48	1	x	x
48	2	x	x
48[a]	2[a]	x	x

[a] Wheat flour that has been tempered two-step for 48 h and kept in room conditions for 30 days.

Grinding Process

Optimally tempered wheat samples were separately milled into flour using a laboratory mill. Cleaned and tempered wheat was first broken by the crushing system, then semolina, coarse bran and some crushed flour were separated from crushed wheat. The semolina obtained as the main product was reduced to the flour by milling in the reduction system and divided into refined fine bran by-products. The separated refined fine bran was passed through the reduction system for a second time to obtain whole flour. These flour samples were used for rheological analyses. Adana-99 and Russian wheat samples were milled at 52.3% and 61.3% "wheat flour" extraction rates, respectively.

Analysis of Wheat Samples

The physical and chemical properties of two different wheat samples used in the study are given in detail in the previous study (Kurt and Dizlek, 2020). Some of the technological properties of flour samples were determined according to AACCI Methods therefore Adana-99 and Russian wheat samples had average 28.5% and 21.6% wet gluten content; 9.2% and 6.7% dry gluten content; 38 and 32 mL sedimentation value; 31 and 20 mL delayed sedimentation value; 337 and 356 s falling number value, respectively.

Measurements of Rheological Characteristics of Wheat Flour Groups

Wheat flour samples were objected to farinograph and extensograph tests with the purpose of determining

important rheological parameters of wheat flours for cereal industry. AACCI Approved Method 54-21.02 (AACCI, 2010) was followed to determine farinograph properties (water absorption [WA], dough development time [DDT], stability time [ST], softening degree [SD], and farinograph quality number [FQN]). The mixing bowl for 300 g flour was used. AACCI Approved Method 54-10.01 (AACCI, 2010) was used to determine extensograph features. For this purpose; resistance to extension (R_5), maximum resistance to extension (R_{max}), EX, ratio (R_{max} EX^{-1}) and energy values in 45th', 90th', and 135th' were determined. An average of the 3 values was used in the discussion of the results. Rheological properties were determined two days after flour milling. Briefly, tempered flour from each treatment group was compared with the flour of non-tempered group (control).

Statistical Analysis

All experiments were carried out in two replicates. Means and standard deviations were determined using Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA). Variance of analysis (ANOVA) was used to obtain conclusion on measured characteristics of wheat and flour samples. Duncan's multiple comparison test was used to determine difference among treatment means at ($P<0.05$). Statistical analysis was performed using the statistical package program developed by the SAS statistical institute (The SAS System for Windows v6.12).

RESULTS and DISCUSSION

Effects of Different Tempering Treatments on Farinograph Properties of Flour

The farinograph test has been a standard tool of the cereal chemists for many years, giving information related to WA and kneading properties of wheat flours (D'Appolonia and Kunerth, 1984; AACCI, 2010; Aydoğan et al., 2012; 2015). The farinograph test is performed to evaluate the behavior of dough against mixing at a specified constant speed with specified water addition (Diósi et al., 2015). Farinograph DDT is an essential indicator of protein quality of flour and strong flour has been reported to have a higher DDT than weaker ones (Karababa and Ozan, 1998). It is reported that the ST is a parameter indicating the flour strength, tolerance of flour to kneading and that strong flours have high ST (Basar et al., 2016).

Farinogram values of flour obtained from Adana-99 and Russian wheat samples with different tempering treatments are summarized in Table 2. In both wheat samples, it was determined that tempering process reduced WA of flour ($P<0.05$). The results showed, among the single-step tempered samples, wheat flour sample which was tempered for 48 h had higher WA than the 24 h tempered wheat; whereas among the 48 h tempered wheat samples, single-step tempered sample had higher WA than two-step tempered sample; among two-step tempered samples, the sample whose flour was rested for 30 days had higher WA. The easier separation of the flour from the bran by tempering treatment, and therefore the easier release of the pure endosperm layer by milling reduced the transition of compounds with high water holding ability such as cellulose and pentosane in the bran layer to flour, and this situation led to a decrease in WA capacity. Also Sünter (2003) reported that, increasing the tempering time from 12 h to 24, 36 and 48 h resulted in a decrease in the farinograph WA values of the flour samples. It was determined that there was no significant difference ($P>0.05$) between two different tempering treatments (single and two-step) in terms of DDT.

Table 2. Effect of tempering treatment on farinograph properties of two wheat varieties[1].
Çizelge 2. Tavlama işleminin iki buğday çeşidinin farinograf özelliklerine etkisi[1].

Wheat Variety (*Buğday Çeşidi*)	Tempering Treatment (*Tavlama Muamelesi*)	Water Absorption (*Su Absorpsiyonu*) (%)	Dough Development Time (*Hamur Gelişme Süresi*) (min) (*d*)	Stability Time (*Stabilite Süresi*) (min) (*d*)	Softening Degree (*Yumuşama Derecesi*) (B.U.)[2]	Farinograph Quality Number (*Farinograf Kalite Sayısı*)
Adana-99	non-tempered (control)	60.6^{a}±0.3	2.4^{a}±0.1	8.4^{d}±0.2	66^{a}±7	92±8
	single-step tempered for 24 h	58.2^{c}±0.2	2.2^{a}±0.0	11.4^{c}±0.3	41^{b}±3	41^{b}±2
	single-step tempered for 48 h	58.9^{b}±0.1	2.3^{a}±0.0	12.6^{b}±0.1	34^{c}±2	51^{a}±3
	two-step tempered for 24 h	58.1^{c}±0.2	2.2^{a}±0.1	12.9^{a}±0.1	31^{c}±3	43^{b}±2
	two-step tempered for 48 h (rested for 30 days)	59.1^{b}±0.3	2.4^{a}±0.1	13.1^{a}±0.2	30^{c}±4	42^{b}±1
Russian	non-tempered (control)	57.6^{a}±0.3	1.7^{a}±0.1	2.7±0.2	68^{b}±7	36^{a}±3
	single-step tempered for 24 h	54.8^{c}±0.2	1.7^{a}±0.0	2.8±0.2	81^{ab}±7	31^{a}±3
	single-step tempered for 48 h	56.2^{b}±0.2	1.7^{a}±0.2	2.3±0.3	94^{a}±9	27^{a}±3
	two-step tempered for 24 h	55.2^{c}±0.2	1.7^{a}±0.0	2.7±0.3	80^{ab}±6	31^{a}±2
	two-step tempered for 48 h (rested for 30 days)	58.3^{a}±0.4	1.7^{a}±0.1	2.8±0.2	60^{c}±2	32^{a}±2

[1] Mean values in the table for the same column and same variety (Adana-99 or Russian) shown with the different superscript letter are significantly different ($P<0.05$). [2] Brabender Unit.

WA is the amount of water required by a given weight of flour to yield dough of given consistency. Farinograph WA is mainly influenced by the properties of flour main components, starch and gluten. It correlated also with dough ST. High WA is desirable. High WA combined with low SD indicates good quality flour, whereas a high WA combined with a high SD indicates poor quality flour. Farinograph ST is correlated with flour strength. Long ST are generally more suited for variety bread production and often require longer kneading times (Aydoğan et al., 2015).

There was no statistically significant difference ($P>0.05$) between the samples of Russian wheat variety in terms of ST, I contrast there was a considerable difference between the ST of samples in Adana-99 wheat variety. Accordingly, the tempering process increased the ST of samples in Adana-99 variety ($P<0.05$). This increase was highest in two-step tempered samples. It was determined that the 48 h tempered samples had a higher ST than the 24 h tempered samples. ST (in full) of tempered samples was higher than the control sample, thus the ST, which is an important farinogram parameter as it shows flour strength and the processing tolerance of the dough (Dizlek and Ozer, 2017a), additionally, it illustrate how tempering process is important. It was found that the different tempering treatments in the experiment caused a meaningful variation among the flour samples of the Adana-99 variety. It was therefore determined that the two-step tempering process significantly improved ($P<0.05$) the most critical farinogram quality parameter (Table 2). However, the same conclusion was not observed with Russian wheat variety. It was also observed that, the ST of the two wheat samples was different due to the diverse characteristics of the wheat varieties. According to this, the ST of the flours belonging to the Adana-99 variety was 3-5 times higher than that of the Russian variety. In accordance with the ST, SD of Adana-99 variety decreased with tempering treatment. The reduction was higher in the 48 h tempered samples. Clearly from the results 48 h tempered samples with a SD halved, relative to the control sample, however, a two-step tempering has a beneficial effect in improving the flour quality. In the Adana-99 variety, although there is a significant difference between the control sample and tempered samples in terms of SD, there is limited difference between the tempered samples. A contradictory result in SD was witnessed in the Russian variety treated wheat samples. A sample with the best SD was the tempered twice for 48 h and the flour was rested for 30 days. A low FQN, has been associated with weak flour, in contrast a high value is associated with strong flour. It is not advisable to evaluate FQN for untampered wheat (D'Appolonia and Kunerth, 1984). Therefore, the FQN of all samples, including the non-tempered samples, were determined during the farinograph measurements, but the control sample was not taken into consideration when comparing the treatments. Values of FQN showed that the Adana-99 variety has superior properties compared to the Russian variety. It has been determined that there is no significant difference between the tempered treatments in terms of FQN (Table 2).

Codina (2010) provides the following information using the Brabender farinograph device for the determination of flour quality. Flours with WA capacity of more than 65% are of very high quality, flours of 60-65% are of high quality, flours of 55-60% are acceptable quality, and flours of less than 55% are unacceptable (inadequate) quality. Flours with DDT of more than 3 min are very high quality, flours with 2-3 min are high quality, flours with 1.5-2 min are acceptable quality, and flours with less than 1.5 min are unacceptable quality. Flours with a ST of more than 8 min are of very high quality, flours with 5-8 min is high quality, flours with 3-5 min are acceptable quality, and flours with less than 3 min are unacceptable quality. Flours with a dough SD of less than 60 B.U. are of very high quality, flours with 60-80 B.U. are high quality, flours with 80-100 B.U. are acceptable quality, and flours with more than 100 B.U. are unacceptable quality. Flours with FQN of more than 65 are very high quality, flours with 50-65 are high quality, flours with 40-50 are acceptable quality, and flours with less than 40 are unacceptable quality. According to the data stated by Codina (2010), Adana-99 sample had acceptable quality in terms of WA and FQN; The Russian sample had acceptable quality in terms of WA, DDT, and SD; it was determined that the Adana-99 had very high quality in terms of ST and SD, whereas Russian variety had low quality in terms of ST and FQN (Table 2). On the other hand, it was reported that (Canadian Grain Commission, 2009) DDT value was lower than 2 min in weak flour, 2-3 min in medium strong flour, 3-5 min in strong flour, and 5-12 min in very strong flour. The ST value was lower than 4 min in weak flour, 4-7 min in medium strong flour, 7-14 min in strong flour, and higher than 14 min in very strong flour. According to these data, Adana-99 wheat flour groups was evaluated as (medium) strong, while the Russian wheat flour groups was evaluated as weak (Table 2).

In the study, while the application of different tempering treatments on the Adana-99 wheat variety resulted in a significant ($P<0.05$) and meaningful modification on the ST and SD of flour samples, it is not possible to mention the same effect on Russian variety. Similarly, Warechowska and co-workers (2016) reported that, in the Astoria and Cytra wheat used in their research, the increase in the amount of moisture (from 12% to 18%) added to the wheat by tempering caused an average of 4 min increase in the

ST and an average of 35 B.U. decrease in the SD of the flour. These values had a statistically significant effect on these criteria, but the effect was insignificant in the Radunia wheat variety. These findings indicate that tempering treatments do not have the same effect on all wheat varieties, so it is useful and necessary to determine the appropriate tempering conditions for each wheat variety. It was observed that when the tempering treatment was applied, gluten quality (ST) of the Adana-99 wheat flour samples sharply increased and the WA capacities of both flour samples reduced according to Table 2 ($P<0.05$).

Effects of Different Tempering Treatments on Extensograph Properties of Dough

Extensograph, draws a unique graph about the dough's resistance against extension and it's EX (D'Appolonia and Kunerth, 1984). The extensograph records a force-time curve for a piece of dough stretched until it breaks. Attributes of force-time curves (extensograms), are used to assess the dough general quality characteristics. In the evaluation of extensogram; R_5, R_{max}, EX, energy, and ratio are the common measurements (AACCI, 2010). The greater the dough ratio and energy values, the greater the fermentation tolerance, gas holding ability, and suitability for processing. Holistically, the extensograph test gives unique clues concerning bread-making, since, determination of the test takes longer period approximately (135 min) as in regular bread-making (Dizlek and Ozer, 2017 a; b).

The data on extensograph measurements is presented in Table 3. Tempering of wheat samples resulted in a significant increase in the dough resistance values ($P<0.05$). This can be seen by examining the values of R_5 and R_{max}. The data on the Adana-99 variety clearly shows that the best treatment is a two-step tempering process. The two-step tempering treatment increased the dough resistance value one of the dough quality indicator, it is one of the critical. It was determined that samples having the best resistance value after two-step tempering treatments were single-step tempering for 48 h, followed by single-step tempering for 24 h. However, it was also observed that even in single-step tempered samples for 24 h dough resistance was significantly higher than what was experienced in non-tempered control sample. However, the positive effect of the tempering process on the dough resistance of the Adana-99 variety was more noticeable than the Russian variety. The dough resistance of the 48-h two-step tempered wheat was 75% higher in R_{max} value and 82% higher in R_5 value than the control sample.

Table 3. Effect of tempering treatment on extensograph properties[1] of wheat flours[2].
Çizelge 3. *Tavlama işleminin buğday unlarının[2] ekstensograf özelliklerine[1] etkisi.*

Wheat Variety (*Buğday Çeşidi*)	Tempering Treatment (*Tavlama Muamelesi*)	R_5 (Resistance to Extension) (*Uzamaya karşı direnç*) (B.U.)[3]	R_{max} (Dough Resistance) (*Hamur Direnci*) (B.U.)[3]	Extensibility (EX) (*Uzayabilme Yeteneği*) (mm)	Ratio (R_{max} EX^{-1}) (*Oran*) (B.U. mm^{-1})	Energy (*Enerji*) (cm^2)
Adana-99	non-tempered (control)	$295^{d}\pm12$	$419^{d}\pm18$	$174^{a}\pm3$	$2.41^{d}\pm0.11$	$99^{d}\pm2$
	single-step tempered for 24 h	$343^{c}\pm12$	$497^{c}\pm17$	$161^{b}\pm3$	$3.07^{c}\pm0.10$	$105^{c}\pm1$
	single-step tempered for 48 h	$398^{b}\pm19$	$573^{b}\pm26$	$165^{b}\pm2$	$3.47^{b}\pm0.15$	$123^{b}\pm2$
	two-step tempered for 24 h	$537^{a}\pm27$	$735^{a}\pm26$	$150^{c}\pm2$	$4.88^{a}\pm0.18$	$139^{a}\pm3$
	two-step tempered for 48 h (rested for 30 days)	$494^{a}\pm29$	$678^{a}\pm27$	$151^{c}\pm1$	$4.47^{a}\pm0.19$	$133^{a}\pm4$
Russian	non-tempered (control)	$331^{c}\pm8$	$385^{c}\pm10$	$127^{a}\pm3$	$3.02^{d}\pm0.07$	$67^{c}\pm2$
	single-step tempered for 24 h	$370^{b}\pm10$	$438^{b}\pm11$	$130^{a}\pm2$	$3.36^{c}\pm0.08$	$76^{b}\pm1$
	single-step tempered for 48 h	$428^{a}\pm13$	$492^{a}\pm18$	$117^{b}\pm3$	$4.21^{a}\pm0.11$	$76^{b}\pm2$
	two-step tempered for 24 h	$374^{b}\pm9$	$455^{ab}\pm16$	$131^{a}\pm3$	$3.46^{c}\pm0.06$	$79^{ab}\pm3$
	two-step tempered for 48 h (rested for 30 days)	$399^{a}\pm14$	$489^{a}\pm18$	$132^{a}\pm3$	$3.70^{b}\pm0.09$	$85^{a}\pm3$

[1] The average values of 45th', 90th', and 135th' measurements were given.
[2] Mean values in the table for the same column and same variety (Adana-99 or Russian) shown with the different superscript letter are significantly different ($P<0.05$).
[3] Brabender Unit.

In contrast to resistance values in the Adana-99 variety, tempering treatment caused a decrease in the EX of the doughs. Accordingly, the doughs belonging to the two times tempered applications with the highest resistance value had the lowest EX value, with respect to the control the EX of these dough decreased by about 14%. On the other hand, it was found that there was a limited variation in terms of the EX values of the doughs in the Russian variety, and just that according to the control sample 1 cm (about 9%) decrease was observed in the dough's EX of the wheat sample which was single-step tempered for 48 h. Expectedly, dough resistance increased but there was a reduction in EX values with the tempering treatment. The ratio values of the dough samples increased significantly ($P<0.05$) compared to the control sample. The increment is more

than twice as much as the control sample for the Adana-99 variety two-step tempered sample for 48 h. The positive effect of tempering treatment was also observed in the Russian variety in terms of ratio values. However, it was determined that the best series in the Russian variety in terms of ratio value emerged after a 48-h single-step tempering treatment. It has been determined that two-step tempered treatments had the highest value in terms of energy value, which is an important indicator of the dough gas holding ability. In the Adana-99 variety, the energy value of the two-step tempered sample for 48 h (without flour resting) was 40% higher than that of the control sample. Depending on the variety properties, the energy value of the dough of Adana-99 non-tempered control sample is approximately 50% higher than the Russian sample of the same feature. In both varieties, the energy values of the dough samples increased parallel with an increase in the number (from 1 to 2) and time (from 24 h to 48 h) of tempering. On this increment, the effect of the number of tempering was found to be more dominant than the tempering time. A similar effect can be mentioned in terms of other extensogram criteria. By examining the findings of the extensograph data together (Table 3); there are very significant differences between the different treatments discussed in the experiment, these differences are more obvious in Adana-99 variety. There is a limited difference between the treatments in the farinographic measurements (Table 2), however it was determined that the difference in the characteristics of the dough, which is an extremely important indicator about the bread quality of the flour, and in this sense, the dough, which is rested for 90, 135 min as in bread making, is observed more clearly in the extensograph measurements. The data of the extensograph measurements showed a high correlation with the ST and SD values of Adana-99 variety, as mentioned above, the top (superior) flour and dough characteristics of two-step tempered treatments, which constitute the basic sheet leg of the study, have been demonstrated. Clearly it can be seen by examining the resistance values (Table 3), particularly in two-step tempering, generally tempering, significantly increased the strength, force and resistance of the dough. Resistance criterion is the most important quality criterion taken into consideration in extensograph measurements (AACCI, 2010). It was observed that the Russian variety having lower bread-making quality than the Adana-99 also improved its bread-making quality through the tempering treatments (dough strength, resistance, gas holding, and fermentation ability increased).

Generally, analytical findings from samples tempered for 48 h are better than samples tempered for 24 h. This situation is thought to result from the use of cold tempering method in the tempering of wheat samples in the study. Özkaya and Özkaya (2005) reported that homogeneous distribution of water in grain during cold tempering requires a relatively long period of time, which may be up to 72 h. The findings obtained from our study are consistent with the findings and/or conclusions of previous researchers. Accordingly, Tekeli (1964) and Diraman (2010) stated that, by the steam-tempering of the sunn pest-damaged wheat, negative conditions in dough processing such as sticky, runny, splay, soft dough characteristics, difficult to shape dough, poor hand, and machine processing ability of dough can be eliminated. Similarly, Posner and Hibbs (1997) reported that, tempering in the temperature range of 36-43 °C prevents the negative properties of dough such as runny and splay. Warechowska and co-workers (2016) declared that the dough belonging to the tempered treated wheat had a more stable structure during kneading.

CONCLUSIONS

In this study, the effects of two-step tempering treatment, on the rheological characteristics and hence bread properties of two different bread wheat were investigated. There was no significant difference between the tempering treatments in the Russian variety in terms of farinograph values, however with an increase in the number and time of tempering in Adana-99 variety, the ST of flour samples increased, SD and WA ability reduced. Moreover, there was a significant difference in extensographic measurements. The findings from extensograph defines the dough testing properties while flour testing properties are defined by farinograph parameters, therefore, both parameters providing more meaningful information in describing bread quality. Extensographic data showed that it is more useful to apply the tempering process to wheat at two-step rather than single-step. In these measurements, the best series compared to other treatments appeared in the 48-h two-step tempered samples. Subsequently, it was concluded that tempering time would be more appropriate for 48 h compared to 24 h. As a result, the tempering treatment resulted in a significant improvement in the rheological properties of flour. This positive effect was more evident in the extensograph measurements compared to farinograph measurements. Two-step tempering treatment significantly increased the resistance, ratio and energy values of the dough. This showed that two-step tempering treatment significantly increased ($P<0.05$) strength, force, resistance, and the ability to retain gas properties of dough, which are the most important bread dough quality parameters. Tempering treatment for 48 h among single-step tempering treatments resulted in a more improved flour quality. As a result; the findings include clues that, the fact that water is added to the wheat in the tempering process in two-

steps improved the flour quality in terms of rheological properties. However, it is clear that further studies are required on this subject.

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Author's Contributions

All authors contributed to the study conception and design. Mustafa Kurt has provided research materials, milled the wheat groups and performed the laboratory work. Halef Dizlek performed the statistical analysis, interpreted the results and drafted the manuscript. The first draft of the manuscript was written by Halef Dizlek. Tempering process of wheat samples and writing - original draft preparation of the manuscript were written by Mustafa Kurt and Halef Dizlek; Writing - review and editing were performed by Halef Dizlek. All authors commented on previous versions of the manuscript. Also all authors read and approved the final manuscript. Halef Dizlek is the Master of Science supervisor of Mustafa Kurt.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Mineral Composition of Elements in Red Lentil and Chickpea Cultivars Grown in Southeast of Turkey

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ABSTRACT

Legumes such as red lentils and chickpeas, which are the most important legumes of the Southeastern Anatolia Region, have a rich content of many mineral substances. However, very little work has been done in this area in the region. In this study, contents of mineral elements such as K, Ca, Mg, Zn, Fe, Cu, Cd, Pb, Cr, As, Se, V, Sn, Mn, Si and Ni were determined by inductively coupled plasma optic emission spectrometry (ICP-OES) method in six red lentil and two chickpea cultivars registered by GAP International Agricultural Research and Training Center (GAPUTAEM). In addition, the element concentrations of the species and the comparison of the element amounts in lentils and chickpeas were evaluated. Element concentrations in lentil varieties were listed in descending order as follows K> Mg> Ca> Fe> Si> Zn> Sn> Mn> Cu> Ni> Cr> Se> V> Cd> Pb> As, while in chickpea cultivars are; K> Ca> Mg> Fe> Zn> Si> Mn> Sn> Cu> Ni> V> Cr> Se> Pb> Cd> As. The study results indicated that registered lentil and chickpea varieties were rich in potassium. On the other hand, calcium content of chickpea cultivars were higher than those of lentils. In addition, toxic heavy metals such as Cd, Pb and As concentrations were determined to be below the limits. The results showed that legumes were very important as mineral resources. Lentils and chickpeas were very similar in terms of macro and micro element contents. Additionally, it was observed that they had a nutritionally safe mineral structure.

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Türkiye'nin Güneydoğusunda Yetişen Bazı Kırmızı Mercimek ve Nohut Çeşitlerinin Mineral Bileşimi

ÖZET

Güneydoğu Anadolu Bölgesinin en önemli bakliyat ürünleri olan kırmızı mercimek ve nohut gibi baklagiller ayrıca mineral madde bakımından zengin bir yapıya sahiptir. Ancak bölgede bu alanda çok az çalışma yapılmıştır. Bu çalışmada, K, Ca, Mg, Zn, Fe, Cu, Cd, Pb, Cr, As, Se, V, Sn, Mn, Si ve Ni gibi mineral elementlerin konsantrasyonları GAP Uluslararası Tarımsal Araştırma ve Eğitim Merkezi (GAPUTAEM) tarafından tescilli altı kırmızı mercimek ve iki nohut çeşidinde indüktif eşleşmiş plazma optik emisyon spektrometresi (ICP-OES) ile belirlenmiştir. Ayrıca türlerin element konsantrasyonlarının belirlenmesi, mercimek ve nohuttaki element miktarlarının karşılaştırılması da değerlendirilmiştir. Mercimek çeşitlerinde element konsantrasyonları azalan sırayla K> Mg> Ca> Fe> Si> Zn> Sn> Mn> Cu> Ni> Cr> Se> V> Cd> Pb> As, nohut çeşitlerinde ise; K> Ca> Mg> Fe> Zn> Si> Mn> Sn> Cu> Ni> V> Cr> Se> Pb> Cd> As şeklinde olmuştur. Araştırmada tescilli mercimek ve nohut çeşitlerinin potasyum elementi yönünden zengin olduğu tespit edilmiştir. Nohut çeşitlerinde ise kalsiyum içeriği mercimek çeşitlerine göre daha yüksek bulunmuştur. Ayrıca toksik ağır metaller Cd, Pb ve As derişimlerinin sınır değerlerin altında olduğu belirlenmiştir. Sonuçlar bize baklagillerin çok önemli mineral

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Mineraller
ICP-OES

kaynakları olduğunu göstermiştir. Mercimek ve nohutun, makro ve mikro element içerikleri bakımından birbirine çok benzer olduğu, ayrıca besin açısından güvenli mineral değerlere sahip oldukları görülmüştür.

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INTRODUCTION

Food legumes grown in almost every region of our country constitute the protein source of Turkish cuisine and especially for low-income families. Lentil (*Lens culinaris*), which is among edible legumes, has an important place in human nutrition due to its high protein content. According to TURKSTAT data; in 2018, 51.5% of legume production was chickpea and 25.3% was red lentil (Anonymous, 2020). Turkey's south-eastern region is knon as the motherland of gene center of legumes. It was reported that 630 thousand tons of chickpea and 310 thousand tons of red lentil were produced in Turkey (TÜİK, 2019). Overaal, 77.5% of the production of red lentils is done in Şanlıurfa, Diyarbakır and Mardin, respectively. Chickpea has an important place in human nutrition thanks to 20-25% protein, 40-60% carbohydrate, 4.5-5.5% fat, phosphorus and calcium in their grains (Babaoğlu, 2013). Dried legumes are rich in minerals. One of the most important issues is the accumulation of toxic metals in the food structure. The concentrations of metals as a result of deposition may rise well above those in water and in the air. The human or animal who receives such a highly toxic metal-containing food can be poisoned. It also has the ability of the human body to accumulate some toxic metals. For example, the half-life of lead in the human body is 1460 days, that of cadmium is 200 days, that of zinc is 933 days (Gündüz, 2004). Minerals in the composition of foods cover a large and complex element group. Many of these are necessary for humans, and especially some trace level elements must be present in the body at certain concentrations. High levels of elements in the body are called macro elements, while those found in small amounts are called micro or trace elements. This distinction was made not by their physiological significance, but by their density. Basic elements provided through food; potassium, sodium, calcium, magnesium, chlorine, sulfur and phosphorus. Some other elements are: iron, copper, iodine, cobalt, selenium, fluoride and zinc. The elements whose nutritional values are not yet known are aluminum, boron, chromium, nickel and tin. Therefore, it is very important to analyze minerals and trace elements of all products. Food and Agriculture Organization (FAO) and World Health Organization (WHO), European Commission (EC) and other regulatory bodies of other countries strictly regulate the allowable concentrations or maximum permitted concentrations of toxic heavy metals in foodstuffs (FAO/WHO 1984; EC, 1989). In the World there are a lot of studies about trace elements in foods. For instance, Ereifej at al. (2001); Salama and Radwan(2005); Momen at al. (2006); Erdoğan at al. (2006); Reyes at al.(2010) studied in this fild. Few studies have been made in this field in Turkey. There has been almost no study about minerals related content of legumes grown in our region. In this study we aimed to determine mineral elements in registerd red lentils and chickpea cultivars grown in Southeast of Turkey and varieties are established for nutrition and food safety. The function of some important elements can be summarized as follows (Table 1).

Calcium: An adult has 1.2-1.5 kg of calcium in the body of which about 99% is in the skeletal system. Although no side effects have been observed in adults taking up to 2.5 grams of calcium per day, doses above this cause various diseases (Baysal, 1996; Bilge, 1998; Keskin, 1987; Vaessen and Kamp, 1990; Erdoğan, 2002).

Magnesium: It is found together with calcium and phosphorus in the structure of bones and teeth.

İron: The amount of iron in the body is 4-5 g. Most of this is found in hemoglobin and myoglobin pigments. When there is not enough iron in the body, the number of blood cells decreases in "iron deficiency anemia" anemia, the amount of hemoglobin is reported to be 15 mg per day for women and 12 mg for adult men (Bilge, 1998; Keskin, 1987; Levy, 1998).

Copper: The amount of copper in the body is around 100-150 mg. 2-5 mg of copper is taken with an average daily diet.

Chromium: Chromium (Cr) is among several essential trace elements. Chromium optimizes insulin function and can have a marked impact on the metabolism of carbohydrates, proteins, and lipids. Chromium has been found to improve in vivo insulin activity, which optimizes tolerance to glucose. In addition, Cr protects the body against arteriosclerosis. (Anonymous, 2021)

Manganese: It is an essential trace element commonly found in plant and animal cells. The human body contains 10-40 mg of manganese. It is at the level of 2-48 mg day^{-1} that can be met by daily nutrition. Safe

and adequate intake of manganese is thought to be 2.0-5.0 mg day^{-1} (Baysal, 1996; Bilge, 1998; Keskin, 1987).

Selenium: Selenium and Vitamin E support each other in terms of antioxidant effects. The recommended daily intake is 50-200 micrograms. 30-150 micrograms is sufficient for children (Özyılmaz, 1999).

Table 1. A person weighing an average of 70 kg per day the amount of elements to be taken (Erdoğan 2002).
Çizelge 1. Günde ortalama 70 kg ağırlığında bir kişinin alması gereken element miktarları (Erdoğan 2002).

Elements	*Amount (mg day^{-1})*	*Elements*	*Amount (mg day^{-1})*
Zn	15	**Co**	0.04
Mn	2.8 (2-5)	**Ni**	0.025
Fe	15 (10-28)	**Cr**	0.05-0.20
Cu	2.5 (2-3)	**Pb**	0.415
Sr	1.6 (0.98-2.2)	**Mg**	300
Ba	1.1 (0.65-1.7)	**Ca**	500

Zinc: The amount of zinc contained in tissues in the human body varies between 2-4 g. The requirement met by a normal diet is 6-22 mg day^{-1}. Zinc is an important part of many enzymes and is found in more than 200 enzymes. Zinc requirement is determined as 12 mg day^{-1} for an adult woman and 15 mg day^{-1} for a man (Bilge, 1998; Onianwa at al., 2001).

Potassium: The concentration of this element in the body is 2 mg g^{-1}. It regulates the osmotic pressure in the cell as it is usually found inside the cell. The amount of potassium taken with a normal diet is 2-5.9 g day^{-1} and the minimum requirement of potassium varies between 1.6-2.0 g per day (Baysal, 1996; Bilge, 1998; Keskin, 1987; Erdoğan, 2002).

Sodium: The sodium content in the body is 1.4 mg g^{-1}. Sodium, together with potassium, is involved in the regulation of the osmotic pressure of the body fluid. Sodium absorption by the body is very fast. Sodium intake of the body under normal conditions varies between 1.7-6.9 g day^{-1} (Bilge, 1998; Keskin, 1987; Erdoğan, 2002).

Silicon: In animal experiments, silicon deficiency causes growth retardation, bone, cartilage and connective tissue disorders. However, no determination has been made regarding silicon deficiency in humans so far.

Vanadium: Firstly, vanadium was found to be essential for bone and tooth development. It is on record that vanadium plays an important role in carbohydrate metabolism.

MATERIAL AND METHOD

Materials

Registred six red lentil varieties (Çağıl, Altıntoprak, Seyran-96, Fırat-87, Yerli Kırmızı, Tigris) and Chickpea cultivars (Diyar-95, ILC-482) were taken from Food Legumes Unit of GAP International Agricultural Research and Training Center. Samples were stored in polipropilen cups until analysis time.

Methods

Digestion procedure: About 0.25 g dried and ground sample was put into burning cup and 8 ml 65% HNO_3, 2 ml 30% H_2O_2 added. The samples were dissolved in a microwave oven (Milestone Smart D) according to program showing at table 2. Samples dissolved and diluted 25 ml volume with ultra pure water. Concentrations were determined with ICP-OES (Thermo ICAP 6300).

Table 2. Sample digestion program for lentil and chicpea cultivars
Çizelge 2. Mercimek ve nohut çeşitleri için örnek çözünürleştirme programı

Step	*Time(min)*	*T (°C)*	*Power(W)*
1	20:00	180	1200
2	20:00	180	1200

Instrumentation

Parameters related to the device and method are shown in the tables below table 3, table 4 and table 5.

Statistical Analysis

The Mann-Wihitney Test, which is one of the nonparametric tests applied between two independent groups to determine whether there is a statistically significant difference between the element concentrations contained in the lentil and chickpea samples. As a result of the test, it was seen that there was a difference between the groups in terms of the mean of Ca, K, Mn, Cu, Ni and Se according to the level of significance ($P < 0.05$).

Correlation between elements was determined by considering the Spearman rho coefficient.

