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Larva parasited by *Apanteles brunnistigma*

Photo: Glay KAÇAR

*Apanteles brunnistigma* tarafından parazitli larva

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Ankara Üniversitesi  
ZİRAAT FAKÜLTESİ

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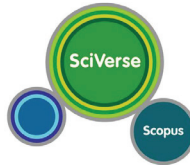
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## Yağ Güllü (*Rosa damascena* Mill.)'nde Distilasyon Ürünlerinin Uçucu Yağ ve Fenolik Madde İçerikleri ile Antiradikal ve Antioksidan Aktiviteleri

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### ÖZET

Yağ güllü (*Rosa damascena* Mill.), sahip olduğu yüksek kalitedeki aromatik bileşenler nedeniyle parfüm ve kozmetik endüstrisinde değerlendirilen en önemli kokulu gül türüdür. Yağ güllünde doğal ve sağlıklı ürün çeşitliliğinin artırılması, kullanım alanlarının genişletilmesi ve damıtma atıklarının değerlendirilmesi amacıyla yürütülen bu çalışmada, taze yağ güllü çiçeklerinin damıtılma sürecinde elde edilen gül yağı ve gül suyu gibi temel ve posa suyu gibi atık damıtma ürünlerinin uçucu yağ ve fenolik madde içerikleri ile antiradikal ve antioksidan aktiviteleri belirlenmiştir. Süleyman Demirel Üniversitesi Gül ve Gül Ürünleri Araştırma ve Uygulama Merkezine (GÜLAR) ait yağ güllü (*Rosa damascena* Mill.) araştırma bahçesinden gül toplama ve damıtma sezonunda (Mayıs ve Haziran) sabah erken saatlerde toplanan taze yağ güllü çiçekleri Clevenger hidro-distilasyon cihazında damıtılarak gül yağı, gül suyu ve posa suyu elde edilmiş ve bu ürünlerde uçucu yağ bileşenleri gaz kromatografisi/kütle spektrometresi (GC/MS) kullanılarak tespit edilmiştir. Ayrıca elde edilen bu distilasyon ürünlerinde fenolik madde ekstraksiyonları da yapılarak, toplam fenolik madde, toplam flavanol ve toplam flavonol içerikleri spektrofotometrik yöntemlerle, fenolik bileşik içerikleri de yüksek performanslı sıvı kromatografisi (HPLC) ile belirlenmiştir. Ekstraktların antioksidan ve antiradikal aktivitelerini belirlemek için de sırasıyla demir indirgeme gücü (FRAP) yöntemi ile 1,1-difenil-2-pikril-hidrazil (DPPH) yöntemi kullanılmıştır. Araştırma sonucunda, gül yağında sitronellol (% 35,27), gül suyu ve posa suyunda ise feniletıl alkolün (sırasıyla % 60,71 ve % 90,32) en önemli uçucu yağ bileşeni olduğu tespit edilmiştir. Fenolik madde içerikleri bakımından bir değerlendirme yapıldığında ise gül yağının zengin bir içeriğe sahip olduğu, posa suyunun da gül suyuna göre daha fazla fenolik bileşik içerdiği belirlenmiştir. Genel olarak gül ürünlerinin yüksek antiradikal ve antioksidan aktiviteye sahip olduğu, ancak distilasyon ürünleri arasında gül yağı ve posa suyunun gül suyuna göre daha yüksek antiradikal ve antioksidan etkiler gösterdiği saptanmıştır. Araştırma sonucunda özellikle distilasyon sürecinde atık ürün olarak elde edilen posa suyunun doğal antioksidan kaynağı olarak ekonomiye kazandırılabilceği öngörülmüştür.

Anahtar Kelimeler: Yağ güllü; Distilasyon ürünleri; Fenolik bileşikler; Antiradikal ve antioksidan aktivite

## Essential Oils and Phenolic Compounds, Antiradical and Antioxidant Activities of Distillation Products in Oil-bearing Rose (*Rosa damascena* Mill.)

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## ABSTRACT

Oil-bearing rose (*Rosa damascena* Mill.) is one of the most strongly scented rose species, producing a high-value aromatic oil, which is used in the fragrance and cosmetic industries. In this research, developing the usage of natural and healthy products of oil-bearing rose and evaluating waste of distillation products were aimed. From this point of view, the main target in the study was to determine the essential oil and phenolic compounds, antiradical and antioxidant activities of rose oil and rose water as basic products, and also residue water as a waste product obtained in the distillation process of fresh oil-bearing flowers. Oil-bearing rose flowers were collected early in the morning hours of the days during the harvesting and processing season (May and June) from the research garden of the Rose and Rose Products Research Application Center (GULAR) at Süleyman Demirel University and then rose oil, rose water and residue water were obtained from the fresh rose flowers by using Clevenger type hydro-distillation apparatus. Essential oil compounds were analysed by gas chromatography/mass spectrometry (GC/MS). After phenolic extractions in the distillation products, total phenolics, flavanol and flavonol contents were analysed by spectrophotometric methods and phenolic compounds were determined by high performance liquid chromatography (HPLC). Antioxidant capacity was assessed by ferric reducing antioxidant power (FRAP) method, and antiradical activity was made by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Appreciation of the total results, citronellol in rose oil (35.27%) and phenylethyl alcohol in rose water and residue water (60.71% and 90.32%, respectively) were the main essential oil compounds. Rose oil had a rich content phenolics. On the other hand rose water contained more phenolic compounds according to the residue water. Antiradical and antioxidant activity were found high in the distillation products of oil-bearing rose. Rose oil and residue water among the distillation products had a higher antiradical and antioxidant activity when compared with rose water. As a conclusion, the results indicate that residue water as a large scale waste product during the hydro-distillation, can be evaluated for natural antioxidant sources to obtain economical gain.

Keywords: Oil rose; Distillation products; Phenolic compounds; Antiradical and antioxidant activity

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## 1. Giriş

Dünyada 150 kadar gül (*Rosa* sp.) türü bulunmasına karşın, bunlardan çok azı koku endüstrisinde değerlendirilmektedir. Özellikle kokulu gül türleri arasında ekonomik değeri en yüksek olan ülkemizde “Isparta gülü” olarak adlandırılan *Rosa damascena* Mill. (Damask gülü) türüdür. Son 125 yılda dünyanın en önemli yağ gülü ve gül ürünleri üretim merkezlerinden birisi haline gelen Isparta yöresinde, yağ gülünün taze çiçeklerinden su distilasyonu ile gül yağı ve gül suyu, *n*-hekzan ekstraksiyonu ile konkret ve konkretten de etil alkol ekstraksiyonu ile absolüt elde edilmektedir (Kürkçüoğlu & Başer 2003; Baydar 2006). Gül çiçeklerinden damıtılması ve ekstraksiyonu ile elde edilen bu ürünler; ilaç, gıda, parfüm ve kozmetik endüstrisinin en değerli hammaddeleri arasında yer almaktadırlar. Her yıl Göller yöresinde yaklaşık 25 bin da yağ gülü plantasyon sahasından ortalama 10 bin ton yağ gülü çiçeği üretilmekte, yörede faaliyet gösteren 20 kadar damıtma ve ekstraksiyon tesisinde

yılda 1.5 ton kadar gül yağı, 2 ton kadar absolüt, 5 ton kadar konkret ve 500 ton kadar konsantre gül suyu ile yaklaşık 30 bin tona yakın atık posa suyu elde edilmektedir (Baydar & Kazaz 2013).

Son yıllarda özellikle tıbbi ve aromatik bitkilerden elde edilen ekstraktların antiradikal ve antioksidan aktiviteleri üzerinde yoğun araştırmalar yapılmaktadır. Çünkü normal metabolizma faaliyetleri sürecinde oksidasyon sonucunda meydana gelen serbest radikaller, hücrelere ve bağışıklık sistemine zarar vererek, özellikle de hücre zarlarının yapısında bulunan lipidlerin peroksidasyonuna neden olmaktadır. İşte enzimatik olan (glutathion peroksidaz, katalaz ve superoksit dismutaz gibi pirmer, glutathion reduktaz ve glukoz-6-fosfat dehidrogenaz gibi sekonder enzimatik defans sistemleri) veya enzimatik olmayan (A, E ve C vitaminleri, kofaktör Q10, selenyum ve çinko gibi mineraller, glutatyon gibi peptidler, flavonol, flavanol ve antosiyanin gibi flavonoidler, karoten ve likopen gibi karotenoidler, gallik asit gibi fenolik asitler ve kateşin ve epikateşin

gibi fenolik maddeler) antioksidanlar, serbest radikalleri kendilerine bağlayıp onları etkisiz hale getirerek, olası doku zararlanmalarını en aza indirmekte, böylece yaşlanmayı geciktirerek (*anti-aging*), olası kalp-damar ve kanser hastalıklarının oluşumunu engellemektedirler (Shebis et al 2013).

Bitkiler aleminde özellikle tıbbi ve aromatik bitkilerde yoğun olarak sentezlenen savunma mollekülleri olarak fenolik maddelerin ve terpenoidlerin güçlü antioksidan etkileri olduğu bilinmektedir (Guimarães et al 2010). Bu nedenle bu tür bitkilerden elde edilen distilasyon ve ekstraksiyon ürünlerinin doğal antioksidan kaynakları olarak aromaterapik ve fitoterapik sağlık ürünlerinde kullanılması giderek önem kazanmaktadır. Bitkilerin antioksidan kapasitesi ve serbest radikal tutma aktivitesi en başta taşıdıkları fenolik madde miktarına ve kompozisyonuna göre değişmektedir (Dimitrios 2006; Göktürk Baydar et al 2007; Siger et al 2008). Yağ gülü dahil bazı gül türlerinde ekstrakt ürünlerinin antioksidan etkileri üzerine bazı araştırmalar yapılmıştır (Biolley et al 1994; Özkan et al 2004; Vinokur et al 2006; Wei & Shibamoto 2007; Ulusoy et al 2009; Yassa et al 2009; Göktürk Baydar & Baydar 2013).

Bu araştırmada, daha önce yapılan araştırmalardan farklı olarak, gül yağı ve gül suyu gibi temel damıtma ürünleri ile birlikte ayrıca önemli bir damıtma yan ürünü olan ancak ekonomik olarak değerlendirilmediği için doğaya salınan posa suyunun uçucu yağ ve fenolik madde içerikleri ile antioksidan ve antiradikal aktivitelerinin belirlenmesi amaçlanmıştır.

## 2. Materyal ve Yöntem

### 2.1. Materyal

Bu araştırmada, bitkisel materyal olarak gül işleme sezonunda (2009 yılı 25 Mayıs tarihinde), Süleyman Demirel Üniversitesi Gül ve Gül Ürünleri Araştırma ve Uygulama Merkezine (GÜLAR) ait gül bahçesinden sabahın erken saatlerinde (saat 7:00-8:00 arasında) toplanan yağ gülü (*Rosa damascena* Mill.) çiçekleri kullanıldı.

### 2.2. Yöntem

#### 2.2.1. Distilasyon ve uçucu yağ bileşenlerinin belirlenmesi

500 g taze gül çiçeği, Clevenger hidro-distilasyon ünitesinin 4 L'lik balonuna doldurulmuş ve üzerine 1.5 L saf su ilave edilmiştir. Damıtma balonundaki suyun kaynamaya başlamasından itibaren 3 saat süreyle damıtma yapılmıştır. Damıtma sonunda su üstünde biriken gül yağı ve yağ altında biriken gül suyu toplanmıştır. Ayrıca hidro-distilasyon balonunda kalan posa sıkılarak posa suyu elde edilmiştir. 100 µL gülyacağı 1 mL n-hekzan içinde çözülmüş ve bundan alınan 1 µL örnek GC/MS cihazına enjekte edilmiştir. Diğer yandan 25 mL gül suyu ve 25 mL posa suyu 1'er mL n-hexan ile 1 gece boyunca sıvı-sıvı ekstraksiyonuna bırakılmış ve uçucu yağları taşıyan üst fazlardan alınan 1 µL örnek GC/MS cihazına enjekte edilmiştir.

Elde edilen gül yağı, gül suyu ve posa suyunun uçucu yağ bileşenleri SDÜ Deneysel ve Gözlemsel Araştırma ve Uygulama Merkezinde bulunan GC/MS (Gas chromatography/Mass spectrometry) cihazında (QP-5050 GC/MS, Quadrapole detektörlü) belirlenmiştir. Kapılar kolon olarak CP-Wax 52 CB (50 m x 0.32 mm, 0.25 µm)'nin kullanıldığı analizlerde, fırın sıcaklık programı dakikada 10 °C artırılarak 60 °C'den 220 °C'ye ulaştırılmış ve 220 °C'de 10 dakika kadar bekleme şeklinde yapılmıştır. Toplam koşuturma süresinin 60 dakika, enjektör sıcaklığının 240 °C ve detektör sıcaklığının 250 °C olarak ayarlandığı bu çalışmada taşıyıcı gaz olarak helyum (20 mL dak<sup>-1</sup>) gazı kullanılmıştır. Uçucu yağ bileşenlerinin tanımlanmasında NIST kütüphanesinden yararlanılmıştır (Stein 1990).

#### 2.2.2. Distilasyon ürünlerinde fenolik madde ekstraksiyonu

Gül yağında fenolik madde ekstraksiyonu için 1 mL gül yağı alınarak 2 mL hekzan içinde iyice çözülene kadar karıştırılmış ve üzerine su:metanol (60:40) karışımından 2 mL eklenmiştir. Santrifüj edildikten sonra ayrılan su:metanol fazı başka bir kapta toplanarak hekzanlı kısım 2 kez daha aynı şekilde ekstrakte edilmiştir. Toplanan ekstraktlardan

su ve metanol, rotary evaporatör yardımıyla uzaklaştırılmış ve elde edilen katı ekstrakt saf metanolde çözüldükten sonra filtre edilerek analizlerde kullanılmıştır. Gül suyu ve posa suyunda fenolik madde ekstraksiyonu ise Tassoni et al (2005) tarafından uygulanan yöntemle yapılmıştır. 60 mL gül suyu ve 60 mL posa suyu ayrı ayrı 30 mL % 5'lik sodyum bikarbonat ve 60 mL etil asetat ile 2 dakika süreyle vortekste karıştırılmış, etil asetatlı faz ayrıldıktan sonra rotary evaporatörde etil asetat uçurulmuştur. Kalıntı saf metanolde çözüldükten sonra filtre edilerek analizlerde kullanılmıştır.

### 2.2.3. Toplam fenolik madde miktarının belirlenmesi

Toplam fenolik madde analizleri Singleton & Rossi (1965) tarafından açıklanan Folin-Ciocalteu reagent (FCR) metodu yardımıyla yapılmıştır. Örneklerin absorbans değerleri PG Instruments (T70 Plus Dual Beam/Arlington, USA) marka spektrofotometre ile 765 nm dalga boyunda okunmuştur. Analizler 5 tekerrürlü olarak, toplam fenolik madde miktarları gallik asit eşdeğeri olarak (GAE) 1 mL gül yağı, gül suyu ve posa suyunda mg olarak ( $\text{mg mL}^{-1}$ ) hesaplanmıştır.

### 2.2.4. Toplam flavanol ve flavonol miktarlarının belirlenmesi

Gül yağı, gül suyu ve posa suyu ekstraktlarında toplam flavanoller, Arnous et al (2001) tarafından açıklanan DMAC (dimetilaminosinnamaldehit) yöntemi ile spektrofotometrik olarak belirlenmiştir. Buna göre 200  $\mu\text{L}$  ekstrakt 1 mL DMAC çözeltisi ile karıştırılmış ve oda sıcaklığında 10 dakika bekletildikten sonra 640 nm'de okunmuştur. Daha sonra kateşin standartı kullanılarak oluşturulan kurve yardımıyla toplam flavanol miktarı kateşin eşdeğeri (KE) olarak 1 mL gül yağı, gül suyu ve posa suyunda  $\mu\text{g}$  ( $\mu\text{g mL}^{-1}$ ) olarak hesaplanmıştır.

Gül yağı, gül suyu ve posa suyu ekstraktlarında toplam flavonoller ise Dai et al (1995) tarafından açıklanan yöntemle göre Neu solusyonu kullanılarak yapılmıştır. Buna göre 100  $\mu\text{L}$  ekstrakt, 900  $\mu\text{L}$  2-aminoetilfenilborinat solusyonuna ilave edilip iyice karıştırılmış ve 410 nm'deki absorbans

değerleri tespit edilmiştir. Daha sonra rutin standardı ile oluşturulan kurveden yararlanılarak toplam flavonollerin miktarı rutin eşdeğeri (RE) olarak 1 mL gül yağı, gül suyu ve posa suyunda  $\mu\text{g}$  ( $\mu\text{g mL}^{-1}$ ) olarak hesaplanmıştır. Toplam flavanol ve flavonol analizleri 5 tekerrürlü olarak yapılmıştır.

### 2.2.5. Fenolik bileşiklerin belirlenmesi

Fenolik bileşikler, Shimadzu marka yüksek performanslı sıvı kromatografisi (HPLC) ile Caponio et al (1999) tarafından açıklanan yöntemle göre analiz edilmiştir. Araştırmada 19 adet fenolik madde standardı (gallik asit, kateşin, klorogenik asit, kafeik asit, epikateşin, siringik asit, *p*-kumarik asit, ferulik asit, viteksin, rutin, hesperidin, apigenin-7-glukozit, rosmarinik asit, eriodiktol, kuersetin, naringin, luteolin, apigenin ve asasetin) ve ekstraktlar 0.45  $\mu\text{m}$ 'lik membran filtreden süzöldükten sonra analizlerde kullanılmıştır. HPLC çalışma koşulları ve gradient programı Göktürk Baydar et al (2011) tarafından belirtilen yöntemle göre yapılmıştır. Veriler Shimadzu Class-VP Chromatography Laboratory Automated Software sistemi kullanılarak analiz edilmiştir. 3 tekerrürlü olarak yapılan analizlerde, fenolik madde miktarları 1 mL gül yağı, gül suyu ve posa suyunda  $\mu\text{g}$  olarak ( $\mu\text{g mL}^{-1}$ ) olarak hesaplanmıştır.

### 2.2.6. Demir indirgeme gücünün belirlenmesi

Demir indirgeme gücü (FRAP), Oyaizu (1986) tarafından açıklanan yöntemle göre belirlenmiştir. Gül yağı için 10 ve 50  $\mu\text{L}$  gül yağına, gül suyu ve posa suyu için 1 ve 5 mL gül suyu ve posa suyuna denk gelecek miktarlarda ekstrakt içeren 2.5 mL hacmindeki örneklerin üzerine 2.5 mL 200 mM sodyum fosfat tampon çözeltisi (pH 6.6) ile 2.5 mL % 1'lik potasyum ferrisiyanidin ilave edilip karıştırılmıştır. 50 °C'de 20 dakika bekletildikten sonra üzerlerine 2.5 mL % 10'luk trikloroasetik asit ilave edilerek 10 dakika süreyle santrifüj edilmiştir. Üst fazdan 5 mL alınıp, üzerine 5 mL deiyonize su ile 1 mL % 0.1'lik demir klorür eklenmiştir. Analizler 5 tekerrürlü olarak yapılmış ve spektrofotometrede 700 nm dalga boyunda absorbans değerleri ölçülerek demir indirgeme gücü saptanmıştır.

### 2.2.7. Antiradikal aktivitenin belirlenmesi

Antiradikal aktivite, Shimata et al (1992) tarafından açıklanan 1,1-difenil-2-pikril-hidrazil (DPPH) yöntemine göre belirlenmiştir. Gül yağı için 10 ve 50 µL gül yağına; gül suyu ve posa suyu içinde 1 ve 5 ml gül ya da posa suyuna eşit miktarlarda ekstrakt içeren örneklerden 1 mL alınarak, üzerlerine 1 mL 0.2 mM DPPH katılmıştır. Karışımlar vorteksle iyice karıştırıldıktan sonra karanlık ortamda ve oda sıcaklığında 30 dakika bekletilmiştir. Analizler 5 tekerrürlü olarak yapılmıştır. Daha sonra spektrofotometrede 517 nm’de okumalar yapılarak antiradikal aktiviteleri Eşitlik 1 yardımıyla hesaplanmıştır.

Antiradikal aktivite (%)= [(kontrolün absorbans değeri-örneğin absorbans değeri)/(kontrolün absorbans değeri)]x100 (1)

## 3. Bulgular ve Tartışma

### 3.1. Distilasyon ürünlerinin uçucu yağ bileşenleri

Bu araştırmada elde edilen gül yağı, gül suyu ve posa suyu örneklerinin uçucu yağ bileşenleri Çizelge 1’de verilmiştir. GC/MS analizlerine göre gül yağının en önemli koku bileşenlerinin; linalool (% 1.15), sitronellol (% 35.27), nerol (% 8.69) ve geraniol (% 21.55) gibi monoterpenik alkoller, nonadesan (% 12.77), 9-nonadesan (% 3.38), eikosan (% 1.58) ve heneikosan (% 6.96) gibi uzun zincirli hidrokarbonlar, metil öjenol (% 2.43) gibi oksit ve eterler, geranil asetat (% 1.89) gibi ester ve aldehitler ile öjenol (% 0.61) gibi fenoller olduğu saptanmıştır. Türk gül yağlarının bileşenleri üzerine yapılan daha önceki araştırmalarda da bulgularımızı destekler nitelikte gül yağını oluşturan en önemli bileşenlerin monoterpen alkoller (geraniol, sitronellol, nerol, linalool), parafinik hidrokarbonlar (nonadesan, 9-nonadesan, eikosan ve heneikosan gibi), fenoller (öjenol gibi), oksit ve eterler (metil öjenol gibi) ile esterler (geranil asetat ve sitronellil asetat gibi) olduğu tespit edilmiştir (Anaç 1984; Başer 1992; Bayrak & Akgül 1994).

Gül suyu ve posa suyunda feniletil alkolün (sırasıyla % 60.71 ve % 90.32) en önemli uçucu yağ bileşeni olduğu tespit edilmiştir (Çizelge 1). Taze

gül çiçeklerinin en belirgin koku molekülü olan feniletil alkol damıtma ürünü gül yağında sadece % 0.69 oranında tespit edilebilmiştir (Çizelge 1). Çünkü damıtma sırasında feniletil alkolün önemli bir kısmı posa suyuna ve gül suyuna geçmektedir. Bu nedenle posa suyundan ve gül suyundan izole edilen gül yağlarının temel koku bileşeni fenil etil alkolüdür (Kürkçüoğlu & Başer 2003; Agarwal et al 2005; Göktürk Baydar & Baydar 2005; Baydar & Kazaz 2013).

### Çizelge 1- Distilasyon ürünlerinin uçucu yağ bileşenleri ve oranları

Table 1- Essential oil compounds and ratios of distillation products

Bileşenler	Geliş zamanı (dakika)	Gül yağı (%)	Gül suyu (%)	Posa suyu (%)
Etanol	8.1	-	1.04	-
Linalool	30.6	1.15	0.98	0.25
Geranil asetat	43.4	1.89	-	-
Sitronellol	43.7	35.27	12.55	3.21
Nerol	45.8	8.69	4.67	-
Feniletil asetat	46.9	0.35	-	-
Geraniol	48.3	21.55	9.22	3.19
Nonadesan	51.7	12.77	0.68	0.13
9-nonadesan	52.1	3.38	0.14	-
Feniletil alkol	52.2	0.69	60.71	90.32
Eikosan	57.0	1.58	-	0.76
Metil öjenol	57.7	2.43	2.27	0.29
Heneikosan	57.8	6.96	-	-
Öjenol	67.8	0.61	3.29	1.85
Trikosan	81.1	1.46	4.45	-
Farnesol	83.7	1.22	-	-

### 3.2. Distilasyon ürünlerinin toplam fenolik madde içerikleri

Gül yağı, gül suyu ve posa suyunun Folin-Ciocalteu reagent (FCR) metodu ile gallik asit eşdeğeri olarak hesaplanan toplam fenolik madde miktarları Çizelge 2’de sunulmuştur. Toplam fenolik madde içeriği gül yağında 2.923 mg mL<sup>-1</sup> olarak bulunurken, gül suyunda 0.054 mg mL<sup>-1</sup>, posa suyunda ise 0.098 mg mL<sup>-1</sup> olarak belirlenmiştir. Posa suyunun gül suyuna göre toplam fenolik madde miktarının daha yüksek



olması, fenolik maddelerin önemli bir miktarının damıtma sırasında posa olarak ayrılan haşlanmış gül çiçeklerinde kaldığını göstermektedir. Nitekim distilasyon sonrası ortaya çıkan posadan elde edilen ekstraktların taze gül çiçeklerinden elde edilen ekstraktların sahip olduğu fenolik maddelerin yaklaşık % 83'ünü koruduğu tespit edilmiştir (Göktürk Baydar et al 2013).

### 3.3. Distilasyon ürünlerinin toplam flavanol ve flavonol içerikleri

Distilasyon ürünlerinin toplam flavanol içeriklerinin yer aldığı Çizelge 2 incelendiğinde, gül yağının 40.952  $\mu\text{g mL}^{-1}$ , gül suyunun 4.491  $\mu\text{g mL}^{-1}$  ve posa suyunun da 37.154  $\mu\text{g mL}^{-1}$  toplam flavanol içerdikleri görülmektedir. Ayrıca distilasyon ürünlerine göre değişimle birlikte, örneklerin 4.493 ile 21.825  $\mu\text{g mL}^{-1}$  arasında değişen miktarlarda toplam flavonol içerdikleri tespit edilmiştir. Bu sonuçlardan, genellikle hiç değerlendirilmeyen posa suyunun 1 mL'sinde bulunan toplam flavanol ve flavonol miktarının 1 mL gül suyunun içerdiği miktarların çok üstünde ve yaklaşık 1 mL gül yağının içerdiği miktara yakın olduğu anlaşılmaktadır.

Nitekim posanın taze gül çiçeklerine çok yakın miktarlarda toplam flavanol ve flavonol içerdiği, bu bileşiklerin büyük bir kısmının distilasyon sonrasında atık olarak ortaya çıkan posada kaldığı belirlenmiştir (Göktürk Baydar et al 2013).

Fenolik bileşiklerin içinde büyük bir aileye sahip olan flavanol ve flavanoller antioksidan özellikleri son derece yüksek olan bileşikler olarak tanınmaktadır (Wang et al 2000). Bu nedenle bu bileşenlerce zengin olan materyaller gıda, sağlık, kozmetik alanında çok değerli materyaller olarak önem kazanmaktadır.

### 3.4. Distilasyon ürünlerinin antiradikal aktiviteleri ile demir bağlama güçleri

DPPH yöntemi, diğer birçok antioksidan yöntemine göre daha kolay ve kısa sürede sonuç veren bir teknik olması nedeniyle antiradikal aktivitenin belirlenmesinde yaygın olarak kullanılan bir metottur (Gülçin et al 2004). Araştırmada, distilasyon ürünleri olarak kullanılan gül yağı, gül suyu ve posa suyunun antiradikal aktiviteleri ile ilgili elde edilen veriler Çizelge 3'de sunulmuştur. Antiradikal aktivitenin belirlenmesinde kullanılan

## Çizelge 2- Distilasyon ürünlerinin toplam fenolik madde, toplam flavanol ve total flavonol içerikleri

Table 2- Total phenolic, total flavanol ve total flavonol contents of distillation products

Distilasyon ürünleri	Toplam fenolik madde miktarı (mg GAE mL <sup>-1</sup> )	Toplam flavanol miktarı ( $\mu\text{g KE mL}^{-1}$ )	Toplam flavonol miktarı ( $\mu\text{g RE mL}^{-1}$ )
Gül yağı	2.923±0.10*	40.952±1.12	21.825±0.00
Gül suyu	0.054±0.01	4.491±0.07	4.493±0.07
Posa suyu	0.098±0.01	37.154±2.30	14.643±2.30

\*, ortalama±standart sapma

## Çizelge 3- Distilasyon ürünlerinin antiradikal aktivite ve demir bağlama güçleri

Table 3- Antiradical activities and ferric reducing powers of distillation products

Distilasyon ürünleri	Konsantrasyon	Radikal bağlama aktivitesi (%)	Demir indirgeme gücü (A)
Gül yağı	10 $\mu\text{L}$	67.42±2.11*	1.12±0.02
	50 $\mu\text{L}$	72.68±2.24	1.97±0.08
Gül suyu	1 mL	53.63±2.11	1.74±0.09
	5 mL	53.78±1.14	2.71±0.03
Posa suyu	1 mL	73.87±3.41	2.78±0.20
	5 mL	82.02±2.42	2.83±0.01

\*, ortalama±standart sapma

DPPH metodu ile yapılan analizler sonucunda, 10 µL gül yağının antiradikal aktivitesi % 67.42 iken, konsantrasyonun 50 µL'ye çıkması ile serbest radikallerin % 72.68'inin bağlandığı belirlenmiştir.

Benzer şekilde Wei & Shibamoto (2007), gül yağının yüksek antioksidan aktiviteye sahip uçucu yağlardan birisi olduğunu, DPPH yöntemine göre antiradikal aktivitesinin 50 µg mL<sup>-1</sup> gibi düşük konsantrasyonlarda bile % 50'nin üzerinde olduğunu tespit etmişlerdir. Yine Yassa et al (2009), yağ gülü çiçeklerinden su distilasyonu ile elde ettikleri gül yağının DPPH yöntemi ile antioksidan etkisinin BHT ve tokoferol (Vitamin E) standartları ile karşılaştırıldığında, her iki standarda göre de daha yüksek radikal tutma aktivitesi gösterdiğini, LC<sub>50</sub> değerinin BHT ve Vitamin E için sırasıyla 110.98 ve 22.72 µg mL<sup>-1</sup> iken, gül yağında 3.54 µg mL<sup>-1</sup> olarak tespit etmişlerdir.

Posa suyunda da gül yağında olduğu gibi konsantrasyon artışına bağlı olarak antiradikal aktivitenin arttığı, miktarın 1 mL'den 5 mL'ye çıkarıldığında aktivitenin de % 73.87'den % 82.02'ye çıktığı belirlenmiştir. Ancak araştırmada gül suyunda konsantrasyon artışının antiradikal aktivite üzerinde belirgin bir fark yaratmadığı da belirlenmiştir (Çizelge 3). Bitki ekstraktlarında antiradikal aktivitenin belirli bir noktaya kadar konsantrasyon artışıyla doğru olarak arttığı, ancak o noktadan sonra konsantrasyon artışının radikalleri bağlama üzerinde önemli bir etkide bulunmadığı daha önce yapılan çalışmalarla da tespit edilmiştir (Göktürk Baydar et al 2007; Ramakrishna et al 2012).

Distilasyon ürünlerinin antioksidan kapasitelerinin de belirlendiği bu araştırmada, demir bağlama gücü yöntemi ile elde edilen verilerin sunulduğu Çizelge 3 incelendiğinde, 10 µL gül yağının absorban değeri (A) 1.12 iken, konsantrasyonun 50 µL'ye çıkmasıyla bu değer 1.97'ye çıktığı tespit edilmiştir. Aynı şekilde 1 mL gül suyu ve gül posasının absorban değerleri sırasıyla 1.74 ve 2.78 iken; miktarın 5 mL'ye çıkmasıyla absorban değerlerinin sırasıyla 2.78 ve 2.83'e yükseldiği görülmüştür. Yüksek absorban değerinin

yüksek antioksidan kapasiteyi gösterdiği bu yöntemde, gül posa suyunun gül suyuna göre daha yüksek antioksidan aktiviteye sahip olduğu, hatta 1 mL posa suyunun 5 mL gül suyundan daha yüksek antioksidan özellikler gösterdiği belirlenmiştir. Araştırmada ayrıca konsantrasyon artışının antioksidan aktiviteyi artırdığı da belirlenmiştir

### 3.5. Distilasyon ürünlerinin fenolik bileşik içerikleri

Distilasyon ürünlerinin içermiş oldukları fenolik bileşiklerin HPLC ile belirlendiği bu araştırmada 19 farklı fenolik bileşik standardı kullanılmıştır. Ancak bu bileşiklerden siringik asit, rutin, apigenin-7-glukosid, rosmarinik asit, luteolin, apigenin ve asasetin hiç bir örnekte tespit edilememiştir. Gül yağında gallik asit, kateşin, klorogenik asit, epikateşin, ferulik asit, hesperidin ve naringin olmak üzere 7 farklı fenolik bileşik tespit edilmiş olup, bu bileşiklerden 16.20 µg mL<sup>-1</sup> ile epikateşin ve 11.84 µg mL<sup>-1</sup> ile hesperidin en fazla bulunan bileşikler olarak belirlenmiştir (Çizelge 4).

### Çizelge 4- Distilasyon ürünlerinin fenolik bileşik içerikleri

Table 4- Phenolic contents of distillation products

Fenolik bileşikler	Distilasyon ürünleri (µg mL <sup>-1</sup> )		
	Gül yağı	Gül suyu	Posa suyu
Gallik asit	3.72±0.28*	-	-
Kateşin	1.08±0.09	-	7.12±0.34
Klorogenik asit	8.70±0.46	-	-
Kafeik asit	-	-	0.03±0.01
Epikateşin	16.20±1.57	-	0.85±0.08
p-kumarik asit	-	-	0.04±0.01
Ferulik asit	0.60±0.04	-	-
Viteksin	-	-	0.16±0.05
Hesperidin	11.84±1.09	-	-
Eridoktiol	-	-	5.06±0.33
Kuersetin	-	-	2.04±0.20
Naringin	1.98±0.15	-	-

\*; ortalama±standart sapma



Gül suyunda yapılan analizlerde incelenen fenolik bileşiklerden hiçbiri dedeksiyon limitinin üzerinde tespit edilemezken; posa suyunda kateşin, kafeik asit, epikateşin, *p*-kumarik asit, viteksin, eridiktol ve kuersetin olmak üzere 7 farklı bileşen elde edilmiştir. Kateşin posa suyunda 7.12 µg mL<sup>-1</sup> ile en fazla bulunan bileşik olarak saptanırken; onu antioksidan özellikleri yüksek bir diğer bileşik olan eridoktiol (5.06 µg mL<sup>-1</sup>) takip etmiştir (Çizelge 4). Yassa et al (2009), *Rosa damascena* çiçeklerinden elde edilen ve çoğunlukla kateşin ve kuersetin gibi flavonoidlerden meydana gelen ekstraktların yüksek antioksidan aktiviteye neden olduklarını tespit etmişlerdir.

Gül posasının gül suyuna göre daha yüksek fenolik madde içermesi ve daha yüksek antiradikal ve antioksidan aktiviteye sahip olması, diğer bazı araştırmalarda da rapor edildiği gibi (Göktürk Baydar et al 2007), bitkisel ürünlerin antioksidan ve antiradikal aktivitelerinin içerdiği fenolik hidroksil gruplarından kaynaklanmaktadır. Örneğin *Lamiaceae* familyasına ait aromatik bitkilerin, özellikle biberiye (*Rosmarinus officinalis*), adaçayı (*Salvia officinalis*), dağçayı (*Sideritis* sp.), oğulotu (*Melissa officinalis*) ve kekik türlerinin (*Origanum* sp., *Satureja* sp., *Thymbra* sp. ve *Thymus* sp.) yüksek antioksidan aktivitesi gösterdikleri, bu tür bitkilerde antioksidan kapasite ve serbest radikal tutma aktivitesinin genellikle rosmarinik asit, luteolin, karnosik asit, kateşin, kuersetin, rutin gibi fenolik ve flavanoid maddelerin varlığı ve onların serbest radikal tutma aktiviteleriyle ilişkili bulunmuştur (Baydar et al 2009).

#### 4. Sonuçlar

Araştırmada uçucu yağ ve fenolik madde içerikleri ile antiradikal ve antioksidan aktivitelerinin incelendiği üç farklı distilasyon ürününden sadece gül yağı ve gül suyu ekonomik olarak değerlendirilmektedir. Ancak distilasyon sonrasında atık ürün olarak ortaya çıkan ve ekonomik anlamda değerlendirilmeyen posa suyunun özellikle fenolik madde içeriğinin gül suyuna oranla daha zengin olduğu, buna bağlı olarak da antiradikal ve antioksidan aktivitelerinin de daha yüksek olduğu tespit edilmiştir. Araştırmadan

elde edilen bu sonuçlar yüksek miktarlarda (yılda yaklaşık 30 bin ton kadar) üretilen ancak ekonomik olarak yararlanılmadığı için doğaya salınan posa suyunun doğal antioksidan kaynağı olarak ekonomiye kazandırılması gerektiğini göstermiştir.

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## Mercimeğe (*Lens esculanta* Moench) Uygulanan Farklı Klor Tuzu ve Dozlarının Kimi İlk Gelişme Özelliklerine Etkisi

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### ÖZET

Bu çalışmada, mercimekte (*Lens esculanta* Moench) farklı klor tuzlarının çimlenme ve fide gelişimi ile fide mineral içeriklerine etkileri araştırılmıştır. Denemede 3'ü yeşil (Ankara Yeşili, Ceren ve Meyveci 2001) ve 3'ü kırmızı (Çiftçi, Kafkas ve Özbek) olmak üzere toplam 6 mercimek çeşidi kullanılmış olup, bunlara CaCl<sub>2</sub>, MgCl<sub>2</sub> ve NaCl tuzlarının 0, 5, 10 ve 20 dS m<sup>-1</sup> EC'lik dozları uygulanmıştır. Laboratuvar ve saksı denemeleri, tesadüf parselleri deneme deseninde üç tekrürlü olarak 20±1 °C'de karanlık koşullarda çimlendirme dolabında yürütülmüştür. Çalışmanın sonuçlarına göre; hem çimlendirme testlerinde hem de saksı denemesinde kök uzunluğu dışında ele alınan tüm özelliklerde (çimlenme oranı, çimlenme indeksi, ortalama çimlenme süresi, fide boyu, fide kuru ağırlığı ile fide N, Na, K, Ca, Mg ve Cl içeriği) uygulamalar arası farklılıklar ile interaksyonlar istatistiki yönden P≤0.01 düzeyinde önemli bulunurken; farklı klor tuzlarındaki doz artışı ile çimlenme oranı (ÇO), çimlenme indeksi (Çİ), fide boyu (FB), kök uzunluğu (KU), fide kuru ağırlığı (FKA) ile fidelerin azot (N), kalsiyum (Ca), magnezyum (Mg) ve potasyum (K) içerikleri azalmış; ortalama çimlenme süresi (OÇS), sodyum (Na) ve klor (Cl) içerikleri artmıştır. Sonuç olarak, Meyveci 2001 ve Çiftçi çeşitlerinin tuz toleransları daha yüksek bulunmuştur.

Anahtar Kelimeler: Mercimek (*Lens esculanta* Moench); Tuz stresi; İlk gelişme faktörleri; Besin maddesi konsantrasyonu

## Effect of Different Chloride Salts and Doses on Early Growth Characters in Lentil (*Lens esculanta* Moench)

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### ABSTRACT

In this study, effects of different chloride salts applied to lentil (*Lens esculanta* Moench) on germination ratio and seedling growth with seedling mineral nutrient contents were investigated. In the experiment, three cultivars of green lentils (Ankara Yeşili, Ceren and Meyveci 2001) and three cultivars of red lentils (Çiftçi, Kafkas and Özbek) were tested. Different chloride salts (CaCl<sub>2</sub>, MgCl<sub>2</sub> and NaCl), and 0, 5, 10 and 20 dS m<sup>-1</sup> EC doses of these salts were applied to

the lentil cultivars. Laboratory and pot experiments were conducted according to completely randomized plots design in a darkened growth cabinet at 20±1 °C with three replications. Differences between applications with interactions of all the characteristics apart from root length in the pot experiments was found statistically significant. Analysis of the data showed that; germination rate, germination index, shoot length, root length, shoot dry weight, nitrogen, calcium, magnesium and potassium content of seedlings decreased with the increasing dose of the different salts of chloride while the mean germination times, concentrations of sodium and chloride in seedling increased.

Keywords: Lentil (*Lens esculanta* Moench); Salt stress; First growth characters; Nutrient concentrations

## 1. Giriş

Kurak ve yarı kurak bölgelerde yağış azlığı nedeniyle tuzların yıkanması yok denecek kadar az olup, buharlaşmanın yüksek olduğu bu tip bölgelerde toprakta ve toprak yüzeyinde tuzlar birikmektedir (Tursun 2008; Anonim 2010). Ülkemizde yapılan arazi etütlerine göre sulanabilir özellikteki 12.5 milyon ha arazinin yaklaşık 1.5 milyon hektarında tuzlu ve sodyumlu topraklar, 2.8 milyon hektarında ise yaş topraklar oluşmuştur (Taş & Öztürk 2011; Öztürk 2015). Bitki yetiştiriciliği yönünden ele alındığında, tuzlu koşullardaki çimlenme ve fide gelişimi dönemi, bitkinin toplam yaşam döngüsündeki en kritik dönem olup (Wang & Shannon 1999; Almansouri et al 2001), bu aşamada topraktaki tuzlar, suyun osmotik basıncını yükselterek tohumlara alınmasını engellemekte veya Na<sup>+</sup> ve Cl<sup>-</sup> iyonlarının zehir etkisinden dolayı tohumun çimlenmesi son derece olumsuz etkilenmektedir (Sadeghian & Yavari 2004). Öte yandan aşırı tuz stresinde büyüyen bitkilerde vejetatif gelişme ile kök büyümesi gerilemekte, solunum ve fotosentez olayları kesintiye uğramakta, enzimlerin etkinliği ile protein sentezinde aksama olmakta, kloroplastlar ve diğer hücrel yapılar zarar görmektedir. İyonik denge bozulduğundan tuz iyonları ile diğer elementler arasındaki rekabete bağlı olarak bitkiler kendileri için gerekli elementleri yeterli miktarda alamamaktadırlar. Yapılan araştırmalarda, tuz stresinden etkilenmeyen ya da göreceli olarak daha az etkilenen bitkilerin dokularında biriken Na<sup>+</sup> ve Cl<sup>-</sup> iyonlarının daha az, prolin miktarının ise daha çok olduğu (Malkoç & Aydın 2003; Karakullukçu & Adak 2008; Tursun 2008); tuz stresi besin maddelerinin alınımına, rekabetine ve dokular arasında taşınmasına da

etki etmekte olduğu belirlenmiştir. Tuz stresine bağlı fosfor (P) alınımındaki azalmaya paralel olarak kalsiyum (Ca) alımı, bitkideki taşınması ve hareketliliğinin de etkilediği azalma bu etkileşime örnek verilebilir (Kuşvuran 2010). Sodyum klorür (NaCl) kaynaklı tuzluluk stresinde sodyum, potasyum (K) iyonlarının alınımını; klor ise nitrat (NO<sub>3</sub>) iyonlarının alınımını önemli ölçüde kısıtlamaktadır. Bitki tür ve çeşitlerinin çimlenme ve gelişmeleri yönünden toprak tuzluluğu ile farklı tuz tiplerine karşı fizyolojik-metabolik olarak gösterdikleri tepkilerinde çok büyük değişimler olabilmektedir (Özcan et al 2000). Nitekim Yıldız & Terzi (2011)'de arpada yaptıkları tuzluluk çalışmasında; bitkinin kök ve gövde dokusunda NaCl konsantrasyonunun artmasıyla Na<sup>+</sup> içeriğinin önemli düzeyde arttığını, K<sup>+</sup> içeriğinin önemli düzeyde azaldığını ve kuru ağırlık ve Na<sup>+</sup>/K<sup>+</sup> oranının bir genotipin tuza toleransında güvenilir bir indeks olarak kullanılabileceğini belirtmişlerdir.

Türkiye kurak ve yarıkurak iklim koşullarına bağlı doğal etmenler ile tuzluluk, erozyon ve çölleşme sorunlarıyla çok fazla ve yaygın olarak karşılaşmaktadır. Yıllık yağış, gerek toplam miktar gerekse yıl içerisinde dağılımı nedeniyle topraktaki tuzların yıkanmasında yeterli olmadığından tuzluluk ve çoraklaşma artmaktadır. Çoraklaşma ile toprak yüzeyini kaplayan bitki örtüsü sınırlanmakta, organik madde azalmakta, suyun ve toprağın fiziksel ve kimyasal özellikleri kötüleşmektedir. Artan osmotik basınç etkisiyle suyun yararıslılığı ve bitki besin maddelerinin alımı olumsuz etkilenmektedir. Ülkesel toprak etütlerine göre 2775115 ha arazide tuzluluk ve drenaj sorunu vardır. Bu toprakların tarımsal üretim maksadıyla kullanılabilmesi tuzlu toprakların ıslahı veya dayanıklı bitki türlerinin ıslah

ve kullanımına bağlıdır. Bu bağlamda bitki tür ve çeşitlerinin de tuza verdikleri tepkiler ile (Sönmez & Beyazgül 2014) tuza dayanıklı tür ve/veya çeşitlerin belirlenmesi ve geliştirilmesi önem kazanmaktadır. Tarla bitkileri içerisinde mercimek, tuzluluğa hassas bitkiler içerisinde değerlendirilmektedir (Yaşar 2003). Bu çalışmada tuzluluğa oldukça fazla duyarlı ve tuz stresinden zarar gören bir baklagil cinsi olan mercimeğin farklı çeşitlerinde değişik klorür tuzları ve dozlarının çimlenme ve bazı fide gelişim özelliklerine etkileri ile fidede Ca, Mg, Na, Cl, K ve N konsantrasyonlarının belirlenmesi amaçlanmıştır.

## 2. Materyal ve Yöntem

Çalışmada deneme materyali olarak 3'ü kırmızı (Kafkas, Çiftçi, Özbek) ve 3'ü yeşil (Meyveci 2001, Ankara yeşili, Ceren) olmak üzere toplam 6 mercimek çeşidi ile 3 farklı klorür tuzunun (NaCl, MgCl<sub>2</sub> ve CaCl<sub>2</sub>) 4 farklı konsantrasyonu (0, 5, 10 ve 20 dS m<sup>-1</sup> EC) kullanılmıştır. Tuz konsantrasyonlarının hazırlanmasında EC metre (Cond 330i WTW marka) kullanılmış olup, hem çimlendirme hem de saksı denemeleri 2012-2013 yıllarında yürütülmüştür. Çimlendirme testlerinde çimlenme oranı (ÇO), çimlenme indeksi (Çİ) ve ortalama çimlenme süresi (OÇS) incelenmiştir. Çimlendirme denemeleri ISTA (2012)'ya göre 21x21 cm boyutlarındaki üç adet çimlendirme kâğıdı arasında ve 20±1 °C'de tamamen karanlık çimlendirme dolabında yürütülmüş olup, deneme 3 tekrarlamalı ve her tekrarlama 50 adet tohum olacak şekilde tesadüf parselleri deneme deseninde kurulmuştur. Her parselde hazırlanan tuz çözeltilerinden her bir çimlendirme kâğıdı başına 10 mL eklenmiş ve rulo kâğıtlar buharlaşmayı engellemek için ağzı kilitli poşetlere konulmuştur. Deneme 10 gün sürmüş ve tuz birikimi olmaması için kâğıtlar 2 günde bir değiştirilmiş, gerektiğinde çimlendirme kâğıtlarına 10'ar mL test solüsyonu eklenmiştir. Her gün yapılan sayımlarda 2 mm kökçük uzunluğuna sahip tohumlar çimlenmiş olarak sayılmıştır (Murillo-Amador et al 2002). Çimlendirme denemesinde 10. gün sonunda, Akıncı & Çalışkan (2010)'ın yöntemine göre çimlenen tohum sayısının toplam tohum sayısına oranı çimlenme oranı (ÇO) olarak;

Wang et al (2004)'ün bildirdiği yöntemle göre her gün çimlenen tohum oranının sayım günlerine bölünmesiyle çimlenme indeksi (Çİ) ve Ellis & Robert (1980)'in belirttiği yöntem ve eşitliğe göre, her gün yeni çimlenen tohum sayısı x çimlenme gün sayısı toplamının toplam çimlenen tohum sayılarına bölünmesiyle ortalama çimlenme süresi (OÇS) hesaplanmıştır. Çimlenme indeksi ve ortalama çimlenme süresi için hergün çimlenen tohumlar sayılmış olup, çimlenmenin sabitlendiği gün son sayım günü olarak değerlendirilmiştir. Deneme sonunda tesadüf olarak seçilen 10 bitkinin fide ve kökleri milimetrik cetvelle ölçülerek fide boyu ve kök uzunluğu mm olarak ve fideler 65 °C'de bir gün etüvde kurutulduktan sonra hassas terazide tartılarak fide kuru ağırlıkları g olarak belirlenmiştir (Kaya et al 2008).

Saksı denemeleri 2.8 L'lik saksılarda torf kullanılarak yapılmıştır. Her saksıya, tuza karşı duyarlı bitkilerin çimlendirme denemeleri için üretilmiş olan 1100 g torf (Mikkskaar) doldurulmuştur. Ortam sıcaklığı 25±3 °C'ye ayarlanmış, denemeler 3 tekrarlamalı ve her tekrarda 25 tohum olacak şekilde yürütülmüştür. Yirmi (20) gün süreyle ihtiyaç duyulduğunda (bire gün arayla her saksıya 100 mL) her saksıya test solüsyonlarından eşit miktarda uygulanmıştır. Saksı denemesi şeklinde yürütülen fide gelişim testlerinde 20. gün sonunda her saksıdan tesadüfen seçilip köklü olarak sökülen 10'ar bitkinin kökleri musluk suyunda dikkatlice yıkanmıştır. Bu işlemden sonra yüzey kurulaması yapıp; fide boyu, kök uzunluğu ve fide kuru ağırlığı belirlenmiştir. Saksı denemelerinden elde edilen bitki örneklerinde, AOAC (1990)'da belirtilen yöntem ve esaslara göre: Azot mikro Kjeldahl yöntemiyle; Na, K, Ca ve Mg Atomik Absorbsiyon Spektrofotometre cihazında (Perkin-Elmer); P vanadomolibdat sarı renk yöntemiyle belirlenmiştir (Kacar & Katkat 2010). Klor (Cl) ise Johnson & Ulrich (1959)'in bildirdiği Mohr metodu ile belirlenmiştir. Denemede elde edilen verilere varyans analizi (ANOVA) yapılmış ve uygulamalar arasındaki farklılıklar P≤0.05 önemlilik düzeyinde Duncan testi ile belirlenmiştir (Düzgüneş et al 1987).



### 3. Bulgular ve Tartışma

Denemede elde edilen verilerle yapılan varyans analizi sonuçlarına göre; ÇO, OÇS, fide boyu, fide kuru ağırlığı özellikleri ile bitkide N, K, Na, Ca, Mg ve Cl içeriği bakımından çeşit x tuz x doz etkileşimini 0.01 düzeyinde; Çİ özelliğinde 0.05 düzeyinde önemli bulunmuştur. Kök uzunluğu özelliğinde ise çeşit ve dozlar ile tuz x tuz dozu etkileşimini  $P \leq 0.01$  düzeyinde önemli olmuştur. Çizelge 1 ve Çizelge 2'de görüldüğü gibi, üç klor tuzunda da tüm çeşitlerde en yüksek çimlenme oranları kontrol uygulamasından elde edilmekle birlikte; 5 dS m<sup>-1</sup> ve 10 dS m<sup>-1</sup> tuz dozlarıyla aynı grupta yer almıştır.

Genel ortalamalar yönünden tuz dozlarındaki artışlara paralel olarak ÇO azalma göstermiş, en düşük oranlar 20 dS m<sup>-1</sup> uygulamalarından alınmıştır. Artan tuz dozlarına göre ÇO azalışları NaCl tuzunda MgCl<sub>2</sub> ve CaCl<sub>2</sub> tuzlarına göre çok daha belirgin olmuştur (Çizelge 1). Çeşitler yönünden incelendiğinde, yeşil mercimeklerde Meyveci 2001 ve Ceren çeşitleri; kırmızı mercimeklerde ise Çiftçi çeşidinin ÇO yönünden her üç tuz tipinde de artan tuz konsantrasyonlarına toleranslarının daha yüksek olduğu söylenebilir. CaCl<sub>2</sub> ve NaCl tuzlarında Ankara Yeşili ile MgCl<sub>2</sub> tuzunda ise Özbek çeşidindeki ÇO artan dozlardan önemli düzeyde etkilenmiş ve en düşük ortalamalara sahip olmuşlardır (Çizelge 1). Birçok araştırmada da; artan tuz stresine bağlı olarak bitki tohumlarında ÇO, çimlenme süresi ve tohum

**Çizelge 1- Farklı klor tuzu ve dozları uygulanan mercimekte çimlenme oranı (%) ve çimlenme indeksi ortalamaları**

Table 1- Mean germination rates and germination index of lentil cultivars exposed to different chloride salts and doses

Tuzlar	Çeşitler	Çimlenme oranı (%)					Çimlenme indeksi				
		Tuz dozları (dS m <sup>-1</sup> )					Tuz dozları (dS m <sup>-1</sup> )				
		0	5	10	20	Ort.	0	5	10	20	Ort.
CaCl <sub>2</sub>	Meyveci 2001	99.3 a	100.0 a	99.3 a	94.7 a	98.3	22.3 a	20.8 ab	19.7 b	17.1 c	20.0
	Ankara Yeşili	97.3 a	93.3 a	95.3 a	49.3 b	83.8	20.8 a	17.0 b	15.5 b	4.7 c	14.5
	Ceren	100.0 a	100.0 a	99.3 a	97.3 a	99.2	22.1 a	21.5 a	19.1 b	13.0 c	18.9
	Kafkas	99.3 a	98.7 a	98.7 a	90.7 b	96.8	20.8 a	19.9 a	18.5 a	8.1 b	16.8
	Çiftçi	100.0 a	100.0 a	100.0 a	97.3 a	99.3	20.7 a	20.1 a	16.4 b	14.1 b	17.8
	Özbek	98.0 a	97.3 a	96.7 a	66.0 b	89.5	16.2 a	12.3 b	10.3 b	4.2 c	10.8
Ortalama		99.0	98.2	98.2	82.6	94.5	20.5	18.6	16.6	10.2	16.5
MgCl <sub>2</sub>	Meyveci 2001	99.3 a	99.3 a	100.0 a	98.0 a	99.2	23.9 a	22.5 ab	21.2 b	17.2 c	21.2
	Ankara Yeşili	96.7 a	100.0 a	96.7 a	71.3 b	91.2	19.4 a	17.9 ab	16.8 b	8.9 c	15.7
	Ceren	99.3 a	99.3 a	98.7 a	98.7 a	99.0	22.4 a	19.8 b	16.6 c	12.6 d	17.9
	Kafkas	100.0 a	98.7 a	98.0 a	84.0 b	95.2	22.7 a	19.3 b	18.3 a	10.4 c	17.7
	Çiftçi	100.0 a	100.0 a	100.0 a	97.3 a	99.3	21.1 a	20.5 a	18.8 a	14.8 b	18.8
	Özbek	98.7 a	97.3 a	97.3 a	55.3 b	87.2	18.3 a	15.5 b	12.9 c	5.2 d	13.0
Ortalama		99.0	99.1	98.5	84.1	95.2	21.3	19.2	17.4	11.5	17.4
NaCl	Meyveci 2001	99.3 a	100.0 a	97.3 a	66.7 b	90.8	21.7 a	20.6 a	19.2	9.9 b	17.9
	Ankara Yeşili	98.0 a	98.0 a	92.7 a	47.3 b	84.0	17.8 a	16.0 ab	14.2	4.8 c	13.2
	Ceren	99.3 a	100.0 a	98.0 a	86.7 b	96.0	19.7 a	17.0 b	14.4	8.7 d	14.9
	Kafkas	100.0 a	100.0 a	98.7 a	87.3 b	96.5	17.3 a	15.9 ab	14.4	7.5 c	13.8
	Çiftçi	99.3 a	98.7 a	98.0 a	88.0 b	96.0	17.8 a	16.8 a	15.4	11.0 b	15.3
	Özbek	98.0 a	96.7 a	95.3 a	76.7 b	91.7	16.7 a	15.3 a	11.5 b	6.2 c	12.4
Ortalama		99.0	98.9	96.7	75.5	92.5	18.5	17.0	14.8	8.0	14.6

**Çizelge 2- Farklı klor tuzu ve dozları uygulanan mercimekte ortalama çimlenme süresi (gün) ve fide boyu (cm) ortalamaları**

Table 2- Mean shoot length and mean germination time of lentil cultivars exposed to different chloride salts and doses

Tuzlar	Çeşitler	Ortalama çimlenme süresi (gün)					Fide boyu (cm)				
		Tuz dozları (dS m <sup>-1</sup> )					Tuz dozları (dS m <sup>-1</sup> )				
		0	5	10	20	Ort.	0	5	10	20	Ort.
CaCl <sub>2</sub>	Meyveci 2001	2.31 a	2.51 ab	2.65 ab	2.85 a	2.58	29.9 a	29.6 a	29.2 a	19.7 b	27.1
	Ankara Yeşili	2.39 b	2.73 ab	3.02 a	2.71 ab	2.71	27.2 a	27.1 a	24.5 b	24.3 b	25.8
	Ceren	2.35 b	2.44 b	2.71 b	3.91 a	2.85	21.1 a	18.1 b	18.4 b	16.9 b	18.6
	Kafkas	2.49 b	2.55 b	2.71 b	5.45 a	3.30	23.1 a	23.1 a	23.1 a	16.7 b	21.5
	Çiftçi	2.55 b	2.56 b	2.82 b	4.25 a	3.05	27.9 a	27.3 a	26.6 a	19.6 b	25.3
	Özbek	2.74 c	3.75 b	3.85 b	4.97 a	3.83	25.1 a	24.5 a	23.9 a	14.7 b	22.1
Ortalama		2.47	2.76	2.96	4.02	3.05	25.7	25.0	24.3	18.7	23.4
MgCl <sub>2</sub>	Meyveci 2001	2.11 b	2.27 b	2.49 ab	2.96 a	2.46	29.8 a	27.9 ab	27.7 b	19.3 c	26.2
	Ankara Yeşili	2.56 a	2.84 a	2.87 a	2.94 a	2.80	25.7 a	24.5 a	24.4 a	16.4 b	22.7
	Ceren	2.28 c	2.61 bc	2.97 b	3.95 a	2.95	19.7 a	19.3 a	18.8 a	15.2 b	18.3
	Kafkas	2.28 b	2.62 b	2.72 b	3.57 a	2.80	24.2 a	23.1 a	22.8 a	18.2 b	22.1
	Çiftçi	2.49 b	2.55 b	2.78 b	3.51 a	2.83	26.7 a	26.4 a	26.2 a	18.9 b	24.6
	Özbek	2.75 b	3.09 b	3.75 a	3.03 b	3.16	24.2 a	24.1 a	22.7 a	12.8 b	20.9
Ortalama		2.41	2.66	2.93	3.33	2.83	25.0	24.2	23.8	16.8	22.5
NaCl	Meyveci 2001	2.37 a	2.54 a	2.69 a	2.38 a	2.50	28.5 a	28.3 a	28.6 a	14.9 b	25.1
	Ankara Yeşili	2.85 ab	3.07 ab	3.15 a	2.59 b	2.92	27.1 a	26.3 a	25.3 a	18.6 b	24.3
	Ceren	2.64 c	2.99 bc	3.41 b	4.49 a	3.38	22.4 a	20.1 b	21.2 ab	11.0 c	18.7
	Kafkas	2.95 c	3.18 bc	3.47 b	5.27 a	3.72	24.0 a	23.8 a	23.0 a	15.9 b	21.7
	Çiftçi	2.85 b	2.97 b	3.19 b	3.83 a	3.21	26.8 a	26.3 a	26.1 a	16.7 b	24.0
	Özbek	2.85 b	2.97 b	3.19 b	3.83 a	3.21	25.2 a	24.9 a	24.1 a	19.6 b	23.5
Ortalama		2.75	2.95	3.18	3.73	3.16	25.7	24.9	24.7	16.1	22.9

canlılığı gibi özelliklerin olumsuz yönde etkilendiği, tohum çimlenmesi ve bitki gelişmesi üzerine farklı tuz tiplerinin etkilerinin değişken olabileceği ve tuz dozlarına toleransın bitki türlerinde hatta aynı tür içindeki çeşitlerde bile değişebileceği belirtilmiştir (Kuşvuran 2010; Sözen et al 2010; Asgharipour & Rafiei 2011; Islam et al 2012; Güldüren & Elkoca 2012).

Çİ yönünden de en yüksek ortalamalar tüm mercimek çeşitlerinde kontrol parsellerinden elde edilmiş olup, artan tuz dozlarına bağlı olarak indeks değerleri önemli ölçüde azalmıştır (Çizelge 1). NaCl tuzunun tüm çeşitlerde çimlenme indeksine olumsuz etkilerinin diğer iki tuz tipinden daha çok olduğu görülmüş, genotipler bakımından

değerlendirildiğinde; Meyveci 2001, Ceren ve Çiftçi'nin Ankara Yeşili, Kafkas ve Özbek'e göre artan tuz stresine karşı daha toleranslı oldukları kanısına varılmıştır. Çalışmamızda kullanılan tuzlar, çözeltilerin osmotik basıncını artırmış olup, çoktan aza doğru MgCl<sub>2</sub>, CaCl<sub>2</sub> ve NaCl şeklinde sıralanmışlardır. Bu sonuçlar; kimi bitkilerde çözeltideki tuzların tohumlar tarafından alınmasını engellediğini veya Na<sup>+</sup> ve Cl<sup>-</sup> gibi iyonların zehir etkisinden dolayı çimlenmeyi olumsuz etkilediğini, tuzlar ve dozları bakımından çeşitler arasında da önemli değişimlerin olabileceğini bildiren Kaya et al (2008) ve Güldüren & Elkoca (2012)'nin sonuçlarıyla benzerlik içindedir. Ortalama çimlenme süresi tohum gücü (vigor)'nün bir ifadesi olup, özellikle stres koşullarında hızlı bir çimlenme potansiyeline sahip



tohumlar, bitkisel üretimde verimi artırma ve bitki sağlığı yönünden önemlidir (Mavi & Demir 2010). Denemede üç tuz tipindeki dozaj artışına bağlı olarak incelenen çeşitlerin ortalama çimlenme süreleri kontrol uygulamasına göre önemli düzeylerde gecikmiş (Çizelge 2), genelde tüm uygulamalarda en geç çimlenme süreleri 20 dS m<sup>-1</sup> uygulamasından elde edilmiştir. CaCl<sub>2</sub> tuzunda Ankara Yeşili, MgCl<sub>2</sub> tuzunda Özbek ve NaCl tuzunda ise Meyveci 2001 ve Ankara Yeşili çeşitlerinde en geç OÇS 10 dS m<sup>-1</sup> dozunda belirlenmiştir. Ortalama çimlenme süresi testinde, çimlenen tohumlar deneme sonuna kadar günlük olarak sayılmaktadır. Özellikle yukarıda belirlenen çeşitlerin tohumlarında az da olsa çimlenme problemleri çıkmıştır. Bu sorun tuz ile ilgili olmayıp, tohum materyali kaynaklıdır. Çizelge 2'nin incelenmesinden anlaşılacağı gibi, OÇS üzerine en çok olumsuz etkinin NaCl tuzunda belirlendiği ve genellikle kırmızı mercimek çeşitlerinin yeşil mercimeklere göre daha geç sürede çimlendiği; yeşil mercimeklere de tane yapısı küçük olan Ceren çeşidinin diğer yeşil mercimek çeşitlerine göre daha geç çimlendiği saptanmıştır. Kaya et al (2008), yaptıkları çalışmalarında; küçük tohumlu nohut (*Cicer arietinum* L.) çeşitlerinin daha çabuk su aldığı ve çimlenme süresinin daha kısa olduğunu, ancak artan tuz dozları ile çimlenme süresinin uzadığını saptamışlardır. Nitekim su alma oranı en yüksek çeşit olan Meyveci 2001 çeşidi ortalama çimlenme süresi yönünden en toleranslı çeşit olmuştur. Okçu et al (2005), bezelyede (*Pisum sativum* L.) artan tuz düzeylerinin, ortalama çimlenme süresini uzattığını ve bu sürelerin çeşitlere göre değişim gösterdiğini belirlemişlerdir. Denemede kullanılan üç tuz tipinde de artan tuz dozlarına bağlı olarak tüm çeşitlerde ortalama FB ve KU önemli düzeyde azalmış ve en düşük FB ve KU 20 dS m<sup>-1</sup> tuz dozlarından elde edilmiştir. FB bakımından, çeşitlerin ortalaması olarak en yüksek tuz dozunda kontrole göre CaCl<sub>2</sub>'de % 27, MgCl<sub>2</sub>'de % 33 ve NaCl'de ise % 38'e yaklaşan oranlarda azalmalar görülmüştür. FB yönünden NaCl tuzunun olumsuz etkisi diğer tuzlara göre daha yüksek olmuştur. Tüm klor tuzları için FB, Meyveci 2001 çeşidinde en fazla; Ceren çeşidinde en az olmuştur (Çizelge 2).