RESULTS and DISCUSSION

Results

In this study, the results are presented in Table 6. As a result of our research on different varieties of red lentil and chickpea varieties the amount of Ca was found in the lentils and chickpeas between 601±5.4-950±41 and 1038±21-1499±15 mg kg^{-1} respectively. The results correspond to those reported prevously for cowpea (Harmankaya et al., 2016), bean (Ceyhan, 2001; Ceyhan et al., 2008; Harmankaya et al., 2009;

Table 3. Determination of Analytical Wavelengths (λ), Detection Limits (LOD) and Quantitation Limits (LOQ), Accuracy Assessment Through the Analysis Element Contents of CRM (GBW10011 wheat flour-trace elements) After Shredding (mean ± S.D, n = 3, µg g^{-1} dry weight)

Çizelge 3. Analitik Dalga Boylarının (λ), Tespit Limitlerinin (LOD) ve Kantitasyon Limitlerinin (LOQ) Belirlenmesi, Çözünme Sonrası SRM Analiz İçeriği (GBW10011 buğday unu-iz elementler) aracılığıyla Doğruluk Değerlendirmesi (ortalama ± SD, n = 3 , µg g^{-1} kuru ağırlık)

Chemical Elements	*Wavelengths (λ nm)*	*LOD (µg g^{-1})*	*LOQ (µg g^{-1})*	*Certified values (µg g^{-1})*	*Confidence Interval Measured (µg g^{-1})*	*Recovery (%)*
Ca	317.933	0.0078	0,0259	340 ± 20	355 ± 18	104.40
K	766.440	0.0084	0.0279	1400 ± 60	1380 ± 85	98.58
Mg	279.553	0.0052	0.0173	450 ± 70	435 ± 62	96.65
Fe	259.940	0.0150	0.0499	18.5 ± 3.1	17,7 ± 4.8	95.60
Zn	206.200	0.0004	0.0013	11.6 ± 0.7	12.1 ± 0.51	104.31
Sn	189.989	0.096	0.319	-	7.356 ±1.36	-
Mn	257.610	0.0023	0.312	5.4±0.3	5.68 ± 0.40	105.18
Cr	267.716	0.028	0.093	0.096±0.014	0.093 ± 0.02	96.87
Cu	327.393	0.0012	0.0039	2.7±0.2	2.64 ± 0.127	97.70
Cd	228.802	0.0018	0.0060	0.018 ±0.04	0.0193±0.004	107
Si	251.611	0.085	0.283	80	75.6±5.31	95
Ni	221.647	0.0056	0.0186	0.06±0.02	0.065±0.91	108.33
V	292.402	0.0098	0.0326	0.034±0.012	0.036±0.018	105.88
Pb	220.353	0.0083	0.0276	0.065±0.024	0.070±0.032	107.69
As	193.696	0.0012	0.0040	0.031±0.005	0.029±0.006	93.54
Se	196.090	0.0095	0.0316	0.053±0.007	0.056±0.012	105.66

Table 4. Instrumental Operating Conditions Using Thermo ICAP 6300 ICP-OES

Tablo 4. Thermo ICAP 6300 ICP-OES Enstrümantal Çalışma Koşulları

Parameter	*Normal*	*Hydride System*
Power	1150 W	1350 W
Pomp speed	50 rpm	30 rpm
Purge gas	Argon	Argon
Coolant Gas		
Flow	12 L/min.	16 L /min.
Auxiliary gas		
Flow	0.5 L/min.	0.5 L/min.
Torch	Axial, Radial	Axial
Auto sampler	Cetac ASX-260	

Table 5. Corelation between variables

Çizelge 5. Değişkenler arasındaki korelasyon

	Ca	*K*	*Mg*	*Zn*	*Fe*	*Si*	*Mn*	*Sn*	*Cu*	*Ni*	*Pb*	*V*	*Cd*	*Cr*	*Se*
Ca	1														
K	-0.214	1													
Mg	0.393	-0,250	1												
Zn	-0.310	0.429	-0.393	1											
Fe	-0.667	0.048	-0.214	0.667	1										
Si	-0.405	0.024	0.286	0.095	0.548	1									
Mn	0.500	-0.524	0.536	-0.833*	-0.714*	-0.357	1								
Sn	-0.095	-0.452	0.143	-0.357	-0.262	0.524	0.071	1							
Cu	-0.548	0.738*	-0.429	0.071	0.119	0.095	-0.262	0.524	1						
Ni	-0.500	0.619	-0.393	0.571	0.571	-0.024	-0.619	-0.119	0.690	1					
Pb	0.317	-0,610	0.00	0.122	0.146	0.024	0.195	-0.415	-0.537	-0.268	1				
V	-0.024	-0,476	-0.107	-0.119	-0.238	-0.048	0.286	-0.095	-0.476	-0.714*	0.293	1			
Cd	-0.443	0.587	-0.607	0.024	-0.168	-0.299	-0.275	0.216	0.635	0.371	-0.798*	-0.084	1		
Cr	-0,494	0.602	-0.631	0.458	0.446	0.012	-0.542	0.060	0.795*	0.916**	-0.136	-0.136	0.394	1	
Se	-0.619	0.619	-0.750	0.095	0.143	0.095	-0.357	0.452	0.929**	0.619	-0.439	-0.436	0.707	0.807*	1

Table 6. Concentrations of Elements in Lentil and Chickpea Cultivars (mean±standart deviation, mg kg^{-1}, dry weight n=3)

Çizelge 6. Mercimek ve Nohut Çeşitlerinde Element Konsantrasyonları (ortalama ± standart sapma, mg kg^{-1}, kuru ağırlık, n = 3)

	Lentils					*Chickpeas*		
Elements	***Çağıl***	***Altıntoprak***	***Seyran-96***	***Fırat-87***	***Yerli Kırmızı***	***Tigris***	***Diyar-95***	***ILC-482***
Ca	601[a]±5.4[b]	783±17	606±7	609±6.2	879±14	950±41	1038±21	1499±15
K	4851±77	5350±13	4996±95	4891±37	5052±4	5441±33	4235±154	3285±102
Mg	863±10	880±24	855±9	848±15	867±21	854±16	-	901±14
Zn	32.4±0.05	34.1±0.07	38.03±0.2	38.2±0.04	40.8±0.03	33.4±0.1	33.7±0.2	29.6±0.02
Fe	73.1±0.4	70.3±8	96.4±10	73.2±9	86±3.3	84.3±0.5	49.0±0.98	52.1±7
Si	33.5±0.7	36.8±3	50.3±1	34.2±2.7	32.5±7	29.7±2	23.3±0.3	35.4±5
Mn	16.3±0.08	14.4±2	14.2±2	12.3±3	12.5±0.5	14.8±0.6	22.2±0.4	20.1±6
Sn	20.0±1.5	30.2±0.3	22.1±4	19.4±4	17.4±1.4	23.4±0.5	18.7±0.4	21.2±1.9
Cu	11.1±0.05	10.4±1.9	11.3±1.7	8.6±2	9.5±1	11.7±0.6	8.3±0.1	7.2±2.5
Ni	2.10±0.01	1.8±0.008	2.27±0.03	2.09±0.03	2.51±0.04	2.12±0.003	1.06±0.03	0.94±0.02
Pb	<0.0025	<0.0025	0.131±0.07	0.070±0.01	0.062±0.02	<0.0025	0.310±0.03	0.080±0.02
V	0.470±0.1	0.710±0.1	0.100±0.006	0.89±0.2	0.054±0.003	0.071±0.01	1.48±0.14	0.20±0.02
Cd	0.031±0.006	0.022±0.006	0.020±0.007	0.030±0.001	0.021±0.004	0.032±0.003	0.020±0.001	0.011±0.006
As	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Cr	0.52±0.007	0.51±0.006	1.63±0.006	0.52±0.005	0.59±0.007	0.71±0.01	0.51±0.01	0.50±0.0036
Se	0.552±0.1	0.475±0.11	0.558±0.06	0.480±0.17	0.437±0.07	0.650±0.02	0.410±0.09	0.316±0.15

a: mean
b:standart deviation

Ceyhan et al., 2014), chickpea (Kahraman et al., 2015) and pea (Harmankaya et al., 2010; Ada et al., 2019; Ceyhan and Simsek, 2021). On the other hand, according to Ereifej et al. (2001), the amount of Ca is given as 423 ± 40, 979 ± 48, 710 mg kg^{-1} in Jordan abd Morocco for lentils, respectively. These values are close to our foundings in this study. At the same time, Mg and K contents were found in the lentils and chickpeas between 848 ± 15 to 880 ± 24 mg kg^{-1}, -901±14 mg kg^{-1}, and 4851±77-5441±33, 3285±102 to 4235±154 mg kg^{-1} respectively. Significant genotype effects were observed for Mg and K contents in bean (Ceyhan, 2001; Ceyhan et al., 2008; Harmankaya et al., 2009; Ceyhan et al., 2014), chickpea (Kahraman et al., 2015) and pea (Harmankaya et al., 2010; Ada et al., 2019; Ceyhan and Simsek, 2021) grown in Konya, Turkey. However, According to Ereifej et al.(2001) the amount of Mg and K has been reported as 129 ± 20, 1190 ± 10 mg kg^{-1}, 381 ± 30, 5480 ± 210 mg/kg in Jordan and Morocco lentils, respectively.

As a result of the correlation analysis, some significant positive and negative relationships were found between the investigated element concentrations in lentil and chickpea seeds. Seed Mn content was negatively correlated with Zn and Fe (respectively r=-0.833, r=-0.714 respectively, P<0.05 for both). In addition to, seed V content with Ni, seed Cd content showed a negative statistically significant correlation with Pb (r=-0.714 and r=-0.798 respectively, P<0.05 both). Seed Se content had a strong positive correlation with Cu (r =0.929, P < 0.01) while seed Cr content had a strong positive correlation with Ni (r=0.914, P < 0.01). Also, Seed K content showed a positive correlation with Cu, and seed Cr content showed a positive correlation with Se and Cu (r=0.738, r=0.807 and r=0.795 respectively, P<0.05 for all three) (Table 5).

The values which was found in this study quite higher than Jordan lentils, whereas to be lower than those in Morocco lentils.

The amount of Fe was found in lentils and chickpeas between 70.3±8-96.4±10 mg kg^{-1}, 49.0±0.98-52.1±7 mg kg^{-1}, respectively. The results were similar to previous reports for cowpea (Harmankaya et al., 2016), bean (Ceyhan, 2001; Ceyhan et al., 2008; Harmankaya et al., 2009; Ceyhan et al., 2014), chickpea (Kahraman et al., 2015) and pea (Harmankaya et al., 2010; Ada et al., 2019; Ceyhan and Simsek, 2021) in Turkey. On the other hand, Ereifej at al.(2001) stated the amount of Fe as 133 ± 20, 119 ± 14, 78 mg kg^{-1} in Jordan, Morocco and FAO lentils, respectively. In this study Fe values was high than Jordan and Morocco lentils. However, It was found to be in line with FAO 's values. The amount of Zn was found in lentils and chickpeas between 32.4±0.05-40.8±0.03 mg kg^{-1}, 29.6±0.02-33.7±0.2 mg kg^{-1}, respectively. Whereas Zn values is given as 62±0.3, 37.3±0.8 mg kg^{-1} in Jordan and Morocco lentils, respectively, according to Ereifej at al.(2001), Mn concentrations were found in lentils and chickpea between 12.5±0.5-16.3±0.08 mg kg^{-1}, 20.1±6-22.2±0.4 mg kg^{-1}, respectively. On the other hand Cd, Cu, Fe, Mg, Mn, and Zn values in lentils is given as 0.50 ± 0.03, 7.7 ± 0.4, 129 ± 5, 2229 ± 59, 19.0 ± 0.8, 58 ± 3 mg kg^{-1}, respectively according to Momen et al. (2006). In the study, heavy metal concentrations such as Cd, Cu, Cr, Ni ve Pb ve Se were found to be a parallel the indicated quantity in the literature such as Salama and Ridwan, (2005), Akinyele and Shokunbi, (2015), Çiçek at al. (2017). At the same time in the study As concentrations were found below the detection limit.

CONCLUSION

As a result, the fact that consumption of lentils and chickpea provides many benefits to human health in terms of mineral balance has been revealed in this study. In general, it can be said that lentils and chickpeas are rich in macro and micronutrient elements, especially elements such as calcium and potassium, and the deficiency of these minerals can be eliminated by consuming them. This study will benefit in terms of food safety both in Turkey and to the people of our region by determining the mineral content in registered lentils and chickpeas. Lentil seeds had higher mean values for K, Zn, Fe, Si, Sn, Cu, Ni, Cr and Se elements. However, chickpea seeds had higher average values in terms of Ca, Mg and Mn. The amount of Ca, K and Se of Tigris cultivar was higher than others. The amount of Cr, Fe and Si in Seyran-96 cultivar, on the other hand, was found to be higher compared to other types. The amount of Mg was found to be close to each other in all varieties. In the products analyzed weren't found concentrations of the toxic levels of elements according to the FAO/WHO and Turkish food Codex.

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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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The article does not need an ethics committee decision.

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Afyonkarahisar İlinde Patates Üretiminin Ekonomik Analizi

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ÖZET

Bu çalışmanın amacı; patates üretimi yapan işletmelerin üretim maliyetlerini ve kârlılık göstergelerini hesaplayarak işletmelerin ekonomik analizini ortaya koymaktır. Çalışma alanı olarak Afyonkarahisar ilinin seçilmesinde Türkiye'nin önemli patates üretim merkezlerinden biri olması etkili olmuştur. Çalışmanın ana materyali 2019 üretim yılında patates üretimi yapan işletmeler arasından tabakalı tesadüfi örnekleme yöntemiyle seçilen 79 işletmeyle görüşülerek elde edilen veriler oluşturmaktadır. İşletmelerde dekara toplam üretim masrafları içerisinde değişen masrafların payı %82.23, sabit masrafların payı ise %17.77 olarak hesaplanmıştır. Değişen masraflar içerisinde en önemli masraf kalemi tohum masrafı olup bunu ilaç, gübre, elektrik ve makine kirası masraflarının takip ettiği belirlenmiştir. Sabit masraflar içerisinde en önemli masraf kaleminin arazi kirası olduğu belirlenmiştir. Patates üretiminde üretim masrafları 3521.53 TL/da, GSÜD 3556.06 TL/da, brüt kâr 660.42 TL/da, net kâr 34.53 TL/da, nispi kâr ise 1.01 olarak hesaplanmıştır. İşletmelerde ortalama patates verimi 3658 kg/da, 1 kg patatesin üretim maliyeti ise 0.96 TL olarak belirlenmiştir. Bölgede patates işletmelerinin maliyetlerinin yüksek, kârlılıklarının düşük olduğu hesaplanmıştır. Patates üretiminde tohum, ilaç, gübre ve enerji kullanımının diğer ürünlere kıyasla daha fazla olduğu belirlenmiş olup girdi fiyatlarında yaşanan artışlar patates üreticilerinin kârlılıklarını düşürdüğü tespit edilmiştir.

Tarım Ekonomisi

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Patates,
Afyonkarahisar,
Ekonomik Analiz,
Maliyet,
Kârlılık

Economic Analysis of Potato Production in Afyonkarahisar Province

ABSTRACT

The aim of this study was to reveal the economic analysis of the enterprises by calculating the production costs and profitability indicators of the enterprises producing potatoes. Afyonkarahisar province, one of the significant potato production centers in Turkey, was opted for this study. The data constitutes from the statistics obtained from 79 enterprises selected through the stratified random sampling method in the 2019 production year. The shares of variable and fixed costs were calculated as 82.23% and 17.77%, respectively, within the production costs. The most significant cost among the variables costs was determined that cost of seed, followed by pesticide, fertilizers, electricity, and renting machinery, respectively, whereas that cost within the fixed costs was the land rent. The production cost of potatoes, gross production value (GPV), gross profit, and net profit in this region were assessed as 3521.53 TRY/decare, 3556.06 TRY/decare, 660.42 TRY/decare, and 34.53 TRY/decare, respectively. The relative profit was determined as 1.01, as well. It was decided that the average yield of potato in these enterprises was 3658 kg/decare, and also the unit cost of potato was 0.96 TRY. It was calculated that potato enterprises in this region possessed high costs while their profitability was low. It was determined that the use of seeds, medicines, fertilizers, and energy in potato production was higher than other agricultural products. Therefore, rising input prices declined the profitability of potato producers.

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GİRİŞ

Dünya nüfusunda her yıl yaklaşık olarak 80 milyon artış yaşanması, gıda arz ve talep denkleminde talebin arza göre daha hızlı yükselmesine neden olmaktadır. Bununla beraber temel gıda ürünü olan patatesin kullanım alanlarının yaygınlaşması patatese olan talebi ve tüketimi hızlı bir şekilde arttırmaktadır. Dünyada en çok üretimi yapılan bitkisel ürünler sıralamasında şeker kamışı, mısır, pirinç ve buğdaydan sonra patates beşinci sırada gelmektedir. 2019 yılı Birleşmiş Milletler Gıda ve Tarım Örgütü'nün (FAO) verilerine göre 17.3 milyon hektar alanda 370 milyon ton patates üretimi gerçekleşmiş olup toplam bitkisel üretim içerisindeki payı %3.96'dır (Anonymous, 2020).

Türkiye'de patates üretimi belli illerde yoğun olarak yapılmakla birlikte hemen hemen bütün illerde yapılmaktadır. Türkiye İstatatistik Kurumu verilerine göre Türkiye'de 2020 yılında 81 ilin 72'sinde patates tarımı yapılmıştır. 2020 yılında toplam 1479935 dekar alanda 5 milyon 200 bin ton patates üretilmiştir (Anonim, 2021). Çalışma bölgesi olan Afyonkarahisar ili üretim alanı bakımından Niğde ilinden sonra 182820 dekar ve %10.36'lık pay ile ikinci sırada yer almaktadır. Üretim miktarı bakımından ise Niğde ve Konya illerinden sonra 551453 ton ve %10.60'lık pay ile üçüncü sıradadır. Afyonkarahisar ilinde çok sayıda çiftçi tarafından üretilen patates, bölgeye sağladığı istihdam ve üretim değeri bakımından önemli bir geçim kaynağıdır.

Patates, içerdiği vitamin ve mineraller açısından besin değeri yüksek, sert iklim koşullarına uyum sağlayabilen ve çeşitli iklim bölgelerinde yetişen temel bir gıda ürünüdür. Patates; birim alandan çok ürün alınabilmesi, üretimin tüketimden çok olduğu dönemlerde depolama imkânının olması, ürün yaşam döngüsünün kısa olması (yaklaşık 100 gün içerisinde yüksek verim) ve küçük ölçekli üreticiler için kısa sürede nakit kaynağına dönüştürülebilmesi nedeniyle üreticiler tarafından tercih edilen bir üründür. Ayrıca patates oldukça çeşitli kullanım alanlarına sahiptir. Taze veya işlenmiş olarak insan beslenmesinde, yumruları direkt olarak veya fabrika artıkları şeklinde hayvan beslenmesinde, bitkisel üretiminin devamlılığını sağlamak için tohumluk olarak ve çeşitli şekillerde gıda sanayinde işlenerek (cips, püre, hazır çorba, un, donmuş vb.) tüketiciler tarafından kullanılmaktadır.

Patates, Cobweb Teoremi'ne (örümcek ağı) konu olan bir üründür. Teoriye göre patates, soğan, sarımsak gibi fiyat dalgalanmalarının çok görüldüğü ürünlerde çiftçiler; bir önceki dönem fiyatlarına bakarak üretimlerine karar vermektedirler. Geçen dönem ürünlerin fiyatları yüksek ise gelecek dönemde de yüksek olacağı beklentisiyle hareket eden ve aynı şekilde düşünen milyonlarca çiftçi üretim planlamalarını bu beklentiye göre yaparak üretimlerini arttırmaktadır. Bu şekilde piyasaya çok fazla ürün arz edilmekte ve ürün fiyatları düşmektedir. Aynı şekilde bir önceki dönemde fiyatlar düşük ise gelecek dönemde de fiyatların düşük olacağı beklentisine giren çiftçiler üretimlerini azaltmakta ya da üretim yapmamaktadırlar. Bu durum piyasada ürün arzının azalmasına neden olmakta ve ürün fiyatlarını yükseltmektedir. Teori üreticilerin bu şekilde üretimlerine karar vermelerinden dolayı ürün fiyatlarında ve miktarında dönemsel ve yıllar bazında dalgalanmaların olduğunu ifade etmektedir (Ezekiel, 1938). Patates hem üretim hem tüketim hem de fiyat bakımından üreticiler ve tüketiciler için önemli bir gıda ürünüdür.

Türkiye'de ve dünyada patates üretimini ve maliyetlerini konu alan çalışmalar yapılmıştır (Rehber ve Erkuş, 1984; Kızıloğlu, 1997; Arıoğlu ve ark., 2006; Yılmaz ve ark., 2006; Birinci ve Küçük, 2006; Tok ve Davran, 2010; Topçu ve ark., 2010; Karsan ve Gül, 2017; Sapkota ve ark., 2020) ancak Türkiye patates üretiminde önemli bir bölge olan Afyonkarahisar ilinde daha önce patates üretiminin ekonomik analizini ve maliyetini konu alan çalışmaya rastlanılmamıştır. Bu çalışmada Afyonkarahisar ilinde patates üretim maliyeti ve kârlılık göstergeleri tespit edilmiştir.

MATERYAL ve METOD

Araştırmanın materyalini, Afyonkarahisar ilinde patates üretiminin yoğun olarak yapıldığı Sandıklı ve Şuhut ilçelerinde, patates üretim faaliyeti yapan 79 işletmeyle anket yapılmıştır. Anket görüşmeleri 2020 yılı Nisan ayında yüz yüze olarak gerçekleştirilmiştir. Dolayısıyla araştırmada kullanılan veriler birincil nitelikteki verilerdir.

Örnekleme Yöntemi

İşletmelerin ana kitlesini Afyonkarahisar ilinde patates üretiminin %53.89'unu ve ekim alanı olarak %55.04'ünü oluşturan Sandıklı ve Şuhut ilçelerinde, bu alanda faaliyet gösteren işletmeler oluşturmuştur. Ana kitleye ait veriler Afyonkarahisar ili Sandıklı ve Şuhut ilçeleri Tarım ve Orman Müdürlükleri kayıtlarından elde edilmiştir. Araştırma bulgularının daha sağlıklı yorumlanması ve işletme genişlik gruplarının daha dengeli bir yapıda oluşturulması için görüşme yapılacak örnek işletme sayısının

belirlenmesinde tabakalı örnekleme yöntemlerinden Neyman Yöntemi kullanılmıştır (Yamane, 2001). Ana kitleyi temsil edecek örnek sayısı eşitlik 1 yardımıyla hesaplanmıştır.

$$n = \frac{(\sum NhSh)^2}{N^2D^2 + \sum NhSh^2} \quad (1)$$

Formülde:
n: örnek hacmini
N: Ana kitledeki toplam birim sayısını
Nh: h. tabakadaki birim sayısını
Sh: h. tabakanın standart sapmasını
Sh^2: h. tabakanın varyansını
D^2: d^2/z^2
d^2: ana kitle ortalamasından izin verilen hata miktarını, (ortalamadan %10 sapma)
z^2: izin verilen güvenlik sınırının dağılım tablosundaki değerini (%99 güven sınırı öngörülmüştür) göstermektedir.

Saptanan örnek genişliğinden hareket edilerek, popülasyondan işletmeler tesadüfi olarak seçilmiştir. Patates üretiminde bulunan işletmeler, patates ekim alanı büyüklüğüne göre üç tabakaya ayrılmıştır. Eşitlik 2 yardımıyla işletmelerin tabakalara göre dağıtımı gerçekleşmiştir (Çiçek ve Erkan, 1996).

$$n_h = \frac{N_h S_h}{\sum N_h S_h} * n \quad (2)$$

Formülde:
Nh: her tabakaya seçilen örnek sayısı,
n: toplam örnek sayısını ifade etmektedir.

Hata payı %10 ve %99 güven aralığında ana kitleyi temsil eden örnek sayısı 79 olarak hesaplanmıştır. Hesaplama sonucuna göre 25.00 dekar ve altında patates alanına sahip işletmeler (20 işletme) I. tabaka, 25.01-75.00 dekar arasındaki işletmeler (13 işletme) II. tabaka, 75.01 ve daha fazla dekar patates alanına sahip işletmeler (46 işletme) III. tabaka olarak tanımlanmıştır (Çizelge 1).

Çizelge 1. Örneklem büyüklüğü
Table 1. Sample size

Grup *(Group)*	Patates ekim alanı (dekar) *(Potato cultivation area (decares))*	Adet (Nh) *(Number (Nh))*	Standart sapma *(Standard deviation)*	Varyans (Variance)	İşletme sayısı *(Number of farms)*
I	1.00-25.00	824	5.97	35.68	20
II	25.01-75.00	379	8.20	67.28	13
III	75.01 +	322	34.69	1203.42	46
Toplam *(Total)*		1525	35.55	1263.52	79

İstatistik Analizler

Anket verileri 2019 üretim dönemini kapsamaktadır. Toplanan verilerden hareketle patates üretiminin maliyetleri ve kârlılıları hesaplanmıştır. Görüşme yapılan işletmeler patates üretiminin yanı sıra farklı üretim kollarında da faaliyet gösterdikleri için işletme masraflarının hesaplanmasında tek ürün bütçe analiz yöntemi kullanılmıştır. Üretim faaliyetinde gerçekleşen masrafların hesaplanmasında 2019 üretim sezonu fiyatları dikkate alınmıştır. Patates üreten işletmelerde patates üretim masrafları (ÜM); değişen masraflar (DM) ve sabit masraflar (SM) olarak iki başlık altında hesaplanmıştır. Değişen masraflar; makine kirası, yabancı işgücü, gübre, ilaç, tohum, elektrik, su, pazarlama, döner sermaye faizi ve diğer değişen masraflar olarak belirlenmiştir. Sabit masraflar ise arazi kirası, aile işgücü ve genel idare giderleri olarak belirlenmiştir. Araştırmada tek ürün bütçe analizi yapıldığından üreticilerin kendilerine ait makineleri ve arazileri kullanmaları halinde, makine ve arazi kirası fiyatları dikkate alınarak hesaplamalar yapılmıştır. Aile işgücü ücret karşılığı ise bölgede kadın ve erkek yabancı işgücü ücretleri esas alınarak belirlenmiştir. T.C. Ziraat Bankası'nın tarım sektörü için uyguladığı bitkisel üretim kredi faizi oranının yarısı (%4.00) göz önünde bulundurularak döner sermaye faizi hesaplanmıştır. Değişen masraflarının toplamının %3.00'ü alınarak genel idare giderleri hesaplanmıştır.

İşletmelerin gayrisafi üretim değeri (GSÜD) ve kârlılık göstergeleri; brüt kâr (BK), mutlak (net) kâr (MK) ve nispi kâr (NK) 3,4,5 ve 6 numaralı eşitlikler yardımıyla hesaplanmıştır (Erkuş, 1979; Açıl ve Demirci, 1984; Rehber, 1993; Erkuş ve ark., 1995; Kıral ve ark., 1999).

Gayrisafi Üretim Değeri = Üretim Miktarı (kg) x Satış Fiyatı (TL/kg) (3)
Brüt Kâr = Gayrisafi Üretim Değeri – Değişen Masraflar (4)
Mutlak Kâr = Gayrisafi Üretim Değeri – Üretim Masrafları (5)
Nispi Kâr = Gayrisafi Üretim Değeri / Üretim Masrafları (6)

BULGULAR ve TARTIŞMA

İşletmeler ortalamasında toplam 212.92 dekar arazi içerisinde en fazla üretim alanının 104.94 dekar (%49.28) ile patates üretim faaliyetinde gerçekleştiği tespit edilmiştir. Tabakalara göre ortalama arazi

miktarı; I. tabakada 84.40 dekar, II. tabakada 116.23 dekar ve III. tabakada 296.13 dekara olarak belirlenmiştir. Patates üretim alanı I. tabakada 16.15 dekar (%19.14), II. tabakada 42.38 dekar (%36.46) ve III. tabakada 161.22 dekar (%54.45) olarak tespit edilmiştir. İşletmelerde patatesin yanı sıra arpa (%29.71), buğday (%12.44), haşhaş (%1.71), vişne (%1.18), şeker pancarı (%1.09), soğan (%1.03), ayçiçeği (%0.89), kuru fasulye (%0.70), anason (%0.42), tritikale (%0.15), yonca (%0.15), barbunya (%0.10), ceviz (%0.06) ve fiğ (%0.04) üretiminin de yapıldığı belirlenmiştir. Ayrıca toplam arazinin %1.05'inin nadasa bırakıldığı tespit edilmiştir.

İşletmelerde üretilen bitkisel ve hayvansal üretim miktarları ile bu ürünlerin fiyatları çarpılarak hesaplanan değere, prodüktif demirbaş artışları eklenerek gayrisafi üretim değeri hesaplanmıştır. İşletme ortalamasına göre toplam GSÜD 488806.26 TL olarak bulunmuştur. Toplam GSÜD'nin %76.36'sı patates üretiminden elde edilirken, %20.68'i diğer bitkisel ürünlerden, %2.97'si ise hayvansal ürünlerden elde edilmektedir. Toplam GSÜD I. tabakada 125628.94 TL, II. tabakada 263302.57 TL ve III. tabakada 710438.76 TL olarak tespit edilmiştir. İşletme gruplarına göre patates GSÜD'nin, toplam GSÜD içerisindeki payı I. tabakada %42.60, II. tabakada %51.29 ve III. tabakada %81.56 olarak tespit edilmiştir. İşletme ölçeği arttıkça, patates GSÜD'nin toplam GSÜD içerisindeki payının da arttığı belirlenmiştir (Çizelge 2).

Engiz (2007) tarafından yapılan çalışmada, toplam GSÜD'i içerisinde patatesin payının %91.20, diğer bitkisel ürünlerin payının %8.94, hayvansal ürünlerin payının ise %2.86 olduğunu bildirmiştir. Araştırma bulgusuna göre farklı bölgede yapılan bu çalışmada GSÜD içerisinde patatesin payı yüksek, diğer bitkisel GSÜD ve hayvansal GSÜD'i payları düşük bulunmuştur.

Rehbber ve Erkuş (1984) yaptıkları çalışmada, toplam GSÜD'i içerisinde patatesin payının %64.48, diğer bitkisel ürünlerin payının %14.83, hayvansal ürünlerin payının ise %10.69 olduğunu bildirmişlerdir. Araştırma bulgusuna göre farklı bölgede yapılan bu çalışmada ise GSÜD içerisinde patatesin ve diğer bitkisel ürünlerin payları düşük, hayvansal GSÜD'inin payı yüksek bulunmuştur.

Çizelge 2. İşletmelerde üretim faaliyetlerine göre gayrisafi üretim değeri
Table 2. Gross production value by production activities in farms

Gayrisafi Üretim Değerleri *(Gross Production Values)*	I		II		III		İşletmeler Ortalaması *(Farms Average)*	
	TL	%	TL	%	TL	%	TL	%
Patates GSÜD *(Potato GPV)*	53515.94	42.60	135055.57	51.29	579427.80	81.56	373161.01	76.36
Diğer bitkisel GSÜD *(Other Agricultural GPV)*	56419.75	44.91	95416.15	36.24	122144.68	17.19	101107.09	20.68
Hayvansal GSÜD *(Animal GPV)*	15693.25	12.49	32830.85	12.47	8866.27	1.25	14538.16	2.97
Toplam GSÜD *(Total GPV)*	125628.94	100.00	263302.57	100.00	710438.76	100.00	488806.26	100.00

Tarım sektöründe üreticiler, politika yapıcılar, sanayiciler, tüccarlar ve üretimden tüketime kadar geçen süreçte sektörde yer alan diğer kesimler için üretim maliyetlerinin bilinmesi önemli bir karar ölçütüdür (Bayramoğlu ve ark., 2021).

İşletmelerde dekara üretim masrafları toplamı 3521.53 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada 4121.02 TL, II. tabakada 3047.89 TL ve III. tabakada 3530.68 TL olarak tespit edilmiştir. Dekara değişen masrafların toplamı 2895.64 TL (%82.23) olarak bulunmuştur. İşletme gruplarına göre değişen masraflar; I. tabakada 3499.39 TL (%84.92), II. tabakada 2594.59 TL (%85.13) ve III. tabakada 2891.78 TL (%81.90) olarak tespit edilmiştir. İşletmelerde değişen masraf unsurları içerisinde en önemli masraf kalemi tohum masrafı olup üretim masrafları içerisindeki payı %25.01 olarak belirlenmiştir. Tohum masrafını sırasıyla %13.55 ile ilaç, %12.82 ile gübre, %10.19 ile elektrik, %7.17 ile makine kirası, %6.98 ile geçici işgücü, %3.16 ile döner sermaye faizi, %2.40 ile pazarlama, %0.39 ile su ve %0.56 ile diğer değişen masraflar takip etmektedir. İşletmelerde dekara sabit masrafların toplamı 625.89 TL (%17.77) olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada 621.63 TL (%15.08), II. tabakada 453.30 TL (%14.87) ve III. tabakada 638.90 TL (%18.10) olarak tespit edilmiştir. İşletmelerde en önemli sabit masraf unsurunun arazi kirası olup, üretim masrafları içerisindeki payı %14.55 olarak belirlenmiştir. Arazi kirası masrafını sırasıyla %2.47 ile genel idari giderleri, %0.75 ile daimi aile işgücü masrafları takip etmektedir (Çizelge 3).

Yılmaz ve ark. (2006) yaptıkları çalışmada, patates üretim faaliyetinde toplam masraflar içerisinde değişen masrafların payının %78.70, sabit masrafların payının %21.30 olduğunu bildirmişlerdir. Patates üretim masrafları içerisinde en önemli masraf unsurlarının; %30.60'lık pay ile tohum, gübre ve ilaç, %18.90'lık pay ile arazi kirası, %12.40'lık pay ile işgücü, %10.60'lık pay ile makine masrafı olduğunu bildirmişlerdir.