KU, çeşitlerin ortalaması olarak 20 dS m<sup>-1</sup> tuz dozunda kontrol parsellerine göre CaCl<sub>2</sub>'de % 41, MgCl<sub>2</sub>'de % 48 ve NaCl ise % 29'u bulan oranlarda azalmış olup, MgCl<sub>2</sub> tuzu en yüksek olumsuz etkiyi göstermiştir (Çizelge 3).

Tuz stresi bitkilerde vejetatif ve generatif büyümeyi farklı şekillerde etkilemektedir (Saab et al 1990). Bitkilerdeki gövde gelişmesi, kök gelişmesine göre tuzluluğa daha fazla duyarlı olup, Delane et al (1982) ve Weimberg et al (1984)'e göre bu tepki, tuz stresi veya su stresi için morfolojik bir adaptasyon şeklinde düşünülmektedir (Saab et al 1990). Çalışma sonuçlarına göre artan tuz dozları ile FB, KU'na göre tuz dozlarından daha fazla etkilenmiştir. Yokoi & Bresson (2002), çalışmalarında, tuz stresinde bitkilerin konsantrasyon artışıyla birlikte, FB'ndaki azalmaların daha önemli olduğunu bildirmişlerdir. Genel olarak kontrol ve 5 dS m<sup>-1</sup> dozları birbirine yakın değerler göstermiştir. Bu durum, bitkinin tuz stresine belli bir düzeye kadar karşı koyabildiği ve bu düzeyden sonra direncinin azaldığını bildiren Egeh & Zamora (1992) ve Özdemir & Engin (1994)'in sonuçlarına uyumludur. Öte yandan fide kuru ağırlığı (FKA) yönünden her üç tuz türünde de tüm çeşitlerde tuz uygulanmamış kontrol parselleri ile 5 dS m<sup>-1</sup> ve 10 dS m<sup>-1</sup> tuz dozu uygulamaları birbirine benzer sonuçlar vermiş ve ortalamalar istatistiksel olarak aynı grupta yer almış; 20 dS m<sup>-1</sup> tuz dozunda ise en düşük FKA ortalamaları saptanmıştır (Çizelge 3). Tuz stresinde toprağın su potansiyeli düşmekte ve bitki köklerinin su alımı engellenmekte ya da iyon zehirlenmesi ortaya çıkmaktadır. Özellikle sodyum iyonları tuza hassas bitkilerde zehirlenme etkisi oluşturmada, ayrıca klor da toksik etki yapmaktadır. Buna bağlı olarak, mitoz bölünme ve enzim aktivasyonunun olumsuz etkilenmesiyle bitki gelişimi ve büyümesi önemli derecede sınırlanmakta; ayrıca, bitki yaş ve kuru ağırlıklarında azalma, klorofil miktarında azalmaya, hücre zarında parçalanmaya neden olmaktadır (Demir & Kocaçalışkan 2001; Kuşvuran 2010). Çizelge 4'te verildiği gibi, üç tuz çeşidinde de artan dozlarla birlikte mercimek genotiplerinde erken fide döneminde bitki azot içeriklerinde sırasıyla %

**Çizelge 3- Farklı klor tuzu ve dozları uygulanan mercimekte kök uzunluğu (cm) ve fide kuru ağırlığı (g) ortalamaları**

Table 3- Mean root length and shoot dry weight of lentil cultivars exposed to different chloride salts and doses

Tuzlar	Çeşitler	Kök uzunluğu (cm)					Fide kuru ağırlığı (g)				
		Tuz dozları (dS m <sup>-1</sup> )					Tuz dozları (dS m <sup>-1</sup> )				
		0	5	10	20	Ort.	0	5	10	20	Ort.
CaCl <sub>2</sub>	Meyveci 2001	16.79	13.77	12.69	9.68	13.23	0.34 ab	0.36 a	0.33 b	0.22 c	0.31
	Ankara Yeşili	16.05	13.03	12.79	9.76	12.91	0.32 b	0.35 a	0.33 ab	0.23 c	0.31
	Ceren	15.07	12.79	11.06	6.78	11.43	0.18 a	0.19 a	0.17 a	0.11 b	0.16
	Kafkas	17.62	17.00	11.58	11.57	14.44	0.23 a	0.22 a	0.21 a	0.14 b	0.20
	Çiftçi	15.50	13.72	13.81	11.63	13.67	0.21 a	0.22 a	0.21 a	0.14 b	0.20
	Özbek	16.47	14.12	13.01	8.43	13.01	0.21 a	0.20 a	0.20 a	0.12 b	0.18
Ortalama		16.25 a	14.07 b	12.49 b	9.64 c	13.11	0.25	0.26	0.24	0.16	0.23
MgCl <sub>2</sub>	Meyveci 2001	14.39	14.05	9.53	9.13	11.78	0.34 a	0.34 a	0.33 a	0.21 b	0.31
	Ankara Yeşili	18.50	17.93	17.29	10.23	15.99	0.34 a	0.34 a	0.35 a	0.18 b	0.30
	Ceren	13.69	13.54	13.24	6.31	11.70	0.19 a	0.19 a	0.19 a	0.10 b	0.17
	Kafkas	16.16	14.36	13.96	8.91	13.35	0.23 a	0.22 a	0.22 a	0.15 b	0.21
	Çiftçi	18.27	16.51	15.68	9.50	14.99	0.22 a	0.23 a	0.22 a	0.14 b	0.20
	Özbek	17.69	12.07	12.20	7.05	12.25	0.19 a	0.22 a	0.21 a	0.12 b	0.19
Ortalama		16.45 a	14.74 b	13.65 b	8.52 c	13.34	0.25	0.26	0.25	0.15	0.23
NaCl	Meyveci 2001	15.02	13.84	11.61	9.31	12.45	0.36 a	0.37 a	0.34 a	0.17 b	0.31
	Ankara Yeşili	16.95	16.23	13.35	12.41	14.74	0.35 a	0.35 a	0.33 a	0.20 b	0.31
	Ceren	11.08	10.61	9.72	8.79	10.05	0.20 a	0.19 a	0.19 a	0.10 b	0.17
	Kafkas	13.43	13.44	11.24	10.88	12.25	0.21 a	0.22 a	0.21 a	0.13 b	0.19
	Çiftçi	16.68	15.23	13.54	11.57	14.26	0.23 a	0.22 a	0.21 a	0.14 b	0.20
	Özbek	12.83	11.72	10.32	8.41	10.82	0.21 a	0.21 ab	0.18 bc	0.17 c	0.19
Ortalama		14.33 a	13.51 a	11.63 b	10.23 b	12.43	0.26	0.26	0.24	0.15	0.23

10.0, % 11.4 ve % 16.3 oranında azalma olmuştur. Demir & Kocaçalışkan (2001), tuza duyarlı fasulye (*Phaseolus vulgaris* L.)’de kök ve yaprak dokularında protein miktarının NaCl uygulaması ile azaldığını; Rai & Singh (1999) ise mercimek çeşidini kullandıkları denemelerinde genotip x tuz interaksiyonlarını önemli bularak, genotiplere göre azot içeriğinin değiştiğini belirlemişlerdir ki elde ettikleri bu sonuçlar, bulgularımızla uyum içindedir. Tuz stresinde bitkilerde aşırı miktarda biriken Na, K alımını Cl ise özellikle NO<sub>3</sub> alımını engelleyerek ve P alımında kontrol kaybına neden olarak bitkilerde iyon dengesizliğine neden olmaktadır. Tuz stresinden etkilenmeyen ya da göreceli olarak az etkilenen bitkilerin dokularında daha az klor iyonu birikmektedir. Tuzluluğa hassas çeşitlerde ise özellikle Na ve Cl iyonlarının yüksek

olduğu ve K iyonunun konsantrasyonunun azaldığı bildirilmektedir (Turan et al 2007; Kuşvuran et al 2008).

CaCl<sub>2</sub> ve MgCl<sub>2</sub> uygulamalarında bitkideki Na içeriği, tuz dozlarındaki artışla azalmakta ancak, incelenen tuz dozları ile kontrol uygulaması arasındaki farklılıklar istatistiksel olarak önem düzeyinde bulunmamaktadır. NaCl tuzu uygulamasında ise Na miktarı kontrole göre % 97.7 oranında önemli artışlar göstermiştir. Ceren ve Kafkas çeşitleri bünyelerine daha az Na almışlardır (Çizelge 4).

Kacar & Katkat (2010), arpa (*Hordeum vulgare* L.)’daki çalışmalarında artan NaCl tuz düzeyinin bitkideki Na konsantrasyonunu artırdığını bulmuş; Dabuxilatu & Motoki (2005), soya fasulyesi

**Çizelge 4- Farklı klor tuzu ve dozları uygulanan mercimekte azot ve sodyum içeriği (%) ortalamaları**

Table 4- Mean nitrogen and sodium concentrations of lentil cultivars exposed to different chloride salt and doses

Tuzlar	Çeşitler	Azot içeriği (%)					Sodyum içeriği (%)				
		Tuz dozları (dS m <sup>-1</sup> )					Tuz dozları (dS m <sup>-1</sup> )				
		0	5	10	20	Ort.	0	5	10	20	Ort.
CaCl <sub>2</sub>	Meyveci 2001	4.48 a	4.51 a	4.42 a	4.11 b	4.38	0.044 a	0.042 a	0.039 a	0.041 a	0.042
	Ankara Yeşili	3.95 a	3.72 b	3.54 c	3.47 c	3.67	0.048 a	0.046 a	0.044 a	0.044 a	0.046
	Ceren	4.04 ab	4.11 a	4.16 a	3.90 b	4.05	0.046 a	0.044 a	0.040 a	0.040 a	0.043
	Kafkas	4.38 a	4.39 a	4.16 b	4.16 b	4.27	0.043 a	0.039 a	0.038 a	0.038 a	0.040
	Çiftçi	4.57 a	4.35 b	4.23 b	4.07 c	4.31	0.044 a	0.042 a	0.039 a	0.040 a	0.041
	Özbek	4.33 a	3.90 b	3.62 c	3.47 d	3.83	0.045 a	0.044 a	0.042 a	0.042 a	0.043
Ortalama		4.29	4.16	4.02	3.86	4.09	0.045	0.043	0.040	0.041	0.042
MgCl <sub>2</sub>	Meyveci 2001	4.48 a	4.58 a	4.56 a	4.12 b	4.44	0.044 a	0.041 a	0.039 a	0.039 a	0.041
	Ankara Yeşili	3.95 a	3.88 ab	3.80 ab	3.42 c	3.76	0.048 a	0.044 a	0.042 a	0.042 a	0.044
	Ceren	4.04 a	4.09 a	3.80 a	3.65 c	3.90	0.046 a	0.041 a	0.041 a	0.038 a	0.042
	Kafkas	4.38 a	4.37 a	4.20 a	3.72 c	4.17	0.043 a	0.040 a	0.036 a	0.036 a	0.039
	Çiftçi	4.57 a	4.23 b	4.16 b	3.96 c	4.23	0.044 a	0.040 a	0.040 a	0.037 a	0.040
	Özbek	4.33 a	4.33 a	3.97 a	3.93 b	4.14	0.045 a	0.041 a	0.042 a	0.041 a	0.042
Ortalama		4.29	4.25	4.08	3.80	4.13	0.045	0.041	0.040	0.039	0.041
NaCl	Meyveci 2001	4.48 a	4.31 b	4.00 b	3.81 d	4.15	0.044 d	1.750 c	1.800 b	2.000 a	1.399
	Ankara Yeşili	3.95 a	3.83 a	3.39 a	3.19 c	3.59	0.048 d	1.715 c	1.965 b	2.290 a	1.505
	Ceren	4.04 a	3.92 a	3.58 a	3.44 c	3.75	0.046 d	1.770 c	1.965 b	1.085 a	1.217
	Kafkas	4.38 a	4.12 b	3.78 b	3.64 d	3.98	0.043 d	1.595 c	1.845 b	2.000 a	1.371
	Çiftçi	4.57 a	4.40 b	4.28 b	3.97 c	4.31	0.044 d	1.815 c	1.970 b	2.045 a	1.469
	Özbek	4.33 a	3.80 b	3.69 b	3.46 c	3.82	0.045 d	1.885 c	2.135 b	2.290 a	1.589
Ortalama		4.29	4.06	3.79	3.59	3.94	0.045	1.755	1.947	1.952	1.425

(*Glycine max* Merrill)'ndeki tuz zararına, yaprak ve kök hücrelerinde biriken Cl<sup>-</sup>'nin; hiyardaki tuz zararının başlıca nedeninin bitkide Na birikimi olduğunu belirtmişlerdir. Kuşvuran et al (2007), Na<sup>+</sup> iyonunu toleranslı genotiplerin bünyelerine daha az, duyarlı olanların ise daha çok aldıklarını bildirmişlerdir. Denememizde, tuz stresiyile birlikte mercimek fidesinin potasyum içeriğinde önemli azalmalar ortaya çıkmış, en yüksek K içeriği tüm çeşitlerde, tuz uygulamasının yapılmadığı kontrol parsellerinden alınmıştır. CaCl<sub>2</sub>, MgCl<sub>2</sub> ve NaCl dozlarındaki artışlara paralel olarak mercimek fidelerinin potasyum içeriği (sırasıyla % 29.6, % 28.9 ve % 31.9) azalmış; tuz stresine uğrayan kök ve gövdelerdeki tuz iyonlarında (Na ve Cl başta olmak üzere) artışlar saptanmış, ancak K ve Ca iyon konsantrasyonlarında belirgin azalmalar olmuştur

(Çizelge 5). Öte yandan artan Na konsantrasyonunun yapraklardaki fotosentez ve transpirasyonu olumsuz yönde etkilediği, Na ve K iyonlarının antagonistik etkisi nedeniyle K eksikliklerinin de ortaya çıktığı gözlenmiş olup, genel olarak Ca ve K, tuz stresinden olumsuz etkilenecek iyon konsantrasyonlarında azalmanın meydana geldiği (Kuşvuran 2010) görüşüne varılmıştır. Kuşkusuz, denemede kullanılan her üç klor tuzunda da doz artışıyla birlikte ve genelde bitki bünyesindeki Ca miktarında azalma ortaya çıkmış; bu bakımdan diğer tuzlara göre NaCl'deki (% 13) Ca konsantrasyonu daha çok olmuş ve bu içerik tüm tuzlarda % 0.997-1.397 şeklinde değişmiştir ve bu bakımdan elde edilen bulgular genel olarak en çok Meyveci 2001 ile en az Özbek çeşitlerinde saptanmıştır (Çizelge 5).

**Çizelge 5- Farklı klor tuzu ve dozları uygulanan mercimekte potasyum ve kalsiyum içeriği (%) ortalamaları**

Table 5- Mean potassium and calcium concentrations (%) of lentil cultivars exposed to different chloride salt and doses

Tuzlar	Çeşitler	Potasyum içeriği (%)					Kalsiyum içeriği (%)				
		Tuz dozları (dS m <sup>-1</sup> )					Tuz dozları (dS m <sup>-1</sup> )				
		0	5	10	20	Ort.	0	5	10	20	Ort.
CaCl <sub>2</sub>	Meyveci 2001	2.79 a	2.34 b	2.27 b	2.08 c	2.37	1.357 ab	1.367 a	1.357 a	1.320 b	1.350
	Ankara Yeşili	2.69 a	2.40 b	1.98 c	1.78 d	2.21	1.397 a	1.380 a	1.397 a	1.380 a	1.389
	Ceren	2.72 a	2.27 b	2.11 c	2.06 c	2.29	1.327 bc	1.360 ab	1.370 a	1.310 c	1.342
	Kafkas	2.65 a	2.16 b	2.07 c	1.93 d	2.20	1.377 a	1.360 ab	1.367 a	1.327 b	1.358
	Çiftçi	2.74 a	2.48 b	2.19 c	1.92 d	2.33	1.397 a	1.377 a	1.360 a	1.277 b	1.353
	Özbek	2.61 a	2.57 a	2.01 b	1.61 c	2.20	1.310 a	1.277 a	1.280 a	1.220 b	1.272
Ortalama		2.70	2.37	2.11	1.90	2.27	1.361	1.354	1.355	1.306	1.344
MgCl <sub>2</sub>	Meyveci 2001	2.79 a	2.41 b	2.18 c	2.00 d	2.35	1.357 a	1.357 a	1.360 a	1.350 a	1.356
	Ankara Yeşili	2.69 a	2.41 b	2.15 c	1.97 d	2.31	1.397 a	1.257 b	1.207 c	1.150 d	1.253
	Ceren	2.72 a	2.31 b	2.10 c	1.95 d	2.27	1.327 a	1.290 b	1.227 c	1.210 c	1.264
	Kafkas	2.65 a	2.19 b	2.04 c	1.97 c	2.21	1.377 a	1.250 b	1.220 b	1.060 c	1.227
	Çiftçi	2.74 a	2.29 b	2.04 c	1.91 d	2.25	1.397 a	1.357 b	1.350 b	1.350 b	1.364
	Özbek	2.61 a	2.23 b	1.93 c	1.74 d	2.13	1.310 a	1.100 b	1.077 b	1.010 c	1.124
Ortalama		2.70	2.31	2.07	1.92	2.25	1.361	1.269	1.240	1.188	1.264
NaCl	Meyveci 2001	2.79 a	2.68 b	2.60 c	1.92 d	2.50	1.357 a	1.257 b	1.240 b	1.120 c	1.244
	Ankara Yeşili	2.69 a	2.33 b	2.30 b	2.06 c	2.35	1.397 a	1.370 ab	1.337 b	1.190 c	1.324
	Ceren	2.72 a	2.33 b	2.14 c	1.98 d	2.29	1.327 a	1.317 a	1.307 a	1.300 a	1.313
	Kafkas	2.65 a	2.14 b	2.10 b	1.86 c	2.19	1.377 a	1.320 b	1.330 b	1.230 c	1.314
	Çiftçi	2.74 a	2.25 b	2.10 c	1.78 d	2.22	1.397 a	1.380 a	1.360 a	1.267 b	1.351
	Özbek	2.61 a	2.03 b	1.78 c	1.43 d	1.96	1.310 a	1.117 b	1.070 c	0.997 d	1.124
Ortalama		2.70	2.29	2.17	1.84	2.25	1.361	1.294	1.274	1.184	1.278

CaCl<sub>2</sub> uygulamasında Ankara Yeşili, MgCl<sub>2</sub> uygulamasında ise Meyveci 2001, NaCl uygulamasında ise Ceren'deki Ca konsantrasyonu bakımından tuz dozları arasındaki farklılıklar önemsiz olarak bulunmuştur. Araştırma bulgularına göre, tuz uygulaması ile bitkilerdeki Ca içeriğinin azaldığı, tuz stresindeki bitkilere dışarıdan verilen özellikle kalsiyum ve potasyumun koruyucu etkisi olduğu belirlenmiştir (Reid & Smith 2000; Cramer 2002; Vicente et al 2004). Yine, Malkoç & Aydın (2003), mısır (*Zea mays* L.) ve fasulye bitkilerinde artan tuz dozunun çeşide bağlı olarak bitki gelişmesini yavaşlattığı, bitkideki kuru madde miktarı ile N, P, K, Ca, Mg, Fe, Mn, Zn ve Cu içeriklerinin genelde azalma gösterdiğini bildirmişlerdir. Nitekim denememizde de tüm tuz çeşitlerinde artan tuz dozları ile bitkideki Mg içeriğinde azalma belirlenmiş; CaCl<sub>2</sub>, MgCl<sub>2</sub> ve NaCl tuzlarındaki Mg içeriğinde azalmaların sırasıyla

% 36.4, % 24.4 ve % 35.9 şeklinde olduğu, bunlardan en çok olanının Mg içeriğinde ve Ceren çeşidinde, en az olanının ise Ankara Yeşili'nde gerçekleştiği bulunmuştur (Çizelge 6). Öte yandan, bunların bitkide kök içi bölgesine aynı taşıyıcılarla (iyonoforlarla) taşınmalarından dolayı, Mg<sup>+2</sup> ile çeşitli katyonlar (K<sup>+</sup> ve Ca<sup>+2</sup>) arasında şiddetli bir rekabet olmakta, ayrıca bunlar, K ve Ca, Mg alımını engelledikleri gibi bitki kökünden diğer organlarına taşınmasını da olumsuz yönde etkilemektedirler. Magnezyumun kökte iç yöreye taşınmasında birçok katyonla aynı taşıyıcıların kullanılması nedeniyle katyonlarla bir rekabet oluşmaktadır. Bu da magnezyum alımında olumsuz etkilere neden olabilmektedir. Bunun dışında magnezyum alımı üzerine bitkinin transpirasyon oranı, bitkinin gelişme durumu, ortam pH'sı ve sıcaklık farkları gibi birçok faktör etkili olmaktadır. Ayrıca bitkilerde klorun aktif absorpsiyon yöntemiyle alınması

nedeniyle Cl alımı ve birikimi daha hızlı olmaktadır. Bu nedenle klorun toksik etkisi de bulunabilir (Schimansky 1981; Kacar & Katkat 2010). Nitekim, Yakıt & Tuna (2006), mısırdaki yaptıkları çalışmalarında, tuzlu ortamlara  $Mg(NO_3)_2$  ve  $MgCl_2$  içeren besin çözeltilerini eklemişler ve kontrol uygulamasına (% 0.34) göre bitkinin Mg içeriğinin ortama  $Mg(NO_3)_2$  uygulamasından (% 0.34) etkilenmediğini, ancak  $MgCl_2$  eklenmesiyle Mg içeriğinin (% 0.20) çok önemli oranda azaldığını belirtmişlerdir. Cl tuzunda da tüm çeşitlerde en düşük Cl konsantrasyonu tuz uygulanmayan parsellerden elde edilmiş, tuz dozlarındaki artışlarla birlikte mercimek çeşitlerinde Cl konsantrasyonu da önemli düzeyde artmıştır. En düşük Cl konsantrasyonu, dozların ortalaması olarak  $CaCl_2$  ve  $NaCl$ 'de Meyveci 2001 ve Çiftçi'de;  $MgCl_2$ 'de ise yine Meyveci 2001'de belirlenmiştir (Çizelge 6).

#### 4. Sonuç

Bu çalışmada farklı klor tuzlarındaki tuz konsantrasyonu artışına bağlı olarak, ÇO, Çİ, FB, KU, FKA ile fidedeki N, Ca, Mg ve K içerikleri azalırken; OÇS, Na ve Cl içerikleri artmıştır. Yeşil mercimeklerden Meyveci 2001, kırmızı mercimeklerden ise Çiftçi çeşidi diğer genotiplere göre tuz tiplerine daha fazla tolerans göstermiştir. Tuzluluk problemlerinin yoğun olduğu tarım topraklarında ekonomik kayıpların azaltılabilmesi için tuzlu topraklarda yetiştirilebilecek uygun bitki tür ve çeşitlerinin seçimi önemlidir. Çalışmada ele alınan özellikler yönünden çeşitlerin tuz tiplerine ve dozlarına tepkileri farklılık göstermiştir. Tuz stresine toleransın belirlenmesinde kullanılacak en az 200 kadar parametre bulunduğu (Kuşvuran 2010) göz önüne alınırsa; daha kesin sonuçlar ve genotipler

**Çizelge 6- Farklı klor tuzu ve dozları uygulanan mercimekte magnezyum ve klor içeriği (%) ortalamaları**  
Table 6- Mean magnesium and chloride concentrations (%) of lentil cultivars exposed to different chloride salt and doses

Tuzlar	Çeşitler	Magnezyum içeriği (%)					Klor içeriği (%)				
		Tuz dozları ( $dS\ m^{-1}$ )					Tuz dozları ( $dS\ m^{-1}$ )				
		0	5	10	20	Ort.	0	5	10	20	Ort.
$CaCl_2$	Meyveci 2001	0.425 a	0.415 a	0.415 a	0.275 b	0.383	0.19 d	2.53 c	2.68 b	2.92 a	2.08
	Ankara Yeşili	0.320 a	0.215 b	0.200 bc	0.185 c	0.230	0.23 d	2.54 c	2.78 b	3.09 a	2.16
	Ceren	0.455 a	0.440 a	0.415 b	0.375 c	0.421	0.24 d	2.50 c	2.92 b	3.03 a	2.17
	Kafkas	0.425 a	0.320 b	0.285 c	0.235 d	0.316	0.20 c	2.77 b	2.84 a	2.89 a	2.18
	Çiftçi	0.325 a	0.310 ab	0.300 b	0.260 c	0.299	0.22 c	2.48 b	2.50 b	2.82 a	2.01
	Özbek	0.455 a	0.305 b	0.285 b	0.200 c	0.311	0.24 d	2.65 c	2.93 b	3.11 a	2.23
Ortalama		0.401	0.334	0.317	0.255	0.327	0.22	2.58	2.78	2.98	2.14
$MgCl_2$	Meyveci 2001	0.425 a	0.445 a	0.395 b	0.370 c	0.409	0.19 c	2.61 b	2.63 b	3.02 a	2.11
	Ankara Yeşili	0.320 a	0.305 ab	0.290 b	0.285 b	0.300	0.23 d	2.80 c	2.94 b	3.23 a	2.30
	Ceren	0.455 a	0.370 b	0.375 b	0.310 c	0.378	0.24 d	2.76 c	2.85 b	3.16 a	2.25
	Kafkas	0.425 a	0.360 b	0.340 b	0.265 c	0.348	0.20 c	2.68 b	2.72 b	3.15 a	2.19
	Çiftçi	0.325 a	0.345 a	0.280 b	0.260 b	0.303	0.22 d	2.51 c	2.79 b	3.22 a	2.19
	Özbek	0.455 a	0.370 b	0.365 b	0.325 c	0.379	0.24 d	2.98 c	3.05 b	3.17 a	2.36
Ortalama		0.401	0.366	0.341	0.303	0.354	0.22	2.72	2.83	3.16	2.23
$NaCl$	Meyveci 2001	0.425 a	0.410 a	0.355 b	0.300 c	0.373	0.19 d	2.34 c	2.44 b	2.52 a	1.87
	Ankara Yeşili	0.320 a	0.310 a	0.235 b	0.160 c	0.256	0.23 d	2.81 c	3.00 b	3.08 a	2.28
	Ceren	0.455 a	0.370 b	0.350 b	0.350 b	0.381	0.24 d	2.62 c	2.78 b	2.94 a	2.15
	Kafkas	0.425 a	0.335 b	0.255 c	0.255 c	0.318	0.20 d	2.53 c	2.66 b	2.92 a	2.08
	Çiftçi	0.325 a	0.280 b	0.260 b	0.235 c	0.275	0.22 d	2.46 c	2.56 b	2.84 a	2.02
	Özbek	0.455 a	0.280 b	0.260 bc	0.240 c	0.309	0.24 d	3.07 c	3.16 b	3.41 a	2.47
Ortalama		0.401	0.331	0.286	0.257	0.319	0.22	2.64	2.77	2.95	2.14



arasındaki farklılıkları daha net ortaya koyabilmek için yeni özellikler ve verim denemelerinin de eklenerek çalışmanın devam ettirilmesinde yarar vardır. Tuz tipleri yönünden değerlendirildiğinde; çimlenme oranı, çimlenme indeksi ve ortalama çimlenme süresi yönünden en fazla olumsuz etkinin NaCl tuzunda, bunu sırasıyla CaCl<sub>2</sub> ve MgCl<sub>2</sub> tuzlarının izlediğini söyleyebiliriz. Fide boyunda MgCl<sub>2</sub>, kök uzunluğunda ise NaCl tuzunun olumsuz etkileri diğer tuz tiplerine göre daha fazla olmuştur. Fide kuru ağırlığı yönünden ise artan tuz dozları fide ağırlığını azaltmakla birlikte tuz tipleri yönünden belirgin bir farklılık oluşmamıştır. Denemede ele alınan üç tuzda da bitkinin mineral içeriği olumsuz yönde etkilenmiştir.

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## Yüksek Çözünürlüklü Uydu Görüntülerinde Farklı Görüntü İşleme Yöntemleri ile Yağ Gülü Parsellerinin Belirlenmesi

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### ÖZET

Bu çalışmada amaç, yüksek çözünürlüklü uydu verisinde iki farklı yöntem kullanılarak yağ gülü (*Rosa damascena*) dikili alanların parsel bazında belirlenebilirliğini araştırmaktır. Çalışma, Türkiye’de yağ gülü üretiminin yoğun olarak yapıldığı Isparta ili Güneykent Belediyesi sınırları içerisinde yürütülmüştür. Çalışmada temel kartografik olarak Quickbird-2 uydu verisi, uydu verilerinin sınıflandırmasında ERDAS ve e-Cognition yazılımları kullanılmıştır. Bu amaçla öncelikle uydu verisinde geometrik düzeltme (rectify), görüntü keskinleştirme (pan-sharp) ve görüntü zenginleştirme (histogram equalization) işlemleri yapılmış, arazide kullanılmak üzere parsellerin yer aldığı 1/5000 ölçekli altlık veriler oluşturulmuştur. Bu veriler araziye oryantasyon amacıyla kullanılmış ve arazi çalışmaları yürütülmüştür. Arazi çalışmalarında parsel bazında Arazi Kullanım Türleri (AKT) belirlenmiş, ArcGIS yazılımı ile sayısal AKT haritası hazırlanmıştır. Daha sonra Quickbird-2 uydu verisinin 4, 3 ve 2 bant kombinasyonunda piksel ve obje tabanlı sınıflama metodları kullanılarak gül parselleri belirlenmiş ve en uygun sınıflandırma metodu seçilmiştir. Kontrollü sınıflandırma yöntemi maksimum olabilirlik karar kuralı algoritması ile yapılan sınıflandırmada oluşturulan tematik haritada gül parsellerinin üretici doğruluğu % 48.72, kullanıcı doğruluğu % 18.63, kappa değeri 0.1539 olarak bulunmuştur. Objeye tabanlı sınıflandırmada ölçek: 100, bütünlük: 0.5, biçim: 0.1 parametreleri kullanılarak gül parsel sınırlarının en iyi belirlendiği, bu yöntem ile oluşturulan tematik haritada gül parsellerinin % 60.78 doğrulukta ayırt edilebildiği belirlenmiştir. Ayrıca objeye tabanlı sınıflandırmada ölçek: 25, bütünlük: 0.5, biçim: 0.1 parametreleri kullanılarak yapılan sınıflamada gül sıralarının ayırt edilebildiği görülmüştür. Yüksek çözünürlüklü uydu verileri kullanılarak yapılan piksel tabanlı sınıflandırmada gül parsellerinin diğer parsellerle karıştığı, bu sınıflama yöntemlerinin gül parsellerinin belirlenmesinde iyi sonuç vermediği, bunun yerine gül parsellerinin daha iyi ayırt edilebildiği objeye tabanlı sınıflandırmanın kullanılabileceği tespit edilmiştir.

Anahtar Kelimeler: Quickbird uydu verisi; Gül tarım alanları; Piksel ve objeye tabanlı sınıflandırma

## Determination of Rose Plantation Using by High Resolution Satellite Imagery

### ARTICLE INFO

Research Article

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## ABSTRACT

The purpose of this study is to investigate mapping of oil rose (*Rosa damascena*) parcels in high-resolution satellite imagery using two different methods. This study was carried out within boundaries of Güneykent Municipality in Isparta in where was produced most of oil rose production in Turkey. Quickbird-2 satellite image was used as base cartographic, and ERDAS and e-Cognition software were used classification of satellite imagery. This purpose; rectify, pan-sharpen and histogram equalization processes were made in the satellite data. The base map including borders of parcel at 1/5000 scale was produced to orient in field work. Then field survey was carried out using these bases. In field survey, Land Use Type (LUT) was determined for each parcel. The digital LUT map was produced using ArcGIS software. Then satellite image was classified to separate the rose parcels. In classification, the methods of pixel and object-based classification were performed on 4, 3 and 2 band combination of Quickbird-2 satellite data. The most appropriate classification method was selected to apply in study. The manufacturer accuracy, user accuracy and kappa value of oil rose class were found respectively as 48.72%, 18.63% and 0.1539 using maximum likelihood decision rule algorithm of supervised classification method. The boundaries of rose parcels were determined best accuracy by using scale: 100, compactness: 0.5, shape: 0.1 parameters in object-based classification. In the thematic maps, accuracy of rose parcels was found 60.78%. In addition, rose rows were separated by using scale: 25, compactness: 0.5, shape: 0.1 parameters. In the pixel-based classification methods, the rose parcels were mixed with other LUT using high-resolution satellite image. These classification methods were not enough successful for determining of rose parcels. But object-based classification methods were found as applicable to identify rose parcels.

Keywords: Quickbird satellite data; Rose agricultural areas; Pixel and object-based classification

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## 1. Giriş

Uydu verilerinin yersel çözünürlüğünün artması ile yeryüzü objeleri daha fazla piksel ile görüntülenmekte daha ayrıntılı olarak kullanıcılara sunulmaktadır. Arazi kullanımı ve arazi örtüsünün uydu görüntüleri ile belirlenmesi amacıyla uygulanan sınıflandırma yöntemleri piksel ve obje temelli olmak üzere iki farklı yaklaşım ile yapılabilmektedir. Bu görüntülerde obje büyüklüğü ile piksel boyutları arasında ortaya çıkan ilişki piksele dayalı sınıflandırma metodlarında bir faktör olarak kullanılmaması nedeniyle obje-tabanlı sınıflandırma yöntemlerine olan ilgi artmıştır (Blaschke 2010). Objeye tabanlı sınıflandırma metodu segmentlere ayırma ve bağlamsal sınıflandırmanın birleşimidir. Objeye dayalı görüntü analizlerinde temel olan ilk adım görüntü segmentasyonudur (Castilla & Hay 2008). Segmentasyon işlemi, objelerin spektral, geometrik, tekstürel ve diğer özelliklerinin sınıflandırılmasına göre düzenlenen görüntünün homojen piksel gruplarına (segmentler) bölünmesidir (Veljanovski et al 2011). Görüntü segmentasyon işleminde uygun parametrelerin seçimi önemli bir aşamadır. Ayrıca segmentasyonun

başarısı için metodun kullanımına bağlı olarak yüksek kaliteli bir görüntü kullanımı en büyük faktörlerden biridir (Yu et al 2006; Lu & Weng 2007; Mallinis et al 2008; Moran 2010). Çok yüksek yersel çözünürlüklü (VHSR) uydular tarafından alınan verilerde objelerin büyüklüğü ve şekilleri, birçok yeni uygulamalara imkân veren obje tabanlı sınıflandırma metodlarıyla daha fazla ayırt edilebilmektedir. Bu yazılımlarda yer alan sınıflandırma algoritmaları AKT (tahıl, orman, meyve bahçeleri gibi), parsel sınırları ve dikim deseninin belirlenmesinde kullanılabilir. Objeye tabanlı sınıflandırma kullanılarak üzüm bağlarında yapılan çalışmalarda sıra (Bobillet et al 2003), dikim şekilleri (Wassenaar et al 2002) ve yaprak yoğunluğu belirlenebilmiş (Bobillet et al 2003), her bir parselin ayrımı mümkün olmuştur. Bağ parsellerinin VHSR uydu görüntülerinde net olarak görülebilen en önemli özelliği dikim deseninin birbirine paralel sıralar halinde olmasıdır (Delenne et al 2008). Bağların dikim deseninde görülen bu özel durum yağ gülü (*Rosa damascena*) parsellerinde de temel özelliğidir. Yağ gülü 1.5-2 m aralıkla sıralar halinde dikilmektedir (GTB 2011; Ersan 2013).

Dünyada gül çiçeği üretimi yapılan en önemli ülkelerden biri Türkiye'dir. Yağ gülü (*Rosa damascena*) ekonomik olarak sadece Isparta ve çevresinde yetişmekte olup, Isparta Gül'ü olarak da bilinmektedir. Üretilen gülden elde edilen yağın %95'i ve gül konkretinin %100'ü ihraç edilmektedir. 2010 yılı verilerine göre ülkemizde 6000 ton gül üretimi yapılmış, 4000 kg ha<sup>-1</sup> verim elde edilmiş, 1000 kg gül yağı, 5000 kg gül konkreti ihracatı ile 7700000 £ gelir sağlanmıştır (GTB 2011).

Isparta gülünün üretim alanlarına ait rakamlar bulunmakta ise de, bu rakamlar bir önceki yıla göre oluşan değişikliklerin tahmininden elde edilmekte ve üretim yapılan alanların konumsal bilgileri bulunmamaktadır. Birçok tarımsal üründe olduğu gibi tarımsal planlamalarda gerekli veriler ve envanterler hazırlanmamıştır. Bu eksiklik ekolojik, ekonomik, sosyal, kültürel ve teknik faktörler, toprak özellikleri, hastalık ve zararlılar gibi tarımsal etkenler ve pazar durumu nedeniyle üretim alanlarındaki konumsal değişikliğin belirlenememesi ve izlenememesi gibi olumsuzluklara yol açmaktadır. Ayrıca sektörün geliştirilmesi ya da yaygınlaştırılması amaçlandığında yatırımın nerelere yapılması gerektiği bilinmemektedir. Bu olumsuzluklar ise ekolojik genişleme sınırlarının aşılması bunun sonucunda gül bahçelerinin gül yağı fabrikalarından uzaklaşması, gülün verim ve kalitesinin düşmesine, gül yağı ve gül ürünleri pazarında rakip ülkelere karşı rekabet gücünün zayıflamasına neden

olmaktadır. Bu sorunların çözümünde ise stratejik üretim planlaması yatmaktadır. Ancak planlamada esas olan temel haritaların üretilmesi gerekli görülmektedir. Yukarıda bahsedilen zorunluluğun yerine getirilmesinde ve sorunların çözümünde ilk basamak mevcut gül üretim alanlarının belirlenmesidir. Ancak bu büyüklükteki bir çalışmanın hızlı, doğru ve ekonomik bir şekilde yürütülebilmesi için uydu verilerinin kullanılması ve bir yöntem geliştirilmesi ile mümkün olabilecektir (Ersan 2013).

Bu çalışmada Quickbird-2 uydu verisi kullanılarak piksel ve obje tabanlı sınıflandırma yöntemleri ile yağ gülü üretimi yapılan parsellerin belirlenebilirliği araştırılmıştır. Yağ gülü üretilen alanların parsel bazında haritalanmasına yönelik bir metot ortaya konulmuş, planlama, yönetim ve izleme çalışmalarının gerçekleştirilebilmesinde gerekli olan bilgi sistemleri için harita niteliğindeki sayısal verilerin oluşturulabilirliği araştırılmıştır.

## 2. Materyal ve Yöntem

### 2.1. Test alanı

Çalışma, gül tarımının yoğun olarak yapıldığı Isparta'nın Gönen ilçesine bağlı Güneykent belediyesi sınırları içerisinde yürütülmüştür (Şekil 1). Güneykent, Gönen ilçesi ve Keçiborlu ilçesi arasında olup Kapı Dağı'nın uzantısı olan Tepelci,



Şekil 1- Çalışma alanının konumu

Figure 1- Location of study area

Erenler ve Demirli dağlarının ortasında Güney'e bakan bir yamaçta kurulmuştur. Güneykent'in rakımı 1250 m'dir. Güney kısmında bulunan ovası Burdur Gölü'ne doğru uzanmaktadır. Akdeniz Bölgesinde bulunmasına rağmen İç Ege ve İç Anadolu Bölgesi iklimleri hüküm sürmektedir. Toprak istekleri yönünden seçici olmaması nedeniyle bölgenin büyük bölümünde gül yetiştirilmektedir (Anonim 2012).

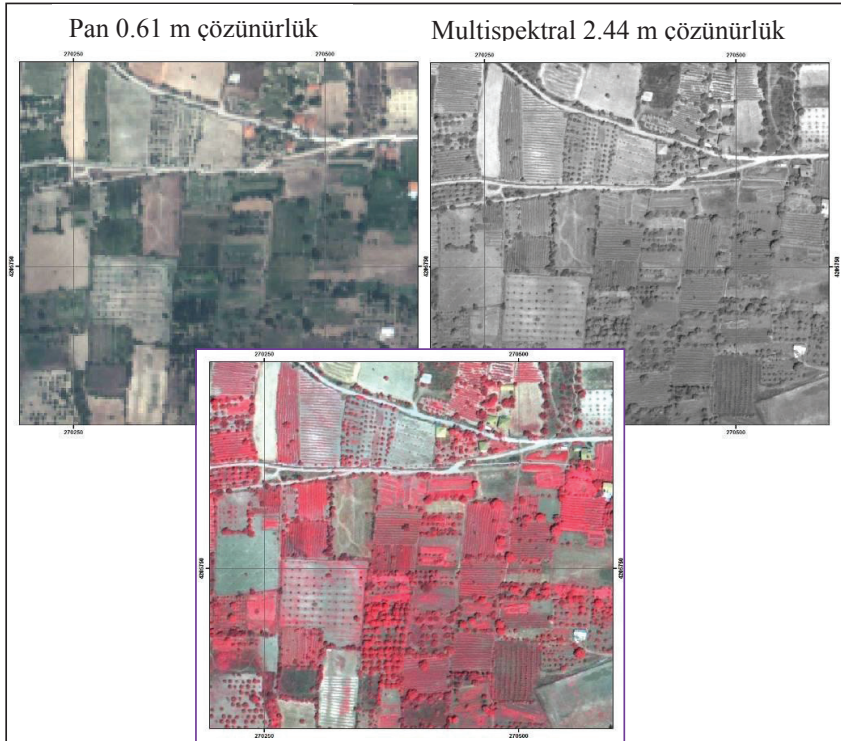
## 2.2. Çalışmada kullanılan uydu verisi ve yazılımlar

Bölgede, gülün fenolojik dönemi Mart ayı sonunda başlamakta ve Ekim ayı sonlarına kadar sürmektedir (Basayığıt et al 2013). Bu nedenle vejetasyon periyodunu kapsayan ve bulutluluğun en az olduğu günler dikkate alınarak 06.08.2006 tarihli 0.61 m pankromatik ve 2.44 m multispektral çözünürlüklü Quickbird-2 uydu verisi kullanılmıştır. 18 Ekim

2001 tarihinde ABD-Kaliforniya eyaletinden fırlatılan Quickbird-2 uydu verisi 11 bit radyometrik, 1-3.5 gün zamansal çözünürlüğe sahip, tarama genişliği 16.5 km'dir (nadir). Spektral bant genişliği pankromatik sensörde VIS/NIR bant 0.45-0.90 um, multispektral sensörde mavi bant 0.45-0.52 um, yeşil bant 0.52-0.60 um, kırmızı bant 0.63-0.69 um, NIR bant 0.76-0.90 um bant genişliğine sahiptir (Anonim 2014). Çalışmada uydu verilerinin işlenmesinde Erdas IMAGINE 9.1, ARCGIS 9.1 ve eCognition Developer Trial 8 yazılımları kullanılmıştır.

## 2.3. Ön çalışmalar

Farklı çeşit ve yoğunluktaki bitki desenlerinden oluşan ve farklı büyüklükte gül bahçelerinin bulunduğu 1x1 km'lik test alanı seçilmiştir. Uydu verisinde geometrik düzeltme, görüntü keskinleştirme (Şekil 2) ve görüntü zenginleştirme



Şekil 2- Çalışma alanının Pan-sharpened görüntüsü (4, 3, 2 bant)

Figure 2- Pan-sharpened image of study area (4, 3, 2 band)

işlemleri yapılmıştır. Çalışmada 4, 3, 2 bant kombinasyonları kullanılarak elde edilen yalancı renk görüntüsünde (false colour) foto yorumlama yapılmıştır. Bu amaçla görüntüdeki doku, desen, ton ve renk özelliklerinin arazi gerçeğine karşılığı belirlenmiş, bu parametrelerden elde edilen bilgilere göre görüntü yorumlanmıştır. Test alanında yer alan tüm parsellerin arazi kontrolleri yapılmış, gül tarım alanları ve diğer AKT'ne ait veriler toplanmıştır. Bu verilerin ArcGIS ortamında sayısallaştırması yapılmış, veri tabanına girilerek sayısal AKT'ni gösteren parsel bazında haritası oluşturulmuştur.

#### 2.4. Görüntü sınıflandırma ve arazi etüdüleri

Uydu verilerinde sınıflandırma işlemlerinde piksel tabanlı sınıflandırma için ERDAS yazılımı kullanılmış. Sınıflandırmada kontrollü sınıflandırma yöntemi seçilmiş, Maksimum Olabilirlik Karar Kuralı algoritması ile görüntü 10 sınıfa ayrılmıştır.

Objeye tabanlı sınıflandırmada ise eCognition yazılımı kullanılmıştır. Yazılımın görüntü segmentasyonu algoritması, spektral ve mekansal ölçütlere göre, birbirine yakın özellikteki pikselleri homojen parçalar olarak gruplandırmıştır. Multiresolution segmentasyon algoritmasında objelerinin boyutuna göre en uygun ölçek parametresinin seçilmesi sınıflandırmanın başarısı açısından çok önemlidir (Smith 2010). Genellikle küçük objelerin ortaya çıkarılmasının amaçlandığı görüntülerde yapılan sınıflandırmalarda küçük ölçek parametre değerleri, büyük objelerin ayırt edilmesi planlanan görüntülerde büyük ölçek parametre değerlerinin kullanımı uygun olmaktadır (Duro et al 2012). Bu işlemden kesin doğru bir segmentasyon seçeneği bulunmamaktadır. Çalışmanın amacına en uygun segmentler oluşturulana kadar çok sayıda deneme yapılmaktadır. Burada önemli olan uygun homojenliği sağlayarak pikselden objeye doğru, uygun yapıyı kurmaktır.

Test alanının Quickbird-2 uydu görüntüsünde 4, 3, 2 bant kombinasyonu kullanılarak farklı biçim, bütünlük ve ölçek parametreleri denenerek segmentasyon işlemleri yapılmış, en uygun parametreler seçilmiştir. Multiresolution segmentation

işleminde biçim faktörü 0.1 ve bütünlük faktörü 0.5 olarak sabit alınmış, ölçek parametresi 100, 75 ve 25 alınarak test alanı görüntüsü segmentlere ayrılmıştır. Gül parsel sınırlarının ayırt edilebildiği en iyi biçim, bütünlük ve ölçek parametresi seçilmiştir. Standart en yakın komşu metoduna göre eğitim sınıfları oluşturulmuş ve sınıflandırılması yapılmıştır.

#### 2.5. Doğruluk değerlendirmesi (accuracy assessment)

Uydu verileri sınıflandırma yazılımlarındaki algoritmalar kullanılarak sınıflandırılmış, görüntüde tesadüfi noktalar seçilmiş, bu noktaların sınıf değerleri (Class value) olarak sayısal AKT haritasından belirlenen bitki desenleri atanmıştır. Tüm noktalar girildikten sonra yazılım ile doğruluk değerlendirmesini yapmak üzere referans verilerle otomatik sınıflandırılmış veriler arasındaki gerçek uyum ve yine referans verilerle rastgele sınıflandırılmış veriler arasındaki şans uyumu arasındaki farkı göstermek için kullanılan kappa değerleri (Nelson 2005) hesaplanmıştır. Kappa (k), iki veya daha fazla gözlem arasındaki kalitatif uyum oranını ölçmek için geliştirilmiş bir testtir (Çelik 2006; Dengiz & Demirağ Turan 2014). Objeye tabanlı metottan üretilen verilerin doğruluğu parsel haritası ile objeye tabanlı sınıflandırma yorumlarına göre yapılmıştır.

### 3. Bulgular

#### 3.1. Test alanının AKT'leri

Test alanına ait AKT'leri Şekil 3'de verilmiştir. Test alanı gül, elma, kiraz, ayva, fındık, ceviz, sebze, badem, karışık meyve bahçeleri, yem bitkisi, çıplak toprak, zayıf bitki örtüsü, anız, geniş yapraklı ağaçlar, kavak, orman ve sazlık alanlardan oluşmaktadır. Çizelge 1'de test alanının AKT'lerine göre dağılımları verilmiştir.

Test alanında en az anız (2.91 da) ve gül2 olarak tanımlanan bakımlı, taç genişliği fazla, sıra arası vejetasyonla kaplı gelişimli gül (5.21), en fazla meyve (70.28 da) parselleri yer almaktadır. Alanda gül olarak sınıflandırılan ve gül3 olarak tanımlanan bakımlı, taç genişliği fazla, sıra arası sürülü orta





**Şekil 3- Çalışma alanının parsel bazlı arazi kullanım türleri (AKT) haritası**

Figure 3- Parcel-based LUT maps of study area

**Çizelge 1- Test alanının AKT'lerine göre dağılımları**

Table 1- Distributes according to LUTs of study area

AKT	Alan (da)	%
Anız	2905.5	0.99
Gül2	5208.7	1.77
Anız2	6622.6	2.25
Yem bitkisi	6669.9	2.27
Yol	15263.3	5.18
Çıplak toprak2	16368.4	5.56
Zayıf bitki örtüsü	19538.5	6.64
Gül1	24516.8	8.33
Sebze	24857.8	8.44
Gül3	33199.9	11.28
Çıplak toprak	68972.2	23.43
Meyve	70279.9	23.87
Toplam	294403.4	100.00

gelişimli gül parsellerinin alanı 33.20 dekadır. Orta gelişimli gül parsellerinin alanı toplam alanın % 11.28'ine karşılık gelmektedir. Gül1 olarak tanımlanan genç, taç genişliği az, sıra arası sürülü az gelişimli gül parsellerinin alanı 24.52 dekadır.

Az gelişimli gül parsellerinin alanı toplam alanın % 8.33'üne karşılık gelmektedir. Çalışma alanında gül parsellerinin toplamı 62.93 dekadır. Bu parsellerin toplam AKT'leri içerisindeki oranı % 21.37'dir.

### 3.2. Gül parsellerinin görsel özellikleri

Foto yorumlama özelliklerine göre, test alanında yer alan kırmızı rengin parlak tonundaki zeminler yem bitkisi ve bazı tek yıllık bitkileri, açık tonunda veya pembemsi renkte düzgün kenarlara sahip zeminler sebze yetiştirilen parsellere karşılık gelmektedir. Kırmızı rengin koyu tonlarında dokulu, sıra arası ve sıra üzeri belirgin topluluklar halinde görülen ve gölgeleri olan bitkiler geniş yapraklı ağaçları, yeşil rengin koyu tonlarında, dokulu birbirine sıkı topluluklar halinde ve geniş gölgeleri bulunan ağaç toplulukları ormanlık alanları göstermektedir. Koyu kırmızı-bordo renklerde birbirine sıkı ince dokulu ince ve uzun gölgeli alanlar kavaklıkları göstermektedir. Mavi rengin açık tonları yol ve anız alanlarını ve açık yeşil zeminler yeni sürülü çıplak toprakları, koyu yeşil zeminler ise çıplak toprakları göstermektedir. Krem-sarı renkli belirgin şekilli (dikdörtgen veya kare) gölgeleri olan objeler binaları, koyu mavimsi



renkte ve gölgeleri olan objeler sera veya hayvan barınaklarını temsil etmektedir.

Meyve ağaçları sıra arası ve sıra üzeri mesafeleri düzenli görülmekte, meyve ağaçları türleri ise sıra üstü ve sıra arası mesafe, taç genişliği ve dikim desenine göre ayırt edilebilmektedir. Tahıl gibi tek yıllık bitkiler zeminin tamamını kaplamakta, çıplak toprak, kuru ot veya nadas gibi AKT'lerinden belirgin olarak ayırt edilebilmektedir. Kavak ise uzunlamasına ince taç genişliği bulunmakta olup kavaklık alanlarda sıkı bir desene sahiptir. Yol ve parsel kenarlarında dikilmiş olan kavakların gölgeleri uydu görüntüsünde üzerine düştüğü parsellerin gerçek görünümünü örtmekte, görsel yorumlama ve sınıflandırma metodlarında doğruluğu azaltmaktadır. Gül tarım alanları ise ton olarak çok yıllık bitkilerle benzerlik göstermekte iken dikim deseniyle belirgin olarak ayırt edilebilmektedir. Eğimli arazilerde eğime dik yönde, düz arazilerde ise kuzey-güney yönünde uzunlamasına paralel sıralar halinde dikim deseni görülmektedir. Bu özellikler piksel tabanlı sınıflandırmanın yanında objelerin spektral, geometrik, tekstürel ve diğer özelliklerine göre sınıflandırma yapan obje-tabanlı sınıflandırma metodu için referans olacak niteliktedir. Çalışma alanında yer alan gül tarım alanlarının morfolojik görünümü ve yorumu Şekil 4'de verilmiştir.

### 3.3. Piksel tabanlı sınıflandırma

ERDAS yazılım ile kontrollü sınıflandırma yöntemi kullanılarak 10 eğitim sınıfı oluşturulmuştur. Eğitim sınıfları kullanılarak kontrollü sınıflandırmada yer alan yöntemlerin her biri için hata matrisi çıkartılmıştır. Test alanına ilişkin sınıflandırma performansına göre en uygun yöntem olarak Maksimum Olabilirlik Karar Kuralı seçilmiştir. Maksimum Olabilirlik Karar Kuralı kullanılarak yapılan sınıflandırma sonucu oluşturulan görüntü Şekil 5'de verilmiştir.

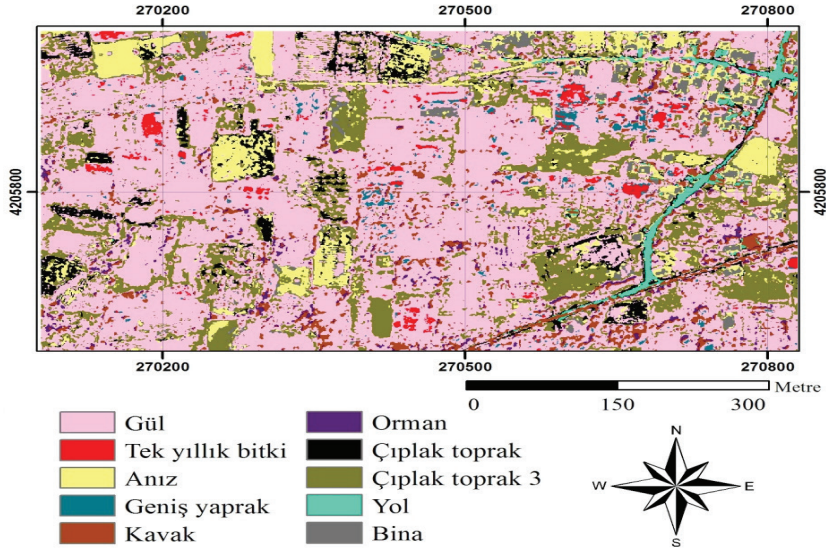
Mavi bantta en yüksek yansımaya değerini yollar en düşük yansımaya değerini ise orman örtüsü vermiştir. Yollar ile birlikte çıplak toprak ve anız örtüsü diğer kullanım türlerinden belirgin olarak ayırt edilmiştir. Yeşil bantta yol, çıplak topraklar, anız ve binaların yansımaları belirgin olarak diğerlerinden ayrılırken tek yıllık bitkiler ile gülün yansımaları yakın değerler göstermiştir. Kırmızı bantta kavak ile orman ve gül ile tek yıllık bitkilerin yansımaları yakın bulunmuştur. Yakın kızıl ötesi bantta ise gülün yansımaları geniş yapraklı bitkilere yakın çıkmıştır.

Eğitim sınıflarında yapılan doğruluk değerlendirmesinde üretici doğruluğu gül parselleri için % 48.72 olarak belirlenmiştir. AKT açısından değerlendirdiğimizde en yüksek doğruluğu orman, anız ve kavak alanları, en düşük doğruluğu geniş yapraklı parseller vermiştir. Sınıfların genel doğruluk değerlendirmesi % 58.04, kappa değerleri (k) 0.5339 olarak bulunmuştur (Çizelge 2). Mutlak

GÜL	
Üst yapı	Çalı formunda, sıra üzerinde uzunlamasına, gölgesi çok az belirgin görünüm
Ton (RGB)	Açık kırmızı-koyu kırmızı arasında ton, bakımsız ve genç bitkilerde koyu-siyahimsi kırmızı, sağlıklı bitkilerde kırmızımsı bir ton
Desen	Eğimli arazilerde eğime dik yönde, düz arazilerde ise kuzey-güney yönünde uzunlamasına paralel sıralar halinde
Doku	Sıkı

Şekil 4- Çalışma alanında yer alan gül tarım alanlarının morfolojik görünümü ve yorumu

Figure 4- Morphological image and interpretation of rose area in location of study area



**Şekil 5- Çalışma alanının piksel tabanlı sınıflandırması**

*Figure 5- Pixel-based classification of study area*

bir ayırım bulunmamasına rağmen, daha önce yayımlanmış çalışmalarda gözlemciler arası uyumu belirten k değeri  $\leq 0.20$  ise önemsiz uyum, 0.21-0.40 ise minimal uyum, 0.41-0.60 ise orta derecede uyum, 0.61-0.80 ise önemli derecede uyum, 0.81-1.00 ise gözlemciler arasındaki uyumun tam

olduğunu gösterir (Çelik 2006; Dengiz & Demirağ Turan 2014). Buna göre çalışmada bulunan üretici doğruluğu ve genel doğruluk için belirlenen kappa değerleri orta derecede uyumlu sınıfın içinde yer almıştır.

**Çizelge 2- Test alanında kontrollü sınıflandırmanın doğruluk değerlendirilmesi**

*Table 2- Accuracy assesment of supervised classification in study area*

<i>Sınıf</i>	<i>Üretici doğruluğu (%)</i>	<i>Kullanıcı doğruluğu (%)</i>	<i>Kappa</i>
Gül	48.72	18.63	0.1539
Tek yıllık bitki	49.73	91.09	0.8911
Geniş yapraklı	37.36	63.73	0.5626
Yol	68.85	82.35	0.7996
Çıplak toprak3	60.91	65.69	0.6154
Çıplak toprak	57.14	66.67	0.6226
Kavak	74.55	40.20	0.3679
Orman	86.54	44.12	0.4112
Bina	62.75	62.75	0.5861
Anız	77.05	46.08	0.4265
Overall Kappa Statistics : 0.5339			
Overall Classification Accuracy: % 58.04			

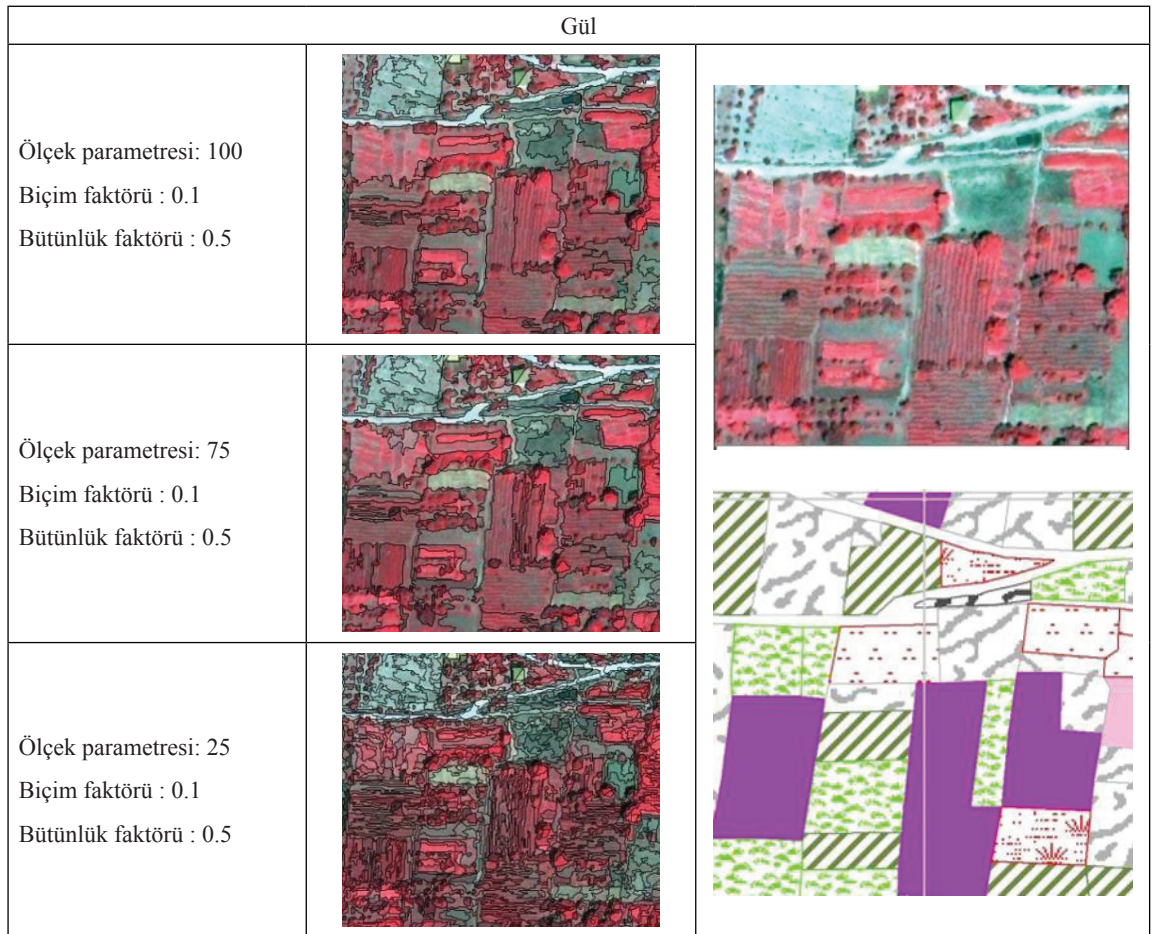
### 3.4. Obje tabanlı sınıflandırma

Test alanının Quickbird-2 uydu görüntüsünde 4, 3, 2 bant kombinasyonu kullanılarak farklı biçim, bütünlük ve ölçek parametreleri denenmiş, parseller segmentlere ayrılmıştır. Multiresolution segmentation işlemi biçim faktörü 0.1 ve bütünlük faktörü 0.5 olarak sabit alınarak ölçek parametresi 100, 75 ve 25 seçilmiştir. Parametre farklılıklarının segmentlere bölme işlemi sonucu oluşan görüntü Şekil 6'da verilmiştir.