Akçapınar (2007) tarafından yapılan çalışmada, patates üretim faaliyetinde toplam masraflar içerisinde değişen masrafların payının %90.06, sabit masrafların payının %9.94 olduğunu bildirmiştir. Patates üretim masrafları içerisinde en önemli masraf unsurlarının; %49.31'lik pay ile tohum, gübre ve ilaç, %25.05'lik pay ile işgücü, %8.26'lık pay ile makine masrafı olduğunu bildirmiştir.

Toplam masraflar içerinde arazi kirasının payları bölgede yapılan diğer çalışma sonuçları ile yakın bulunmuştur. Ayrıca işgücü masraflarının payı düşük, tohum, gübre ve ilaç masraflarının payları yüksek bulunmuştur. Bu durum çalışmalar arasında zaman farkının olması ile ve yıllar itibariyle patates üretiminde işgücü ihtiyacının azalıp, tohum, gübre ve ilaç maliyetlerinin artmış olmasıyla açıklanabilir.

Çizelge 3. İşletmelerde patates üretim masrafları
Table 3. Potato production costs in farms

Maliyet (Cost)	I		II		III		İşletmeler Ortalaması *(Farms Average)*	
	TL/da	%	TL/da	%	TL/da	%	TL/da	%
Tohum *(Seed)*	566.26	13.74	592.57	19.44	915.77	25.94	880.67	25.01
İlaç *(Pesticide)*	475.05	11.53	373.97	12.27	484.73	13.73	476.99	13.55
Gübre *(Fertiliser)*	596.63	14.48	480.80	15.77	442.87	12.54	451.38	12.82
Elektrik *(Electricity)*	799.69	19.41	355.92	11.68	339.83	9.63	358.82	10.19
Makine kirası *(Machine rental)*	348.54	8.45	309.06	10.15	244.51	6.92	252.86	7.17
Geçici işgücü *(Temporary labour costs)*	372.48	9.04	256.96	8.43	239.52	6.78	245.86	6.98
Pazarlama *(Marketing)*	179.85	4.36	92.92	3.05	79.76	2.26	84.53	2.40
Su *(Irrigation)*	26.30	0.64	24.69	0.81	12.26	0.35	13.57	0.39
Diğer değişen masraflar *(Other variable costs)*	0.00	0.00	7.91	0.26	21.32	0.60	19.60	0.56
Döner sermaye faizi *(The interest in working capital)*	134.59	3.27	99.79	3.27	111.21	3.15	111.36	3.16
Değişen Masraflar *(Variable costs)*	**3499.39**	**84.92**	**2594.59**	**85.13**	**2891.78**	**81.90**	**2895.64**	**82.23**
Arazi kirası *(Land rent)*	428.48	10.40	342.74	11.25	528.74	14.98	512.47	14.55
Genel idari giderleri *(General administration expenses)*	104.98	2.54	77.84	2.55	86.75	2.46	86.86	2.47
Aile işgücü *(Permanent-Family labour)*	88.17	2.14	32.72	1.07	23.41	0.66	26.56	0.75
Sabit Masraflar *(Fixed cost)*	**621.63**	**15.08**	**453.30**	**14.87**	**638.90**	**18.10**	**625.89**	**17.77**
Üretim Masrafları *(Production costs)*	**4121.02**	**100.00**	**3047.89**	**100.00**	**3530.68**	**100.00**	**3521.53**	**100.00**

İşletmelerde patates üretiminde gayrisafi üretim değeri dekara 3556.06 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada 3313.68 TL, II. tabakada 3186.43 TL ve III. tabakada 3594.08 TL olarak tespit edilmiştir. GSÜD'nden değişen masrafların çıkartılmasıyla hesaplanan brüt kâr işletmelerde dekara 660.42 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada -185.71 TL, II.

tabakada 591.84 TL ve III. tabakada 702.30 TL olarak tespit edilmiştir (Çizelge 4). I. tabakadaki işletmelerin dekara brüt kâr değerinin negatif olması patates üretiminde değişen masrafları bile karşılayamadıkları ve zarar ettiklerini göstermektedir.

GSÜD'nden toplam üretim masrafların çıkartılmasıyla hesaplanan net kâr işletmelerde dekara 34.53 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada -807.34 TL, II. tabakada 138.54 TL ve III. tabakada 63.40 TL olarak tespit edilmiştir (Çizelge 4). I. tabakadaki işletmeler patates üretim faaliyeti için yaptığı masrafın altında gelir elde etmektedir.

GSÜD'nin toplam üretim masraflarına bölünmesiyle hesaplanan nispi kâr işletmelerde ortalama 1.01 olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada 0.80, II. tabakada 1.05 ve III. tabakada 1.02 olarak tespit edilmiştir. Nispi kâr değeri patates üretiminde bir birimlik masraf karşılığında elde edilen üretim değerini ifade etmektedir. İşletme ortalamasına göre patates üretimi için 1.00 TL'lik masraf karşılığında 1.01 TL'lik üretim değeri elde edildiği ve 0.01 TL'lik kâr elde edildiği belirlenmiştir. I. tabakadaki işletmelerin patates üretimi için ortalama 1.00 TL'lik masraf karşılığında 0.80 TL'lik üretim değeri elde edildiği ve 0.20 TL zarar ettikleri tespit edilmiştir (Çizelge 4).

İşletmelerde patates verimi dekara 3658.00 kg olarak belirlenmiştir. Dekara patates verimi I. tabakada 3800.31 kg, II. tabakada 3309.44 kg ve III. tabakada 3677.70 kg olarak tespit edilmiştir (Çizelge 4). Türkiye İstatistik Kurumu verilerine göre, 2019 yılında Afyonkarahisar'da patates verimi dekara 3745 kg olarak bildirilmiştir (Anonim, 2021). Araştırma bulgusu ile araştırma bölgesinin TÜİK verileri birbirine yakın bulunmuştur.

GSÜD'nin üretim miktarına bölünmesiyle hesaplanan patates satış fiyatı işletmelerde kilogram başına ortalama 0.97 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada 0.87 TL, II. tabakada 0.96 TL ve III. tabakada 0.98 TL olarak tespit edilmiştir. İşletme ölçeği arttıkça işletmelerde patates satış fiyatının arttığı belirlenmiştir (Çizelge 4). Türkiye İstatistik Kurumu verilerine göre 2019 yılında patatesin kilogram fiyatı Türkiye'de ortalama 1.85 TL, Afyonkarahisar ilinde ise 1.69 TL olarak bildirilmiştir (Anonim, 2020).

Toplam üretim masraflarının üretim miktarına bölünmesiyle hesaplanan patates maliyeti işletmelerde kilogram başına ortalama 0.96 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada 1.08 TL, II. tabakada 0.92 TL ve III. tabakada 0.96 TL olarak tespit edilmiştir (Çizelge 4).

Çizelge 4. Patates üretiminde maliyet ve kârlılık
Table 4. Cost and profitability in potato production

Maliyet ve Kârlılık *(Costs and Profit)*	I	II	III	İşletmeler Ortalaması *(Farms Average)*
1. GSÜD (TL/da) *(GPV (TRY/daa)*	3313.68	3186.43	3594.08	3556.06
2. Değişen Masraflar (TL/da) *(Variable costs (TRY/daa)*	3499.39	2594.59	2891.78	2895.64
3. Sabit Masraflar (TL/da) *(Fixed costs (TRY/daa)*	621.63	453.30	638.90	625.89
4. Toplam Masraflar (TL/da) *(Total cost (TRY/daa)*	4121.02	3047.89	3530.68	3521.53
5. Brüt Kâr (TL/da) (1-2) *(Gross profit (TRY/daa))*	-185.71	591.84	702.30	660.42
6. Net Kâr (TL/da) (1-4) *(Net profit (TRY/daa))*	-807.34	138.54	63.40	34.53
7. Nispi Kâr (1/4) *(Relative profit)*	0.80	1.05	1.02	1.01
8. Verim *(Yield)*	3800.31	3309.44	3677.70	3658.00
9. Satış fiyatı (TL/kg) (1/8) *(Sale price (TRY/kg))*	0.87	0.96	0.98	0.97
10. Üretim maliyeti (TL/kg) (4/8) *(Production cost (TRY/kg))*	1.08	0.92	0.96	0.96
11. Kâr marjı (TL/kg) (9-10) *(Profit margin (TRY/kg))*	-0.21	0.04	0.02	0.01

Patatesin kilogram satış fiyatından kilogram maliyeti çıkartılarak hesaplanan kâr marjı işletmeler ortalamasında 0.01 TL olarak hesaplanmıştır. İşletme gruplarına göre; I. tabakada -0.21 TL, II. tabakada 0.04 TL ve III. tabakada 0.02 TL olarak tespit edilmiştir (Çizelge 4). İşletmeler ortalamasında kâr marjının çok düşük olduğu ve birim satış fiyatı ile birim üretim maliyetinin neredeyse başa baş oldukları belirlenmiştir. I. tabakadaki işletmelerin kâr marjı negatif olarak hesaplanmış ve bu gruptaki işletmelerin zarar ettikleri belirlenmiştir. Bunun

sebebi olarak I. tabakadaki işletmelerin patates verimi yüksek olmasına karşın, diğer işletme gruplarına göre değişen masraflarının yüksek, patates satış fiyatının ise düşük olmasıyla açıklanabilir. Bu gruptaki işletmelerde değişen masraf unsurları içerisinde özellikle elektrik ve gübre masraflarının yüksek olduğu tespit edilmiştir.

SONUÇ ve ÖNERİLER

Patates ekim alanı ve üretim miktarı açısından Türkiye'nin önemli bir bölgesi olan Afyonkarahisar ilinde patates üretim faaliyetinde bulunan 79 adet patates işletmesinin 2019 üretim dönemi verilerini kapsayan bu çalışmada patates üretim masrafları ve kârlılık göstergeleri belirlenerek ekonomik analizi yapılmıştır.

İncelenen işletmelerde patates üretim masrafları 3521.53 TL/da olarak hesaplanmıştır. Patatesin 1 kg maliyeti 0.96 TL, ortalama satış fiyatı ise 0.97 TL/kg olarak tespit edilmiştir. Kâr marjı, 1 kg patates için 0.01 TL'dir. Patates üretim faaliyetinden elde edilen gayri safi üretim değeri 3556.06 TL/da, brüt kâr 660.42 TL/da, net kâr 34.53 TL/da ve nispi kâr ise 1.01 olarak hesaplanmıştır. Patates işletmelerinin kârlılık göstergelerinin düşük olmasının sebebi üretim maliyetlerinin yüksek olmasıdır. Patates üretiminde tohum, ilaç ve gübre kullanımı diğer ürünlere kıyasla daha fazladır. Ayrıca sulama; elektrik veya akaryakıt kullanarak yapılmaktadır. Bu nedenle girdi fiyatlarında yaşanan artışlar patates üreticilerinin kârlılıklarını düşürmektedir.

Bunun yanı sıra patates fiyatlarının yıllara göre dalgalı seyir izlemesi üreticileri ve tüketicileri etkilemektedir. Hem patates fiyatlarındaki dalgalanmalar, hem üretimde kullanılan girdi fiyatlarının artması, hem de uygun bir girdi bileşimi sağlayarak üretim yapılmaması işletmelerin kârlılıklarını düşürmektedir. Bu bakımdan üreticilerin girdi maliyetleri düşürülerek üretim masraflarının azaltılması ve patates fiyatlarında piyasa denge fiyatının sağlanması bölgede patates üretiminin sürdürülebilirliği açısından büyük önem arz etmektedir.

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Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Elma Üretiminin Ekonomik Analizi: TRB1 Bölgesi Örneği

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ÖZET

Bu çalışmanın amacı, TRB1 Bölgesinde elma üretiminin yoğun olarak yapıldığı Bingöl, Elâzığ ve Malatya illerindeki elma üretiminde girdi kullanımı, üretim maliyeti ve yıllık faaliyet sonuçlarının karşılaştırmalı olarak belirlenmesidir. Çalışmanın ana materyalini, TRB1 illerindeki elma üreten tarım işletmeleri arasından tabakalı tesadüfi örnekleme metoduna göre seçilen 223 işletmeden yüz yüze gerçekleştirilen anketlerden elde edilen 2018-2019 üretim dönemine ait verileroluşturmuştur. İncelenen işletmelerde elma üretim maliyeti ve karlılığının belirlenmesinde tek ürün bütçe analizi kullanılmıştır. Çalışma sonuçlarına göre; üreticilerin ortalama tarım deneyimi 19 yıl, elma yetiştiriciliği deneyimi 16 yıl olarak belirlenmiştir. işletmelerin elma arazisi büyüklüğü 28.02 da'dır. İncelenen işletmelerde ortalama elma verimi 627.65 kg da, 1 kg elmanın üretim maliyeti 1.04 ₺'dir. Elmanın ortalama satış fiyatı 1.16 ₺ kg, ürün net karı ise 0.12 ₺ kg'dır. Dekara elma üretim değeri 728.07 ₺, brüt kar ise 343.27 ₺'dir. İşletmelerde dekara elma net karı 73.77 ₺, nispi karı ise 1.11 olarak bulunmuştur.

Tarım Ekonomisi

Araştırma Makalesi

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Anahtar Kelimeler
TRB1 bölgesi
Brüt kar
Net kar
Elma verimi

Economic Analysis of Apple Production: Case of TRB1 Region

ABSTRACT

The aim of this study was to determine the use input in apple production, calculate the production cost per 1 kg of apples, decide the annual activity results and to analyze them comparatively, by making profitability and productivity analyzes for apple growing areas of Bingöl, Elâzığ and Malatya. The main material of the study was obtained by face-to-face surveys conducted during the 2018-2019 production period covering 223 enterprises producing apples using stratified random sampling method. Single product budget analysis was used to determine the production cost and profitability of apple enterprises. According to the results of the study; on average, the general agricultural experience of the apple growers was approximately 19 years and the cultivation experience was 16 years. While the average size of apple land was calculated as 28.02 decare (da) for the investigated enterprises. While the average apple yield was 627.65 kg da in the enterprises surveyed, the cost of apple per kg was calculated as 1.04 ₺. The average sales price was 1.16 ₺ kg and the profit margin provided per kilogram was 0.12 ₺. The production value per da was determined as 728.07 ₺ and the gross profit was 343.27 ₺. The net profit per da was found to be 73.77 ₺ and the proportional profit was 1.11 in the apple farming.

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GİRİŞ

Elma (Malus domestica), gülgiller (Rosaceae) familyasına ait kültürü yapılan ve besin değeri çok yüksek olan bir meyvedir. Bütün dünyaya Orta Asya'dan yayılmıştır. Tarih boyunca yapılan kültür çalışmalarıyla 1000 farklı elma çeşidi üretildiği

tahmin edilmektedir. Elma, Türkiye'de iyi gelir getiren meyve türlerinden birisidir. Üretimi oldukça iyi düzenlenmiş yerlerde ve bakım şartlarının iyi olduğu durumlarda meyve verimi ortalama 3000 kg da'a kadar çıkmaktadır. Türkiye, kişi başına 20 kg elma tüketimi ile dünyada en fazla elma tüketen ülkeler arasında yer almaktadır.

Meyvecilik sektörü, özellikle son yıllarda gelişen yetiştiricilik sistemleri sayesinde dünyada ve Türkiye'de hızla artan bir ivmeyle değer kazanmıştır. Özellikle yumuşak çekirdeklilerde sağlanan üretim sistemlerindeki reformlar, yetiştiricileri bu klasik sistem yerine bodur anaçlarla üretim yapmaya itmiştir. Böylece üretimde problem olan masraflar azaltılmış, birim alana dikilebilecek ağaç sayısı artırılarak daha fazla verim sağlanmış ve artan gıda gereksinimi karşılanmaya çalışılmıştır (Efecan, 2006).

Dünya meyve üretiminin yaklaşık %7.5'ini oluşturan elma, meyve üretiminde muz ve karpuzdan sonra 3 üründür.Avrupa Birliği (AB)'nin 2019 yılındaki üretimi 15 milyon ton, ABD üretimi ise yaklaşık olarak 5 milyon ton olarak gerçekleşmiştir. Dünya toplam elma üretiminin yarısından fazlası (%54) Çin tarafından karşılanmaktadır (Anonim, 2020). Türkiye 2019 yılında 3618.752 kg elma üretim miktarıyla dünya elma üretiminde Çin, AB ve ABD'den sonra 4. sırada yer almaktadır (Çizelge 1). Elma ihracatında Çin 1. sırada iken, bu ülkeyi AB ve ABD izlemekte, Türkiye ise elma ihraç eden önemli 10 ülke arasında yer almaktadır. Elma ithalatında 1. sırada Rusya yer alırken, AB 2. sırada, Irak 3. sırada yer almaktadır. Türkiye elma ihracatını en fazla Irak'a yaparken, elma ithalatını ise en fazla Kuzey Kıbrıs Türk Cumhuriyetinden yapmıştır. Türkiye'de 2019 yılı itibariyle toplam elma üretim alanı 1.7 milyon da olup, Niğde İli 235 bin da ile 1. sıradadır. Karaman ise 9.2 milyon adet ile en fazla meyve veren ağaca sahip il olarak kayıtlara geçmiştir. Türkiye'nin toplam elma üretim alanlarının yarıdan fazlası (%52) Niğde, Isparta, Karaman, Antalya ve Konya illerinden oluşmaktadır. Türkiye'de 2019 yılında 3.6 milyon ton olan toplam elma üretiminin 732 bin ton'u Isparta'da, 486 bin ton'u Karaman'da ve 438 bin ton'u ise Niğde'de gerçekleşmiştir. (Anonim, 2020).

Çizelge 1. Dünya ve Türkiye 2019 yılı elma verileri
Table 1. Apple data World and Turkey in 2019.

Veriler Data	Dünya World	Türkiye Turkey
Alan[1] (bin ha)	4904	174.439
Verim[1] (ton ha)	17565	20745
Üretim[2] (bin ton)	70964	3626
Tüketim[2] (bin ton)	58877	2413
İthalat[2] (bin ton)	5764	151
İhracat[2] (bin ton)	5921	1176

Kaynak: [1]: Anonim, 2020a; [2]: Anonim, 2020b

Meyvecilik TRB1 bölgesinde önemli bir bitkisel üretim faaliyeti olmakla birlikte, Kayısı, Üzüm ve Elma yetiştiricilik açısından önemli ürünler olarak ilk sıralarda yer almaktadır. Meyvecilik sektörü TRB1 Bölgesinin bitkisel üretim katma değerinin yükseltilmesinde lokomotif bir alt sektör görevi yüklenmektedir. TRB1 Bölgesinin Türkiye elma üretimi içindeki payı %1,6'dır. Elazığ ilinde meyve veren ağaç başına 237 kg verim alınırken, bunu 226 kg ile Malatya ve 155 kg ile Bingöl ilinin takip ettiği belirlenmiştir (Çizelge 2).

Çizelge 2. Türkiye ve TRB1 Bölgesi illerinde 2019 yılı elma yetiştiriciliğine ait veriler
Table 2. Turkey and TRB1 Area provinces of apple cultivation data in 2019.

Elma verileri Data of apple	**Türkiye Turkey**	**Bingöl Bingol**	**Elazığ Elazig**	**Malatya Malatya**
Meyve veren yaşta ağaç sayısı (Adet)	61288.452	313211	305954	717618
Meyve vermeyen yaşta ağaç sayısı (Adet)	15004.981	85124	188268	46592
Toplu meyveliklerin alanı (Dekar)	1746.404	11760	16388	28954
Üretim miktarı (Ton)	3625.960	11811	13776	31360
Verim (kg Meyve veren ağaç)	293	155	237	226

Kaynak: Anonim, 2019

Tarımsal üretimde girdi kullanım miktarı, maliyet ve gelirin belirlenmesi üreticiler ve ekonomi politikası yapıcıları için mikro düzeyde önem arz etmektedir. Tarımsal ürün maliyet çaalışmalarının sonuçları fiyat politikalarının saptanmasında hükümetlerin yararlandıkları önemli araçlardır. Ayrıca tarımsal ürün maliyetleri; fiziki üretim girdilerinin kullanım düzeyi, işgücü planlaması, finansman programlarının yapılması, ürün bütçelerinin ve yatırım projelerinin hazırlanması gibi planlama faaliyetlerinde sıklıkla kullanılmaktadır (Özalp ve Yılmaz, 2013). Bu bağlamda bu çalışmanın amacı, TRB1 Bölgesindeki Bingöl, Elâzığ ve Malatya illerinde elma üretiminde girdi kullanımı, elmaüretim maliyeti ve yıllık faaliyet sonuçlarının karşılaştırmalı olarakincelenmesidir.

MATERYAL ve YÖNTEM

Materyal

Araştırmanın ana materyalini, TRB1 Bölgesi'nde Bingöl, Elâzığ ve Malatya illerinde elma üreticiliği yapan işletmelerden yüz yüze gerçekleştirilen anketlerden sağlanan veriler oluşturmuştur. Çalışma, Tarım ve Orman Bakanlığı kayıtları, Türkiye İstatistik Kurumu (TÜİK) ve Tarım ve Orman Örgütü (FAO) tarafından yayımlanan istatistik verileri ve

daha önceden yapılmış konuyla alakalı tez çalışmaları, araştırma makaleleri, araştırma raporları gibi ikincil verilerle de desteklenmiştir. Anket çalışması, 2019 yılı Nisan- Ağustos döneminde yapılmış olup, işletmelerin elma üretim faaliyetine dair veriler 2018-2019 üretim dönemini kapsamaktadır.

Yöntem

Örneklemede kullanılan yöntem

Malatya, Elazığ ve Bingöl İl Tarım ve Orman Müdürlüklerinden, ilçeler ve ilçelere bağlı köylerin isimleri ve bu köylerdeki tarım işletmesi sayıları ve işletme büyüklüklerine ait veriler temin edilmiştir. Anket uygulanacak tarım işletmesi sayısının hesaplanmasında tabakalı tesadüfi örnekleme metodundan yararlanılmıştır (Güneş ve Arıkan, 1988; Çiçek ve Erkan, 1996).

Araştırma bölgesindeki tarım işletmelerinin arazi büyüklüklerinin dağılımına göre 1-50 dekar olanlar (birinci grup), 51-100 dekar olanlar (ikinci grup), 101 dekardan daha fazla olanlar (üçüncü grup) olmak üzere üç tabaka olarak belirlenmiştir. Anket yapılan işletme sayısı, tabakalı tesadüfî örnekleme yöntemine göre %10 hata payı ve %95 güven aralığında 223 adet olarak belirlenmiştir.

$n = (\Sigma Nh.Sh)^2/N^2.D^2+\Sigma (Nh.Sh^2)$

$D^2 : (d/Z)^2$

$n = Nh\ Sh*n/\Sigma\ Nh\ Sh$ (1)

Formülde:

d: öngörülen sapma miktarı

Z: Serbestlik derecesine ait çizelge değeri

N_h : Tabakalarda yer alan işletme sayısı

S_h : Tabakalara ait standart sapma

S_h^2 : Tabakalara ait varyans

N : Populasyon hacmi

n_i : Tabakadaki örnek sayısı

n : Örnek hacmi

Tesadüfi sayılar tablosu esas alınarak anket yapılan işletmelerin seçimi gerçekleştirilmiştir. Araştırma kapsamında Bingöl İline bağlı 8 köyde 18 adet, Elazığ İline bağlı 29 köyde 73 adet, Malatya İline bağlı 24 köyde 132 adet olmak üzere toplam 223 adet anket yapılmıştır.

Verilerin analizi ve değerlendirilmesinde kullanılan yöntem

Çalışmada, sosyo ekonomik özelliklerin belirlenmesi için gerçekleştirilen hesaplamalarda işletmelerin mevcut işgücünü tespit ederken, Erkek İşgücü Birimi (EİB) dikkate alınmıştır. Erkek iş gücü birimine dönüştürmede, (Açıl, 1980) tarafından belirlenen katsayılar kullanılmıştır.

İşletmelerin ekonomik analizinde kullanılan yöntem

İncelenen işletmelerinin masraflarının hesaplanmasında tek ürün bütçe analiz yöntemi kullanılmıştır (Kıral ve ark., 1999).

Elmanın üretim maliyetlerinin saptanmasında uygulanan yöntem

Elma üretim maliyeti, iki aşamada hesaplanmıştır. İlk aşamada elma bahçelerinin tesis masrafı hesaplanarak elma bahçesinin tesis masrafları amortisman payı bulunmuştur. İkinci aşama ise bir üretim döneminde yapılan üretim masrafı hesaplanmıştır. Üretim döneminde yapılan masraflar; değişken masraflar ve sabit masraflar olarak sınıflandırılmıştır. Değişken masraflar; gübreleme, ilaçlama, budama, hasat, sulama, meyve seyreltme, toprak işleme, çapalama ve bunların işçilik döner sermaye faizi masraflarını kapsamaktadır. Sabit masraflar ise; çıplak arazi değerinin faizi, genel idari gideri, tesis sermayesi faizi ve tesis masrafları amortisman payını kapsamaktadır.

Maliyet analizinde üretim masrafları içinde bulunan tesis masrafları amortisman payı, çıplak arazi değerinin %5'i ve genel idare giderleri, maliyet kalemi olarak gösterilmektedir. Çiftçi ve aile bireylerinin ailedeki işgücü ücret karşılıkları, cari dönem itibariyle ve bölgedeki yabancı işgücüne ödenen ücret dikkate alınarak hesaplanmıştır (Kıral ve ark., 1999).

Kira bedeli olarak incelenen bölgede çıplak arazi değerinin %5'i alınmıştır (Kıral ve ark., 1999). İncelenen bölge için çıplak arazi değeri, hem anket yapılan üreticilerin beyanları hem de ilgili İl Tarım ve Orman Müdürlükleri verilerinden ortalama olarak 2800 ₺ olarak kabul edilmiştir.

Bitkisel üretim için T.C. Ziraat Bankası'nın açtığı krediler için uygulanan faiz oranı üzerinden döner sermaye faizi hesaplanabilmektedir. Döner sermaye faizi hesaplanırken, değişken masrafların üretim dönemine yayılma durumunun oldukça homojen olduğu varsayımı kabul edilerek, değişken masrafların yarısı üzerinden faiz uygulanır (Güneş ve Arıkan, 1988).

Döner sermaye faizi bitkisel üretim için %14 olarak belirlenmiş ve bunun yarı değeri %7 kullanılmıştır. Genel idare giderleri, değişken masraflar toplamının %3'ü alınarak hesaplanmaktadır (Karagölge, 1996; Kıral ve ark., 1999).

Tesis masrafları yıllık amortisman payı, tesis dönemi (4 yıl) boyunca yapılan toplam tesis masraflarının elma bahçesinin ekonomik ömrüne (45 yıl) bölünerek elde edilmiştir. Tesis sermayesi faizi ise toplam tesis masrafları yarı değerine %5 faiz uygulanarak hesaplanmıştır (Karagölge, 1996; Karaçayır, 2010).

Amortisman payı=(Ortalama tesis masrafı (₺))/(Ekonomik ömür (Yıl)) (2)

Elma üretim değeri, elma verimi ile satış fiyatının çarpımı sonucu hesaplanmıştır. Üretim değerinden, değişen masraflar çıkarılarak brüt kar, üretim değerinden, üretim masraflarının çıkarılmasıyla net

kar tespit edilmiştir.

$$Elma\ Gayrisafi\ Üretim\ Değeri\ (₺/da) = Elma\ verimi\ (kg/da)\ x\ Elma\ satış\ fiyatı\ (₺/da)$$
$$Brüt\ Kar\ (₺/da) = Üretim\ Değeri - Değişen\ Masraflar$$
$$Net\ Kar\ (₺/da) = Üretim\ Değeri - Üretim\ Masrafları$$
$$Brüt\ Kar\ Marjı = \frac{Brüt\ Kar}{Üretim\ Değeri} x100$$
$$Net\ Kar\ Marjı = \frac{Net\ Kar}{Üretim\ Değeri} x100$$
$$1\ kg\ Elmanın\ Maliyeti\ (₺/kg) = \frac{Üretim\ masrafları\ toplamı\ (₺/da)}{verim\ (kg/da)}$$
$$1\ kg\ Elmanın\ Net\ Karı\ (₺/kg) = Satış\ fiyatı - 1\ kg\ elmanın\ maliyeti$$
$$Nispi\ Kar = Gayrisafi\ Üretim\ Değeri/Üretim\ Masrafları \quad (3)$$

Üretim işlemleri; toprak hazırlığı, dikim, bakım ve hasat ve pazarlama olarak üç ana başlık altında incelenir ve bu işlemlere ait masraflar değişken masraf olarak kabul edilir. Toprak hazırlığı ve dikim masrafları, sürüm ve dikim işlemleri esnasında oluşan masraflardır. Gübreleme, ilaçlama ve eğer yapılıyorsa sulama gibi masraflar bakım masraflarını oluşturmaktadır. Pazarlama masrafları ise nakliye ve işçilik masraflarının toplamıdır (Kıral ve ark., 1999).

Bitkisel üretim faaliyetinde işletmede kullanılan ya da işletme dışından satın alınan tohumun veya fidenin üretici fiyatıyla (çiftlik avlusu fiyatı) bedeli hesaplanmıştır.

İşletmede kullanılan tarımsal ilaçlar satın alındığından ilaçların işletmeci tarafından mal edildiği fiyatları ile kullanılan ilaç miktarının çarpılması sonucu ilaç masrafları hesaplanmıştır.

Kullanılan traktör ve ekipmanların tükettiği akaryakıt ve yağ miktarı birim satış fiyatlarıyla çarpılarak masraflar hesaplanmıştır (İnan, 2008).

BULGULAR VE TARTIŞMA

Anket Yapılan Üreticilerin Sosyo-Demografik Özellikleri

İncelenen işletme sahiplerinin tamamının erkek olduğu belirlenmiştir. Anket yapılan işletmecilerin yaşlarının 23 ile 70 arasında değiştiği ve ortalamasının 48.9 olduğu belirlenmiştir. İşletmecilerin %85.3'ünün 41-60 yaş aralığında, %9.6'sının 20-40 yaş aralığında ve %5.1'inin ise 61 yaş ve üstü grubunda olduğu belirlenmiştir. Anket yapılan tüm iller itibariyle işletmecilerin daha çok 41-60 yaş grubunda olduğu sonucu belirlenmiştir. İlkokul mezunu olan işletmecilerin oranı %65.9, ortaokul mezunu olan işletmecilerin oranı %14.5, lise mezunu olan işletmecilerin oranı %14.1, okuryazar olan işletmecilerin oranı %3.7 ve üniversite mezunu olan işletmecilerin oranı ise %1.8 olarak belirlenmiştir. İncelen işletmelerin aile birey sayısı 2 ile 7 kişi arasında değişmekte ve ortalama olarak 4.13 olarak hesaplanmıştır. Bingöl ilinde incelenen işletmelerin %27.8'inin 4 kişilik, %27.6'sının ise 6 kişilik ailelerden, Elâzığ ilindeki işletmelerin %51.4'ünün ve Malatya ilindeki işletmelerin ise %61.7'sinin 4 kişilik ailelerden oluştuğu belirlenmiştir. Genel ortalama olarak işletmelerin %46.9'unda ailedeki birey sayısının 4 kişi olduğu sonucuna ulaşılmıştır. Yetiştiricilerin genel ortalama itibariyle genel tarım deneyimi yaklaşık olarak 19 yıl, elma yetiştiriciliği deneyimi ise 16 yıl olarak belirlenmiştir. Bingöl ilindeki yetiştiricilerin hem genel tarım hem de elma tarımında Elâzığ ve Malatya illerine göre yıl olarak daha fazla deneyimli oldukları görülmüştür. Dünyada daha önce yapılmış olan çalışmalarda bulunan değerlere bakıldığında, elma yetiştiriciliği süresini (Alemu ve ark., 2017) Gana'da 10.2 yıl, (Osmanı ve Kambo, 2019) ise Arnavutluk'ta 11.40 yıl, olarak belirlenmiştir Elma yetiştirime süresi Gana ve Arnavutluk ülkelerine göre yüksek bulunmuştur.

İncelenen işletmelerde erkek iş gücü biriminin (EİB) arazi büyüklüğüne bağlı olarak artış gösterdiği ve işletmeler ortalamasında 2.57, arazi büyüklüğü 41 da ve üzerinde olan işletmelerde, 3.05, 21-40 da olan işletmelerde 2.75 ve 1-20 da olan işletmelerde ise 2.28 olduğu belirlenmiştir. Arazi büyüklüğü itibariyle işletmelerin erkek iş gücü birimi ortalamaları arasındaki farklar istatistiki olarak önemli bulunmuştur ($P<0.05$) (Çizelge 3).

Çizelge 3. İncelenen işletmelerin ortalama iş gücü varlığı
Table 3. Average workforce asset of the examined enterprises

Arazi büyüklüğü (da) Land size (da)	Ortalama (Mean)	Standart sapma (Standard deviation)	Standart hata (Standard error)
1-20	2.28[a]	0.75	0.06
21-40	2.75[b]	0.61	0.08
41 ve üzeri	3.05[c]	0.75	0.10
İşletmeler Ortalaması	2.57	0.78	0.05

[a,b,c]: aynı sütunda farklı harfler ile gösterilen ortalamalar arasındaki fark istatistiki olarak önemlidir.

İller itibariyle toplam işletme arazisinin ve elma arazisinin büyüklük değerleri Çizelge 4'te verilmiştir. Toplam işletme arazisinin büyüklüğü Bingöl için ortalama 47.17, Elâzığ için 61.30 ve Malatya ili için ise 65.61 da olarak tespit edilmiştir. Toplam işletme büyüklüğü incelenen işletmelerde ortalama 62.71 da olarak hesaplanmıştır. Malatya ilindeki işletmelerin toplam işletme arazisi büyüklüğü ile Elâzığ ilindeki işletmelerin toplam işletme arazisi büyüklüğünün, Bingöl ilindeki işletmelere göre oldukça yüksek olduğu sonucu ortaya çıkmıştır. İncelenen işletmeler için elma arazisinin büyüklüğü ortalama 28,02 da olarak hesaplanırken, bu değer Malatya için 33.67, Elâzığ için 21.96 ve Bingöl için ise 11.22 olarak bulunmuştur. Toplam işletme arazisi içinde elma arazisinin payı %44.6 olarak belirlenmiştir. Yapılan anova analizinde varyansların homojen olarak dağıldığı test edilmiş ve incelenen işletmeler için hem toplam işletme arazisi hem de elma arazisine ait ortalamalar arasındaki farkların istatistiki olarak önemli olduğu belirlenmiştir. (Gül, 2005) tarafından Antalya'da yapılan çalışmada, işletme arazisi ortalama olarak 64.73 da, elma arazisi 12.46 da olarak belirlenmiş ve elma arazisinin toplam işletme arazisi içindeki payı %28.47 olarak hesaplanmıştır. Isparta, Karaman ve Niğde illerinde (Gül, 2006) tarafından yapılan çalışmada elma arazisi ortalama 15.78 da olarak belirlenmiştir. Antalya ilinde (Aydoğmuş ve Yılmaz, 2010) tarafından tüm işletmeler ortalaması itibariyle elma arazisi büyüklüğü 7.78 da olarak hesaplanmıştır. Konya'da (Karaçayır, 2010) tarafından yapılan çalışmada, toplam işletme arazisi büyüklüğü işletmeler ortalaması için 123.08 da, elma arazisi büyüklüğü ise 93.26 da olarak hesaplanmış ve elma arazisinin toplam işletme arazisi içindeki payı %75,7 olarak saptanmıştır. Konya'da (Kanat ve ark., 2017) tarafından yapılan başka bir çalışmada ise elma arazisinin büyüklüğü 10 da olarak hesaplanmıştır. Arnavutluk'ta yapılan bir çalışmada, elma arazisi büyüklüğü ortalama 12.87 da olarak hesaplanmıştır (Osmanı ve Kambo, 2019). (Ma ve ark., 2018) Çin'de yaptıkları çalışmada elma arazilerinin büyüklüğünü ortalama olarak 5.07 da olarak bildirmişlerdir.

Çizelge 4. İller itibariyle toplam işletme arazisi ve elma arazisine ait ortalama değerler
Table 4. Average values of the total farm land and apple land by provinces

İller (Provinces)	Sayı	Ortalama elma arazi genişliği (da)*** (Average apple land width)	Ortalama işletme arazi genişliği (da)* (Average total farm land size)	Elma Arazisinin Toplam Arazi İçindeki Payı (%)* (Share of Apple Land in Total Land)
Bingöl	18	11.22^{a}±1.380	47.17^{a}±7.274	23.78^{a}±3.615
Elazığ	73	21.96^{b}±1.369	61.30^{b}±3.691	35.82^{a}±1.819
Malatya	132	33.67^{c}±1.484	65.61^{b}±2.732	51.31^{b}±1.048
Genel	223	28.02±1.105	62.71±2.120	44.68±1.052

*:p<0.10, ***:p<0.001.
^{a,b,c}: Aynı sütunda farklı harfler ile gösterilen ortalamalar arasındaki fark istatistiki olarak önemlidir.
±: Ortalamalara ait standart hata değerleri

İncelenen İşletmelerde Elma Üretim Maliyeti

İncelenen işletmelerde elma bahçesi tesis dönemine ait masraflar ve dağılımı Çizelge 5'de verilmiştir. Elma bahçesinin tesis masrafları toplamı 1896.3 ₺ da olarak saptanmıştır. Toplam tesis masraflarının %63.95'ini değişen masraflar, %36.05'ini ise sabit masraflar oluşturmaktadır. Birinci yılda arazi hazırlama, dikim ve fidan maliyetlerinin ve sulama sisteminin olması birinci yıl değişen masrafların fazla olmasının en büyük etkeni olarak yorumlanabilir. (Özalp ve Yılmaz, 2013) Antalya'da inceledikleri nar işletmeleri için tesis dönemi toplam masrafı 2533.47 ₺ da, toplam değişen masrafları 1630.05 ₺ da, sabit masrafları ise 903.42 ₺ da olarak hesaplamışlardır. Toplam değişen masrafların toplam tesis masrafları içindeki payı %65.34, toplam sabit masrafların toplam tesis masrafları içindeki payı ise %35.66 olarak belirlenmiştir. Antalya'da yapılan başka bir çalışmada da nar üretimi tesis maliyeti 2673.34 ₺ da olarak belirlenmiştir (Kaya, 2009).

İncelenen işletmelerde elma üretiminde sürümün genellikle pullukla yapıldığı belirlenmiştir. Sürüm için harcanan aile iş gücü ortalama 1.75 saat, makine çeki gücü ise ortalama 1.58 saat olarak hesaplanmıştır. İşletmelerde toplam işgücü isteği 36.54 saat da, makine çekigücü ise 7.68 saat da olarak hesaplanmıştır (Çizelge 6). Elma üretiminde iş gücü miktarı (Gül, 2005) tarafından Isparta, Karaman ve Niğde illerinde yapılan araştırmada 103.47, makine çeki gücü ise 4.64, (Demircan ve ark., 2005) tarafından Isparta'da yapılan çalışmada ise iş gücü miktarı 103.61 ve makine çeki gücü ise 5.61 saat da olarak bildirilmiştir. (Karaçayır, 2010) tarafından yapılan çalışmada, elma üretiminde toplam iş gücü isteği 106, makine çeki gücü isteği ise 7 saat/da olarak belirlenmiş ve incelenen işletmeler için elma üretiminde yoğun iş gücü kullanıldığı sonucuna varılmıştır. (Aydoğmuş ve Yılmaz, 2010) Antalya'da elma üretiminde iş gücü kullanımını ortalama 110.30 saat da, makine çeki gücünü ise 5.44 saat da olarak tespit etmişlerdir. Isparta'da yapılan bir çalışmada, işgücü 23.85, çeki gücü ise 4.7 saat da olarak bulunmuştur (Erdoğan ve ark., 2016).

Çizelge 5. Elma üretimi birim alana tesis masrafları ve dağılımı (₺/da)
Table 5. Plant costs and distribution per unit area of apple production (₺/da)

Masraf kalemleri Expense items	**1.yıl 1st year Tutar value**	**2.yıl 2nd year Tutar value**	**3.yıl 3rd year Tutar value**	**4.yıl 4th year Tutar value**	**Toplam değer (₺) Total value (₺)**	**Yüzde (%) Percentage (%)**
Arazi hazırlama						
Patlatma	30.0				30.0	1.2
Sürüm	45.0				45.0	1.9
Diskaro	50.0				50.0	2.1
Fidan	165.0				155.0	7.1
Dikim maliyeti						
Yer belirleme	2.5				2.5	0.1
Çukur açma	15.0				15.0	0.6
Dikim	12.5				12.5	0.5
İlaçlama	16.4	27.5	38.9	49.9	122.8	5.7
Çapalama	20.0	22.4	22.4	25.5	90.3	3.9
Gübreleme						
Hayvan gübresi	41.6				41.6	1.8
NPK	20.0	25.6	35.0	55.8	126.4	5.9
Toprak işleme	40.0	40.0	40.0	40.0	160.0	6.9
Budama	5.0	12.5	15.0	18.5	51.0	2.2
Sulama sistemi	200.0				200.0	8.6
Sulama bedeli	22.5	22.5	30.5	35.0	110.5	4.7
Değişen masraflar toplamı	685.5	150.5	181.8	224.7	1212.6	63.95
Yatırım sermayesinin faizi (%7)	47.9	10.5	12.7	15.7	84.8	15
Genel idare masrafı (%3*4)	20.5	4.5	5.4	6.7	38.9	6.9
Çıplak Arazi Değerinin Faizi (140*4)	140	140	140	140	560.0	24.2
Sabit masraflar toplamı					683.7	36.05
Tesis masrafı toplamı					**1896.3**	**100.00**
Tesis Masrafları amortisman payı	1896.3/45				**42.1**	

Çizelge 6. Elma işletmelerinde işgücü ve çeki gücü istekleri
Table 6. Workforce and pull power requests in apple businesses

Tarımsal İşlemler Agricultural Operations	İşgücü İstekleri Labor Requests		Makine Çekigücü İstekleri Machine Power Requirements	
	Saat/da Hours/da	Oran (%) Percantage	Saat/da Hours/da	Oran (%) Percantage
Toprak hazırlığı	1.75	4.78	1.58	20.57
Çapalama	2.00	5.47	1.50	19.53
Gübreleme	2.00	5.47	-	
İlaçlama	5.00	13.70	3.15	41.03
Sulama	3.00	8.20	1.00	13.02
Budama	5.00	13.70	-	
Ot biçme	2.14	5.85	-	
Hasat	8.00	21.9	-	
Taşıma	0.45	1.23	0.45	5.85
Meyve seyreltme	7.20	19.70	-	
Toplam	36.54	100.00	7.68	100.00

İncelenen elma işletmelerinde değişen masraflar toplamı 384.8 ₺, sabit masraflar toplamı ise 269.5 ₺ olarak hesaplanmış ve üretim masrafları toplamı ise 654.3 ₺ olarak belirlenmiştir. Toplam üretim masrafları içerisinde değişen masrafların oranı %58.9 iken sabit masrafların oranı %41.1 olarak bulunmuştur. Değişen masraflar içerisinde ise %13.3 oran ile su ve sulama masrafı (su elektrik ücreti) ilk sırada gelirken, onu %8 ile ilaç ve ilaçlama masrafının takip ettiği belirlenmiştir. Sabit masraflar içerisinde ise çıplak arazi değerinin faizinin oranı %21.3 olarak tespit edilmiştir (Çizelge 7). İncelenen işletmelerde ürün sigortası yaptırılmadığı belirlenmiştir.

Çizelge 7. Elma üreten işletmelerde birim alana üretim masraf unsurları ve dağılımı
Table 7. Production cost elements and distribution per unit area in apple producing enterprises

Masraf unsurları **Cost elements**	**Değer (₺)** **Value (₺)**	**Oran (%)** **Percantage (%)**
Geçici işgücü	50.70	8.9
Toprak işleme	8.83	1.3
Çapalama ve ot temizliği	15.50	2.3
Budama	35.68	5.3
Meyve seyreltme	13.50	2.0
Gübre ve gübreleme	24.17	3.6
İlaç ve ilaçlama	52.50	8.0
Su ve sulama masrafı	87.50	13.3
Hasat	13.87	2.1
Pazarlama	35.40	5.4
Makine kirası	25.40	3.8
Döner sermaye faizi (%7)	21.80	3.3
A-Değişen Masraflar Toplamı	**384.8**	**58.9**
Genel İdari Giderleri (A*%3)	10.00	1.5
Çıplak Arazi Değerinin Faizi	140.00	21.3
Daimi aile iş gücü	30.00	4.5
Tesis masrafları amortisman payı	42.10	6.4
Tesis sermayesi faizi	47.40	7.2
B- Sabit masraflar toplamı	**269.5**	**41.1**
C-Üretim masrafları toplamı (A+B)	**654.3**	**100.00**
D-Elma verimi (kg da)	**627.65**	
1 kg elma maliyeti (₺) (C/D)	**1.04**	

İncelenen İşletmelerde Elma Üretiminin Karlılık Durumu

Tarımsal ürün fiyatlarının belirlenmesinde kullanılan en önemli kriterlerden birisi, üretim sırasında yapılan masrafların ortaya konularak maliyetinin bulunmasıdır. Üreticinin karlı olabilmesi ve üretimine devam edebilmesi için, gelir gider arasındaki dengenin korunması gerekmektedir. İncelen işletmelerdeki elma üretiminin karlılık durumu, Çizelge 8'de verilmiştir. Ortalama elma verimi 627.65 kg da, 1 kg elmanın maliyeti 1.04 ₺'dir. Araştırma bölgesinde elmanın ortalama satış fiyatı 1.16 ₺ kg, kilogram başına sağlanan kar marjı 0.12'dir. Dekara düşen üretim değeri 728.07 ₺, brüt kar ise 343.27 ₺'dir. Brüt karın üretim değeri içindeki payı ise %47.10 olarak belirlenmiştir. İncelenen elma işletmelerinde dekara net kar 73.77 ₺, üretim değeri içindeki payı ise %10.10'dur. Elma üretiminde yapılan 1 ₺'lik masrafa karşılık 0.12 ₺ kar elde edilmiştir. İncelenen işletmelerde nispi kar 1.11 olarak hesaplanmış, incelenen işletmeler için elma üretiminde 1 birim masrafa karşılık elde edilen üretim değerinin 1.11 olduğu sonucu belirlenmiştir. İl Tarım ve Orman Müdürlüğü (2012)'nün çalışmasında; TRB1 bölgesinin ortalama elma verimi 1185 kg da, elma satış fiyatı 1,08 ₺ kg, toplam masrafı 460 ₺ da, gayrisafi üretim değeri 1284 ₺ da ve brüt karı ise 824 ₺ da olarak belirtilmiştir. Bu bulgularla mevcut çalışmanın bulguları karşılattırıldığında, bölgedeki elma veriminin yarı yarıya düştüğü ve elma satış fiyatının neredeyse hiç değişmediğinden dolayı bu durumun elma üretim değerinde ciddi bir azalışa sebep olduğu sonucu ortaya çıkmıştır. Ayrıca üreticilerin yeterince gelir kazanmadığını düşündüğü için elma yetiştiriciliğine masraf yapmaktan kaçınmalarına rağmen, üretim değerindeki düşüş brüt karıda etkilemiştir. (Gül, 2005) tarafından Antalya ilinde elma verimi ortalama 2426.43 kg da, net kar 119.31 ₺ kg, üretim maliyeti 161.26 ₺ kg, satış fiyatı 0.28 ₺ kg ve oransal kar 1.74 olarak belirlenmiştir. Antalya'da yapılan bir çalışmada net kar değeri; bodur elma bahçelerinde 0.77, yarı bodur elma bahçelerinde 0.05 ve çöğür elma bahçelerinde ise 0.12 ₺ olarak, brüt kar değeri işletmeler ortalamasında 1922, net kar değeri ise 1215 ₺ da olarak hesaplanmıştır (Aydoğmuş ve Yılmaz

2010). Karaman'da (Karaçayır, 2010)'ın yapmış olduğu çalışmada, elma verimi 1975 kg da, değişen masraflar 590.46 ₺ da, üretim masrafları 736.2 ₺ da, Brüt kar 1107.26 ₺ da ve elma maliyeti ise 0.37 ₺ kg olarak tespit edilmiştir. (Erdoğan ve ark., 2016) tarafından Isparta'da yapılan çalışmada, verim değeri 4877.07 kg da, satış fiyatı 0.87 ₺, üretim değeri 4243.05 ₺ da, değişen masraflar 729.88 ₺ da ve brüt kar ise 3513.17 ₺ da olarak hesaplanmıştır. (Kanat ve ark., 2017) tarafından Konya'da yapılan çalışmada oransal kar yarı bodur elma işletmelerinde 1.56, bodur elma işletmelerinde ise 2.21 olarak belirlenmiştir. Ayrıca bu çalışmada diğer bölgelerde yapılan benzer çalışmalarda nisbi karın Isparta'da 1.63, Karaman'da 1.71, Amasya'da 1.37, Tokat'ta 1.18 ve İçel'de 1.36 olarak hesaplandığı bildirilmiştir (Demircan ve ark., 2005). (Tekin, 2019) Denizli'de elma işletmelerinde yaptığı çalışmada, brüt karı 1499.97, net karı 510.20, 1 kg elma maliyetini 0.94 ₺ kg ve kar marjını ise 0.19 olarak hesaplamıştır. Isparta ve karaman illerinde yapılan çalışmada elma üretiminde nispi kar 1.42, net kar 1412.59 ₺ da ve brüt kar ise 2409.46 ₺ da olarak hesaplanmıştır (Bayav ve Karlı, 2020).

Çizelge 8. İncelenen işletmelerde elma üretiminin karlılık durumu
Table 8. Profitability of apple production in the examined enterprises

Masraf ve Gelir Unsurları	Elma arazisi genişlik grupları (da)			
	1-20	21-40	41+	İşletmeler Ort.
Verim (kg da)	630.35	715.79	543.18	627.65
Satış Fiyatı (₺ da)	1.25	0.92	1.31	1.16
Üretim Değeri (₺ da)	787.93	658.52	711.56	728.07
Değişen Masraflar (₺ da)	442.27	390.51	324.29	384.80
Üretim Masrafları (₺ da)	700.70	600.48	665.71	654.30
1kg ürün Üretim Masrafları (₺ kg) (ÜM/Verim)	1.11	0.83	1.22	1.04
Brüt Kar (₺ da) (ÜD-DM)	345.66	268.01	387.27	343.27
Net Kar (₺ da) (ÜD-ÜM)	87.23	58.04	45.85	73.77
Oransal Kar (₺ da) (ÜD/ÜM)	1.12	1.09	1.06	1.11
Brüt Kar Marjı (Brüt Kar/ÜD)*100	43.8	40.6	54.4	47.10
Net Kar Marjı (Net Kar/ÜD)*100	11	8.8	6.4	10.10
1 kg Ürün Net Kârı (₺ kg)	0.14	0.09	0.09	0.12

SONUÇ VE ÖNERİLER

Elma tarımı ile uğraşan bireylerin genelde orta yaş grubunda (41-60 yaş) ve inceleme alanında okuryazarlık oranının Türkiye ortalamasının üzerinde olduğu sonucuna varılmıştır. Yetiştiricilerin genel ortalama itibariyle genel tarım deneyimi yaklaşık olarak 19 yıl, elma yetiştiriciliği deneyimi ise 16 yıl olarak belirlenmiştir. Hem genel tarım hem de elma tarımında Bingöl ilindeki yetiştiricilerin Elâzığ ve Malatya illerine göre daha fazla deneyimli oldukları belirlenmiştir. Elma yetiştiriciliği deneyim süresinin genel tarım deneyim süresi içindeki payı %82 olarak hesaplanmıştır. İncelenen işletmelerde elma yetiştiriciliği yapılan alanın, %45.3 oranında 1-20 da, %35 oranında 21-40 da ve %19.7 oranında ise 41 da ve üzerinde olduğu belirlenmiştir. Bingöl ve Elâzığ'da bulunan elma yetiştiricilerinin özellikle küçük arazi varlığında elma yetiştirmeye odaklandığı, Malatya ilinde ise elma yetiştiriciliğinin arazi varlığı bakımından yayıldığı belirlenmiştir. Malatya ilinde Elâzığ ve Bingöl'e göre daha büyük arazi varlığında elma yetiştirildiği sonucu ortaya çıkmıştır. İncelenen işletmeler için elma arazisinin büyüklüğü ortalama 28,02 da olarak hesaplanırken, bu değer Malatya için 33.67, Elâzığ için 21.96 ve Bingöl için ise 11.22 da olarak tespit edilmiştir. Elma arazisinin, toplam işletme arazisi içindeki payı %44.6 olarak hesaplanmıştır. İncelenen işletmelerde, ortalama elma verimi 627.65 kg/da olarak gerçekleşirken, 1 kg elmanın maliyeti 1.04 ₺ olarak hesaplanmıştır. Elmanın ortalama satış fiyatı 1.16 ₺ kg, kilogram başına sağlanan ürün net karı ise 0.12 olarak belirlenmiştir. Dekara düşen üretim değerinin 728.07 ₺, brüt karın ise 343.27 ₺ olduğu belirlenmiştir. İncelenen elma işletmelerinde dekara net kar 73.77 ₺, oransal kar ise 1.11 olarak bulunmuştur. Araştırma sonuçlarına göre; elma üretiminde en önemli masraf kaleminin işgücü masraflarından oluştuğu belirlenmiştir. Özellikle hasat zamanında fazla işgücüne gereksinim duyulması, yabancı işgücü temininde bölgede yaşanan zorluklar ve yabancı işgücü ücretlerinin yüksek olması elma üreticilerinin sorunları arasında yer almaktadır. Araştırmanın genel sonucu olarak; yetiştiriciler elma üretimi ile ilgili ekimden hasata kadar gerekli olan üretim bilgilerini diğer üreticilerden öğrenerek bu işe başladıklarını ancak, elmanın satış fiyatının düşük olması ve alıcı sayısının az olmasından dolayı elma üretimini bırakmak istediklerini beyan etmişlerdir. Ortaya çıkan sonuçlar ışığında aşağıdaki öneriler belirlenmiştir;

Elma yetiştiriciliğinde kalite arttırılmalı ve maliyetler düşürülmelidir.

Üreticilerin kooperatifleşme seçeneği ile organize bir

şekilde pazara girmesi sağlanmalıdır.

Modern tarım ile ilgili bilgilendirilmeler yapılmalıdır.

Üreticilerin pazarlamaya yönelik fiyat istekleri dikkate alınmalıdır.

Üreticilerin ürünleri için ihracat olanakları arttırılmalıdır.

Üreticilere, tesis masraflarında destek sağlanması ve üreticilerin pazar talepleri doğrultusunda yeni çeşitlerle bahçe tesis etmesi teşvik edilmelidir.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

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Economic Analysis of Dairy Cattle Farms in Izmir Province of Türkiye

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ABSTRACT
The aim of this study was to cover the economic analysis of dairy cattle farms in Turkey. The research data were obtained from face-to-face surveys of 102 dairy farms selected from the İzmir province of Turkey by stratified random sampling method in 2014. Farmers are divided into three groups according to their number of cows. According to the results of the study, as the farm size increase, daily milk yield per dairy cattle, milk production in the lactation period, and milk sales price increase. There are also positive relationships between farm size and forage planting area and the proportion of forage planting area within the total planting area. Moreover, the most common forage of cattle breeders in the province was the silage corn with 48.7%. Fixed costs accounted 12.6% of total cost and fall sharply as farm size increases. Feed costs accounted for a remarkably high proportion of total variable costs (85.1%). The Benefit-Cost Ratio was 1.84, which was increasing with the farm size.

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ÖZET
Bu çalışmanın amacı, Türkiye'deki süt sığırcılığı işletmelerinin ekonomik analizini kapsamaktır. Araştırma verileri 2014 yılında İzmir ilinde tabakalı rastgele örnekleme yöntemi ile seçilen 102 süt işletmesinden yüz yüze anketlerinden elde edilmiştir. İşletmeler sahip oldukları süt sığırı sayısına göre üç gruba ayrılmıştır. Araştırma sonuçlarına göre, işletme büyüklüğü arttıkça, süt sığırı başına günlük süt verimi, laktasyon döneminde süt üretimi ve süt satış fiyatı artmaktadır. İşletme büyüklüğü ile yem bitkisi ekim alanı ile yem bitkisi ekim alanının toplam ekim alanı içindeki oranı arasında da pozitif yönlü bir ilişkiler vardır. Ayrıca bölgedeki büyükbaş hayvan yetiştiricileri tarafından üretilen en yaygın yem bitkisi %48.7 ile silajlık mısırdır. Sabit maliyetler toplam maliyetin %12.6'sını oluşturmakta ve işletme genişliği büyüdükçe artıkça azalma eğilimi göstermektedir. Yem maliyetleri, toplam değişken maliyetlerin oldukça yüksek bir oranını (%85.1) oluşturmaktadır. Fayda-Maliyet Oranı 1.84 olarak hesaplanmış ve bu oran işletme büyüklüğüne göre artış göstermektedir.

Tarım Ekonomisi

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INTRODUCTION

Milk and dairy products are the most important nutrients required for people to grow, develop and live a healthy life (Gorska-Warsewicz, 2019 and Visioli and Strata, 2014). Therefore, dairy products are so important and cannot be ignored for the continuation of human life. Studies show that 1 liter of milk can meet the entire calcium and phosphorus needs of adults. Therefore, it is necessary to increase and support milk and milk production in order to increase the nutritional level of the society and to create better quality and sustainable food chain. In addition, milk and dairy production directly or indirectly contribute to the country's economy such as increasing national income, providing raw materials for many industries, increasing employment and export (Tandoğan, 2006; Karakaya and Akbay, 2014).

In order to improve dairy cattle farming and provide

sustainability, the sector must be examined economically. In plans and programs, it should be ensured that the profitability of the farms engaged in dairy cattle activities should be increased and enterprises with greater potential should be established. Studies should be carried out periodically to inform the farmers about the instability in milk prices and the prevention of unstoppable increases in feed costs and the use of the correct breeds (Ilban, 2010).

In Turkey, especially in recent years, some studies have shown that the desired efficiency will be achieved (Tokmak, 2009). As of 2019, the average milk yield per lactation period in dairy cattle in Turkey was 3158 kg. The average milk yield in Izmir province is 3745 kg (TÜİK, 2020). The main reason for this is that almost all of the cattle breeds in the province are from the culture and culture hybrid race (Uygur, 2015).

The feed is one of the most important factors affecting the cost of farmers in dairy cattle. based on 2014 TUIK data; in Turkey, 3.4% of the planting area of feed crops was in Izmir province. This rate has also shown that there was enough roughage for Izmir. The most important plant that makes up the roughage is silage corn. In Turkey, 11% of silage corn is produced in Izmir (TÜİK, 2020). According to 2001 TUIK data; in İzmir, 2.4% of the agricultural farmers engaged in only livestock, while 63.0% made both livestock and crop production. As of 2014; the share of animal production value of Izmir in Turkey was 4.7%. (Gucer, 2014). The size of feed industry in Izmir has increased over the years. Izmir province meets 8.1% of its mixed feed requirement. There were 21 mixed feed factories in Izmir in 2014 (TÜİK, 2015). There were 130 dairy processing facilities in Izmir, which provide 15.0% of the milk sold in the dairy processing plant in Turkey (Gucer, 2014; Akbay and Akdoğan, 2020).

The main purpose of this study was to determine the economic analysis of dairy cattle farms in İzmir. The three specific objectives of this study were to investigate the production structure of dairy farms, to search roughage and concentrated feed amounts given to cattle, veal and heifer by farm size groups, and to analyze cost, returns and profit of milk production of dairy farms.

MATERIAL and METHOD

The main material of the research consisted of data obtained from the agricultural enterprises engaged in dairy cattle breeding in İzmir province in 2014 through face to face survey. As the main population of the study, districts with the highest number of dairy cattle farms were selected in İzmir. Considering the cattle milked in dairy cattle farms, the survey was conducted with the farmers having at least 5 dairy cattle.

In determining the main mass of the research, districts where dairy cattle farming are carried out in Izmir province, are concentrated. Considering the cattle provided in dairy cattle, the survey was conducted with the farms with 5 heads and more of the dairy cattle. The sample size (n) was calculated by stratified random sampling method (Yamane, 2001):

$$n = \frac{N\sum N_h S_h^2}{N^2 D^2 + \sum N_h S_h^2} \qquad D^2 = \frac{E^2}{t^2} \quad (1)$$

where, N is the number of farmers in the main population, N_h is the number of farmers in each stratum, S_h is standard deviation in each stratum, D^2 is appropriate variance, E is the amount of error allowed from the population average, t is the value of the allowed confidence interval in the distribution table t. The sample size was determined as 102 with 95% confidence interval and 5% error. Based on the number of dairy cows including dry cows but excluding calves, heifers, and bulls, farms were classified into three groups as 5-14 heads (Group 1), 15-29 heads (Group 2), 30+ heads (Group 3). The number of farms in these groups is distributed as 47, 23 and, 32 respectively. For the purposes of the study, descriptive statistics, F-test and Chi-Square test were used to analyze the data.

Total cost is calculated with the sum of variable and fixed cost; Gross Profit (UK) is found by subtracting variable costs (labor costs, feed costs, veterinary vaccination and medicine costs, etc.) from gross production value. Net profit is calculated by subtracting the total cost from total income; per liter milk cost is calculated by dividing the total cost by the total amount of milk sold.

Inventory asset exchange (IAE) includes heifers, calves, and bulls in the farms. In the calculation of inventory asset exchange, the animal value was calculated by taking the difference between the year-end values of the animals in the farms and the beginning of year's values (at fixed prices). Those older than six months (calves) are included in the inventory change. Those younger than six months are considered as subordinate income as calf income. IAE is obtained with the help of the equation:

IAE = (End of period animal value + sold animal value + slaughtered animal value) - (per year animal value + purchased animal value)

If the result is negative (-), it is evaluated as an expense item, namely, inventory value decrease. If the result is positive (+), it is considered as operating income, namely, an increase in inventory value (Kıral et al., 1999).

RESEARCH FINDINGS and DISCUSSION

Socio-demographic and Economic Characteristics of Dairy Farms

The dairy animal production status of farmers is given in Table 1. The average number of dairy cattle per farm

was 26 heads, lactation period (milking time) was 267 days, average daily milk yield per cow was 21.4 kg, the amount of milk obtained in a lactation period was 5711.9 lt, and the milk sales price was 1.07 TL/lt. Moreover, the annual milk income was 121816.6 TL, the number of fattened cattle was 13.4, and the total meat sold was 3347.7 kg/year. All farms are milking twice a day. All of the dairy cattle breeds in the farms are cultural breeds. Approximately 86% of the farms have Holstein breeds, while the remaining farms have both Holstein and other culture breeds. There is no statistical difference between farm size groups according to the percentage of Holstein breeds owned by the farms.

Table 1. Production status of dairy farms
Çizelge 1. Süt işletmelerinin yapısı ve üretim durumu

	Average	Standard Deviation
Number of dairy cattle (head)	25.70	26.44
Milking time (days)	266.91	35.34
Cow milk yield (kg/head/day)	21.40	3.26
Lactation yield (kg/head/year)	5711.87	236.52
Milk sales price (TL/lt)	1.07	0.08
Number of cattle (units)	13.36	19.11
Meat sold (kg/year)	3347.66	5 387.37
Total meat value sold (TL/year)	58715.18	959334.33
Milk income (TL/year)	121816.57	112876.45
Calf income (TL/year)	20270.91	7694.83

Both quantitative estimation and F-test results illustrated a positive and statistically significant difference between dairy farm size groups and the number of dairy cattle, milk yield, milk production in a lactation period, and milk sales price (Table 2). According to the LSD confidence interval test; while the difference in milk yield was caused by the first group, the difference in milk sales price was determined from the third group. As the farm sizes increase, the number of dairy cattle in the farms, daily milk yield per dairy cattle, milk production in the lactation period and milk sales price increase ($P<0.01$).

Table 2. The number of dairy cattle, milk yield and prices by farms size group
Çizelge 2. İşletme genişlik gruplarına göre süt sığırlarının sayısı, süt verimi ve süt satış fiyatları

Farm size groups (number of milk cows)	Number of dairy cattle (head)	Milk yield (lt/head/day)	Lactation days (lt/head/day)	Lactation yield (lt/head/ lactation)	Milk sales price (TL/lt)
1st Group (5-14 head)	8.60^{c}	20.26^{b}	266.87	5405.57^{c}	1.04^{c}
2nd Group (15-29 head)	22.04^{b}	22.26^{a}	265.43	5908.47^{b}	1.07^{b}
3rd Group (30+head)	52.44^{a}	22.47^{a}	268.41	6024.88^{a}	1.13^{a}
Average	25.70	21.40	266.91	5761.87	1.07
F-Test	59.938*	5.931*	0.038	6.021*	19.29*
(P value)	(0.000)	(0.004)	(0.962)	(0.000)	(0.000)

*: Statistically important at a 1% significance level.

Use of Forage Crops in Farms

According to results, 71.3% of the producers applied different feed rotations to their animals in the region. The averages daily feed amounts given to the dairy cattle, calves and heifers of the farms were researched and explained in Table 3. According to results by farm size groups, Group 3 enterprises give concentrated feed (factory feed) to cattle, calf and heifer more than other farms.

The production of maize silage has been increasing gradually in recent years as it increases milk yield and decreases the production cost in dairy cattle. Considering the daily corn silage amount given at the farms; The farm group that gives the most corn silage is the third group with 18.3 kg/head/day for the cow, while the second group for the calf (9.7 kg/head/day) and the second group for the heifer (10.6 kg/head/day).

Another noteworthy point in the table is that the farms that give the most Straw to cattle, veal and heifers are the ones in the second group. Moreover, the farms that give the highest alfalfa to the cattle are the first group farms, while the farms that give the highest alfalfa to the calf and heifers are the second group.

Producers were asked if they produced roughage feed for their animals, and according to the answers received, 92.20% of the dairy cattle farms produced roughage feed. According to previous researches, 90.70% of the dairy cattle farms in Izmir province (Uygur, 2015) and 32.80% of the dairy cattle farms in Kahramanmaras province (Ayman, 2014) produced roughage feed.

Total cultivated area and forage plantation areas by farm groups are given in Table 4. As the scales of the farms grow, the total and forage crops cultivation areas of the businesses tend to increase. When the relationship between farm groups and the total farm

Table 3. Feed amounts given to cattle, veal and heifer for one day by farm groups
Çizelge 3. İşletme genişlik grupları itibariyle dana ve düveye bir günlük verilen yem miktarları

The amount of daily feed given to a head of cattle per day as of the operating groups					
Farms size groups	Cattle Daily Feed Amounts (kg)				
	Straw	Hay	Alfalfa	Corn Silage	Factory Feed
1st Group (5-14 head)	4.3	3.4	4.1	14.1	6.5
2nd Group (15-29 head)	5.7	4.3	3.2	17.2	6.9
3rd Group (30+head)	4.0	2.7	2.7	18.3	7.6
Feed amounts given for a daily head calf as of farm groups					
Farms size groups	Calf Daily Feed Amounts (kg)				
	Straw	Hay	Alfalfa	Corn Silage	Factory Feed
1st Group (5-14 head)	3.1	2.1	2.0	8.5	4.2
2nd Group (15-29 head)	4.2	3.8	2.2	9.7	4.9
3rd Group (30+head)	3.0	2.0	1.7	8.7	5.5
Feed amounts given for a head heifer per day as of farm groups					
Farms size groups	Heifer Daily Feed Amounts (kg)				
	Straw	Hay	Alfalfa	Corn Silage	Factory Feed
1st Group (5-14 head)	3.4	2.1	3.3	8.8	3.7
2nd Group (15-29 head)	4.2	3.8	3.6	10.6	3.6
3rd Group (30+head)	2.9	2.3	2.1	10.0	4.3

area owned by farmers was tested by Anova, significant differences were found between the groups. The average total area of farms was 79.52 da and the forage planting area was 39.46 da. According to the LSD confidence interval, as farm size getting increases, the total planting area and also forage planting area are statistically getting increase ($P<0.01$). On the other hand, the proportion of the forage planting area within the total planting area was 49.6%. The second farm group had the highest forage planting area with 62.2%. But the shares by farm size groups were not statistically different ($P>0.05$).