Test alanı için uygulanan ölçek parametresi: 100, biçim faktörü: 0.1, bütünlük faktörü: 0.5 olarak

yapılan Multiresolution Segmentasyon işlemi gül parsellerinin ayırt edilebildiği en uygun parametreler olarak bulunmuştur. Test alanında bu parametreler kullanılarak obje tabanlı sınıflama uygulanmıştır (Şekil 7).

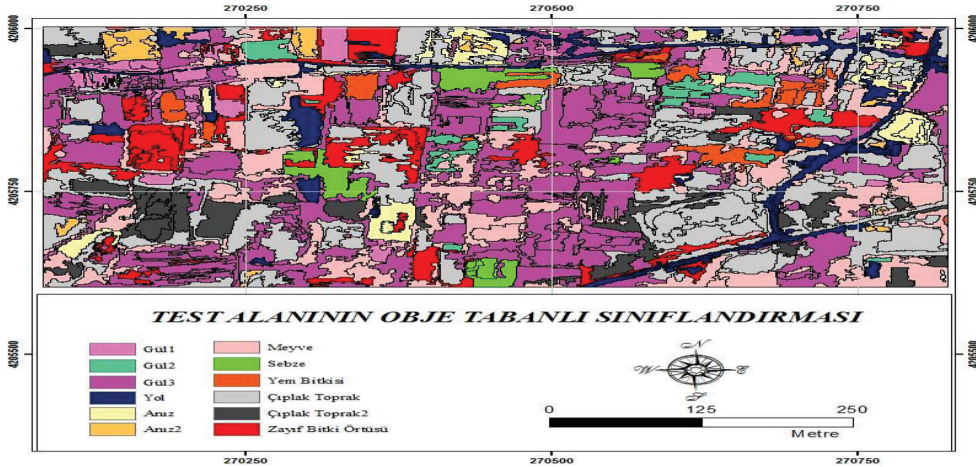
Test alanının obje tabanlı sınıflandırmasında en yüksek doğrulukta (% 60.78) Gül3 parselleri vermiştir. Gül3 parsellerinin yer yer meyve parselleri ile karıştığı görülmüştür. Meyve ağaç yapraklarının gül yaprakları ile yakın yansıma göstermesinin yanı sıra Gül3 parsellerinin taç genişliği ve sıra arası mesafe dağılımının bir meyve parselindeki



**Şekil 6- Çalışma alanında değişik ölçekler kullanılarak yapılan segmentasyon işlemi**

*Figure 6- Segmentation process that was made by using different scale in study area*





**Şekil 7- Çalışma alanının obje tabanlı sınıflandırması (ölçek parametresi: 100, biçim faktörü: 0.1, bütünlük faktörü: 0.5)**

*Figure 7- Object-based classification of study area (scale parameters: 100, shape factor: 0.1, compactness factor: 0.5)*

taç genişliği ve sıra arası mesafe dağılımına benzer oran göstermesi nedeniyle bu parsellerinin karışmasına neden olmuştur. Gül1 ve Gül2 alanları ise sırası ile % 29.09 ve % 25.00 doğruluk değerleri vermiştir. Gül1 parsellerinin düşük doğruluk vermesinde temel neden sıra arasının sürülü olması ve genç gül fidanlarından oluşmasıdır. Bu nedenle Gül1 parselleri çıplak toprak ve bazı bakımsız meyve parselleri ile karıştığı gözlemlenmiştir. Gül2 alanlarının en düşük doğruluk değeri vermesinde ise spektral yansımalarındaki karışmadan kaynaklanmaktadır. Bu parsellerdeki gül fidanları bakımlı olmakla birlikte toprak yüzeyinin yabancı otlarla kaplı olması nedeni piksellerin ortalama yansımalarının meyve parsellerinin pikselleri ile benzer yansıma göstermesi ve oluşturulan sınıflarda karışmasına neden olduğu belirlenmiştir.

#### 4. Tartışma ve Sonuç

Gül parsellerinin belirlenmesinde ölçek değeri: 100, bütünlük değeri: 0.5, biçim: 0.1 parametreleri kullanılarak yapılan obje tabanlı sınıflandırmanın maksimum olabilirlik karar kuralı ile yapılan kontrollü sınıflandırma metoduna göre daha iyi sonuç verdiği, obje tabanlı sınıflamada ölçek: 25,

bütünlük: 0.5, biçim: 0.1 parametreleri kullanılarak yapılan sınıflamanın ise gül sıralarını ayırt edebildiği sonucuna varılmıştır.

Son zamanlarda objeye dayalı yapılan sınıflandırma çalışmalarında çoklu görüntü segmentasyon ölçekleri (multiple image segmentation scales) kullanılmıştır (Yan et al 2006; Myint et al 2011; Whiteside et al 2011; Duro et al 2012). Gül üretilen alanların parsel bazında belirlenmesi amaçlandığında küçük alanlar için görsel yorumlama teknikleri piksel temelli sınıflama yöntemlerine göre daha başarılı olmaktadır. Çünkü bir parseli oluşturan temel örtü tipi altında bulunan çıplak toprak, yabancı otlar, doğal örtü durumu eğitim için seçilen alanların yansıma ortalamalarını birbirine yaklaştırmaktadır. Bunun sonucunda ise gül parselleri kimi zaman çıplak toprakla kimi zaman vejetasyonla kimi zaman ise diğer alanlar ile karışmaktadır. Ayrıca gül bitkilerinin yaşı, taç genişliği, sıra arası ve sıra üzeri mesafeleri ve budama şekli de piksellerin yansıma ortalamalarını etkileyen faktörlerdir. Bu nedenlerden dolayı yüksek çözünürlüklü uydu verilerinde piksel yansıma değerlerine bağlı sınıflandırma yöntemleri kaba çözünürlüklü uydu verilerine göre daha düşük

doğruluk vermektedir. Nitekim Devi & Krishna (2012) LISS 4 görüntülerde uyguladıkları piksel ve obje-tabanlı sınıflandırma çalışmalarında su, meyve bahçesi, kum, nadas, çeltik, çalı ve yapı olarak 7 eğitim sınıfı oluşturmuş, tüm alan için belirlenen başarı derecesinde obje tabanlı sınıflandırma % 87.5, piksel tabanlı sınıflandırma % 78.1 doğruluk vermiştir. Objeye-tabanlı metodların yüksek çözünürlüklü veriler için arazi yapısı ve görüntünün kalitesine bağlı olarak kabul edilebilir sonuçlar verdiğini belirtmişlerdir.

Öte yandan piksellerin yansıma değerleri kullanılarak gül bitkilerinin tek tek ayırt edilmesi mümkün olabilmektedir. Bu yöntemde ise gül bitkileri arasında kalan çıplak toprak, yabancı otlar, doğal örtü durumu ve tek yıllık çayır örtüleri gibi diğer yansıma unsurları başka AKT sınıfları içerisinde yer almaktadır. Bunun sonucunda da sınıf ayırımı yapılamayan karışık örtü tiplerinden oluşan bir haritalama üniteleri ortaya çıkmakta, parsel bazında sınıflandırma mümkün olmamaktadır. Devi & Krishna (2012) yaptıkları çalışmalarında objeye dayalı sınıflandırmanın piksele dayalı sınıflandırmadan daha doğru sonuçlar verdiğini, piksele dayalı metodların piksellerin sadece spektral bilgilerini kullandığı, dolayısıyla oluşturulan tematik haritalarda tek piksellerden oluşan dağınık sınıfların meydana geldiğini belirtmiştir.

Sonuç olarak; yüksek çözünürlüklü uydu verilerinde yapılan piksel tabanlı sınıflamada gül parsellerinin diğer parsellerle karıştığı, bu sınıflama yöntemlerinin gül parsellerinin belirlenmesinde çalışmadığı, bunun yerine bu tür özel bitkilerin sınıflandırılmasında obje tabanlı sınıflamanın kullanılabilmesi ve obje tabanlı sınıflamaların bitki indisleri ile birleştirilerek farklı özellikteki gül parsellerin tanımlanabileceği belirlenmiştir.

Ayrıca obje tabanlı sınıflandırma ile elde edilen tematik harita katmanlarının CBS ortamına entegre edilebilir olması konumsal özelliklerinde kullanılarak yoruma dayalı bilgilerin üretilmesine olanak sağlamaktadır (Wassenaar et al 2000).

## Teşekkür

Bu çalışma Süleyman Demirel Üniversitesi Bilimsel Araştırma Projeleri Yönetim Birimi Başkanlığı 3257-YL2-12 No'lu Proje ile desteklenmiştir.

### Kısaltmalar ve Semboller

AKT	Arazi kullanım türleri
k	Kappa
LUT	Land use types
NIR	Near infrared reflectance
VHSR	Çok yüksek yersel çözünürlüklü
VIS	Visible infrared spectral

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## Inheritance of Some Agronomic Characters in Pea

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### ABSTRACT

This study was conducted by using Jinks-Hayman type analyses on four pea genotypes (PS4009, PS3053, PS3073 and Reyna) and their population of the complete diallel reciprocal crosses in order to examine the genetic structure. According to the results of all diallel crosses, environmental variance (E) and additive gene variance (D) was not significant. All the features of the dominant gene variances were higher than the additive gene variances ( $H_1$ ) and  $D-H_1$  value was found to be negative. In the population, dominant genes were found significant for all the characteristics except number of seed per pod. Result of  $W_r$ ,  $V_r$  graphics indicated partial dominance for the number of seed per pod, and over dominance for the rest of the characters studied.

Keywords: Agronomic characters; Diallel; Inheritance; Pea

## Bezelyede Bazı Agronomik Özelliklerin Kalıtımı

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### ÖZET

Bu araştırma, dört bezelye genotipi (PS4009, PS3053, PS3073 ve Reyna) ile bunların tam diallel melezlerinden oluşturulan populasyonun Jinks-Hayman tipi analiz kullanarak genetik yapısını incelemek amacı ile yapılmıştır. Diallel melez sonuçlarına göre incelenen tüm özelliklerde çevre varyansı (E) ve eklemeli gen varyansı (D) önemsiz bulunmuştur. Tüm özelliklerinde dominant gen varyansları ( $H_1$ ) eklemeli gen varyanslarından yüksek olup, buna paralel olarak  $D-H_1$  değerleri ise negatif bulunmuştur. Populasyonda baklada tane sayısı üzerine resesif genler, bitki boyu, bakla sayısı, yüz tane ağırlığı, bakla verimi ve tane verimi özelliklerine ise dominant genlerin daha etkili olduğu tespit edilmiştir.  $W_r$ ,  $V_r$  grafikleri sonucu baklada tane sayısı için kısmi dominantlık, incelenen diğer özelliklerde ise aşırı dominantlık saptanmıştır.

Anahtar Kelimeler: Agronomik özellikler; Diallel; Kalıtım; Bezelye

## 1. Introduction

Sources of proteins have undeniable importance in human nutrition. As a leguminous plant, peas are considered a good source of protein and contain 20-30% protein; sufficient carbohydrates; rich in calcium, iron, phosphorus and vitamins (Ceyhan et al 2011). Considering worldwide consumption, human nutrition were made of 22% vegetable proteins, 7% carbohydrates and animal nutrition of 38% proteins and 5% carbohydrates are derived from edible legumes (Sehirali 1988; Adak et al 2010). Thus, peas have a big importance on canned and frozen food industry as a human nutrition.

Success in developing varieties depends on range of variation and right selection from the variations. Pea breeders often make crosses to create new variations. But lack of labor, time, high costs etc. are limiting factors to make numerous crosses. That's why breeders need to choose accurate parental lines to avoid extra expenses and long working hours. Success rate could be higher if the genetic inheritance of parental lines would be identified earlier with different methods. Breeder needs to know effects of the genes on the agronomic characters of interests. Inheritance of genes governing the trait of interest determines the generation for selection in breeding a self-pollinated plant species like peas (Ceyhan & Avci 2004).

Various methods are used in determination of the genetic parameters. With the help of these genetic parameters, information is obtained about the status of the population, the value of a feature to breeding and the effectiveness of the selection. Diallel crossing is one of these methods (Ceyhan & Kahraman 2013). Seed yield and its genetic parameters and heritability were the subject of many researches (Snoad & Arthur 1974; Ceyhan et al 2008; 2014b; Ceyhan & Kahraman 2013).

In this work, a 4x4 complete diallel reciprocal combinations of parents and populations was studied by using Jinks-Hayman type analysis to identify and the genetic structure of agricultural features.

## 2. Material and Methods

In this study, three pure pea lines (PS4009, PS3053, PS3073) and Reyna a commercial variety were

used. The study was conducted at Selcuk University experimental field in 2012 and 2013. The parental lines were planted on 8 March 2012, followed by five consecutive plantings at 10 days intervals in order to coincide flowering times of the genotypes. For the ease of crossing, parental lines were planted in 3x1 m rows 25 cm apart. The four genotypes were crossed in a 4x4 complete diallel reciprocal according to Eser (1974) and Ceyhan (2003) in 2012.

Soil analysis was made from the soil that was taken from 0-30 cm depth. The results revealed that soil was a clay-loam, light alkaline (pH= 7.8), low in organic matter (1.20%), and has an EC ( $\mu\text{S cm}^{-1}$ ) of 189, available  $\text{P}_2\text{O}_5$  of 10.74 mg  $\text{kg}^{-1}$ , Fe of 1.28 mg  $\text{kg}^{-1}$ , and exchangeable  $\text{K}_2\text{O}$  of 219.96 mg  $\text{kg}^{-1}$ , Zn of 2.16 mg  $\text{kg}^{-1}$ , Cu of 0.82 mg  $\text{kg}^{-1}$ , Mn of 4.95 mg  $\text{kg}^{-1}$ , and Ca of 5863 mg  $\text{kg}^{-1}$ .

The study was conducted in Konya where summers are hot and dry and winters are cold. During the vegetation in 2013 for 5 months (March through July) average temperature was 19.5 °C, total rain 63.4 mm, humidity 39.7%.

Parental lines and crosses were planted by hand on March 2013 as three rows 2 m in length with a 50 cm row spacing and 20 cm plant spacing, each plot had 36 seeds which were planted 5 cm deep. Trials were made by "Randomized Block Design" in 3 replicates at Selcuk University Agricultural Department Trial Station. Plots were fertilized with 15 kg DAP (Diammonium phosphate 18-46%) at planting (Ceyhan 2003; Ceyhan et al 2014a). To reinforce the germination after the planting, sprinkle irrigation was used, and by drip irrigation system twice during growing period. Plots were hand-weeded and harvesting was made by hand between 03 and 14 July 2013.

At the parental lines and  $F_1$  plants, plant height, number of pod, number of seed per pod, 100 seed weight, pod yield and seed yield were studied (Ceyhan 2003). Mean values were used to analyze the variation. In the study, the traits under the genetic control were analyzed by Jinks & Hayman (1953) diallel method. After detecting significant statistical differences between parents and among the crosses, validity of hypothesis was checked (Hayman 1954).

The parameters for genetic variation and regression analysis of  $W_r$  and  $V_r$  values of parents were made according to Jinks & Hayman (1953) and Hayman (1954) diallel analysis method. Statistical analysis was made by using TARPOGEN statistical package programme (Özcan & Açıkgöz 1999).

### 3. Results and Discussion

Analysis of variance results were shown in Table 1. To apply the Jinks-Hayman type analysis, the validity of the assumption was checked first. All the measurement made from 4x4 peas complete diallel reciprocal were found to be significant at  $P= 0.01$

level. Results indicated the presence of large enough variation in order to proceed to advanced genetic analysis (Jinks & Hayman 1953; Hayman 1954; Jinks 1954; Ceyhan & Kahraman 2013).

To apply Jinks-Hayman type diallel analysis some of the assumptions have to be valid. Validation of these assumptions (Yıldırım et al 1979) was checked in 2 ways. The  $W_r$ ,  $V_r$  variance analysis results are given in Table 2. The F values deduced from  $W_r$ - $V_r$  variance analysis of  $F_1$  populations was found not significant, indicating that  $W_r$ ,  $V_r$  variation differences are uniform. Thus, means of the three replications were used to do genetic analysis.

**Table 1- Mean sum of squares of the measurements**

Çizelge 1- İncelenen özelliklere ait kareler ortalaması

Sources	SD	Plant height	Number of pod	Number of seed per pod	100 seed weight	Pod yield	Seed yield
Block	2	178.938	1.021	0.023	3.559	0.764	2.710
Genotype	15	357.950**	144.421**	0.728**	38.138**	78.894**	78.914**
Error	30	94.071	9.888	0.196	2.558	5.708	3.935

\*\* ,  $P<0.01$

**Table 2- Sum of squares for  $W_r$ ,  $V_r$  variances**

Çizelge 2- İncelenen özelliklere ait  $W_r$ ,  $V_r$  varyans analizi kareler ortalaması

Sources	SD	Plant height	Number of pod	Number of seed per pod	100 seed weight	Pod yield	Seed yield
Replication	2	4307.043	118.231	0.079	61.171	5.788	57.197
Series	3	8162.156	1368.434	0.138	219.802	151.596	174.993
Error	6	14365.334	155.353	0.062	95.980	105.576	71.583

$W_r$ ,  $V_r$  regression values detected with regression analysis and t values calculated for  $b= 1$  hypothesis are presented at Table 3. The average regression values and t values obtained from  $b= 1$  hypothesis showed that  $b= 1$  hypothesis is suitable.

#### 3.1. Genetic structure of the population

Variance components estimated by Jinks-Hayman method and standard errors are shown in Table 4. In this research environmental variance and additive gene variance were found to be not significant. This result shows that selection would not be successful for the traits studied, similar

**Table 3- Regression values calculated from means ( $W_r$ , $V_r$ ) of the blocks and t values calculated for  $b= 1$  hypothesis**

Çizelge 3- Bloklar üzerinden alınmış ortalama ( $W_r$ ,  $V_r$ ) değerleri arasındaki regresyon katsayıları ve  $b= 1$  hipotezi için hesaplanan t değerleri

Features	b	SH	$t_1$
Plant height	0.503	0.253	1.965
Number of pod	0.704	0.289	1.025
Number of seed per pod	0.449	0.193	2.853
100 seed weight	1.203	0.624	-0.325
Pod yield	0.769	0.351	0.658
Seed yield	0.978	0.666	0.034

**Table 4- Variance components of the 4x4 complete diallel pea cross populations**

Çizelge 4- 4x4 tam diallel bezelye melez populasyonlarında bazı agronomik özelliklerin genetik varyans komponentleri ve bunlar arasındaki oranlar

Genetic parameters	Plant height	Number of pod	Number of seed per pod	100 seed weight	Pod yield	Seed yield
E	33.13±55.33	3.11±22.65	0.06±0.23	0.87±9.08	1.80±7.54	1.29±8.10
D	19.07±123.71	49.64±50.65	0.33±0.51	42.12±20.30	18.00±16.86	7.97±18.11
F	5.18±317.82	40.58±130.13	-0.15±1.31	46.15±52.15	26.65±43.31	20.42±46.54
H <sub>1</sub>	620.63±359.61	207.69±147.25	1.11±1.48	53.84±59.01	117.07±49.01	122.26±52.65
H <sub>2</sub>	561.47±331.95	202.13±135.92	1.51±1.36	44.13±54.47	97.67±45.24	100.94±48.61
D-H <sub>1</sub>	-601.56±322.89	-158.06±132.21	-0.78±1.33	-11.72±52.99	-99.07±44.01	-114.29±47.28
(H <sub>1</sub> /D) <sup>1/2</sup>	5.71	2.05	1.84	1.13	2.55	3.916
(H <sub>2</sub> /4H <sub>1</sub> )	0.23	0.24	0.34	0.21	0.21	0.20
KD/KR	1.05	1.50	0.77	2.88	1.82	1.972
h <sup>2</sup>	433.83±225.16	282.43±92.19	0.40±0.92	0.02±36.95	161.97±30.69	144.78±32.97
K	1.77	1.40	0.27	1.01	1.66	1.43
GH	0.52	0.70	0.39	0.77	0.75	0.78
DH	0.03	0.22	0.18	0.79	0.16	0.07

results were reported before (Sarawat et al 1994; Ceyhan & Kahraman 2013).

Of all the traits studied, dominant gene variances (H<sub>1</sub>) was higher than the additive gene variances, and D-H<sub>1</sub> was negative (Table 4). Similar results were reported elsewhere (Snoad & Arthur 1974; Sarawat et al 1994; Girase & Deshmukh 2000; Ceyhan & Kahraman 2013).

F value that shows direction of recessive and dominant alleles was found to be positive except for the number of pod (Table 4). This indicates dominant and additive gene effects of alleles are together and in increasing direction (Yildirim et al 1979; Ceyhan & Kahraman 2013). Positive F value shows that dominant alleles outnumber the recessive alleles. For the number of seed per pod negative F value opposite effect of above mentioned condition (Yildirim et al 1979; Ceyhan & Kahraman 2013).

The average degree of dominance (H<sub>1</sub>/D)<sup>1/2</sup> indicates the degree of the domination in a population (Table 4). All the data collected in this study, (H<sub>1</sub>/D)<sup>1/2</sup> value was higher than 1 that may mean the population may have over dominance

(Snoad & Arthur 1974; Ceyhan et al 2008; 2014a; 2014b; Ceyhan & Kahraman 2013).

Dominant and recessive alleles (H<sub>2</sub>/4H<sub>1</sub>) showed similar frequencies for seed yield (0.20) and number of seed per pod (0.34) (Table 4). This may indicate that dominant and recessive alleles frequencies are close to each other (0.5), and multiplication of dominant and recessive alleles frequencies would be around 0.25, a high enough value for successful phenotypic selection (Ceyhan & Kahraman 2013).

Ratio of dominant alleles to recessive alleles (KD/KR) for number of seed per pod was lower than 1 (0.77), indicating number of recessive alleles outnumber dominant alleles (Table 4). For the remaining characters studied, KD/KR value was higher than 1, a strong indication that genes operating on these traits are dominant (Ceyhan & Kahraman 2013). Another factor that was examined in this research which, this means features manages. Therefore in research population, except pea count other features were more dominant than recessive genes.

For the effective number of genes (K), all the traits, except number of seed per pod, had K>1

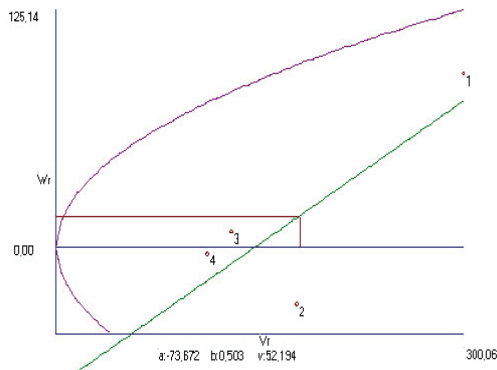


values (Table 4). This may show that value of K depends largely on dominance. It was reported that K value is associated with dominance and K is underestimated in cases where there is weak dominant effect on traits (Jinks 1954; Yildirim 1974; Ceyhan & Kahraman 2013).

Narrow sense (DH) and broad sense heritabilities (GH) have also been evaluated (Table 4). Low DH values were evident on all traits but 100 seed weight, indicating a low response to selection. The DH for 100 seed weight was 0.79, which would be rather responsive to selection.

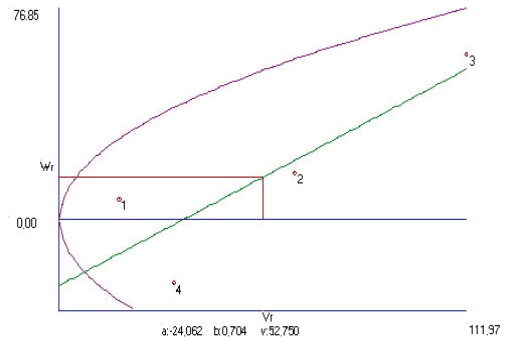
### 3.2. Analysis of $W_r$ , $V_r$ graphics

The  $W_r$  and  $V_r$  values of parents derived from the means of the 3 blocks are presented in Figure 1 to 6. The regression line for number of seed per pod cut through the  $W_r$  on positive side (above 0), indicating presence of a partial dominance for the trait. For all other traits studied, the regression line cut  $W_r$  on the negative region, showing over dominance are in effect. Ceyhan & Kahraman (2013) reported that hundred seed weight was under the control of partial dominant genes while seed yield, plant height, and number of pod were under control of over dominant genes.



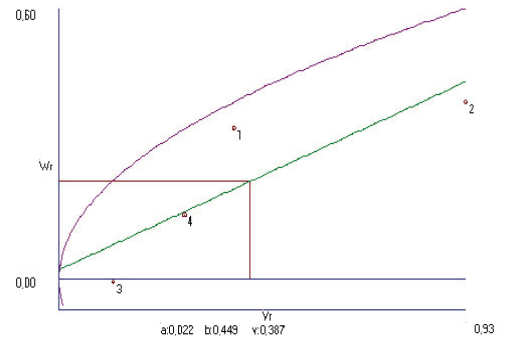
**Figure 1-  $W_r$ ,  $V_r$  values for the plant height of parents**

Şekil 1- Bitki boyu için ebeveynlere ilişkin  $W_r$ ,  $V_r$  değerleri



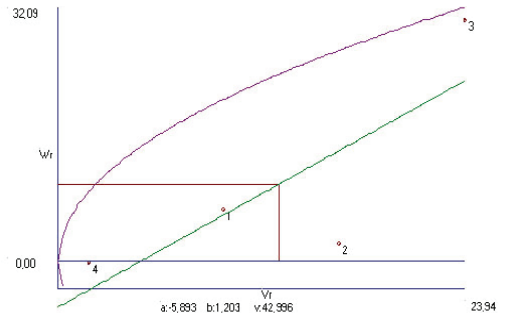
**Figure 2-  $W_r$ ,  $V_r$  values for the number of pod per plant of parents**

Şekil 2- Bakla sayısı için ebeveynlere ilişkin  $W_r$ ,  $V_r$  değerleri



**Figure 3-  $W_r$ ,  $V_r$  values for number of seed per pod of parents**

Şekil 3- Baklada tane sayısı için ebeveynlere ilişkin  $W_r$ ,  $V_r$  değerleri



**Figure 4-  $W_r$ ,  $V_r$  values for hundred seed weight of parents**

Şekil 4- Yüz tane ağırlığı için ebeveynlere ilişkin  $W_r$ ,  $V_r$  değerleri

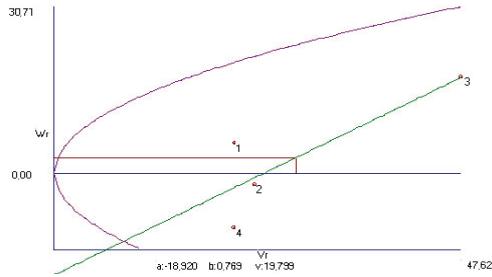


Figure 5-  $W_r$ ,  $V_r$  values for pod yield of parents

Şekil 5- Bakla verimi için ebeveynlere ilişkin  $W_r$ ,  $V_r$  değerleri

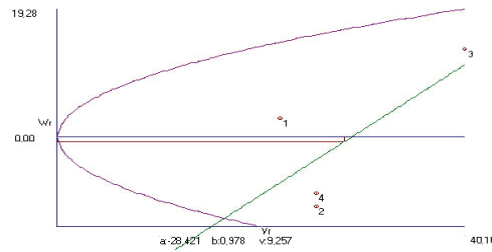


Figure 6-  $W_r$ ,  $V_r$  values for the seed yield of parents

Şekil 6- Tane verimi için ebeveynlere ilişkin  $W_r$ ,  $V_r$  değerleri

At the parental lines shortest plant height were 33.33 cm for PS4009 and PS3073 lines while tallest were Reyna with 46.33 cm. And the shortest hybrid was 42.33 cm (PS3053 x PS3073), and the tallest was 69.00 cm (PS4009 x PS3053) (Table 5).

In terms of plant height, there is no epistatic effect in parental genotypes PS4009, PS3073, and Reyna because they were close to the regression line, while PS3053 was far from regression line, indicative of an epistatic effect in action. Because PS4009 was far from the origin, it transfers recessive gene to crosses. A negative correlation ( $r = -0.613$ ) was found between the hypothetical dominance ( $W_r + V_r$ ) of parents and the average value of the ancestors.

When average number of pod of parents was examined, PS3073 parent had the lowest with 9.00 and Reyna the highest with 26.33. While the PS4009 x PS3053 cross had 21.67 the lowest, and Reyna x PS3053 cross 36.67 the highest (Table 5).

There is an epistatic effect for number of pod in PS4009 and Reyna. The crosses originating from parents with high pot numbers resulted with higher pod numbers. The correlation between hypothetical

Table 5- Agronomic values of the 4x4 full diallel pea cross populations

Çizelge 5- 4x4 tam diallel bezelye melez populasyonlarında bazı agronomik özelliklere ait değerler

Genotypes	Plant height	Number of pod	Number of seed per pod	100 seed weight	Pod yield	Seed yield
PS4009	33.33 <sup>d</sup>	19.33 <sup>fg</sup>	4.95 <sup>ab</sup>	14.49 <sup>de</sup>	14.51 <sup>c</sup>	12.55 <sup>c</sup>
PS3053	42.67 <sup>cd</sup>	17.00 <sup>g</sup>	5.45 <sup>a</sup>	16.55 <sup>cd</sup>	15.53 <sup>c</sup>	13.90 <sup>de</sup>
PS3073	33.33 <sup>d</sup>	9.00 <sup>h</sup>	5.00 <sup>ab</sup>	18.45 <sup>bc</sup>	13.52 <sup>c</sup>	11.74 <sup>e</sup>
Reyna	46.33 <sup>bed</sup>	26.33 <sup>edef</sup>	4.14 <sup>bc</sup>	28.97 <sup>a</sup>	22.70 <sup>cd</sup>	18.28 <sup>ed</sup>
Crosses						
PS4009 x PS3053	69.00 <sup>a</sup>	21.67 <sup>efg</sup>	4.98 <sup>ab</sup>	19.91 <sup>bc</sup>	20.91 <sup>d</sup>	19.48 <sup>c</sup>
PS4009 x PS3073	49.33 <sup>abcd</sup>	25.00 <sup>def</sup>	5.06 <sup>ab</sup>	18.66 <sup>bc</sup>	23.13 <sup>bcd</sup>	21.37 <sup>bc</sup>
PS4009 x Reyna	63.67 <sup>abc</sup>	27.67 <sup>bcde</sup>	4.12 <sup>bc</sup>	19.90 <sup>bc</sup>	23.14 <sup>bcd</sup>	20.65 <sup>bc</sup>
PS3053 x PS4009	65.00 <sup>ab</sup>	26.67 <sup>cde</sup>	4.47 <sup>abc</sup>	20.88 <sup>b</sup>	24.83 <sup>bcd</sup>	22.31 <sup>bc</sup>
PS3053 x PS3073	42.33 <sup>cd</sup>	28.67 <sup>bcde</sup>	4.19 <sup>bc</sup>	19.37 <sup>bc</sup>	23.56 <sup>bcd</sup>	21.06 <sup>bc</sup>
PS3053 x Reyna	43.67 <sup>bed</sup>	34.33 <sup>ab</sup>	3.51 <sup>c</sup>	12.21 <sup>c</sup>	20.95 <sup>d</sup>	13.14 <sup>e</sup>
PS3073 x PS4009	46.00 <sup>bed</sup>	28.33 <sup>bcde</sup>	4.58 <sup>ab</sup>	17.99 <sup>bcd</sup>	23.60 <sup>bcd</sup>	21.05 <sup>bc</sup>
PS3073 x PS3053	44.67 <sup>bed</sup>	30.67 <sup>abcd</sup>	4.26 <sup>bc</sup>	21.05 <sup>b</sup>	28.17 <sup>ab</sup>	24.89 <sup>b</sup>
PS3073 x Reyna	44.33 <sup>bed</sup>	29.67 <sup>abcd</sup>	4.35 <sup>bc</sup>	21.26 <sup>b</sup>	27.29 <sup>abc</sup>	24.95 <sup>b</sup>
Reyna x PS4009	51.67 <sup>abcd</sup>	27.33 <sup>bcde</sup>	4.48 <sup>abc</sup>	20.46 <sup>b</sup>	25.19 <sup>bcd</sup>	22.64 <sup>bc</sup>
Reyna x PS3053	64.00 <sup>abc</sup>	36.67 <sup>a</sup>	5.03 <sup>ab</sup>	17.90 <sup>bcd</sup>	32.65 <sup>a</sup>	29.88 <sup>a</sup>
Reyna x PS3073	54.67 <sup>abcd</sup>	33.33 <sup>abc</sup>	4.23 <sup>bc</sup>	19.33 <sup>bc</sup>	27.57 <sup>abc</sup>	24.59 <sup>b</sup>

dominance and mean values of parents was negative ( $r = -0.947$ ). This shows that they carry the dominant genes of high pot number.

When number of seed per pod was examined, Reyna had 4.14 peas the lowest, and PS3053 had 5.45 peas the highest counts. The PS3053 x Reyna cross possessed 3.51 peas while PS4009 x PS3073 cross had 5.06 peas per pod (Table 5).

For the number of seed per pod, the parents PS4009 and PS3073 had epistatic effects. Analysis ( $W_r + V_r$ ) indicated that the lower number of peas per pod was dominant in action ( $r = 0.582$ ).

The 100 seed weight ranged from 14.49 g (PS4009) to 28.97 g (Reyna) where PS3053 x Reyna cross had the lowest 100 seed weight with 12.21 g and PS3073 x Reyna cross the highest with 21.26 g (Table 5). The lower hundred seed weight trait (i.e. smaller seed size) was found to be dominant ( $r = 0.845$ ).

When approximate pod yield was examined, PS3073 parent and PS4009 x PS3053 cross had the lowest pod yields with 13.52 g and 22.91 g while Reyna parent and Reyna x PS3053 cross the highest with 22.70 g and 32.65 g, respectively (Table 5). Genetic analysis ( $W_r + V_r$ ) showed that high pod yield was dominant ( $r = -0.701$ ).

The seed yield of parents ranged from 11.74 g (PS3073) to 18.28 g (Reyna) and crosses from 13.14 g (PS3053 x Reyna) to 29.88 g (Reyna x PS3053) (Table 5). All of the parents except the PS4009 showed epistatic effect for seed yield. The parents with PS4009 and Reyna transferred dominant genes to the crosses while the parent with PS3073 transferred recessive gen. The negative correlation ( $r = -0.633$ ) between the theoretical dominance ( $W_r + V_r$ ) and means of the values in the population implied having dominant genes of the parents that are high yielded.

#### 4. Conclusions

A large enough genetic variation exists for the traits studied in the population. It was concluded that recessive genes for number of seed per pod, and

dominant genes for plant height, number of pod, 100 seed weight, pod yield and seed yield are in action. The presence of high DH for hundred seed weight shows that genetic gains are possible at early generation selection for the trait.

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## A Study of Some Physical Properties of Lentil and Wild Oat Weed and Parameters Affecting the Separation of Wild Oat Weed from Lentil by a Gravity Table Separator

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### ABSTRACT

The present study measured physical properties of lentil and wild oat weed; mass of 1000 seeds, true density, porosity and coefficient of static friction. A gravity table separator was used to separate wild oat weeds from lentil seeds. The gravity table separator had five adjustable parameters; longitudinal slope, latitudinal slope, amplitude of oscillation, frequency of oscillation and air velocity. The effect of these parameters was investigated in order to maximize the separation of wild oat weed from lentil. Results of tests indicated that an increase in latitudinal slope of the table from 0.5° to 1° and longitudinal slope from 1.5° to 2° resulted in increased separation of wild oat weed from lentil. At longitudinal slope of 2°, latitudinal slope of 1°, amplitude of oscillation of 5 mm, frequency of oscillation of 400 cycles min<sup>-1</sup>, and air velocity of 5.7 m s<sup>-1</sup>, the separation percentage was 37%. Finally, after determination of the most suitable settings for amplitude of oscillation and air velocity; using the information on longitudinal slope, latitudinal slope and frequency of oscillation of the table was used to calculate mathematical relations of separation percentage of wild oat weed from lentil clumps using Datafit Software.

Keywords: Gravity separator; Physical properties; Lentil; Wild oat

## Mercimek ve Yabani Yulaf Tohumlarının Kimi Fiziksel Özellikleri ve Gravite Masa Selektörü ile Yabani Yulaf Tohumlarının Mercimek Tohumlarından Ayrılmasını Etkileyen Özellikler Üzerine Bir Araştırma

### ESER BİLGİSİ

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### ÖZET

Bu çalışmada, mercimek ve yabani yulaf tohumlarının 1000 tane ağırlığı, gerçek yoğunluğu, porozitesi ve statik sürtünme katsayıları belirlenmiştir. Yabani yulaf tohumlarının mercimek tohumlarından ayrılmasında gravite masa selektörü



kullanılmıştır. Gravite masa selektörü ayarlanabilir boylam açısı, enlem açısı, amplitüd titreşim, titreşim frekansı ve hava hızı olmak üzere 5 özelliğe sahiptir. Yabani yulaf tohumunun mercimek tohumundan ayrılmasını en iyi hale getirmek üzere bu özelliklerin etkisi araştırılmıştır. Çalışmadan elde edilen sonuçlar masaya göre enlem açısının  $0.5^\circ$  ila  $1^\circ$ , boylam açısının ise  $1.5^\circ$  ila  $2^\circ$  artırılmasının yabani yulaf tohumlarının mercimek tohumlarından ayrılmasını artırdığını göstermiştir. Boylam açısının  $2^\circ$ , enlem açısının  $1^\circ$ , amplitüd titreşiminin 5 mm, titreşim frekansının 400 titreşim  $\text{dak}^{-1}$  ve hava hızının  $5.7 \text{ m s}^{-1}$  olması durumunda ayırma % 37 olmuştur. Sonuç olarak; en uygun amplitüd titreşim ve hava hızı ayarları belirlendikten sonra enlem ve boylam açı bilgileri Datafit programı kullanılarak yabani yulaf tohumlarının mercimek tohumlarından ayrılmasında matematik ilişkiler hesaplanmıştır.

Anahtar Kelimeler: Gravite selektörü; Fiziksel özellikler; Mercimek; Yabani yulaf

## 1. Introduction

Lentil (*Lens culinaris medic*) is an important and highly nutritious crop belonging to the family of legumes (Kaur et al 2014). Lentil is cultivated worldwide but competition with weeds is a problem affecting production and can reduce performance by more than 80% (Dhuppar et al 2013; Joshi et al 2013). Wild oat (*Avena ludoviciana*) is a major weed in lentil cultivation. Extensive research has been done to increase the purity of seeds and harvested beans. Some such research established physical and aerodynamic properties of crops. For example, Falconer (2003) reports a comparative study on traditional and modern methods of gravity separation for seed purity developed by a gravity separator. The investigation covered advantages, disadvantages, principles and variables of both methods. The study concluded that selection for the most appropriate separator for specific use should be based on empirical experiments, although analysis of parameters is essential for optimization of the separating operation in continuous production (Falconer 2003). In another study, Hollatz & Quick (2003) reported that feeding rates low, aerodynamic separation of grain from straw happening on the screen and at high feed rate of fine particles in the sieve to form a negative factor, loss of separation increases. In another study, Rasekh (2006) was used a gravity separator apparatus for separating of sunn pest-damaged wheat from undamaged wheat. Influence of (longitudinal and latitudinal slopes, oscillation frequency and amplitude and velocity of air) machine table parameters to obtain of maximum

separation of sunn pest-damaged wheat from undamaged wheat have been studies. Results showed that increase of latitudinal slope of table from  $0.5^\circ$  to  $2^\circ$  and reduction of longitudinal slope from  $5^\circ$  to  $3^\circ$ , along the frequency of oscillation 450 cycle  $\text{min}^{-1}$  and air velocity  $8 \text{ m s}^{-1}$ , increased separation of sunn pest-damaged wheat. In another study, Innocentini et al (2009) were experimentally investigated the dehulling process of cracked soybeans. The mean sauter diameter ( $d_{vs}$ ) of as-received material was 2.70 mm, with an average proportion of 95% meats and 5% hulls for a moisture content of 11.8%. The true densities of hulls and meats were 1090 and 1267  $\text{kg m}^{-3}$ , with  $d_{vs}$  of 2.11 and 2.74 mm, respectively. Hulls were mostly elutriated around  $2.7\text{-}4.5 \text{ m s}^{-1}$  and meats around  $9.1\text{-}13.7 \text{ m s}^{-1}$ . The overlap of terminal velocity profiles required a combination of pneumatic and sieving operations for optimized separation. The influence of particle concentration on continuous dehulling was investigated for three solid-to-air ratios (W/Q). The procedure that maximized particle separation was a sequence of pneumatic dehulling with  $v_s = 7.4\text{-}9.1 \text{ m s}^{-1}$  and  $W/Q = 1.05 \text{ kg}_{\text{solids}} \text{ m}^{-3} \text{ air}^{-1}$ , followed by screening of lifted material with sieve ASTM no. 6 and a final pneumatic separation of small hulls and meats at  $v_s = 3.9\text{-}4.1 \text{ m s}^{-1}$ . An industrial scale pneumatic dehuller was built and tested for  $W = 6973 \text{ kg h}^{-1}$ ,  $v_s = 7.6\text{-}8.2 \text{ m s}^{-1}$  and  $W/Q = 0.97 \text{ kg}_{\text{solids}} \text{ m}^{-3} \text{ air}^{-1}$ . The efficiency of the pneumatic device to remove hulls from the cracked soybean was very high, with the recovery of meats with purity around 99%. Kashi (2009) managed to separate wild oat weed from wheat by a gravity separator. The device

had 5 adjustable parameters (the table settings of latitudinal and longitudinal slope, oscillation frequency, oscillation amplitude and air velocity). It was reported that the maximum level of separation was 56.24% based on settings of longitudinal slope 4°, latitudinal slope 1°, frequency of oscillation of 450 cycles min<sup>-1</sup>, and oscillation amplitude of 7 mm. Results also showed a significant effect of crop type and humidity level on the physical properties of seeds of wheat and wild oat weed. Kashi (2009) maintained that density values of wild oat weed and wheat were 0.902 and 1.327 g cm<sup>-3</sup>, respectively and that bulk density evaluations were 0.439 and 0.810, respectively (Kashi 2009). In the research of Mollazade et al (2009) some physical and mechanical properties of cumin seed were obtained as moisture content variations from 7.24% to 21.38% d.b. Their results showed that increasing of moisture content was caused to increase the seed length (5.14 to 5.58 mm), width (1.33 to 1.55 mm), thickness (0.97 to 1.05 mm), arithmetic mean diameter (2.48 to 2.73 mm), and coefficient of static friction on the three surfaces: glass (0.48 to 0.77), galvanized iron sheet (0.36 to 0.73), and plywood (0.57-0.69). However, bulk density was found to decrease from 447.66 to 369.88 kg m<sup>-3</sup>, and rupture force, rupture energy along with seed length and width were found to decrease from 83.74 to 56.17 N, 132.95 to 84.47 N, 50.66 to 27.52 mJ, and 67.8 to 33.36 mJ, respectively. Many studies have also been done to determine physical and aerodynamic properties of agricultural crops including chickpea (Konak et al 2002), lentil (Amin et al 2004), raw and parboiled paddy (Reddy & Chakraverty 2004), sunflower (Gupta et al 2007), flaxseed (Coskuner & Karababa 2007), kokum (Sonawane et al 2014) and wild sunflower seeds (Perez et al 2007). The study of physical properties and separation of wild oat weed from lentil by a gravity separator is of extreme importance. Since no study has been done to date, in this work physical and aerodynamic properties of lentil and wild oat weed are investigated. Also, the effects of different parameters of a gravity separator and their influence on the separation of wild oat weed from lentil seeds are evaluated.

## 2. Material and Methods

In the present research, lentil samples were taken from farms in Ardebil Province (Bileh-Savar cultivar) and transferred to the seed technology laboratory at the University of Tehran. The initial moisture content of the lentil seeds and wild oat weed was determined by oven drying at 103 °C for 72 h (Bagherpour et al 2010). Physical properties of lentil and wild oat weed were calculated as follows. The mass of 1000 seeds and wild oat weed was measured using a digital balance with a measurement accuracy of 0.01 g. The true density of lentil and wild oat weed ( $P_t$ ) was determined by the fluid displacement method (toluene) by a certain mass of lentil and wild oat weed and using a graduated cylinder, according to Equation 1 (Varnamkhasti et al 2008).

$$P_t = \frac{m_t}{V_t} \quad (1)$$

Where;  $P_t$ , true density (g cm<sup>-3</sup>);  $m_t$ , sample mass (g);  $V_t$ , volume of the displaced fluid (cm<sup>3</sup>).

The bulk density of lentil and wild oat weed ( $P_b$ ) was determined by measuring the weight obtained from pouring lentil seeds and wild oat weeds from a height of 15 cm into a 500 mL glass beaker, according to Equation 2 (Owolarafe et al 2007).

$$P_b = \frac{m_b}{V_b} \quad (2)$$

Where;  $P_b$ , bulk density (g cm<sup>-3</sup>);  $m_b$ , bulk mass (g);  $V_b$ , volume of the used cylinder (cm<sup>3</sup>).

The porosity percentage of lentil and wild oat weed was calculated using bulk density and true density from Equation 3 (Aydin 2003).

$$\varepsilon = \left[ 1 - \frac{P_b}{P_t} \right] \times 100 \quad (3)$$

Where;  $\varepsilon$ , porosity.

The coefficient of static friction of lentil and wild oat weed was measured by an inclinometer and through frictional surfaces of galvanized iron sheet and particle board. The crop bulk was poured into a thin

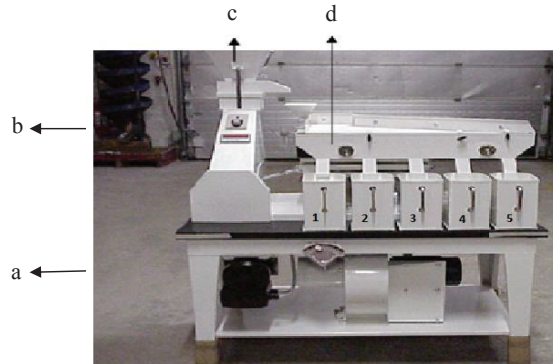
can with no lid or bottom end and placed on a surface. The can edge had no contact with the surface of the table and only the seeds inside were in contact with the surface under investigation. Tilt angle of the surface was increased gradually, where the device slope was measured and read with an accuracy of 1° once the slightest slip was observed in the can containing the materials. The coefficient of static friction was calculated by Equation 4 (Mohsenin 1986).

$$\mu_s = \tan \phi \quad (4)$$

Where;  $\mu_s$ , coefficient of friction;  $\phi$ , angle of tilt in degrees.

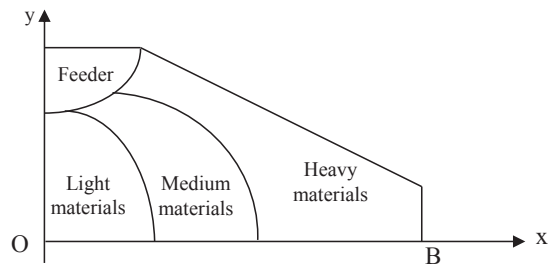
Tests were conducted to evaluate seed properties as follows; weight of 1000 seeds, true density, bulk density, porosity, and coefficient of static friction. Tests were done on lentil seeds and wild oat weed seeds based on a completely random design. The bulk of evaluated lentil had a purity of 68%, whereas wild oat weed accounted for 43% of the external materials together with the lentil bulk. A Laboratory Gravity Separator Type LA-K (Westrup A/S Denmark) was used to separate wild oat weed from lentil seeds (Figure 1). In this machine, table settings were as follows; longitudinal slope parameters (0-6° (in x-axis direction in Figure 2)), latitudinal slope (0-3°), air velocity (0-13.3 m s<sup>-1</sup>), frequency of oscillation (0-500 cycles min<sup>-1</sup>), and amplitude of oscillation (5, 7, 9, 11 mm), these settings were all adjustable. Similarly, the instrument had 5 boxes whereby, through proper adjustment, the heavier material was transferred toward the right side of the table (box number 5) and lighter material moved toward the left side (box number 1). Through proper adjustment of the main parameters of the instrument, the wild oat weed seeds were separated from the lentil seeds.

Figure 2 shows that materials of different weights were accommodated in different parts. The materials were poured onto the instrument table from inside the tank. The table height was increased in a positive direction of x (longitudinal slope) and positive direction of y (latitudinal slope). Thus, the right-side higher corner of the table (point S) had



**Figure 1- Gravity table separator; a, air velocity; b, frequency of oscillation; c, feeder; d, table surface of gravity separator**

*Şekil 1- Gravite masa selektörü; a, hava hızı; b, titreşim frekansı; c, besleyici; d, gravite selektörü masa yüzeyi*



**Figure 2- A schematic vertical view of the surface of the table and the approximate range of movement of materials on that surface**

*Şekil 2- Masa yüzeyinin şematik dikey görünümü ve materyallerin yüzeyde yaklaşık hareket mesafeleri*

the maximum height and the left-side lower corner of the table (point O) had the minimum height. The materials were free to leave the table along the OB side. The table had a reciprocating motion along the x-axis and then the underneath airstream hit the materials on the table. In response to table vibration and air force, the lighter materials floated above the heavier materials and were further separated due to longitudinal and latitudinal slopes of the table at the left-side lower corner. The heavier parts remained in contact with the table and were transferred over the longitudinal slope (the right-side lower corner of the

table) and were free to leave the table at that point in response to reciprocating motion of the table

In order to determine effects of parameters of table settings longitudinal slope, latitudinal slope and the frequency of oscillation on the separation percentage of wild oat weed from lentil seeds, tests were done according to a fully random, factorial design with the table settings at; latitudinal slope 0.5°, 1°, and 1.5°, longitudinal slope at 1.5°, 1.75°, and 2°, and frequency of oscillation at 380, 400, and 420 cycles min<sup>-1</sup>. Next, by having the desirable values of the three parameters obtained from this experiment, the instrument was set at the best values of these 3 parameters; longitudinal slope, latitudinal slope, and oscillation frequency. The impact of the parameters related to four air velocity settings of 3.6, 4.5, 5.7, and 6.6 m s<sup>-1</sup> and two amplitude frequencies of 5 and 7 mm, investigated in a completely randomized design with five replications for each test. In each test, some lentil seeds were collected together with external materials from the output of light seeds (box number 1). Then using an electronic seed counter, five groups of seed which each group containing 100 seeds were counted and selected. Next, each single seed was observed and the average for separation percentage of wild oat weed seeds from lentil seeds was determined for each replication and thus in each test. After determination of the most suitable table settings for amplitude of oscillation and air velocity using the information on longitudinal slope, latitudinal slope, and frequency of oscillation, the relations related to

calculation of the separation percentage of wild oat weed from lentil were derived by Datafit Software. Data analysis and comparison of means were done by Dunkin multi-domain mean comparison test using MSTAT-C Software.

### 3. Results and Discussion

Results for some physical properties of lentil and wild oat weed are shown in Table 1. Also the initial moisture content of the lentil seeds was 9.2% (wb) and wild oat weed was 10.1%.

Table 1 shows the results of the mean comparison data obtained from measuring such some of physical properties as mass of 1000 seeds, true density, bulk density, porosity and coefficient of static friction of lentil seeds and wild oat weed. As the mean mass of 1000 seeds of the lentil seeds had the maximum value (57.3 g) that the reason for this result is that the lentil seeds is larger than wild oat weed, the mean bulk density of the lentil seeds had the maximum value (1.214 g cm<sup>-3</sup>) that the reasons for this result are more sphericity, more regular grain placement, and smaller holes, the mean true density of the lentil seeds had the maximum value (0.782 g cm<sup>-3</sup>) that the reason for this result is the difference in cell structure and texture between the lentil seeds and wild oat weed, the mean porosity of wild oat weed had the maximum value (37.2%) that the reason for this result is that wild oat weed had the lower bulk density than lentil seeds, the mean the static coefficient of friction of wild oat weed had the maximum value

**Table 1- The results of mean comparison of some physical properties of lentil seeds and wild oat seed**

*Çizelge 1- Mercimek ve yabani yulaf tohumlarının kimi fiziksel özelliklerine ait ortalama değerler*

<i>Physical properties</i>	<i>Lentil seed</i>	<i>Wild oat seed</i>
Mass of 1000 seeds (g)	57.3 a*	5.69 b
True density (g cm <sup>-3</sup> )	1.214 a	0.857 b
Bulk density (g cm <sup>-3</sup> )	0.782 a	0.538 b
Porosity (%)	35.59 b	37.2 a
Coefficient of static friction (galvanized iron sheet (degrees))	0.39 a	0.32 b
Coefficient of static friction (particle board (degrees))	0.37 b	0.40 a

\*, different letters show significant differences at probability level of 1%



for surfaces of galvanized iron sheet (0.40%) and shows that the mean bulk density of the lentil seeds had the maximum value for surfaces of particle board (0.39%) that the reason for the difference in the quality of the frictional contact surfaces is the quality of wild oat weed and lentil seeds, and the different appearance of lentil seeds and wild oat weed. The geometric properties of a variety of products including amaranth seeds and faba bean grains were also examined (Mwithiga & Sifuna 2006; Jannatizadeh et al 2008). Like the results achieved by Bagherpour et al (2010), results of this study showed lentil mass of 1000 seeds (56.1 to 64.1 g), true density (1.194 to 1.330 g cm<sup>-3</sup>), bulk density (0.68 to 0.79 g cm<sup>-3</sup>), porosity (40.6 to 43.05%), and coefficient of static friction (galvanized steel (33%) and plywood (17%)) in moisture content from 8% to 20% (wb) (Bagherpour et al 2010).

The results of variance analysis of the factorial design with three factors of table settings; (longitudinal slope at 3 levels, latitudinal slope at 3 levels, and frequency of oscillation of the Table at 3 levels) are demonstrated in Table 2.

**Table 2- The results of variance analysis of separation percentage of wild oat weed from lentil with the factors including longitudinal slope, latitudinal slope, and frequency of oscillation**

*Çizelge 2- Enlem ve boylam açıları ile titreşim frekansı faktörlerine bağlı olarak yabani yulaf tohumlarının mercimek tohumlarından ayrılma oranına ilişkin varyans analiz sonuçları*

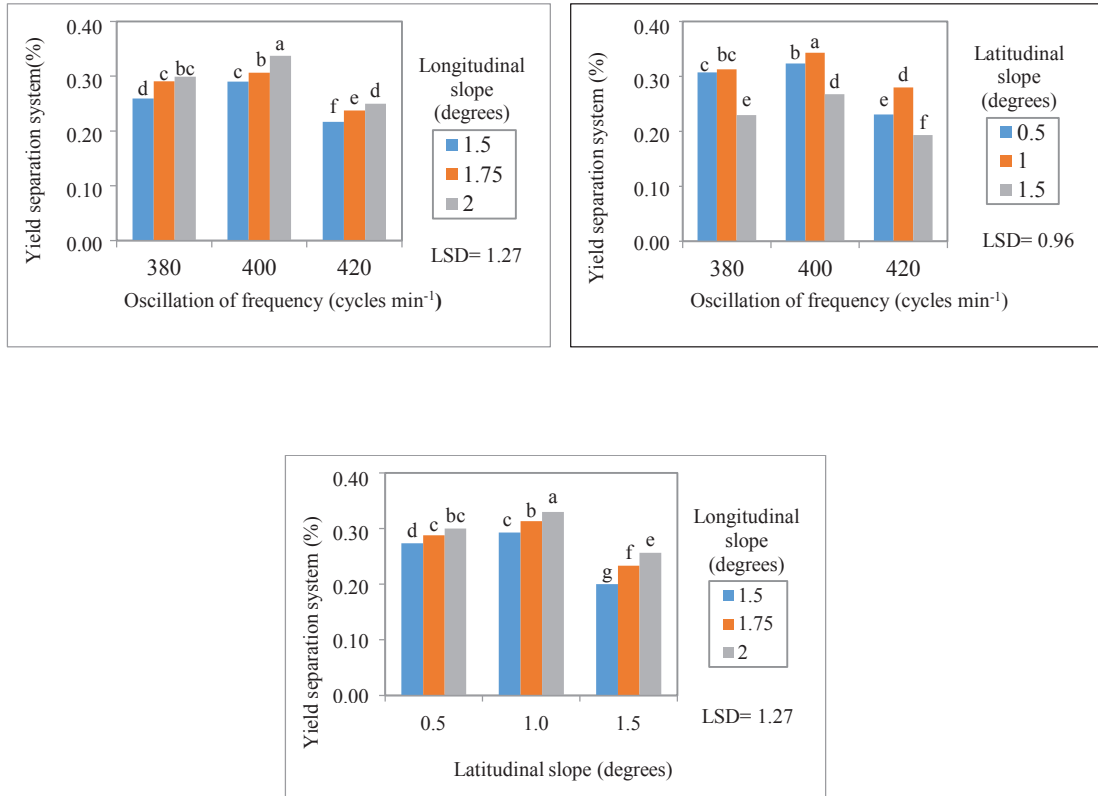
Source	Degrees of freedom	Mean square
Longitudinal slope (LO)	2	366.326**
Latitudinal slope (LA)	2	1590.770**
LO X LA	4	35.452**
Frequency of oscillation (F)	2	1353.170**
LO X F	4	24.358*
LA X F	4	94.607**
LO X LA X F	8	12.504 <sup>ns</sup>
Error	108	190
Total	134	

\*\* , significant at P<0.01; \* , significant at P<0.05; ns, not significant; CV= 4.8%

Table 2 shows the main effect of table settings; oscillation of frequency, latitudinal slope, and longitudinal slope, the mutual binary effect of latitudinal and longitudinal slope, the mutual binary effect of the latitudinal slope and the frequency of oscillation significant at a 1% and the mutual binary effect of the longitudinal slope and the frequency of oscillation significant at a 5%. However, the mutual triple effect of oscillation frequency, longitudinal slope, and latitudinal slope was not significant. Figure 3 demonstrates the mean comparison of two-fold interactive effects (the mutual binary effect of longitudinal and latitudinal slope of the table, the mutual binary effect of longitudinal and frequency of oscillation, and the mutual binary effect of latitudinal slope and frequency of oscillation).

Figure 3 illustrates that increased longitudinal slope from 1.5 to 2°, increased latitudinal slope from 0.5 to 1°, and elevated frequency of oscillation from 380 to 400 cycles min<sup>-1</sup> resulted in the development of separation percentage of wild oat weed along with lentil seeds. This was evident under all circumstances. The conditions were, frequency of oscillation was set at 400 cycles min<sup>-1</sup>, longitudinal slope was 2°, and latitudinal slope was 1°, the distribution of materials on the table was more homogeneous and resulted in better flotation of wild oat weed and lentil seeds and thus led to increased separation by the gravity separator. Furthermore, at the frequency of 420 cycles min<sup>-1</sup>, movement of seeds on the table surface was not homogenous, demonstrating decreased separation compared with the frequency of 400 cycles min<sup>-1</sup> because of disturbance in the movement of materials on the surface of the table. The results shown in Figure 3 demonstrate that at settings of longitudinal slope of 2°, latitudinal slope 1°, and frequency of oscillation 400 cycles min<sup>-1</sup>, the maximum separation was 37%. Results of Rasekh (2006) showed demonstrate that at settings of increase of latitudinal slope of table from 0.5° to 2° and reduction of longitudinal slope from 5° to 3°, along the frequency of oscillation 450 cycle min<sup>-1</sup> and air velocity 8 m s<sup>-1</sup>, increased separation percentage of sun pest-damaged wheat (Rasekh 2006).





**Figure 3- The results of the mean comparison of mutual binary effects; a, longitudinal slope and frequency of oscillation; b, latitudinal slope and frequency of oscillation; c, longitudinal slope and latitudinal**

*Şekil 3- Karşılıklı ikili değerlerin karşılaştırma sonuçları; a, boylam açısı ve titreşim sıklığı; b, enlem açısı ve titreşim sıklığı; c, boylam açısı ve enlem açısı*

The results of variance analysis of factorial design with two factors; table oscillation at two levels of 5 and 7 mm, and air velocity at 4 levels, 3.6, 4.5, 5.7 and 6.6 m s<sup>-1</sup> and at optimal conditions, the longitudinal slope of 2°, latitudinal slope of 1°, and frequency of oscillation of 400 cycles min<sup>-1</sup>, at the completely randomized design at 5 replications, are indicated in Table 3.

Table 3 clarifies that the main impact of table settings for air velocity and amplitude of oscillation together with the dual interactive effects of air velocity and amplitude of oscillation was significant at 1% level.

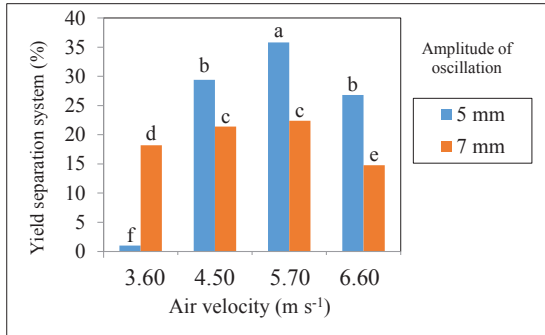
**Table 3- The results of variance analysis of separation percentage of wild oat weed from lentil with the factors of air velocity and amplitude of oscillation**

*Çizelge 3- Hava hızı ve amplitüd titreşime bağlı olarak yabani yulaf tohumlarının mercimek tohumlarından ayrılma oranına ilişkin varyans analizi sonuçları*

Source	Degree of freedom	Mean square
Amplitude of oscillation (AO)	1	164.0**
Air velocity (AV)	3	2147.7**
AV X AO	3	1544.5**
Error	32	86.8
Total	39	

\*\* , significant at less than 1% probability level; CV= 7.76%

Figure 4 shows the results of means comparison of the dual impact of air velocity and the amplitude of oscillation of the table.



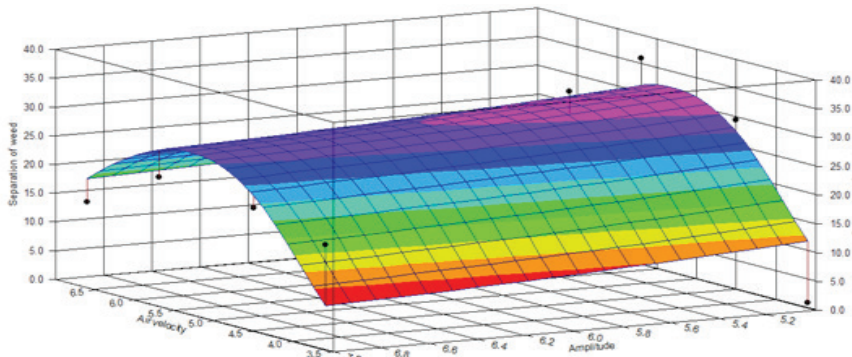
**Figure 4- The results of the mean comparison of mutual binary effects of air velocity and the amplitude of oscillation of the table for separation percentage of wild oat weed from lentil, LSD= 2.85**

Şekil 4- Hava hızı ve amplitüde titreşim karşılaştırma sonuçlarına göre yabancı yulaf tohumlarının mercimek tohumlarından tohumlarından ayrılma oranı, LSD= 2.85

Figure 4 demonstrates that the best settings for air velocity and amplitude of oscillation for separation of wild oat weed from lentil seeds were 5.7 m s<sup>-1</sup> and 5 mm, respectively. This was is due to the fact that under the stated conditions, distribution of seeds on the surface of the table (the height of seeds

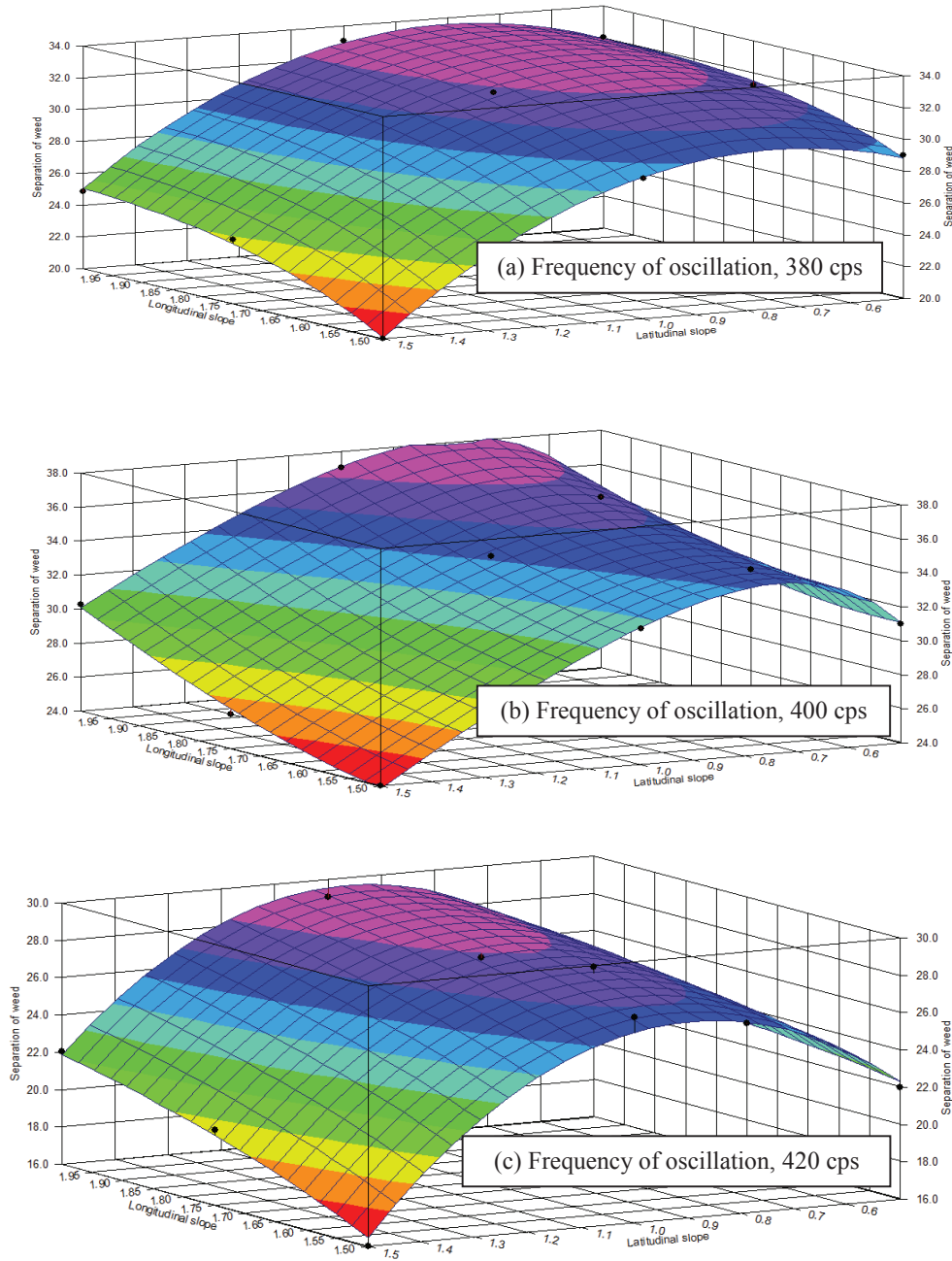
on the table) was more homogenous, because first, negative impact of the mutual triple effect of length, slope and frequency was the lowest and second, for a longer time on the surface of the table, thus the distribution of materials on the table Therefore, in response to the wind force blown on to the seeds from under the table, the flotation improved, and in turn, resulted in increased separation of wild oat weed from lentil seeds. Note that at the amplitude of oscillation of 7 mm, transference of materials towards the right side of the table output edge was halted and the surface of the table was not fully covered by the fed materials, this deceased separation. Results of Kashi (2009) showed that maximum level of separation of wild oat weed from wheat occurred under the mentioned conditions (the longitudinal slope of table 4°, latitudinal slope of table 2°, frequency of oscillation 450 cycle min<sup>-1</sup>, air velocity 5.7 m s<sup>-1</sup> and amplitude of oscillation of 7 mm) (Kashi 2009).

Figure 5 and 6 show results obtained from the values of air velocity (5.7 m s<sup>-1</sup>), amplitude of oscillation (5 mm), frequency of oscillation (380, 400, and 420 cycles min<sup>-1</sup>), longitudinal slope (1.5, 1.75, and 2°), and latitudinal slope (0.5, 1, and 1.5°), together with the most suitable mathematical relation, for determination of the separation percentage of wild oat weed from the lentil using Datafit Software.



**Figure 5- The extent of separation of wild oat weed from lentil bulk with air velocity and amplitude of oscillation of the table**

Şekil 5- Amplitüde titreşimi ve hava hızına göre yabancı yulaf tohumlarının mercimek tohumlarından ayrılma durumu



**Figure 6-** The extent of separation of wild oat weed from the lentil bulk with the longitudinal and latitudinal slopes under the conditions oscillation of frequency of the table

*Şekil 6- Titreşim frekansına bağlı olarak enlem ve boylam açılarına göre yabani yulaf tohumlarının mercimek tohumlarından ayrılma durumu*

Figure 5 shows that amplitude of oscillation (5 mm) and air velocity (5.7 m s<sup>-1</sup>) resulted in increased separation of wild oat weed from the lentil bulk.

Similarly, Figure 6 (a-c), indicate that increased longitudinal slope from 1.5° to 2°, latitudinal slope from 0.5°-1°, and frequency of oscillation from 380 to 400 cycles min<sup>-1</sup> led to better separation of wild oat weed from the lentil bulk. The results Figure 5 and Figure 6, shown demonstrate that at settings of air velocity 5.7 m s<sup>-1</sup>, amplitude of oscillation 5 mm, frequency of oscillation 400 cycles min<sup>-1</sup>, longitudinal slope 2°, and latitudinal slope 1°, the maximum separation percentage of wild oat weed from lentil bulk was 37%. This was is due to the fact that under the stated conditions, distribution of seeds on the surface of the table was more homogenous. Therefore, in response to the wind force blown on to the seeds from under the table, the flotation improved, and in turn, resulted in increased separation of wild oat weed from lentil seeds. Results of Rasekh (2006) showed that under the mentioned conditions (dimensionless number  $\frac{V}{a\omega} = 152.3$  (A dimensionless number  $(V/a\omega)$  which shows ration of intertie force of air current

blown to lentil to force arising from oscillation, was considered in ratio of separation.), latitudinal slope of table 2° and longitudinal slope 3°) the maximum separating percentage of sunnpest-damaged wheat from undamaged wheat was 95.14% (Rasekh 2006).

Table 4 summarizes the mathematical models predicted under different conditions of longitudinal slope of table ( $x_1$ ), latitudinal slope of table ( $x_2$ ), oscillation of frequency of table, amplitude of oscillation of table ( $x_3$ ), and air velocity ( $x_4$ ) with a high determination of coefficient.

#### 4. Conclusions

Physical properties obtained for lentil were as follows; weight of 1000 seeds (57.03 g), true density (1.214 g cm<sup>-3</sup>), bulk density (0.782 g cm<sup>-3</sup>), coefficient of static friction (galvanized iron sheet (0.394 degrees) and particle board (0.37 degrees)), porosity (35.59%), and the wild oat weed including weight of 1000 seeds (5.69 g), true density (0.857 g cm<sup>-3</sup>), bulk density (0.538 g cm<sup>-3</sup>), coefficient of static friction (galvanized iron sheet (0.32 degrees) and particle board (0.40 degrees)), porosity (37.2%).

**Table 4- The predicted mathematical models (longitudinal slope ( $x_1$ ), latitudinal slope ( $x_2$ ), air velocity ( $x_3$ ) and amplitude of oscillation ( $x_4$ ))**

*Çizelge 4- Boylam açısı ( $x_1$ ), enlem açısı ( $x_2$ ), hava hızı ( $x_3$ ) ve amplitüid titreşimini ( $x_4$ ) tahmin için matematik modeller*

	Predicted mathematical models	R <sup>2</sup>
Amplitude of oscillation (mm) and air velocity (m s <sup>-1</sup> )	$Y = -392.1 + \frac{5475.7}{x_4} - \frac{23505.9}{x_4^2} + \frac{31830.8}{x_4^3} + \frac{70.87}{x_3}$	0.60
380	$Y = 13.1 + \frac{132.6}{x_1} + 34.1x_2 - \frac{124.6}{x_1^2} - 17.7x_2^2 - 11\frac{x_2}{x_1}$	0.99
Frequency of oscillation (cycles min <sup>-1</sup> )	400 $Y = 46.85 - 24.3x_1 - 19.6(\ln(x_2)) + 9.6x_1^2 - 20.1(\ln(x_2^2)) = 4.9x_1(\ln(x_1))$	0.99
420	$Y = -5.9 + \frac{32.1}{x_1} + 64.7x_2 - \frac{23.8}{x_1^2} - 27.2x_2^2 - 24.3\frac{x_2}{x_1}$	0.98

Increased latitudinal slope of the table from 0.5° to 1° and longitudinal slope of table from 1.5° to 2° result in increased separation percentage of wild oat weed from lentil seeds.

Under the mentioned conditions (oscillation frequency setting of 400 cycle min<sup>-1</sup>, amplitude of oscillation of the Figure 3 mm, air velocity 5.7 m s<sup>-1</sup>, latitudinal slope 1° and longitudinal slope 2°), the maximum separation percentage was 37%. In this case, the output lentil seeds contained the least amount of wild oat weeds.

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## Physicochemical and Sensory Characteristics of Winter Yoghurt Produced from Mixtures of Cow's and Goat's Milk

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### ABSTRACT

Winter yoghurt is one of the most popular dairy products in Van and Hatay region of Turkey. It is made of different kinds of milk, and known as “cooked yoghurt” or “salted yoghurt” due to its high solids content and long shelf life. In this study, two different concentration methods were used to produce traditional winter yoghurt. Some physical (firmness and cohesiveness, *L*-, *a*- and *b*- values), chemical (total solid, fat, protein, pH, lactic acid, salt) and sensory analyses (color, odor, consistent and flavor) were made to determine the effect of production methods and storage on winter yoghurt samples at the 1<sup>st</sup>, 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> days. Significant differences were found between sensory, rheological properties and color values of winter yoghurts however no significant differences were found between physicochemical properties of the samples. Especially, winter yoghurts produced from 100% goat milk were the more appreciated samples.

Keywords: Winter yoghurt; Concentrated yoghurt; Goat's milk; Salt; Traditional dairy products

## İnek ve Keçi Sütü Karışımlarından Üretilen Kış Yoğurtlarının Fizikokimyasal ve Duyusal Karakteristikleri

### ESER BİLGİSİ

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### ÖZET

Kış yoğurdu Türkiye'nin Van ve Hatay illerinde en meşhur süt ürünlerinden biridir. Çeşitli sütlerden üretilen kış yoğurdu yüksek kurumadde içeriği ve uzun raf ömründen dolayı “pişmiş yoğurt” veya “tuzlu yoğurt” olarak bilinmektedir. Çalışmada kış yoğurdunun geleneksel olarak üretilmesi amacıyla iki farklı koyulaştırma yöntemi uygulanmıştır. Farklı üretim yöntemlerinin ve depolama süresinin kış yoğurdu örnekleri üzerine etkilerini belirlemek amacıyla 1., 30., 60. ve 90. günlerde bazı fiziksel (sertlik ve yapışkanlık, *L*-, *a*- ve *b*- değerleri), kimyasal (kurumadde, yağ, protein, pH, laktik asit, tuz) ve duyusal (renk, koku, kıvam ve lezzet) analizler yapılmıştır. Kış yoğurdu örneklerinin duyusal ve

reolojik özellikleri ile renk değerleri arasında önemli farklılıklar bulunmuş, bununla birlikte örneklerin fizikokimyasal özellikleri arasındaki farklar önemsiz olarak tespit edilmiştir. Özellikle % 100 keçi sütünden üretilen kış yoğurtları daha çok beğenilmiştir.

Anahtar Kelimeler: Kış yoğurdu; Konsantre yoğurt; Keçi sütü; Tuz; Geleneksel süt ürünleri

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## 1. Introduction

Elimination of yoghurt whey is one of the most important factors for keeping quality and extending the shelf-life of yoghurt. Traditional and new methods have been used in removing yoghurt whey for the manufacture of strained yoghurt. The strained yoghurt produced by using traditional method is preferred by consumers due to its sensory properties in Turkey. However modern methods of ultrafiltration and centrifugation have been employed to produce strained yoghurts (Tamime et al 1991; Şenel et al 2011). Many strained/concentrated milk products are manufactured by different methods in other countries. They are known as labneh or lebneh in the Middle East, leben zeer in Egypt, skyr in Iceland, chakka and shirkland in India, than or tan in Armenia and Ymer in Denmark (Nergiz & Seçkin 1998; Şenel et al 2011). Several types of strained/concentrated yoghurt have been produced traditionally in Turkey. These products are known as kurut, torba yoghurt, tulum yoghurt, kese yoghurt, peskuten and winter yoghurt. It is recommended that these yoghurts made from different types of milk may be considered an important source of phosphorus, calcium, magnesium, selenium and zinc over the regular yoghurts and whey products and, have a relatively high content of total solids (Güler & Sanal 2009; Kesenkaş 2010; Şenel et al 2011).

Among them, winter yoghurt is one of the most popular varieties of traditional dairy product manufactured in Hatay, Van and Sivas regions in Turkey. It has high total solid content and long shelf life. There are two procedures in the production of winter yoghurt. In the first procedure the set-type yoghurt is boiled (cooked) and then salt is added. The second procedure was the method which yoghurt whey is removed by using cloth bag, boiled, and then salt is added in order to shorten cooking time (Güler & Park 2009).

Because of its peculiar flavor and nutritional properties and its recognition as a healthy food, goat's milk attracts attention by dairy industry. Some properties of goat's milk are known to be advantageous compared with other milk varieties such as higher tolerance by allergic children and the high proportion of smaller fat globules, which provide better digestibility (Queiroga et al 2013).

Thus the aim of this study was to investigate the availability of goat milk for winter yoghurt production and to assess some quality parameters and sensory acceptability of this traditional product made from different mixtures of goat's and cow's milk, by two different concentration methods. For this purpose the difference between samples and the effect of storage period on physical, chemical and sensorial properties of winter yoghurts were investigated throughout 90 days storage.

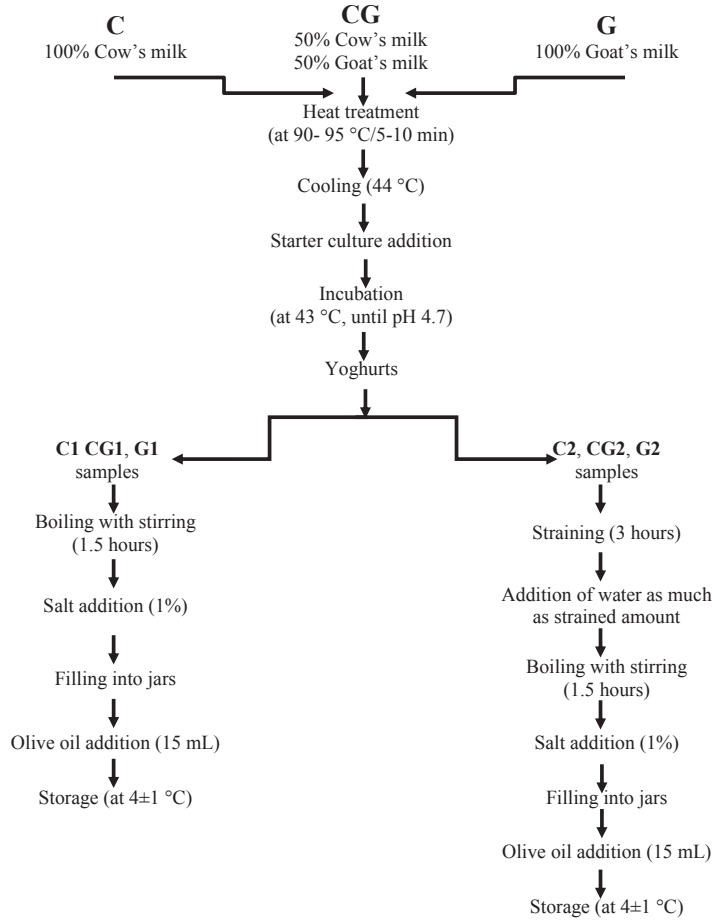
## 2. Material and Methods

### 2.1. Materials

Cow's and goat's milk was obtained from Ege University, Faculty of Agriculture and transferred directly to the pilot dairy plant of the Department of Dairy Technology (İzmir, Turkey). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (MYE 96-98 Maysa Istanbul, Turkey) were used as yoghurt starter cultures in accordance with the manufacturer's recommendation. Food grade NaCl (Estuz, Eskisehir, Turkey) was used for salting.

### 2.2. Winter yoghurt production

Winter yoghurt, samples were made by a traditional method in the pilot dairy plant (Figure 1). Six different yoghurt samples were made from 100% cow milk, 50-50% cow and goat milk and 100% goat



**Figure 1- Production of winter yoghurt samples**

*Şekil 1- Kış yoğurdu örneklerinin üretimi*

milk with using two different methods. Samples were grouped as C1: Winter yoghurt made with 100% cow milk only by boiling, C2: Winter yoghurt made with 100% cow milk by straining and boiling, G1: Winter yoghurt made with 100% goat milk only by boiling, G2: Winter yoghurt made with 100% goat milk by straining and boiling, CG1: Winter yoghurt made with 50% cow-50% goat milk only by boiling, CG2: Winter yoghurt made with 50% cow-50% goat milk by straining and boiling. All winter yoghurts were filled to glass jars (100 mL) after cooling and their upper surfaces were coated with olive oil. Winter yoghurts were stored at 4±1 °C for 90-days.

### 2.3. Physicochemical analyses

The total solids, fat, total protein, pH and lactic acid contents of cow's and goat's milk were determined according to the Association of Official Analytical Chemist methods (AOAC 2003). The total solid contents of samples were determined by gravimetric method using oven drying at 105 °C for 3 h (AOAC 2003). Protein was measured by Kjeldahl method. Fat and salt content were determined by the Gerber method and the titration method as described in Turkish standards, respectively (TSE 1999; 2001). The pH was determined with a pH meter (Hanna

Instruments, Portugal). The textural characterization (firmness and cohesiveness) of samples was carried out by using a Texture Analyzer (Brookfield Texture Analyzer TA-CT3, Middleboro, USA) with a 38 mm diameter probe (TA4/1000). The penetration of 10 mm was determined at following speeds: pre-test 2 mm s<sup>-1</sup>, test 1 mm s<sup>-1</sup> and post-test 10 mm s<sup>-1</sup>. A colorimeter (CR-300, Minolta Co., Japan) was used to determine whiteness/blackness (L-), red/greenness (a-), and yellow/blueness (b-) values of the strained yoghurts.

#### 2.4. Sensory evaluation

Sensory properties of yoghurt samples were evaluated according to Altuğ & Elmacı (2011). Sensory panel was carried out by six panelists who are members of the Dairy Technology Department. Samples were evaluated in terms of color, odor, consistent and flavor with using 5-point scale. Samples were left at the room temperature for 15 minutes and then they were served with a glass of water and an unsalted cracker to the panelists.

#### 2.5. Statistical analysis

One way analysis of variance (ANOVA) and Duncan's Multiple Range Test were applied in order to determine the differences between winter yoghurts and to estimate the effect of storage on samples. Also multivariate general linear model (GLM) ANOVA was carried out to investigate the two-way interactions (production method x storage). All statistical analyses were performed using the statistical software SPSS® 15.0 for Windows. In all cases, the 0.05 probability level was considered. All experiments and analyses were completed in duplicate.

### 3. Results and Discussion

The mean values for the chemical composition of cow's and goat's milk used to produce winter yoghurt was given in Table 1. The non-fat solid, fat, protein and lactic acid contents of goat's milk were found to be higher than cow's milk. Total solid, fat contents and pH values of the goat's milk were found to be higher than reported by Güler (2007) and Güler & Park (2009).

**Table 1- Chemical composition of raw cow and goat milk used in production**

Çizelge 1- Üretimde kullanılan çiğ keçi ve inek sütünün kimyasal kompozisyonu

	NFS <sup>1</sup> (g 100 g <sup>-1</sup> )	Fat (g 100 g <sup>-1</sup> )	Protein (g 100 g <sup>-1</sup> )	pH	Titration acidity (Lactic acid, %)
Cow milk	8.90±0.10	3.30±0.05	3.38±0.04	6.70±0.03	0.145±0.01
Goat milk	9.10±0.05	4.80±0.05	3.45±0.00	6.68±0.02	0.158±0.02

<sup>1</sup>, non-fat total solids

The total solid, fat and salt content of winter yoghurts were determined only at first day of storage (Table 2). Total solid of the yoghurt produced in our study varied between 25.36% and 36.53%. Differences between total solid contents of samples were significant (P<0.05) while CG1 and CG2 samples had closer results. Levels of total solids increased during winter yoghurt-making process. This is due to the removing of whey and evaporation of water as a result of cooking, and also to addition of salt. Especially total solid content were higher than those reported by Gönç &

Oktar (1973), Güler (2007), Güler & Park (2009) and Köse & Ocak (2011).

The fat content of winter yoghurts changed between 5.65% and 11.40%. Differences in the fat content between all samples were significant (P<0.05). It was found that fat contents of winter yoghurt samples produced only by boiling were significantly higher than others which were produced by straining and boiling but with the same milk mixture. On the other hand, it was seen that G1 and G2 sample which produced with goat's milk had higher fat content than C1 and C2 sample which

**Table 2- Chemical composition of winter yoghurt samples***Çizelge 2- Kış yoğurdu örneklerinin kimyasal kompozisyonu*

	Total solids (%)	Fat (%)	Salt (%)
C1	31.74±1.25 <sup>b</sup>	6.65±0.07 <sup>c</sup>	3.85±0.07 <sup>b</sup>
C2	25.36±0.73 <sup>a</sup>	5.65±0.07 <sup>a</sup>	4.05±0.07 <sup>c</sup>
G1	36.53±1.27 <sup>d</sup>	11.40±0.00 <sup>f</sup>	4.65±0.07 <sup>d</sup>
G2	34.51±2.10 <sup>cd</sup>	9.70±0.14 <sup>e</sup>	4.05±0.07 <sup>c</sup>
CG1	33.89±0.74 <sup>bc</sup>	9.05±0.07 <sup>d</sup>	2.95±0.07 <sup>a</sup>
CG2	32.75±1.09 <sup>bc</sup>	6.35±0.21 <sup>b</sup>	4.10±0.00 <sup>c</sup>

C1, winter yoghurt made with 100% cow milk only by boiling; C2, winter yoghurt made with 100% cow milk by straining and boiling; G1, winter yoghurt made with 100% goat milk only by boiling; G2, winter yoghurt made with 100% goat milk by straining and boiling; CG1, winter yoghurt made with 50% cow-50% goat milk only by boiling; CG2, winter yoghurt made with 50% cow-50% goat milk by straining and boiling; a-d, means in the same column with different superscripts among samples significantly differ (P<0.05)

produced with cow's milk. It was thought that these results depend substantially on the composition of milk.

As shown in Table 2 differences in the salt content between all samples of winter yoghurts were significant (P<0.05). The salt content of winter

yoghurts changed between 2.95% and 4.65%. The variation between salt contents can be attributed to evaporation differences during boiling. Güler (2007) indicated that salt content was not changed from the beginning to the end of storage in salted yoghurt samples which were added 2% salt. In our study, salt content of winter yoghurts were similar with Gönç & Oktar (1973), Biçer et al (1995) and Şahan & Say (2003).

### 3.1. pH and acidity

pH values and the changes occurred during the storage is given in Table 3. Production method and storage affected pH values and also a significant interaction was detected (P<0.05). It can be seen that at the beginning and the end of the storage the lowest pH value was determined for C1, while the highest pH value was determined for G1 at the beginning and for G2 on 90<sup>th</sup> day of the storage. During the storage, increases and decreases in pH values was determined, this irregular pH changes were also determined by Köse & Ocak (2011) in winter yoghurt which was stored for 180 days. Furthermore similar pH values were also found by Nergiz & Seçkin (1998), Kırdar & Gün (2002) and Ersöz et al (2011). The highest lactic acid value was

**Table 3- Acidity of winter yoghurt samples during 90-days storage***Çizelge 3- Kış yoğurdu örneklerinin 90 günlük depolama sırasındaki asitlik değerleri*

	Samples	Days			
		1	30	60	90
pH	C1*	3.93±0.06 <sup>aZ</sup>	3.95±0.01 <sup>bZ</sup>	3.46±0.02 <sup>aX</sup>	3.77±0.06 <sup>Y</sup>
	C2	4.15±0.00 <sup>bZ</sup>	4.12±0.03 <sup>cZ</sup>	3.65±0.07 <sup>bX</sup>	3.85±0.07 <sup>Y</sup>
	G1	4.23±0.04 <sup>bZ</sup>	4.08±0.02 <sup>eY</sup>	4.15±0.07 <sup>dYZ</sup>	3.93±0.04 <sup>X</sup>
	G2	4.19±0.02 <sup>bY</sup>	4.19±0.00 <sup>dY</sup>	4.22±0.03 <sup>dY</sup>	3.94±0.05 <sup>X</sup>
	CG1	4.00±0.07 <sup>aY</sup>	3.69±0.01 <sup>aX</sup>	3.99±0.05 <sup>cY</sup>	3.80±0.00 <sup>X</sup>
	CG2	4.11±0.02 <sup>bZ</sup>	4.12±0.03 <sup>cZ</sup>	3.58±0.02 <sup>abX</sup>	3.93±0.04 <sup>Y</sup>
Titration acidity (%)	C1	1.98±0.01 <sup>c</sup>	1.97±0.01 <sup>c</sup>	1.98±0.00 <sup>c</sup>	1.88±0.04 <sup>c</sup>
	C2	1.27±0.00 <sup>aX</sup>	1.36±0.00 <sup>aZ</sup>	1.32±0.00 <sup>aY</sup>	1.34±0.01 <sup>aYZ</sup>
	G1	2.24±0.00 <sup>dY</sup>	1.60±0.09 <sup>bX</sup>	2.39±0.00 <sup>fZ</sup>	2.25±0.03 <sup>dYZ</sup>
	G2	1.95±0.02 <sup>cXY</sup>	1.98±0.01 <sup>eYZ</sup>	2.01±0.02 <sup>dZ</sup>	1.92±0.00 <sup>cX</sup>
	CG1	2.35±0.04 <sup>eZ</sup>	2.17±0.01 <sup>dX</sup>	2.27±0.00 <sup>eY</sup>	2.23±0.00 <sup>dXY</sup>
	CG2	1.59±0.02 <sup>b</sup>	1.51±0.08 <sup>b</sup>	1.55±0.00 <sup>b</sup>	1.62±0.02 <sup>b</sup>

\*, for C1, C2, G1, G2, CG1 and CG2 please refer to Table 2; means in the same row with different superscripts upper-case letter (X, Y and Z) significantly differ (P<0.05); means in the same column with different superscripts lower case letter (a-f) among yoghurt samples significantly differ (P<0.05)



found for G1 on the 60<sup>th</sup> day of the storage, while initially the lowest lactic acid value was found for C2. As with the pH values irregular increases or decreases were determined in lactic acid content. Lactic acid amounts of the products vary depending on the activity of culture flora, non-fat solid and fat content of yoghurt samples. A similar nonlinear content of lactic acid amounts were reported by Köse & Ocak (2011). Moreover average lactic acid content is consisted with the studies of Atamer et al (1988), Kırdar & Gün (2002), Ersöz et al (2011) and Mısırlılar et al (2012).

### 3.2. Textural characteristics

Table 4 shows the textural properties of winter yoghurt samples during 90 days of storage. Storage time significantly affected the firmness of winter yoghurt samples except for sample G1 and CG2, and the differences between samples were also significant at all storage periods ( $P<0.05$ ). There was also a significant ( $P<0.05$ ) production method x storage interaction. The firmness values in all samples showed a decrease on 60<sup>th</sup> day however increased again on 90<sup>th</sup> day of storage. Köse & Ocak (2011) reported similar fluctuations in firmness values in

winter yoghurt samples. Seçkin & Özkılınç (2011) also observed a decrease in firmness of concentrated yoghurt samples on 14<sup>th</sup> day and then an increase on the 21<sup>st</sup> day, and they attributed this to increased water holding capacity of proteins with storage.

Moreover the firmness of yoghurt depends on the total solid content of the product and also on protein-protein interactions (Ekinci & Gürel 2008). Our results are in accordance with this phrase because winter yoghurt samples of higher total solids (Table 2) generally had more firmness and cohesiveness values. On the other hand the difference between cohesiveness values of samples were significant at all periods but the storage period was only effective on sample G2, CG1 and CG2 ( $P<0.05$ ). The interaction effect on cohesiveness values were also significant ( $P<0.05$ ). According to the findings obtained from winter yoghurt samples produced only by boiling (C1, G1, CG1), cohesiveness values were generally higher than those of produced by straining and then boiling (C2, G2, CG2).

### 3.3. Color

There is very little information about the *L*, *a*, *b* parameters of goat's milk and fermented milk products

**Table 4- Textural properties of winter yoghurt samples during 90-days storage**

*Çizelge 4- Kış yoğurdu örneklerinin 90 günlük depolama sırasındaki tekstürel özellikleri*

	Samples	Days			
		1	30	60	90
Firmness (g)	C1*	516.25±43 <sup>bY</sup>	536.75±11 <sup>bY</sup>	390.75±15 <sup>dX</sup>	629.00±7 <sup>bcZ</sup>
	C2	256.50±35 <sup>aX</sup>	531.50±93 <sup>bY</sup>	462.25±25 <sup>eY</sup>	422.50±38 <sup>abY</sup>
	G1	427.25±88 <sup>b</sup>	727.75±107 <sup>c</sup>	313.75±39 <sup>c</sup>	702.75±193 <sup>bc</sup>
	G2	251.50±38 <sup>aX</sup>	502.75±68 <sup>abY</sup>	268.75±30 <sup>bcX</sup>	600.25±47 <sup>bcY</sup>
	CG1	568.75±91 <sup>bY</sup>	631.25±8b <sup>cY</sup>	229.00±21 <sup>abX</sup>	725.75±176 <sup>cY</sup>
	CG2	189.25±37 <sup>a</sup>	347.75±44 <sup>a</sup>	179.75±21 <sup>a</sup>	240.25±59 <sup>a</sup>
	Cohesiveness (g)	C1	27.25±7 <sup>ab</sup>	45.25±13 <sup>b</sup>	22.00±3 <sup>a</sup>
C2		10.75±15 <sup>a</sup>	24.00±7 <sup>a</sup>	13.25±1 <sup>a</sup>	13.25±1 <sup>a</sup>
G1		59.49±48 <sup>ab</sup>	207.00±62 <sup>c</sup>	86.00±16 <sup>c</sup>	222.75±95 <sup>c</sup>
G2		79.75±3 <sup>bX</sup>	162.50±27 <sup>bcY</sup>	74.50±0 <sup>cX</sup>	158.95±14 <sup>bcY</sup>
CG1		170.75±12 <sup>cY</sup>	179.75±30 <sup>cY</sup>	70.50±13 <sup>cX</sup>	204.00±38 <sup>cY</sup>
CG2		68.25±11 <sup>bX</sup>	95.50±7 <sup>abY</sup>	49.00±0 <sup>bX</sup>	64.25±14 <sup>abXY</sup>

\*, for C1, C2, G1, G2, CG1 and CG2 please refer to Table 2; means in the same row with different superscripts upper-case letter (X, Y and Z) significantly differ ( $P<0.05$ ); means in the same column with different superscripts lower case letter (a-c) among yoghurt samples significantly differ ( $P<0.05$ )

produced by using goat's milk. The color values of winter yoghurt samples, which play an important role in consumer acceptance, are shown in Table 5.

Whiteness in fluid milk results from the presence of colloidal particles, such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum (Garcia-Perez et al 2005). Production method and storage time (except for sample C1) showed no effect to *L* values (whiteness/lightness values) of winter yoghurts. The interaction effect of these two factors on all color values was also insignificant. In the first day of the storage while the highest *L* value was determined for CG1 sample, the lowest was determined for G1. In the 90<sup>th</sup> day of the storage while the highest value was determined for CG2 sample, the lowest value was determined for G1 sample also at the beginning of the storage. Besides, the whiteness values of all samples increased throughout the storage. The differences

of whiteness/lightness values of yoghurts arise from fragmentation of fat globule diameters due to the result of continuous mixing during processing; furthermore it shows these values can be different for the dairy products produced by using goat's milk. Yazıcı & Akgün (2004) determined higher *L* values of low-fat torba yoghurts than high-fat torba yoghurts; moreover they found that usage of fat substitute does not affect the *L* values of samples. The negative *a* value (greenness) was determined for all samples and the differences between samples were significant at 1<sup>st</sup>, 30<sup>th</sup> and 90<sup>th</sup> days of storage ( $P<0.05$ ). The storage was significantly affected *a* values of the samples ( $P<0.05$ ) except for sample C1 and G2. In the first day of the storage while the highest *a* value was found for G1 sample, the lowest was found for CG1. At the end of the storage, the highest value was determined for G1 again, whereas the lowest was determined for G2. These differences occurring in *a* value of samples may be

**Table 5- Color values of winter yoghurt samples during 90-days storage**

*Çizelge 5- Kış yoğurdu örneklerinin 90 günlük depolama sırasındaki renk değerleri*

		Days			
Samples		1	30	60	90
<i>L</i>	C1*	70.70±2.40 <sup>X</sup>	64.96±3.39 <sup>X</sup>	69.60±4.21 <sup>X</sup>	82.03±1.52 <sup>Y</sup>
	C2	72.53±3.51	73.10±3.86	69.87±9.22	85.15±0.70
	G1	69.33±4.85	76.52±10.23	66.59±4.56	72.77±1.70
	G2	72.23±7.86	75.44±6.05	83.41±1.79	83.87±1.52
	CG1	80.24±9.58	71.05±4.13	73.46±4.55	85.15±0.43
	CG2	74.79±7.53	76.34±4.67	76.52±4.93	85.35±1.42
	<i>-a</i>	C1	-1.26±0.41 <sup>bc</sup>	-2.34±0.09 <sup>c</sup>	-1.89±0.62
C2		-1.71±0.09 <sup>abcW</sup>	-2.80±0.03 <sup>bY</sup>	-3.02±0.02 <sup>X</sup>	-1.96±0.10 <sup>bcZ</sup>
G1		-0.98±0.26 <sup>eY</sup>	-2.41±0.04 <sup>bcX</sup>	-2.92±0.24 <sup>X</sup>	-1.38±0.31 <sup>eY</sup>
G2		-1.69±0.74 <sup>abc</sup>	-3.26±0.07 <sup>a</sup>	-3.01±0.42	-2.70±0.43 <sup>a</sup>
CG1		-2.62±0.25 <sup>aXY</sup>	-3.29±0.26 <sup>aX</sup>	-3.25±0.13 <sup>X</sup>	-2.28±0.25 <sup>abY</sup>
CG2		-2.18±0.21 <sup>abZ</sup>	-3.39±0.09 <sup>aX</sup>	-3.26±0.52 <sup>XY</sup>	-2.42±0.31 <sup>abYZ</sup>
<i>+b</i>		C1	12.72±0.48 <sup>cX</sup>	13.15±0.69 <sup>cX</sup>	12.25±0.82 <sup>X</sup>
	C2	8.95±0.46 <sup>a</sup>	8.78±0.08 <sup>a</sup>	9.55±0.91	10.68±0.46
	G1	11.16±0.61 <sup>b</sup>	13.06±1.04 <sup>c</sup>	11.68±0.98	13.70±0.55
	G2	9.32±0.48 <sup>a</sup>	11.23±0.68 <sup>b</sup>	11.46±0.31	14.87±3.86
	CG1	10.95±0.91 <sup>b</sup>	10.77±0.39 <sup>b</sup>	10.74±0.47	11.95±0.68
	CG2	8.21±0.46 <sup>a</sup>	9.09±0.07 <sup>a</sup>	9.53±1.18	10.82±1.55

\*, for C1, C2, G1, G2, CG1 and CG2 please refer to Table 2; means in the same row with different superscripts upper-case letter (W-Z) significantly differ ( $P<0.05$ ); means in the same column with different superscripts lower case letter (a-c) among yoghurt samples significantly differ ( $P<0.05$ )

due to the lack of a fully homogenous distribution of the olive oil which was added according to the production method. Yazıcı & Akgün (2004) have reported using fat substitute does not affect *a* value, but storage time effects. When *b* values (yellowness/blueness) were analyzed, it was seen that significant differences were occurred on the 1<sup>st</sup> and 30<sup>th</sup> day of the storage ( $P < 0.05$ ). While the highest *b* value was determined for C1 initially, the lowest was determined for C2 at the end of storage. Additionally for all samples, *b* values have increased during the storage but this was found statistically insignificant except for sample C1. Because of the production method, heat treatment at the high temperature causes caramelisation of

lactose which is the main carbohydrate of milk, and leads maillard reaction by interacting reactions with proteins. This effects yellowness values of samples, too. Yazıcı & Akgün (2004) reported increasing the fat proportion of Torba yoghurt samples give rise to the *b* values, as well as the storage time effects the *b* values.

#### 3.4. Sensory properties

The sensory properties of winter yoghurt samples were given in Table 6. In winter yoghurt samples, statistically no significant changes were determined in production method and storage regarding color-appearance and odor. Furthermore the interaction effect of these two factors on sensory properties

**Table 6- Sensory properties of winter yoghurt samples during 90-days storage**

*Çizelge 6- Kış yoğurdu örneklerinin 90 günlük depolama sırasındaki duyuusal özellikleri*

	Samples	Days			
		1	30	60	90
Color - appearance	C1*	4.00±0.47	3.17±0.23	3.75±0.35	3.66±0.71
	C2	3.50±0.71	3.83±0.24	4.35±0.03	4.08±0.35
	G1	4.83±0.24	3.75±0.59	4.39±0.38	4.08±0.82
	G2	4.66±0.00	4.67±0.47	4.69±0.45	4.25±0.12
	CG1	4.00±0.00	4.00±0.47	4.25±0.35	4.00±1.18
	CG2	3.83±0.71	4.50±0.23	4.42±0.12	4.75±0.35
Odor	C1	4.00±0.47	3.33±0.00	4.13±0.18	4.00±0.00
	C2	4.16±0.71	4.50±0.23	4.17±0.94	4.16±0.71
	G1	4.83±0.24	3.75±0.83	4.29±0.06	4.16±0.00
	G2	4.33±0.47	4.17±0.23	4.33±0.47	4.50±0.23
	CG1	4.13±0.18	4.17±0.23	4.19±0.26	4.25±0.35
	CG2	4.00±0.94	4.50±0.23	4.17±0.94	3.75±0.83
Consistency	C1	3.00±0.00 <sup>a</sup>	3.58±0.11	3.75±0.12	3.50±0.71
	C2	2.83±0.2 <sup>4a</sup>	4.08±0.11	3.67±0.23	3.75±0.59
	G1	4.83±0.24 <sup>c</sup>	4.33±0.47	4.75±0.35	4.08±0.35
	G2	4.67±0.47 <sup>c</sup>	4.67±0.47	4.58±0.59	4.00±0.00
	CG1	3.75±0.35 <sup>b</sup>	4.00±0.23	4.17±0.23	4.00±0.47
	CG2	3.83±0.24 <sup>b</sup>	4.25±0.12	4.25±0.35	4.50±0.24
Flavor	C1	3.42±0.12 <sup>a</sup>	2.92±0.12	3.69±0.26	3.41±0.35
	C2	3.29±0.06 <sup>a</sup>	3.75±0.59	3.75±0.59	3.58±0.59
	G1	4.13±0.18 <sup>bc</sup>	3.92±0.59	4.17±0.23	3.67±0.94
	G2	4.71±0.06 <sup>d</sup>	4.33±0.47	4.25±0.35	4.00±0.00
	CG1	3.71±0.06 <sup>ab</sup>	3.92±0.12	3.79±0.65	3.67±0.94
	CG2	4.31±0.44 <sup>cd</sup>	4.41±0.35	3.33±0.47	3.83±0.47

\*, for C1, C2, G1, G2, CG1 and CG2 please refer to Table 2; means in the same column with different superscripts lower case letter (a-d) among yoghurt samples significantly differ ( $P < 0.05$ )

of winter yoghurt samples was also insignificant. The reason for G1 and G2 samples produced from goat's milk getting high color-appearance points on the 1<sup>st</sup> day of storage was the whiter color of goat's milk. The excessive darkening of the color in the further days of the storage was assessed negatively by the panelists. Winter yoghurt has a unique, characteristic odor. Panelists have detected the olive oil odor most as the foreign odor which was used for protecting the samples. Throughout the storage process, our samples received an acceptable appreciation, and this is an important criterion in terms of being free of foreign odors. In a previous similar study (Ocak et al 1998), panelists have detected a burnt odor. Our experiences during the conduct of this research showed that intensity of the burner flame, Benmari technique and continuous stirring during cooking process have prevented this defect. Regarding consistency, the production method was found to be statistically effective only on the 1<sup>st</sup> day of the storage ( $P<0.05$ ). The changes in consistency points of our samples in our research is thought to result from the milk type and total solid content of the milk, acidity of the samples and the changes occurred during cooking. Similar results were also reported in studies by Coşkun & Şimşek (1994), Akın & Konar (1997), Ocak et al (1998), Say & Şahan (2002) and Tokatlı (2011).

One of the most important criteria that determine the quality of yoghurt is flavor and aroma. Raw material quality, faults occurring during production and storage cause undesired flavor and aroma. Regarding flavor, production method statistically have a significant effect on the 1<sup>st</sup> day of the storage ( $P<0.05$ ), this difference was not determined on the other days of the storage. During the sensory analysis throughout the storage, the highest flavor point was received by G2 sample. Winter yoghurt samples produced from cow's milk (C1 and C2) received lower points throughout the whole storage. Uysal & Gönç (1998) have determined that the total points received by torba yoghurt produced from goat's milk were higher than those received by torba yoghurts produced from cow's milk.

#### 4. Conclusions

The boiling/cooking process increased the total solids content of winter yoghurt samples about 2-2.5-fold in comparison with the regular yoghurt. Especially, in the further days of the storage, the samples that were produced from 100% goat's milk were found to have higher quality regarding both sensory and rheological properties. Therefore, it could be assumed that goat milk can be used easily to produce traditional winter yoghurt. In addition modern production methods, high quality raw milk, pure culture usage and appropriate storage conditions can increase the safety, quality and the marketing potential of the winter yoghurt.

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## Evaluation of Some Physical, Chemical and Sensory Properties of Kasar Cheese and Its Processed and Analogue Types

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### ABSTRACT

The objective of this study was to evaluate the changes in physical, chemical and sensory properties of the kasar cheese and its processed and analogue types during 90-day storage. Aroma-active compounds in the cheeses were identified by gas chromatography-olfactometry. Sensory properties of the cheeses were determined by descriptive sensory analysis. Differences in basic composition of the cheeses were found. There were also significant differences among the cheeses in terms of meltability, oil separation index,  $L^*$  and  $a^*$  values. Whey, cooked, creamy and fermented were the most intense sensory descriptors in the cheeses analyzed on days 1 and 30. However, waxy and animal intensities were much higher in analogue cheese than others. 18 aroma-active compounds were determined in the cheeses during 90 day storage. Diacetyl, acetic acid, butyric acid, 2-/3-methyl butyric acid, 2-acetyl-2-thiazoline,  $\beta$ -ionene, 2-phenylethyl alcohol, maltol, *p*-cresol, sotolon,  $\delta$ -decalactone and  $\gamma$ -dodecalactone were some of the characteristic aroma-active compounds. Kasar cheese was the most liked cheese while analogue cheese was the least liked cheese by consumers.

Keywords: Analogue; Kasar and processed cheeses; Flavor

## Kaşar Peyniri, Eritme ve Taklit Tiplerinin Bazı Fiziksel, Kimyasal ve Duyusal Özelliklerinin Değerlendirilmesi

### ESER BİLGİSİ

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### ÖZET

Bu çalışmanın amacı, kaşar, eritme kaşar ve taklit kaşar peynirlerinin, 90 günlük depolama süresince fiziksel, kimyasal ve duyusal özelliklerinde meydana gelen değişiklikleri değerlendirmektir. Peynirlerin aroma-aktif bileşenleri gaz kromatografi-olfaktometri ile saptanmıştır. Peynirlerin duyusal özellikleri tanımlayıcı duyusal analiz ile belirlenmiştir. Peynirlerin temel bileşenlerinde farklılıklar bulunmuştur. Peynirler arasında eriyebilirlik, yağ ayrılma indeksi,  $L^*$  ve

*a\** değerleri açısından önemli farklılıklar gözlenmiştir. Peynirlerde 1. ve 30. günde peynir altı suyu, pişmiş, kremamsı ve fermente en yoğun tanımlayıcı terimlerdir. Ancak taklit peynirde, mumsu ve hayvanımsı aroma yoğunlukları diğer peynirlerden daha fazladır. Peynirlerde 90 günlük depolama süresince 18 aroma-aktif bileşen belirlenmiştir. Karakteristik aroma-aktif bileşenlerin bazıları; diasetil, asetik asit, bütirik asit, 2-/3-metil bütirik asit, 2-asetil-2-tiazolin, β-iyonen, 2-feniletıl alkol, maltol, *p*-kresol, sotolon, δ-dekalakton ve γ-dodekalakton'dur. Kaşar tüketiciler tarafından en çok sevilen peynir olurken, taklit Kaşar ise en az beğenilen peynir olmuştur.

Anahtar Kelimeler: Taklit; Kaşar ve eritme peynirler; Lezzet

## 1. Introduction

Many types of processed and analogue cheeses have entered to the market due to increase in the usage of cheese as a major ingredient in various foods (pizza, hamburger etc.) and food service (salad bar etc.) over the past few decades (Awad et al 2004; Kapoor & Metzger 2008). It was emphasized that processed cheese was the leader (243 million kg) in the total super market in the USA (Kapoor & Metzger 2008). Processed cheese is made by mixing of cheese curd and other ingredients such as emulsifying salts, water, salts etc., while analogue cheese is produced by the mixtures of dairy and/or non-dairy proteins (rennet casein, soy protein) and dairy fat or vegetable oils (corn, sunflower oils). During the production of both cheeses, heating and mixing processes are applied to obtain a homogeneous cheese structure with an extended shelf life. Functional properties of both cheeses (meltability, microstructure, viscosity, stretching ability etc.) are the most important factors for the performance of the cheese which contribute to the taste and the aesthetic appeal of the food during preparation and consumption. Functional properties of both cheeses were influenced by the ingredients used in the cheese formulation and processing conditions (Bachmann 2001; Guinee 2002; Kapoor & Metzger 2008).

Kasar cheese is a pasta filata-type cheese and consumed in a large scale after the White cheese in Turkey. It is estimated that 80,000 tons of Kasar cheese is produced in Turkey annually. Kasar cheese is commonly made from pasteurized cow milk in industrial scale. However, traditionally it can be made from the mixture of sheep's and cow's milk (Atasoy 2008; Hayaloglu 2009; Yasar & Guzeler

2011). Scalding and kneading are the most important stages to form characteristic properties of the cheese. Kasar cheese has smooth, dry and yellowish rind, homogenous structure without gas holes, its flavor is piquant and slightly salty (Ucuncu 2004). Nowadays, processed cheese technology has been used in the production of Kasar cheese. Two approaches have been commonly used for this purpose, the first one is the addition of melting salts instead of scalding of the curd after coagulation of milk by rennet enzyme. The other one is using rennet casein and other additives (melting salts, cream, vegetable oil, water etc.) instead of pasteurized milk (Koca & Metin 2004; Balkir & Metin 2011). Both types of cheeses are commonly used in pizzas, prepared foods, salad, sandwich, pasta, sauces and toast.

The effects of processing parameters, storage conditions and packaging types on physical and chemical properties of Kasar cheese had been extensively studied (Sert 2004; Tarakci & Kucukoner 2006; Var et al 2006; Urkek 2008; Yasar & Guzeler 2011). However, its processed and analogue types have not been discussed in terms of chemical, physical and sensory properties and characteristic aroma-active compounds in details. The objectives of this study were to evaluate and compare the changes in physical, chemical and sensory properties of the kasar cheese and its processed and analogue types during storage.

## 2. Material and Methods

### 2.1. Materials

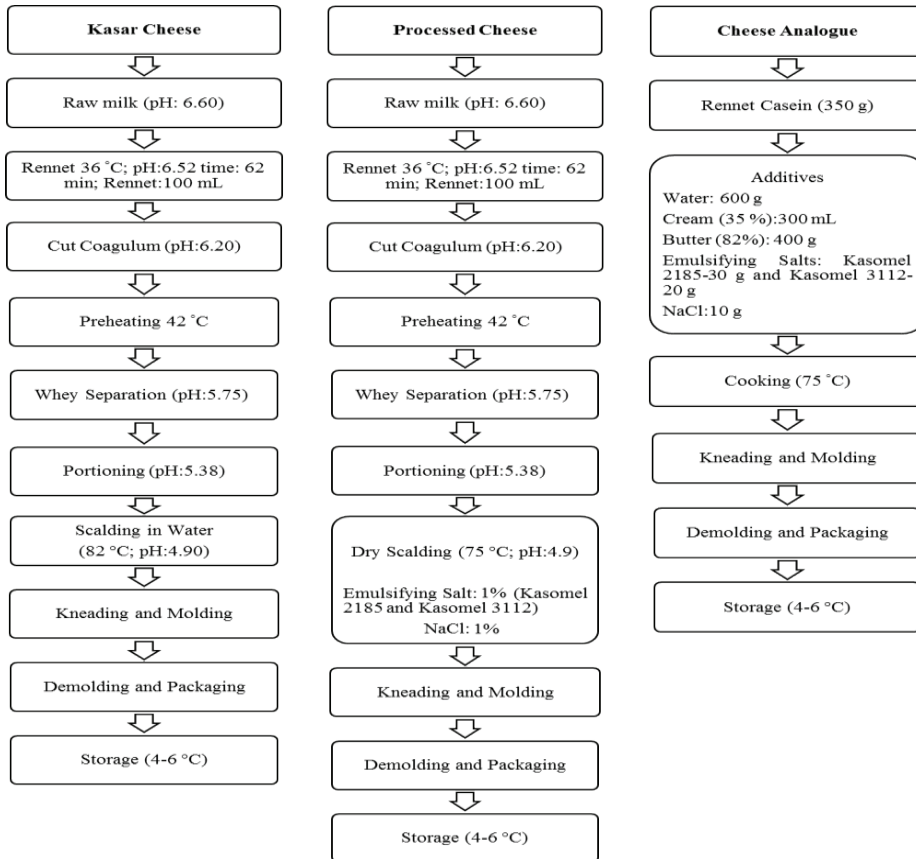
Three types of cheese formulation were evaluated in the present study. They were kasar cheese,

processed cheese and cheese analogue. All cheeses were produced in a dairy plant located in Biga Vocational School, Department of Dairy Technology, (Canakkale Onsekiz Mart University, Canakkale, Turkey). In production of the cheeses, cow milk was obtained from milk producers in Biga region. Rennet casein (DSE 7943, 90 mesh) and melting salts (Kasomel™ 2185, Kasomel™ 3112) were obtained from Fonterra (Rellingen, Germany) and Ozgocerler Dairy Company (Adana, Turkey), respectively. All chemicals used in this study were analytical and chromatographic grades (Merck, Darmstadt, Germany).

## 2.2. Cheese making procedure and composition of cheeses

Kasar cheese was produced by following the traditional production steps. Processed and analogue cheeses were produced by in-house production procedures. The production steps of the cheeses were given in Figure 1.

Titrateable acidity (% lactic acid), pH, dry matter (%) and ash (%) contents of the cheeses were determined by Bradley et al (1992). Nitrogen (%) content of the cheeses was determined by AOAC (2000). Fat (%) content was determined by Gerber method (NEN 1969). Mohr method was used for determination of salt (%) (Metin 2006).



**Figure 1- Cheese production steps**

*Şekil 1- Peynir üretim aşamaları*

### 2.3. Physical analysis

Hardness and melting properties of cheeses were evaluated. Hardness of cheeses was determined by TA.XTPlus texture analyzer (Stable Micro Systems Ltd, Surrey, UK) with 2 mm diameter cylinder P2 probe (stainless steel). The hardness test was carried out on 15×15×15 mm of cheese sample at 20 °C. Test parameters were; pre-test speed of 1.0 mm s<sup>-1</sup>, test speed of 2.0 mm s<sup>-1</sup>, post-test speed of 1.0 mm s<sup>-1</sup>. Hardness value of cheese, which was expressed a maximum force required to compress the cheese, was calculated from TPA curve using Texture Exponent software (Okpala et al 2010). Melting test was determined by two methods (Schreiber test and tube test) (Koca & Metin 2004). Oil separation index of cheeses was also determined (Awad et al 2004). Minolta Cr-400 (Minolta Co. Ltd, Tokyo, Japan) was used to measure *L\**, *a\**, and *b\** color parameters of cheese samples. Color measurements were taken from three different locations of each sample (ISO 2008).

### 2.4. Analysis of aroma-active compounds

Solid phase microextraction (SPME) procedure was used for extraction of aroma compounds (Pawliszyn 2012). Five grams of grated cheese samples were placed in 40 mL amber colored and screw top vial with hole cap PTFE/silicon septa (Supelco, Bellafonte, US) and then 1 g of NaCl was added. Samples were kept at 40 °C in water bath for 30 min to improve mass transfer between cheese matrix and headspace. 2 cm-50/30 µm DVB/Carboxen/PDMS stable flex SPME fiber (Supelco, Bellafonte) was inserted at a depth of 2 cm for extraction of volatile compounds from headspace for 15 min.

Aroma-active compounds of cheese samples were determined by using gas chromatography-olfactometry (GCO) (Agilent Technologies, HP 6890 GC, Wilmington, De, USA). Sniffing was achieved on a polar capillary column (HP-INNOWAX (30m length x 0.25 mm id x 0.25 µm film thickness (d<sub>f</sub>); J & W Scientific, Folsom, CA, USA). The GC oven temperature was programmed from 40 °C to 200 °C at a rate of 10 °C min<sup>-1</sup>, with initial hold of 3 min and final hold time of 20 min. The FID and sniffing port

were maintained at the temperatures of 250 °C and 200 °C, respectively. Post peak intensity method was used for determination of aroma intensities by using 10-point scale anchored on the left with 'not' and on the right with 'very' (Van Ruth 2001). Aroma-active compounds were identified by comparing retention indices (RI) and odor quality of unknowns with those of authentic standards analyzed at the same experimental conditions by sniffer during GCO procedure. Retention indices of aroma-active compounds were calculated by using n-alkane series (Van den Dool & Kratz 1963).

### 2.5. Sensory analysis

Sensory descriptors of the cheese samples were developed by using Spectrum™ method. Four female and two male panelists (26-42 years of age) were selected from Department of Food Engineering at Canakkale Onsekiz Mart University to identify flavor attributes of cheese samples. 60 hours of training sessions were performed to familiarize descriptive terms. The terms used to define taste and flavor were shown in Table 1. Panelists quantified the intensities of the attributes using 15-point scale anchored on the left with 'not' and on the right with 'very'. Panelists used water to rinse their mouth (Meilgaard et al 1999). Consumer test was conducted by using seven point hedonic scale to determine liking of cheese samples by 48 panelists (Meilgaard et al 1999).

### 2.6. Statistical analysis

Analysis of variance (two way ANOVA) was conducted to determine the differences among the cheeses with respect to chemical, physical and sensory properties (Sheskin 2000). The ANOVA model is shown in Equation 1.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk} \quad (1)$$

Where;  $Y_{ijk}$ , observed or measured value of k<sup>th</sup> the cheese in cheese type i and storage time j;  $\mu$ , general population mean;  $\alpha_i$ , effect of cheese type (i= 1, 2, 3);  $\beta_j$ , effect of storage time (j= 1, 2, 3);  $(\alpha\beta)_{ij}$ , effect of cheese type by storage time interaction;  $e_{ijk}$ , random error term.

**Table 1- Descriptive terms used for sensory evaluation of cheeses**

Çizelge 1- Peynirlerin duyuşal deęerlendirmesinde kullanılan tanımlayıcı terimler

Descriptors	Definitions	References*
Cooked	Cooked milk related aromatics	Heated milk (85 °C 10 min)
Whey	Whey related aromatics	Fresh whey
Creamy/milk fat	Milk fat related aromatics	Cream
Waxy	Wax related aromatics	Wax
Animal	Sheep, cow etc. related aromatics	Na-caseinate
Fermented	Fermented milk related aromatics	Yogurt
Nutty	Hazelnut or peanut related aromatics	Roasted hazelnut
Sour	Taste sensation elicited by acids	0.05% w v <sup>-1</sup> citric acid solution
Salty	Taste sensation elicited by salts	0.02% w v <sup>-1</sup> NaCl solution
Sweet	Taste sensation elicited by sugars	2% w v <sup>-1</sup> sugar solution
Umami	Chemical feeling factor elicited by certain peptides and nucleotides	0.5% w v <sup>-1</sup> monosodium glutamate solution

\*, reference adapted from Meilgaard et al (1999)

The Tukey honestly significant difference (HSD) test was used for separating means Cheese samples were also evaluated by Multidimensional scaling method (MDS) in terms of sensory differences of cheese samples. Results of consumer test were evaluated by non-parametric Kruskal-Wallis and Friedman tests. Dunn test was used for determination of significant differences in non-parametric tests. The results of differences from control test which evaluated by Welch because of the results did not meet the prerequisites (homogeneity of variance and equality of variance) for ANOVA (Sheskin 2000) Minitab for Windows (version 13.0), SPSS 2006 (SPSS 2006) for Windows (version 17.0), NCSS 2007 and MSTAT-C statistical packages were used for all statistical analyses.

### 3. Results and Discussion

#### 3.1. Basic composition

Basic composition of cheeses was determined on the first day of storage. Chemical composition of the cheeses was shown in Table 2. While significant differences were observed among the cheeses in terms of protein (%), fat (%) and ash content (%) ( $P < 0.05$ ), there were no significant differences in total dry matter (%) and salt content (%) of the cheese samples. The highest protein content was observed in kasar cheese, whereas the lowest value was observed in analogue cheese ( $P < 0.01$ ). Moreover, the highest fat and ash contents were determined in analogue cheese, but both values were the lowest in kasar cheese. Our findings were supported by

**Table 2- Composition (mean±SE) of cheese samples**

Çizelge 2- Peynir örneklerinin kompozisyonu (ortalama±standart hata)

Composition (%)	Kasar cheese	Processed cheese	Cheese analogue	P value
Dry matter	50.68±0.97	48.75±3.20	50.74±0.61	0.74
Protein	26.86±0.61 <sup>A*</sup>	21.49±1.15 <sup>B</sup>	17.98±0.01 <sup>B</sup>	0.01
Fat	21.50±0.25 <sup>B</sup>	24.00±1.00 <sup>B</sup>	29.75±1.00 <sup>A</sup>	0.01
Salt	1.16±0.13	0.89±0.14	0.87±0.01	0.26
Ash	3.24±0.69 <sup>B</sup>	3.55±0.16 <sup>AB</sup>	4.15±0.02 <sup>A</sup>	0.04

\*, means in the same attribute followed by different superscripts (A-B) letter represent significant differences among cheeses ( $P < 0.05$ ); SE, standard error



other researchers (Piska & Stetina 2004; Sert 2004; Dimitreli et al 2005; Curuk 2006; Yasar 2007; Balkir & Metin 2011). While Karademir-Sanlı (2006) indicated that dry matter of kasar cheese changed between 46.03-49.66%, Koca (2002) showed dry matter content of low fat kasar cheese in between 45.13-52.0%. In a study conducted by Balkir & Metin (2011), imitatiton fresh kasar cheese was prepared from casein, caseinates and soy protein. The cheese had 46.34-52.22% of dry matter, 22.99-27.49% of fat, 20.05-24.32% of total protein, 1.19-2.15% of salt and 3.18-3.77% of ash.

Changes in pH and titratable acidity were shown in Table 3. Significant interaction was determined between storage time and cheese type in terms of pH and titratable acidity. pH values of kasar cheese was lower than other cheese samples during the storage (P<0.05). Specifically anoluge cheese had higher pH values than other two cheeses. For titratable acidity, processed cheese samples had higher values than other cheeses until 30 day storage. Similar

pH results were reported by Bunka et al (2009) and Urkek (2008) for kasar cheese and processed cheese. However, higher titratable acidities were reported for kasar and analogue cheese by other researchers (Gursoy 2000; Oztekin 2003; Oksuztepe et al 2009). Oksuztepe et al (2009) showed that titratable acidity of kasar cheese was average 0.42% (lactic acid), whereas, Oztekin (2003) reported that titratable acidity of kasar cheese changed between 0.80-0.87% (lactic acid).

### 3.2. Color measurements

Color measurements of cheese samples were shown in Table 4. Specifically,  $L^*$  and  $b^*$  values are important color parameters for cheeses. No significant interaction between cheese type and storage time was determined for color values (P>0.05). Cheese type had significant effect on  $L^*$  (P= 0.02) and  $a^*$  (P= 0.01) values.  $L^*$  values of processed and analogue cheeses were higher than kasar cheese. The highest  $a^*$  value was determined in processed cheese. Cheese analogue had the lowest  $a^*$  value (Table 4).