In his research for Aydın, Nizam (2006) reported that the average total land of farmers is 128.31 da, the forage planting area within the total area was 101.15 da (78.83%), Şahin et al. (2001) reported that, for the province of Adana, the average total land assets of the farmers was 132.3 da and the share of the forage planting area in the total area was only 12.1 da (9.1%).

Table 4. Feed plant areas of farmers as of farm groups
Çizelge 4. İşletme genişlik gruplarına göre işletmelerin yem bitkileri alanları

Farm size groups	Total area (da)	Forage production area (da)	The proportion of forage planting area within total planting area (%)
1st Group (5-14 head)	38.36[c]	22.41[c]	58.40
2nd Group (15-29 head)	73.04[b]	45.43[b]	62.20
3rd Group (30+head)	144.63[a]	79.88[a]	55.20
Average	79.52	39.46	49.62
F-test	18.386*	12.920*	1.586
(P-value)	(0.000)	(0.000)	(0.653)

*: Statistically important at a 1% significance level.

In dairy cattle farms, silage corn is produced more than other products because producers want to obtain affordable and quality feed (TÜİK, 2014). While Aegean Region is at the top of the silage maize production in Turkey, this research determined that the most common forage of cattle breeders in İzmir province was the silage corn with 48.7%. The least cultivated forage in the total forage cultivation area is vetch with 1.60%. The second most planted forage by the farmers was barley with 12.7% followed by Karamba with 11.2%. Karamba plant has been one of the forages that producers have preferred after corn silage in recent years due to its protein for dairy cattle and its use as green feed in winter. Moreover, the share of turnip plant, which balances milk yield in dairy cattle in winter and is used as a green feed plant, is 6.6% in the feed plant cultivation area (Table 5). Dairy farm owners also grow vegetables (potatoes, cucumbers, tomatoes) and olives in the area left over from forage crops. The silage corn cultivation rate of the farmers in the study area was found to be higher than the finding (42.10%) predicted by Nizam (2006)

for Aydın province.

Looking at the current warehouse conditions for the roughage and concentrated feed of dairy cattle farms; the average roughage of the first group of farmers was 24.42 tons, and the concentrated feed was 4.58 tons. In third group farms, the average available roughage was 135.34 tons, and concentrated feed was 34.54 tons (Table 6). In the research area, the existing warehouse assets increased in proportion to the dairy farm size. When the relationship between farm size groups and the roughage stock owned by farms was tested with ANOVA, significant differences were found between the groups (P<0.01). According to the LSD confidence interval, the amount of roughage was statistically increasing as the farm size increases.

Table 5. Forage crops grown by agricultural farms (%)
Çizelge 5. Tarımsal işletmeler tarafından yetiştirilen yem bitkileri (%)

Products	Percentages
Corn Silage	48.7
Barley	12.7
Karamba	11.2
Clover	8.1
Tourniquet	6.6
Wheat	6.1
Oats	3.0
Triticale	2.0
Vetch	1.6

Table 6. Roughage and concentrated feed amounts of farmers by farm size groups
Çizelge 6. İşletme genişlik gruplarına göre işletmelerin kaba ve konsantre yem miktarları

Farm size groups	Presence of roughage feed (tons)	Roughage feed (TL)	Presence of concentrated feed (tons)	Concentrated feed (TL)
1st Group	24.42[c]	7391.32[c]	4.58	4293.10
2nd Group	109.02[b]	28658.89[b]	24.21	10131.67
3rd Group	135.34[a]	36484.07[a]	34.54	12931.67
Average	78.85	21467.47	19.58	8572.82
F-test	8.461*	9.414*	1.816	1.668
(P value)	(0.000)	(0.000)	(0.170)	(0.196)

*: Statistically important at a 1% significance level.

Table 7 illustrates the situation of farmers meeting their own needs from their roughage production. According to results, 24.8% of dairy farmers had met all of their own needs, 25.7% most of them, 30.70% half of them, and only 3.0% did not meet their own needs.

Table 7. The status of meeting the need of roughage produced by the dairy cattle farmers
Çizelge 7. Süt sığırcılığı işletmelerinde üretilen kaba yemin ihtiyacı karşılama durumu

Degree	Number of dairy farms	%
Never	3	3.00
Very little of it	16	15.80
Half	32	30.70
Most	26	25.70
Entire	25	24.80
Total	102	100.00

Economic Analysis of Dairy Production

Variable costs depending on the production amounts of farms during the year consist of feed, labor, veterinary, vaccine, and drug costs, etc. (Tokmak, 2009). The variable costs of the farms in the research area are shown in Table 8. Variable costs consisting of feed costs, barn cleaning costs, milking costs, foreign labor costs, veterinary drug cost, vaccinations, artificial insemination costs, heating, cooling and lighting costs, disinfection costs, were 33059,6 TL in the first group, 70644,6 TL in the second group, 195604,7 TL in the third group and the average of all farms was 104980,0 TL. Animal insurance costs were not included in the variable costs because only one agricultural enterprise in the group insured their animals. When we look at farm groups; as the number of animals on the farm increases, variable costs also increases. Feed costs have the biggest share in variable costs.

Variable costs per animal milked by farm groups are given in Table 9. Variable costs were found to be 3628.9 TL in small farm group, 3235.7 TL in middle farm group, and 3637.7 TL in large farm group, a sizeable advantage. Fixed costs were 1102.3 TL in the first group, 577.8 TL in the second group and 398.5 TL in the third farm group. As farm size grows, fixed costs decrease and variable costs decreases for second farm size group but increases for large farm size group. Therefore, there may be significant economies of scale in dairy milk production. Feed costs account for a large proportion of total costs across farm sizes, but the average feed costs do not appear to be a source of scale economies, as they do not fall sharply with farm size. Fixed costs fall sharply as farm size increases, suggesting that large farms use their equipment and structure more effectively. As a part of fixed costs, labor costs per animal also fall quite sharply by farms size group. Larger farms can also minimize the idle time of farm equipment.

Table 8. Average variable costs by farm size group (TL)
Çizelge 8. İşletme genişlik gruplarına göre ortalama değişken maliyet (TL)

Type of Costs	1st farm size group (5-14 head)	2nd farm size group (15-29 head)	3rd farm size group (30+ head)	Average
Feed costs	26589.2	57537.3	169755.4	89200.1
Barn cleaning	403.8	1615.3	2676.6	1643.3
Milking costs	486.5	1296.7	2578.1	1523.3
Veterinarian, medicine and vaccine	2930.8	5680.0	12109.4	7205.7
Artificial insemination	829.2	1856.3	3062.5	1991.5
Heating, cooling and lighting	1560.8	2507.3	4512.5	2956.8
Costs of disinfection	259.2	151.7	296.3	236.0
Total variable expenses	33059.5	70644.6	194990.8	104756.7
Total fixed costs	10042.2	12614.1	21360.3	15034.6
Total Cost	43101.7	83258.7	216351.1	119791.3

Table 9. Variable costs per animal by farm size groups (TL/head)
Çizelge 9. İşletme genişlik gruplarına göre hayvan başına değişken maliyetler (TL/Baş)

Type of Costs	1st farm size group (5-14 head)	2nd farm size group (15-29 head)	3rd farm size group (30+ head)	Average
Feed costs	2918.7	2635.4	3166.9	3010.8
Barn cleaning	44.3	74.0	49.9	55.5
Milking costs	53.4	59.4	48.1	51.4
Veterinarian, medicine and vaccine	321.7	260.2	225.9	243.2
Artificial insemination	91.0	85.0	57.1	67.2
Heating, cooling and lighting	171.3	114.8	84.2	99.8
Costs of disinfection	28.5	6.9	5.5	8.0
Total variable expenses	3628.9	3235.7	3637.7	3535.9
Total fixed costs	1102.3	577.8	398.5	507.5
Total Cost	4731.3	3813.5	4036.2	4043.4

The share of each cost on total variable costs is illustrated in Table 10. As a result of the research, feed costs constitute the highest cost among all farm groups (85.15%), this percentage increases proportionally from the first group (80.43%) to the third group (87.06%). Increases in feed prices have substantial effects on costs. This result is similar to finding by MacDonald et al. (2007) found that feed costs account for a large share of total costs across farm sizes.

On the other hand, veterinarian, medicine and vaccine cost (8.87%) constitutes the second important cost type, and as the farm size groups, the percentage share of veterinarian, medicine and vaccine cost decreases as the farm size increases. At the same time, share of heating, cooling and lighting cost and disinfection costs decrease as farm size increases. But there was no parallel changing between farm size and barn cleaning, artificial insemination.

In the researched dairy farms, the total income, total costs, gross and net profit per milked animal according to the farm size groups are given in Table 11. Gross profit is an important criterion in terms of using scarce resources and determining competition in farms (Özüdoğru, 2012). Therefore, by comparing the gross profit values per animal milked in the researched farms, the second group of farms is determined to be more successful by using their production tools more efficiently, while the average per animal milked gross profit was 3885.27 TL. Total costs are declining as farm size increases. The average total income per animal was 7421.21 TL and the net profit was 3377.79 TL on average. Moreover, there was a statistical difference in total revenue by farm size group. Besides, total income for large farms were 12.7% above small farms but costs for the small farms were 17.2% above large farms. Because of the cost advantage of large farms, gross and net profit of these farms was 26.1% and 69.6% higher than small farms. Benefit-Cost Ratio is 1.84 and seems highly feasible. This ratio indicates that dairy farming in the region is economically efficient and beneficial. Moreover, the Benefit-Cost Ratio is increasing with the farm size. These results are similar to the results observed by MacDonald et al. (2007), Kumawat et al. (2014) and Datta et al. (2019). For example, MacDonald et al. (2007) found that the cost advantages of larger size allow large farms to be profitable, on average, even while most small farms were unable to earn enough to replace their capital. The profit of 1 liter of raw milk was found as 0.44 TL 0.60 TL , and 0.67 TL for first, second and the third group farms, respectively.

Table 10. The share of each cost types on total variable costs by farm size groups (%)
Çizelge 10. İşletme genişlik gruplarına göre her bir maliyet türünün toplam değişken maliyetlerdeki oranı (%)

Type of Costs	1st farm size group (5-14 head)	2nd farm size group (15-29 head)	3rd farm size group (30+ head)	Average
Feed costs	80.43	81.45	87.06	85.15
Barn cleaning	1.22	2.29	1.37	1.57
Milking costs	1.47	1.84	1.32	1.45
Veterinarian, medicine and vaccine	8.87	8.04	6.21	6.88
Artificial insemination	2.51	2.63	1.57	1.90
Heating, cooling and lighting	4.72	3.55	2.31	2.82
Costs of disinfection	0.78	0.21	0.15	0.23
Total variable expenses	100.00	100.00	100.00	100.00

Table 11. Average total income, total cost, gross and net profit per milking animal by farm size groups (TL/Head)
Çizelge 11. İşletme genişlik gruplarına göre ortalama gelir, maliyet, sağılan hayvan başına brüt ve net kar (TL/ Baş)

Farm size groups	Total Revenue	Total Cost	Gross Profit	Net Profit	Benefit-Cost Ratio
1st Group (5-14 head)	7013.14	4731.25	3384.22	2281.89	1.48
2nd Group (15-29 head)	7257.09	3813.49	4021.36	3443.60	1.90
3rd Group (30+head)	7906.62	4036.23	4268.88	3870.39	1.96
Average	7421.21	4043.42	3885.27	3377.79	1.84
F-test	4.505*	3.808*	1.975	7.205*	4.557*
(P value)	(0.014)	(0.026)	(0.145)	(0.001)	(0.013)

*: Statistically important at a 5% significance level.

CONCLUSSION and RECOMENDATIONS

The main objective of this study was to determine the economic analysis of dairy cattle holdings. Turkey milk production is rapidly shifting to larger dairy farms. The results proved that the productivity and profitability of dairy farming are positively affected by the size of the dairy farm. Large dairy farms have substantial cost advantages over smaller ones. Large dairy farms in the region were much more likely to use new technologies to increase their income and profit. Results showed that large dairy farms had many advantages on milk yield, milk prices, and high production of feed planting, lower cost, higher return, and profit. Given this, production should continue to shift towards large dairy farms. It was determined that 92.20% of the farmers produced roughage itself. However, their production does not meet all their needs. It was observed that farmers with small-scale enterprises had difficulty in producing roughage because they did not have enough land. Farmers should be provided with rental land by the Provincial Directorates of Agriculture or they should provide quality feed supply at an affordable price. It should be noted that the feed inputs provided to small agricultural farms are of high quality and affordable prices so that the farmers will go to increase their livestock and grow their farms. Most of the producers apply different but unconscious rations to dairy cattle. Since feed cost constitutes the most expensive item of a dairy farm, with the application of ration, feed costs decrease, and the yield per animal increases. In particular, all the farms dealing with dairy cattle breeding should be informed about the ration application by technical staff and the application should be ensured.

The higher the yield obtained from the unit animal, the higher the profit of the activity produced. For this reason, regular records should be kept for dairy cattle and these records should also be checked by the Breeding Union or Agricultural District Directorates. Cattle with higher productivity in regularly recorded cattle are transferred to the next generation, so that the yield can be increased. Moreover, in order to achieve high productivity at low cost in enterprises, ration application should be applied in feeding. The instability of coarse and concentrated feed prices, high prices and low milk prices put the animal enterprises in the region in a difficult situation. This instability in the market must be eliminated and the state should support the farmers in this regard.

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Researchers Contribution Rate Declaration Summary

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

Conflicts of Interest

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Ecological and Phytogeographical Status and Species Composition of the Phytoplankton in the Gulf of Aqaba (Red Sea)

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ABSTRACT
One hundred and seven samples were collected from sea surface from 5 stations along the Jordanian coast of the Gulf of Aqaba. Overall,188 species were identified under six phytoplankton classes. Dinoflagellates dominate sixty percent of the total species. Diatoms constituted 38% and other groups represented by 2%. The geographical distribution of the identified species, 37% cosmopolitan, 28% boreal-tropical, 17% tropical, 11% tropical-subtropical, 4% boreal, 2% arcto-boreal and% 1 was determined as subtropical. According to ecological distribution, 88% of the species were marine and 12% marine-brackish origin. Also, 80% of the species were of pelagic origin, and 20% are benthic origin species. The phytoplankton species composition, phytogeographic and ecological distribution and species origins were presented from 2007 through 2008.

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Akabe Körfezi (Kızıldeniz) Fitoplanktonunun Ekolojik ve Fitocoğrafik Durumu ve Tür Kompozisyonu

ÖZET
Aqaba Körfezi Ürdün kıyılarından 5 istasyondan deniz yüzey suyundan 107 örnek toplanmıştır. Genel olarak, 6 fitoplankton sınıfına ait 188 fitoplankton türü tespit edilmiştir. Toplam tür sayısının %60'ı dinoflagellatlar tarafından domine edilmiştir. Toplam tür sayısının %38'ini diatomlar ve %2'sini diğer gruplar oluşturmuştur. Tespit edilen türlerin coğrafi dağılımı; % 37 kozmopolit, % 28 boreal-tropikal, % 17 tropikal, % 11 tropikal-subtropikal, % 4 boreal, % 2 arkto-boreal ve % 1 subtropikal olarak belirlenmiştir. Ekolojik dağılıma göre türlerin % 88'i deniz, % 12'si deniz-acı su kökenlidir. Ayrıca türlerin % 80'i pelajik kökenlidir ve % 20'si bentik kökenli türlerdir. 2007-2008 döneminde fitoplankton tür kompozisyonu, fitocoğrafik ve ekolojik dağılımı ve türlerin kökenleri sunulmuştur.

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INTRODUCTION
The Gulf of Aqaba, where the world's amazing tropical coral reefs are located, is a particular marine area of the coastal countries and the world. However, the Gulf is under high pressure from different sources such as urban and industrial pollution, shipping, port activities and tourism (Mergner, 1981; Walker and Ormond, 1982; Abu-Hilal, 1987; Abu-Hilal and Badran, 1990; Abelson et al., 1999). There is a total of 13 km of coral reefs along the 27 km long Jordanian coastline (Lazar et al., 2008). This intermediate and around the reefs is surrounded by seagrass (UNEP/IUCN, 1998). Around 30-40% of untouched natural areas along the coastline have been destroyed and transformed into a port and industrial zone in the last 25 years (Abu-Hilal, 1997). Besides, Aqaba Port was declared as a "Special Economic Zone" in 2001. Industrial and port activities continue to increase since this process (Khalaf and Kochzius, 2002).

Four significant changes highlighting the threat of

eutrophication were observed in the region: (1) dissolved inorganic nitrogen in the deep-water pool, (2) deep oxygen depletion, (3) increased growth rate of macroalgae in between coral reefs (4) and increased organic content in the sediment. These findings emphasize the dangers encountered in future eutrophication in the Gulf (Genin, 2005).

Studies on Red Sea phytoplankton composition are very few. The low number of phytoplankton species in the Red Sea and the representation of a large part of the composition (> 95) with ultraplankton (8µm) are characteristic when compared to other seas (Lindell and Post, 1995; Post et al., 2002; Al-Najjar et al., 2007). In previous studies to reveal the composition, a limited number of samples were collected in the short term. The phytoplankton species composition was ignored in most studies (Weikert, 1987; Sommer, 2000; Sommer et al., 2002).

The clear/clean seawater is one of the most critical assets of the Gulf of Aqaba's coastal populations. Because of these waters' high quality, the world-renowned coral reef community has survived for thousands of years. It is a unique hot-spot of biodiversity and a repository and refuge of threatened species.

However, there is overwhelming evidence that the Gulf of Aqaba's reefs have been declining both in live coral cover and in the biodiversity of corals and associated biota in recent years. The concomitant changes in the phytoplankton, whose structure and function are significant to the survival of coral reefs, are also important.

This study provides a taxonomic evaluation of phytoplankton species composition in the Gulf of Aqaba using microscopic analyses. In addition to that, the phytogeography and ecological status were revealed.

MATERIALS and METHOD

Sampling Area

The Gulf of Aqaba lies between the Sinai Peninsula and the Arabian coast and is a part of the great Syrian-East Africa Rift (Gregory, 1921) (Figure 1). There are two major basins in the Gulf: the northern one extending south to Nuweiba with a maximum depth of 1000 m, and the southern one, rising to the Straits of Tiran with a maximum depth of 1800 m. The water mean annual temperature of the is 23.0 ^{o}C. Due to the exceptionally intense evaporation (average 200 cm per year) (Godeaux, 1986), the salinity exceeds oceanic salinity and research values of 41.0-42.0 ‰. Surface water salinity increases progressively from south to the north. The Gulf of Aqaba is considered oligotrophic based on chlorophyll-*a* values (0.024-0.522 mg m^{-3}) and primary productivity measurements (36 cg m^{-2} year-1) (Oren, 1970; Azov, 1986; Berman et al., 1986; Kimor, 1990; Sommer et al., 2002; Badran et al., 2005; Al-Najjar et al., 2007). 5 stations were selected to represent Jordan's continental shelf in Aqaba Bay (Figure 1).

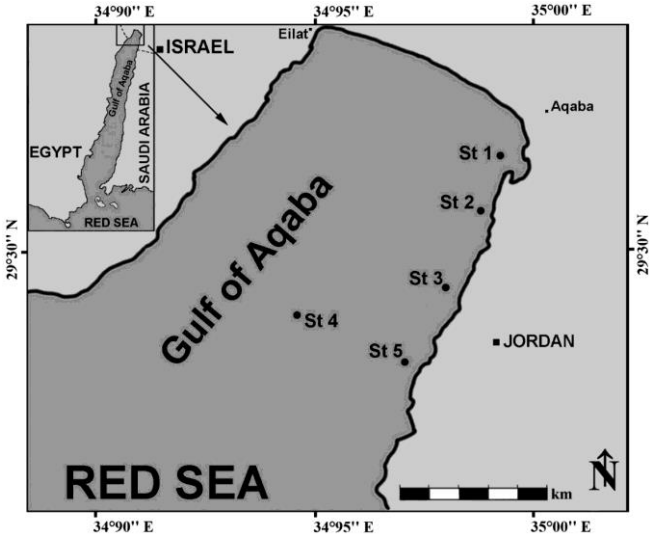

Figure1. Location of the sampling stations in the study area

Şekil 1. İstasyonların çalışma alanındaki konumları

Phytoplankton Sampling and Analyzing

A total of 107 samples were collected from sea surface at five stations along the Gulf of Aqaba's Jordanian coast from January to December 2007 and January to October 2008 (Figure 1). Phytoplankton samples were taken from surface from each station by using Niskin type universal water sampler (5 l). Sea water samples fixed with acidic-lugol iodine solution (2.5-3.0 cc 1^{-1}) and transported to the laboratory (Sukhanova, 1978).

A Sedwick-Rafter counting chamber was used for the micro-phytoplanktonic species with a cell diameter over 15 µm. Cell numbers (l) were counted under Nikon Eclippse E600 at various magnifications. Nano-phytoplanktonic species with a cell diameter <15 µm were mounted on glass slides (0.01 ml) and examined under the same microscope at high magnifications.

The following sources were used for species identification: Cupp,(1943), Kiselev (1950), Proshnika-Lavienko (1955), Rampi and Bernhard (1980), Senichkina (1986), Hillebrand et al. (1999). Taxonomy of species is organized according to AlgaeBASE (Guiry and Guiry, 2021). To establish the ecological and phytogeographical characteristics of the phytoplankton the following sources were used: Heimdal (1989), Medlin and Priddle (1990), Makarevich and Larionov (1992) and Druzhkov and Makarevich (1999).

RESULTS

Species Composition

The phytoplankton species collected at the sampling

stations in 2007-2008 is given in Table 1. A total of 188 species was determined in which Bacillariophyceae was represented by 28 genera and 35 species; Coscinodiscophyceae was represented by 11 genera and 20 species; Mediophyceae was represented by 13 genera and 17 species; Dinophycaeae by 21 genera and 112 species; Coccolithophyceae by 1 genus and 1 species and Dictyochophyceae was represented by 2 genera and 3 species. From the species diversity, 60% were represented by dinoflagellates, 38% by diatoms and 2% by the other classes.

When the number of phytoplankton species were examined at the genus level, it was found that dinoflagellates contained the genera with the highest number of species. The highest number of species are from the Dinophyceae class: *Tripos* (29 species), *Protoperidinium* (18 species), *Oxytoxum* (12 species), *Dinophysis* (7 species), *Prorocentrum* (7 species), *Gonyaulax* (5 species) and *Histioneis* (4 species); from the Bacillariophyceae class: *Licmophora* (3 species); from the Coscinodiscophyceae class: *Actinoptychus* (2 species), *Coscinodiscus* (3 species) and *Rhizosolenia* (3 species); from the Mediophyceae class: *Chaetoceros* (4 species) have been identified.

Table 1 Taxonomic composition of the Jordan shores of the Gulf of Aqaba (Red Sea) in 2007-2008 (Phyto-Geographical Group-PG, C: Cosmopolitan species, A-B: Arcto-boreal species, B: Boreal species, B-T: Boreal-tropical species, T: Tropical species, T-ST: Tropical-subtropical species, ST: Subtropical species; Ecological Group-EG, M: Marine species, MB: Marine and Brackishwater species; Geographical Origin-GO, b: benthic-originated species, p: pelagic-originated species; Prevailing field-PF, P: Pelagial, B: Benthal, m: Mobil substratum, h: Hard substratum)

Çizelge 1. Akabe Körfezi (Kızıl Deniz) 2007-2008 dönemi taksonomik kompozisyonu (Fito-Coğrafik Grup-PG, C: Kozmopolit tür, A-B: Arkto-boreal tür, B: Boreal tür, B-T: Boreal-tropikal tür, T: Tropikal tür, T-ST: Tropikal-subtropikal tür, ST: Subtropikal tür; Ekolojik Grup-EG, M: Denizel tür, MB: Denizel ve Acısu tür; Coğrafik Köken-GO, b: bentik orijinli tür, p: pelajik orijinli tür; Bulunuş Alanı-PF, P: Pelajik, B: Bentik, m: Hareketli substratum, h: Sert substratum)

SPECIES LIST	Sampling Years		Phytogeographical-Ecological Status		
	2007	2008	PG	EG-GO	PF
BACILLARIOPHYCEAE	-	+	C	M-b	PBm,h
Achnanthes adnata Bory 1822	-	+	C	M-b	PBm,h
Achnanthes armillaris (O.F.Müller) Guiry 2019	+	+	C	M-b	PBm,h
Amphora lineolata Ehrenberg 1838	+	+	T-ST	MB-b	PBm,h
Campylodiscus neofastuosus Ruck & Nakov 2016	+	+	T-ST	M-b	PBm,h
Campylodiscus sp.	+	-	T-ST	M-b	PB
Coronia decora (Brébisson) Ruck & Guiry 2016	+	+	T-ST	M-b	PB
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin 1964	+	+	C	M-b	PBm,h
Diploneis interrupta (Kützing) Cleve 1894	+	+	C	MB-b	PBm,h
Diploneis sp.	+	-	C	MB-b	PBm,h
Fragilaria sp.	+	-	C	MB-b	PBm,h
Grammatophora marina (Lyngbye) Kützing 1844	-	+	C	M-b	PBm,h
Halamphora coffeiformis (C.Agardh) Mereschkowsky 1903	-	+	C	MB-b	PBm,h
Licmophora ehrenbergii (Kützing) Grunow 1867	+	+	C	MB-b	PBm,h
Licmophora flabellata (Greville) C.Agardh 1831	+	+	C	M-b	PBm,h
Licmophora gracilis (Ehrenberg) Grunow 1867	+	+	C	M-b	PBm,h
Lyrella lyroides (Hendey) D.G.Mann 1990	+	+	T-ST	MB-b	PBm,h
Mastogloia sp.	+	-	T-ST	MB-b	PBm,h
Meuniera membranacea (Cleve) P.C.Silva 1996	-	+	C	MB-b	PBm,h
Navicula sp.	+	-	C	MB-b	PBm,h
Nitzschia longissima (Brébisson) Ralfs 1861	+	+	B	M-b	Bh
Nitzschia tenuirostris Mer.	+	+	B	M-b	Bh
Petrodictyon gemma (Ehrenberg) D.G.Mann 1990	+	+	T-ST	M-b	PBm,h
Plagiodiscus nervatus Grunow 1867	+	-	C	M-p	PBm
Pleurosigma angulatum (J.T.Quekett) W.Smith 1852	+	+	C	MB-b	PBm
Stenopterobia heribaudii (Playfair) Playfair	+	+	C	M-b	PB
Striatella unipunctata (Lyngbye) C.Agardh 1832	+	+	C	M-b	PB
Surirella hybrida Grunow 1881	+	-	T-ST	M-b	PBm,h
Synedra sp.	+	-	C	M-b	PBm
Tabellaria fenestrata (Lyngbye) Kützing 1844	+	-	C	M-p	PBm
Thalassionema nitzschioides (Grunow) Mereschkowsky 1902	-	+	C	M-p	PBm,h
Thalassiothrix longissima Cleve & Grunow 1880	-	+	A-B	M-p	P
Trachyneis aspera (Ehrenberg) Cleve 1894	+	+	C	M-b	PBm

SPECIES LIST	Sampling Years		Phytogeographical-Ecological Status		
	2007	2008	PG	EG-GO	PF
Tryblionella compressa (Bailey) Poulin 1990	+	+	B-T	M-p	PB,m
Ulnaria ulna (Nitzsch) Compère 2001	-	+	C	M-b	PBm
COSCINODISCOPHYCEAE					
Actinocyclus octonarius Ehrenberg 1837	+	+	B-T	M-p	PBm
Actinoptychus senarius (Ehrenberg) Ehrenberg 1843	+	+	B-T	M-p	PBm
Actinoptychus splendens (Shadbolt) Ralfs 1861	+	+	B-T	M-b	PBm
Asterolampra marylandica Ehrenberg 1844	-	+	C	M-p	P
Asteromphalus flabellatus (Brébisson) Greville 1859	-	+	C	M-p	P
Coscinodiscus centralis Ehrenberg 1839	+	+	B-T	M-p	PBm
Coscinodiscus perforatus Ehrenberg 1844	+	+	B	M-p	PBm
Coscinodiscus radiatus Ehrenberg 1840	+	+	C	M-b	PBm,h
Guinardia delicatula (Cleve) Hasle 1997	+	+	T-ST	M-p	PBm
Guinardia flaccida (Castracane) H.Peragallo 1892	+	+	T-ST	M-b	PBm
Melosira sp.	+	-	B-T	M-b	Bm,h
Neocalyptrella robusta (G.Norman ex Ralfs) Hernández-Becerril & Meave del Castillo 1997	+	-	B-T	M-b	Bm,h
Proboscia alata (Brightwell) Sundström 1986	+	+	C	M-p	P
Proboscia indica (H.Peragallo) Hernández-Becerril 1995	-	+	B-T	M-p	P
Pseudosolenia calcar-avis (Schultze) B.G.Sundström 1986	+	+	B-T	M-p	P
Rhizosolenia imbricata Brightwell 1858	+	+	B	M-p	P
Rhizosolenia setigera Brightwell 1858	+	-	B	M-p	P
Rhizosolenia styliformis T.Brightwell 1858	+	+	B	M-p	P
Triceratium favus Ehrenberg 1839	+	+	B-T	MB-p	P
Triceratium sp.	+	-	B-T	MB-p	P
MEDIOPHYCEAE					
Auliscus sculptus (W.Smith) Brightwell 1860	-	+	C	M-b	PBm
Bacteriastrum delicatulum Cleve 1897	-	+	B-T	M-p	P
Biddulphia alternans (Bailey) Van Heurck 1885	+	-	C	M-p	P
Chaetoceros brevis F.Schütt 1895	-	+	ST	M-p	P
Chaetoceros decipiens Cleve 1873	+	+	A-B	M-p	P
Chaetoceros lauderi Ralfs ex Lauder 1864	-	+	T-ST	M-p	P
Chaetoceros lorenzianus Grunow 1863	-	+	B-T	M-p	PB,m
Climacodium frauenfeldianum Grunow 1868	-	+	B-T	M-b	PB,m
Hemiaulus hauckii Grunow ex Van Heurck 1882	+	-	B-T	M-p	P
Lampriscus shadboltianum (Greville) Peragallo & Peragallo 1902	+	+	B-T	MB-p	P
Leptocylindrus danicus Cleve 1889	+	+	C	M-p	PBm
Odontella aurita (Lyngbye) C.Agardh 1832	-	+	B-T	MB-p	PB,m
Skeletonema costatum (Greville) Cleve 1873	-	+	C	M-p	PB,m
Terpsinoë americana (Bailey) Ralfs 1861	+	-	B-T	MB-p	P
Thalassiosira eccentrica (Ehrenberg) Cleve 1904	+	+	A-B	M-p	PBh
Thalassiosira leptopus (Grunow) Hasle & G.Fryxell 1977	-	+	C	M-b	B,m
Toxarium undulatum Bailey 1854	-	+	C	M-p	P
DINOPHYCEAE					
Acanthogonyaulax spinifera (Murray & Whitting) H.W.Graham 1942	-	+	B-T	M-p	P
Actiniscus pentasterias (Ehrenberg) Ehrenberg 1844	+	+	C	M-p	P
Amphisolenia bidentata B.Schröder 1900	+	+	C	M-p	P
Ceratocorys armata (Schütt) Kofoid 1910	+	+	T	M-p	P
Ceratocorys gourretii Paulsen 1937	+	+	T	M-p	P
Ceratocorys horrida Stein 1883	+	+	T	M-p	P
Corythodinium constrictum (F.Stein) F.J.R.Taylor 1976	+	+	T	M-p	P
Corythodinium milneri (G.Murray & Whitting) F.Gómez 2017	+	-	T	M-p	P
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III 1966	+	-	B-T	M-p	P
Dinophysis argus (Stein) Abé 1967	-	+	C	M-p	P