**Table 3- pH and titratable acidity (% lactic acid) (mean±SE) of cheeses during storage**

Çizelge 3- Depolama boyunca peynirlerin pH ve titrasyon asitliği (% laktik asit) (ortalama±standart hata)

Day	Kasar cheese		Processed cheese		Cheese analogue	
	pH	Titratable acidity	pH	Titratable acidity	pH	Titratable acidity
1	5.52±0.06 <sup>Ac*</sup>	0.43±0.33 <sup>Bb</sup>	5.97±0.06 <sup>ABb</sup>	0.73±0.04 <sup>Ba</sup>	7.39±0.02 <sup>Aa</sup>	0.41±0.10 <sup>Bb</sup>
30	5.07±0.05 <sup>Bc</sup>	0.52±0.18 <sup>Ab</sup>	5.80±0.02 <sup>Bb</sup>	1.33±0.13 <sup>Aa</sup>	6.93±0.09 <sup>Ba</sup>	0.51±0.11 <sup>Ab</sup>
90	5.63±0.07 <sup>Ac</sup>	0.35±0.21 <sup>Ca</sup>	6.03±0.01 <sup>Ab</sup>	0.43±0.01 <sup>Ca</sup>	6.37±0.01 <sup>Ca</sup>	0.44±0.04 <sup>Ba</sup>
P value	0.01		0.01		0.01	

\*, means in the same attribute during storage followed by different superscripts (A-C) letter represent significant differences (P≤0.05) and means in the same attribute of different cheeses in the same day followed by different lowercase letters (a-c) represent significant differences (P≤0.05); SE, standard error

**Table 4- Color values (mean± SE) of cheese samples during storage**

Çizelge 4- Depolama boyunca peynir örneklerinin renk değerleri (ortalama±standart hata)

Day	Kasar cheese			Processed cheese			Cheese analogue		
	$L^*$ value	$a^*$ value	$b^*$ value	$L^*$ value	$a^*$ value	$b^*$ value	$L^*$ value	$a^*$ value	$b^*$ value
1	83.38±0.05	-3.31±0.17	15.78±1.37	83.33±2.95	-4.11±0.03	16.75±0.40	83.09±0.96	-2.67±0.15	18.07±4.26
30	81.28±0.53	-2.79±0.34	13.37±1.18	82.17±0.98	-3.36±0.02	15.00±0.38	84.15±0.22	-2.09±0.09	15.97±2.84
90	80.95±0.49	-4.48±0.09	17.32±0.20	86.68±1.22	-3.98±0.44	17.03±0.11	88.14±0.00	-3.42±0.29	20.68±3.44
Overall	81.86±0.35 <sup>C*</sup>	-3.52 ± 0.20 <sup>B</sup>	15.49 ± 0.91	84.06±1.71 <sup>B</sup>	-3.81 ± 0.16 <sup>A</sup>	16.26 ± 0.29	85.12 ± 0.39 <sup>A</sup>	-2.72 ± 0.17 <sup>C</sup>	18.24 ± 3.51

\*, means in the same color attribute in different cheeses followed by different superscripts (A-C) represent significant differences (P≤0.05); SE, standard error

$L^*$ ,  $a^*$  and  $b^*$  values determined in natural, process and analogue cheeses in the present study are consistent with the findings of other studies (Gursoy 2000; Awad et al 2004; Firat 2006; Dervisoglu & Gul 2011). Awad et al (2004) found that  $L^*$ ,  $a^*$  and  $b^*$  values of processed Ras cheese changed between 82.9-85.8, 3.9-4.1 and 20.1-21.4, respectively. In a study by Gursoy (2000), it was found that analogue cheeses prepared with 10% soya milk had -3.22, -3.03, -2.24 of  $a^*$  values in 1<sup>st</sup>, 30<sup>th</sup> and 45<sup>th</sup> days of storage.

### 3.3. Textural properties of cheeses

Table 5 shows the changes of textural properties of cheese samples during storage. Meltabilities determined by tube (P= 0.01) and by Schreiber test (P= 0.01), oil separation indexes (P= 0.02) and hardness of the cheeses (P= 0.01) were significantly affected by cheese type. The lowest hardness was determined in kasar cheese while processed cheese had the highest hardness. The lowest meltability scores were determined in cheese analogue. Moreover, the lowest oil separation index was found in processed cheese. Kasar cheese and analogue

cheese samples have similar oil separation indexes (P>0.05, Table 5). Similar results were reported by Koca (2002), Koca & Metin (2004) and Altan et al (2005).

### 3.4. Aroma-active compounds of cheeses

Total 18 aroma compounds were defined in cheese samples (Table 6). Diacetyl, acetic acid, butyric acid, 2-acetyl-2-thiazoline, 2-phenylethyl alcohol, maltol, sotolon,  $\delta$ -decalactone and  $\gamma$ -dodecalactone had higher intensities than other aroma compounds in the cheeses. While acetic acid was determined at high intensities in processed cheese samples on days 1 and 90, it was determined at very low intensity (0.5) in analogue cheese in the first day of storage.  $\beta$ -Ionene was only found in analogue cheese. Maltol intensity was higher in kasar cheese than processed cheese on day 30.  $\gamma$ -dodecalactone was only found at higher intensity in processed cheese on day 90.  $\delta$ -Decalactone was determined in all cheese samples. However, the intensity of  $\delta$ -decalactone was higher in analogue cheese than the other cheeses on days 30 and 90. These might be results of some ingredients (cream and butter) used to produce analogue cheese.

**Table 5- Textural properties (mean $\pm$ SE) of cheese samples during storage**

Çizelge 5- Depolama boyunca peynir örneklerinin tekstürel özellikleri (ortalama $\pm$ standart hata)

Day	Hardness (gram force)	Schreiber test (mm)	Tube test (cm)	Oil separation index
<i>Kasar cheese</i>				
1	4.83 $\pm$ 0.27	17.88 $\pm$ 1.50	13.82 $\pm$ 2.13	152.9 $\pm$ 76.5
30	4.79 $\pm$ 0.20	21.13 $\pm$ 2.00	12.25 $\pm$ 0.70	133.8 $\pm$ 16.2
90	4.56 $\pm$ 0.06	18.50 $\pm$ 1.62	12.72 $\pm$ 0.47	197.1 $\pm$ 14.7
Overall	4.72 $\pm$ 0.18 <sup>C*</sup>	19.17 $\pm$ 1.70 <sup>A</sup>	12.93 $\pm$ 1.1 <sup>A</sup>	161.26 $\pm$ 35.80 <sup>A</sup>
<i>Processed cheese</i>				
1	78.85 $\pm$ 3.85	17.00 $\pm$ 0.25	9.65 $\pm$ 4.10	38.24 $\pm$ 2.94
30	64.4 $\pm$ 2.09	18.81 $\pm$ 1.94	12.90 $\pm$ 1.15	58.82 $\pm$ 5.88
90	50.82 $\pm$ 2.38	15.63 $\pm$ 3.13	7.90 $\pm$ 3.20	82.4 $\pm$ 41.2
Overall	64.69 $\pm$ 2.77 <sup>A</sup>	17.14 $\pm$ 1.77 <sup>A</sup>	10.15 $\pm$ 2.81 <sup>A</sup>	59.82 $\pm$ 16.67 <sup>B</sup>
<i>Cheese analogue</i>				
1	38.16 $\pm$ 3.17	0.65 $\pm$ 0.219	0.43 $\pm$ 0.22	70.59 $\pm$ 5.88
30	22.50 $\pm$ 9.83	0.44 $\pm$ 0.437	0.70 $\pm$ 0.15	60.29 $\pm$ 4.41
90	21.08 $\pm$ 0.75	4.63 $\pm$ 4.25	2.18 $\pm$ 0.92	179.4 $\pm$ 14.7
Overall	27.24 $\pm$ 4.66 <sup>B</sup>	1.91 $\pm$ 1.63 <sup>B</sup>	1.10 $\pm$ 0.43 <sup>B</sup>	103.42 $\pm$ 8.33 <sup>AB</sup>

\*, means in the same texture attribute in different cheeses followed by different superscripts (A-C) letter represent significant differences (P $\leq$ 0.05); SE, standard error

Increases in intensities of butyric acid, 2-/3-methyl buytric acid and 2-acetyl-2-thiazole were higher in Kasar and analogue cheeses than processed cheese during storage. The intenisty of unknown 3 with spicy flavor decreased in all cheese samples during storage. Aroma-active compounds determined in the cheese samples were reported by several other studies (Karagul-Yuceer et al 2001; Qian & Reineccius 2002; Carunchia-Whetstine et al 2003; Avsar et al 2004; Awad et al 2004; Mahajan et al 2004; Karagul-Yuceer et al 2009; Vitova et al 2012). They have a great contribution on flavor of many dairy products. Ethlyl pentanoate, *p*-cresol, maltol, sotolon, 2-acetyl-2-thiazoline and  $\delta$ -decalactone were found as aroma-active compounds in Cheddar cheese (Avsar et al 2004). Hayaloglu (2009) investigated volatile composition of kasar cheese at different stages of ripening. Acetic acid, butyric

acid, pentanoic acid, 2-phenylethyl alcohol and *p*-cresol were also determined in Kasar cheese similar to our findings. Gunecer & Karagul-Yuceer (2011) determined similar aroma-active compounds in Circassian cheese by GCO analysis. However, the intensities of  $\gamma$ -dodecalactone, *p*-cresol, 2-phenylethyl alcohol and 2-/3-methyl buytric acid were found to be higher in Circassian cheese than our cheese samples. Also, acetic acid was observed at higher intensity in processed cheese compared to Circassian cheese.

3.5. Descriptive sensory analysis of cheeses

Eleven descriptive terms were developed by panelists (Table 1). Among the cheese samples, analogue cheese became inedible due to deterioration at 90 days storage. Sensory evaluation of cheese samples was conducted at 1<sup>st</sup> and 30<sup>th</sup> days

**Table 6- Aroma active compounds of cheese samples during storage**

*Çizelge 6- Depolama boyunca peynir örneklerinin aroma aktif bileşenleri*

Aroma	RI <sup>a</sup>	Aroma quality <sup>b</sup>	Aroma intensity <sup>c</sup>								
			Kasar cheese			Processed cheese			Cheese analogue		
			D1	D30	D90	D1	D30	D90	D1	D30	D90
Diacetyl	<1100	Creamy/fat	0.6	1.1	0.3	0.8	1	1	-	1.2	1.3
Ethyl pentanoate	1171	Fruity	0.3	-	0.4	-	-	-	-	-	-
Unknown 1	1278	Sour	2	1.3	2.3	1.3	0.7	1.8	-	0.4	1.5
Methional	1392	Potato/metallic	0.5	-	-	0.9	-	1	0.6	-	-
Acetic acid	1435	Sour	5	0.4	-	6	-	6	0.5	-	-
Unknown 2	1480	Mushroom/metallic	-	1.3	0.8	-	0.9	-	-	0.5	-
Butyric acid	1538	Acid	0.3	1.3	6	-	3	-	0.3	1	6
2-/3-methyl butyric acid	1603	Sour/vinegar	0.9	-	2.3	-	1	0.9	1.5	0.9	4.3
2-acetyl-2-thiazoline	1717	Popcorn	0.4	-	3.5	1.5	-	5.8	-	-	3.5
Pentanoic acid	1733	Sour	-	-	-	-	3.3	-	-	0.8	-
b-ionene	1866	Herbaceous	-	-	-	-	-	-	0.8	0.8	1
2-phenylethanol	1927	Rose/Sour	-	0.8	0.9	-	0.8	0.8	0.4	-	-
Maltol	1988	Burnt sugar	1.3	4.5	0.5	0.7	-	0.8	2.5	2.3	1.2
<i>p</i> -cresol	2012	Animal	-	-	-	-	-	1.8	-	0.4	-
Unknown 3	2087	Spicy	1.8	-	-	1.8	-	-	1.8	0.7	-
Sotolon	2139	Burnt sugar	-	2	0.8	-	1.8	1.5	1	1.5	1.3
$\delta$ -decalactone	2244	Flower/lactone	1.8	3	2	0.9	1.5	3	1.8	2.5	2.3
$\gamma$ -dodecalactone	2380	Sweet/lactone	-	1.5	1.5	-	1	2.3	-	0.8	1.4

<sup>a</sup>, retention indices is on INNOWAX column (J &W Scientific, Folsom, CA, USA); <sup>b</sup>, aroma quality determined on olfactory port; <sup>c</sup>, mean aroma intensities (post-peak intensity, 10-point scale) on INNOWAX column; odour intensities represent the mean of each sample evaluated in duplicate by sniffer; D1, day 1; D30, day 30; D90, day 90

of storage. Significant interaction was determined between storage time and cheese type in terms of whey and animal like terms ( $P \leq 0.05$ ). At the first day of storage, it was found that whey flavor intensity was lower in kasar cheese analogue than other cheese samples while animal like flavor intensity was higher than other cheese samples (Table 7). Karagül-Yüceer et al (2003) found that rennet casein had an animal like/wet dog flavor which was associated its volatile compounds such as hexanoic acid, indole and guaiacol and *p*-cresol. For this reason, higher animal like flavor in Kasar cheese analogues prepared by rennet casein could be ascribed to characteristics of rennet casein.

Table 8 shows the effects of cheese type on nutty, waxy, cooked, creamy, salty, sour and umami intensities perceived by panelists. Nutty intensities of natural kasar and processed cheeses were higher than analogue cheese ( $P \leq 0.05$ ). Nutty intensities of kasar cheese and processed cheese were higher than analogue cheese. Cheese type affected waxy aroma note ( $P \leq 0.05$ ). It was found that intensity

of waxy flavor was higher in cheese analogue than other cheese samples. This might be related to the composition of cheese analogue. It was emphasized that waxy flavor in cheese associated medium chain fatty acid such as 4-methyl octanoic and 4-ethyl octanoic acid by Delahunty & Drake (2004). Intensities of umami taste were lower in all cheese samples. In practice, kasar cheese had higher intensity of umami taste than other cheese samples. The intensities of cooked and creamy in cheese samples were found to be between 2.40-3.21 and 2.90-3.79, respectively. Similar results were reported by other researchers (Karagül-Yüceer et al 2007; Adhikari et al 2009). Adhikari et al (2009) determined that intensities of cooked and creamy flavors in processed cheese ranged between 2.20-2.54 and 3.54-4.33 respectively. Karagül-Yüceer et al (2007) determined that sour, sweet and umami tastes in Ezine cheese ranged between 2.31-4.28, 0.53-1.25 and 0.53-1.40, respectively.

Significant effects of storage were determined on the intensities of fermented flavor and sweet taste of the

**Table 7- Intensities (mean±SE) of whey and animal like aroma in cheese samples during storage**

Çizelge 7- Peynirlerde depolama boyunca peyniraltı suyu ve hayvansı aroma yoğunlukları (ortalama±standart hata)

Day	Kasar cheese		Processed cheese		Cheese analogue	
	Whey	Animal	Whey	Animal	Whey	Animal
1	3.31±0.15 <sup>Aa*</sup>	0.04±0.04 <sup>Ab</sup>	2.77±0.19 <sup>Aa</sup>	0.00 <sup>Bb</sup>	0.41±0.13 <sup>Bb</sup>	6.87±0.39 <sup>Aa</sup>
30	2.75±0.09 <sup>Aa</sup>	0.04±0.04 <sup>Ab</sup>	1.94±0.25 <sup>Bb</sup>	0.40±0.14 <sup>Ab</sup>	1.58±0.39 <sup>Ab</sup>	2.65±0.64 <sup>Ba</sup>
P value	0.01		0.01		0.01	

\*, means in the same attribute during storage followed by different superscripts (A-B) represent significant differences ( $P \leq 0.05$ ); means in the same attributes of different cheeses followed by different lowercase (a-b) letters represent significant differences ( $P \leq 0.05$ ); SE, standard error; mean expresses aroma intensity after testing, 10-point scale

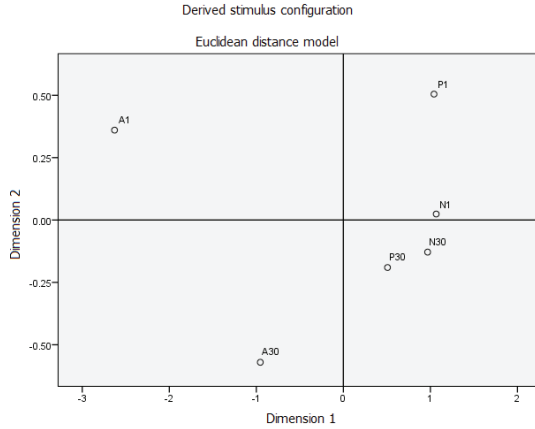
**Table 8- Intensities (mean±SE) of sensory descriptors in cheese samples**

Çizelge 8- Peynir örneklerinde duyusal tanımlayıcıların yoğunlukları (ortalama±standart hata)

Samples	Nutty	Waxy	Cooked	Creamy	Salty	Sour	Umami
Kasar cheese	0.64±0.11 <sup>A*</sup>	0.02±0.02 <sup>B</sup>	3.21±0.14	2.90±0.14	1.56±0.23	1.43±0.20	0.80±0.39
Processed cheese	0.52±0.17 <sup>A</sup>	0.54±0.32 <sup>B</sup>	2.83±0.32	3.79±0.42	1.28±0.16	1.12±0.18	0.29±0.11
Cheese analogue	0.02±0.02 <sup>B</sup>	3.67±0.38 <sup>A</sup>	2.40±0.30	3.12±0.48	1.42±0.19	0.93±0.13	0.37±0.12
P value	0.01	0.01	0.63	0.16	0.63	0.16	0.30

\*, means in the same attribute of different cheeses followed by different superscripts (A-B) represent significant differences ( $P \leq 0.05$ ); SE, standard error; mean expresses aroma intensity after testing, 10-point scale

cheese samples ( $P= 0.03$ ). On the 30<sup>th</sup> day of storage, intensities of fermented flavor ( $1.41\pm 0.28$ ) and sweet taste ( $1.45\pm 0.17$ ) were higher than the first day of storage. When the examined all sensory descriptors of cheese samples by multidimensional scaling methods, it can be said that kasar cheese and processed cheese had similar sensory characteristics (Figure 2).



**Figure 2- Multidimensional scaling map of cheese samples in terms of descriptive sensory properties during storage (A, analogue cheese; P, processed cheese; N, kasar cheese (1:1<sup>st</sup> day of storage 30:30<sup>th</sup> day of storage)), dimension 1 and 2 were established for cheese samples based on all sensory properties by MDS analysis**

Şekil 2- Depolama boyunca tanımlayıcı duyuşal özellikler bakımından peynir örneklerine ait çok boyutlu ölçeklendirme haritası (A, taklit peynir; P, eritme peynir; N, kasar peyniri (1:1. gün, 30:30. gün)) boyut 1 ve 2 tüm duyuşal özellikler dikkate alınarak peynir örnekleri için MDS analizi ile oluşturulmuştur

### 3.6. Consumer acceptance test

The panelists evaluated color/appearance, texture, flavor and overall liking of the cheese samples (Table 9). Based on consumer test, significant differences were observed among the cheeses in terms of flavor attribute ( $P= 0.01$ ). However no significant differences were determined among the cheeses in terms of appearance ( $P= 0.50$ ) and texture acceptance ( $P= 0.08$ ). Kasar cheese had the highest flavor acceptance score while the analogue cheese had the lowest flavor score. Color/appearance and texture scores of the cheese samples varied between 4.81-5.14 and 4.37-4.95, respectively. Moreover, kasar cheese had the highest preference than the other samples. However, the lowest preference score was determined in analogue cheese. These results confirmed that consumers prefer kasar cheese to processed cheese and analogue cheese. Similar results were also indicated by other studies (Gursoy 2000; Koca 2002; Karademir-Sanlı 2006). Gursoy (2000) showed that process cheese prepared with 10% soya milk had lower score than kasar cheese in terms of appearance/color, texture and flavor characteristics.

## 4. Conclusions

Differences in some chemical, physical, sensory properties and aroma characterization of kasar, processed and analogue cheeses were determined. There were significant differences among the cheeses in terms of protein, ash and fat contents, and variations were determined among the cheeses based on pH and titratable acidities during storage.

**Table 9- Consumer acceptance and ranking test scores (mean $\pm$ SE) of cheeses**

Çizelge 9- Peynirlerin tüketici kabul ve sıralama test puanları (ortalama $\pm$ standart hata)

Samples	Color/Appearance	Texture	Flavor	Ranking test
Kasar cheese	5.14 $\pm$ 0.13	4.95 $\pm$ 0.16	5.14 $\pm$ 0.15 <sup>A*</sup>	1.62 $\pm$ 0.11 <sup>C</sup>
Processed cheese	4.89 $\pm$ 0.14	4.62 $\pm$ 0.14	4.43 $\pm$ 0.17 <sup>B</sup>	2.00 $\pm$ 0.11 <sup>B</sup>
Cheese analogue	4.81 $\pm$ 0.17	4.37 $\pm$ 0.20	3.66 $\pm$ 0.20 <sup>C</sup>	2.35 $\pm$ 0.10 <sup>A</sup>
P value	0.50	0.08	0.01	0.01

\* , means in the same sensory attribute of different cheese samples followed by different superscript letters (A-C) represents significant differences ( $P\leq 0.05$ ); SE, standard error; mean expresses ranking scores, 7-point scale



No significant changes were determined in  $L^*$ ,  $a^*$  and  $b^*$  values, hardness, oil separation and melting properties in cheeses during 30 days of storage. 18 aroma-active compounds were determined in the cheeses during 90 day storage. Diacetyl (butter), unknown 1, acetic acid (vinegar), butyric acid (cheesy), 2-/3-methyl butyric acid (sour, dirty), 2-acetyl-2-thiazoline (popcorn),  $\beta$ -ionene (herbal), 2-phenylethyl alcohol (rose), maltol (burnt sugar),  $p$ -cresol (animal like), sotolon (burnt sugar),  $\delta$ -decalactone (flower, peach) and  $\gamma$ -dodecalactone (sweet) were some of the characteristic aroma-active compounds in the cheeses. 'Whey', 'cooked', 'creamy', 'salty' and 'sour' were determined as the most intense sensory descriptors of the cheeses. 'Animal like' and 'waxy' aroma intensities were higher in analogue cheese than the other cheeses. Kasar cheese was the most liked cheese while analogue cheese was the least liked one.

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## The Influence of the Extract Obtained from Giant Red Shrimp (*Aristaeomorpha foliacea*) Shells on Chemical Properties of Cold-Stored Anchovy (*Engraulis encrasicolus*)

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### ABSTRACT

This study aims to determine the effects of extract obtained from giant red shrimp (*Aristaeomorpha foliacea*) shells on the changes in chemical parameters of anchovy (*Engraulis encrasicolus*) during 18 days of refrigerated storage. Butylated hydroxytoluene (BHT) was used for the comparison of antioxidant effects. The investigation of changes in fish during refrigerated storage indicated that lipid oxidation significantly increased ( $P<0.05$ ). Compared to control group, BHT and different rates of shell extract were determined to have significant effects on prevention of oxidation. Comparison of total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), peroxide, free fatty acids and pH values indicated that the most positive result was found in the BHT added group, which was followed by the groups containing 0.5% and 0.1% of shell extracts, and control group. In this study, it was concluded appropriate to use natural shell extracts besides synthetic antioxidants during the storage of fish.

Keywords: Giant red shrimp; *Aristaeomorpha foliacea*; Shrimp shell extract; Anchovy; Shelf life; Cold storage

## Kırmızı Dev Karides (*Aristaeomorpha foliacea*) Kabuklarından Elde Edilen Ekstraktın Soğukta Depolanan Hamsinin (*Engraulis encrasicolus*) Kimyasal Özellikleri Üzerine Etkisi

### ESER BİLGİSİ

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### ÖZET

Bu çalışmada, kırmızı dev karides (*Aristaeomorpha foliacea*) kabuklarından elde edilen ekstraktın, hamsi (*Engraulis encrasicolus*)'nin buzdolabında 18 gün depolanması süresince kimyasal parametrelerinde meydana gelen değişimler üzerine etkisinin belirlenmesi amaçlanmıştır. Antioksidan etkiyi kıyaslamak amacıyla butillendirilmiş hidroksi toluen (BHT) kullanılmıştır. Depolama süresince balıklarda meydana gelen değişimler incelendiğinde, süreyle birlikte lipit

oksidasyonunun önemli ( $P<0.05$ ) ölçüde arttığı tespit edilmiştir. Kontrol grubuyla kıyaslandığında, uygulanan BHT ve farklı oranlardaki kabuk ekstraktlarının oksidasyonun önlenmesi üzerine olumlu etkileri gözlenmiştir. Toplam uçucu bazik azot (TVB-N), tiyobarbiturik asit (TBA), peroksit, serbest yağ asitleri ve pH değerleri kıyaslandığında; uygulama grupları içerisinde en olumlu sonuç BHT eklenen grupta bulunurken bunu sırasıyla % 0.5 ve % 0.1 oranında kabuk ekstraktı içeren gruplar ve kontrol grubu izlemiştir. Bu çalışmada balıkların depolanması esnasında sentetik antioksidanların yanı sıra kabuktan elde edilen doğal ekstraktın da kullanılmasının uygun olacağı belirlenmiştir.

Anahtar Kelimeler: Kırmızı dev karides; *Aristaeomorpha foliacea*; Karides kabuğu ekstraktı; Hamsi; Raf ömrü; Soğuk depolama

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## 1. Introduction

Shrimp catching is important not only in Turkey but also in international fishery. In Turkey, significant amounts of shrimp are hunted in Mediterranean, Aegean and Marmara coasts. Male, jumbo, Karabiga, red and pink shrimp take place among the whole hunted shrimps. In recent years, the economic importance of giant red shrimp (*Aristaeomorpha foliacea*) increased with the raise in the interest towards hunting in deep water species. While they are hunted with bottom trawl and served for fresh consumption, most of them are processed in seafood processing plants and exported.

With the advanced technology in shrimp processing, the assessment of shrimp wastes has been a crucial matter. The wastes of shrimps which are removed from the meat parts in processing factories comprise approximately 40-56% of the whole product (İbrahim et al 1999; Gildberg & Stenberg 2001; Sachindra et al 2006). This rate varies by the species of shrimp and approximately 34% of the total weight consists of head and 14% consists of shell (Binsan et al 2008). These wastes include very valuable bioactive compounds (antioxidants, carotenoids, chitin, peptone, amino acids, peptides, proteins, minerals, enzymes, lipid, flavor compounds and other useful nutrients). They are used as addition agent in foods and also utilized as a protein source in aquaculture and stockbreeding (Gagne 1993; İbrahim et al 1999; Binsan et al 2008).

Chitin and its derivate chitosan rank at the top of the list of bioactive compounds. Approximately

20-30% chitin can be obtained from Crustacea shells. One of the most important compounds that make shrimp shells such valuable is the natural antioxidants. The isolation and identification of natural antioxidants in shell wastes is an important stage for the assessment of shell wastes. A few studies conducted on antioxidant features of shrimp shells so far have researched the characterization and effects of these features on some fish species. In studies conducted so far, it is reported that waste extracts of *Penaeus monodon* species assessed as a potential bioactive substance in shrimp processing factories in Thailand have antioxidant activity (Dajsipun et al 2000); in the extract obtained from *Pandulus jordani* shells, the substance with antioxidant feature is phenol compounds (Seymour et al 1996); once again, in extract obtained from *Pandulus jordani* shells, polar compounds are responsible for antioxidant activity (Li et al 1994) and this extract has positive effects on quality during the storage of some fish (Li et al 1998). However, the number of research conducted on antioxidant feature of shrimp shells is limited with the above mentioned studies and further detailed studies are needed on this subject. For this reason, the present study aims to determine the effects of different concentrations of the shell extract obtained from giant red shrimp, discarded as waste products in sea product processing factories, on chemical properties of anchovy, highly important for Turkish fishery sector, stored in refrigerator.



## 2. Material and Methods

### 2.1. Materials

In present study, shell materials were obtained from the wastes of giant red shrimp (*Aristaeomorpha foliacea*). Fresh samples of shrimp processing discards including intact cephalothorax and abdominal exoskeleton were collected from a local shrimp factory. Representative shrimp wastes were selected, put in polystyrene boxes and stored at -20 °C before and during transportation to the laboratory. Shells were completely separated from the shrimp wastes in laboratory and washed in pure water. BHT was used as a commercial antioxidant.

### 2.2. Preparation of crude extract

Shrimp shells were extracted with ethanol according to the method of Li et al (1998). The mixture was blended until homogeneous with a blender. The slurry was filtered through a funnel using Whatman filter paper (GF/C, Schleicher & Schuell) to remove debris. The filtrate was evaporated to dryness. The dried crude shell extract was resolved by water as a solution dip anchovy treatment. BHT was used as a commercial antioxidant.

Antioxidant activity, total phenol compound and total carotenoid content of the giant red shrimp shells were found as 45.84%, 17.87 mg 100 g<sup>-1</sup> and 20.31 mg 100 g<sup>-1</sup>, respectively (Küçükgülmez & Çelik 2013).

### 2.3. Fish sample preparation

The anchovy (*Engraulis encrasicolus*) (average weight and length: 12.28±1.71 g and 11.74±0.62 cm, respectively) were purchased from a local fish market (total 17 kg anchovy). They were stored in ice an insulated box and transferred to the laboratory. After the viscera, gills and bone were removed, the anchovy samples were divided into four groups (4 kg each).

Fish fillet samples were dipped into the following solutions as different treatments for 5 min; control containing only distilled water, 0.1% and 0.5% (w v<sup>-1</sup>) crude antioxidant solutions and 0.005% BHT solution. These concentrations of crude antioxidant solution and BHT were determined based on the

results of a preliminary study made in accordance with the studies of Li et al (1998) and Soyer (1995). Subsequently, fish samples were put into styropor dishes, covered with stretch films, and then stored in the refrigerator (2.7 °C). Experiment was performed with two-parallels. Analyses were made every other three day for 18 days.

### 2.4. Chemical analysis

Total volatile basic nitrogen (TVB-N) was determined on steam distillation using the Kjeldahl distillation apparatus and titration (Antonocopoulos 1973). Thiobarbituric acid (TBA) number was determined using the method of Tarladgis et al (1960), expressed as mg malondialdehyde kg sample<sup>-1</sup> using a conversion factor of 7.8. The peroxide value (PV) was determined by the acetic acid-chloroform method (AOCS 1990) and expressed as meq O<sub>2</sub> kg lipid<sup>-1</sup>. The free fatty acids (FFA) content of the lipid was determined volumetrically using aqueous sodium hydroxide (0.25 N) and phenolphthalein indicator (1% ethanol) according to IAFMM (1987) method. The pH was determined from homogenates of minced fish and distilled water in a ratio of 1:10 (w v<sup>-1</sup>) by using a digital pH meter (Lima Dos Santos et al 1981).

### 2.5. Statistical analysis

The SPSS (SPSS Inc., Chicago, IL, USA) software was used for the statistical analysis. Comparisons among groups were made using one-way analysis of variance (ANOVA), and significant differences were determined by Duncan's multiple range tests at 5% confidence level.

## 3. Results and Discussion

### 3.1. Total volatile basic nitrogen (TVB-N)

TVB-N values of various anchovy groups are presented in Table 1. TVB-N values of all groups increased significantly during storage period and TVB-N values of the group including 0.5% shell extract and of the group with added BHT were found much lower compared to other groups (P<0.05). As TVB-N amount in fish is associated with bacterial deterioration and activity of endogenic enzymes,

TVB-N analysis is one of the most commonly used methods for the detection of fish freshness (Vareltzis et al 1997). At the end of the microbial activity, some volatile bases emerge as a result of degradation of protein or non-protein nitrogenous compounds (Yerlikaya et al 2005). While TVB-N amount is low in fresh fish, it increases by the deterioration of fish during the storage period. Generally, samples including 25 mg 100 g<sup>-1</sup> TVB-N are considered as “very good”, 30 mg 100 g<sup>-1</sup> as “good”, 35 mg 100 g<sup>-1</sup> as “marketable” and higher than 35 mg 100 g<sup>-1</sup> as “spoiled” (Varlık et al 1993). Accordingly, in the present study, the control group and the other group including 0.1% shell extract exceeded the consumability limit on the 15<sup>th</sup> day of refrigerated storage and those groups with 0.5% shell extract and added BHT exceeded this limit on 18<sup>th</sup> day in terms of TVB-N value. These results indicate the positive

effects of the extract of the used shrimp shells and BHT on TVB-N value of fish. Similar to the present study, other studies which used different plant materials as natural antioxidant source reported that TVB-N value of fish is affected in positive direction (Nam & Ahn 2003; Pazos et al 2005).

### 3.2. Thiobarbituric acid (TBA)

TBA changes in anchovy groups are presented in Table 2. TBA values of all groups statistically significantly increased during refrigerated storage (P<0.05). In general assessment of TBA results, it is found that the TBA values of the group with added BHT during the storage period were significantly lower than other groups (P<0.05). This positive effect was followed by the group with the added 0.5% shell extract.

**Table 1- TVB-N changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (mg 100 g<sup>-1</sup>)**

Çizelge 1- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince TVB-N değişimleri (mg 100 g<sup>-1</sup>)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	16.93±2.02 <sup>a,1*</sup>	17.01±0.81 <sup>a,1</sup>	17.14±0.87 <sup>a,1</sup>	17.11±0.89 <sup>ab,1</sup>
3	18.89±0.68 <sup>b,2</sup>	18.01±1.43 <sup>ab,12</sup>	16.10±2.07 <sup>a,1</sup>	16.79±0.04 <sup>a,12</sup>
6	20.40±0.81 <sup>bc,3</sup>	19.43±0.15 <sup>b,2</sup>	16.64±0.06 <sup>a,1</sup>	16.91±0.40 <sup>a,1</sup>
9	21.37±0.54 <sup>c,2</sup>	21.86±1.66 <sup>c,2</sup>	20.40±0.49 <sup>b,12</sup>	18.68±0.09 <sup>b,1</sup>
12	25.62±0.69 <sup>d,3</sup>	22.78±0.63 <sup>c,2</sup>	23.40±0.43 <sup>c,2</sup>	20.79±0.75 <sup>c,1</sup>
15	35.42±0.59 <sup>e,3</sup>	35.61±1.18 <sup>d,3</sup>	27.20±0.62 <sup>d,2</sup>	24.77±0.55 <sup>d,1</sup>
18	43.48±1.03 <sup>f,3</sup>	40.06±1.17 <sup>e,3</sup>	37.24±0.86 <sup>e,2</sup>	35.75±1.51 <sup>e,1</sup>

\*. data are expressed as means±standard deviation; different letters within the column denote significant differences (P<0.05); different number within the row denote significant differences (P<0.05)

**Table 2- TBA changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (mg MDA kg<sup>-1</sup>)**

Çizelge 2- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince TBA değişimleri (mg MDA kg<sup>-1</sup>)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	1.67±0.38 <sup>a,1*</sup>	2.10±0.14 <sup>a,1</sup>	1.95±0.17 <sup>a,1</sup>	1.68±0.38 <sup>a,1</sup>
3	2.77±0.30 <sup>b,2</sup>	2.67±0.40 <sup>b,2</sup>	2.36±0.05 <sup>a,12</sup>	2.03±0.02 <sup>a,1</sup>
6	4.70±0.19 <sup>c,3</sup>	4.87±0.04 <sup>c,3</sup>	4.12±0.30 <sup>b,2</sup>	3.70±0.02 <sup>b,1</sup>
9	6.45±0.23 <sup>d,3</sup>	6.59±0.01 <sup>d,3</sup>	5.93±0.66 <sup>c,2</sup>	4.15±0.19 <sup>c,1</sup>
12	7.01±0.03 <sup>e,3</sup>	7.67±0.09 <sup>e,3</sup>	6.15±0.25 <sup>d,2</sup>	4.94±0.35 <sup>d,1</sup>
15	8.62±0.31 <sup>f,4</sup>	8.10±0.36 <sup>f,3</sup>	6.53±0.34 <sup>e,2</sup>	5.70±0.24 <sup>e,1</sup>
18	9.08±0.35 <sup>g,3</sup>	8.67±0.16 <sup>g,2,3</sup>	7.12±0.31 <sup>f,2</sup>	6.20±0.14 <sup>f,1</sup>

\*. data are expressed as means±standard deviation; different letters within the column denote significant differences (P<0.05); different number within the row denote significant differences (P<0.05)

TBA value is a commonly used indicator for the determination of lipid oxidation of fish (Sallam 2007; Turhan et al 2009). The detection of TBA amount is based on the malondialdehyde measurement which determines the secondary oxidation products in association with the fish deterioration (Al-Bandak et al 2009). It is reported that consumability limit of the TBA value which is used to determine the rancidity level of oils is between 7 and 8 mg MDA kg<sup>-1</sup> (Varlık et al 1993). According to this assessment, the group with added BHT and the group including 0.5% shell extract did not exceed this value during the storage period while control group and the group including 0.1% shell extract exceeded this value on 15<sup>th</sup> day of the storage. In this study which aims to assess shell extracts as natural antioxidant source, TBA values being an indicator of lipid oxidation support this view. In their study which is very similar to the present study, Li et al (1998) researched the natural antioxidant obtained from shrimp shells of the *Sebastolobus alascanus* fish stored in refrigerator on TBA amount and they obtained similar results.

The effects of natural antioxidants of fish on lipid oxidant are a recently noticeable issue. TBA analyses are widely conducted in order to detect these effects. TBA analysis was used to determine the effect of natural extracts obtained from various plants on lipid oxidation during the storage of various fish species and it is highlighted that these extracts decrease lipid oxidant which is a similar

finding to the present study (Yasin & Abou-Taleb 2007; Selmi & Sadok 2008; Al-Bandak et al 2009).

### 3.3. Peroxide value

The changes in peroxide values of anchovy groups during the storage period are presented in Table 3. Peroxide value statistically significantly increased in all groups by storage period (P<0.05). This increase in peroxide values is an indicator of oxidative rancidity in fish lipids. As fish meat involves high amounts of polyunsaturated fatty acids which can be easily oxidized and lead to high level of peroxide, peroxide levels increase by the storage level (Yasin & Abou-Taleb 2007).

It is observed that in all groups, peroxide level reached the highest level on 15<sup>th</sup> day of storage and started to decrease on 18<sup>th</sup> day. It is assumed that the decrease in peroxide level at last stages of the storage may result from the degradation of hydroperoxides being a secondary oxidation product. Because, peroxide analysis is measured with hydroperoxide formation and it gives good results at initial stages of oxidation.

Many studies conducted on the natural and commercial antioxidant usage so as to delay lipid oxidation, investigated the changes of peroxide levels and it is reported that peroxide levels of groups with the added natural and commercial antioxidant were lower than the control group during the storage

**Table 3- Peroxide changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (meq O<sub>2</sub> kg<sup>-1</sup>)**

Çizelge 3- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince peroksit değişimleri (meq O<sub>2</sub> kg<sup>-1</sup>)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	0.61±0.10 <sup>a,1*</sup>	0.85±0.44 <sup>a,1</sup>	0.87±0.51 <sup>a,1</sup>	0.66±0.00 <sup>a,1</sup>
3	2.13±0.43 <sup>b,1</sup>	1.75±0.37 <sup>a,1</sup>	1.50±0.33 <sup>b,1</sup>	1.68±0.42 <sup>b,1</sup>
6	3.74±0.45 <sup>c,2</sup>	3.13±0.33 <sup>b,2</sup>	3.57±0.69 <sup>c,2</sup>	2.19±0.07 <sup>bc,1</sup>
9	6.82±1.44 <sup>d,3</sup>	6.62±0.18 <sup>d,3</sup>	4.14±0.30 <sup>c,2</sup>	2.44±0.23 <sup>c,1</sup>
12	7.14±0.38 <sup>d,3</sup>	6.28±0.71 <sup>d,2</sup>	6.70±0.15 <sup>d,23</sup>	3.37±0.07 <sup>d,1</sup>
15	10.59±1.44 <sup>e,3</sup>	9.14±1.13 <sup>e,3</sup>	7.70±0.87 <sup>e,2</sup>	5.38±0.64 <sup>e,1</sup>
18	5.94±0.62 <sup>d,2</sup>	5.21±0.50 <sup>c,2</sup>	3.53±0.45 <sup>c,1</sup>	5.30±0.33 <sup>e,2</sup>

\*, data are expressed as means±standard deviation; different letters within the column denote significant differences (P<0.05); different number within the row denote significant differences (P<0.05)

period which is a similar finding to the present study (Sallam 2007; Yasin & Abou-Taleb 2007; Al-Bandak et al 2009). To conclude, peroxide level of fish meat significantly differs by fish species, initial peroxide level, processing and storage conditions, storage period and the type of the added antioxidant.

### 3.4. Free fatty acids

Free fatty acid levels of anchovy groups are presented in Table 4. Free fatty acid levels statistically significantly increased in all groups during the 18-day storage period (P<0.05).

Although no statistically significant difference was observed between groups at initial periods of storage, free fatty acid values of the anchovy group with added BHT were found statistically lower

than other groups (P<0.05) which was followed by the group including 0.5% shell extract, the group including 0.1% shell extract and lastly the control group. Free fatty acids which emerge as a result of the hydrolysis of the lipids in fish stored or frozen in refrigerator are important for the development of rancidity (Chaouqy et al 2008). In the present study, the positive effects of shell extract and BHT on this value are remarkable as well. In a similar study conducted on the effects of commercial antioxidants and vacuum packaging on free fatty acids, similar results were obtained (Soyer 1995).

### 3.5. pH

pH changes in anchovies during the storage period are presented in Table 5. pH values of all groups

**Table 4- Free fatty acid changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (oleic acid %)**

Çizelge 4- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince serbest yağ asitleri değişimleri (% oleik asit)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	3.21±0.05 <sup>a,2*</sup>	3.17±0.35 <sup>a,2</sup>	1.93±0.52 <sup>a,1</sup>	2.51±0.46 <sup>a,12</sup>
3	3.44±1.08 <sup>a,1</sup>	3.12±0.06 <sup>a,1</sup>	3.12±0.47 <sup>b,1</sup>	2.55±0.29 <sup>a,1</sup>
6	4.68±0.85 <sup>b,2</sup>	3.95±0.10 <sup>a,1</sup>	3.91±0.01 <sup>bc,1</sup>	3.56±0.10 <sup>ab,1</sup>
9	5.30±0.17 <sup>b,2</sup>	4.43±0.32 <sup>b,1</sup>	4.17±0.04 <sup>c,1</sup>	4.16±0.16 <sup>b,1</sup>
12	6.69±0.17 <sup>c,4</sup>	5.66±0.08 <sup>c,3</sup>	5.08±0.00 <sup>d,2</sup>	4.32±0.25 <sup>bc,1</sup>
15	7.22±0.09 <sup>cd,4</sup>	6.03±0.02 <sup>c,3</sup>	5.49±0.38 <sup>e,2</sup>	4.53±0.32 <sup>bc,1</sup>
18	8.09±1.12 <sup>d,3</sup>	6.84±0.50 <sup>d,2</sup>	6.00±0.33 <sup>e,2</sup>	4.74±0.07 <sup>c,1</sup>

\*, data are expressed as means±standard deviation; different letters within the column denote significant differences (P<0.05); different number within the row denote significant differences (P<0.05)

**Table 5- pH changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage**

Çizelge 5- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince pH değişimleri

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	6.08±0.02 <sup>a,12*</sup>	6.04±0.02 <sup>a,1</sup>	6.06±0.01 <sup>ab,1</sup>	6.11±0.01 <sup>b,2</sup>
3	5.99±0.01 <sup>a,1</sup>	6.00±0.04 <sup>a,1</sup>	6.01±0.05 <sup>a,1</sup>	6.02±0.05 <sup>a,1</sup>
6	6.16±0.07 <sup>b,1</sup>	6.20±0.01 <sup>b,1</sup>	6.13±0.07 <sup>b,1</sup>	6.12±0.05 <sup>b,1</sup>
9	6.34±0.07 <sup>c,2</sup>	6.30±0.05 <sup>c,12</sup>	6.29±0.04 <sup>c,12</sup>	6.20±0.05 <sup>c,1</sup>
12	6.63±0.09 <sup>d,2</sup>	6.67±0.03 <sup>de,2</sup>	6.47±0.01 <sup>d,1</sup>	6.38±0.00 <sup>d,1</sup>
15	6.97±0.06 <sup>e,4</sup>	6.80±0.01 <sup>e,3</sup>	6.56±0.01 <sup>e,2</sup>	6.41±0.01 <sup>d,1</sup>
18	7.18±0.03 <sup>f,4</sup>	7.00±0.06 <sup>f,3</sup>	6.77±0.02 <sup>f,2</sup>	6.50±0.01 <sup>e,1</sup>

\*, data are expressed as means±standard deviation; different letters within the column denote significant differences (P<0.05); different number within the row denote significant differences (P<0.05)

decreased on 3<sup>rd</sup> day of the storage and then started to increase again by storage period. pH value is at low levels initially due to the fact that glycogen turns into lactic acid at post-mortem glucoses stage of fish (Şengör et al 2000). At following stages of the storage, oxido-reduction balance is disturbed due to enzymes and bacteria; changes occur in concentration of free hydrogen and hydroxide ions and thus pH value increases. pH value of fresh fish is 6.0-6.5; consumability value is 6.8-7.0 (Varlık et al 1993; Turhan et al 2001). According to this assessment and considering the pH results of the present study, it is found that pH values of control group and the groups including 0.1% shell extract exceeded the critical limit 6.8-7.0 on 15<sup>th</sup> day of storage while the group including 0.5% shell extract and the groups with added remained behind the critical limit during the storage. Different studies reported that pH value of fish meat differs by fish species, hunting type, fish processing technology, storage conditions and contamination of microorganism and this is not an absolute criterion for the freshness or quality yet should be considered as a supportive factor to other parameters (Selmi & Sadok 2008).

#### 4. Conclusions

The shell wastes which are not utilized in seafood processing factories in Turkey have a great potential. The assessment of these wastes is very important not only for seafood factories but for other industries. Although there are many studies on the assessment and economic use of wastes of shrimp and other shellfish in the world, the number of studies on their antioxidant feature is very limited. In this scope, to obtain natural antioxidant from wastes of giant red shrimp shells and thus to contribute to economy with wastes through obtaining food protecting materials which are safe for human health and to prevent the environmental damage caused by the unused wastes show the importance of the present study. Consequently, it was concluded that the extract isolated from shrimp shells could be used during the cold storage of fish fillets instead of synthetic antioxidants.

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## Effects of Some Properties of Drive Tires Used in Horticultural Tractors on Tractive Performance

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### ABSTRACT

Many studies on tractive performance of tractor tires have been carried out to solve soil-wheel interaction problems in the last decades. The purpose of this study is to experimentally determine effects on tractive performance of radial and bias-ply drive tires at three different tire lug heights, axle loads and inflation pressures. The experiments were carried out in stubble field conditions. To obtain sufficient performance data, a new single wheel tester was designed and manufactured. Travel reduction, net traction ratio and tractive efficiency varied from 3.3% to 34.1%, 0.24 to 0.93 and from 0.27 to 0.78 respectively depending on drawbar pull. The effects of tire type, lug height, dynamic axle load and inflation pressure on tractive efficiency were found significant ( $P<0.01$ ) by the performed variance analysis and LSD tests. Radial tires provide better tractive performance compared with bias-ply tires. The tractive efficiency increased especially with increasing dynamic axle load and decreased with increasing tire inflation pressure. Tire dynamic axle load was the major contributory factor on tractive performance as compared with other independent variables. For a given drawbar pull, it was observed that tractive efficiency of radial tire can be maximized by selecting appropriate levels of lug height, dynamic axle load and inflation pressure.

Keywords: Dynamic load; Inflation pressure; Lug height; Tire; Tractive efficiency; Tractive performance

## Bahçe Traktörlerinde Kullanılan Muharrik Lastiklerin Bazı Özelliklerinin Çeki Performansına Etkileri

### ESER BİLGİSİ

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### ÖZET

Yıllardır, toprak-tekerlek etkileşimi sorunlarını çözmek için traktör lastiklerinin çeki performansı üzerine birçok çalışma yürütülmüştür. Bu çalışmanın amacı, bahçe traktörlerinde kullanılan üç farklı profil yüksekliğine sahip radyal ve çapraz kathi muharrik lastiklerin çeki performansına, aks yükünün ve lastik iç basıncının etkilerini deneysel olarak belirlemektir.

Denemeler anız tarla koşullarında yürütülmüştür. Belirtilen şartlar altında, yeterli performans verileri oluşturabilmek için, yeni bir tek tekerlek deney düzeneği tasarlanmış ve imal edilmiştir. Çeki kuvvetine bağlı olarak; patinaj değerleri % 3.3 ile % 34.1, net çeki oranı değerleri 0.24 ile 0.93 ve çeki verimliliği değerleri 0.27 ile 0.78 arasında değişmiştir. Yapılan varyans analizi ve LSD testi sonuçları; lastik tipi, profil yüksekliği, dinamik aks yükü ve lastik iç basıncının, çeki verimliliği üzerindeki etkisinin önemli olduğu göstermiştir ( $P < 0.01$ ). Radyal lastikler çapraz katlı lastiklere göre daha iyi çeki performansı sağlamışlardır. Çeki verimliliği, özellikle dinamik aks yükünün artırılmasıyla artmış, lastik iç basıncı artırıldıkça çeki verimi azalmıştır. Dinamik aks yükünün çeki performansı üzerindeki etkisi diğer bağımsız değişkenlere göre daha büyük olmuştur. Çeki verimliliği, radyal lastiklerin uygun profil yüksekliği, aks yükü ve iç basıncının seçilmesiyle artırılabilceği gözlenmiştir.

Anahtar Kelimeler: Çeki performansı; Çeki verimi; Dinamik yük; İç basınç; Lastik; Profil yüksekliği

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## 1. Introduction

The main power supply of agricultural production is tractor. Due to increasing production of vegetables and fruits, small and powerful horticulture tractors are widely used in agricultural production. Tires are an important part of off-road vehicles; hence the study of its structural and working characteristics are of fundamental importance. The tractive characteristics of tire depend on tire geometry (weight, diameter, section height), tire type (radial, bias-ply), lug design, inflation pressure, dynamic load on the axle, soil type and ground conditions.

Net traction ratio (NTR) and tractive efficiency (TE) are two important factors of tractive performance (Burt et al 1980). Bashford et al (1993) compared the dynamic traction ratio and the tractive efficiency of a tractor equipped with three different sized rear tires at different inflation pressures and on two different surfaces. As a result, the best tractive performance was observed at the lowest inflation pressure.

Generally bias-ply and radial tires are used for agricultural tractors. As for radial tires, much research has been conducted, and it has already been found that lowering inflation pressure increases tractive performance (Lee & Kim 1997). Many studies have demonstrated that there are some advantages in using radial tractor tires over the bias-ply tires. These advantages are due to the construction of radial tires (Al-Hamed et al 1994). Looking at the performance level of the tire types, tractive performance of radial tires is higher than the

performance of the bias-ply tire types. Bias-ply tires show lower efficiency of power distribution than radial tires (Turner 1995).

To take advantage of power generated by the tractor, proper axle loads are necessary considering travel reduction limits. Effects of inflation pressure and dynamic load on soil compaction for a forwarder tire were investigated by McDonald et al (1996). Reductions in inflation pressure at a given dynamic load tended to decrease bulk density, soil cone index, and rut depth. Decreases in these variables tended to be affected more from dynamic load than inflation pressure. Çarman & Aydın (2002) reported that dynamic load increased drawbar pull at the rate of 32% at travel reduction of 17%. The tractive efficiency tended to increase as static load increased for a given inflation pressure (Elwaleed et al 2006).

Adjusting tire inflation pressure has been used as a method for improving tractive performance of agricultural tractors. Adjusting inflation pressure has recently attracted attention of many researchers. It is known that optimum tractive performance of a driving tire can be obtained by adjusting the inflation pressure of the tire based on soil conditions (Lee & Kim 1997). A lot of researches have been conducted where the effects of inflation pressure for different tire sizes were investigated. A decrease in inflation pressure results in an improvement tractive performance of the tire. Jun et al (2004) reported that the net traction and the tractive efficiency of the forwarder tire decreased with increasing inflation pressure at constant dynamic load.

Machines for operating a single wheel for traction and soil compaction research are commonly known as single tire testers or single wheel testers. Some single tire testers are large enough to accommodate the largest agricultural tractor drive tires currently available, which are up to about 2090 mm overall diameter. Other single tire testers are designed to accommodate a smaller tire, such as a garden tractor rear tire with an overall diameter of about 600 mm (Way 2009). Various designs of single wheel traction research machines have been developed and used. Machines for operating a single tire on soils in soil bins include those described by Pope (1971), Raheman & Singh (2003), Kawase et al (2006), Yahya et al (2007) and Tiwari et al (2009). There are some disadvantages of them. Soil bins, where the soil is brought to the tester, have been used to acquire a significant amount of data; however, the majority of these tests were conducted with low wheel speeds and wheel slips, and soils used in outdoor or indoor bins do not perform like those found in the field. This lack of data leaves tractor pullers with a trial and error approach to the tractive performance of the vehicle (Upadhyaya et al 1986). At asphalt, concrete, stabilized, stubble field or plowed field conditions, single wheel testers designed to overcome the disadvantages of soil bin used in experiments in order to carry out tractive performance tests, are completely dependent to tractor or semi-independent. Single wheel traction research machines for operating a single tire in a field include those described by Upadhyaya & Wulfsohn (1989), Monroe & Burt (1989), Schmulevich et al (1996), Ferhadbegović et al (2005) and Way (2009). The single wheel tester in our work was selected among others in the literature because of its capability as a tire and can be subjected to performance tests for different types of grounds with this single wheel tester. Also, this single wheel tester was designed for the first time in our country and was used in tractive performance experiments.

In the performance experiments encountered in the literature survey, tests are conducted on relatively big diameter tires of powerful tractors used in big farms. Trials for tires used for horticultural

tractors are less in number. The novel part of our study is that it is done for the tires of horticultural tractors. Because it was thought that the dependent and independent parameters in former studies were insufficient in providing a proper view of tire tractive performance, increased number of parameters are accounted in the experiments.

There is very little information about tractive performance of tires based on wheel speed and travel reduction conditions of small structures such as horticulture tractors. Therefore a new single wheel tester was designed and manufactured to create sufficient data under these conditions. With single wheel tester, it was studied to determine tractive performances of two different tire types for three different tire lug heights, three different axle loads and three different tire inflation pressures in stubble ground conditions. Experiments were conducted by mounting single wheel tester to a three point hitch of an agricultural tractor (Ekinci 2011).

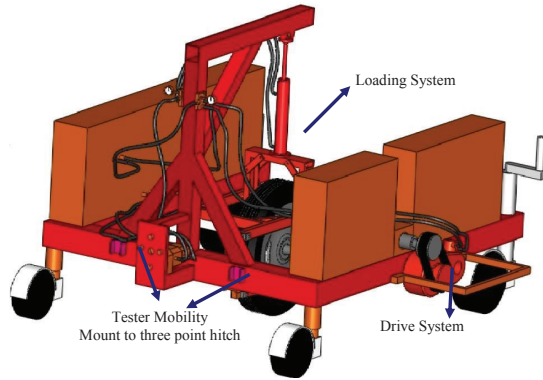
## 2. Material and Methods

### 2.1. Facilities and equipment

In this study, a new single wheel tester (traction setup) was designed and manufactured for determining tractive performance of tractor tire. This mechanism is capable of controlling peripheral speed of tire, wheel travel reduction and dynamic wheel load sufficiently. Designed and manufactured single wheel tester can carry out performance measurements of up to 10 kN axle load and 3.5 kN m torque (Ekinci 2011).

The single wheel tester used in tractive performance experiments consists of three sections (Figure 1). The first part is traction section. It is connected to three point linkage mechanism of tractor. This part provides mobility to experimental setup, which pushes symmetry axis to tractor (traction carriage).

The second section is driving system. It transfers motion from hydraulic motor driven by hydraulic pump connected to PTO shaft of the tractor to test tire. Power transmission via the hydraulic pump



**Figure 1- Single wheel tester design concept**

*Şekil 1- Tek tekerlek deney düzeneğinin tasarım konsepti*

and motor combination is provided. The pump was attached to the PTO using a planetary step up gearbox. This planetary gearbox has 1:3 speed increments and was attached to the 540 rpm PTO shaft on the tractor. A variable tandem pump was chosen for this application. The maximum displacement of the pump is  $32 \text{ cm}^3 \text{ rev}^{-1}$ . The operating pressure of this pump is 175 bar and pressure limited using pressure regulating valve. The selected motor is a fixed displacement motor with a maximum output torque of 119 Nm. Maximum speed is 1210 rpm and the motor displacement is  $50 \text{ cm}^3 \text{ rev}^{-1}$ . Hydraulic motor drives the test wheel. The tire driving system is powered with a 30:1 speed reducing gearbox through a flexible coupling when running in the

driving test mode. In order to determine torque, a transducer with the capacity of 3000 Nm was used between gearbox output and the wheel axis input. Input torque to the wheel axle was measured by this torque transducer and recorded in a data logger. Joint shaft was used between these two points in order to intercept the axis eccentricity which is caused by preformed deflections on tire depending on changing dynamic load and internal pressure of tire. Table 1 shows the specifications of load-cells and transducer.

Third section is loading system which consists of a hydraulic piston cylinder mechanism and applied dynamic load to test tire. The hydraulic tandem pump has dual-stage and first stage of the pump was used in the driving system. The second stage operates the cylinder of loading system. The Loading system consists of pressure regulating valve and a hydraulic cylinder which is capable of developing 50 kN force at 25000 kPa pressure. Hydraulic cylinder was connected to upper carrier wheel carriage and dynamic wheel load was transferred from the frame to the tire under test via a hydraulic cylinder connected to an S-type load cell. A 3/8" hose was used in this circuit with JIC 37° fittings used for the connections. Eight 50 kg concrete blocks were mounted to the frame to provide an increased load to the tire as the cylinder rod was extended. With the included weight of the frame, the tester was able to provide 8 kN wheel load.

**Table 1- Specification of loadcells and transducer**

*Çizelge 1- Yük hücreleri ve torkmetrenin teknik özellikleri*

Torque transducer		Loadcell	
Specification	Values	Specification	Values
Measurement range	2000~5000 Nm	Capacity	2500 kg
Max axial load	4800 N	Sensitivity	$3.0 \pm 0.003 \text{ mV V}^{-1}$
Max radial load	1950~4000 N	Input resistance	$400 \pm 20 \Omega$
Supply voltage	12V DC $\pm 10\%$	Output resistance	$352 \pm 3 \Omega$
Option speed rev. max	up to 10 000 rpm	Insulation resistance	$\geq 5000 \text{ M}\Omega$
Current consumption	Approximately 160 mA	Ultimate overload	180% F S
Voltage output	0 to $\pm 10\text{V}$	Excitation	10~12 DC



Two types of tires, bias-ply and radial tires, were used during the experiments. The tires had three different lug heights which were  $h_{Lb1}=18$ ,  $h_{Lb2}=14$ ,  $h_{Lb3}=10$ ,  $h_{Lr1}=30$ ,  $h_{Lr2}=24$ ,  $h_{Lr3}=14$  mm. Table 2 shows the values of the parameters for the tire manufacturer.  $h_{Lb}$ : Bias-ply lug height,  $h_{Lr}$ : Radial lug height.

**Table 2- Specifications of test tires**

Çizelge 2- Test lastiklerinin teknik özellikleri

Specification	Values	
	Bias-ply	Radial
Tire type	7.50-18	7.50R18
Ply rating	8	-
Tire section height (mm)	205	205
Tire overall diameter (mm)	860	860
Tire carrying capacity (kg)	950	900
Load index and speed symbol	106 A6	104 A8

## 2.2. Experimental procedure

Prior to the test, the dynamic rolling radius of the tires were determined on a concrete road surface under unloaded condition. Grease was plastered onto lug on tire which contacted the ground. Tire was rotated four cycles at lower velocity through a straight line in test ground. Distance between the first contact trace and the last contact trace was measured and divided by  $8\pi$  to obtain the dynamic rolling radius. Dynamic radii were determined for the specific experimental conditions for every profile height, dynamic axle load and inflation pressure.

In order to determine the actual forward speed of the wheel for each test, elapsed time between start and finish point of the test area with 100 m distance was measured via a chronometer. Wheel revolution (n) was measured through a magnetic sensor which was on torque transducer to determine the theoretical velocity.

The variables recorded for each test were ( $W$ ) dynamic axle load on wheel ( $P_i$ ) tire inflation pressure ( $F_t$ ) drawbar pull, ( $T$ ) input torque to the axle, ( $V_a$ ) actual velocity and ( $V_t$ ) theoretical velocity.

The traction parameters used to describe the tractive performance are described in Equations 1 to 5.

$$\text{Tire travel reduction (S), } S = \left(1 - \frac{V_a}{V_t}\right) \cdot 100 \quad (1)$$

$$\text{Drawbar power, } P_D = F_t \cdot V_a \quad (2)$$

$$\text{Axle power (kW), } P_a = \frac{T \cdot n}{9549} \quad (3)$$

$$\text{Tractive efficiency, } TE = \frac{P_D}{P_a} \quad (4)$$

$$\text{Net traction ratio, } NTR = \frac{F_t}{W} \quad (5)$$

The tests were conducted on stubble field ground. In order to check the uniformity of the bed conditions, a few important soil parameters such as penetration resistance of a soil, soil shearing strength, moisture content and surface roughness were measured before starting the experiment (Çarman 1997). Soil ground conditions of stubble field are given in Table 3.

**Table 3- Soil parameters of stubble field**

Çizelge 3- Anız tarlanın toprak parametreleri

Parameter	Value
Texture	Clay-loam
Clay (%)	38.00
Silt (%)	38.00
Sand (%)	24.00
Gravimetric moisture content (%) (0-20 cm)	12-13
Penetration resistance (MPa) (0-20 cm)	2.74
Shearing strength (kN m <sup>-2</sup> )	31.74
Surface roughness (%)	6.09

Surface relief was measured using surface profilemeter. This consisted of a set of vertical rods, spaced at 2.5 cm intervals, sliding through an iron bar of 100 cm length. The soil surface roughness was calculated by using the Kuipers equation (Equation 6).

$$R_d = 100 \cdot \log S_d \quad (6)$$

Where;  $S_d$ , standard deviation.

Penetrometers which can measure up to 80 cm depth at each 1 cm distance were used for measuring soil penetration resistance as MPa. Measurements were taken from 0-20 cm depth. Soil shear testing device was used in order to determine the soil shearing strength ( $\tau$ ) which has 10 cm diameter and 12 cm height. Torque arm with measuring range of 0-80 Nm was impaled on shear vane. The maximum torque was obtained via soil shear testing device while shearing strength was obtained by the Equation 7 (Okello 1991).

$$\tau = T / \left[ \pi \cdot d_k^2 \left( h_k / 2 + d_k / 6 \right) \right] \quad (7)$$

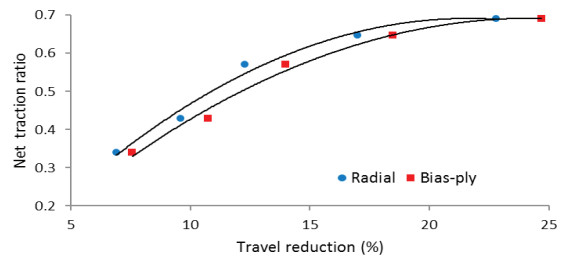
Where;  $d_k$ , paddle switch device diameter and  $h_k$ , paddle switch device blade height.

Traction tests were conducted at velocities of about 5 km h<sup>-1</sup> under stubble field surface conditions which has 100 m distance test area using bias-ply and radial tires having three different lug heights. Axle loads were approximately 5 kN per wheel for horticulture tractors. As experiments were planned by considering these data, axle load was determined respectively as 3.5, 5 and 6.5 kN. Here, values of  $\pm 30\%$  of the 5 kN were taken into account. Axle loads were applied on to the tire by means of a hydraulic cylinder. Dynamic axle load was measured with a loadcell between hydraulic cylinder and wheel carriage. The experiments were conducted at three different tire inflation pressures of 230, 260 and 290 kPa. These inflation pressures varied at  $\pm 12\%$  of the inflation pressure recommendation by tractor manufacturers. These tractor manufacturers recommend inflation pressures for the practical inflation values that local farmers use in horticulture tractor. Every test combination was conducted at five different hand brake levels of the test tractor, and thus five different brake forces were attained. The measurements were repeated 5 times at each dynamic load and inflation pressure. Test tire was made to move via driving system of single wheel test setup mounted on the tractor and brake was

applied to the setup by means of hand brake lever. Four parallel linkages were connected between the main carriage and wheel carriage through load cells. The parallel arms used eliminate the change in vertical reaction force. Drawbar pull is measured using four load cells offset by a vertical distance and symmetric with respect to the tire. Drawbar pull value which was imported via those four load cells was recorded to data taker. Drawbar pull, forward velocity, wheel torque and wheel rotational speed were measured and stored in the data logger. Variance analyses of manipulated variables such as tire type, lug height, axle load and inflation pressure were done in order to determine their effects on tractive efficiency values. MINITAB program was used to obtain the results of variance Analysis and LSD tests were done via ANOVA module.

### 3. Results and Discussion

Two types of tires were each tested at three different lug heights, three levels of inflation pressure and three different vertical loads in stubble field. Two performance characteristics were compared as TE and NTR. The data collected for each experiment was analyzed in a spreadsheet to develop simple relationships between tire type and NTR. The plotted graphs (Figure 2) show that the tire NTR increased with the travel reduction, but the rate of the increase also decreased with increasing travel reduction.



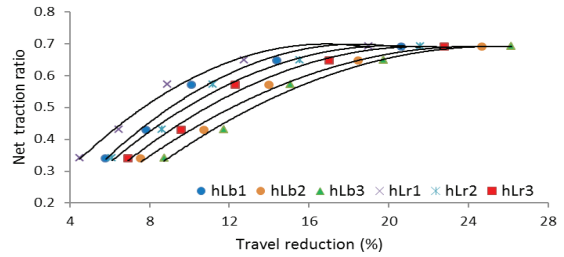
**Figure 2- Net traction ratio as a function of travel reduction for the bias-ply with lug height of 14 mm and radial tire with lug height of 14 mm**

*Şekil 2- 14 mm profil yüksekliğine sahip çapraz katlı ve radyal lastik için patinajın bir fonksiyonu olarak net çeki oranı*

Wheel torque and net traction increase with travel reduction or slip, because initial travel reduction is mainly due to elastic deformation of the tire tread. Further increase in wheel torque and net traction results in part of the tire tread sliding on the ground. At all combinations of three levels of inflation pressure and three different vertical loads, bias-ply tires with lug height of 14 mm developed the same NTR at approximately 10% more travel reduction ratio than radial tires with the same lug height. Depending on the structural and operational characteristics of drive tire, minimum travel reduction value was found to be 3.3% and maximum travel reduction value was 34.1%. Upadhyaya & Wulfsohn (1989) and Bashford & Kocher (1999) found that radial tires performed better than bias ply tires.

At same travel reduction ratio, maximum NTR was 0.69 for  $h_{Lr1}$  lug height of tire while maximum NTR was 0.58 for  $h_{Lb3}$  lug height. Travel reduction ratio for radial tire decreased by 8% when lug height of tire was increased by 71%. Also, travel reduction ratio for radial tire decreased by 25% when lug height of tire was increased by 114%. For bias-ply tire, when lug height of tire was increased by 40% and 80%, travel reduction ratio decreased respectively by 7% and 28%. Although increased lug height increased the hold on tire to soil, decreased lug height increased travel reduction because the tire did not hold on sufficiently to the soil. The results showed that the tire with high lugs can develop higher NTR than the smooth tire. The effect of lug height on the NTR is shown in Figure 3. Plackett (1984) found that as lug height increased, size of the contact area increased, leading to a reduction in mean ground pressure. Thus, NTR increased when lug height of tire was increased. Nakashima et al (2007) stated that higher lugs tend to yield higher net traction.

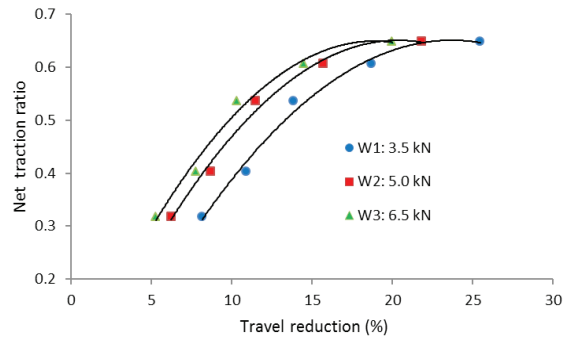
Figure 4 indicates the effects of travel reduction at variable dynamic load on net traction, respectively. For a given dynamic load, net traction increased as travel reduction increased. During the experiments, average travel reduction values decreased by 21% for bias-ply tires and by 18% for radial tires depending on increased axle load of 86%. Dynamic load affected soil-tire contact pressures differently



**Figure 3- Net traction ratio as a function of travel reduction for six lug heights of tires**

*Şekil 3- Lastiklerin altı profil yüksekliği için patinajın bir fonksiyonu olarak net çeki oranı*

for the different transducer locations. Results from previous research for radial-ply tractor drive tires indicate that an increase in dynamic load caused a decrease in normal stresses on lugs at the lug middle and tire centerline positions and an increase in normal stress at the outside edge of the lug, on firm soil (Burt et al 1980). Çarman & Aydın (2002) observed that NTR and TE showed an increase of 22% and 6% respectively when they increased about 50% of the dynamic load.

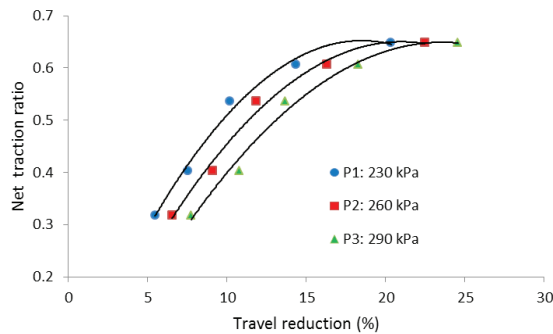


**Figure 4- Net traction ratio as a function of travel reduction for three different dynamic loads**

*Şekil 4- Üç farklı dinamik yükü için patinajın bir fonksiyonu olarak net çeki oranı*

The highest value of 0.65 of NTR was reached at inflation pressure of 230 kPa while using bias-ply and radial tires. By increasing tire inflation to 290 kPa, the NTR decreased to 0.62 at same travel

reduction ratio. Figure 5 shows the graph of tire NTR for the three inflation pressures (230, 260 and 290 kPa). When tire inflation pressure was increased from 230 to 260 kPa, average travel reduction values increased by 27% for both bias-ply and radial tires. In the slip range of approximately 20-30%, the course follows a linear part, where increase of drawbar pull causes the increase of slip loss. The positive effect of lowering inflation pressure on the improvement of tractive performance was evident when forward velocity was 5 km h<sup>-1</sup>. Jun et al (2004) claimed that in height inflation pressure and low dynamic load, distributions of contact pressures across tire width were highly non-uniform and pressures were concentrated at the middle of lug. Correct combinations of dynamic load and inflation pressure were used; however, the distributions of contact pressure were relatively uniform. Therefore, net traction and tractive efficiency of tire decreased with increasing inflation pressure at constant dynamic load. At constant inflation pressure, net traction and tractive efficiency increased with increasing dynamic load.

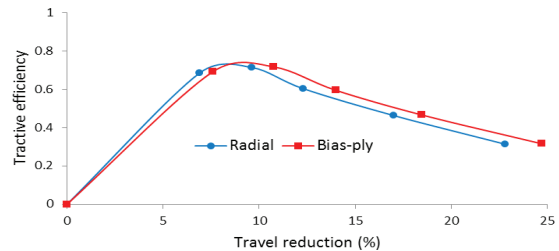


**Figure 5-** Net traction ratio as a function of travel reduction for three different inflation pressures

*Şekil 5- Üç farklı iç basınç için patinajın bir fonksiyonu olarak net çeki oranı*

Depending on changing structural and working characteristics of drive tire, tractive efficiency values were determined in a range of 0.27-0.78 at changing travel reduction. The greatest tractive efficiency for both tires was obtained at the lowest

inflation pressure of 230 kPa and the highest dynamic load of 6.5 kN. Tractive efficiency of the radial tire was greater at all combinations. Tractive efficiency of radial tire with lug height of 14 mm was 2% more than bias-ply tires with lug height of 14 mm. The greatest difference in terms of tractive efficiency between the radial and bias-ply tire was obtained at 230 kPa inflation pressure, 6.5 kN and 4.6% travel reduction. A certain amount of travel reduction of radial tires was obtained at lower levels than bias-ply tires for same axle loads and inflation pressures. Tractive efficiencies as a function of travel reduction for both radial and bias-ply tires are shown in Figure 6. Analysis of variance (ANOVA) was developed showing effects of tire type, lug height, axle load and inflation pressure interactions on tractive efficiency (Table 4). As appreciated from Table 4, effect of both tire types on tractive efficiency was found to be important ( $P < 0.01$ ). Sümer & Sabancı (2005) indicated that radial-ply tires provided a slight advantage over bias-ply tires. Overall tractor efficiency was increased by 3.44%, while specific fuel consumption was decreased by 3.08% on average with radial-ply tires compared to bias-ply tires.



**Figure 6-** Effect of tire type on tractive efficiency at varies travel reduction levels

*Şekil 6- Farklı patinaj seviyelerinde lastik tipinin çeki verimi üzerine etkisi*

Particularly, increasing of lug height has a great effect on tractive efficiency on stubble ground. When lug height was increased by 71% for radial tires, the tractive efficiency increased by 4%. Likewise, when lug height of bias-ply tire was increased by 40%, the tractive efficiency increased by 5% (Figure

**Table 4- Variance analysis and LSD tests which were carried out for tractive efficiency values at stubble field conditions**

Çizelge 4- Anız tarla şartlarındaki çeki verim değerlerinin varyans analizi ve LSD testleri

VS	DF	SS	MS	F- Statistics
Tire size ( $T_s$ )	1	0.0072558	0.0072558	93.34**
Lug height ( $h_L$ )	2	0.0152749	0.0076375	98.25**
Dynamic load (W)	2	0.0124292	0.0062146	79.95**
$T_s * W$	2	0.0001478	0.0000739	0.95 <sup>ns</sup>
$h_L * W$	4	0.0010557	0.0002639	3.40 <sup>ns</sup>
Inflation pressure ( $P_i$ )	2	0.0029860	0.0014930	19.21**
$T_s * P_i$	2	0.0000603	0.0000302	0.39 <sup>ns</sup>
$h_L * P_i$	4	0.0003757	0.0000939	1.21 <sup>ns</sup>
$W * P_i$	4	0.0003275	0.0000819	1.05 <sup>ns</sup>
$T_s * W * P_i$	4	0.0001965	0.0000491	0.63 <sup>ns</sup>
Error	26	0.0020210	0.0000777	
Total	53	0.0421306		

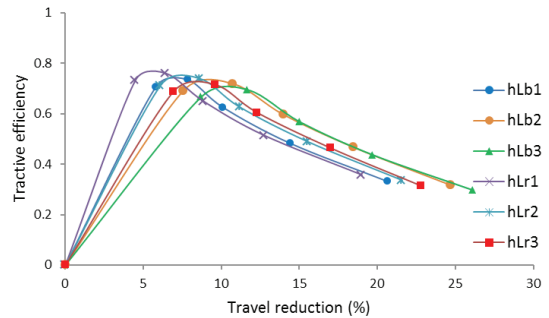
\*\*, (P<0.01); <sup>ns</sup>, not significant

$T_s$	$h_L$	W	$P_i$
(Bias-ply) 0.557 <sub>a</sub>	( $h_{L1}$ ) 0.590 <sub>a</sub>	( $W_1$ ) 0.552 <sub>a</sub>	( $P_{i1}$ ) 0.575 <sub>a</sub>
(Radial) 0.581 <sub>b</sub>	( $h_{L2}$ ) 0.571 <sub>b</sub>	( $W_2$ ) 0.568 <sub>b</sub>	( $P_{i2}$ ) 0.571 <sub>a</sub>
	( $h_{L3}$ ) 0.546 <sub>c</sub>	( $W_3$ ) 0.587 <sub>c</sub>	( $P_{i3}$ ) 0.561 <sub>b</sub>

LSD (5%): 0.004	0.005	0.005	0.005
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7). Maximum tractive efficiency obtained for all combinations was 0.78 at  $h_{Lr1}$  lug height of radial tire while minimum tractive efficiency was obtained 0.27 at  $h_{Lb3}$  lug height of bias-ply tire. Effect of lug height on the tractive efficiency was found to be important (P<0.01).

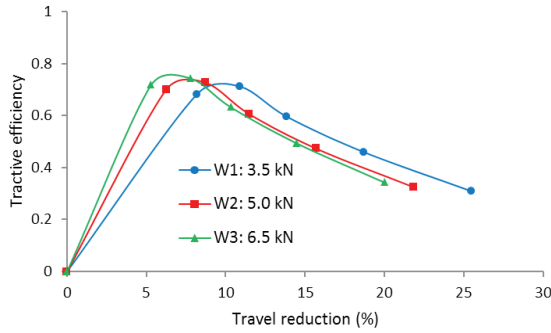
Depending on increased dynamic axle load, the average tractive efficiency values increased by 6%. When dynamic load was increased from 3.5 kN to 5 kN, the tractive efficiency increased by 2.5%. Dynamic load increased the tractive efficiency by 3.5% when it was increased from 5 kN to 6.5 kN. Increased dynamic load improved adherence of tire to soil and decreased travel reduction. Figure 8 shows the effect on tractive efficiency of different dynamic axle loads. Effect of dynamic axle load on the tractive efficiency was found to be important (P<0.01). There were no significant differences between W2 and W3. Similar reports concerned with tractive performance

**Figure 7- Effect of lug height on tractive efficiency at varies travel reduction levels**

Şekil 7- Farklı patinaj seviyelerinde profil yüksekliğinin çeki verimi üzerine etkisi

parameters in the literature confirm the discovered trends in this investigation (Çarman & Aydın 2002; Elwaleed et al 2006; Yahya et al 2007).

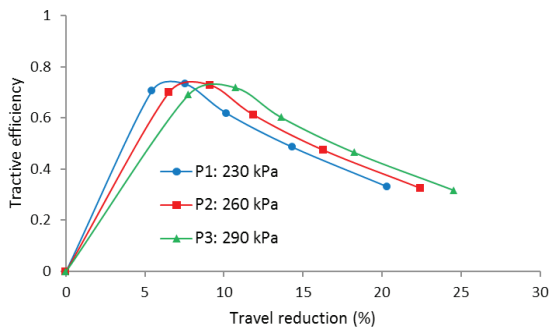




**Figure 8- Effect of dynamic load on tractive efficiency at varies travel reduction levels**

*Şekil 8- Farklı patinaj seviyelerinde dinamik yükün çeki verimi üzerine etkisi*

Tire tractive efficiency curves are shown in Figure 9. Figure 9 shows that tractive efficiency of tire increases suddenly with increasing travel reduction to a maximum value and then gradually decreases with the increase in travel reduction. When inflation pressure was reduced from 290 kPa down to 260 kPa; the tractive efficiency increased by 2% with the same amounts of travel reduction. Similarly, the tractive efficiency increased by 1.2% when inflation pressure was decreased from 260 to 230 kPa. Smerda & Cupera (2010) found that reducing tire inflation of appropriate tire types can improve the drawbar characteristics and, consequently, fuel consumption.



**Figure 9- Effect of inflation pressure on tractive efficiency at varies travel reduction levels**

*Şekil 9- Farklı patinaj seviyelerinde iç basıncın çeki verimi üzerine etkisi*

## 4. Conclusions

As conclusions, effects on travel reduction and tractive efficiency values of manipulated variables such as tire type, lug height, inflation pressure and axle load can thus be summarized as follows.

At all combinations of the variables, radial tires developed more NTR than bias-ply tires. Accordingly, the axle power requirement is 2.7% higher than has occurred. The radial tire had greater tractive efficiency than the bias-ply tire except in one combination of three levels of lug height, three dynamic loads and three inflation pressures selected in the study. Tire tractive efficiency increased suddenly with increasing travel reduction to a maximum value and then gradually decreased with increase in travel reduction.

Travel reduction ratio increased when lug height of tire was decreased. When lug height was increased the tractive efficiency increased for both tires. Tire with high lugs can develop higher tractive force than the smooth tire.

For a given dynamic load, net traction increased as travel reduction increased. During the experiments, depending on axle load increase of 86%, average travel reduction values decreased by 21% for bias-ply tires and by 18% for radial tires. Depending on increased dynamic axle load, the average tractive efficiency values increases by 6%.

The highest value of NTR (0.65) was reached at inflation pressure of 230 kPa while using bias-ply and radial tires. By increasing tire inflation to 290 kPa, the NTR decreased to 0.62 at same travel reduction ratio. When inflation pressure is reduced from 290 kPa to 230 kPa, the tractive efficiency tends to increase by 3%.

The single wheel tester can be used for testing the effects of parameters such as dynamic loads, lug heights and tire inflation pressure on tractive performances of the tire.

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## Phytochemical Analysis, Antioxidant and Antibacterial Activities of Four Lamiaceae Species Cultivated in Barnyard Manure

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### ABSTRACT

The present study was conducted to determine essential oil yields, essential oil compositions, total phenolics, antioxidant and antibacterial activities of organic manure-treated medicinal plants of *Salvia officinalis* L. (sage), *Lavandula angustifolia* L. (lavender), *Melissa officinalis* L. (lemon balm) and *Origanum vulgare* ssp. *hirtum* (origano). Essential oil yields of investigated medicinal plants varied between 0.06±0.01%-3.43±0.06%. The 1,8-cineol (15.285±0.003%), viridiflorol (12.095±0.003%) and cis-thujone (12.200±0.003%) were the major essential oil components in *S. officinalis* L. Linalool (22.400±0.003%), 1,8-cineol (8.215±0.003%), linalyl acetate (7.900±0.003%) and lavadulyl acetate (7.690±0.003%) were the major components in *L. angustifolia* L. Citronellal (14.515±0.003%), geranial (13.050±0.003%) and β-caryophyllene (12.385±0.003%) were the major components in *M. officinalis* L. and carvacrol (65.080±0.003%) was the major component in *O. vulgare* ssp. *hirtum*. The highest total phenolics content and antioxidant activity were observed in *M. officinalis*. The best antibacterial activity against *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 35218 bacteria was observed in *O. vulgare* ssp. *hirtum*.

Keywords: *Salvia officinalis* L.; *Lavandula angustifolia* L.; *Melissa officinalis* L.; *Origanum vulgare* ssp. *hirtum*; Medicinal and aromatic plants

## Ahır Gübresinde Yetiştirilen Dört Lamiaceae Türünün Fitokimyasal Analizleri, Antioksidant ve Antibakteriyel Aktiviteleri

### ESER BİLGİSİ

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### ÖZET

Bu çalışmada organik (ahır gübresinde) yetiştirilen *Salvia officinalis* L. (Tıbbi adaçayı), *Lavandula angustifolia* L. (İngiliz lavantası), *Melissa officinalis* L. (Oğul otu) ve *Origanum vulgare* ssp. *hirtum* (İstanbul kekiği) tıbbi bitkilerinin uçucu

yağ oranları, yağ bileşenleri, toplam fenolik içerikleri, antioksidant ve antibakteriyel aktiviteleri araştırılmıştır. Çalışma sonucunda incelenen bitkilerde uçucu yağ oranının % 0.06±0.01-% 3.43±0.06 arasında değişim gösterdiği belirlenmiştir. Uçucu yağ bileşenleri olarak: *S. officinalis* L.'te 1,8-cineol (% 15.285±0.003), viridiflorol (% 12.095±0.003) ve cistujone (% 12.200±0.003); *L. angustifolia* L.'de linalool (% 22.400±0.003), 1,8-cineol (% 8.215±0.003), linalyl acetate (% 7.900±0.003) ve lavadulyl acetate (% 7.690±0.003); *M. officinalis* L.'te citronellal (% 14.515±0.003), geranial (% 13.050±0.003) ve β-caryophyllene (% 12.385±0.003); *O. vulgare* ssp. *hirtum*'de carvacrol (% 65.080±0.003) tespit edilmiştir. En yüksek toplam fenolik içerik ve antioksidant aktivite *Melissa officinalis* L.'te görülmüştür. Kullanılan *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 ve *Escherichia coli* ATCC 35218 bakterilerine karşı en iyi antibakteriyel aktiviteyi *Origanum vulgare* ssp. *hirtum*'un gösterdiği belirlenmiştir.

Anahtar Kelimeler: *Salvia officinalis* L.; *Lavandula angustifolia* L.; *Melissa officinalis* L.; *Origanum vulgare* ssp. *hirtum*; Tıbbi ve aromatik bitkiler

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## 1. Introduction

Increasing infection risks stemming from antibiotic-resistant microorganism have made the discovery of new and natural antimicrobial substances the focus of various researches. What is more, various synthetic food additives for preservative purposes created serious concerns on sensitive and conscious consumers. Such concerns have brought about the concepts of organic food or organic agriculture. The expectations of conscious consumers have encouraged and even forced the producers and service providers to use natural preservatives. Then, a need has arisen for researchers to investigate and test efficiency of various plants against microorganisms. Antibacterial impacts of various plant extracts microorganisms and especially on food pathogens have been supported by several researchers.

As it is well known, reactive oxygen species, singlet oxygen, superoxide radicals, hydrogen peroxide, hydroxyl radicals and nitric oxide are unstable and extremely reactive compounds. Oxidative stress-induced reactive oxygen species are blamed to be the indicators of development and progress of various cardiovascular diseases. Antioxidants prevent negative impacts of free radicals and reactive oxygen species and protect the body. Today, BHT, BHA, propyl gallate and tert butyl hydroquinone are the most common synthetic antioxidants. However, reliability of these synthetic

antioxidants are argued because of their toxic and carcinogenic effects and resultant liver injury. Therefore, discovery of new, reliable and unharmed antioxidants from natural resources have become the most common research topic (Birman 2012).

Lamiaceae species are now cultivated worldwide, mainly to be used as culinary and medicinal herbs and they are widely studied as natural antioxidant sources since they are relatively rich in polyphenols (Cuvelier et al 1994). The Lamiaceae species of *Salvia officinalis* L. (sage), *Lavandula angustifolia* L. (lavender), *Melissa officinalis* L. (lemon balm), *Origanum vulgare* ssp. *hirtum* (origano) cultured in this study are popular herbal teas and essential-oil containing drugs. Their therapeutic actions are assigned to biologically active polyphenol components, such as flavonoids and phenolic acids, which possess antioxidant activities. They are naturally grown in Turkey and commonly used by local people in treatments of various diseases. However, culture of these plants is scarcely any. Therefore, they are commonly collected from their natural habitats and marketed then. Collection usually starts with the fresh shoots through the early development stages and such a collection results in excessive damage to plants. Damaged plants are then not able to develop efficient seeds and ultimately they experience various problems for their survival. Thus, for the preservation of natural plant cover and plant genetic sources, culture environments should be created for these plants under such pressures.

A common standardization also plays a significant role for the trade of these plants. Standardized production will then be possible only with the culture and breeding of species.

The primary objectives of the present study are to prevent genetic erosion in country flora; to grow high yield and quality medicinal plants and to raise an awareness on fertilizer utilization which has not been fully comprehended by Turkish farmers and to improve organic fertilizer (manure) use over agricultural fields. In sustainable agriculture, organic fertilizers not only supply plant nutrients but also improve soil organic matter contents. Thus, the objective is to encourage the use of organic fertilizers over cultivated lands. Another objective of the present study is to determine the essential oil yields, essential oil compositions, total phenolics, antioxidant and antibacterial activities of four medicinal and aromatic plants of Lamiaceae family *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum* cultivated with organic barnyard manure.

## 2. Material and Methods

The seedlings supplied from Field Crops Central Research Institute of the General Directorate of Agricultural Researches and Policies constituted the primary materials of the present study. Experiments were conducted in randomized block design with three replications over 1500 m<sup>2</sup> area in Kürtün town of Gümüşhane Province. Average climatic data were recorded for years 2010-2013 as follows: 10.4 °C temperature; 39.51 mm precipitation; 64.0% relative humidity (Anonymous 2015). Experimental fields have sandy-clay-loam soil texture with slightly alkaline characteristics (pH 7.20). Soils were classified as unsaline (0.8%) and found to be sufficient in phosphorus (with available phosphorus content of 84.91 kg ha<sup>-1</sup>).

While selecting plant species, the significant plants for regional development, the ones suitable for regional ecology and with high value-added were taken into consideration. A month before plantation of seedlings, 15 ton ha<sup>-1</sup> decomposed manure were applied. Maintenance and care practices were

regularly implemented based on climate conditions and 15 ton ha<sup>-1</sup> manure was also applied in autumn of every year (in November). No chemicals were used in experiments.

*S. officinalis* L., *L. angustifolia* L., *M. officinalis* L. and *O. vulgare* ssp. *hirtum* were harvested at full bloom stage in a sunny day at noon time of the year 2013. Plants were dried at shade and made ready for laboratory analyses.

### 2.1. Isolation of the essential oil (essential oil preparation)

Essential oil analyses were carried out in accordance with TS 8882 method. About 20 g sample was taken from dried plants of each species and placed into glass Clevenger flasks. About 200 mL (about ten times of sample weight) distilled water was added and samples were then subjected to hydro-distillation for about 3 hours. The essential oil accumulated on top and separated from the rest of the sample. The amount was recorded in ml from the graduated section of the flask and weights were then used to calculate percent essential oil yields.

### 2.2. Gas chromatography-mass spectrometry/flame ionization detector (GC-MS/FID)

The essential oil composition of samples was analyzed by gas chromatography (Agilent 7890A) coupled with flame ionization detector and mass spectrometry (Agilent 5975C) with capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm). Essential oils were diluted 1:50 ratio with hexane. GC-MS/FID analysis was carried out at split mode of 50:1. Injection volume and temperature were adjusted as 1 µL and 250 °C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 1 mL min<sup>-1</sup>. The oven temperature was programmed as follow; 60 °C for 10 minutes, increased at 20 °C minute<sup>-1</sup> to 250 °C, and held at 250 °C for 8 minutes. MS spectra were monitored between 35-450 amu and the ionization mode used was electronic impact at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas, and components were identified by WILEY, NIST and FLAVOR libraries.



### 2.3. Extraction of samples

Dry samples were extracted by methanol at three steps according to Cai et al (2004). Briefly, approximately 5 g of the arils were extracted twice with 10 mL of pure methanol for 1 hour, 10 mL for 30 minutes, and then with 5 mL for 30 minutes in an ultrasonic bath at room temperature.

### 2.4. Determination of total phenolic content

Spectrometric method defined by Spanos & Wrolstad (1990) was employed to determine total phenolic substance. About 100  $\mu$ L sample were into a tube and 900  $\mu$ L distilled water was added. Then, 5 mL 0.2 N Folin-Ciocalteu solution (10 times diluted with distilled water) and 4 mL saturated sodium carbonate solution (75 g L<sup>-1</sup>) were added into samples and tubes were completely vortexed and left in dark for 2 hours. The extracts were combined and phenolic content of these extracts were measured at 765 nm by using UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed as gallic acid equivalent (mg GAE g<sup>-1</sup>) by using standard calibration curve of this phenolic compound.

### 2.5. Determination of antioxidant activity

Antioxidant activities (AA) of the samples were determined by DPPH method (Lafka et al 2007). Antioxidant capacity of these extracts (same as total phenolic matter extraction procedure) was measured at 515 nm by UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Results were calculated as inhibition capacity (IC<sub>50</sub>). % inhibition values (swiping effects of samples on DPPH radical) were calculated by using the Equation 1.

$$\% \text{ Inhibition} = [(A_{\text{DPPH}} - A_{\text{extract}}) / A_{\text{DPPH}}] \times 100 \quad (1)$$

Where;  $A_{\text{DPPH}}$ , absorbance of the control reaction;  $A_{\text{extract}}$ , absorbance in the presence of tested extracts;  $A_{\text{DPPH}}$ , absorbance value of 0.1 mL methanol+3.9 mL DPPH solution;  $A_{\text{extract}}$ , absorbance value of samples after 30 minutes; reset solution, pure methanol.

Percent inhibition values obtained from samples at different concentrations and concentration

values were inserted into graphs and effective concentration inhibiting DPPH effects by 50% (EC<sub>50</sub>) was calculated for each sample (Lafka et al 2007).

### 2.6. Determination of antibacterial activity

The test organisms included gram-positive *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and gram-negative *Echerichia coli* ATCC 29213. All ATCC bacterial strains were obtained from BATEM Microbial Culture Collection, Antalya, Turkey. The bacteria were grown in the Müller Hinton Agar (MHA) at 37 °C and maintained on Müller Hinton Agar plate at 4 °C. In vitro antibacterial activity was examined for essential oil obtained from *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum* traditionally used as medicinal plants. Antibacterial activities of these essential oils were evaluated by disc diffusion method (CLSI 2006). For all the bacterial strains, overnight cultures were grown in MHA and they were adjusted to an inoculation size of 0.5 McFarland 10<sup>8</sup> CFU mL<sup>-1</sup> for inoculation of the agar plates. 100  $\mu$ L of bacterial culture suspension was spread on MHA. Then the bacteria were spread over MHA with a sterile swab. Then, sterile filter paper disc was soaked into 10  $\mu$ L of essential oil and blank disc (for sterilization control) and antibiotic disc were placed on it. Based on sensitivity characteristics of each bacterium, standard antibiotic discs selected from CLSI were used as positive control treatments. Bacteria were incubated at 37 °C overnight. After an incubation period of 24 h at 37 °C, antibacterial activity was evaluated by inhibition zones of bacterial growth. Three replications of each test were performed. The results are presented as average zone of inhibition of all the bacterial strains of ATCC.

### 2.7. Statistical analysis

The Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. Data sets were analyzed with one-way ANOVA and means were compared

with Tukey's post-hoc test. The Tukey test results were displayed in the form of letters. Parameters were displayed as mean±standard error of the mean (SEM). The alpha level was set at 5%. The statistical analysis was performed using Minitab 17 statistical software.

### 3. Results and Discussion

Descriptive statistics for essential oils and essential oil components of *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L. and *O. vulgare* ssp. *hirtum* plants are provided in Tables 1-5. The descriptive statistics and results of Tukey's post-hoc test at  $P<0.05$  for common components (essential oil, total phenolic content, antioxidant activity, antibacterial activity) are provided in Tables 6-8. Results of ANOVA carried out to compare the major components are provided in Table 9.

#### 3.1. Essential oils and chemical composition of essential oils

Essential oils contents of *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum*, and *S. officinalis* L. were respectively observed as  $0.73\pm 0.04\%$ ,  $0.06\pm 0.01\%$ ,  $3.43\pm 0.06\%$  and  $1.40\pm 0.04\%$  (Table 5). The differences between essential oil yields of the plants were found to be significant ( $P<0.05$ ). Tukey's test revealed the highest essential oil yields in *O. vulgare* ssp. *hirtum* and the lowest in *M. officinalis* L. ( $P<0.05$ ).

Bouaziz et al (2009) reported the essential oil yields of *S. officinalis* L. as 0.72%, and Ben Taarit et al (2010) as 0.66%. Mirjalili et al (2006) reported the essential oil yields of cultured *S. officinalis* L. in Iran as between 0.20-0.90%. Current finding for the essential oil yield of *S. officinalis* L. ( $1.40\pm 0.04\%$ ) were higher than those reported in earlier studies. Environmental and agronomic practices may result in variations in essential oil yields (Chope & Terry 2009). Seidler-Lozykowska et al (2013) reported the essential oil yield of *M. officinalis* L. as 0.05%, (Padova) as 0.44% and (Warsaw), Carnat et al (1998) as between 0.02-0.3%. The current findings for essential oil yield of *M. officinalis* L. were

similar to those presented in earlier studies. Porto et al (2009) used the HD method of extraction and reported the essential oil yield of *L. angustifolia* L. as between 0.5-1.02% and Yazdani et al (2013) as between 0.25-2.0%. Milos et al (2000) reported the essential yield of *O. vulgare* ssp. *hirtum* as 2.9%.

De Martino et al (2009) reported the essential yield of *O. vulgare* ssp. *hirtum* collected from three different regions as between 2.35-3.15%. Current essential oil yields of *O. vulgare* ssp. *hirtum* were similar with those earlier ones. In the present study, 32 major components were identified in essential oils of *S. officinalis* L. (Table 1). The major components of the essential oil were identified as 1,8-cineol ( $15.285\pm 0.003\%$ ), viridiflorol ( $12.095\pm 0.003\%$ ), cis-thujone ( $12.200\pm 0.003\%$ ),  $\beta$ -pinene ( $9.410\pm 0.003\%$ ),  $\alpha$ -pinene ( $6.310\pm 0.003\%$ ). In essential oil of *L. angustifolia* L., 40 components were identified (Table 2). Linalool ( $22.400\pm 0.003\%$ ), 1,8-cineol ( $8.215\pm 0.003\%$ ), linalyl acetate ( $7.900\pm 0.003\%$ ), lavadulyl acetate ( $7.690\pm 0.003\%$ ) were identified as the major components. In essential oil of *M. officinalis* L., 15 components were identified (Table 3) and citronellal ( $14.515\pm 0.003\%$ ), geranial ( $13.050\pm 0.003\%$ ),  $\beta$ -caryophyllene ( $12.385\pm 0.003\%$ ) were the major components. In essential oil of *O. vulgare* ssp. *hirtum*, 21 components were identified (Table 4). The major component was carvacrol ( $65.080\pm 0.003\%$ ) and it was followed by thymol ( $10.490\pm 0.003\%$ ),  $\gamma$ -terpinene ( $7.340\pm 0.003\%$ ), para-cymene ( $5.315\pm 0.003\%$ ). In previous studies, carvacrol (64.06%) was identified as the major component of essential oil of *O. vulgare* (Stupar et al 2014). Karamanos & Sotiropoulou (2013) reported the carvacrol content of essential oil of *O. vulgare* ssp. *hirtum* as between 56.46-84.88% based on plant organs, seasons and treatments and carvacrol was followed by  $\pi$ -cymene (4.19-21.4%) and  $\alpha$ -pinene (0.11-1.88%). The results of the present study agree with the results of previous works. In a previous report, Stupar et al (2014) indicated the major components of *L. angustifolia* as linalool (37.61%) and linalool acetate (34.86%). Oh (2013) reported linalool and linalyl acetate contents

**Table 1- The essential oil composition of *S. officinalis* L. (%)**Çizelge 1- *S. officinalis* L. 'in uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
cis-salvene	10.16	0.210±0.000	0.000	0.210-0.210
α-pinene	13.03	6.310±0.006	0.010	6.300-6.320
α-thujene	13.18	0.180±0.000	0.000	0.180-0.180
Camphene	14.90	3.100±0.000	0.000	3.100-3.100
β-pinene	16.74	9.410±0.012	0.020	9.390-9.430
Myrcene	19.14	0.685±0.003	0.005	0.680-0.690
α-terpinene	19.96	0.260±0.000	0.000	0.260-0.260
Limonene	20.81	1.120±0.000	0.000	1.120-1.120
1,8-cineol	21.27	15.285±0.009	0.015	15.27-15.30
cis-β-ocimene	22.28	0.650±0.000	0.000	0.650-0.650
γ-terpinene	22.85	0.510±0.000	0.000	0.510-0.510
para-cymene	23.95	0.230±0.000	0.000	0.230-0.230
α-terpinolene	24.43	0.185±0.003	0.005	0.180-0.190
cis-thujone	30.02	12.20±0.006	0.010	12.19-12.21
trans-thujone	30.69	4.200±0.000	0.000	4.200-4.200
cis-Sabinene hydrate	31.13	0.210±0.000	0.000	0.210-0.210
α-copaene	32.42	0.175±0.003	0.005	0.170-0.180
Camphor	33.40	3.265±0.003	0.005	3.260-3.270
Linalool	33.70	0.445±0.003	0.005	0.440-0.450
bornyl acetate	35.26	0.590±0.017	0.030	0.560-0.620
terpinen-4-ol	35.79	0.400±0.006	0.010	0.390-0.410
β-caryophyllene	35.97	5.700±0.000	0.000	5.700-5.700
delta-terpineol	37.82	0.260±0.006	0.010	0.250-0.270
α-humulene	38.19	5.125±0.003	0.005	5.120-5.130
α-terpineol	38.54	0.480±0.006	0.010	0.470-0.490
γ-murolene	38.60	0.405±0.003	0.005	0.400-0.410
Borneol	38.77	7.225±0.020	0.035	7.190-7.260
delta-cadinene	40.51	0.380±0.000	0.000	0.380-0.380
Caryophyllene oxide	46.91	0.365±0.003	0.005	0.360-0.370
humulene epoxide II	48.30	0.510±0.000	0.000	0.510-0.510
Viridiflorol	49.10	12.095±0.003	0.005	12.09-12.10
Manool	52.83	7.835±0.032	0.055	7.780-7.890

of *L. angustifolia* species respectively as between 30.3-38.7% and between 48.0-53.7%. Current major components of the essential oil of *L. angustifolia* L. were a bit lower than the earlier ones. The observed differences may probably be due to use of different parts of plant for analysis, different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition (Ahmadvand

et al 2013). Argyropoulos & Muller (2014) reported the major components of *M. officinalis* L. essential oil as citro-nellal (17.9±1.8%), neral (12.4±2.2%), geranial (16.1±2.7%). Seidler-Łożkowska et al (2013) indicated citral (neral+geranial) as the major component of the essential oil obtained from *M. officinalis* L. leaves and reported the citral contents as between 10.13% (Bonn)-35.83% (Bratislava). Present findings comply with these

earlier values. Bouaziz et al (2009) indicated the major components in essential oils of *S. officinalis* L. as  $\beta$ -thujone (17.76%), 1,8-cineole (eucalyptol) (16.29%), camphor (14.19%),  $\alpha$ -thujone (7.41%), transcaryophyllene (5.45%), viridiflorol (4.63%). Ben Taarit et al (2010) reported the major components

in essential oils of the control plants of *S. officinalis* L. as  $\beta$ -thujone (23.43%), camphor (17.60%), 1,8-cineole (13.83%), viridiflorol (9.36%). The current findings were similar with the findings of the other researchers.

**Table 2- The essential oil composition of *L. angustifolia* L. (%)**

*Çizelge 2- L. angustifolia* L. uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
$\alpha$ -pinene	13.03	0.585±0.003	0.005	0.580-0.590
Camphene	14.90	0.730±0.000	0.000	0.730-0.730
$\beta$ -pinene	16.73	1.085±0.003	0.005	1.080-1.09
Myrcene	19.14	0.540±0.000	0.000	0.540-0.540
Limonene	20.81	3.350±0.000	0.000	3.350-3.350
1,8-cineol	21.25	8.215±0.003	0.005	8.210-8.220
cis- $\beta$ -ocimene	22.28	0.380±0.000	0.000	0.380-0.380
trans- $\beta$ -ocimene	23.03	0.495±0.003	0.005	0.490-0.500
3-octanone	23.20	0.715±0.003	0.005	0.710-0.720
meta-cymene	23.83	0.675±0.003	0.005	0.670-0.680
para-cymene	23.93	1.405±0.003	0.005	1.400-1.410
1-Octen-3-ol acetate	28.02	1.895±0.003	0.005	1.890-1.900
cis-Linalool oxide	30.46	1.760±0.012	0.020	1.740-1.780
trans-Linalool oxide	31.45	1.250±0.000	0.000	1.250-1.250
Camphor	33.40	5.125±0.003	0.005	5.120-5.130
Linalool	33.71	22.40±0.000	0.000	22.40-22.40
linalyl acetate	34.20	7.900±0.000	0.000	7.900-7.900
bornyl acetate	35.27	0.995±0.014	0.025	0.970-1.020
lavandulyl acetate	35.75	7.690±0.023	0.040	7.650-7.730
terpinen-4-ol	35.79	0.685±0.003	0.005	0.680-0.690
Lavandulol	37.78	0.610±0.006	0.010	0.600-0.620
Cyrtone	38.27	3.690±0.012	0.020	3.670-3.710
$\alpha$ -terpineol	38.54	2.020±0.000	0.000	2.020-2.020
Borneol	38.76	4.830±0.023	0.040	4.790-4.870
Eucarvone	39.11	0.740±0.006	0.010	0.730-0.750
neryl acetate	39.32	0.980±0.000	0.000	0.980-0.980
Carvone	39.99	0.660±0.000	0.000	0.660-0.660
geranyl acetate	40.16	2.435±0.003	0.005	2.430-2.440
$\gamma$ -cadinene	40.69	0.985±0.003	0.005	0.980-0.990
Nerol	41.28	0.450±0.000	0.000	0.450-0.450
cumin aldehyde	41.35	1.965±0.003	0.005	1.960-1.970
Geraniol	42.46	1.050±0.006	0.010	1.040-1.060
meta-cymen-8-ol	42.54	0.725±0.003	0.005	0.720-0.730
para-cymen-8-ol	42.72	0.540±0.000	0.000	0.540-0.540
Caryophyllene oxide	46.91	3.735±0.003	0.005	3.730-3.740
1,10-di-epi-Cubenol	48.44	0.435±0.003	0.005	0.430-0.440
para-cymen-7-ol	49.16	0.500±0.000	0.000	0.500-0.500
epi- $\alpha$ -cadinol	51.01	5.360±0.006	0.010	5.350-5.370
Unidentified		0.425±0.061	0.105	0.320-0.530

### 3.2. Total phenolic content

Total phenolic contents of the present study are provided in Table 6. Total phenolic contents varied between  $16.480 \pm 0.087$ - $76.110 \pm 1.030$  mg GAE g<sup>-1</sup> dw with the highest value in *M. officinalis* L. and the lowest value in *L. officinalis* L. ( $P < 0.05$ ). In previous literatures, total phenolic compound of *L. officinalis* L. extract was reported as 76.8 mg GAE g<sup>-1</sup> dw (Rabiei et al 2014). Lin et al (2012) reported the total polyphenols of *M. officinalis* L. as  $175.15 \pm 11.02$  mg g<sup>-1</sup> dw in frozen dry sample extracts and as  $164.13 \pm 12.02$  mg g<sup>-1</sup> dw in hot air dry sample extracts. Barros et al (2013) reported the total phenolic contents of *M. officinalis* L. grown under field conditions and in vitro conditions respectively as 59.59 mg g<sup>-1</sup> and 30.21 mg g<sup>-1</sup> of infusion. Ben Farhat et al (2013) estimated the total phenolic contents spectrophotometrically and reported that the values ranged from (67.67-72.02 mg GAE g<sup>-1</sup> dw) for *S. argentea* extracts to (112.93-161.37 mg GAE g<sup>-1</sup> dw) for *S. officinalis* samples. Salem et al (2013) reported the phenolic contents of non-treated sage leaves as  $36.5 \pm 2.35$  mg GAE g<sup>-1</sup> fw. Chun et al (2005) reported the total phenolic content in water extracts of the clonal oregano as

52.8 mg g<sup>-1</sup> dw compared to 39.4 mg g<sup>-1</sup> dw in the commercial sample. Martins et al (2014) indicated that decoction presented the highest concentration of flavonoids (75.25 mg g<sup>-1</sup> decoction) and total phenolic compounds (98.05 mg g<sup>-1</sup> decoction) for *O. vulgare* L. and it was followed by infusion and hydroalcoholic extracts, respectively. Total phenolic contents obtained from *M. officinalis* L., *O. vulgare* ssp *hirtum*, *S. officinalis* L. and *L. angustifolia* L. of the present study were slightly different from those earlier reported ones. The differences between the current and previous findings were probably because of differences in harvest times, climate, cultural practices and/or plant genetics. Plant genetics and cultural practices may significantly affect phenolic contents and thus they play significant roles in nutritional values of the food stuff (Yang et al 2007; Ozgen et al 2008).

### 3.3. Antioxidant activity

In the present study, antioxidant activities were determined by using DPPH method and the values varied between  $0.930 \pm 0.023$ - $6.140 \pm 0.058$  g<sup>-1</sup> DPPH (Table 7). *M. officinalis* L. had the highest antioxidant activity ( $0.930 \pm 0.023$  g<sup>-1</sup> DPPH) and

**Table 3- The essential oil composition of *M. officinalis* L. (%)**

*Çizelge 3- M. officinalis* L. uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
1-octen-3-ol	30.41	0.760±0.000	0.000	0.760-0.760
β-caryophyllene	33.35	12.385±0.032	0.055	12.33-12.44
Citronellal	31.78	14.515±0.026	0.045	14.47-14.56
α-humulene	38.18	1.210±0.006	0.010	1.200-1.220
α-copaene	32.41	1.015±0.003	0.005	1.010-1.020
β-bourbonene	33.35	0.890±0.000	0.000	0.890-0.890
Methyl citronellate	34.43	2.090±0.000	0.000	2.090-2.090
β-copaene	39.32	3.645±0.026	0.045	3.600-3.690
Geranial	39.69	13.05±0.023	0.040	13.01-13.09
δ-cadinene	40.51	1.110±0.017	0.030	1.080-1.140
humulene epoxide II	48.30	1.685±0.003	0.005	1.680-1.690
Fokienol	50.45	1.160±0.029	0.050	1.110-1.210
epi- α-cadinol	51.02	0.955±0.014	0.025	0.930-0.980
α-cadinol	52.59	1.615±0.009	0.015	1.600-1.630
Unidentified		4.230±0.000	0.000	4.230-4.230



it was respectively followed by *O. vulgare* ssp. *hirtum* ( $1.895 \pm 0.006$  g g<sup>-1</sup> DPPH), *S. officinalis* L. ( $1.895 \pm 0.020$  g g<sup>-1</sup> DPPH) and *L. angustifolia* L. ( $6.140 \pm 0.058$  g g<sup>-1</sup> DPPH) ( $P < 0.05$ ). Sahin et al (2004) employed the free radical scavenging activity and lipid oxidation inhibition in *O. vulgare* ssp. *vulgare* extracts and studied the essential oils in vitro. The researchers reported the order of diphenylpicrylhydrazine with IC<sub>50</sub> as  $9.9 \pm 0.5$  and  $19.8 \pm 0.5$  µg mL<sup>-1</sup>, respectively. Skotti et al (2014) investigated the antioxidant activity of some medicinal and aromatic plants by using DPPH method and reported the antioxidant activities as between  $1.31 \pm 0.01$ - $3.16 \pm 0.06$  mol trolox mL<sup>-1</sup> for *O. vulgare*, as between  $3.03 \pm 0.09$ - $6.34 \pm 0.05$  mol trolox mL<sup>-1</sup> for *M. officinalis* L. and as between  $0.34 \pm 0.01$ - $1.64 \pm 0.01$  mol trolox mL<sup>-1</sup> for *S. officinalis*. Kaliora et al (2014) used DPPH method and reported that the infusion of dittany had highest antioxidant activity against the sage. In previous literatures investigating the essential

oils of *Origanum* species, thymol and carvacrol were reported to have high antioxidant activity (Barrata et al 1998; Milos et al 2000; Ruberto & Barrata 2000; Puertes-Mejia et al 2002). Although present findings are somehow similar to results of those earlier studies, differences in extraction and antioxidant activity methods, climate, soil, environmental factors, diseases and pesticide treatments, harvest time, drying and storage methods and plant parts used in analyses may significantly affect the antioxidant activity of plants (Bergonzi et al 2001; Wang & Zheng 2001). There are several studies indicating the relationships between antioxidant activity and phenolic contents of the plants (Ruberto & Barrata 2000; Dorman et al 2004; Cai et al 2006; Canadanović-Brunet et al 2008). In the current study, positive correlation was also found between total phenolic content and antioxidant activity in all plant extracts (Table 9).

**Table 4- The essential oil composition of *O. vulgare* ssp. *hirtum* (%)**

Çizelge 4- *O. vulgare* ssp. *hirtum* uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
1-octen-3-ol	30.41	0.590±0.000	0.000	0.590-0.590
β-caryophyllene	35.96	3.105±0.003	0.005	3.100-3.110
α-humulene	38.18	0.230±0.000	0.000	0.230-0.230
α-pinene	13.03	0.560±0.000	0.000	0.560-0.560
α-thujene	13.18	0.910±0.000	0.000	0.910-0.910
Myrcene	19.14	1.540±0.000	0.000	1.540-1.540
α-phellandrene	19.31	0.180±0.000	0.000	0.180-0.180
α-terpinene	19.96	1.175±0.003	0.005	1.170-1.180
Limonene	20.81	0.210±0.000	0.000	0.210-0.210
β-phellandrene	21.27	0.200±0.000	0.000	0.200-0.200
γ-terpinene	22.86	7.340±0.006	0.010	7.330-7.350
3-octanone	23.20	0.113±0.038	0.053	0.075-0.150
para-cymene	23.96	5.315±0.003	0.005	5.310-5.320
cis-Sabinene hydrate	31.12	0.380±0.000	0.000	0.380-0.380
trans-Sabinene hydrate	33.95	0.150±0.000	0.000	0.150-0.150
carvacrol mehyl ether	35.81	1.075±0.003	0.005	1.070-1.080
Borneol	38.76	0.250±0.000	0.000	0.250-0.250
Thymol	50.92	10.490±0.012	0.020	10.47-10.51
Carvacrol	51.74	65.080±0.017	0.030	65.05-65.11

**Table 5- The essential oil yield of the plants (%)**

Çizelge 5- Bitkilerin uçucu yağ verimi (%)

Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Lavandula angustifolia</i> L.	0.73±0.04	0.07	0.67-0.80	C
<i>Melissa officinalis</i> L.	0.06±0.01	0.01	0.05-0.07	D
<i>Origanum vulgare</i> ssp. <i>hirtum</i>	3.43±0.06	0.10	3.33-3.53	A
<i>Salvia officinalis</i> L.	1.40±0.04	0.07	1.33-1.47	B

\*, different letters represent groups with significant differences (P&lt;0.05)

**Table 6- The total phenolic content of the plant extracts (mg GAE g<sup>-1</sup>)**Çizelge 6- Bitki ekstraktlarının toplam fenolik içeriği (mg GAE g<sup>-1</sup>)

Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Lavandula angustifolia</i> L.	16.480±0.087	0.15	6.33-16.63	D
<i>Melissa officinalis</i> L.	76.110±1.030	1.78	74.33-77.89	A
<i>Origanum vulgare</i> ssp. <i>hirtum</i>	70.690±1.090	1.88	68.81-72.57	B
<i>Salvia officinalis</i> L.	63.275±0.915	1.59	61.69-64.86	C

\*, different letters represent the groups with significant differences (P&lt;0.05)

**Table 7- The antioxidant activity of the plant extracts (IC<sub>50</sub> (g g<sup>-1</sup> DPPH))**Çizelge 7- Bitki ekstraktlarının antioksidant aktivitesi (IC<sub>50</sub> (g g<sup>-1</sup> DPPH))

Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Lavandula angustifolia</i> L.	6.140±0.058	0.10	6.04-6.24	A
<i>Melissa officinalis</i> L.	0.930±0.023	0.04	0.89-0.97	C
<i>Origanum vulgare</i> ssp. <i>hirtum</i>	1.890±0.006	0.01	1.88-1.90	B
<i>Salvia officinalis</i> L.	1.895±0.020	0.04	1.86-1.93	B

\*, different letters represent the groups with significant difference (P&lt;0.05)

### 3.4. Antibacterial activity

Antibacterial activity against *S. aureus* ATCC 43300, *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212 and *E. coli* ATCC 35218 bacteria varied between 8.00±0.00-52.00±0.58 mm (Table 8). The highest antibacterial activity against the entire bacteria was observed in *O. vulgare* ssp. *hirtum* (P<0.05). Entire plants also exhibited relatively high antibacterial activity against *S. epidermidis* ATCC 12228 bacteria (P<0.05). Stagos et al (2012) using agar well-diffusion assay, tested the ability of Lamiaceae species to inhibit the growth of *S. aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) bacteria. The results showed that five out of seventeen extracts had

antibacterial activity against *S. aureus* but none against *P. aeruginosa*. Voda et al (2003) indicated that antifungal activity of oxygenated essential oil components vary based on the type and position over aromatic chain. Moon et al (2006) investigated the efficiency of five *Lavandula* species against various microorganism strains and reported that essential oils of these plants had antibacterial effects against *S. aureus*, metisiline-resistant *S. aureus* and *E. coli*, but hydrosol and water-extracts of these species were not able to exhibit antibacterial effects against the tested strains. Lin et al (2004) carried out in vitro studies and indicated distinctive antibacterial effects of water-extracts of *Origanum vulgare* against *L. monocytogenes*. Friedman et al (2002) carried out an antibacterial activity study with 96

**Table 8- The antibacterial activity of the plant extracts (mm)**

Çizelge 8- Bitki ekstraktlarının antibakteriyel aktivitesi (mm)

Bacteria	Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Staphylococcus aureus</i> ATCC 43300	<i>Lavandula angustifolia</i> L.	18.525±0.303	0.53	18.00-19.05	B
	<i>Melissa officinalis</i> L.	16.875±0.072	0.13	16.75-17.00	C
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	41.025±0.563	0.98	40.05-42.00	A
	<i>Salvia officinalis</i> L.	13.500±0.289	0.50	13.00-14.00	D
<i>Staphylococcus aureus</i> ATCC 29213	<i>Lavandula angustifolia</i> L.	13.500±0.289	0.50	13.00-14.00	C
	<i>Melissa officinalis</i> L.	17.000±0.000	0.00	17.00-17.00	B
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	32.500±0.289	0.50	32.00-33.00	A
	<i>Salvia officinalis</i> L.	11.000±0.000	0.00	11.00-11.00	D
<i>Staphylococcus epidermidis</i> ATCC 12228	<i>Lavandula angustifolia</i> L.	38.000±0.577	1.00	37.00-39.00	B
	<i>Melissa officinalis</i> L.	15.000±0.000	0.00	15.00-15.00	D
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	52.000±0.577	1.00	51.00-53.00	A
	<i>Salvia officinalis</i> L.	25.000±0.577	1.00	24.00-26.00	C
<i>Enterococcus faecalis</i> ATCC 29212	<i>Lavandula angustifolia</i> L.	16.025±0.014	0.03	16.00-16.05	B
	<i>Melissa officinalis</i> L.	11.000±0.000	0.00	11.00-11.00	D
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	24.250±0.144	0.25	24.00-24.50	A
	<i>Salvia officinalis</i> L.	21.500±0.289	0.50	21.00-22.00	C
<i>Escherichia coli</i> <sup>a</sup> ATCC 35218	<i>Lavandula angustifolia</i> L.	10.000±0.000	0.00	10.00-10.00	
	<i>Melissa officinalis</i> L.	13.000±0.000	0.00	13.00-13.00	
	<i>Melissa officinalis</i> L.	29.000±0.000	0.00	29.00-29.00	
	<i>Salvia officinalis</i> L.	8.000±0.000	0.00	8.00-8.00	

\*, different letters represent the groups with significant difference (P<0.05); <sup>a</sup>, statistical analyses were not performed since the replications are not different

**Table 9- Total phenolics, essential oil, antioxidant activity and antibacterial activity**

Çizelge 9- Toplam fenolik, uçucu yağ, antioksidant ve antibakteriyel aktivite

Parameters	Source	DF	SS	MS	F	Significance
Essential oil (%)	Between groups	3	19.130	6.3768	1348.4	0.000***
	Within groups	8	0.0378	0.0047		
	Total	11	19.168			
Total phenolic (mg GAE g <sup>-1</sup> dw)	Between groups	3	6700.0	2233.3	967.07	0.000***
	Within groups	8	18.480	2.3100		
	Total	11	6718.5			
Antioxidant activity (IC50 (g g <sup>-1</sup> DPPH))	Between groups	3	48.810	16.270	5035.2	0.000***
	Within groups	8	0.0259	0.0032		
	Total	11	48.836			
ATCC 43300	Between groups	3	1414.9	471.62	1264.5	0.000***
	Within groups	8	2.9800	0.3700		
	Total	11	1417.8			
ATCC 29213	Between groups	3	838.50	279.50	2236.0	0.000***
	Within groups	8	1.0000	0.1300		
	Total	11	839.50			
ATCC 12228	Between groups	3	2319.0	773.00	1030.7	0.000***
	Within groups	8	6.0000	0.7500		
	Total	11	2325.0			
ATCC 29212	Between groups	3	312.19	104.06	1329.4	0.000***
	Within groups	8	0.6300	0.0800		
	Total	11	312.82			

\*\*\*, significant according to ANOVA (P<0.001)

essential 23 oil components and reported efficient activity of cinnamaldehyde, thymol, carvacrol and eugenol against *E. coli*, *Salmonella enterica* and *L. Monocytogenes*. Researchers also indicated relatively higher antimicrobial activity of Oreganol against gram-positive and negative bacterial pathogens. Current findings comply with the results of those earlier studies. Considering those earlier studies, it can be stated herein that essential oils of medicinal plants had higher antibacterial activity than the other extracts like water, methanol, ethanol and hexane (Ahmad et al 1998; Eloff 1998).

#### 4. Conclusions

Together with widespread utilization of natural additives in food industry, the interest in natural antioxidants of the plants also increased day by day. Therefore, investigation of natural antioxidants has become a popular research topic, recently. Current findings revealed that essential oil plants of *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum* cultured with organic manure could reliably be accepted as natural antioxidant sources and these plants could also reliably be used in pharmaceutical and food industries to prevent the effects of reactive oxygen species and to reduce the risks of cardiovascular diseases. It was observed in this study that essential oils of these plants exhibited antibacterial effects against *S. aureus*, *S. epidermidis*, *E. faecalis* and *E. coli* bacteria. Thus, they can be used in treatment of infectious diseases caused by resistant microbes. In addition, the data in the present study are supporting the use of these plants as tea or additive in foods, and traditional remedies for the treatment of infectious diseases.

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## Doğu Akdeniz Bölgesi Zeytin Bahçelerinde *Palpita unionalis* ile İki Önemli Parazitoiti (*Apanteles brunnistigma* ve *Trichogramma evanescens*) Arasındaki İlişki

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### ÖZET

Zeytin fidantırtılı, *Palpita unionalis* (Hüb.) (Lepidoptera: Carambidae) zeytinin özellikle yaprak ve sürgün gibi yeşil aksamını ve yüksek popülasyonlarda meyveleri tüketerek zarar veren önemli bir türdür. Türkiye’de bu zararlının yerli parazitoitlerinin parazitlenme oranı bilinmemektedir. Bu amaçla, *P. unionalis* ile larva parazitoiti *Apanteles brunnistigma* Abdinbekova (Hymenoptera: Brachonidae) ve yumurta parazitoiti *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) arasındaki ilişki ile parazitlenme oranları tespit edilmiştir. Çalışma, Doğu Akdeniz Bölgesi zeytin bahçelerinde 2009-2010 yılları arasında Erzin (Hatay), Kadirli (Osmaniye), Tarsus (Mersin) ve Sarıçam (Adana)’da dört zeytin bahçesinde yürütülmüştür. İki parazitoitin örnekleme, iki yıl boyunca periyodik olarak Nisan ayından Kasım ayı sonuna kadar haftada bir, Aralık ayından Mart ayı sonuna kadar iki haftada bir yapılmıştır. *Trichogramma evanescens* tarafından parazitlenen zararlı yumurtaları, iki yıl süresince Nisan sonundan Aralık ayı sonuna kadar yedi-sekiz ay boyunca tespit edilmiştir. *T. evanescens* için parazitlenme oranı iki yıl için Erzin’de % 13.8-100, Kadirli’de % 11.1-100, Tarsus’da % 9.1-100 ve Sarıçam’da % 1.4-100 arasında bulunmuştur. *P. unionalis*’in larva parazitoiti olan *A. brunnistigma*’nın iki yıl boyunca Mayıs sonu-Kasım sonu arasında beş-altı ay aktif olduğu tespit edilmiştir. *A. brunnistigma*’nın parazitlenme oranı iki yıl için Erzin’de % 0.5-100, Kadirli’de % 2.6-33.3, Tarsus’da % 5.3-14.3 ve Sarıçam’da % 1.4-10.2 arasında bulunmuştur.

Anahtar Kelimeler: Zeytin; *Palpita unionalis*; *Trichogramma evanescens*; *Apanteles brunnistigma*; Parazitlenme oranı

## Interaction Between Two Important Parasitoids (*Apanteles brunnistigma* and *Trichogramma evanescens*) and Their Host *Palpita unionalis* in Olive Groves of the Eastern Mediterranean Region

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## ABSTRACT

Olive leaf moth, *Palpita unionalis* (Hüb.) (Lepidoptera: Carambidae) is an important species which damages by consuming green organs of olive trees such as leaves and twigs and fruits at high population. It has not been completely known the parasitism rate of its native parasitoids in Turkey. For this aim, the study was conducted to determine the interaction between olive leaf moth, *Palpita unionalis* (Hüb.) (Lepidoptera: Carambidae) and its larval parasitoid, *Apanteles brunnistigma* Abdinbekova (Hymenoptera: Brachonidae) and egg parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), as well as their parasitism rates. The study was conducted on four olive groves in Erzin (Hatay), Kadirli (Osmaniye), Tarsus (Mersin) and Sarıçam (Adana) in the eastern Mediterranean region between 2009 and 2010. Both parasitoids were checked weekly from April to November and fortnightly rest of the months during two years. Parasited eggs were detected between the end of April and December during seven and eight months for both years. The parasitism rate caused by *T. evanescens* was calculated between 13.83 and 100% in Erzin, 11.1 and 100% in Kadirli, 9.1 and 100% in Tarsus, 1.4 and 100% in Sarıçam for two years. *Apanteles brunnistigma* which is larva parasitoid of *P. unionalis* was determined to be actived between at the end of May and November during five and six months for two years. The parasitism rate of *A. brunnistigma* was calculated between 0.5 and 100% in Erzin, 2.6 and 33.3% in Kadirli, 5.3 and 14.3% in Tarsus, 1.4 and 10.2% in Sarıçam.

Keywords: Olive; *Palpita unionalis*; *Trichogramma evanescens*; *Apanteles brunnistigma*; Parasitism rate

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## 1. Giriş

Türkiye, 8,260,915 da ekim alanı ve 1,330,438 ton zeytin üretimi ile dünyada dördüncü sırada yer almaktadır. Zeytin, Akdeniz havzasının doğal bitki örtüsü içinde yer almaktadır. Tüm dünyada bulunan 900 milyon zeytin ağacının % 98'i Akdeniz havzasında bulunmaktadır (FAO 2013; TUİK 2014). Akdeniz Bölgesi'nde Tarım Bakanlığı'nın teşviki ile İl Özel İdareler ve özel teşebbüsler tarafından temin edilen fidanlarla 2001 yılından sonra zeytin üretim alanlarında hızlı bir artış olmuştur. Akdeniz Bölgesi'nde 2001 yılında 548,420 da alanda 174,742 ton zeytin üretimi yapılırken, 2014 yılında ekim alanı 1,374,278 da, üretim ise 437,677 ton olarak gerçekleşmiştir. 2001 yılında çalışmanın yürütüldüğü Adana'da 35,410 da alanda 10,894 ton; Hatay'da 310,360 da alanda 55,786 ton; Mersin'de 66,660 da alanda 65,308 ton ve Osmaniye'de 14,090 da alanda 3,952 ton zeytin üretimi yapılırken, 2014 yılında Adana'da ekim alanı 118,309 da, üretim 49,768 ton; Hatay'da ekim alanı 517,029 da, üretim 131,647 ton, Mersin'de ekim alanı 377,756 da, üretim 114,162 ton ve Osmaniye'de ekim alanı 123,049 da, üretim 55,390 ton olarak gerçekleşmiştir (TUİK 2014).