SPECIES LIST	Sampling Years		Phytogeographical-Ecological Status		
	2007	2008	PG	EG-GO	PF
Dinophysis acuminata Claparède & Lachmann 1859	+	-	B	M-p	P
Dinophysis amandula (Balech) Sournia 1973	+	+	C	M-p	P
Dinophysis caudata W.S.Kent 1881	+	-	B-T	M-p	P
Dinophysis doryphorides (P.A.Dangeard) Balech 1967	+	+	B-T	M-p	P
Dinophysis fortii Pavillard 1924	+	+	B-T	M-p	P
Dinophysis sphaerica F.Stein 1883	+	-	C	M-p	P
Diplopsalis lenticula Bergh 1881	-	+	C	MB-p	P
Fragilidium sp.	+	-	B-T	M-p	PB,m
Gonyaulax birostris Stein 1983	+	+	B-T	M-p	P
Gonyaulax monacantha Pavillard 1916	+	+	B-T	M-p	P
Gonyaulax polygramma F.Stein 1883	+	+	B-T	M-p	P
Gonyaulax scrippsae Kofoid 1911	+	+	B-T	M-p	P
Gonyaulax spinifera (Claparède & Lachmann) Diesing 1866	+	+	B-T	M-p	P
Gyrodinium helveticum (Penard) Y.Takano & T.Horiguchi 2004	+	+	C	M-p	P
Gyrodinium britannia Kofoid & Swezy 1921	-	+	C	M-p	P
Gyrodinium fusiforme Kofoid & Swezy 1921	+	+	T, A-B	M-p	P
Gyrodinium spirale (Bergh) Kofoid & Swezy 1921	-	+	B-T	M-p	P
Histioneis elongata Kofoid & J.R.Michener 1911	+	+	T	M-p	P
Histioneis joergensenii J.Schiller 1928	+	-	T	M-p	P
Histioneis longicollis Kofoid 1907	+	-	T	M-p	P
Histioneis sphaeroidea Rampi 1947	-	+	T	M-p	P
Lingulodinium polyedra (F.Stein) J.D.Dodge 1989	+	+	B-T	M-p	PB,m
Ornithocercus magnificus F.Stein 1883	+	-	T	M-p	P
Ornithocercus quadratus Schütt 1900	+	-	T	M-p	P
Oxytoxum caudatum Schiller 1937	+	-	T	M-p	P
Oxytoxum depressum J.Schiller 1937	-	+	C	M-p	P
Oxytoxum globosum Schiller 1937	+	-	B-T	M-p	P
Oxytoxum longiceps Schiller 1937	+	-	T	M-p	P
Oxytoxum longum J.Schiller 1937	+	+	T	M-p	P
Oxytoxum minutum Rampi 1941	+	-	T	M-p	P
Oxytoxum mitra (F.Stein) Schröder 1906	+	-	T	M-p	P
Oxytoxum parvum J.Schiller 1937	+	+	B-T	M-p	P
Oxytoxum rampii Sournia 1973	+	-	T	M-p	P
Oxytoxum sceptrum (F.Stein) Schröder 1906	-	+	B-T	M-p	P
Oxytoxum scolopax F.Stein 1883	+	+	B-T	M-p	P
Oxytoxum tesselatum (F.Stein) Schütt 1895	+	+	T	M-p	P
Parahistioneis acutiformis Rampi 1947	+	-	T	M-p	P
Phalacroma mitra F.Schütt 1895	+	+	C	M-p	P
Phalacroma porodictyum F.Stein 1883	+	-	C	M-p	P
Phalacroma rapa F.Stein 1883	+	-	C	M-p	P
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & J.R.Michener 1911	+	+	C	M-p	P
Podolampas bipes F.Stein 1883	+	+	T	M-p	P
Podolampas palmipes Stein 1883	+	+	T	M-p	P
Podolampas spinifera Okamura 1912	+	+	T	M-p	P
Prorocentrum cordatum (Ostenfeld) J.D.Dodge 1976	+	+	B-T	M-p	P
Prorocentrum lima (Ehrenberg) F.Stein 1878	+	+	C	M-p	PB,h
Prorocentrum maximum (Gourret) J.Schiller 1931	+	+	B-T	M-p	P
Prorocentrum micans Ehrenberg 1834	+	+	C	M-p	PB,h
Prorocentrum obtusum Ostenfeld 1908	+	+	C	M-p	PB,h
Prorocentrum rotundatum J.Schiller 1918	+	-	B-T	M-p	P
Prorocentrum triestinum J.Schiller 1918	-	+	B-T	M-p	P
Protoperidinium bipes (Paulsen) Balech 1974	-	+	C	M-p	P
Protoperidinium brochii (Kofoid & Swezy) Balech 1974	+	+	C	M-p	P

SPECIES LIST	Sampling Years		Phytogeographical-Ecological Status		
	2007	2008	PG	EG-GO	PF
Protoperidinium cerasus (Paulsen) Balech 1973	+	+	C	MB-p	P
Protoperidinium claudicans (Paulsen) Balech 1974	+	+	C	MB-p	P
Protoperidinium conicum (Gran) Balech 1974	+	-	C	M-p	P
Protoperidinium crassipes (Kofoid) Balech 1974	+	+	B-T	M-p	P
Protoperidinium curvipes (Ostenfeld) Balech 1974	+	+	B-T	M-p	P
Protoperidinium depressum (Bailey) Balech 1974	+	+	C	M-p	P
Protoperidinium diabolus (Cleve) Balech 1974	-	+	T-ST	M-p	P
Protoperidinium divergens (Ehrenberg) Balech 1974	-	+	C	M-p	P
Protoperidinium elegans (Cleve) Balech 1974	-	+	C	M-p	P
Protoperidinium excentricum (Paulsen) Balech 1974	+	+	B-T	M-p	P
Protoperidinium granii (Ostenfeld) Balech 1974	+	+	C	MB-p	P
Protoperidinium pallidum (Ostenfeld) Balech 1973	+	-	C	MB-p	P
Protoperidinium pellucidum Bergh 1881	+	+	C	MB-p	P
Protoperidinium saltans (Meunier) Balech 1973	+	-	B-T	M-p	P
Protoperidinium steinii (E.G.Jørgensen) Balech 1974	+	+	B-T	M-p	PB,m
Protoperidinium thulesense (Balech) Balech 1974	+	-	B-T	M-p	P
Pyrocystis elegans Pavillard 1931	+	-	B-T	M-p	P
Pyrocystis fusiformis C.W.Thomson 1876	+	+	C	M-p	P
Pyrocystis lunula (Schütt) Schütt 1896	+	+	C	M-p	P
Scrippsiella acuminata (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling 2015	+	+	B-T	M-p	PB,m
Tripos arietinus (Cleve) F.Gómez 2013	+	+	T	M-p	P
Tripos belone (Cleve) F.Gómez 2013	+	-	T	M-p	P
Tripos candelabrum (Ehrenberg) F.Gómez 2013	+	+	T-ST	M-p	P
Tripos carriensis (Gourret) Hallegraeff & Huisman 2013	+	-	T-ST	M-p	P
Tripos compressus (Gran) F.Gómez 2013	+	-	T-ST	M-p	P
Tripos contortus (Gourret) F.Gómez 2013	+	-	T-ST	M-p	P
Tripos declinatus (G.Karsten) F.Gómez 2013	+	-	T-ST	M-p	P
Tripos deflexus (Kofoid) Hallegraeff & Huisman 2020	+	-	C	M-p	P
Tripos extensus (Gourret) F.Gómez 2013	+	-	T	M-p	P
Tripos furca (Ehrenberg) F.Gómez 2013	+	+	C	M-p	PB,m
Tripos fusus (Ehrenberg) F.Gómez 2013	+	+	C	M-p	PB,m
Tripos gibberus (Gourret) F.Gómez 2013	+	-	T-ST	M-p	P
Tripos hexacanthus (Gourret) F.Gómez 2013	+	-	T-ST	M-p	P
Tripos horridus (Cleve) F.Gómez 2013	+	+	B-T	M-p	P
Tripos incisus (Karsten) F.Gómez 2013	-	+	B-T	M-p	P
Tripos kofoidii (E.G.Jørgensen) F.Gómez 2013	+	-	ST	M-p	P
Tripos limulus (Pouchet) F.Gómez 2013	-	+	B-T	M-p	P
Tripos lineatus (Ehrenberg) F.Gómez 2013	+	+	B-T	M-p	P
Tripos longissimus (Schröder) F.Gómez 2013	+	+	T	M-p	P
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman 2020	+	+	C	M-p	P
Tripos massiliensis (Gourret) F.Gómez 2013	+	+	B-T	M-p	P
Tripos minutus (E.G.Jørgensen) F.Gómez 2013	+	-	T	M-p	P
Tripos muelleri Bory 1826	+	+	C	M-p	PB,m
Tripos pavillardii (E.G.Jørgensen) F.Gómez 2013	+	-	T	M-p	P
Tripos pentagonus (Gourret) F.Gómez 2013	+	+	T	M-p	P
Tripos platycornis (Daday) F.Gómez 2013	-	+	T	M-p	P
Tripos ranipes (Cleve) F.Gómez 2013	+	+	T	M-p	P
Tripos teres (Kofoid) F.Gómez 2013	+	+	T-ST	M-p	P
Tripos trichoceros (Ehrenberg) Gómez 2013	+	+	T-ST	M-p	P
COCCOLITHOPHYCEAE					
Emiliania huxleyi (Lohmann) W.W.Hay & H.P.Mohler 1967	+	+	C	M-p	PBm,h
DICTYOCHOPHYCEAE					

SPECIES LIST	Sampling Years		Phytogeographical-Ecological Status		
	2007	2008	PG	EG-GO	PF
Dictyocha fibula Ehrenberg 1839	+	+	C	M-p	PBm,h
Octactis speculum (Ehrenberg) F.H.Chang, J.M.Grieve & J.E.Sutherland 2017	+	+	C	M-p	PBm,h
Octactis octonaria (Ehrenberg) Hovasse 1946	+	+	B-T	M-p	PBm,h

Phytogeographical and Ecological Compositions

65% of the species belonging to the Bacillariophyceae class have been determined as cosmopolitan species, 23% tropical-subtropical species, 6% boreal species, 3% boreal-tropical species and 3% arcto-boreal species.

50% of the species belonging to the Coscinodiscophyceae class have been determined as boreal-tropical species, 20% cosmopolitan species, 20% tropical species and 10% tropical-subtropical species.

41% of the species belonging to the Mediophyceae class have been determined as boreal-tropical species, 35% cosmopolitan species, 12% arcto-boreal species, 6% tropical-subtropical species and 6% subtropical species.

30% of the species belonging to the Dinophyceae have been determined as cosmopolitan species, 30% boreal-tropical species, 29% tropical species, 9% tropical-subtropical species, 1% boreal species and 1% subtropical species.

The only sampled species belonging to the Coccolithophceae class, *Emiliania huxleyi* is a cosmopolitan species.

67% of the species belonging to the Dictyochophyceae have been determined as cosmopolitan species and 33% boreal-tropical species.

Different types of phytoplankton classes are examined and have been identified as marine species according to ecological groups. 69% of the species belonging to the Bacillariophyceae class are marine species and 31% are marine-brackish species, 90% of the species belonging to the Coscinodiscophyceae class are marine species and 10% are marine-brackish species, 82% of the species belonging to the Mediophyceae class are marine species and 18% are marine-brackish species, 95% of the species belonging to the Dinophyceae class are marine species and 5% are marine-brackish species and the species belonging to the Coccolithophceae and Dictyochophyceae classes have been identified as marine species (Table 1).

When phytoplankton classes are evaluated according to their geographical origins, it has been determined that 86% of Bacillariophyceae species are benthic originated species and 10% are pelagic originated species, 75% of Coscinodiscophyceae species are pelagic originated species and 25% are benthic originated species and 82% of Mediophyceae species are pelagic originated species and 25% are benthic originated species. All species belonging to dinoflagellates, coccoliths and silicoflagellates have been identified as pelagic originated species.

The phytogeographic status of the phytoplankton groups was examined, it was found that 47% of diatoms, 30% of dinoflagellates and 75% of the other groups were represented by cosmopolitan species. Marine species dominate 78% of diatoms, 95% of dinoflagellates and all other groups. It was determined that 53% of the species belonging to the diatom group as benthic originated species, and the species belonging to dinoflagellates and other groups were determined as pelagic originated species (Table2).

Table 2 The phytogeographical and ecological characteristics of the phytoplankton groups (C: Cosmopolitan species, A-B: Arcto-boreal species, B: Boreal species, B-T: Boreal-tropical species, T: Tropical species, T-ST: Tropical-subtropical species, ST: Subtropical species; M: Marine species, MB: Marine and Brackishwater species; p: pelagic-originated species, b: benthic-originated species)

Çizelge 2. Fitoplankton gruplarının fitocoğrafik ve ekolojik özellikleri (C: Kozmopolit tür, A-B: Arkto-boreal tür, B: Boreal tür, B-T: Boreal-tropikal tür, T: Tropikal tür, T-ST: Tropikal-subtropikal tür, ST: Subtropikal tür; M: Denizel tür, MB: Denizel ve Acısu tür; p: pelajik-orijinli tür, b: benthic-orijinli tür)

Phytoplankton Groups	% Phyto-Geographical Group							T
	C	A-B	B	B-T	T	T-ST	ST	%
Diatom	47	4	8	25	-	15	1	100
Dinoflagellate	30	-	1	30	29	9	1	100
Other	75	-	-	25	-	-	-	100
Phytoplankton Groups	% Ecological Group		T	% Geographical Origin				T
	M	MB	%	p		b		%
Diatom	78	22	100	47		53		100
Dinoflagellate	95	5	100	100		-		100
Other	100	-	100	100		-		100

DISCUSSION and CONCLUSION

The limited number of phytoplankton species composition studies conducted in Gulf of Aqaba and the Red Sea are given in Figure 2. Halim (1969) identified 209 phytoplankton species (125 dinoflagellates, 84 diatoms) covering the entire Red Sea. Dowidar et al. (1978) detected 224 species (111 dinoflagellates, 112 diatoms) in their studies off the coast of Saudi Arabia. In another study conducted off the coast of Saudi Arabia (Shaikh et al., 1986), 283 species (110 dinoflagellates, 137 diatoms) were reported. Madkour et al. (2007) detected 181 phytoplankton species (117 dinoflagellates, 60 diatoms) in their study in the northern Red Sea, which includes the Gulf of Aden and the Gulf of Aqaba. In the study, 184 phytoplankton species (113 dinoflagellates, 71 diatoms) were identified, including 148 (97 dinoflagellates, 51 diatoms) in 2007 and 131 (76 dinoflagellates, 55 diatoms) in 2008. In 2007-2008, 4 species were identified from other groups.

The number of phytoplankton species identified in this study has more than the number of species (Total: 137, 49 diatoms, 88 dinoflagellates) detected in Gulf of Aqaba in Madkour et al.'s (2007) study. Also, compared to other studies, the number of species in this study is less than the previous studies. The number of dinoflagellate species found in this study is in parallel with previous studies. The decrease in the total number of species is due to the number of diatom species identified in this study. The low number of diatom species compared to other reviews can be explained by the method of this study. In previous studies, samples were taken with plankton net and at the same time, sampling was made from the whole water column. The samples were taken from the surface water in this study using Niskin bottle. Studies carried out in the Red Sea have shown that species diversity decreases towards the north (Halim, 1969; Weikert, 1987).

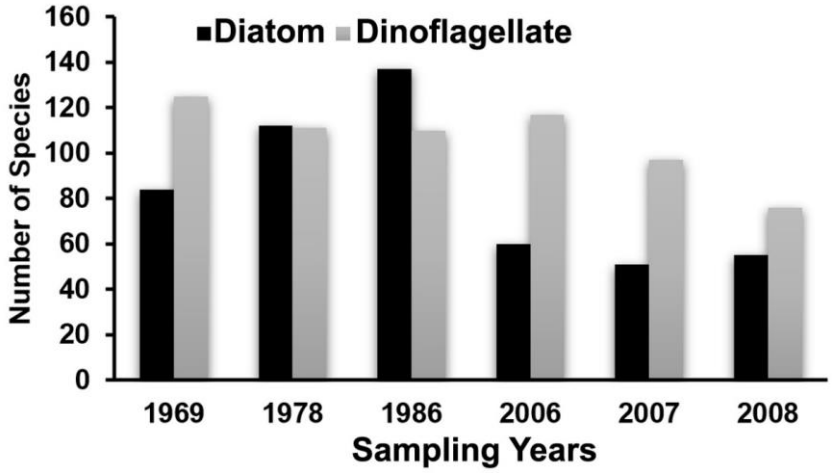

Figure 2. Diatom-dinoflagellate species numbers determined in studies conducted in the Red Sea (1969: Halim, 1969; 1978: Dowidar et al., 1978; 1986: Shaikh et al., 1986; 2006: Madkour et al., 2007; 2007-2008: This study)

Şekil 2. Kızıldeniz'de yapılan çalışmalarda belirlenen diatom-dinoflagellat tür sayıları (1969: Halim, 1969; 1978: Dowidar ve ark., 1978; 1986: Shaikh ve ark., 1986; 2006: Madkour ve ark., 2007; 2007-2008: Bu çalışma)

In studies conducted in the Red Sea, the low number of diatom species has been characterized as typical. Halim (1969) reported that the number of diatom species decreased dramatically, especially in warm periods (June-October period), and even was not encountered from time to time. Madkour et al. (2007) also reported that the number of diatom species decreased especially in the late spring-early autumn periods. This study is also in parallel with other studies. It is striking that the number of diatom species is scarce, especially during the 2008 hot period. Studies have reported that the qualitative and quantitative scarcity of diatom and dinoflagellate species is due to limited nutrient input and grazing, especially on diatoms (Sommer, 2000; Sommer et al., 2002; Al-Najjar et al., 2007). Especially in experimental studies, the scarcity of diatom and dinoflagellate species was attributed to the movements of the nutrient cycle in the gulf from the bottom to the surface and from the surface to the bottom (Sommer, 2000).

A genus with the highest number of species were determined as *Tripos*, *Protoperidinium*, *Oxytoxum*, *Dinophysis*, *Prorocentrum*, *Gonyaulax*, *Licmophora*, *Actinocyclus*, *Coscinodiscus*, *Rhizosolenia* and *Chaetoceros* have been identified in this study. Diatoms tend to collapse into deep water when turbulence in the body of water decreases and nutrients are depleted. Dinoflagellates can stay in the

euphotic zone because of their movements, and so, explaining its high diversity in surface waters (Spector, 1984). Dinoflagellates have adapted very well to the high temperature values of the Red Sea (especially in the summer period) (Halim, 1969). Nutrient increase and vertical stratifications triggered by winter monsoons do not affect dinoflagellates because of their high adaptability to the region's environmental conditions (Halim, 1969; Sommer, 2000; Al-Najjar, 2007). *Neoceratium* (*Tripos*) genus is the most crucial dinoflagellate genus in tropical waters (Dowidar, 1983). It has been determined that the *Neoceratium* genus has the highest number of species in all studies conducted in the region (Halim, 1969; Dowidar, 1983; Madkour et al., 2007). Madkour et al. (2007) stated that the genera *Neoceratium*, *Protoperidinium*, *Chaetoceros*, *Rhizosolenia* and *Nitzschia* are dominant in terms of number of species. Subra Rao and Al-Yamani (1998) reported that the most critical genera they identified in their study in the Persian Gulf were *Chaetoceros*, *Coscinodiscus*, *Rhizosolenia*, *Neoceratium*, *Protoperidinium* and *Prorocentrum*.

When the prevalence of species in the community was examined spatially and temporally, it was found that the following were observed as constant species, including *Coscinodiscus perforatus*, *Thalassiosira eccentrica*, *Dictyocha fibula*, *Ceratocorys armata*, *Gymnodinium helveticum*, *Gyrodinium fusiforme*, *Tripos arietinus*, *Tripos furca*, *Tripos lineatus*, *Tripos macroceros*, *Tripos teres*, *Phalacroma rotundatum*, *Prorocentrum micans*, *Protoperidinium steinii*, *Pyrocystis fusiformis*, *Scrippsiella acuminata*, *Triceratium favus* and *Emiliania huxleyi*. This result shows remarkable similarities with the constant species that Madkour et al. (2007) obtained from samples taken from many stations in the Red Sea, including the Gulf of Aqaba, between 2005-2006. Halim (1960) reported the *Nitszchia delicatissima*, *Leptocylindrus danicus, Hermesinum adiraticum, Chaetoceros affinis, Cerataulina bergoni, Chaetoceros decipiens, Biddulphia rhombus*, *Exuviaella cordata, Skeletonema costatum, Chaetoceros curvisetus, Hemiaulus sinensis, Chaetoceros costatus, Rhizsolenia hebetata semispina* and *Chaetoceros socialis* species detected in study on the coasts of Egypt in 1956 as the constant species of the region. Kimor et al. (1987) reported that the species belonging to the *Rhizosolenia-Chaetoceros* genus, *Thalassiothrix frauenfeldii* and *Gymnodinium* sp. species are constant species that show distribution in layers close to surface water.

The results of this study were similar to the phytogeographic composition obtained in previous studies in the region. In our research, most of the phytoplankton species were found to be cosmopolitan species (> 50%), besides boreal-tropical species and subtropical species were also determined. Most of the identified species are marine species. In previous studies, more than 60% of the species composition consists of cosmopolitan, tropical, and subtropical species characteristic of the Indo-Pacific Region (Halim, 1960; 1969, Dowidar et al., 1978; Shaikh et al., 1986; Madkour et al., 2007).

The dynamics of phytoplankton compositions are significantly influenced by the physical and chemical properties of the water column (Lindell and Post 1995; Labiosa et al., 2003; Laiolo et al., 2014). The effect of the Gulf trophic level, including the mesotrophic conditions in winter and oligotrophic conditions in summer and autumn, is crucial to phytoplankton groups relative changes.

The thermal stratification and low nutrient level suppress the small-scale organisms such as *Emiliania huxleyi* throughout the year and revealing that these organisms are more advantageous in low nutrient concentration in surface waters. This condition may be due to high nutrient uptake efficiency, large surface: small volume structures, or low nutrient requirements (Genin et al., 1995; Al-Qutob et al., 2002; Mackey et al., 2007).

Studies on large groups of phytoplankton should be included in the scope of long-term monitoring programs to control the increasing eutrophication pressure in the Gulf of Aqaba and on the coral reefs, which are very important for the region. In addition to this, it is necessary to regularly monitor not only along the Jordanian part of the Gulf but also in all Gulf of Aqaba's territorial waters.

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Author's Contributions

Fatih Şahin: Conception and design of study, Acquisition of data, Analysis and interpretation of data, Writing-original draft. Levent Bat: Conception and design of study, Writing-review & editing. Dilek Ediger: Conception and design of study, Writing-review & editing. Tarıq Al-Najjar: Conception and design of study, Acquisition of data, Writing-review & editing.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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The Effects of Chitosan on Aluminium Accumulation in the Gill, Liver and Muscle of Freshwater Fish (*Oreochromisniloticus*)

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ABSTRACT
In this study, accumulation of aluminium (Al) in the gill, liver and muscle of *Oreochromis niloticus* were determined following exposure to Al (0, 1, 2 and 4 ppm) alone and in combination with chitosan (10 ppm) for 7, 14 and 21 days. Aluminium concentrations in the tissues were measured by ICP-MS. There were no fish mortality and apparent morphological or behavioural changes after 21 days of exposure duration. Al concentrations of the tissues increased significantly ($P<0.05$) in both Al alone exposures and Al+chitosan combination exposures and following order was found in Al accumulation among the tissues; Gill>Liver>Muscle. Data also showed that chitosan significantly ($P<0.05$) reduced the accumulation of Al in the tissues. This study suggests that chitosan may be used as an effective chelate in Al contaminated waters and emphasizes Al burdens in commercial fish species from contaminated waters for human health point of view.

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Alüminyum'un *Oreochromis niloticus*'un Solungaç, Karaciğer ve Kas dokularındaki Birikimi ile Kitosan'ın Doku Alüminyum Birikimi Üzerine Etkileri

ÖZET
Bu çalışmada, alüminyum (Al)'un tek başına (0, 1, 2 ve 4 ppm) ve kitosan (10 ppm) ile birlikte etkilerine 7, 14 ve 21 gün süre ile maruz kalan *Oreochromis niloticus*'un solungaç, karaciğer ve kas dokularında Al birikimi belirlenmiştir. Dokulardaki alüminyum düzeyleri ICP-MS ile ölçülmüştür. Yirmi bir gün sonunda balıklarda ölüm ve belirgin bir morfolojik değişim veya davranışsal bozukluk görülmemiştir. Hem tek başına Al etkilerinde hem de Al+kitosan kombinasyonlarında dokuların Al düzeyleri anlamlı ($P<0.05$) olarak artarken, dokular arasında Al birikimini bakımından şu sıralamada görülmüştür; Solungaç>Karaciğer>Kas. Veriler ayrıca kitosan'ın dokularda Al birikimini önemli ölçüde ($P<0.05$) azalttığını da göstermiştir. Bu çalışma, kitosan'ın Al ile kirlenmiş sular için etkili bir şelat olarak kullanılabileceğini öne sürmekte ve Al kirlenmesinin olduğu alanlarda insan tüketimi açısından balık dokularındaki Al yükünün toksisitesini vurgulamaktadır.

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INTRODUCTION

Developments in science and technology have increased the use of heavy raw metal materials in industry and consequently resulting an increasing their discharges into aquatic systems. Because the aquatic systems are the main receivers for contaminants released to the environment, the environmentalists worry about the increase in the contaminant loads of waters. The uncontrolled discharges of wastes to the aquatic environments can

cause significant changes in the physical and chemical qualities of waters and finally affect the health of aquatic biosystem (Sidra et al., 2018). Some metals such as Cd, Hg, Pb and As do not have any known function in biological systems and they can be very toxic to fish even at low concentrations. On the other hand, fish need the essential metals (Cu, Zn, Fe, Mn, Mo, Co, Se) at trace levels for their metabolisms. However, even the essential metals can be toxic to fish above certain threshold levels (Abbasi and Khayatzadeh, 2012).

Al is one of the most abundant metal in the earth's crust and used widely in different areas of industry including automotive, aerospace, packaging, construction materials, catalysts, ammonium nitrate explosives and waste water treatment plants. Al can directly enter to the aquatic ecosystems via various discharges and also indirectly enter the aquatic systems by washing up the soil and rocks due to the acid rains (Wood et al., 2012). Studies conducted with aquatic organisms showed that Al inhibited the active ion uptake by inhibiting ATPases and altered the ion regulation, causing development disorder and reduced swimming performance (Authman, 2011; Azmat et al., 2012). It was also shown that Al can cause the lipid peroxidation and alter the biochemical and haematological parameters in the blood (Camargo et al., 2009).

In the aquatic systems, some organic and inorganic materials such as EDTA (Ethylene diamine tetra acetic acid), NTA (Nitrilo tri acetic acid), DTPA (Diethylene triaminepenta acetic acid), DFO (Deferroxamine), DFP (Deferiprone), zeolite, clinopylolite and chitosan are widely used as a chelate. Chitosan is a linear amino polysaccharide obtained from deacetylation of chitin in the exoskeleton of crustaceans and arthropods in alkaline environment. It has been determined that chitosan has the capacity to strongly adsorb heavy metals such as Hg, Cu, Ni and Zn (McKay et al., 1989). Chitosan has low toxicity, biological applicability, easy to obtain and low cost. These characteristics of chitosan could make it widely used remediation material in contaminated aquatic systems (Samarakoon et al., 2003).

Freshwater fish *O. niloticus*is widely distributed in fresh waters in the tropical and subtropical climate zone. It has high resistant to diseases, salinity and pollutants that make them desirable culture fish. Therefore, tilapias are widely consumed fish species in the tropical and subtropical regions. However, resistance to pollutants may cause great amounts of metal accumulations in their tissues that end up in the feed of humans. Because the chronic exposures of subletal concentrations of metals cause their accumulations in fish tissues and chitosan is an effective chelate in removing metals from the aquatic systems, this work was undertaken to investigate the chronic accumulation of Al in tissues of *O. niloticus* and effects of chitosan in tissue accumulation of Al.

MATERIALS and METHODS

In this study, freshwater fish *O. niloticus* (13.0 ± 3.0 cm in length and 35.0 ± 1.2 g in weight) were used for the experiments. Fish were obtained from the Aquaculture Unit of the Faculty of Fisheries (Mersin University) and transported to the laboratory where the experiments were carried out (the Basic Sciences Research Laboratory). Fish were kept in glass aquariums (40x100x40 cm) for one month until they were adapted (12 h light/ 12 h dark) to the experimental conditions. Some physical and chemical characteristics of experimental waters were measured daily and presented in Table 1. In the experiments, 5 fish were randomly allocated to 7 glass aquariums (40x100x40 cm) for each experimental period (7, 14 and 21 days) and a total of 21 aquariums (105 fish in total) were used for all experiments. About 120 L of 1, 2 and 4 ppm Al solutions were added in the first three out of 7 aquaria and the other three aquaria was filled with the 1, 2 and 4 ppm alüminium solutions together with 10 ppm chitosan solutions. The last aquarium was filled with the same amount (120L) of tap water and used as control. Sublethal concentrations of aluminium (0, 1, 2, 4 ppm Al) were determined using our preliminary studies and also literature data guided in determination of the test concentrations (Noureen, 2017; Canli et al., 2018; Canli and Canli, 2020). Fish were exposed to Al concentrations alone and also together with 10 ppm chitosan. An acetic acid solution (1%) was used in preparing the stock solution of chitosan (Aldrich, GR, Deacetylation ≥ 75). The aquaria of control fish did not contain Al or chitosan. Experiments were carried out using the semi-static test protocol, renewing exposure media every day. Fish were fed once a day with a commercial fish food (Pellets No: 2, Izmir, Turkey) during the experiments, serving them approximately 2% of their total biomass at the same day of water renewal.

Table 1. Some physical and chemical properties of water in experimental aquariums
Çizelge 1. Deney akvaryumlarındaki suyun bazı fiziksel ve kimyasal özellikleri

Temperature	22 ± 1 °C
Total alkalinity	331 ± 0.5 mg $CaCO_3$/L
Total harness	259.3 ±5.82 ppm $CaCO_3$
Dissolved Oxygen	6.6 ± 0.5 mg/L
pH	7.4 ± 0.7

Heavy Metal Analysis

At the end of the exposure periods, fish were removed from exposure aquariums and anesthetized with an anesthetic substance MS 222 (Tricaine methane - sulphonate 75 mL/L) (Cicik et al., 2004). They were

rinsed with tap water and dissected using clean equipment. Liver, gill and muscle tissues were taken out carefully from each fish and put into petri dishes and placed in an oven set at 150 °C. The tissues were kept in the oven for 48 hours until they reached to constant weights. Then, the tissues were weighed to the nearest mg and put into the digestion tubes. All the tissues were digested in 2 ml of nitric acid (HNO_3, 65%, s.g. 1.40, Merck) and 1 ml perchloric acid ($HClO_4$, 60%, s.g. 1.53, Merck) mixtures on a hot plate set to 120 °C for 3 h (Muramoto, 1983). Digested samples were transferred to polyethylene tubes and ultrapure water was added onto them to obtain a final volume of 10 ml. Al levels in the tissues were measured using an ICP-MS and IAEA-407 (International Atomic Energy Agency) samples prepared from fish tissue homogenate were used as reference material to check the validity of the measurements (IAEA, 2003).

Statistical Analyses

A statistical package program SPSS v.16.0 (IBM Corp., Armonk, NY, USA) was used to analyse data. Before statistical tests, the homogeneity of variance was checked. One-way ANOVA test was first applied to data and significant ($P<0.05$) results were re-analysed by post-hoc tests (SNK) to estimate groups differing from controls (see Figure 1, 2, 3).

RESULTS and DISCUSSION

The present data demonstrated that significant ($P<0.05$) accumulation of Al occurred in the gill, liver and muscle of *O. niloticus* comparing to controls (Figs. 1, 2, 3). Additionally, tissue accumulation of Al increased in relation to increases in exposure concentrations and exposure periods. However, the presence of chitosan in the exposure mediums reduced significantly ($P<0.05$) the accumulation of Al in the tissues (Figs. 1, 2, 3). The present data demonstrated that chitosan affected the uptake of Al by fish, possibly reducing bioavailability of Al and suggested that it could be an effective chelate for Al contaminated waters. As it is well known, heavy metal accumulation in fish tissues is determined as a results of the uptake, excretion, storage and transformation processes which are regulated by homeostatic mechanisms. However, continuous exposures to metals deactivate homeostatic mechanisms and accumulation increases in tissues, eventually causing toxic effects (Javed and Usmani, 2017). The gill plays vital roles in the uptake of metals as it is responsible for the respiration and osmoregulation in fish and is also the main target organ for toxic substances (Heath, 1995). The effects of different chelates in Al accumulation by *Cyprinus carpio* (Muramoto, 1981). The authors demonstrated that exposure of fish to the subletal concentrations of different Al compounds ($AlCl_3.6H_2O$ and $Al_2(SO_4)_3.18H_2O$) increased Al accumulation in tissues, though Al levels in tissues reduced sharply when fish were exposed Al and chelates (EDTA or NTA) combinations. Their findings were similar to the present data, as the highest accumulation occurred in the gill following exposure to Al alone and together with chitosan (Figure 1A). This is apparently due to the fact that the gills are in direct interaction with exposure waters, so that the gill accumulate more Al compared to the other tissues. The other reason for this might be the retention of Al by binding to sialic acid residues in the mucus covering the gill surface (Muramoto, 1981).

Effects of toxic xenobiotic on mortality in the aquatic organisms vary depending on various factors such as toxic potential of xenobiotic, duration and concentration of exposure, chelates, temperature and biology of species in concern. Although there was no fish mortality in the present study, Rao and Kumar (2014) demonstrated that there was fish mortality (*Channa punctatus*) following exposure to 0.001 M Al on the 30th day and 80% of the fish died at the end of 60th of exposure period. In a study conducted with *O. niloticus*, the 96 hour LC50 value for Al was found to be 68.03±0.86 mg/L and the lethal concentration was 111.00±10.02 mg/L (Noureen, 2017). Chelates used to remove pollutants from the medium can also cause fish mortality if they are used in high levels. The lethal effects of EDTA (0.5 g/L) in *O. niloticus* (Janes et al., 1998) and chitosan (0.075, 0.75 ppm) in *Oncorhynchus mykiss* (Bullock et al., 2000) were evident, as the fishes died in a few days. However, no fish mortality occurred following exposures to Al alone or together with 10 ppm chitosan up to 21 days in the present study, indicating the concentrations of both Al and chitosan were not in the range of lethal concentrations for tilapias.

Fish react to changing environmental conditions by changing their behaviour. There were behavioural changes such as impaired swimming movements, lack of food intake, orientation to the aquarium surface and increase in operculum movements in studies conducted with *O. mykiss* (Rod et al., 1992) and *Brachydanio rerio* (Anandhan and Hemalatha, 2008). In the present study, similar behavioural changes were observed in fish after exposure to Al and Al+chitosan combinations at the beginning of the experiments, but these changes returned to normal at the end of the exposures. This can be attributed to the response of fish to changing environmental conditions and adaptation.