Zeytin bahçelerinde zarara neden olan önemli türlerden biri olan Zeytin fidantırtılı, *Palpita unionalis* (Hüb.) (Lepidoptera: Carambidae), zeytinlerin özellikle yaprak, sürgün ve meyvelerinde beslenerek zarara neden olmaktadır. Zararlıının konukçuları arasında bulunan Oleaceae familyasından özellikle zeytin, kurtbağı ve yaseminde önemli zarara neden olduğu kaydedilmiştir (Avidov & Rosen 1961; Balachowsky et al 1972; Tzanakakis 2003). Subtropik bölgelerde geniş bir alanda dağılım gösteren *P. unionalis*, Fransa ve Java'da yaseminin önemli bir zararlısı olmasına karşın, Akdeniz ülkelerinde ise zeytinin önemli bir zararlısı olduğunu bildirmiştir (Tzanakakis 2003). Son yıllarda Akdeniz Bölgesi'nde yeni kurulan zeytin bahçelerinin artmasıyla birlikte, *P. unionalis*'in önemli zarara neden olduğu kaydedilmiştir (Kaçar & Ulusoy 2012). *Palpita unionalis* zeytinin özellikle yaprak ve sürgünlerinde oburca beslenip, yeşil aksamı tüketerek bitkinin gelişmesini engellemektedir. Zararlı popülasyonunun yüksek olduğu dönemde fındık büyüklüğündeki yeşil meyvelerin etli kısmıyla beslenerek, meyvelerin zarar görmesine ve dökülmesine neden olmaktadır. Ülkemizin değişik bölgelerinde bulunduğu bildirilen *P. unionalis*'in, Marmara ve Doğu Akdeniz Bölgesi'nde son yıllarda

yapılan çalışmalarda zeytin bahçelerinde önemli zarara neden olduğu bildirilmiştir (Nizamlioğlu & Gökmen 1964; İyriboz 1968; Atay 2005; Kovancı et al 2006; Kumral & Kovancı 2008; Kaçar & Ulusoy 2012; 2013; Yılmaz 2012; Yılmaz & Genç 2013). İsrail, İtalya, Mısır ve Suriye’de yapılan çalışmalarda; zararlının parazitoitleri arasında Hymenoptera takımından *Apanteles lacteicolor* Vier., *A. laevigatus* Ratz., *A. obscurus* Nees, *A. syleptae* Ferriere, *A. xanthostigmus* (Hal.), *Chelonus* sp. ve *Dolichogenidea trachalus* (Nixon) (Braconidae), *Brachymeria aegyptiaca* Masi., *B. euploae* (Westwood) (Chalcididae), *Elasmus steffani* Viggiani (Eulophidae) ve *Xanthopimpla punctata* (Fabricius) (Ichneumonidae), *Trichogramma brassicae* Bezdenko, *T. bourarachae* Pintureau & Babault, *T. cacociae* Marchal, *T. cordubensis* Vargas & Cabello, *T. euproctidis* Girault, *T. evanescens* Westwood, *T. oleae* Voegelé & Pointel, *T. nerudai* Pintureau & Gerding ve *T. nr. pretiosum* Riley (Trichogrammatidae) ve Diptera takımından *Ctenophorocera pavida* Meig., *Nemorilla maculosa* Meigen (Tachinidae), *Syrphus corllae* F. (Syrphidae) türleri bulunmaktadır (Avidov & Rosen 1961; Triggiani 1972; Badawi et al 1976; El-Sherif et al 1977; Fodale & Mule 1990; Fodale et al 1990; Pinto et al 1994; 1995; El-Khawas et al 2000; Nasr et al 2002; Herz & Hassan 2003; Hegazi et al 2004; 2005; Herz & Hassan 2006; Lababidi & Haj Hammoud 2008). Türkiye’de ise *P. unionalis*’in üzerinde 9 adet parazitoit ve 21 adet predatör olmak üzere toplam 30 adet faydalı türünün bulunduğu, larva parazitoiti *A. brunnistigma* Abdinbekova’nın konukçuları arasında bu zararlının ilk defa kaydedildiği, ek olarak *T. evanescens*’inde önemli bir yumurta parazitoiti olduğu bildirilmiştir (Kaçar & Ulusoy 2011).

Biyolojik mücadelede üç temel yaklaşımdan birincisi mevcut doğal düşmanların korunması, ikincisi etkinliklerinin artırılması, üçüncüsü doğal düşman popülasyonunun çoğaltılarak, desteklenmesi ve sonucuda doğal düşmanların ithal edilmesidir (Öncüler 1998). Bunlardan mevcut doğal düşmanların korunması ve etkinliklerinin artırılması amacıyla, doğada var olan canlı baskı unsurlarının zararlı popülasyonlar üzerindeki etkinliğinin

bilinmesi en önemli unsurdur. Elde edilen veriler doğal düşmanların doğada sürdürülebilirliğini sağlama, etkinliğini artırma ve daha sonra yapılacak çalışmaların kapsamını oluşturacak olan yerli faydalı türlerin kitle halinde üretilerek salıverilme çalışmalarına öncülük etmesi bakımından temel bir çalışmadır. Bu çalışmayla zararlının yumurta ve larvaları ile doğal düşmanları arasındaki ilişkilerin belirlenmesi ve doğada var olan doğal düşmanların etkinliklerinin ve potansiyellerinin saptanması amaçlanmıştır.

## 2. Materyal ve Yöntem

Bu çalışma, 2009-2010 yılları arasında Adana’nın Sarıçam, Hatay’ın Erzincan, Mersin’in Tarsus ve Osmaniye’nin Kadirli ilçelerinde bulunan dört zeytin bahçesinde yürütülmüştür (Çizelge 1). Sarıçam ilçesindeki bahçenin üç yanı zeytin bahçeleriyle çevrili olup, batısında ilk yıl buğday, sonraki yıl ayçiçeği tarımı yapılmış, doğusunda bir dere etrafında doğal bir bitki örtüsü ile çevrili alan ve zeytinlikler yer almaktadır. Erzincan ilçesindeki bahçenin dört yanında zeytin bahçeleri bulunmaktadır. Ayrıca bahçenin doğusuna doğru yükselti artmakta ve zeytin bahçesinden sonra yabancı zeytin ve çalimsı bitkilerin yoğun olarak bulunduğu korunmuş çoğunlukla makiliklerin oluşturduğu küçük orman alanı yer almaktadır. Tarsus’da bulunan bahçenin batısında üzüm bağı ile karışık diğer bir zeytin bahçesi, kuzeyinde ise bir incir bahçesi ile güneyinde ise çok küçük bir maki alan ve sonrasında bağ alanları ve doğusunda ise zeytin bahçesi yer almaktadır. Kadirli’deki bahçenin ise üç yanında zeytin bahçeleriyle çevriliyken, doğusunda susam ve buğday tarımı yapılmıştır. Ayrıca güneyinde bir akarsu ve küçük çalılık alanlar yer almaktadır. Tüm bahçeler eğimli olup, güneye bakmaktadır. Bütün bahçelere her yıl 15 Şubat’tan sonra budama ve gübreleme uygulaması yapılmıştır. Hasat ve budamadan sonra Bordo bulamacı ile fungal etmenlere karşı koruyucu ilaçlama şeklinde yürütülmüştür. Erzincan’da ilk yıl karakoşnil problemi nedeniyle Şubat ayında kışlık yağ uygulaması yapılmıştır. Gübrelemeler suni ve hayvan gübresi

**Çizelge 1- Doğu Akdeniz Bölgesi'nde *Palpita unionalis*'in larva parazitoiti *Apanteles brunnistigma*'nın ve yumurta parazitoiti *Trichogramma evanescens* popülasyon takibi yapılan bahçelere ait bilgiler**

Table 1- The information of groves which population dynamic of larva parasitoid, *Apanteles brunnistigma* and egg parasitoid, *Trichogramma evanescens* of *Palpita unionalis* in eastern Mediterranean region

İl	İlçe	Köy	Çeşit	Yaş (yıl)	Alan (da)
Adana	Sarıçam	Hocalı	Gemlik	8-4	25
Hatay	Erzin	Gökdere	Gemlik	8-4	15
Mersin	Tarsus	Ulaş	Gemlik	5	35
Osmaniye	Kadirli	Topraktepe	Gemlik	6	30

olarak verilmiştir. Erzin, Kadirli ve Sarıçam'da bulunan bahçelerde salma sulama yapılırken, Tarsus'da damlama sulama ile 15 Haziran'dan sonra her 15-20 günde bir Eylül ayı sonuna kadar devam edilmiştir. Çalışmanın yürütüldüğü bahçeler kimyasal uygulamaların az tercih ettiği üreticiler arasından seçilmiştir. Bu nedenle, Erzin'deki zeytin üreticisinin ikinci yıl iyi tarım uygulamalarına geçmesini kolaylaştırmıştır.

Çalışmada, *Palpita unionalis* (Lepidoptera: Carambidae)'in larva parazitoiti *Apanteles brunnistigma* Abidinbekova (Hymenoptera: Brachonidae) ve yumurta parazitoiti *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)'in popülasyon gelişimi ve parazitlenme oranları belirlenmiştir. Arazi çıkışlarında dört bahçenin her birinde tesadüfî olarak 25 ağaç seçilmiştir. Her bir ağacın farklı yönlerindeki 25-30 cm'lik toplam 100 sürgünde kontrol yapılarak, sayımlar yapılmıştır. Sayımlara Nisan-Kasım ayları arasında haftada bir, Aralık-Mart ayları arasında on beş günde bir olacak şekilde iki yıl boyunca devam edilmiştir. Sayımlar süresince *T. evanescens* tarafından parazitlenen *P. unionalis*'in yumurtalarının renk değiştirerek, siyah bir renk aldığı görülmüştür (Şekil 1a, b, c). *Palpita unionalis*'in yumurtaları laboratuvarında kültüre alınmıştır. Zararlının yumurtalarının parazitlenme oranını belirlemek amacıyla; sürgünlerdeki yumurtaların sayıları kontrol edilmiş, parazitli ve parazitli olmayan yumurtaların sayıları kaydedilmiştir. Böylece *T. evanescens*'in *P. unionalis* yumurtaları üzerindeki % parazitlenme oranı belirlenmiştir. Elde edilen ergin parazitoit örneklerinin teşhisleri Dr. Bernard C. J. PINTUREAU (UMR INRA/INSA de Lyon, France)

tarafından *T. evanescens* türü olarak teşhis edilmiştir. Zararlının larvalarının parazitlenme oranını belirlemek amacıyla, aynı bahçelerden tesadüfî olarak alınan yüz sürgündeki larvaların sayıları kaydedilmiştir. Yumurtadan yeni çıkan ilk dönem ve son dönem larvalar hariç, tüm diğer dönemdeki larva örnekleri laboratuvara getirilerek zeytin sürgünleri üzerinde kültüre alınmıştır. Tüm larvalar ergin veya parazitoit ergini elde edilene kadar örnekler takip edilerek % parazitlenme oranı belirlenmiştir (Şekil 1d, e, f). *Apanteles brunnistigma* örnekleri Dr. Papp JENO (Department of Zoology, Hungarian Natural History Museum, Hungary) tarafından teşhis edilmiştir. Bu çalışma 25 °C'de % 60±5 nem ve 14:10 saat aydınlatmalı odalarda yürütülmüştür. Bu işlemler her arazi çıkışında ve her bir zeytin bahçesi için ayrı ayrı yapılmıştır.

### 3. Bulgular ve Tartışma

#### 3.1. *Trichogramma evanescens*'in *Palpita unionalis* yumurtaları üzerindeki popülasyonu

Erzin (Hatay)'de bulunan bahçede 2009 yılında *T. evanescens* tarafından parazitli yumurtalar 6 Temmuz ile 15 Aralık tarihleri arasında, 2010 yılında ise 31 Mayıs ile 10 Kasım tarihleri arasında belirlenmiştir. 2009 yılında en fazla parazitli yumurta sayısı 7 ile 8. aylar arasında tespit edilmiştir. Zararlının yumurtalarının en yüksek sayıya ulaştığı 24 Ağustos'da 54 yumurtada parazitlenme oranı % 83.3 olduğu belirlenmiştir. 2010 yılında ise en fazla parazitli yumurta sayısı 6 ile 8. aylar arasında bulunmuştur. Zararlının yumurtalarının en yüksek sayıya ulaştığı 16 Haziran'da 115 yumurtada



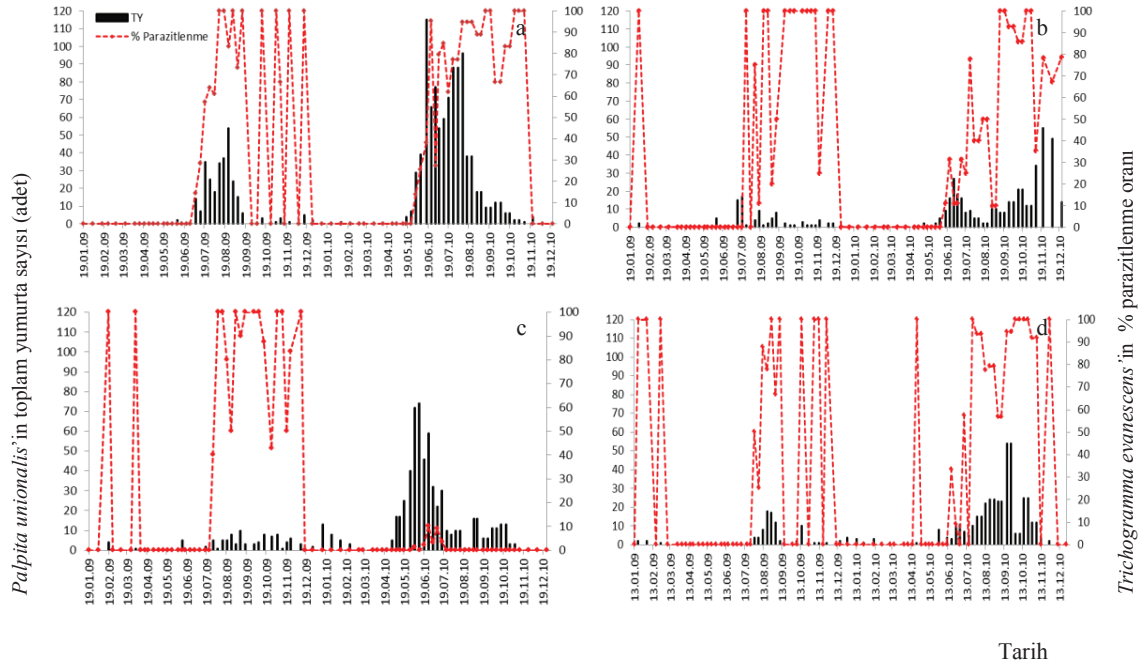


**Şekil 1- *Trichogramma evanescens*'in tarafından parazitli yumurtalar (a ve b) ve parazitoitin ergini (c) ve *Apanteles brunnistigma* tarafından parazitli larva (d), parazitoitin pupası (e) ve ergini (f) (fotoğraflar ilk yazar tarafından çekilmiştir)**

*Figure 1- The eggs (a and b) were parasitized by Trichogramma evanescens and its adult (c), the larva (d) was parasitized by Apanteles brunnistigma, its parasitoid pupa (e) and adult (f) (the photos were taken by first author)*

parazitlenme oranı % 38.2 olarak bulunurken 9 Ağustos'da 96 yumurtada bu oran % 94.7'ye ulaşmıştır (Şekil 2a). Kadirli (Osmaniye)'de 2009 yılında, ilk parazitli yumurtaların belirlendiği 18 Şubat'tan 27 Temmuz'a kadar geçen zaman aralığında parazitli yumurta bulunamamıştır. Ancak, bu tarihten 15 Aralık tarihine kadar her hafta parazitli yumurta belirlenmiştir. Sonraki yılda ise 16 Haziran'dan 22 Aralık tarihine kadar parazitli yumurtalar kaydedilmiştir. 2009 yılında zararlı yumurtalarının en yüksek sayıya ulaştığı 17 Temmuz'da 9 yumurtada % 11.1 ve 14 Eylül'de 8 yumurtada % 50 parazitlenme belirlenirken, 2010'da 7 Aralık'ta 55 yumurtada parazitlenme oranı % 8.2 olarak belirlenmiştir (Şekil 2b). Sarıçam (Adana)'da 2009 yılında ilk parazitli yumurtalar 2 Şubat'da daha sonra 1 Nisan'da görülmesine rağmen, bu tarihten 29 Temmuz'a kadar tekrar parazitli yumurta tespit edilememiştir. Bu tarihten 11 Kasım'a kadar olan sürede parazitli yumurtalar

bulunmuştur. 2010 yılında ise parazitli yumurtalar 25 Haziran ile 24 Aralık tarihleri arasında belirlenmiştir. İlk yıl zararlı yumurtalarının en yüksek sayıya ulaştığı 9 Eylül'de 10 yumurtada % 90 parazitlenme, ikinci yıl ise 6 Haziran'da 72 yumurtada % 1.3 ve 25 Haziran'da 59 yumurtada % 10.1 olduğu belirlenmiştir. İki yıllık çalışma sonucuna göre, parazitoitin Haziran başından Aralık ayı sonuna kadar 6 ile 7. aylar arasında aktif olduğu görülmüştür (Şekil 2c). Tarsus (Mersin)'de ilk parazitli yumurtalar 2009 yılında 29 Temmuz ile 25 Kasım arası, 2010 yılında ise 22 Nisan'da belirlenmiş, ancak bu tarihten 18 Haziran'a kadar tekrar tespit edilememiştir. 18 Haziran ile 26 Kasım arasında parazitli yumurtalar haftalık olarak kaydedilmiştir. *Trichogramma evanescens* tarafından parazitlenen en yüksek sayıdaki zararlı yumurtalarına ilk yıl 19 Ağustos'da 18 yumurtada % 77.7, ikinci yıl 17-24 Eylül'de 54 yumurtada % 94.4 parazitlenme oranı belirlenmiştir. İki yıl



**Şekil 2- Erzin (Hatay) (a), Kadirli (Osmaniye) (b), Tarsus (Mersin) (c) ve Sarıçam (Adana) (d)'da *Palpita unionalis*'in 2009 ve 2010 yılları arasında toplam yumurta sayısı (TY) ve *Trichogramma evanescens*'in % parazitlenme oranı**

Figure 2- The % parasitism range of *Trichogramma evanescens* and the total number of eggs of *Palpita unionalis* in Erzin (Hatay) (a), Kadirli (Osmaniye) (b), Tarsus (Mersin) (c) and Sarıçam (Adana) (d) between 2009 and 2010

boyunca, parazitoitin Nisan sonundan Aralık ayı sonu arasında 7-8 ay aktif olduğu belirlenmiştir (Şekil 2d).

*Trichogramma evanescens* için parazitlenme oranı Erzin'de % 13.8-100 (ilk yıl en düşük 6 Temmuz'da % 14.3 ile en yüksek 10 Ağustos'da ve sonrasında % 100, ikinci yıl en düşük 31 Mayıs'da % 13.8 ile en yüksek 13 Eylül ve sonrasında % 100 parazitlenme), Kadirli'de % 11.1-100 (ilk yıl en düşük 17 Ağustos'da % 11.1 ile en yüksek 2 Şubat'da ilk defa % 100 parazitlenme görülmesine karşın süreklilik oluşturmamış, 27 Temmuz'dan sonra muhtelif tarihlerde de % 100 parazitlenme görülmüştür, ikinci yıl ise en düşük 16 Haziran'da % 11.1 ile en yüksek 13 Eylül'de ve sonrasında % 100 parazitlenme), Tarsus'da % 25-100 (ilk yıl en

düşük 5 Ağustos'da % 25 ile en yüksek 19 Ocak ve 2-25 Şubat'da % 100 parazitlenme olmasına karşın süreklilik oluşturmamış, 26 Ağustos'dan sonra muhtelif tarihlerde de % 100 parazitlenme, ikinci yıl en düşük 25 Haziran'da % 9.1 ile en yüksek 22 Nisan'da % 100 parazitlenme) ve Sarıçam'da % 1.4-100 (ilk yıl en düşük 20 Temmuz'da % 40 ile en yüksek 18 Şubat'ta ve sonrasında 1 Nisan'dan sonra da muhtelif tarihlerde % 100 parazitlenme, ikinci yıl en düşük 4 Haziran'da % 1.4 ile en yüksek 25 Haziran'da % 10.2 parazitlenme) bulunmuştur (Şekil 2 a, b, c, d).

İki yıl boyunca yapılan çalışmalarda *T. evanescens*'in dört zeytin bahçesinde de parazitlenme oranlarının yukarıda belirtilen dönemlerde yüksek olduğu ortaya çıkmıştır. Bu parazitoitin inceleme

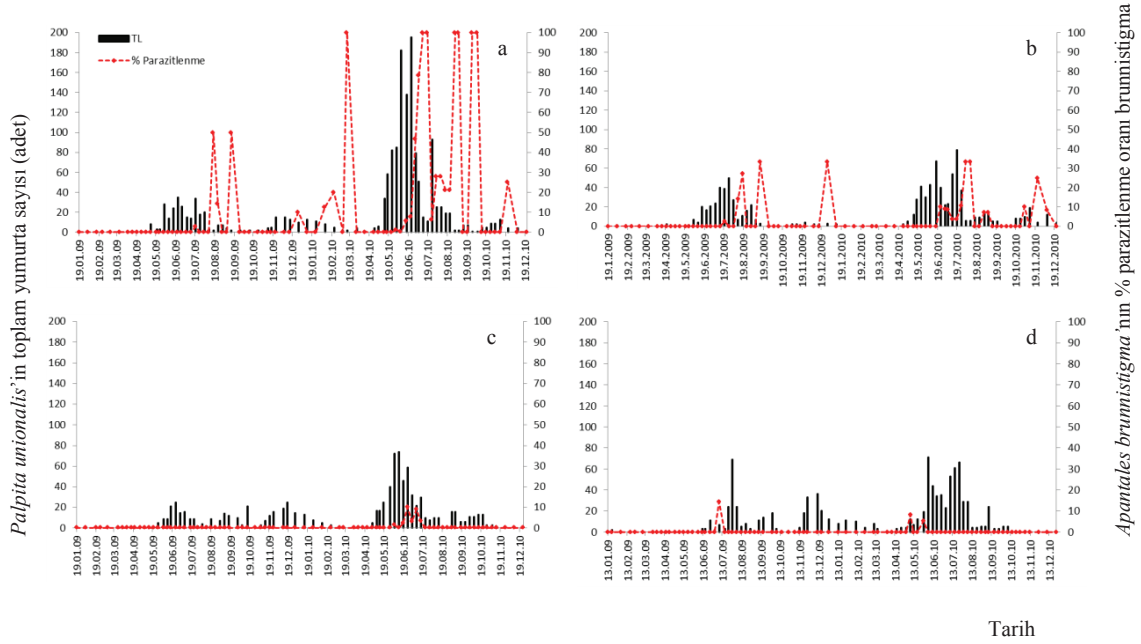
yapılan bahçelerde zararlıyı baskı altına tutan etmenlerden biri olduğu görülmüştür. Nitekim, Mısır'da *P. unionalis*'in yumurtaları üzerinde *Trichogramma*'nın otuzdan fazla yeni biyotipinin parazitlenme etkisi araştırılmış, bunlardan ticari olarak üretilen *T. brassicae* Bezdenko ve *T. evanescens*'in en yüksek parazitlenme oranına sahip olduğu bildirmişlerdir (Herz & Hassan 2003). Mısır'da yapılan diğer bir çalışmada ise zeytin bahçelerinde *P. unionalis*'de bulunan *T. bourarachae pintureau*, *T. cordubensis* Vargas and Cabello, *T. nr. pretiosum* Rile ve *T. cacociae* Marchall türlerinden oluşan yerli *Trichogramma* türlerini toplamışlardır. Bu türlerin tümünün Haziran-Ağustos aylarında konukçu yumurtalarını parazitlediğini, zararlının zarar yapacak popülasyonunu engellemek için yeterli seviyeye ulaşamadığını bildirmişlerdir (Hegazi et al 2005).

### 3.2. *Apanteles brunnistigma*'nin *Palpita unionalis*'in larvaları üzerindeki popülasyonu

*Palpita unionalis*'in larva parazitoiti olan *A. brunnistigma*'nın dört zeytin bahçesindeki parazitlenme oranları ayrı ayrı belirlenmiştir. Erzin'de 2009 yılında *A. brunnistigma* 20 Temmuz ile 14 Eylül arasında yedi hafta boyunca, 2010 yılında ise 31 Mayıs ile 22 Kasım tarihleri arasında 21 hafta boyunca belirlenmiştir. İlk yıl zararlının larvalarının en yüksek sayıya ulaştığı 22 Haziran'da 35 larvada % 0 ve 20 Temmuz'da 34 larvada % 2.9 parazitlenme belirlenirken, ikinci yıl 23 Haziran'da 195 larvada % 7.7 ve bu oranın muhtelif tarihlerde % 100'e ulaştığı görülmüştür. İki yıl süresince parazitoitin 5 ile 6. aylar arasında varlığını bahçe içerisinde sürdürdüğü belirlenmiştir (Şekil 3a). Kadirli'de *A. brunnistigma*'nın zararlı üzerinde 2009 yılında 27 Temmuz ile 15 Aralık arasında 5 hafta boyunca, 2010 yılında 23 Haziran ile 7 Aralık tarihleri arasında 13 hafta boyunca bulunduğu belirlenmiştir. İki yıl boyunca parazitoitin beş ay süresince bahçede varlığını sürdürdüğü tespit edilmiştir. İlk yıl zararlının larvalarındaki parazitlenme oranı % 2.7'den, ikinci yıl % 33.3'lere ulaşmıştır (Şekil 3b). Sarıçam'da 2009'da *A. brunnistigma* belirlenemezken, 2010 yılında ise 4

Haziran ile 16 Temmuz tarihleri arasında yaklaşık altı hafta boyunca bahçede varlığı belirlenmiş ve parazitlenme oranı % 1.4 ile % 10.2 arasında olduğu belirlenmiştir (Şekil 3c). Tarsus'da 2009 yılında *A. brunnistigma* yalnızca 8 Temmuz'da bir hafta, 2010'da yıl ise 7-28 Mayıs tarihleri arasında iki hafta süresince tespit edilebilmiştir. İlk yıl parazitlenme oranı 8 Temmuz'da % 14.2 olduğu, ikinci yıl 7 Mayıs'da % 8.3 ve 28 Mayıs'da % 5.3 olduğu kaydedilmiştir (Şekil 3d). *Apanteles brunnistigma* için parazitlenme oranı Erzin'de ilk yıl 20 Temmuz'da en düşük parazitlenme % 2.3 ile en yüksek 17 Ağustos'da % 50, ikinci yıl 31 Mayıs'da % 0.5 ve ilk defa 15 Mart ve sonrasında 12 Temmuz'da ve muhtelif tarihlerde % 100 parazitlenme, Kadirli'de ilk yıl en düşük 20 Temmuz'da % 2.6 ve en yüksek 14 Eylül'de % 33.3, ikinci yıl en düşük 12 Temmuz'da % 3.8 ve en yüksek 2 Ağustos'da % 33.3, Tarsus'da ilk yıl sadece 8 Temmuz'da % 14.3, ikinci yıl en düşük 28 Mayıs'da % 5.3 ve en yüksek 7 Mayıs'da % 8.3, Sarıçam'da ilk yıl parazitlenme oranı % 0, ikinci yıl en düşük 4 Haziran'da % 1.4 ve en yüksek 25 Haziran'da % 10.2 bulunmuştur (Şekil 3a, b, c, d).

Sonuç olarak, *A. brunnistigma*'nın dört ildeki zeytin bahçelerinde varlığı bilinmekle birlikte, 31 Mayıs-22 Kasım arasında 5 ile 6. aylar arasında aktif olduğu belirlenmiştir. Erzin'de parazitoitin diğer bahçelerden daha etkin olduğu bulunmuştur. Bu bahçede *A. brunnistigma*'nın 2008'de üç ay süresince bulunduğu, 2009'da bu sürenin iki katına çıktığı belirlenmiştir. Ayrıca aynı bahçede *A. brunnistigma*'nın tüm yıl boyunca muhtelif tarihlerde görüldüğü ve etkinliğini de artırdığı da tespit edilmiştir (Şekil 3a). Bu bahçede ikinci yıl herhangi bir insektisit uygulaması yapılmamıştır. Bu durumun parazitoidlerin sayısını ve etkinliğini artmasına yol açan nedenlerden biri olduğu düşünülmektedir. Ayrıca, çalışmanın yürütüldüğü bahçeye yakın alanda bir orman alanının bulunması, burada alternatif konukçusu olabilecek yabani zeytin ve çalimsı bitkilerin varlığı, parazitoidlerin yaşama alanı bulmasına ve sonraki yılda da etkinliğinin artmasına yardımcı olduğu kanısına varılmıştır.



**Şekil 3- Erzin (Hatay) (a), Kadirli (Osmaniye) (b), Tarsus (Mersin) (c) ve Sarıçam (Adana) (d)'da 2009 ve 2010 yılları arasında *Palpita unionalis* toplam larva sayısı (TL) ve *Apanteles brunneistigma*'nın % parazitlenme oranı**

Figure 3- The % parasitism range of *Apanteles brunneistigma* and the total number of larvae of *Palpita unionalis* in Erzin (Hatay) (a), Kadirli (Osmaniye) (b), Tarsus (Mersin) (c) and Sarıçam (Adana) (d) between 2009 and 2010

Nitekim, Kütük & Yiğit (2011) parazitoit ve predatör türlerin, içinde buldukları ekosistemde varlıklarını yıl boyunca belirli bir yoğunluk düzeyinde sürdürebilmelerinin önem taşıdığını, av veya konukçularının bulunmadığı zamanlarda doğal düşmanlara doğada uygun alternatif av ve konukçular ile uygun kış barınakları sağlanması ile mümkün olabildiğini bildirmişlerdir. Diğer ülkelerde yapılan çalışmalarda bu parazitoitin *P. unionalis*'in üzerinde herhangi bir konukçu kaydı olmamasına karşın farklı larva parazitoitlerle ilgili kayıtlar bulunmaktadır. Suriye'de *Dolichognida trachalus* (Nixon) (Hymenoptera, Braconidae)'un arazi koşullarında parazitlenme oranının % 6 ile 85 arasında olduğunu kaydetmişlerdir (Lababidi & Hej Hammoud 2008). Mısır'da *Apanteles syleptae* F. (Hymenoptera, Braconidae)'nın Haziran'da en yüksek oranda (% 92) parazitlediğini ve *P.*

*unionalis*'in birey sayılarını kontrol etmede önemli bir rol oynadığını bildirmişlerdir (El-Sherif et al 1977). İtalya'da *A. xanthostigmus* (Hal.) (Hymenoptera, Braconidae)'ın zararlının ikinci ve üçüncü dönem larvalarını % 30-35 oranında parazitlediğini tespit etmişlerdir (Fodale & Mule 1990; Fodale et al 1990). Bu ülkede yapılan başka bir çalışmada *A. syleptae*'nin parazitlenme yüzdesinin Ekim ayında en yüksek seviyeye (% 73) ulaştığını ve bahar ile yaz aylarında çok düşük oranda bulunduğunu kaydetmişlerdir. Yine aynı çalışmada, *P. unionalis* ile mücadele eden genellikle *A. syleptae* gibi doğada mevcut olan doğal düşman aktiviteleri ile kültürel önlemlerin zararlıyı kontrol altında tutabildiğini bildirmişlerdir (Pinto et al 1994; 1995).



#### 4. Sonuçlar

*Trichogramma evanescens* tarafından parazitlenen *P. unionalis*'in yumurtalarına Erzin'de Mayıs sonu-Aralık ortası, Kadirli'de Şubat ile Haziran ortası ile Aralık sonu, Tarsus'da Ocak, Şubat, Nisan ve Haziran ayının ikinci yarısında ile Kasım sonunda ve Sarıçam ise Şubat, Nisan ve Haziran başı ile Aralık sonunda tespit edilmiştir. Sonuç olarak, *T. evanescens*'in popülasyonu Nisan sonundan Aralık ayı sonuna kadar yedi-sekiz ay aktif olduğu belirlenmiştir. *T. evanescens*'in dört zeytin bahçesinde de parazitlenme oranlarının yüksek olduğu bulunmuştur. *T. evanescens* için parazitlenme oranı iki yıl süresince Erzin'de % 13.8-100, Kadirli'de % 11.1-100, Tarsus'da % 9.1-100 ve Sarıçam'da % 1.4-100 olmuştur. *A. brunnistigma* tarafından parazitlenen *P. unionalis* larvalarına Erzin'de Şubat, Mart ayları ile Mayıs sonu-Aralık sonunda, Kadirli'de Haziran sonu-Aralık sonu, Tarsus'da sadece Temmuz ve Mayıs'da, Sarıçam'da ise ilk yıl tespit edilemeyen parazitoit, ikinci yılda Haziran başından Temmuz ortasına kadar belirlenmiştir. *P. unionalis*'in larva parazitoiti olan *A. brunnistigma*'nın iki yıl boyunca Mayıs sonu-Kasım sonu arasında beş-altı ay aktif olduğu tespit edilmiştir. *A. brunnistigma* parazitlenme oranı iki yıl süresince Erzin'de % 0.5-100, Kadirli'de ilk yıl % 2.6-33.3, Tarsus'da % 5.3-14.3 ve Sarıçam'da % 1.4-10.2 bulunmuştur. Sonuç olarak, *P. unionalis* ile mücadelede bu iki parazitoitin zeytin bahçelerinde varlığını sürdürülebilmesi ve etkinliğini artırması için, doğal düşmanların korunarak, desteklenmesi çalışmalarına ihtiyaç duyulmaktadır. Bunun için tarım ilaçlarının ve uygulama şekillerinin bu parazitoitlere olumsuz etkisi üzerine çalışmaların yürütülerek, sonuçlar üreticilerle paylaşılmalıdır. Bu kapsamda doğal düşmanlara zararı olmayan veya az zararlı kimyasalların tespiti, parazitoitlerin bahçede varlığını sürdürmesi için alternatif çiçekli bitkilerin belirlenmesi çalışmalarına öncelik verilmelidir. Bu çalışma, tarımda sürdürülebilirliği sağlamak için konvansiyonel tarıma alternatif olan biyolojik mücadele ve organik tarımda kullanılabilecek önemli sonuçlar içermektedir.

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## Kışlık Ekmeklik Buğday Çeşitlerinin Azot Etkinliklerinin Belirlenmesi

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### ÖZET

Tarımsal üretimde istenen verim düzeyine ulaşmada önemli bir faktör olan azot (N) gübrelemesinin etkin bir şekilde yapılabilmesi sürdürülebilir verim ve çevre kalitesi açısından önem taşımaktadır. Bu çalışma, kimi ekmeklik buğday çeşitlerinin azot etkinliklerinin belirlenmesi amacıyla, Süleyman Demirel Üniversitesi araştırma ve deneme alanında 2010-11 ve 2011-12 yıllarında, tekrarlamalı olarak kuru tarım koşullarında yürütülmüştür. Denemede dört ekmeklik buğday (Adana-99, Bezostaja-1, Esperia ve Tosunbey) çeşidi 0, 7.5 ve 12.5 kg N da<sup>-1</sup> olacak şekilde 3 farklı N dozu kullanılarak karşılaştırılmıştır. Azot dozları, çalışmada incelenen özelliklerden geri kazanım etkinliği hariç tümünde etkili olmuştur. Uygulanan 12.5 kg da<sup>-1</sup> N dozu, 7.5 kg da<sup>-1</sup> N dozuna göre verim, protein içeriği ve tepki indeksini artırırken, agronomik ve fizyolojik etkinlik değerlerini azaltmıştır. En yüksek tane verimi ilk yıl 261.9 kg da<sup>-1</sup> ile Esperia, ikinci yıl 211.1 kg da<sup>-1</sup> ile Adana-99 çeşitlerinden elde edilmiştir. Tepki indeksi her iki yılda en yüksek Adana-99 çeşidinde olmuştur.

Anahtar Kelimeler: Azot dozları; Azot etkinliği; Ekmeklik buğday; Tepki indeksi

## Determination of Nitrogen Efficiency in Winter Bread Wheat Cultivars

### ARTICLE INFO

Research Article

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### ABSTRACT

An important factor for achieving the desired yield level in agriculture production is effective nitrogen fertilization in terms of sustainable yield and environment quality. This research was conducted to determine the nitrogen efficiency of some bread wheat varieties at Süleyman Demirel University research and experimental area in the periods 2010-11 and 2011-12 under rainfed conditions. Four bread wheat varieties (Adana-99, Bezostaja-1, Esperia and Tosunbey) in the experiment were compared using three different N doses (0, 75 and 125 kg ha<sup>-1</sup> N). Nitrogen doses affected to all in examined parameters, except apparent recovery fraction. 125 kg ha<sup>-1</sup> N dose increased the yield, protein content and

the response index while value of agronomic and physiological activities decreased compared to 75 kg ha<sup>-1</sup> N dose. The highest grain yield in the first year was obtained from Esperia cultivar with 2619 kg ha<sup>-1</sup> and Adana-99 cultivar with 2111 kg ha<sup>-1</sup> in the second year. The highest response index was found in Adana-99 cultivar in the both years.

Keywords: Nitrogen doses; Nitrogen efficiency; Bread wheat; Response index

## 1. Giriş

Buğday dünya ve ülkemiz nüfusunun birincil besin kaynağı olması, yetiştirici gelirinin genel olarak buna bağlı olması, bazı arazilerde alternatifsiz olması gibi nedenlerle en önemli tahıldır. Yetiştirilmesinde, hem çevresel faktörler hem de kültürel faaliyetler birbirine yakın düzeyde etkili olmaktadır. Buğday üretiminde etkili en önemli iklimsel faktörlerin, yağış ve ortalama hava sıcaklığı olduğu bilinmektedir (Özcan et al 2011). Ekim ve gübreleme işlemleri ise kültürel faaliyetlerin ana ögesini oluşturmaktadır.

Azot (N), bitkilerin büyüme ve gelişmesi için en önemli besin maddesi olmasının yanında, girdi maliyetleri açısından da büyük yer tutmaktadır. Ayrıca azotlu gübrelerin üretimi için duyulan yüksek enerji ihtiyacı ve kullanılan fazla azotlu gübrenin hem ekonomik hem de çevresel etkileri bu besin elementinin önemini daha da artırmaktadır. Bu nedenlerdir ki, N gübrelenmesi dünyada bilim insanları tarafından en çok araştırılan konuların başında gelmektedir. Bitkiler kullandıkları azotu esas olarak organik maddeden, inorganik gübrelerden, yağış ve biyolojik fiksasyon gibi farklı kaynaklardan sağlamaktadır (Müftüoğlu & Demirel 1998). Topraktaki N ise bitki kullanımı, denitrifikasyon (Olson et al 1979), toprak mikroorganizmaları tarafından kullanım ve yüzey akışı (Burkhart & James 1999) gibi farklı yollarla azalmaktadır. Bitkilerin kullanabilecekleri azotu farklı kaynaklardan sağlaması büyük bir avantaj olmakla birlikte, yukarıda sözü edilen çok çeşitli yollarla kayıplarının da gerçekleşmesi, N bütçesini oluşturmada temel güçlüğü oluşturmaktadır.

Yapılan bir çalışma, dünyada tahıllarda azot kullanım etkinliğinin % 33 oranında olduğunu göstermiştir (Raun & Johnson 1999). Gelişmekte olan ülkelerde bu oran daha düşük gerçekleşmektedir

(Dobermann 2005). Azotlu gübrelerin söz konusu bu düşük kullanım etkinliklerinin başlıca nedenlerinden birisi, yetersiz gübreleme stratejileridir (Mullen et al 2003). Bitkilerin gübre olarak kullanılan azottan faydalanma etkinlikleri toprak tipi (Takahashi et al 2007), gübreleme zamanı (Delogu et al 1998; Melaj et al 2003; Geçit & Çakır 2006; Lopez-Bellidio et al 2006), çeşit (Kanampiu et al 1997; Gouis et al 2000), meteorolojik faktörler (Melaj et al 2003), toprak nemi (Fernandez & Laird 1959; Melaj et al 2003), kök yapısı (Garnett et al 2009) ve toprak pH'sı (De Datta & Buresh 1989) gibi faktörlere bağlıdır. Toprakların pH değerinin 7.5 ve üzerinde olduğu durumlarda NH<sub>3</sub> kaybının çok hızlı olduğu ve uygulanan gübrenin % 50'den fazla kısmının kayıp olduğu bilinmektedir (De Datta & Buresh 1989; Timsina & Connor 2001). Meteorolojik faktörler sadece azot mobilizasyonunu ve fotosentezle alımını değil, gübre olarak uygulanan azotun toprakta kökler tarafından alımını da etkiler (Melaj et al 2003). Bunun yanında, kuru tarım koşullarında, maksimum verim için gerekli olandan fazla N kullanımının, toprak-bitki sistemi tarafından toprakta inorganik N birikmesini engellediği de belirtilmektedir (Raun & Johnson 1995). Artan N dozlarına paralel olarak, toprak ve bitkiden oluşan kayıpların da arttığı, dolayısı ile N etkinliğinin azaldığı bilimsel bir gerçektir (Harper et al 1987; Kanampiu et al 1997; Lees et al 2000). Fazla gübre kullanımından kaçınmak, bitkisel üretimde N etkinliğini artırmada tek olası yol olarak gözükmektedir (Kanampiu et al 1997).

Yürütülen bu çalışmanın amacı, bazı ekmeklik buğday çeşitlerinin azotlu gübre uygulamalarına tepkilerini belirleyerek, çalışmanın yürütüldüğü bölge için gübre-verim arasındaki ilişkiyi ortaya çıkarmak ve olası yüksek azotlu gübre

kullanımlarının önüne geçerek hem ekonomik hem de çevresel katkıları bilimsel olarak ifade etmektedir.

## 2. Materyal ve Yöntem

Araştırma Süleyman Demirel Üniversitesi, Tarımsal Araştırma ve Uygulama Merkezi deneme alanında 2010-11 ve 2011-12 yıllarında tekrarlamalı olarak, kuru tarım koşullarında yürütülmüştür. Denemede Adana-99, Bezostaja-1, Esperia ve Tosunbey ekmeçlik buğday çeşitleri kullanılmıştır. Ekim işlemi ilk yıl 10 Ekim 2010, ikinci yıl 24 Ekim 2011 tarihinde uygun toprak tavinada nadasa bırakılan tarlaya yapılmıştır. Metrekareye 500 bitki gelecek şekilde tohum miktarı hesaplanmıştır. Altı sıralı parsel ekim mibzeri ile 4 metre uzunlukta parseller oluşturulmuştur. Ekimle birlikte dekara 6 kg saf fosfor gelecek şekilde triple süper fosfat gübresi toprağa ekimden önce karıştırılarak uygulanmıştır. Azot 0, 7.5 ve 12.5 kg N da<sup>-1</sup> olacak şekilde ayarlanmış ve yarısı ekimle birlikte, kalan kısmı ise Feekes büyüme skalasına göre 4.0'da (Large 1954) amonyum nitrat olarak toprak yüzeyine uygulanmıştır. Yabancı ot mücadelesi herbisitle yapılmış, litrede 480 g 2,4 D aside eşdeğer Isooctylester içeren ESTER H ilacı kullanılmıştır.

Deneme alanı siltli killi toprak bünyesinde, kuru iken sert, nemli iken gevrekçtir. Organik maddesi (% 1.3) düşük, pH'sı (8.1) yüksek, drenajı iyidir. Bölge Akdeniz iklimi ile İç Anadolu karasal İklimi geçit kuşağında yer almaktadır. Yıllık ortalama yağış miktarı 537 mm'dir. Denemenin ilk yılında ortalamaya göre daha düşük (489 mm) yağış düşmesine rağmen, yağışın yetiştirme periyoduna dağılımı homojen ve uzun yıllar ortalamasına benzer olmuştur. İkinci yıl ise ortalamalardan daha yüksek (575 mm) yağış miktarı kaydedilmesine rağmen bu yağışın aylara dağılımı oldukça düzensiz olmuştur (Çizelge 1).

Hasat döneminde parseller biçilerek bitkiler 1-2 gün kurumaya bırakılmıştır. Tüm bitki parsel verimleri alındıktan sonra, harman edilerek tane verimleri alınmıştır. Tane ve tane hariç bitkinin tüm kısımları parçalanıp öğütülerek SDÜ Ziraat Fakültesi Toprak Bilimi ve Bitki Besleme Bölümü laboratuvarında azot içerikleri belirlenmiştir. Bu değerden faydalanılarak dekardan kaldırılan N miktarları belirlenmiştir.

Azot etkinliği ile ilgili parametrelerin belirlenmesinde Eşitlik 1-4'den faydalanılmıştır (Novoa & Loomis 1981; Moll et al 1982; Delogo et al 1998).

### Çizelge 1- Isparta ilinin denemenin yürütüldüğü döneme ve uzun yıllara ait iklim verileri\*

Table 1- The climate data of experimental years and long-term period in the province of Isparta

Aylar	Ortalama sıcaklık (°C)			Ortalama toplam yağış (mm)		
	2010-2011	2011-2012	Uzun yıllar ortalaması	2010-2011	2011-2012	Uzun yıllar ortalaması
Ekim	12.7	11.3	12.9	79.1	50.4	37.6
Kasım	11.2	4.2	7.4	13.6	0.2	46.5
Aralık	6.2	2.5	3.5	84.2	37.0	84.5
Ocak	3.0	-0.5	1.9	34.6	148.0	72.4
Şubat	3.7	0.5	2.8	51.8	88.6	65.5
Mart	6.4	4.9	6.1	50.4	20.8	53.8
Nisan	10.2	11.8	10.7	54.7	53.2	56.2
Mayıs	14.4	14.5	15.6	43.1	107.4	50.4
Haziran	19.5	22.5	20.2	62.2	18.1	29.6
Temmuz	24.7	25.4	23.6	1.8	0.8	14.9
Ağustos	24.0	22.8	23.2	0.6	34.6	10.5
Eylül	20.0	20.2	18.6	13.2	16.4	15.4
Ortalama/Toplam	13.0	11.7	12.2	489.3	575.5	537.5

\*, Isparta meteoroloji istasyonu kayıtları

$$\text{Agronomik etkinlik (AE; kg kg}^{-1}\text{)} = [Tv(N_x) - Tv(N_0)]/N_x \quad (1)$$

$$\text{Fizyolojik etkinlik (FE; kg kg}^{-1}\text{)} = [Tv(N_x) - Tv(N_0)]/[Nv(N_x) - Nv(N_0)] \quad (2)$$

$$\text{Geri kazanım etkinliği (GKE; \%)} = [(Nv(N_x) - Nv(N_0))/N_x] * 100 \quad (3)$$

$$\text{Tepki indeksi (TI; kg kg}^{-1}\text{)} = Tv(N_x)/Tv(N_0) \quad (4)$$

Burada;  $Tv$ , tane verimi ( $\text{kg da}^{-1}$ );  $Nv$ , bitkinin toprak üstü aksamınca kaldırılan azot miktarı ( $\text{kg da}^{-1}$ );  $N_x$ , uygulanan azot dozu ( $\text{kg}$ );  $N_0$ , azot uygulanmayan parsel verimi.

Tesadüf blokları deneme desenine göre 3 tekerrürlü olarak yürütülen çalışmada elde edilen veriler SAS istatistik paket programında analiz edilmiş, ortalamalar arası farkın önemliliği Duncan testine göre belirlenmiştir.

### 3. Bulgular ve Tartışma

Çizelge 2’de tane verimi üzerine yılların, N dozlarının, çeşitlerin ve N dozu x çeşit interaksiyonlarının istatistiki olarak önemli düzeyde etkili olduğu görülmektedir. Yıllar arasında verim bakımından önemli farklılıklar mevcuttur. Söyle ki, birinci yıl verimi ( $231.4 \text{ kg da}^{-1}$ ) ikinci yıla ( $192.4 \text{ kg da}^{-1}$ ) göre % 17 daha yüksek gerçekleşmiştir. Azot dozları her iki yılda da verimi önemli şekilde

#### Çizelge 2- Kışlık ekmeklik buğday çeşitlerinde azot dozlarının tane verimi ve protein içeriğine etkileri

Table 2- Effect of nitrogen doses on grain yield and protein content of winter bread wheat cultivars

N dozları ( $\text{kg da}^{-1}$ )	Çeşitler	Tane verimi ( $\text{kg da}^{-1}$ )		Protein içeriği (%)	
		2010-11	2011-12	2010-11	2011-12
0	Adana-99	112.3 g **	140.0 e**	9.4 de**	10.3 ab**
	Bezostaja-1	160.0 ef	138.3 e	9.0 e	9.7 b
	Esperia	166.3 ef	139.3 e	9.7 cde	9.5 b
	Tosunbey	157.6 f	134.6 e	10.4 bcde	9.8 b
7.5	Adana-99	176.4 e	225.0 bc	10.5 bcd	10.5 ab
	Bezostaja-1	230.1 d	216.3 bc	11.0 abc	10.3 ab
	Esperia	268.9 c	188.9 d	10.5 bcd	9.8 b
	Tosunbey	275.5 c	194.7 d	9.7 cde	10.0 b
12.5	Adana-99	295.2 b	268.3 a	11.6 ab	11.4 a
	Bezostaja-1	283.6 bc	220.7 bc	12.0 a	10.7 ab
	Esperia	350.5 a	231.2 b	12.0 a	10.2 ab
	Tosunbey	300.6 b	211.8 c	11.3 ab	10.5 ab
Ortalama		231.4 A**	192.4 B	10.6 <sup>öd</sup>	10.2
N dozları ( $\text{kg da}^{-1}$ )	0	149.1 c**	138.1 c**	9.6 c*	9.8 b*
	7.5	237.7 b	206.2 b	10.4 b	10.1 ab
	12.5	307.5 a	233.0 a	11.7 a	10.7 a
Çeşitler	Adana-99	194.6 d**	211.1 a**	10.5 <sup>öd</sup>	10.7 a*
	Bezostaja-1	224.6 c	191.8 b	10.7	10.2 ab
	Esperia	261.9 a	186.5 bc	10.7	9.8 b
	Tosunbey	244.6 b	180.3 c	10.4	10.1 ab
CV (%)		9.1	11.7	9.3	7.2

\*, % 5; \*\*, % 1 seviyesinde istatistiki olarak önemli; öd, önemli değil



etkilemiştir. En yüksek tane verimi 12.5 kg N da<sup>-1</sup> dozunda (birinci yıl 307.5, ikinci yıl 233.0 kg da<sup>-1</sup>), en düşük ise beklendiği şekilde kontrol parsellerinde (ilk yıl 149.1 ikinci yıl 138.1 kg da<sup>-1</sup>) elde edilmiştir. Çeşitler arasında ilk yıl en yüksek verim Esperia çeşidinde (261.9 kg da<sup>-1</sup>), en düşük Adana-99 çeşidinde (194.6 kg da<sup>-1</sup>) bulunurken, ikinci yıl en yüksek verim Adana-99 çeşidinde (211.1 kg da<sup>-1</sup>), en düşük verim ise Tosunbey çeşidinde (180.3 kg da<sup>-1</sup>) belirlenmiştir. Her iki yılın interaksyonları arasında en yüksek tane verimi (350.5 kg da<sup>-1</sup>) 2011 yılında 12.5 kg da<sup>-1</sup> azot dozunda ve Esperia çeşidinde, en düşük (112.3 kg da<sup>-1</sup>) ise aynı yıl N uygulanmayan parselde ve Adana-99 çeşidinde bulunmuştur. Diğer değerler bunlar arasında sıralanmıştır.

Bilindiği üzere, kuru tarım şartlarında verimi belirleyen en etkili faktörlerin başında yağış ve sıcaklık gelmektedir (Lees et al 2000). Yapılan bir çalışmada buğday bitkisinin toplam su tüketiminin 512 mm, Mayıs ayı su tüketiminin ise 199 mm olduğu bulunmuştur (Aran et al 2008). Bu sonuç, kuru tarım koşullarında bahar aylarında düşen yağışın (Nisan-Mayıs) buğday verimi üzerine etkisini açıkça ortaya koymaktadır. Ancak düşen yağış miktarı kadar, bitki gelişim periyodundaki kritik dönemlere dağılımı da oldukça önemlidir. İlk yıl düşen yağış miktarı toplamda daha az olmasına rağmen, yetiştirme periyodundaki aylık dağılımlarının daha düzenli ve uzun yıllar ortalamasına daha yakın olduğu görülmektedir (Çizelge 1). İkinci yılın Kasım ayında çok az yağış düşmesi, yeni çimlenen bitkilerin yeterince gelişmeden kışa girmesine neden olmuştur. Bu da, Adana-99 çeşidi hariç, diğer çeşitlerin agronomik özelliklerini olumsuz etkilemiştir. Buna ilave olarak, Ocak ayının da uzun yıllara göre daha soğuk geçmesi kısmen don zararına neden olmuş ve bu durum verime olumsuz etki yapmıştır. İkinci yılın Haziran ayındaki ortalamalara göre 3 °C daha yüksek olan sıcaklık, sahillerde daha iyi performans gösteren Adana-99 çeşidini verim yönünden olumlu etkilemiştir.

Yıllar arasında protein içeriği açısından önemli farklar bulunmadığı halde, N dozlarının protein içeriği üzerinde etkili olduğu Çizelge 2'de

görülmektedir. Protein içeriği mevcut azot varlığı ile ilgili olduğundan, en düşük değerler azot uygulanmayan parsellerde, en yüksek değerler ise 12.5 kg N da<sup>-1</sup> dozunda belirlenmiştir. Protein miktarı bakımından, ilk yıl çeşitler arasında fark bulunmazken, ikinci yıl en yüksek protein içeriği % 10.7 ile Adana-99, en düşük değer ise % 9.8 ile Esperia çeşidinde bulunmuştur. Her iki yılda da, N dozu x çeşit interaksyonu oldukça önemli bulunmuş, yüksek protein değerleri yüksek N dozlarında belirlenirken, birinci yılda Bezostaja-1 ve Esperia (% 12.0), ikinci yılda ise Adana-99 çeşidinde (% 11.4) bulunmuştur. En düşük protein içeriği değerleri azot uygulanmayan parsellerde, ilk yıl Bezostaja-1 çeşidinde (% 9.0), ikinci yıl ise Esperia çeşidinde (% 9.5) bulunmuştur (Çizelge 2).

Bilindiği gibi, çimlenmeden başaklanmaya kadar bitkilerin kuru madde oluşturması ve biriktirmesinde N başlıca rolü oynamaktadır (Austin et al 1977; Heitholt et al 1990). Verim ve tane protein içeriğinin belirlenmesinde en önemli faktörün genetik özellikler olduğu belirtilmektedir (Otteson et al 2007). Ancak azotun çevresel stres faktörleri nedeniyle her zaman aynı derecede etkili olmamakla birlikte, verim ve protein içeriği üzerine olumlu etkisi konusunda şüphe yoktur (Spiertz & Ellen 1978; Lees et al 2000; Lopez-Bellido & Lopez-Bellido 2001; Çiftçi & Doğan 2013). Tahıl üretimi için azotlu gübrelemenin verim ve kalite üzerine etkilerini çalışmamızın ne kadar önemli olduğu, N<sub>0</sub> dozundaki düşük verim (149.1 ve 138.1 kg da<sup>-1</sup>) ve protein içeriklerinden (% 9.6 ve 9.8) anlaşılmaktadır. Elde edilen verimler dikkate alındığında 7.5 kg da<sup>-1</sup> N dozunun maksimum verim için yeterli olması beklenirdi, ancak gübrelerin uygulandığı dönemlerde düşük yağışlardan dolayı azotun yeteri kadar çözülüp bitki tarafından alınamaması ve buharlaşarak atmosfere karışması şeklinde N kayıplarına neden olduğu ve azot alımını düşürdüğü tahmin edilmektedir.

Agronomik etkinlik (AE) üzerine yılların, çeşitlerin, azot dozu x çeşit interaksyonları ile ikinci yıl azot dozlarının etkisi % 1 düzeyinde önemli olmuştur (Çizelge 3). İlk yıl (12.2) ikinci yıla (8.4) göre % 31 oranında daha yüksek AE

değeri belirlenmiştir. İlk yıl kullanılan N dozları AE üzerine etkili olmazken, ikinci yıl 7.5 kg N da<sup>-1</sup> dozunda daha yüksek AE değeri belirlenmiştir. Çeşitlerin AE değerleri her iki yılda da birbirlerinden farklılıklar göstermiş, ilk yıl Esperia ve Tosunbey çeşitlerinde, ikinci yıl ise Adana-99 çeşidinde daha yüksek değerler tespit edilmiştir (Çizelge 3). Her iki yılın değerlendirilmesinde en yüksek AE (15.7) ilk yıl 7.5 N kg da<sup>-1</sup> dozu x Tosunbey çeşidi interaksyonunda, en düşük ise ikinci yıl 12.5 kg N da<sup>-1</sup> dozu x Tosunbey çeşidinde belirlenmiştir.

Çizelge 3'te görüleceği üzere, fizyolojik etkinlik değerleri yıllara, çeşitlere, N dozu x çeşit interaksyonlarına ve ikinci yıl N dozlarına göre değişiklik göstermiştir. İlk yıl daha düşük olan FE,

ikinci yıl daha yüksek bulunmuştur. Azot dozlarının ise FE üzerine etkisi yalnız ikinci yıl belirgin olmuş, 7.5 kg N da<sup>-1</sup> dozunda daha yüksek bulunmuştur. Çeşitlerin N dozlarına göre FE değerleri belirgin farklılıklar göstermiştir. İlk yıl Tosunbey ve Esperia, ikinci yıl Adana-99 çeşitleri daha yüksek FE değerlerine sahiplerken, ilk yıl Bezostaja-1, ikinci yıl Esperia ve Tosunbey çeşitleri daha düşük değerler göstermişlerdir. Azot dozu x çeşit interaksyonları 19.7 (ilk yıl 7.5 kg N da<sup>-1</sup> x Bezostaja-1) ile 41.2 (ilk yıl 7.5 kg N da<sup>-1</sup> x Tosunbey) arasında değerler almış, ikinci yıl da kısmen daha homojen bir görüntü vermiştir.

Geri kazanım etkinliği (GKE) yıllar, yıl, çeşit ve azot dozu x çeşit interaksyonları bakımından farklı olmuştur. İlk yıl (% 42.4), ikinci yıla (% 25.3) göre

### Çizelge 3- Kışlık ekmeklik buğday çeşitlerinde azot dozlarının agronomik etkinlik, fizyolojik etkinlik, geri kazanım etkinliği ve tepki indeksi üzerine etkileri

Table 3- Effect of nitrogen doses on agronomic efficiency, physiological efficiency, apparent recovery fraction and response index of winter bread wheat cultivars

N dozları (kg da <sup>-1</sup> )	Çeşitler	Agronomik etkinlik (kg kg <sup>-1</sup> )		Fizyolojik etkinlik (kg kg <sup>-1</sup> )		Geri kazanım etkinliği (%)		Tepki indeksi (kg kg <sup>-1</sup> )	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
7.5	Adana-99	8.5 d**	11.3 a**	22.2 d**	38.2 a**	39.7 abc**	28.2 <sup>öd</sup>	1.6 e**	1.6 b**
	Bezostaja-1	9.3 d	10.3 a	19.7 d	37.3 a	48.1 a	28.6	1.4 f	1.5 bc
	Esperya	13.7 b	6.6 b	37.0 ab	32.2 ab	36.9 c	21.9	1.6 e	1.3 d
	Tosunbey	15.7 a	8.0 b	41.2 a	31.5 b	37.8 bc	25.5	1.7 d	1.4 cd
12.5	Adana-99	14.6 ab	10.4 a	30.3 c	36.3 ab	48.3 a	28.4	2.6 a	1.9 a
	Bezostaja-1	9.9 cd	6.6 b	20.7 d	24.3 c	48.2 a	27.4	1.8 d	1.6 b
	Esperia	14.7 ab	7.3 b	32.0 bc	34.3 ab	46.6 ab	21.5	2.1 b	1.7 b
	Tosunbey	11.4 c	6.2 c	34.0 bc	29.2 bc	33.8 c	21.4	1.9 c	1.6 b
Ortalama		12.2A**	8.4 B	29.7 B*	32.9 A	42.4 A**	25.3 B	1.8 A*	1.6 B
N dozları	7.5	11.8 <sup>öd</sup>	9.1 a**	30.1 <sup>öd</sup>	34.8 a*	40.6 <sup>öd</sup>	26.1 <sup>öd</sup>	1.6 b**	1.5 b**
	12.5	12.7	7.6 b	29.2	31.0 b	44.2	24.6	2.1 a	1.7 a
Çeşitler	Adana-99	11.6 b**	10.8 a**	26.3 b**	37.2 a**	44.0 a*	28.3a**	2.1 a**	1.8a**
	Bezostaja-1	9.6 c	8.5 b	20.2 c	33.2 ab	48.1 a	28.0a	1.6 c	1.6 b
	Esperia	14.2 a	7.1 c	34.5 a	30.8 b	41.7 ab	23.5b	1.9 b	1.5 b
	Tosunbey	13.6 a	7.0 c	37.8 a	30.3 b	35.8 b	21.7b	1.8 b	1.5 b
CV (%)		6.3	7.9	6.7	7.8	5.2	7.5	11.4	7.8

\*, % 5; \*\*, % 1 seviyesinde istatistiki olarak önemli bulunmuştur; öd, önemli değil

% 40 gibi daha yüksek oranda GKE belirlenmiştir. Yine ilk yıl diğer çeşitlerde birbirine yakın değerler bulunurken, Tosunbey çeşidinde daha düşük GKE belirlenmiştir. İlk yıl N dozu x çeşit etkisi bakımından önemli farklılıklar bulunmuş, değerler % 48.3 (12.5 kg N da<sup>-1</sup> x Adana-99) ile % 33.8 (12.5 kg N da<sup>-1</sup> x Tosunbey) arasında sıralanmıştır (Çizelge 3). Artan verim ile N kullanım etkinliği arasında doğrusal bir ilişki olduğunu söylemek güçtür. Ancak verimin düşük olduğu yıllarda, çalışmamızda olduğu gibi N etkinlikleri de (AE, GKE, TI) düşük olmaktadır (Lopez-Bellido & Lopez-Bellido 2001; Lopez-Bellido et al 2006; Kara 2010). Agronomik etkinlik uygulanan azota karşılık bitkinin verimi artırma yeteneği olarak ifade edilmekte ve FE ile GKE'nin çarpımı olarak ifade edilmektedir (Novoa & Loomis 1981; Craswell & Godwin 1984). Uygulanan N dozu arttıkça, toprak-bitki sisteminde meydana gelen N kayıpları da buna paralel olarak artmaktadır (Lees et al 2000; Bozkurt et al 2001; Hawkesford 2014). Artan N dozuna bağlı olarak gerçekleşen ikinci yıldaki AE ( $N_{7.5} = 9.1$ ,  $N_{12.5} = 7.6$ ) ve FE ( $N_{7.5} = 34.8$ ,  $N_{12.5} = 31.0$ ) değerlerindeki azalma da bu nedene bağlanabilir. Ancak bu durum, iklimsel şartların daha uygun olduğu veya birbirine yakın N dozlarında, çalışmamızın ilk yılında olduğu gibi net şekilde ortaya çıkmaması yapılan çalışmalardan elde edilen sonuçlarla da uyumludur (Delogu et al 1998; Lopez-Bellido & Lopez-Bellido 2001). Bilindiği gibi, başaklanmadan sonra buğdayın N alımı yüksek sıcaklık, N varlığı ve toprak nemi gibi çevresel faktörlerle yakından ilgilidir (Papakosta & Gagjanas 1991). Kök ve kök çevresi özelliklerinin etkili olduğu GKE uygulanan gübrenin faydalarını belirlemede daha kullanışlıdır (Novoa & Loomis 1981). Bu durumda Delogu et al (1998) çalışmalarında olduğu gibi artan N dozuna bağlı olarak GKE'nin artış göstermesi beklenmektedir. İlk yıl belirlenen artışa ( $N_{7.5} = 40.6$ ,  $N_{12.5} = 44.2$ ) rağmen ikinci yıl ( $N_{7.5} = 26.1$ ,  $N_{12.5} = 24.6$ ) GKE'de azalma olmuş, fakat her iki N dozundaki farklılıklar istatistik olarak önemli olmamıştır. Geri kazanım etkinliği, Lopez-Bellido et al (2006) tarafından belirtildiği ve çalışmamızda elde edilen sonuçlardan da görüldüğü üzere, yıllar bazındaki tane verimine bağlı olarak önemli değişimler göstermektedir.