Heavy metals absorbed through the gills are primarily transported to the liver via the circulatory system. The liver is a metabolically active organ in which toxic substances are detoxified (Heath, 1995). Azmat et al. (2012) found that there were significant accumulations in the liver of fishes (*Catla catla, Labeo rohita, Cirrihinus mrigala*) after exposure to Al. Similarly, the present data also demonstrated that there were

significant accumulations of Al in the tissues of *O. niloticus* following exposure to Al alone and in combination with chitosan (Fig 2). Accumulation of great amount of Al in the liver may result from the retention of Al in the liver by binding to metal-binding proteins such as metallothionein and glutathione.

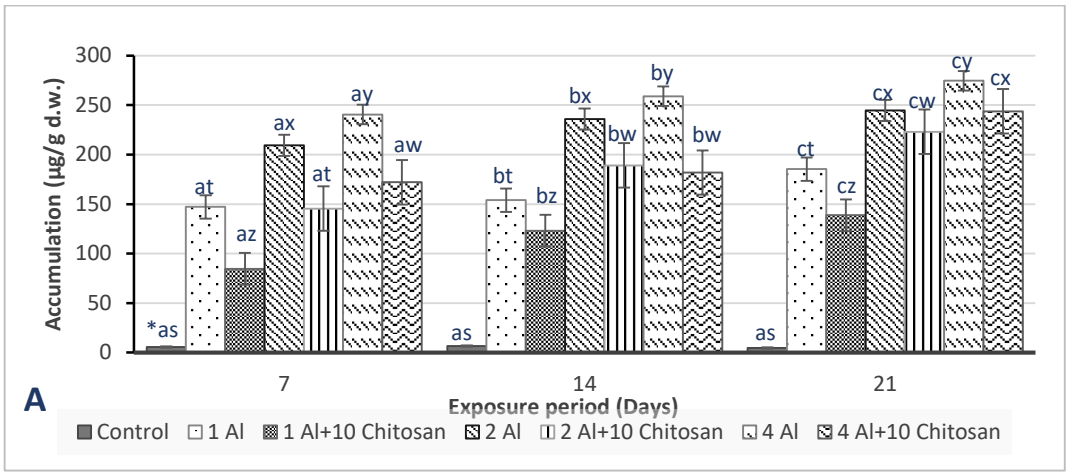

Figure 1. Accumulation of aluminium in the gill tissues of *O. niloticus* following exposure to Al alone and Al+chitosan mixture for 7, 14 and 21 days.

*=SNK; Letters s, t, x, y, w, z and a, b, c show differences among concentrations and exposure periods at a given tissue. Data shown with different letters are significant at the P<0.05 level.

Şekil 1. Al'un tek başına ve kitosan ile birlikte 7, 14 ve 21 gün sürelerle etkisinde O. niloticus'un solungaç dokularındaki birikim düzeyleri.

**=SNK; s, t, x, y, w, z derişimleri; a, b, c harfleri etkide kalma süreleri arasındaki farklılıkları gösterir. Farklı harflerle gösterilen veriler arasında P<0.05 düzeyinde istatistik ayrım vardır.*

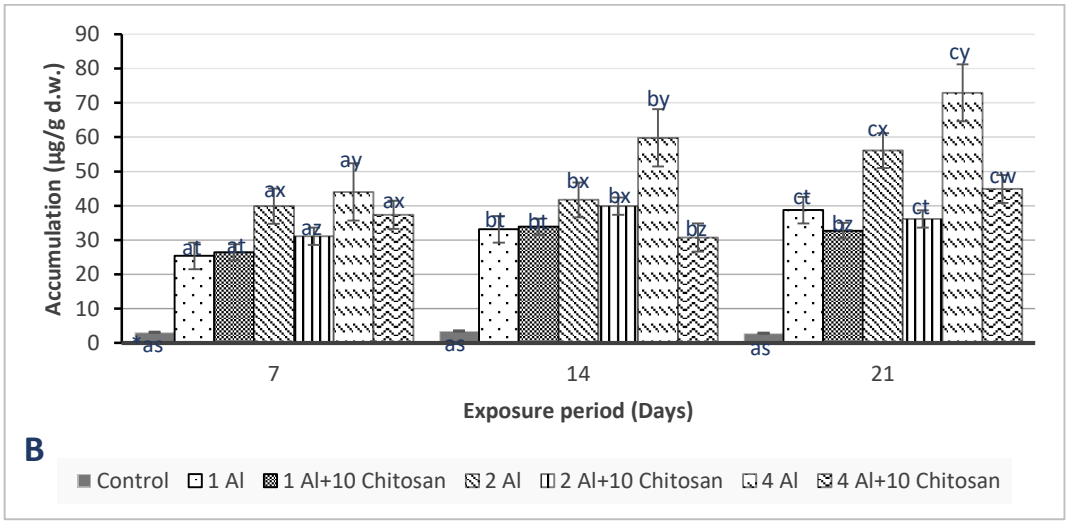

Figure 2. Accumulation of aluminium in the liver tissues of *O. niloticus* following exposure to Al alone and Al+chitosan mixture for 7, 14 and 21 days.

*=SNK; Letters s, t, x, y, w, z and a, b, c show differences among concentrations and exposure periods at a given tissue. Data shown with different letters are significant at the P<0.05 level.

Şekil *2. Al'un tek başına ve kitosan ile birlikte 7, 14 ve 21 gün sürelerle etkisinde O. niloticus'un karaciğer dokularındaki birikim düzeyleri.*

**=SNK; s, t, x, y, w, z derişimleri; a, b, c harfleri etkide kalma süreleri arasındaki farklılıkları gösterir. Farklı harflerle gösterilen veriler arasında P<0.05 düzeyinde istatistik ayrım vardır.*

As it is well known, the muscle is the main consumable part of fish by humans. Although muscle tissue is not an active in terms of metal accumulation, it is very important for public health due to the transport of metals to the diet of humans through the food chain. It was determined that muscle tissue of *B. rerio* showed the lowest Al accumulation following exposure to Al (5.69 and 17.08 ppm) up to 28 days (Anandhan and Hemalatha, 2009). Similarly, Al nanoparticles accumulated in *O. niloticus*, lowest accumulation occurring in muscle tissues (Abdel-Khalek et al., 2020). Similarly, the present data also demonstrated that the lowest Al accumulation occurred in muscle tissue (Figure 3) compared to the gill and liver, suggesting relatively lower metabolic activity of the muscle.

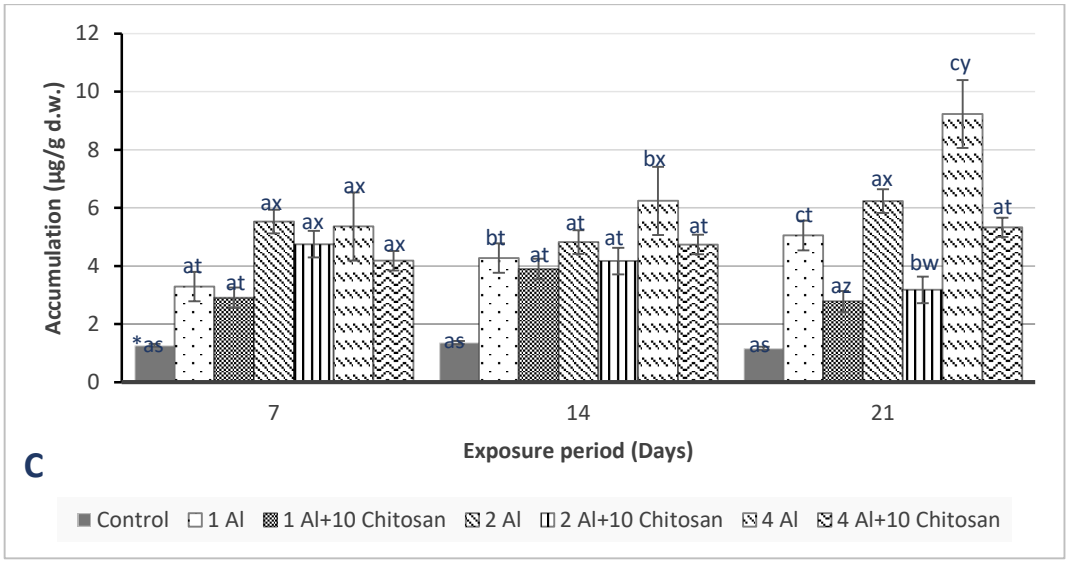

Figure 3. Accumulation of aluminium in the muscle tissues of *O. niloticus* following exposure to Al alone and Al+chitosan mixture for 7, 14 and 21 days.

*=SNK; Letters s, t, x, y, w, z and a, b, c show differences among concentrations and exposure periods at a given tissue. Data shown with different letters are significant at the P<0.05 level.

Şekil 3. Al'un tek başına ve kitosan ile birlikte 7, 14 ve 21 gün sürelerle etkisinde O. niloticus'un kas dokularındaki birikim düzeyleri.

**=SNK; s, t, x, y, w, z derişimleri; a, b, c harfleri etkide kalma süreleri arasındaki farklılıkları gösterir. Farklı harflerle gösterilen veriler arasında P<0.05 düzeyinde istatistik ayrım vardır.*

Chitosan is a very strong adsorbent for heavy metals due to the nitrogen content of amino groups (Qin et al., 2003). The effects of adsorbents on metal toxicity differ. It has been determined that while chitosan increases copper accumulation in the gill tissue in *Clarias gariepinus*, it reduces the accumulation in the liver (Tunçsoy et al. 2016). Similarly, it has been determined that DFO and DFP in *C. mrigala* reduced Al accumulation in liver, kidney, gill and muscle tissues (Sivakumar and Khatiwada, 2012). The present data are in accord with the literature data, as chitosan reduced the accumulation of Al in the gill, liver and muscle tissues. This shows that chitosan forms a complex with Al and prevents its binding to active surfaces and finally reduces the uptake by fish.

CONCLUSION

The effects of Al (1, 2 and 4 ppm) alone and together with chitosan (10 ppm) in different exposure periods (7, 14 and 21 days) resulted significant accumulation of Al in the gill, liver and muscle tissues of *O. niloticus*. Highest accumulation occurred in the gill, while the lowest accumulation was in the muscle. The effect of Al together with chitosan reduced the accumulation of Al in the tissues compared to Al alone exposures. Finally, this study suggests that chitosan may be used as an effective chelate in contaminated waters.

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Authors' Contributions

Authors declares the contribution of the author's is equal.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The ethics committee approval was obtained from Mersin University Animal Experiments Local Ethical Committee by decision number 14/41 dated 04/11/2016.

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Biberiye Yaprağı Ekstraktının Yonca Otunun Rumen Fermentasyonu, Metan ve Mikrobiyal Protein Üretimine Etkisi

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ÖZET

Bu çalışma biberiye yapraklarından elde edilen ekstraktın yonca otunun *in vitro* metan (CH_4) üretimine, gerçek sindirim derecesine (GSD), taksimat faktörüne (TF), mikrobiyal proteinine(MP) ve mikrobiyal proteinin sentezleme etkinliğine (MPSE) etkisini saptamak için düzenlenmiştir. Biberiye ekstraktı yonca otunun *in vitro* gaz, CH_4, GSD, TF, MP ile MPSE değerlerlerini önemli derecede etkilemiştir (P<0.001). Yonca otunun *in vitro* gaz üretimi 92.80 ml ile 109.16 ml/500 mg kuru madde (KM) arasında değişmiştir. Ağustos ayında hasat edilen biberiye ekstraktı ve kurutularak ekstraksiyon yapılan grup hariç diğer gruplarda *in vitro* gaz üretimini önemli düzeyde artırmıştır (P<0.001). Biberiye ekstraktı CH_4 üretimini hem ml, hem de % olarak önemli derecede artırmış ve sırasıyla; 14.66-25.36 ml ile %15.76- %25.36 arasında saptanmıştır. Biberiye ekstraktı yonca otunun GSD'ni önemli derecede düşürmüş ve GSD %67.07-%72.50 arasında bulunmuştur. Biberiye ekstraktı ilavesi mikrobiyal protein üretimini ve sentezleme etkinliğini önemli derecede azaltmıştır (P<0.001). Mikrobiyal protein üretimi 85.56 mg ile 140.20 mg arasında olmuştur. Eylül ayında elde edilmiş taze biberiye ekstraksiyonu en düşük MP ve MPSE neden olduğu saptanmıştır. Sonuç olarak biberiye ekstraktının mikrobiyal protein, sindirim derecesi ve CH_4 üretimindeki olumsuz etkisinden dolayı ruminant rasyonlarında kullanımı önerilmemektedir.

Zootekni

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Anahtar Kelimeler
Biberiye ekstraktı
Gaz üretimi
Metan üretimi
Sindirim

Effect of *Rosmarinus officinalis* Leaves on the Fermentation and Methane Production of Alfalfa Hay

ABSTRACT

The current experiment was conducted to determine the effect of rosemary extract on in vitro methane production, tru digestibility, partitioning factor, microbial protein and efficiency of microbial protein production of alfalfa hay. Rosemary extract significantly (P<0.001) affected the *in vitro* gas production, methane production, true digestibility, partitioning factor, microbial protein and efficiency of microbial protein of alfalfa hay (P<0.001). The gas production ranged from 92.80 ml to 109.16 ml/500 mg dry matter. The gas production significantly increased with supplementation of rosemary extract except for that of supplemented with dry rosemary extract obtained by drying and that of in August (P<0.001). The highest gas production was obtained in group that supplemented with fresh rosemary extract obtained in September. Rosemary extract significantly increased the methane production in both ml or %. The methane production in both ml and % ranged from 14.66 to 25.36 ml and %15.76 to 25.36, respectively. Rosemary extract significantly decreased true digestibility of alfalfa hay. The true digestibility ranged from %67.07 to 72.50. Supplementation of rosemary extract significantly decreased microbial protein production and efficiency of microbial protein production. Microbial protein ranged from 85.56 to 140.20 mg. The lowest microbial protein production and efficiency of microbial protein production were obtained in group of supplemented with fresh rosemary extract obtained in September. As a conclusion,

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rosemary extract is suggested to use in ruminant diets to manipulate the ruminal fermentation due to negative effect on microbial production, digestibility and methane production.

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GİRİŞ

Hayvan beslemede antibiyotiklerin kullanımının yasaklanması sonrası, antibiyotiklere alternatif olacak ve insan sağlığına zararlı olmayan sekonder metabolitleri üzerinde durulmuştur. Bu alanda yapılan çalışmalarla bitki ekstraklarının ruminant beslenmede yem katkı maddesi olarak kullanımının önünü açmıştır (Patra ve ark. 2006; Agarwal ve ark. 2006; Salem 2019; Sinsz ve ark, 2019). Bu alanda yürütülen çalışmaların temel amacı hayvanın performansını olumsuz etkilemeden, rumen fermantasyonunu modifiye etmek, CH_4 üretimini azaltmak, enerji ve proteinin kullanım etkinliğini artırmaktır (Patra ve ark. 2006; Agarwal ve ark. 2006; Salem 2019; Sinsz ve ark, 2019). Ruminant hayvanların ürettikleri enterik CH_4 gazı küresel ısınmaya neden olan karbondioksitten (CO_2) sonra ikinci önemli sera gazıdır (Van Nevel and Demeyer, 1996). Enterik CH_4 üretimi sera gazı emisyonunu artırma yanında, ruminant hayvanlarda önemli düzeyde enerji kaybına neden olduğu bildirilmektedir (Jonhson ve Jonhson 1995). Rasyona tanen, saponin ve esansiyal yağ ilavesiyle ruminantlarda enterik CH_4 üretimini azaltmaya yönelik çalışmalar artmıştır (Wina ve ark. 2005; Temizkan ve ark. 2011; Jayanegara ve ark. 2015; Zhou ve ark. 2020). Son zamanlarda enterik metanı azaltmak için hazırlanması ve kullanımı kolay bitki eksraklarıda ruminant rasyonlarında kullanılmaya başlanmıştır. Bitki ekstraklarının saponin, terponoids, fenolikler, fenolik glikosidler, tanin, alkoloidler ve esansiyel yağlar içerdiği ve rumen fermentasyonunu değiştirme potansiyeline sahip olduğu yapılan çalışmalarla ortaya konmuştur (Salem ve ark. 2011; Bodas ve ark. 2012). Tıbbi ve aromatik bitki olan biberiye (*Rosmarinus officinalis L.*) bitkisi esansiyel yağ ve tanin bakımından zengin (Damianova ve ark. 2010) olmasından dolayı rumen fermantasyonunu değiştirmek için önemli bir aday olduğu düşünülmüştür. Bu araştırma; biberiye ekstraktının ruminantlarda rumen fermentasyonu, CH_4 üretimi ile rumen mikrobiyal biyokitlesine etkisini saptamak amacıyla düzenlenmiştir.

MATERYAL ve METOD

Yem ve Rumen Sıvısı Mataryeli

Araştırmanın yem materyalini çiçeklenme dönemi hasat edilmiş ve 105°C'de etüvde 24 saat süreyle kurutulmuş ve 1 mm elekten geçirilerek öğütülmüş yonca otu (*Medicago sativa*) oluşturmuştur. Rumen sıvısı rumen kanüllü 3 baş koçtan alınmış ve rumen sıvısı alınan koçların bakım ve beslenmesinde etik ilkere uyulmuştur. Çalışmanın yapılabilmesi için KSÜ Hayvan Deneyleri Yerel Etik Kurulundan (28.07.2017 tarih ve 2017/5-1 sayılı toplantı) izin alınmıştır.

Biberiye Ekstraktının Hazırlanması

Biberiye bitkisinin Ağustos-Eylül aylarının yaş ve gölgede kurutulmuş halinin yaprakları ayrı ayrı 5 g tartılıp vida kapaklı cam şişenin içine 50 ml %20' lik metanol-etanol karışımı ile birlikte karıştırılarak konulmuştur. Üç gün boyunca bekletilen karışım biberiye bitkisinden süzülerek elde edilmiştir.

In Vitro Gaz ve Metan Üretiminin Belirlenmesi

Yonca otunun *in vitro* gaz üretimi Menke ve ark. (1979)'nın uyguladıkları tekniğe göre yapılmıştır. 0.5 g yonca otu 100 ml cam şırıngalara konmuş ve üzerine 40 ml tamponlanmış rumen sıvısı ilave edilmiştir. Hazırlanan bu şırıngalara Ağustos ve Eylül aylarının elde edilen biberiye ekstraklarından 1 ml ilave edilmiş ve 39°C'de 24 saat inkübasyona bırakılmıştır. Rumen sıvısı 1.5-2 yaşlarında fistül takılmış 55-60 kg ağırlığındaki iki koçdan alınmıştır. Alınan rumen sıvısı 1:2 oranında tampon çözeltisiyle karıştırılmıştır. Koçlar yaşama payının 1.25 katı düzeyinde beslenmişlerdir. Koçlara ad-libitum su verilmiştir.

Üretilen gazın CH4 içeriği İnfared metan analiz cihazıyla saptanmıştır (Goel ve ark. 2008). 24 saat inkübasyon süresi sonunda şırınga içeriği kullanılarak mikrobiyal biyokitle üretimi Blümmel ve ark. (1997)'nın bildirdikleri yönteme göre saptanmış ve aşağıdaki eşitlikler ile hesaplanmıştır. Yonca otunun gerçek sindirilebilir kuru madde (g) (GSKM), gerçek sindirim derecesi (GSD), taksimat faktörü (TF), mikrobiyal protein (MP), mikrobiyal sentezleme etkinliği (MPSE) değerleri Blummel ve ark, (1997) bildirdiği metoda göre yapımıştır.

GSKM (mg) = İnkübe edilen KM (mg) – Kalan KM (mg)
GSD (%) = (GSKM / İnkübe edilen KM)
TF = GSKM / GÜ
MP (mg/g KM) = GSKM – (GÜ X 2,2 mg/ml),
MPSE = (GSKM – (GÜ X 2.2 mg/ml))/GSKM.

Kimyasal Analizler

Yonca yonca otunun kuru madde (KM), ham kül (HK),

ham protein (HP), ham yağ (HY), içerikleri AOAC (1990)' a göre belirlenmiştir. Yonca otunun asit deterjan fiber (ADF) ve nötr deterjan fiber (NDF) içeriği ise Van Soest (1991)' in uyguladığı yönteme göre saptanmıştır.

İstatistik Analizler

Araştırmadan elde edilen verilerin istatistiki olarak değerlendirilmesinde ortalamalar arasındaki farklılıkların saptanmasında varyans analizi (General Linear Model) (Statistica, 1996), görülen farklılıkların önem seviyelerinin belirlenmesinde ise Tukey çoklu karşılaştırma testinden yararlanılmıştır (Snedecor ve Cochran, 1967).

BULGULAR ve TARTIŞMA

Çiçeklenme dönemi hasat edilmiş yoncanın besin madde kompozisyonları

Çiçeklenme döneminde hasat edilmiş yonca otunun kimyasal kompozisyonu Çizelge 1' de verilmiştir.

Çizelge1. Çiçeklenme dönemi hasat edilen yoncanın kimyasal kompozisyonu

Table 1. Chemical composition of alfalfa hay harvested during flowering

Besin maddeleri	%
Kuru Madde (KM)	92.6
Ham Kül (HK)	9.5
Ham Protein (HP)	16.5
Ham Yağ (HY)	1.2
Nötral Deterjan Lif (NDF)	46.3
Asit Deterjan Lif (ADF)	29.9

Bu çalışmada kullanılan yonca otunun kuru madde, ham kül, ham protein ve nötral deterjan lif içeriği Kamalak ve ark. (2011) bildirdiği değerlerle uyumlu, asit deterjan lif ve ham yağ içeriği daha düşük bulunmuştur. Bu farklılığın yoncanın hasat zamanı ile yetişme bölgesindeki farklılıklardan kaynaklandığı söylenebilir.

Biberiye eksraktının yonca otunu fermentasyon parametrelerine ve sindirim derecesi üzerine etkisi

Biberiye eksraktının yonca otunun fermentasyon ve sindirim derecesi üzerine etkileri saptanmış ve Çizelge 2'de verilmiştir. Biberiye eksraktı yonca otunun *in viro* gaz üretimi, CH_4 üretimini, gerçek sindirim derecesini, mikrobiyal protein üretimini ve mikrobiyal protein sentezleme etkinliğini önemli düzeyde etkilemiştir (P<0.001). Bu çalışmada, fermentasyon sonucunda yonca otunun *in vitro* gaz üretimi 92.80 ml ile 109.16 ml/500 mg KM arasında değişmiştir. Agustos ayında hasat edilen biberiye ekstraktı, hasat edilen ve kurutularak ekstraksiyon yapılan grup hariç önemli derecede *in vitro* gaz üretimini artırmıştır. Eylül ayında hasat edilen ve taze biberiye ekstraksiyonu en yüksek gaz üretime neden olmuştur. Biberiye ekstraktı CH_4 üretimini hem ml olarak hem de %'de olarak önemli derecede artırmış olup fermentasyona tabi tutulan yoncanın CH_4 üretimi sırasıyla 14.66 ml ile 25.36 ml arasında, %15.76 ile %25,36 bulunmuştur. Metan üretimi kontrol grubu hariç yaklaşık iki katına çıkmıştır.

Çizelge 2. Biberiye eksraktının yonca otunun rumen fermantasyon parametreleri ve sindirim derecesine etkisi

Table 2. The effect of rosemary extract on the rumen fermentation parameters and digestion degree of alfalfa.

Parametreler	K	BAY	BAK	BEY	BEK	Ö.D.
Gaz (ml)	92.80^{c}±3.46	100.13^{bc}±3.21	96.80^{bc}±4.73	109.16^{a}±1.53	104.46^{ab}±2.08	***
CH_4 (ml)	14.66^{b}±0.51	25.36a±0.50	23.02^{a}±1.22	24.86^{a}±1.57	24.20^{a}±1.38	***
CH_4 (%)	15.76^{c}±0.07	25.36^{a}±1.29	23.80^{ab}±0.34	22.76^{b}±1.15	23.20^{ab}±1.13	***
GSD (%)	72.50^{a}±0.62	69.73^{ab}±1.37	67.07^{b}±1.05	69.06^{b}±0.87	68.13^{b}±1.26	***
TF	3.73^{a}±0.13	3.30^{b}±0.19	3.30^{b}±0.16	3.00^{b}0.07	3.10^{b}±0.03	***
MP (mg)	140.20^{a}±6.99	110.20^{b}±15.75	105.82^{bc}±10.76	85.56^{c}±6.91	92.50^{bc}±4.28	***
MPSE (%)	40.73^{a}±2.05	33.26^{b}±3.95	33.00^{b}±3.34	26.25^{c}±1.77	28.69^{bc}±0.65	***

*^{abc}Aynı simgeye sahip ve aynı satırda yer alan ortalamalar arasında fark yoktur (P>0,05), Ö.D: Önemli düzeyi, GSD: Gerçek sindirim derecesi (%), TF: Taksimat faktörü, MP: Mikrobiyal protein üretimi (mg), MPSE: Mikrobiyal protein sentezleme etkinliği (%), *** P<0.001.*

Biberiye ekstraktı yonca otunun gerçek sindirim derecesini önemli derecede düşürmüştür (P<0.001). Yonca otunun gerçek sindirim derecesi %67.07 ile %72.50 arasında bulunmuştur. Ağustos ayında hasat edilen ve yaş olarak elde edilen biberiye ekstraktı hariç diğer biberiye ekstraktları yoncanın gerçek sindirim derecesini düşürmüştür. Farklı zaman ve şekilde elde edilen biberiye ekstraktı yonca otunun TF değerini de önemli derecede düşürmüştür. Yoncanın TF değeri 3.00 ile 3.73 arasında değişmiştir. Biberiye ekstraktı ilavesi MP önemli derecede azaltmıştır (P<0.001). Mikrobiyal protein üretimi 85.56 mg ile 140.20 mg arasında olmuştur. Eylül ayında elde edilen taze biberiye ekstraktının en düşük mikrobiyal protein sentezlemesine neden olmuştur. Eylül ayında elde edilen taze biberiye ekstraktın mikrobiyal protein sentezini düşürmüştür.

Bu çalışmada kullanılan biberiye ekstraksiyonu hem *in vitro* gaz üretimi hem de CH_4 üretimini önemli miktarda artırmıştır (P<0.001). Benzer şekilde Patra

ve ark. (2006) farklı bitkisel ekstraklarla yaptığı çalışmada benzer sonuçlar bulmuşlardır. *İn vitro* gaz ve CH_4 üretimindeki artışın ekstraklar içerisinde bulunan suda çözünebilir karbonhidratlardan kaynaklandığını bildirmişlerdir (Patra ve ark. 2006). Çözeltide kullanılan metanol ve etanolün *in vitro* gaz ve CH_4 üretimine etkisi olabileceği hakkında bilgi verilmemiştir Bu çalışmada ekstraksiyon işleminde kullanılan metanol ve etonol arkealar tarafından CH_4 üretiminde kullanabileceği organik maddelerdir. Arkealar metanolu direk CH_4 üretiminde kullanılmasına rağmen, etanolu önce asetik asite dönüştürüp, daha sonra asetik asiti CH_4 üretiminde kullanılabilmektedir (van Lier ve ark. 2008). Bu çalışmada kullandığımız biberiye ekstraktı hem metanol hem de etanolu içermektedir. Hem metanol hem de etanol arkelar tarafından fermente olduğundan dolayı sonuçların doğru bir şekilde elde edilmesi için her muamele grubu için spesifik körler oluşturulmuştur. Her muamele grubundan spesifik körlerden elde edilen *in vitro* gaz ve CH_4 üretim değerleri düşülmesine rağmen muamele gruplarının gaz ve CH_4 üretimleri kontrol grubuna göre önemli derece yüksek bulunmuştur. Gaz üretimindeki artışın ekstrak ve yemin fermentasyonu sonucu oluşan asetik asit miktarının artmasından kaynaklandığı söylenebilir. Toplam *in vitro* gaz üretimi ile rumende oluşan asetik asit arasında yakın ilişki olduğu, asetik asit üretiminin *in vitro* gaz üretimini artırdığı, propionik asitin ise düşürdüğü bildirilmektedir (Getachew ve ark. 1998). Bu çalışmada, CH_4 üretimindeki artış miktarı, gaz üretimindeki artıştan çok daha yüksek bulunmuştur. Metan üretimindeki artış ekstraktan ziyade içinde bulunan ve çözücü olan metanol ve etanoldan kaynaklandığı düşünülmektedir. Arkealar metanolu hem direk, hem de metanol ve H_2 iyonlarını kullanarak CH_4 ürettikleri bildirilmektedir (Jonhson ve Jonhson 1995; Leahy ve ark., 2010; Poulsen ve ark., 2012; Knapp ve ark., 2014;). Biberiye ekstraktıyla birlikte arkealara metanol verilmesi arkea sayısında başlangıçta çok hızlı bir artışa neden olduğu ve zaman geçtikce bu farklılığın kapanmadığı ve bundan dolayı ekstrakt ilavesinin kontrol grubuna göre daha fazla CH_4 üretimine yol açtığı düşünülmektedir. Ayrıca ekstrak içerisinde bulunan etanol metajonik bakteriler için direk kullanılan bir substrat olmadığından dolayı önce asetik asite dönüştürülmesi gerekmektedir. Etanolun asetik asit dönüşmesi sırasında açığa çıkan hidrojen iyonu (H^+), inkübasyonda kullanılan tampon çözeltiyle reaksiyona girerek CO_2 açığa çıkmasına neden olarak *in vitro* gazın üretimini artırmış olacağı söylenebilir.

Bu çalışmada kullanılan biberiye ekstraktları yonca otunun *in vitro* sindirim derecesini düşürmüştür. Benzer şekilde bazı bitki ekstrakların yemlerin sindirim derecesinin düşürdüğü yapılan çalışmalarla ortaya konmuştur (Patra ve ark., 2006; Agarwall ve ark., 2009). Özellikle bitkisel ekstrakların rumendeki selülozik bakterilerin faaliyetlerini engellediği, buna bağlı olarak yemlerin sindiriminin azaldığı bildirilmektedir (Patra, 2006). Bitkisel ekstraklar içerisinde bulunan esansiyal yağlar, tanen ve diğer aktif bileşikler sindirim enzimlerinin aktivitesini düşürerek, yemlerin sindirim derecesinin düşmesine yol açtığı bildirilmektedir (Patra ve ark., 2006, 2010).

Biberiye ekstraktları yonca kuru otunun *in vitro* TF, MP ve MPSE değerlerini önemli düzeyde etkilemiştir ($P<0.001$). Yukarıda sıralanan parametrelerin *in vitro* gaz üretim miktarı ile yakın ilişkili olduğu söylenebilir. Çalışmada kullanılan biberiye ekstraktları hem gerçek sindirim derecesini düşürmesi, hem de *in vitro* gaz üretimini artırması nedenilye, hesaplanan MP, TF ve MPSE'nin de önemli derecede düşmesine neden olmuştur ($P<0.001$).

Biberiye ekstraktının kullanılması *in vitro* MP'ni düşürmüştür. Bu durum rumende MP'ni sınırlayarak ruminantların performansını düşüreceği söylenebilir. Buradan hareketle biberiye ekstraktlarının anti-proteolitik potansiyellerinin saptanmasının önemli olduğuda söylenebilir. Bu şekilde mikrobiyal protein sentezindeki kayıpların bypass proteinle telafi edilip edilmeyeceği ortaya konabilir.

SONUÇ ve ÖNERİLER

Biberiye ekstraktlarının yonca otunun *in vitro* gaz ve CH_4 artırdığı, GSD, TF, MP ve MPSE düşürdüğü saptanmıştır. Bundan dolayı biberiye ekstraktının anti-metanojenik özelliğe sahip olmadığı ve ruminantlarda anti-metanojenik olarak kullanılmasının fayda sağlamayacağı kanısına varılmıştır. Bununla birlikte, düşük MP ve GSD, biberiye ekstraktının anti-mikrobiyel etkisinin olduğunu göstermektedir. Ayrıca ekstraksiyonda kullanılan metanol ve etanolun *in vitro* inkübasyonda kullanılmadan önce ekstraktan bir şekilde uzaklaştırılması yoluyla daha güvenilir sonuçlar elde edileceği söylenebilir. Ayrıca biberiye ekstraktının anti-preteolik etkisinin saptanmasına yönelik olarak *in vivo* ve *in situ* çalışmalara gereksinim olduğuda söylenebilir.

TEŞEKKÜR

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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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Determination of Milk Production Characteristics, Phenotypic, Genetic and Environmental Trends in Jersey Cattle

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ABSTRACT
This study was conducted to determine the effects of environmental factors on milk yield traits of Jersey breed cattle reared at the Karakoy State Farm located in Samsun Province of Turkey, as well as the phenotypic, genetic and environmental trends in relation to 305 day milk yield. The Wombat software was used to estimate heritability and breeding values for milk yield. The effects of parity, season and year factors on actual and 305 day milk yields were found to be statistically significant (P<0.01). The mean values of lactation length, actual and 305 day milk yields of Jersey cattle were found as 310 ± 5 days, 4462 ± 90 kg and 4183 ± 70 kg, respectively. A phenotypic trend of 29.97 kg year^{-1}, genetic trend of 18.71 kg year^{-1} and environmental trend of 11.26 kg year^{-1} were estimated. The heritability of 305 day milk yield was 0.344. The overall results of this study showed that an improvement in Jersey cattle reared at the Karakoy State Farm between the years 2006 and 2014 was provided remarkably on the basis of phenotypic, genetic and environmental trends and the enterprise had a good genotype and a good management.

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ÖZET
Bu çalışmada Karaköy Tarım İşletmesinde Yetiştirilen Jersey Irkı sığırlarda gerçek ve 305 günlük süt verimini etkileyen çevre faktörleri ile 305 günlük süt verimine ait bazı genetik parametreler, çevresel, genetik ve fenotipik yönelimler hesaplanmıştır. Genetik parametrelerden olan kalıtım derecesinin ve damızlık değerlerin hesaplanmasında Wombat istatistik paket programı kullanılmıştır. Verim yılları ve mevsimin, gerçek ve 305 günlük süt verimi üzerine etkisi çok önemli bulunmuştur (P<0.01). Araştırmada Jersey sığırlarına ait ortalama laktasyon süresi 310 ± 5 gün, gerçek süt verimi 4462 ± 90 kg ve 305 gün süt verimi ise 4183 ± 70 kg olarak tespit edilmiştir. İşletmede yıl başına fenotipik yönelim 29,97 kg year^{-1}, genetik yönelim 18,71 kg year^{-1} ve çevresel yönelim ise 11.26 kg year^{-1} olarak tahmin edilmiştir. Üçyüzbeş günlük süt verimine ait kalıtım derecesi (h^2) 0,344 olarak saptanmıştır. 2006-2014 yılları arasında Karaköy Devlet Çiftliği'nde yetiştirilen Jersey sığırlarında fenotipik, genetik ve çevresel eğilimler bazında dikkate değer bir gelişme sağlandığını ve işletmenin iyi bir genotip ve iyi bir yönetime sahip olduğunu göstermiştir.

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INTRODUCTION

Jersey's origin is from Jersey Island which is located between France and the United Kingdom. Jersey cattle were brought from the USA to the Samsun-Karakoy state farm in the year 1958, and then, imported from the United Kingdom and Denmark (Eliçin et al., 1991). Jersey breed are reared densely in Black Sea Region of Turkey. Most particularly, this breed had a high adaptability considerably under environmental conditions of central and eastern Black Sea Region over time and therefore it was used by the region farmers for breeding purpose. The cattle breed used for crossbreeding and pure breeding have been reared officially for pure breeding purpose in Karaköy Agricultural Enterprise connected with general directorate of agricultural enterprises in Turkey (Çankaya and Ünalan, 2008).

Jersey cattle is originated from *Bos longifrons (brachyceros).* The desired weight in mature Jersey cows is 400-500 kg. Mature Jersey bulls weigh approximately 600-725 kg. Jersey's milk is superior in fat and dry matter to other breeds with a fat percentage range of 4 to 8%. On average, the breed produces 3800-4600 kg with the fat percentage of 5.3%. Butter production costs cheaper due to high fat percentage in its milk dry matter. The milk of the breed, preferred for obtaining fat and cream, has a high carotene amount; therefore, its milk color is yellow. The dry matter-fat ratio in the milk is high for cheese making or concentrated milk production (Özhan et al., 2001). Birth weights of Jersey calves range from 19.8 to 23.3 kg. In the first periods of their lifetime, their weight gains are low, and they are not appropriate for young cattle fattening. Their meat is also not in good quality or delicious (Özbeyaz et al., 1997). A large quantity of data for breeding studies is needed, and the number of subgroups in the obtained data is generally unbalanced. In this context, classical estimation methods could not meet the requirements in unbalanced datasets. Software used in evaluation of animal datasets can estimate variance components, heritability and phenotypic, genetic and environmental correlations between traits by providing agreement of genetic and statistical models with the data. By means of software, the breeding values of animals may also be estimated (Akbaş, 1998).

Recent developments in computer technologies offer the opportunity of constructing statistical models evaluating animals as a factor and using nonlinear converge techniques in estimating variance components. Accordingly, Wombat (a tool for mixed model analyses in quantitative genetics by the restricted maximum likelihood function) is a popular program performing simultaneous analysis of random and fixed factors and using techniques that reduce possible data losses. With each passing day, more attention on the program has emerged. Heritability may be estimated in the event of knowing the error variance and variance between animals within the scope of an animal model. The Wombat program enables analysts to estimate the effect sizes of factors by solving random (animal, dam and sire, etc.) and fixed factors simultaneously and then the breeding values of animals by selecting the best linear unbiased prediction option (BLUP). The program estimates converging variance components of random factors, such as animal, dam and sire, on the basis of the Restricted Maximum Likelihood algorithm (REML). Therefore, prior variance values from previous studies facilitate the program's work in estimating variance components. Otherwise, a convergence operation cannot be performed in the event that these prior values are not close to actual values (Tekerli et al., 2014).

To date, various methods have been used to estimate the genetic parameters of 305 day milk yield and the phenotypic, genetic and environmental trends in Turkey. Environmental trend has been computed by the regression of differences obtained from successive yields of cows on calving years, whereas phenotypic trend has been calculated by the regression of standardized yields of cows on calving year (Kaygısız 1996; Aydın et al., 1998; Musani and Mayer 1997). Afterwards, the REML, DFREML and MTDFREML methods have been employed (Ahmad et al., 2001; Leitona and Zeledon, 2008; Rehman et al., 2008, Bakır and Kaygısız, 2009, Çetin and Koç, 2011; Missanjo et al., 2011; Katok and Yanar, 2012; Şahin et al., 2014;, Demirgüç 2015; Selvi and Yanar, 2016). Nowadays, the Wombat software developed based on the REML procedure by Meyer (2011) has been used by Şahin (2012) and Tekerli et al., (2014).

There is no information about the estimation of phenotypic, genetic and environmental trends for the 305 day milk yield on Jersey cattle breed reared in Turkey. Hence, the aim of this study was to determine the effects of environmental factors on milk yield traits (actual and 305 day milk yields) of Jersey breed cattle reared at the Karakoy State Farm located in the Black Sea Region of Turkey and estimate the phenotypic, genetic and environmental trends in relation to 305 day milk yield in the past decade.

MATERIALS and METHODS

The material of the study comprised milk yield records of Jersey breed cattle reared at the Karakoy State Farm located in Samsun province of Turkey between the years 2005 and 2014.

In this study, 704 lactation records of 215 cows belonging to 26 sires were evaluated. In the herd management of Jersey breed cattle reared at the Karakoy State Farm, the computer-aided Westfalia

Dairy Plan has been used as a herd management program to remove problems resulting from human errors in order to make better evaluations on Jersey cows. Thanks to this program, the individual data of animals were recorded manually and automatically. The records of the cattle used in this study were obtained from the computerized herd management program. Cows available in the enterprise have been milked twice a day, i.e., in the morning and evening.

In this study, macro environmental factors, i.e., calving year, calving season and parity, were considered to be able to affect actual and 305 day milk yield traits. The SPSS software was used to determine the influential factors for the traits (SPSS 2004). The mean separation was determined for significant environmental factors by Duncan's multiple comparison test (Yildiz et al., 2011).

The statistical model used for the analysis of variance was as follows:

$Y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl}$

Where,

Y_{ijkl} = i^{th} parity, j^{th} calving season, kth calving year, l^{st} animal effect,

μ = Population mean,

a_i = i^{th} parity effect (i = 1, 2, ….7),

b_j = j^{th} calving season effect (j = 1^{st} winter, 2^{nd} spring, 3^{rd} summer, 4^{th} fall), c_k = k^{th} calving year effect (k = 2006, 2007, … 2014), and

e_{ijkl} = random error with zero mean and variance, σ_e^2.

To estimate the phenotypic trends for 305 day milk yield traits and the effects of environmental factors (parity and calving season), the following statistical model was used in the SPSS statistical package program. 305 day milk yields were standardized according to the determined effect sizes. The statistical model built with these purposes may be written as follows:

$Y_{ijk} = \mu + a_i + b_j + e_{ijk}$

Where;

Y_{ijk} = i^{th} parity,

j^{th} calving season,

k^{th} cow's standardized 305 day milk yield amount,

μ = Population mean, a_i = i^{th} parity effect (i = 1, 2, ...,7),

b_j = j^{th} calving season effect (j = 1^{st} winter, 2^{nd} spring, 3^{rd} summer, 4^{th} fall), and

e_{ijk} = random error with zero mean and variance σ_e^2.

The genetic trend for 305 days milk yield was obtained by calculating the regression between the birth years of the animals and the milk yield averages. The phenotypic trend was obtained by calculating the regression between the calving years of the animals and the average breeding value. Phenotypic trends were calculated by regression of standardized 305-day milk yields by years using the SPSS statistical program. With the objective to determine the genetic trends in this study, the Wombat statistics program developed by Meyer (2011) was utilized.

The following regression equation was used to estimate the phenotypic trend and genetic trend.

$Y_i = a + bx_i + e_i$

Y_i =i^{th} calving year milk yield (for phenotypic trend) or i^{th} birth year breeding value (for genetic trend)

i=2006, 2007, …2014 (calving years), or i = 2003, 2004, 2011 (Birth years)

a : Constant,

bx_i: phenotypic trend or genetic trend

e_i : random error with zero mean and variance σ_e^2

The Windows version of Wombat and user notes can be downloaded from http:/didgeridoo.une.edu.au/km/wombat.php (Meyer 2011). A "pedigri file" from which the entire pedigree of the animals will be extracted, the "parameter file" that constitutes the syllabus of the work to be done, and the "data file" containing the data of the individuals have been prepared. Wombat.exe and these files have been transferred to the same folder.

First, the data set file was created. The pedigree and data files to be used in this program are prepared as follows (Figure 1).

Figure 1. Pedigree Star Program placed in Excel program

Şekil 1. Excel programına yerleştirilen Pedigri Yıldızı Programı

- It is ensured that the sire and dam numbers are smaller than the cow itself. For this purpose, the sire are renumbered (as 1-26). Dam numbers have been renumbered (as 27-241).

-The numbers given to individuals (cows) in the enterprise must be greater than the dam and sire numbers. In this study, the individual numbers were not changed because they were already greater than the dam and sire numbers.

-The pedigri file was created as pedigri.xls file in Excel program.

By pressing the Ctrl + Z key, the "Pedigree Star" program in Figure 1 was started. A pedigree file was created from here.

Then, the corrected data file and pedigree files were saved as two different files in Windows Notepad (txt) format using the save as option. In the first lines of both pedigree and data files, abbreviations are written with the symbol "#" and indicating the data names.

Pedigri and Data files were saved as Windows Notepad (Figure 2 and 3). While creating the parameter file, the data name for each lactation was created with the extension (kitap1.par) as follows (Figure 4).

Table 1. Data file
Çizelge 1. Veri dosyası

birey	baba	ana	dyıl	mevkod	byıl	lakkod	Laksure (gün)	Tsv (kg)	Usv (kg)
Cow number	sire number	dam number	birth year	season code	calving year	parite	lactation length (day)	total milk yield (kg)	305 daily milk yield (kg)
464	1	27	2003	2	2005	1	378	4698	3752
476	1	28	2003	2	2005	1	301	1655	1655
494	1	29	2003	3	2005	1	267	3976	3976
519	1	29	2003	4	2005	1	294	4130	4130
547	1	31	2003	2	2006	1	347	4196	3621
555	2	32	2003	4	2005	1	377	2836	2291
596	1	33	2003	1	2006	1	332	4066	3845
648	1	34	2004	4	2006	1	314	3376	3313
666	3	35	2004	1	2006	1	304	5094	5094
703	1	36	2004	1	2007	1	326	4226	4009
796	4	41	2005	2	2007	1	293	5070	5070
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
66991	19	241	2010	2	2012	1	280	3462	3462

Just after the model statement, the animal itself was written as a random factor, one line down and one column inside, and the expression NRM (numerator relationship matrix) was included with a space distance in case of the pedigree file showing kinship relations. Similarly, RAN for random factors; FIX prefix was used for fixed factors. The TRAIT pre-statement was used to report the examined feature to the program. The model is finished with the END MODEL statement.

Prediction of genetic parameters, breeding values and genetic trend with Wombat program

The following sequence is followed as an example for the prediction of genetic orientation.

1. In the first study, after creating a folder named "milk yield records", the files Kitap1.dat, Kitap1.ped, and Kitap1.par were copied to this folder.

2. A folder has been created for each lactation (kitap1, kitap2, kitap3,.....kitap7). In order to run Wombat on the command line, the CMD program has been transferred to the milk yield records folder.

3. To run the CMD program, write wombat kitap1 on the command line and run the program with the enter key. The desired information was entered in the program and the process was continued until the end.

4. After the process is finished, the SumEstimate.out file is opened from the kitap1 folder. Heritability data for the first lactation were obtained from here.

5. In order to calculate breeding values, RnSoln_ana.dat and RnSoln_Individual.dat files were created in the folder by giving the wombat --blupkitap1 command to the blup program in the Wombat

command line. Among these files, BLUP values (breeding values), standard errors, hit degrees and relatedness degrees of the animals were seen in the RnSoln_Individual.dat file.

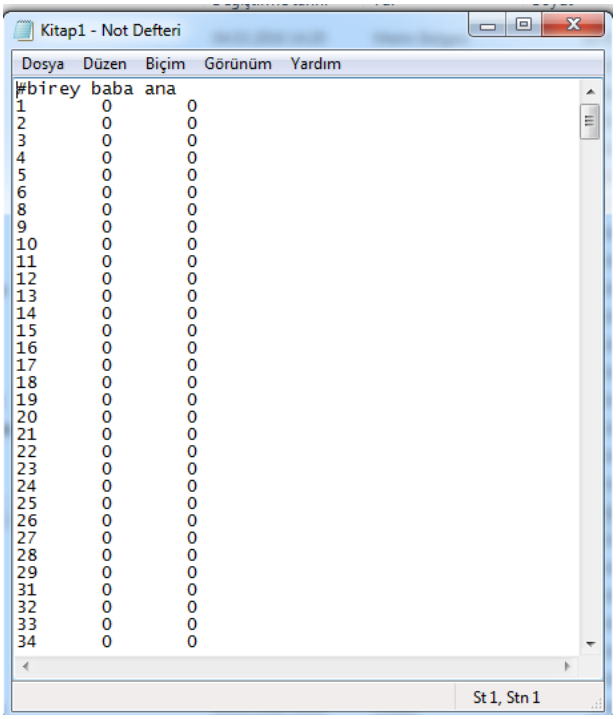

Figure 2. Ped (Pedigri) file (Kitap1.ped)
Şekil 2. Ped (Pedigri) dosyası (Kitap1.ped)

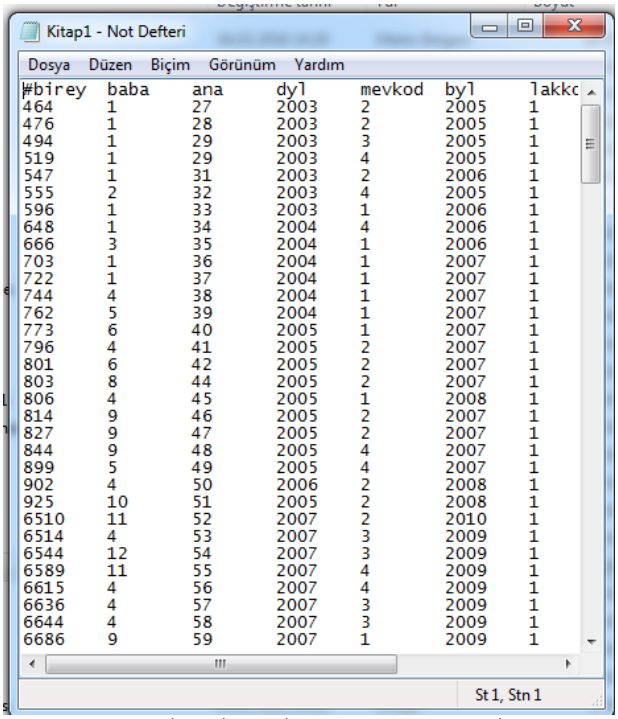

Figure 3. Dat (data) file (sample; Kitap1.dat)
Şekil 3. Dat (veri) dosyası (örnek; Kitap1.dat)

6. Breeding values were calculated separately for seven lactations as stated above than combined in an excel file and average breeding values were calculated for each animal.

7. Genotypic trend was estimated by regression of mean breeding values to "year of birth" in the SPSS statistical program.

The effect sizes of the factors were estimated by solving random (animal, dam and sire, etc.) and fixed factors simultaneously, and then, breeding values of animals by selecting the BLUP option in the 305 day milk yield trait with the help of the Wombat program (Tekerli et al., 2014; Kabakcı 2017). Preparation of pedigree, parameter and data files is explained in detail by Kabakcı (2017) with examples. In addition, genetic parameters and breeding values estimation with Wombat were explained in detail by Kabakcı. Presenting the method here will increase manuscript volume.

Genetic trend was calculated by the regression of breeding values on the birth years of the cows with SPSS statistic program. Heritability and breeding values were estimated by the Wombat program (Tekerli et al., 2014; Kabakcı 2017).

Environmental trend was estimated using the phenotype = genotype + environmental formula. First, the phenotypic and genetic trends were estimation. Second, these values were applied to the formula. Finally, the genetic trend value was subtracted from the phenotypic trend value and thus the environmental trend was calculated.

RESULTS and DISCUSSION

Environmental Factors Affecting Actual and 305 Day Milk Yield Traits

The results of least squares means with standard errors and multiple comparison test of lactation length, actual and 305 day milk yield traits in Jersey cattle are given in Table 1.

The effects of parity, calving year and calving season on actual and 305 day milk yield traits were found significant ($P < 0.01$), which was in agreement with those reported by several authors (Bashir et al., 2008; Lateef et al., 2008; Lemma et al., 2009; Teke and Akdag, 2010, Ünalan and Çankaya, 2010;2012; Missanjo et al., 2011).

The mean lactation length of Jersey cattle reared at the Karakoy State Farm was found as 310 ± 5 days, which was within the limits of the 297 - 323 days given in the relevant literature. (Tahtabiçen, 2008; Lemma et al., 2009; Şahin, 2009; Ünalan and Çankaya, 2010;2012, Kul, 2013; Fernando et al., 2016). It could also be suggested that this figure was a good value for the enterprise. When calving years in lactation length were examined, small fluctuations were observed. The longest lactation length was recorded in the year 2011 with 324 days, whereas the shortest lactation length

with 248 days was obtained in the year 2014. When the effects of the season factor on lactation length were evaluated, it was determined that cows in winter seasons were milked for a longer time (322 days) in comparison to other seasons.

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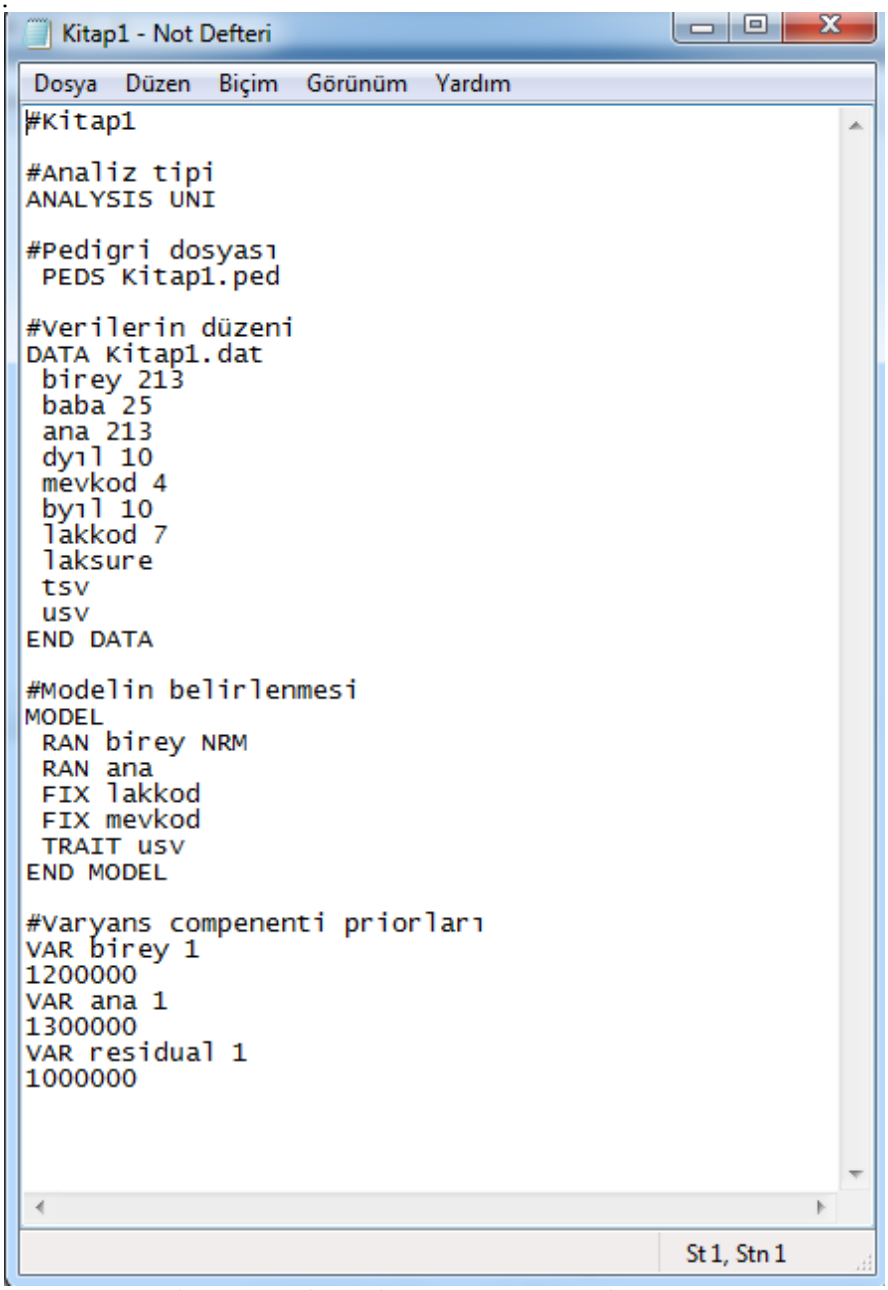

Figure 4. Par (parameter) file (sample Kitap1.par)
Şekil 4. Par (Parametre) dosyası (örnek Kitap1.par)

Actual milk yield mean value of the Jersey cattle was estimated at 4462 ± 90 kg. When actual milk yield was examined based on parity, it reached the peak point (4721 ± 135 kg) at the third lactation, and it started to decrease from the fourth lactation on. When change in actual milk yield was examined in reference to calving years, actual milk yield was recorded on the lowest level (3910 ± 52 kg) in the year 2005, but it reached the highest level with the mean value of 4926 ± 93 kg in the year 2013. When change in actual milk yield was evaluated based on calving season, it was seen that higher milk yield was obtained in the winter season.

In this study, 305 day milk yield reached the peak level by increasing from the first lactation to the third lactation. In agreement with those reported by Nyamushamba et al., (2014), the mean value of 305 day milk yield in the Jersey cattle was 4183 ± 70 kg. The lowest 305 day milk yield amount was recorded in the year 2005, while the highest was obtained in the year 2013 (Table 1). The 305 day milk yield amount recorded in winter was relatively higher than those recorded in other seasons, and lower 305 day milk yield

was found in summer. Due to the fact that the Karakoy State Farm is located in the Black Sea Region of Turkey, the warm-rainy winter season could have positively affected milk yield.

Table 1. Results of least squares means, standard errors and multiple comparison test of lactation length, actual and 305 day milk yield traits in Jersey cattle

Çizelge 1. Jersey sığırlarda laktasyon süresi, gerçek süt verimi, 305 günlük süt verimine, ait en küçük kareler ortalamaları, standart hataları ve çoklu karşılaştırma testi sonuçları

Variables *Değişkenler*	N	Lactation Length (day) *Laktasyon süresi (gün)* $\overline{X} \pm S_{\overline{x}}$	Actual Milk Yield (kg) *Gerçek süt verimi (kg)* $\overline{X} \pm S_{\overline{x}}$	305 Day Milk Yield (kg) *305 Gün Süt Verimi* $\overline{X} \pm S_{\overline{x}}$
Parity		NS	**	**
1	213	313 ± 4	4103 ± 87^{b}	3771 ± 68^{b}
2	157	308 ± 5	4616 ± 104^{a}	4360 ± 81^{a}
3	108	305 ± 6	4752 ± 122^{a}	4548 ± 96^{a}
4	69	312 ± 8	4693 ± 150^{a}	4394 ± 117^{a}
5	65	315 ± 8	4713 ± 156^{a}	4370 ± 122^{a}
6	45	304 ± 10	4348 ± 187^{b}	4121 ± 146^{b}
7	47	312 ± 9	4232 ± 184^{b}	3903 ± 144^{b}
Calving Year		**	**	**
2006	12	319 ± 17^{b}	4305 ± 342^{b}	4082 ± 267^{c}
2007	20	312 ± 13^{b}	4438 ± 266^{ab}	4148 ± 207^{bc}
2008	38	319 ± 10^{b}	4286 ± 195^{ab}	3904 ± 152^{bc}
2009	52	316 ± 9^{b}	4454 ± 168^{ab}	4166 ± 131^{abc}
2010	69	306 ± 7^{b}	4549 ± 144^{ab}	4293 ± 113^{abc}
2011	87	324 ± 6^{b}	4830 ± 128^{a}	4477 ± 100^{a}
2012	143	323 ± 5^{b}	4636 ± 104^{ab}	4276 ± 81^{abc}
2013	182	322 ± 5^{b}	4926 ± 93^{a}	4580 ± 73^{a}
2014	101	248 ± b	4023 ± 119^{b}	3960 ± 93^{bc}
Season		**	**	**
Winter	147	322 ± 6^{b}	4789 ± 114^{a}	4463 ± 89^{a}
Spring	176	316 ± 5^{b}	4548 ± 104^{a}	4247 ± 81^{a}
Summer	208	304 ± 5 ^{b}	4237 ± 98^{b}	3960 ± 76^{b}
Fall	173	299 ± 5 ^{b}	4401 ± 106^{ab}	4168 ± 83^{a}
Overall Mean	704	310 ± 4	4494 ± 72	4210 ± 56

** : P < 0.01 ; * : P < 0.05, $\overline{X} \pm S_{\overline{x}}$: mean and standard error of mean, NS: Non significant

$^{a, b, c, d}$: Means in same columns with different superscripts are significantly different on the level of P < 0.05 or P < 0.01

Phenotypic Trend

Phenotypic trend is named as the change provided in a particular of period of time in a yield trait. Phenotype is composed of two parts i.e. genotype and environment. "Environmental trend" is described as the change that joint effects of all environmental factors affecting quantitative yield traits showed according to years, and "genetic trend" is expressed as the effect degree that genetic improvement studies conducted in order to improve the studied yield traits indicated according to years (Kaygısız, 2000).

In the estimation of phenotypic trends, the year factor was excluded from the studied linear model due to the fact that the regression of 305 day milk yield on years was accounted for. The effect sizes of the environmental factors that influenced the 305 day milk yield trait in the Jersey cattle are given in Table 2.

The coefficients including the obtained regression parameters are presented in Table 3.

The regression equation was obtained by taking the regression of standardized milk yields to yield year. The phenotypic trend was estimated at 29.97±17,10 kg year^{-1}, with the help of the regression prediction equation. These findings were consistent with those reported by Palmer et al., (1972) and Musani and Mayer (1997). In the relevant literature, there is no published information on the estimation of the phenotypic, genetic and environmental trends of Jersey cattle reared in Turkey.

Genetic Trend

The annual genetic trend was calculated as 18.71 ± 7.34 kg year^{-1} (Table 3), indicating that the genetic capacity of the sires used for breeding purposes was good. These estimates were higher than those reported by previous studies (Musani and Mayer, (1997) 0.8 kg, Leiton and Zeledon, (2008) 7.95 kg and Şahin, (2009) 5.90 kg).

Table 2. The effect sizes of environmental factors that influenced the 305 day milk yield trait in Jersey cattle
Çizelge 2. Jersey sığırlarda 305 günlük süt verimini etkileyen çevre faktörleri ve etki miktarları

Variables *Değişkenler*	N	305 day milk yield (kg) *305 gün süt verimi (kg)* $\overline{X} \pm S_{\overline{x}}$	Effect sizes *Etki miktarları*
Overall Mean	704	4290 ± 41	
Parity		**	
1	213	3848 ± 63[b]	-442
2	157	4494 ± 74[a]	204
3	108	4597 ± 89[a]	306
4	69	4517 ± 111[a]	226
5	65	4516 ± 116[a]	226
6	45	4101 ± 139[b]	-189
7	47	3959 ± 135[b]	-331
Season		**	
Winter	147	4501 ± 81[a]	211
Spring	176	4291 ± 74[a]	1
Summer	208	4056 ± 65[b]	-234
Fall	173	4312 ± 73[a]	22

[a, b, c, d]: Means in same columns with different superscripts are significantly different on the level of P < 0.05 or P < 0.01
**: P<0.01; *: P<0.05
$\overline{X} \pm S_{\overline{x}}$: mean and standard error of mean

Table 3. The coefficients including the obtained regression parameters from the 305 daily standardized milk yield and Breeding Values.
Çizelge 3. 305 günlük standardize edilmiş süt verimi ve damızlık değerlerinden elde edilen regresyon parametrelerini içeren katsayılar

Regression equations	a	b	R^2	Signification
Fenotypic Trend				
Y=-56003.07 + 29.97 X	-56003.07 ± 34390.14	29.97 ± 17.10	0.004	NS
Genetic Trend				
Y= -37549.89 + 18.71 X	-37549.89 ± 14740.02	18.71 ± 7.34	0.008	*

Y: predicted milk yield (kg), a: constant, b: linear coefficient for year or breeding value
NS: Non significant, *:P<0.05

To estimate the genetic trend, the regression of the breeding values of the cows on calving years was taken, and the regression prediction equation obtained from this data was as follows (Table 4).

The variation of breeding values by years is presented in Table 4. It is seen that the highest average breeding value belongs to 51 Jersey cows born in 2008 (247,322±41.004 kg), while the lowest average breeding value belongs to 47 cows born in 2004 (-154.648±42.713 kg) (Table 4).

Breeding values of 305 days milk yield were found to be negative in some years and positive values in some years. However, it can be said that the breeding value is positive and high indicates that the breeder selection in the herd is made accurately. It is known that an individual inherits half of his genotype from his mother and the other half from his father, but these halves are passed on to the offspring by chance. Since the parents can be heterozygous for many genes, the genes passed on to the offspring can be good or bad. In other words, the fact that an individual's parents have good yield records does not necessarily require that the offspring be highly productive (Özhan et al. 2001)

Environmental Trend

The environmental trend, which is defined as the difference between the phenotypic and genetic trends, was estimated at 11.26 kg year^{-1}. The estimates of the phenotypic, genetic and environmental trends reflected that the managerial conditions were on a sufficient level. The environmental trends estimated from the Jersey cows were in agreement with Musani and Mayer (1997) who found the value of 14.6 kg year^{-1}, but higher than those reported by Palmer et al. (1972) and Njubi et al. (1993) (-14.0 and 32.2 kg year^{-1}).

Genetic Parameters

The genetic parameters of the Jersey cattle reared at the Karakoy State Farm were used to estimate heritability values (Table 5). The mean heritability estimate of 0.344 was recorded for the Jersey cattle (Table 5). These heritability estimates for the 305 day

milk yield trait were in agreement with those reported by several authors (Makuza et al., 2001, Şahin 2004; Şahin 2009, Ünalan and Çankaya 2010), whereas different results were reported by some authors including Leiton and Zeledon (2008) as 0.21 h^2, Missanjo et al., (2011) as 0.30 h^2 and Banga (1992) as 0.54 h^2.

Table 4. Variation of breeding values by birth years
Çizelge 4. Damızlık değerlerin doğum yıllarına göre değişimi

Birth Year *Doğum Yılı*	n	Breeding Values** *Damızlık değerler*	95% Confidence Interval %95 Güven Sınırları Lower Bound *En Düşük*	Upper Bound *En Yüksek*
2003	58	18.474 ± 38.450^{cd}	-57.018	93.967
2004	47	-154.648 ± 42.713^{e}	-238.511	-70.786
2005	67	-7.667 ± 35.775^{cd}	-77.906	62.572
2006	122	24.512 ± 26.511^{cd}	-27.540	76.563
2007	88	-24.039 ± 31216^{cd}	-85.327	37.249
2008	51	247.322 ± 41.004^{a}	166.816	327.829
2009	70	73.997 ± 35.000^{bc}	5.280	142.715
2010	140	-40.599 ± 24.748^{d}	-89.189	7.992
2011	61	128.299 ± 37.493^{b}	54.686	201.911
Grand Mean		29.517 ± 11.751	6.446	52.588

**:P<0.01

Table 5. Heritability calculated for 305 daily milk yield in parity
Çizelge 5. Laktasyon sırasına göre 305 gün süt verimi için hesaplanan kalıtım dereceleri

Parity *Laktasyon sırası*	h^2 (Heritability) *Kalıtım derecesi*
1	0.346
2	0.345
3	0.344
4	0.344
5	0.343
6	0.343
7	0.343
Mean	0.344

CONCLUSIONS

The overall results of this study reflected that a significant improvement was recorded in the phenotypic, genetic and environmental trends for the Jersey cattle reared at the Karakoy State Farm located in the Black Sea Region of Turkey in the period of 2005-2014. In this study, the estimated positive phenotypic, genetic and environmental trends showed that the Karakoy State Farm had a good herd management process. The enterprise has taken a significant task in elite cattle breeding, especially in presenting elite cattle to farmers. Application of the available herd management process should be sustained identically for many years, in accordance with the phenotypic, genetic and environmental improvements provided by years.

Conflict of Interest

The authors declare that they do not have any competition and any conflicts of interest.

Author Contributions

The data of the study was prepared by DK. Statistical analyzes were made by DK and RA. This study was written by DK as a PhD thesis. This research has been updated and rewritten by DK and RA. Authors declare the contribution of the authors is equal.

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