Uygulanan azota karşı verimde meydana gelen değişimleri gösteren tepki indeksi (TI) üzerine yıllar, azot dozları, çeşitler ve azot dozu x çeşit etkisi önemli olmuştur (Çizelge 3). İlk yıl ikinci yıla göre, 12.5 kg N da<sup>-1</sup> dozunda 7.5 kg N da<sup>-1</sup> dozuna göre, Adana-99 çeşidinde ise diğer çeşitlere göre daha yüksek TI değerleri bulunmuştur. Azot dozu x çeşit etkisinde en yüksek değer (2.6) ilk yıl 12.5 kg N da<sup>-1</sup> dozu x Adana-99 çeşidinde belirlenirken, en düşük değer ise (1.3) ikinci yıl 7.5 kg N da<sup>-1</sup> dozu x Esperia çeşidinde belirlenmiştir.

Kuru tarım şartlarında TI'nin uzun yıllar değerleri 1 ile 4.1 arasında değişmektedir (Johnson & Raun 2003). Geniş yelpazede dağılım, iklim şartlarının verim üzerindeki etkisinden kaynaklanmaktadır. Aynı zamanda düşük TI'nin sebepleri arasında mineralizasyon ve yağmurla ilave edilen N da olabileceği düşünülmektedir (Mullen et al 2003). Çalışmamızda verimin daha iyi olduğu ilk yıl denemesinde (1.8), ikinci yıla (1.6) oranla daha yüksek TI bulunmuştur (Çizelge 3). Tepki indeksinin hesaplama yöntemi gereği artan N dozuna bağlı olarak verimin arttığı noktaya kadar artması beklenmektedir. Çalışmamızda her iki yılda da N dozları arasındaki fark önemli olmuş, ilk yıl ( $N_{7.5} = 1.6$ ,  $N_{12.5} = 2.1$ ) bu fark oldukça belirgin olmuştur. Çeşitler arasında ise en yüksek TI her iki yılda da Adana-99 çeşidinde belirlenmiştir.

Çeşitlerin yıllık değişen verim değerleri agronomik etkinliklerini de değiştirmiştir. Çeşitler arasındaki verim (Otterson et al 2007), protein içeriği (Kara 2010) ve azot etkinlikleri (Baligar & Fageria 2001; Kara 2010) arasındaki farkların genetik ve fizyolojik yapıdan kaynaklanmakla birlikte çevresel faktörlerden oldukça etkilendiği düşünülebilir.

#### 4. Sonuçlar

Bu araştırmanın iki yıllık sonuçlarına göre; her iki yılda da azot dozu arttıkça tane verimi ve protein içeriği artmış, en yüksek tane verimi 12.5 kg da<sup>-1</sup> azot dozunda Adana-99 ve Esperia çeşitlerinde, tanede protein oranı içeriği ise yine aynı N dozunda Adana-99, Bezostaja-1 ve Esperia çeşitlerinde elde edilmiştir. En yüksek agronomik ve fizyolojik

etkinlik 7.5 kg da<sup>-1</sup> N dozunda Adana-99 ve Tosunbey çeşitlerinde, geri kazanım etkinliği ve tepki indeksi ise 12.5 kg da<sup>-1</sup> N dozunda Adana-99 çeşidinde belirlenmiştir.

Sonuç olarak; yıllar arasında yağış dağılımına bağlı olarak tane verimi ve azot alım etkinliğine çeşitlerin tepkisi farklı olmuş, Isparta koşullarında bu çeşitlerin yetiştirilebileceği, ancak tarımsal üretimde nihai hedef olan verim bakımından Esperia ve Adana-99 çeşitleri öne çıkmıştır.

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## Variability and Correlation of Yield and Forage Quality in Alfalfa Varieties of Different Origin

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### ABSTRACT

Alfalfa is the most important forage plant species. There are numerous alfalfa varieties in the world with improved yield potential and forage quality. Many of them have also been created in the region of Southeast Europe, using various breeding techniques. This investigation was carried out in central Serbia and it included 15 alfalfa varieties of different origin (nine from USA and six from Europe). The objective was to determine their yield in each cut and total dry matter yield (TDMY) in the second (A1) and third (A2) year of exploitation, as well as forage quality: the content of mineral matter (MM), crude protein (CP), crude fiber (CF), crude fat matter (CFM) and nitrogen-free extract (NFE). Investigated varieties exhibited high total variability in dry matter yield (DMY) (A1: CV= 13.19%; A2: CV= 9.33%). DMY variability was higher with the varieties from USA (A1: CV= 13.65%; A2: CV= 9.92%) than with those from Europe (A1: CV= 6.67%; A2: CV= 8.01%). Varieties from USA also proved more variable in crude protein (CP) content than European varieties (CV= 6.27% and CV= 2.68%, respectively). Differences between the investigated varieties (genotypes) also influenced total variability of forage quality parameters, with total CV ranging from 5.07% for CP to 10.48% for CF. Dry matter yield (DMY) had significant positive correlation with CP ( $r=0.344$ ), CF ( $r=0.342$ ) and CFM ( $r=0.306$ ), and the strongest correlation, although negative, was between CF and NFE ( $r=-0.917$ ).

Keywords: Alfalfa; Varieties; Dry matter yield; Forage quality; Variability

## Farklı Orijinlere Sahip Yonca Genotiplerinin Verim ve Ot Kalitesindeki Değişimler ve Korelasyonları

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### ÖZET

Yonca önemli bir yem bitkisi türüdür. Dünyada, yüksek verim ve ot kalitesine sahip çok sayıda yonca çeşidi vardır. Farklı ıslah yöntemleriyle Güneydoğu Avrupa'da ıslah edilmiş yonca çeşitleri de vardır. Orta Sırbistan'da yapılmış olan

bu çalışmada farklı orijinlere sahip (9 adedi Amerika ve 6 adedi Avrupa) 15 yonca çeşidi kullanılmıştır. Bu çalışmanın amacı; tesisin ikinci (A1) ve üçüncü (A2) yılında her biçimde yonca genotiplerinin verim ve toplam kuru madde verimi (TKMV) yanında mineral madde (MM), ham protein (HP), ham lif (HL), ham yağ madde (HY) ve azotsuz ekstrakt (AE) içeriklerini belirlemektir. Yonca genotipleri kuru madde verimleri (KMV) açısından yüksek toplam değişkenlik göstermişlerdir (A1: CV= % 13.19; A2: CV= % 9.33). KMV değişkenliği, Amerika orijinli genotiplerde (A1: CV= % 13.65; A2: CV= % 9.92) Avrupa orijinli genotiplere (A1: CV= % 6.67; A2: CV= % 8.01) göre daha yüksek olmuştur. Amerika orijinli genotipler, ham protein (HP) açısından da Avrupa orijinli genotiplere göre daha yüksek değişkenlik göstermişlerdir (sırasıyla CV= % 6.27 ve CV= % 2.68). Genotipler arası farklılık; HP için toplam CV % 5.07'den HL için % 10.48'e değişecek şekilde ot kalitesindeki toplam değişkenliği de etkilemiştir. Kuru madde verimi (KMY) ile HP ( $r=0.344$ ), HL ( $r=0.342$ ) ve HYM ( $r=0.306$ ) arasında önemli olumlu ilişki belirlenirken HL ile AE ( $r=-0.917$ ) arasında önemli olumsuz ilişki belirlenmiştir.

Anahtar Kelimeler: Yonca; Genotip; Kuru madde verimi; Ot kalitesi; Değişkenlik

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## 1. Introduction

Alfalfa is grown in all agricultural regions in more than 80 countries on all continents, on an area of over 33 million hectares (Ivanov 1988). In the Northern hemisphere, it is grown up to 69 °N latitude (Scandinavian countries), and up to the latitudes of 45 °S (New Zealand) and 55 °S (Argentina and Chile) in the Southern hemisphere (Ivanov 1988). In addition to high alfalfa forage yield, its advantage lies in different possibilities of exploitation: as fresh, hay, silage, haylage and/or for grazing. Regardless of the way of use, it retains its high nutritive value. All of this gives it the epithet of “the queen of forage crops”. Alfalfa is a perennial, pollinated plant species with high genetic complexity, which complicates its breeding (Brummer 2004; Robins et al 2008). Creation of new alfalfa varieties is a very time-consuming process of selecting plants with improved qualities in several cycles, in order to produce varieties significantly better than others (resistance to cold or drought, higher yield, better nutritive value). There are numerous varieties in the world, created with one or several positive characteristics, but they often have different adaptability to environmental conditions. In the region of Southeast Europe, adequate methods of selection and breeding have created many alfalfa varieties with great genetic yield potential and other positive characteristics (Nešić et al 2005; Schitea et al 2007; Tucak et al 2008; 2009; Radović et al 2009; Petcu et al 2009). However, the main testing of

each alfalfa variety takes place on individual farms, resulting in the spread or reduction of its growing area. Some varieties have been completely pushed out of production after a very short time, while others have been produced for decades.

The main objective of this study was testing of 15 alfalfa varieties of different origin (USA and central and southeast Europe), during the most productive years of their exploitation (the second and third), to find their potential for dry matter yield (DMY) and five most important parameters of forage quality. The other objective was to determine the correlation between the examined characteristics.

## 2. Material and Methods

The trial was set up in central Serbia (43°34' N, 21°12' E, 150 m a.s.l.) in April 2005 (year A0), by conventional sowing of alfalfa in soil suitable for its growth (pH 6.95, 3.4% humus, 0.21% total nitrogen). The soil was also well supplied with phosphorus (21.2 mg 100 g<sup>-1</sup> of soil) and potassium (25.2 mg 100 g<sup>-1</sup> of soil). The trial was carried out using the conventional method of completely randomized block system in four replications (the cultivars were sown at spacing: 20 cm-10 rows per plot 4×2 m in size). During the second (2006-A1) and third (2007-A2) year of their exploitation, 15 alfalfa varieties of different origin (nine from USA and six from central and southeast Europe, Table 1) were tested for green forage yield per each cut in a basic plot, which

was then recalculated into dry matter yield (DMY, t ha<sup>-1</sup>). The trial was carried out in dry-land conditions and the mowing of each cut was performed when 20-30% of alfalfa plants were in the flowering stage (Stanisavljević 2006). For each investigated variety, the quality of forage dry matter was determined using Weende system of analysis, in four cuts (I-IV) of each investigation year (A1 and A2). The following quality parameters were determined. Mineral matter (MM, g kg<sup>-1</sup>), using the dry ashing method at 550 °C. Crude proteins (CP, g kg<sup>-1</sup>), using the Kjeldahl method (AOAC 1990), crude fiber (CF, g kg<sup>-1</sup>), using the Weende method (AOAC 1990), crude fat matter (CFM, g kg<sup>-1</sup>), using the Soxhlet extraction method (AOAC 1990), nitrogen-free extract (NFE, g kg<sup>-1</sup>), by subtraction the values of previous parameters from 100 (AOAC 1990).

**Table 1- Origin of investigated alfalfa varieties (*Medicago sativa* L.)**

*Çizelge 1- Çalışmada kullanılan yonca (Medicago sativa L.) genotiplerinin orijinleri*

Variety	Origin
Mecca III	USA
Dynamic	USA
Pointer	USA
Weston-4	USA
DKA 50-18-4	USA
WL-625 HQ	USA
Tru test	USA
Ameri stand	USA
Integrity	USA
K-22	Europe-Serbia
K-28	Europe-Serbia
Pop. Užice	Europe-Serbia
Vali	Europe-Slovakia
Synteza 1	Europe-Slovakia
OS-95	Europe-Croatia

Obtained results were analyzed by ANOVA (Analysis of Variance). Medium effect of factors (year, genotype, cut and their interaction) was assessed by F-test. Tukey's multiple range test and coefficients of variation (CV, %) were used for detection of differences between treatments.

Correlation between investigated parameters was determined by Pearson correlation coefficients (r). Data were processed by program STATISTICA, version 8 (StatSoft Inc, Tulsa, OK, USA).

### 3. Results and Discussion

#### 3.1. Dry matter yield (DMY)

It was determined by statistical analysis that all three factors (year, variety-genotype and cut) had significant influence ( $P \leq 0.001$ ) on dry matter yield (DMY). The following significant interactions were also determined; variety×cut ( $P \leq 0.001$ ), as well as year×variety, year×cut and year×variety×cut ( $P \leq 0.01$ ) (Table 2).

For significant impact year and cut and their interactions on DMY of alfalfa indicate (Albayrak & Turk 2013). During the two years of the trial, the amount of precipitation and air temperature greatly varied (Table 3 and 4). This was especially evident during the growth of each year's second cut, when the varieties responded differently. It was indicated by significant interactions; variety×year and variety×cut. Therefore, there is the possibility of choosing a variety according to agro-ecological conditions.

Average dry matter yield (DMY) in the first cut was higher by 2.83 t ha<sup>-1</sup> in the year A1 than in the year A2. Contrary to that, DMY in the second cut was higher by 2.94 t ha<sup>-1</sup> in the year A2 than in the year A1 (Table 5). This difference can be attributed to the influence of precipitations, which is in accordance with the results obtained by (Delić et al 2007). In the third and fourth cut of the year A2, DMY was respectively lower by 0.18 and higher by 0.80 t ha<sup>-1</sup> than in the third and fourth cut in the year A1. This also indicates the influence of weather conditions on yield generation and its distribution between cuts (Table 3, 4 and 5). DMY variations, dependent on different variety genotypes, in four cuts of each of the two years (A1 and A2) were as follows: A1: I-3.64 t ha<sup>-1</sup>, II-1.46 t ha<sup>-1</sup>, III-1.30 t ha<sup>-1</sup> and IV-1.10 t ha<sup>-1</sup>, and A2: I-2.79 t ha<sup>-1</sup>, II-2.42 t ha<sup>-1</sup>, III-1.76 t ha<sup>-1</sup> and IV-2.22 t ha<sup>-1</sup>. High variability

**Table 2- F-test statistical probabilities for medium effect of three factors on yield and forage quality***Çizelge 2- Verim ve ot kalitesine genotip, biçim ve yıl etkisine ilişkin F-test sonuçları*

Source (factors)	df	DM yield	Forage quality (g kg <sup>-1</sup> DM)				
			MM	CP	CF	CFM	NFE
Year	1	***	ns	ns	ns	ns	ns
Variety (genotype)	14	***	*	*	*	*	*
Cut	3	***	ns	ns	ns	ns	ns
Year×variety	14	**	ns	ns	ns	ns	ns
Year×cut	3	**	ns	ns	ns	ns	ns
Variety×cut	42	***	ns	ns	ns	ns	ns
Year×variety×cut	42	**	ns	ns	ns	ns	ns

ns, not significant; \*, significant at P&lt;0.05; \*\*, significant at P&lt;0.01; \*\*\*, significant at P&lt;0.001

**Table 3- Precipitation for central Serbia (mm)***Çizelge 3- Orta Sırbistan'ın yağış durumu (mm)*

Year	Months												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
2006	37	44	146	58	34	71	20	76	45	37	24	60	652
2007	30	54	54	3	107	66	8	62	89	117	114	41	745
1968-2008	38	36	43	57	70	76	60	47	51	50	55	53	636

**Table 4- Mean monthly air temperatures for central Serbia (°C)***Çizelge 4- Orta Sırbistan'ın aylık ortalama sıcaklık durumu (°C)*

Year	Months												Annual average
	1	2	3	4	5	6	7	8	9	10	11	12	
2006	-2.0	1.4	5.4	12.8	16.3	19.9	22.4	20.9	17.8	12.9	6.5	2.3	11.4
2007	5.0	6.0	9.2	12.4	18.3	22.6	24.9	23.4	15.5	10.7	4.0	0.5	12.7
1968-2008	-0.1	2.1	6.8	11.6	16.4	19.9	21.6	21.3	16.8	11.5	6.4	1.4	11.3

of DMY is also indicated by its dependence on the variety genotype, taking into account both years of exploitation-total CV ranged from 11.37% (II cut) to 28.38% (IV cut) (Table 5).

It is in accordance with the results obtained by Stanisavljević et al (2012) on DMY variability in two localities during four-year period (CV= 17.1% and 25.7%). In those parts of the years with more favourable agro-ecological conditions (I and II cut in both A1 and A2), the following varieties exhibited an increased DMY potential: Vali (24.58 t ha<sup>-1</sup>), Synteza 1 (24.19 t ha<sup>-1</sup>), K-22 (23.78 t ha<sup>-1</sup>), Integrity (23.43 t ha<sup>-1</sup>) and OS-95 (23.02 t ha<sup>-1</sup>),

which makes them more suitable for use in May, June and July. In periods of worse agro-ecological conditions (precipitation amount and temperature), (III and IV cut of both A1 and A2), the increased DMY potential was recorded in varieties: Vali (10.73 t ha<sup>-1</sup>), WL-625 HQ (10.58 t ha<sup>-1</sup>) and Weston (10.17 t ha<sup>-1</sup>) (Table 5). Yield distribution between cuts is also very important, especially if alfalfa forage is used immediately after mowing. On annual basis, all the investigated varieties (genotypes) had an influence on high DMY total variability (A1: CV= 13.19%, A2: CV= 9.33%). DMY variability in the varieties from USA was higher (A1: CV= 13.65%,

**Table 5- DMY (t ha<sup>-1</sup>) of alfalfa varieties in four cuts of the years A1 and A2**Çizelge 5- Yonca genotiplerinin yıllara (A1 ve A2) ve biçim dönemlerine göre kuru madde verimleri (t ha<sup>-1</sup>)

Year	Variety	Cut			
		I	II	III	IV
A1	Mecca III	5.49 f*	2.69 e-g	2.77 bc	1.27 d-f
	Dynamic	5.35 f	2.63 fg	2.80 a-c	1.44 c-e
	Pointer	5.65 f	2.82 ef	2.32 bc	1.12 ef
	Weston	5.45 f	2.60 fg	2.42 bc	1.57 b-d
	DKA 50-18	6.17 ef	3.01 d-f	2.82 a-c	1.12 ef
	WL-625 HQ	5.40 f	3.05 c-f	2.69 bc	1.53 b-d
	Tru test	6.95 de	2.35 g	2.98 a-c	1.63 b-d
	Ameri stand	7.89 bc	3.47 ab	2.84 a-c	1.83 a-c
	Integrity	7.55 cd	3.74 ab	3.51 a	2.11 a
	K-22	7.48 cd	3.81 a	2.49 bc	1.50 b-e
	K-28	7.49 cd	3.35 bc	2.21 c	1.01 f
	Pop Užice	8.47 ab	3.45 a-c	2.81 a-c	1.36 d-f
	Vali	8.99 a	3.36 bc	3.01 ab	1.88 ab
	Synteza 1	7.75 b-d	3.63 ab	2.59 bc	1.48 b-e
	OS-95	8.06 bc	3.51 ab	2.76 bc	1.50 b-e
	Average	7.94	3.16	2.73	1.49
	Total CV (%)	17.94	14.58	11.55	20.07
A2	Mecca III	4.25 de	5.71 c-f	1.72 e	1.87 ef
	Dynamic	4.80 c-e	5.26 ef	2.24 de	1.39 f
	Pointer	4.47 de	5.41 d-f	2.10 e	1.98 d-f
	Weston	4.88 c-e	5.07 f	2.81 b-d	3.37 ab
	DKA 50-18	4.39 de	5.75 c-f	3.48 a	2.31 c-e
	WL-625 HQ	3.95 e	5.45 d-f	3.47 a	2.89 a-c
	Tru test	6.24 a	5.24 ef	3.21 ab	3.58 a
	Ameri stand	5.67 a-c	5.79 c-f	2.84 bc	2.09 d-f
	Integrity	5.62 a-c	6.52 bc	2.29 cd	1.76 ef
	K-22	6.14 ab	6.35 bc	2.09 e	2.20 c-e
	K-28	5.11 b-d	5.92 c-e	1.73 e	1.36 f
	Pop. Užice	4.14 de	7.49 a	2.04 e	2.41 c-e
	Vali	5.54 a-c	6.69 b	3.08 ab	2.76 b-d
	Synteza 1	6.74 a	6.07 b-d	2.17 e	1.88 ef
	OS-95	4.73 c-e	6.72 b	3.01 ab	2.48 c-e
	Average	5.11	6.10	2.55	2.29
	Total CV (%)	16.54	11.37	23.73	28.38

\*, means followed by the same letter are not significantly different at P≤0.01

A2: CV= 9.92%) than in the varieties from Europe (A1: CV= 6.67%, A2: CV= 8.01%) (Table 6 and Figure 1). The highest average DMY (of A1 and A2) was recorded in Vali variety (17.66 t ha<sup>-1</sup>). It was followed by varieties: Integrity, OS-95, Ameri

stand, Synteza 1, Užice, Tru test and K-22, with the most difference in average DMY of only 0.52 t ha<sup>-1</sup>. Investigating 17 alfalfa varieties and populations, Radović et al (2009) reported on the difference in DMY of 6.27 t ha<sup>-1</sup>, which is in accordance with



our results. Contrary to that Tomić et al (2005) did not find any significant difference in DMY between investigated alfalfa varieties, but all of them were of blue Pannonian-type and therefore in closer genetic relation than the varieties investigated in this study. Higher DMY was in correspondence with higher

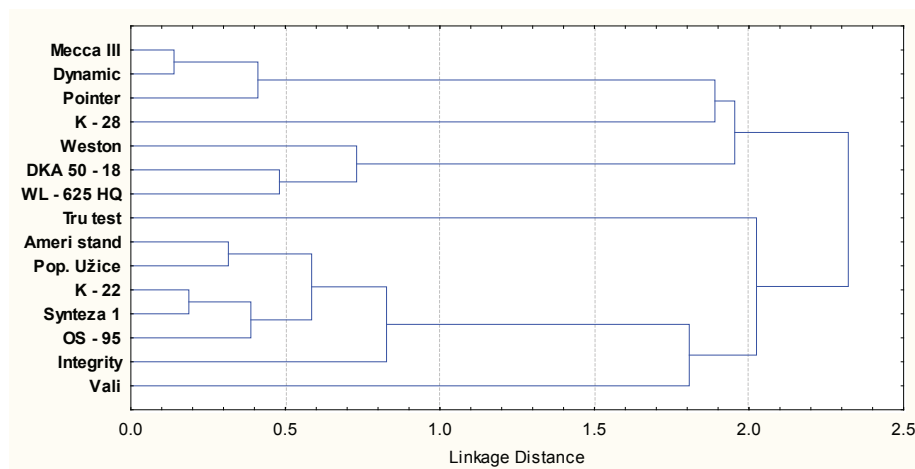
content of CP, CF and CFM ( $r=0.344$ ,  $r=0.342$ ,  $r=0.306$ ;  $P\leq 0.001$ ), while it was in negative correlation with MM ( $r=-0.510$ ,  $P\leq 0.001$ ). According to Tucak et al (2008), there was not a significant correlation between DMY and CP content ( $r=0.04$ ) as there was between DMY and CP yield ( $r=0.91$ ,  $P\leq 0.001$ ).

**Table 6- Annual DMY ( $t\ ha^{-1}$ ) in alfalfa varieties**

*Çizelge 6- Yonca genotiplerinin yıllara göre kuru madde verimleri ( $t\ ha^{-1}$ )*

Variety	A1	A2	Average (A1, A2)
Mecca III	12.22 e*	13.55 e	12.89 c
Dynamic	12.22 e	13.69 d-e	12.96 c
Pointer	11.91 e	13.96 b-e	12.94 c
Weston	12.04 e	16.13 a-e	14.09 bc
DKA 50-18	13.12 c-e	15.93 a-e	14.53 bc
WL-625 HQ	12.67 d-e	15.76 a-e	14.22 bc
Tru test	13.91 b-e	18.27 a	16.09 ab
Ameri stand	16.03 a-c	16.39 a-c	16.21 ab
Integrity	16.91 ab	16.19 a-d	16.55 ab
CV (%)	13.65	9.92	10.14
K-22	15.28 a-e	16.78 a-c	16.03 ab
K-28	14.06 b-e	14.12 c-e	14.09 bc
Pop. Užice	16.09 a-c	16.08 a-e	16.09 ab
Vali	17.24 a	18.07 a	17.66 a
Synteza 1	15.45 a-d	16.86 ab	16.16 ab
OS-95	15.83 a-d	16.94 a	16.39 ab
Average	14.33	15.91	15.13
CV (%)	6.67	8.01	7.13
Total CV (%)	13.19	9.33	10.11

\*, means followed by the same letter are not significantly different at  $P\leq 0.01$



**Figure 1- Dendrogram of total dry matter yield of alfalfa varieties**

*Şekil 1- Yonca genotiplerinin toplam kuru madde dendrogramı*

### 3.2. Forage quality

The variety (genotype) factor had significant effect on forage quality parameters ( $P \leq 0.05$ ) (Table 2, Figure 2), which were not significantly affected by the other factors or their interactions (Table 2). There are many reports on the potentialities of breeding in improving the alfalfa forage quality (Ridy & Brummer 2002; Lamb et al 2006; Štrbanović 2010; Milić et al 2014). Average mineral matter content (MM) of  $85.8 \text{ g kg}^{-1}$  DM was determined by analysis. Depending on the variety, MM content ranged from  $82.8 \text{ g kg}^{-1}$  DM (Synteza 1) to  $104.6 \text{ g kg}^{-1}$  DM (Integrity), so it differed most by  $21.8 \text{ g kg}^{-1}$  DM (total CV= 8.09%). Mineral matter had significant correlation, although negative, only with CF ( $r = -0.364$ ,  $P \leq 0.001$ ).

By decomposing MM into components (Hintz et al 1985) determined correlation between calcium and CP ( $r = 0.40$ ,  $P \leq 0.01$ ) and between phosphorus and CP ( $r = 0.26$ ), which was positive, but not significant. The highest recorded CP content was  $215 \text{ g kg}^{-1}$  DM (Tru test and Pointer), and the lowest was  $174 \text{ g kg}^{-1}$  DM (Mecca III). Depending on the variety, CP content varied most by  $41 \text{ g kg}^{-1}$  DM, and the varieties from USA exhibited higher variability (CV= 6.27%) than those from Europe

(CV= 2.68%). According to Keskin et al (2009) the biggest difference in CP content between 12 investigated varieties was  $37 \text{ g kg}^{-1}$  DM. Altınok & Karakaya (2002) reported it was  $30 \text{ g kg}^{-1}$  DM between eight investigated varieties and Geleti et al (2014) reported the biggest difference in CP content of  $14.1 \text{ g kg}^{-1}$  DM between five investigated varieties. A little higher difference recorded in this study could be attributed to greater heterogeneity of the investigated varieties (Table 7 and 8). Crude protein (CP) was in negative correlation ( $r = -0.393$ ,  $P \leq 0.001$ ) with crude fiber (CF) and in positive ( $r = 0.424$ ,  $P \leq 0.001$ ) with crude fat matter (CFM) (Table 8), which is in accordance with the results obtained by (Katić et al 2005). Stanisavljević (2006) also reported on negative correlation between CP and CF ( $r = -0.409$ ,  $P \leq 0.05$ ). Differences in CF content caused by different variety genotypes ranged to the maximum of  $99 \text{ g kg}^{-1}$  DM, so that total variability of this parameter was high (CV= 10.48%). Average CF content was  $275 \text{ g kg}^{-1}$  DM, while according to (Heuze et al 2013), CF content ranged from 201 to  $315 \text{ g kg}^{-1}$  DM, depending on plant phenophase. The strongest correlation, although negative ( $r = -0.917$ ) was between CF and NFE, which is in accordance with the results obtained by Stanisavljević (2006) ( $r = -0.607$ ). Negative correlation was also determined

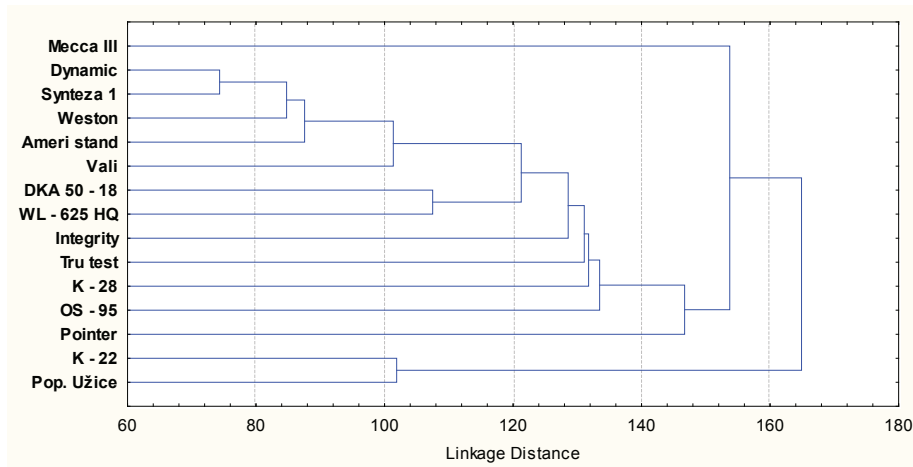


Figure 2- Dendrogram of forage quality of alfalfa varieties

Şekil 2- Yonca genotiplerinin ot kalitesi dendrogramı

between CF and CFM ( $r = -0.227$ ,  $P \leq 0.05$ ). Investigated varieties had the average CFM content of  $24.3 \text{ g kg}^{-1} \text{ DM}$ . This is in accordance with the results obtained by (Marković et al 2008), although the results obtained by (Katić et al 2005) indicated that there was not any significant difference in CFM content between varieties. Considering similar agro-ecological conditions, these contradictory results could be explained by different variety genotypes.

Maximum difference in NFE content between the investigated varieties was  $101 \text{ g kg}^{-1} \text{ DM}$ , with variability of this parameter of 6.74% (total CV). Stanisavljević (2006) determined the maximum difference in NFE content of only  $2.9 \text{ g kg}^{-1} \text{ DM}$  between three domestic (NS-H-11, NS Slavija and ZA-83) and one French variety (Europe), which could also be explained by the influence of genetic constitution of investigated varieties.

**Table 7- Forage quality parameters for different alfalfa genotypes; average of A1 and A2 (I-IV harvests each)**

Çizelge 7- Yonca genotiplerinin 2 yıl (A1 ve A2) ve 4 hasat (I-IV) ortalaması olarak ot kalite özellikleri

Varieties	MM	CP	CF	CFM	NFE
Mecca III	85.8 bc*	174 e	301 a-c	19.2 b	407 a-d
Dynamic	89.8 bc	201 a-d	271 c-e	25.9 a	413 b-d
Pointer	100.4 ab	215 a	225 g	23.0 ab	437 ab
Weston	98.6 a-c	196 b-d	260 d-f	26.7 a	418 bc
DKA 50-18	94.4 a-c	198 b-d	272 c-e	23.1 ab	413 bc
WL-625 HQ	103.8 a	196 b-d	244 e-g	25.5 a	431 a-c
Tru test	84.1 c	215 a	282 ed	23.2 ab	396 de
Ameri stand	90.7 a-c	199 b-d	289 b-d	25.3 a	396 de
Integrity	104.6 a	210 ab	259 d-f	24.2 a	403 cd
CV (%)	8.01	6.27	8.70	9.36	3.47
K-22	87.0 bc	201 a-d	229 fg	25.1 a	458 a
K-28	83.9 c	193 c-d	285 bc	24.4 a	404 cd
Pop. Užice	89.0 a-c	203 a-d	316 ab	25.3 a	362 f
Vali	87.1 bc	206 a-c	297 a-c	26.0 a	369 ef
Synteza 1	82.8 c	200 b-d	277 cd	24.8 a	406 cd
OS-95	98.6 a-c	191 d	324 a	22.7 ab	357 f
Average	85.8	200	275	24.3	405
CV (%)	5.92	2.68	10.97	4.21	8.95
Total CV (%)	8.09	5.07	10.48	7.66	6.74

\*, means followed by the same letter are not significantly different at  $P \leq 0.01$

**Table 8- Coefficients of correlation (r) between investigated parameters (n= 120; 2 years; 15 varieties; four cuts)**

Çizelge 8- İncelenen özellikler arası (n= 120, 2 yıl, 15 genotip, 4 biçim) ait korelasyon katsayıları (r)

DMY ( $t \text{ ha}^{-1}$ )	Forage quality parameters ( $\text{g kg}^{-1} \text{ DM}$ )				
	MM (II)	CP (III)	CF (IV)	CFM (V)	NFE (VI)
I	-0.140 <sup>ns</sup>	0.344***	0.342***	0.306***	-0.510***
II		0.160 <sup>ns</sup>	-0.364***	0.099 <sup>ns</sup>	0.151 <sup>ns</sup>
III			-0.393***	0.424***	0.068 <sup>ns</sup>
IV				-0.227*	-0.917***
V					0.043 <sup>ns</sup>

ns, not significant; \*, significant at  $P \leq 0.05$ ; \*\*\*, significant at  $P \leq 0.001$

#### 4. Conclusions

In this two-year trial, conducted in central Serbia, the investigated varieties originated from different regions of USA and Europe. Several varieties from each region had superior potential for high dry matter yield (DMY): Vali, Integrity, Ameri stand, OS-95, Synteza 1, Užice, Tru test and K-22, with DMY ranging from 16.03 to 17.66 t ha<sup>-1</sup>. Varieties from USA exhibited higher DMY variability (A1: CV= 13.65% and A2: CV= 9.92%) than European varieties (A1: CV= 6.67% and A2: CV= 8.01%). Variability of crude protein content (CP), the most important parameter of forage quality, was also higher in USA varieties than in those from Europe (CV= 6.27% and CV= 2.68%, respectively). Total variability of forage quality parameters ranged from 5.07% (CV) for crude protein (CP) to 10.48% (CV) for crude fiber (CF). The strongest correlation, although negative ( $r = -0.917$ ), was between CF content and nitrogen-free extract (NFE), while DMY was in significant positive correlation with CP ( $r = 0.344$ ), CF ( $r = 0.342$ ) and crude fat matter content (CFM) ( $r = 0.306$ ). The obtained results could be helpful when choosing alfalfa varieties, in this or similar region, for conventional growing and/or for selection and breeding of varieties with good potential for high dry matter yield (DMY) and forage quality.

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## The Assessment of Genetic Diversity of *Venturia inaequalis* Isolates Obtained from Turkey and Europe by Molecular Markers

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### ABSTRACT

Apple scab (*Venturia inaequalis* (Cke) Wint) is the main disease of apple trees not only in the world but also in Turkey. The disease can cause severe yield losses in all Turkish apple orchards. Genetic diversity and relatedness of apple scab (*Venturia inaequalis* (Cke) Wint) isolates collected from Turkey and Europe were investigated in this study through molecular markers. RAPD, ISSR, SSR and SRAP markers were used in the molecular studies. Results obtained by marker system revealed that isolates were quite separated from each other and it is appeared to exist a variation between them. Genetic relatedness between the isolates are very close to each other, and difference among the groups is not significant due to host specificity and geographical location. The groups are consisted of more number of isolates when SSR and SRAP markers are used compared with ISSR and RAPD markers. Using SSR and SRAP markers are preferable to provide more informative outcomes because of ease of use, repeatability and specificity.

Keywords: Apple; Genetic difference; ISSR; RAPD; SRAP; SSR; *Venturia inaequalis*

## Avrupa ve Türkiye’den Toplanan *Venturia inaequalis* İzolatlarının Genetik Farklılığının Moleküler Markırlar ile Değerlendirilmesi

### ESER BİLGİSİ

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### ÖZET

Elma kara lekesi hastalığı (*Venturia inaequalis* (Cke) Wint) dünyada olduğu gibi ülkemizde de elmanın ana hastalığıdır. Hastalık, yurdumuzda elma yetiştirilen tüm bahçelerde önemli ürün kaybına neden olabilmektedir. Bu çalışmada moleküler markırlar ile Türkiye’den ve Avrupa’dan toplanan Elma kara lekesi izolatlarının genetik farklılıkları ve akrabalık ilişkileri araştırılmıştır. Moleküler çalışmalarda RAPD, ISSR, SSR ve SRAP markırları kullanılmıştır. Markır

sistemlerinden elde edilen sonuçlara göre, izolatların tamamı birbirinden ayrılmış ve bunlar arasında bir varyasyon olduğu görülmüştür. Genetik benzerlikler birbirine yakın bulunmuş, gruplar arasında konukçu seçimi ve coğrafik konum yönünden farklılıklar oluşmamıştır. SSR ve SRAP markırlarında gruplar daha toplu oluşmuş ve uygulamanın kolaylığı, tekrarlanabilirliği ve spesifikliğı bakımından daha bilgi verici bulunmuştur.

Anahtar Kelimeler: Elma; Genetik farklılık; ISSR; RAPD; SRAP; SSR; *Venturia inaequalis*

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## 1. Introduction

Apple scab caused by *Venturia inaequalis* is a fungal disease and mostly resulted from filamentous fungus. It is among the most significant threats to commercial orchards located in cool and precipitated regions of the world (MacHardy 1996). Lesions caused by pathogenic infections are commonly superficial and developed between the cuticle and the cell wall of epidermal layers (MacHardy et al 2001). Therefore they are not life threatening. However, such lesions significantly reduce the market values of apples, thus considered as serious concerns for commercial growers (MacHardy et al 2001). Generally fungicides are applied several times throughout the growing season to prevent apples from *V. inaequalis* (Soriano et al 2009).

Virulence and evolutionary structures of the pathogen should be well-comprehended to develop new cultivars resistant to *V. inaequalis*. Gladioux et al (2008) and Tenzer & Gessler (1997) analyzed microsatellite profiles of *V. inaequalis* samples collected from five different continents and indicated the origin of the fungus as Central Asia and reported the widespread of the fungus worldwide. Barbara et al (2008) carried out AFLP analysis on isolates taken from different cultivars and reported significant differences in virulence of the fungus even in the same orchard established with different apple cultivars in UK, but Xu et al (2008) was not able to observe significant differences in isolates taken from different cultivars or regions of China. Xu et al (2009) indicated the need for more knowledge about inner-population variability effecting host pathogen co-evolution.

Molecular techniques and new analytical methods have been developed for better understanding of population biology of plant pathogens (Taylor et al 1999a; 1999b; 2000). Multi-locus DNA datasets are

now used in phylogenetic and population genetic methods to find out the species and populations and to identify their mating system and population structure (Taylor et al 1999a).

Currently, molecular markers are also employed in researches on population structure of *V. inaequalis*. In the studies carried out on population genetics of *V. inaequalis*, neutral markers of four *V. inaequalis* populations from Switzerland (Tenzer & Gessler 1997) and 11 populations from the other European countries (Tenzer & Gessler 1999) were evaluated to investigate relationship between the geographic population structure and host origin. Those findings revealed a high level gene flow in both short and long-distances in Switzerland (Tenzer & Gessler 1997) and throughout Europe (Tenzer & Gessler 1999).

Gladioux et al (2008) carried out a broad study including 1,273 isolates from 28 orchards of 5 continents with microsatellite markers to identify the origin of *V. inaequalis*. Researchers gathered isolates under 17 clusters representing distinct groups of geographic locations. Analysis of Molecular Variance (AMOVA) revealed about 88% variation within populations. Depending on such a high genetic variation and random association of alleles from different microsatellite loci, researchers concluded that regular sexual recombination occurred in all sampled sites.

While Sierotzki et al (1994), Sierotzki & Gessler (1998), Tenzer & Gessler (1999), Melounova et al (2004) and Padder et al (2011) used RAPD markers in molecular studies related to *Venturia inaequalis*, Tenzer et al (1999), Boehm et al (2003), Guérin et al (2004) and Xu et al (2008) used SSR markers in similar studies. However, ISSR and SRAP markers haven't been used in molecular characterization of *V. inaequalis*.

The present study was conducted to investigate the genetic diversity and relatedness of 54 *Venturia inaequalis* genotypes through RAPD, ISSR, SSR and SRAP molecular markers.

## 2. Material and Methods

### 2.1. Isolation

*Venturia inaequalis* isolates were isolated as single spore from apple orchard at different ages and types (Sierotzki et al 1994). Isolates of *V. inaequalis* were obtained from infected apple tree leaves were washed with sterile distilled water. Spore suspensions were adjusted in sterile distilled water to  $15 \times 10^3$  conidia  $\text{mL}^{-1}$  and maintained on agar surface to obtain pure culture in petri dishes. Petri dishes were kept at 20 °C for 24 h in the dark, and the conidia were transplanted to Potato Dextrose Agar (PDA) medium. Conidia on the PDA were taken from petri dishes and ground in liquid nitrogen using a mortar and pestle. About 100 mg of fungal powder was added to centrifuge cups and used in DNA isolation. DNA isolation was performed with

a genomic QIAGEN® DNA extraction kit (OIGEN, Roche) along with the recommendations of producer firm. Single spore isolates of 43 *Venturia inaequalis* supplied from 22 provinces of Turkey and 11 from 5 different European countries in April and June when the disease is active were used in this study (Table 1).

### 2.2. PCR analysis

For PCR analyses, a total of 24  $\mu\text{L}$  PCR mixture was prepared with 1xPCR buffer solution (50 mM KCl, 10 mM Tris HCl, 25 °C, pH 9.0, 1% Triton X-100), 2.5 mM  $\text{MgCl}_2$ , 0.2 mM dNTP, 0.8  $\mu\text{M}$  primer/primers and 30-50 ng (1  $\mu\text{L}$ ) DNA with 1.25 U Taq DNA polymerase (Takara Ex Taq) enzyme. For DNA amplification, the prepared PCR mixture was placed into PCR device (BioRad, C1000 Thermal Cycler USA) and gene amplification was performed. All isolates were screened through by selecting 11 RAPD primers (Tenzer & Gessler 1997; Meleunova et al 2004), 6 ISSR primers (Baysal et al 2009), 7 SSR primers (Tenzer et al 1999) and 14 SRAP primer combinations (Li & Quiros 2001).

**Table 1- List of samples collected from European countries and different provinces of Turkey**

*Çizelge 1- Türkiye'nin farklı şehirlerinden ve Avrupa ülkelerinden toplanan örneklerin listesi*

No	Sample no	No	Sample no	No	Sample no
1	03D09st	19	32EgHR09st	37	53ID10kr
2	05M09a1	20	32GeBag09st	38	55M09a
3	05M09a2	21	32EnTst1209st	39	59M09go
4	07E109st	22	32EnTst709s	40	59M09st
5	07E110st	23	33M09st	41	60M09st
6	17Br09st	24	33M10an	42	70M09st
7	17B109st	25	33Mut09st	43	77AE10st
8	17L09ga	26	38Y10st	44	AlKln10kr(Germany/Cologne)
9	17Lap09st	27	38YS09st	45	BelAE10kr(Belgium/Resc. Inst.)
10	17MS09st	28	42BS09st	46	BelBrM10kr(Belgium, Brussel)
11	17Un09st	29	42BS10st	47	BelBrO10kr(Belgium/Oaslt)
12	20COm09go	30	42E10st	48	BelGe10kr(Belgium/Gent)
13	20Om09st	31	43To09br	49	BelLux10se(Belgium-Luxemburg)
14	20Om09st2	32	43To09go	50	Bos210kr(Bosnia/University)
15	22Lb10pl	33	43To09gr	51	Bos310kr(Bosnia/University)
16	24CK10st	34	43To09st	52	Hol10kr(The Netherland)
17	24M09a	35	48U109go	53	HolRot10se(The Netherland/Rotterdam)
18	26MhIS10st	36	50M09st	54	LuxM10se(Luxemburg/Center)

PCR products of RAPD, ISSR and SRAP markers were separated by electrophoresis method in Tris boric acid EDTA (TBE) buffer solution by using 2% Agar (Sigma, A5093 Agarose) and PCR products of SSR markers by using 2% High Resolution Agar (LONZA Metaphor Agarose). Then dyed with ethidium bromide (0.5 mg mL<sup>-1</sup>) and imaged in a gel imaging system (Gel Logic 200 Imaging System) under ultraviolet light and scored as 0 indicating the absence of a band and 1 indicating existence of a band.

Resultant data were analyzed through NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y. USA) software. Similarity indexes were calculated in accordance with Dice (1945) method and dendrograms was created in accordance with UPGMA (Unweighted Pair-Group Method with Arithmetic Average) method (Rohlf 1993). With all these analyses, variation and similarity levels among investigated scab isolates were identified and characteristics of genetic structure were put forth.

### 3. Results and Discussion

Phylogenetic analyses of 54 isolates were performed by using RAPD, ISSR, SSR and SRAP markers to identify genetic relatedness of apple scab single

spore isolates supplied from different production sites.

With RAPD markers, number of bands per primer was found to be 5.9 and number of polymorphic band was found to be 3.9. The highest number of band (8 bands) was observed in OPG05 and M2 RAPD primers. The greatest number of polymorphic band (7 bands) was observed in M2 primer and polymorphism ratio was identified as 88%. The highest Polymorphism Information Content (PIC) value (0.51) was observed again in OPG05 and M2 RAPD primers and general average was observed as 0.42. RAPD marker-based dendrograms revealed that scab isolates separated into an initial group with a ratio of 0.83 and it was followed by several sub-groups. A distinct difference was not observed in host-specificity and geographic origin (Figure 1). With regard to relativeness, the samples 50M09st and 42BS09st were the closest ones (0.97) to each other. In general, European isolates formed collectively and genotypic similarity was around 0.84.

Melounova et al (2004) identified the genetic similarity with RAPD markers as 79%. The value was quite close to the similarity (88%) observed in this study with RAPD markers. Padder et al (2011) also carried out phylogenetic analyses with RAPD molecular markers and were not able to identify

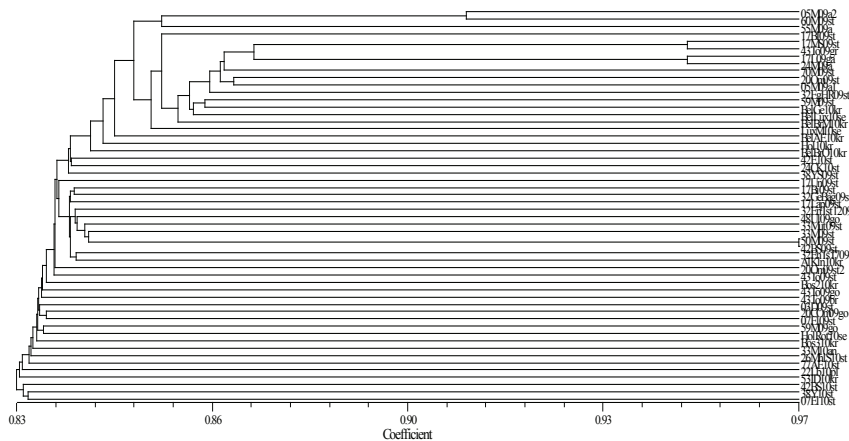


Figure 1- Phylogenetic dendrograms for *Venturia inaequalis* isolated based on RAPD markers

Şekil 1- RAPD markerler kullanılarak *Venturia inaequalis* için elde edilen filogenetik dendogramlar

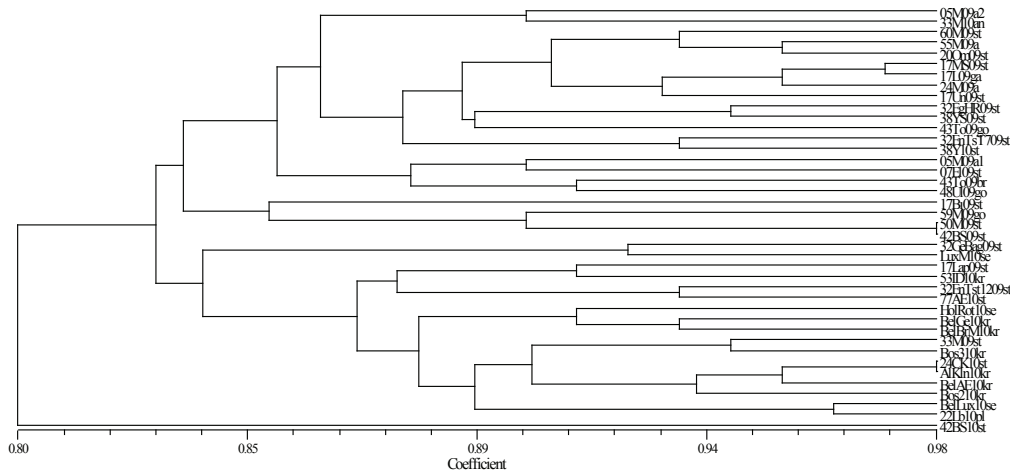
distinctive relationships among the groups with regard to geographical and host specificity. Current findings were parallel to those earlier ones.

With ISSR makers, average number of band per primer was found to be 6.2 and average number of polymorphic band was identified as 4.2. The highest number of band was observed in UBC 885 ISSR primer (8 bands) and the highest number of polymorphic band was observed in the same primer (7 bands) and polymorphism was identified as 88%. The greatest Polymorphism Information Content was observed in UBC 887 ISSR primer (0.47) and general average was identified as 0.36. ISSR marker-based dendrograms (Figure 2) revealed that samples were separated into two groups with a ratio of 0.80. The first group was formed only by the isolate taken from Konya-Beyşehir (42BS10st) and the rest formed the second group. The second group was divided into sub-groups with 0.83 similarity ratio and the isolates 50M0st and 42Bs09st, the isolate 24CK10st and German isolate AIKln10kr were identified as close relative with each other with a genetic similarity ratio of 0.98. The use of ISSR markers in identification of genetic diversity in *V. inaequalis* isolates haven't been reported, yet. However, Baysal et al (2009) performed genetic race

separation in *Fusarium oxysporum* f. sp. *lycopersici* by using 6 ISSR primer markers. Researchers obtained 23 bands (average 3.83 bands per primer) with a band widths between 190 bp and 900 bp and identified the separation capacity as  $\sum \delta: 1.08 \pm 0.59$ . Of the ISSR primers, UBC 880 (1.56), UBC 886 (1.54) and UBC 887 (1.52) were identified as the most effective primers in separation.

Yalim (2005) in a study selected 10 ISSR primers based on polymorphism ratio and produced 75 bands of which 51 were polymorphic. As an indicator of polymorphism level, the researcher identified the highest PIC value as 0.844 and the lowest as 0.354 with an average value of 0.611. In this study, the least PIC value (0.36) was observed in analyses carried out with ISSR markers. Such findings were found to be parallel to those earlier ones. In this marker system, it was observed that variation among genotypes came from a single source and variations were not observed based on geographic or host origin.

With SSR markers, average number of band per SSR primer pair was observed as 1.9 and average number of polymorphic band was observed as 1.7. The highest PIC value (0.71) was observed in 1tc1a and 1tc1b SSR primer pair and general average



**Figure 2- Phylogenetic dendrograms for *Venturia inaequalis* isolated based on ISSR markers**

Şekil 2- ISSR markerler kullanılarak *Venturia inaequalis* için elde edilen filogenetik dendogramlar



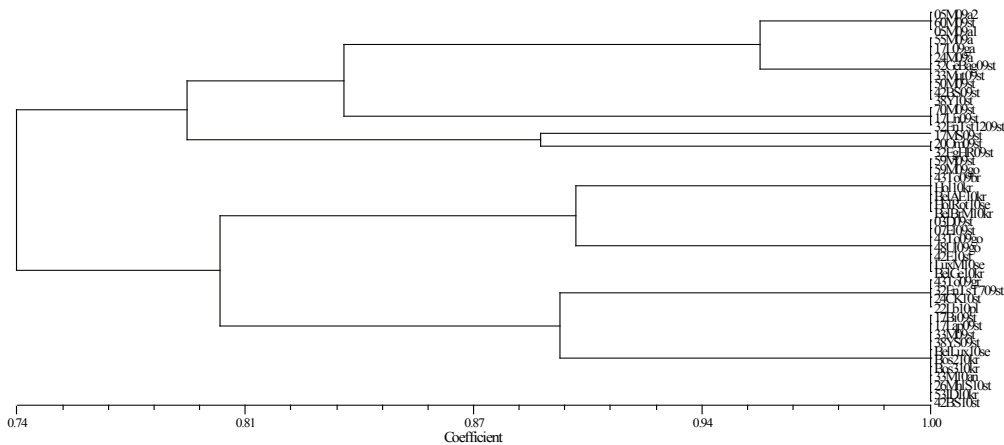
was observed as 0.56. In SSR-based dendrograms, genetic similarity of entire genotypes varied between 0.57-1.00. The dendrograms (Figure 3) revealed 2 groups with a similarity ratio of 0.74. These two main groups were then separated into 7 sub-groups with a relatedness ratio of 0.81 within themselves. Considering the genetic similarities between the sub-groups, it was observed that even European isolates were placed separately in sub-groups and distinctive differences were not able to be observed between the isolates. Populations were separated better and groups were clearer with SSR markers.

Similarly, Tenzer et al (1999) carried out a study screened 350 *V. inaequalis* isolates collected from 11 European countries with 7 SSR markers. Researchers identified genetic diversity as between 0.52-0.96 and population diversity as between 0.28-0.49. On the other hand, Guérin et al (2004) obtained 21 microsatellite (SSR) markers from *Venturia inaequalis* isolate and analyzed 44 isolates to identify host-pathogen and geographical differences. Each primer pair was found to be polymorphic and average 9.1 alleles (2-24) were obtained from the loci.

Current findings did not reveal distinctive genetic differences among the isolates. The differences

among the isolates were probably because of inner-race variations.

As it was in ISSR primer system, the use of SRAP markers in identification of genetic diversity in *V. inaequalis* isolate haven't been also reported before. However, Baysal et al (2009) carried out genetic race separation of *Fusarium oxysporum* f. sp. *lycopersici* by using 4 SRAP primer markers and reported higher separation capacity ( $\Sigma\hat{c}$ : 1.28±0.97) for ISSR markers. Phylogenetic dendrograms created based on SRAP markers is presented in Figure 4. Similar to analyses with SSR markers, more clear outcomes were obtained with SRAP markers. The dendrograms revealed 2 main groups with a relatedness ratio of 0.66. Of these groups, the first one included only 1 isolate (17B109st) and the rest were placed in the second group. The second group was then separated into 2 sub-groups with a relatedness ratio of 0.77. The first sub-group was composed of 33M09st and 38Y10st and the samples supplied from Bosnia were placed in the second sub-group with a relatedness ratio of 0.94. The isolates 17MS09st and 24M09a and the isolates from The Netherlands (Hol10kr) and Belgium (BelAE10kr) were found to be the closest isolates with a relatedness ratio of 0.96. A low level variation was observed among the samples and



**Figure 3- Phylogenetic dendrograms for *Venturia inaequalis* isolated based on SSR markers**

*Şekil 3- SSR markerler kullanılarak Venturia inaequalis için elde edilen filogenetik dendogramlar*

the dendrograms revealed the similarity levels of the genotypes as between 0.54-0.96. The average number of band per primer was observed as 6.5 and number of polymorphic band was observed as 4.8. The highest PIC value was observed in Em5Me10 primer combination (0.76) and general average was observed as 0.54. Current findings were similar to earlier reports of the researchers and SRAP markers were found to be more effective in separation than RAPD and ISSR markers.

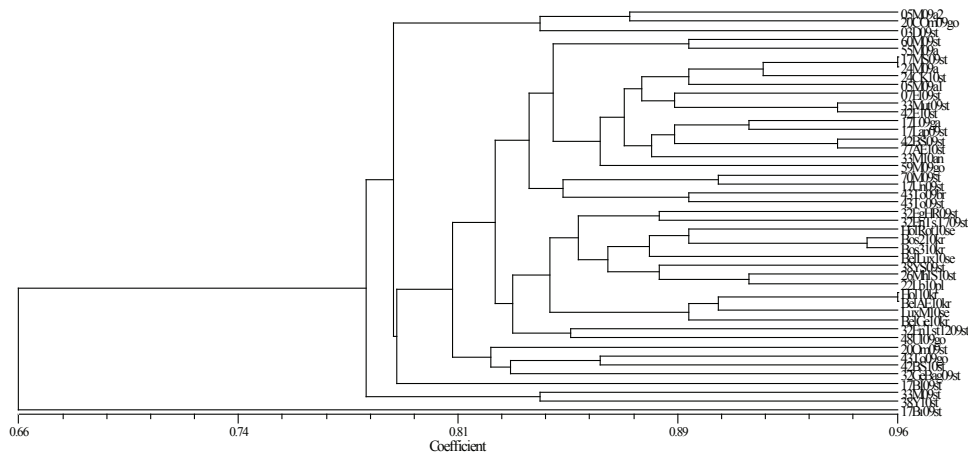
Current findings pointed out that the analyses carried out with SRAP markers may reveal a regional relationship. Also, SRAP markers were used for the first time worldwide in molecular analyses of *V. inaequalis* isolates and SRAP markers were found to be quite promising in such analyses.

Boehm et al (2003) investigated the genetic diversity in *V. inaequalis* (Cke.) Wint. populations with neutral markers and indicated that genetic diversity was mostly related to ecological conditions of the places from where isolates supplied in Israel. The researchers identified that the populations supplied from coastal sections of the country were quite uniform and the populations supplied from Golan Heights were quite diverse. The researchers also indicated that this pathogen did not reproduce

well in regions with mild winters and existed in such regions as clonal lineages.

Tenzer & Gessler (1999) investigated the genetic diversity among 11 *V. inaequalis* populations supplied from five European countries through allele frequencies of 18 random amplified polymorphic DNA markers and the internal transcribed spacer region of the ribosomal DNA. The diversity within each population was observed as between 0.26-0.33. The average differentiation among populations was identified as 0.11 and populations were isolated by distance ( $r^2=0.50$ ,  $P<0.01$ ). Such results indicated an extensive short-distance gene flow in Europe and dispersal over longer distances enough to prevent differentiation due to genetic drift (Tenzer & Gessler 1999).

Xu et al (2008) pointed out the significance of identification of pathogen population structure for breeding resistant cultivars. The researchers supplied *V. inaequalis* isolates from China, India and the United Kingdom to investigate the differences in pathogen populations through AFLP markers. The researchers reported significant differences in *V. inaequalis* populations supplied from China and the United Kingdom and insignificant differences in populations supplied from China.



**Figure 4- Phylogenetic dendrograms for *Venturia inaequalis* isolated based on SRAP markers**

Şekil 4- SRAP markerler kullanılarak *Venturia inaequalis* için elde edilen filogenetik dendogramlar

Guérin & Le Cam (2004) indicated that *V. inaequalis* population structure might be greatly influenced by genetic background of host cultivars. The recent breakdown of the Vf resistance gene by *V. inaequalis* in France allowed the comparison of *V. inaequalis* populations and consequently identification of resistant and susceptible cultivars. The researchers genotyped 133 isolates of *V. inaequalis* from Vf cultivar Judeline and non-Vf cultivars Petit Jaune, Juliana, and Judor from a commercial orchard through AFLPs and four microsatellite loci and indicated significantly lower genetic diversity for *V. inaequalis* populations than for non-Vf cultivars. The researchers also reported that isolates from Vf cultivars were belong to the same clonal lineage.

Guérin & Le Cam (2004) investigated three susceptible apple cultivars and one cultivar carrying the Vf gene by using AFLPs and four microsatellite loci. Xu et al (2008) analyzed *V. inaequalis* populations supplied from different apple cultivars from the UK, China and India and reported significant differences between populations of Asia and Europe based on cluster analysis of more than 100 isolates. The researchers also indicated significant differences among the isolates supplied from three apple cultivars (cvs. Cox, Bramley, Worcester) of a single orchard in the UK. Xu et al (2008) in the same study reported that *V. inaequalis* populations within China (Shaanxi Province) were much more homogenous and no population differentiation was detected on isolates obtained from different cultivars (cvs. Gala, Fuji and Qingquan).

Tenzer et al (1999) used 7 microsatellite markers with (TC)<sub>n</sub> and (AAC)<sub>n</sub> repeats and tested them on 350 *V. inaequalis* isolates. The researchers reported that three markers with the (TC)<sub>n</sub> motif out of seven were highly diverse with Nei's expected genetic diversity value of between 0.52-0.96. Guérin et al (2004) developed 21 new polymorphic microsatellite markers to be used genetic analyses of *V. inaequalis* isolates. All above mention studies were capable of detecting high level of genetic diversity for *V. inaequalis*.

#### 4. Conclusions

ISSR and SRAP markers were used for the first time in this study for molecular characterization of *V. inaequalis* isolates. The groups were better clustered with SSR and SRAP markers and were more informative considering the easy use, repeatability and specificity of the markers.

Chemical control practices may sometimes create new races resistant to fungicides. Commercial cultivars are sensitive to pathogen most of the time since an obstruction is not experienced in spread of disease based on sensitivity of the cultivar, a specificity is not also observed based on the host pathogen (Kaymak et al 2008). Whenever the orchards are established with resistant cultivars, the fungus will experience a powerful obstruction and will try to specialize to adapt itself to changing conditions. Then in time, resistant cultivars will turn into sensitive ones. An effective use of gene sources will allow the identification of new resistance genes and provide significant contributions in control of the fungus.

Considering the entire results obtained from marker system together, it was observed that all of the isolates were separated from each other and a variation was observed among them. The present study is the first study in this subject matter and further more comprehensive and detailed studies will reveal significant information about diversity and distribution of scab races.

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## Structural Analysis of Field Sprayer Booms

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### ABSTRACT

In this paper, structural analysis of a 21 meters wide field sprayer boom, designed for precision agriculture applications, was conducted with finite element analysis. G-programming language, a data acquisition board and an inductive force transducer were used to measure the forces acting on the boom arms. An experimental setup, developed in a laboratory environment, was able to measure and record various forces in 5 milliseconds intervals. An ANSYS model was developed to analyze the forces recorded during laboratory experiments. Steel (SAE/AISI St 42) and aluminum (ISO AIMg2.5) materials for the sprayer boom structure were used in the analyses. Based on the analysis results, using aluminum for active boom suspension system for field sprayers produced more favorable structural results than steel.

Keywords: Transient analysis; Aluminum; Steel; G-Programming; Finite element analysis; ANSYS

## Bir Tarla Pülverizatör Buminun Yapısal Analizi

### ESER BİLGİSİ

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### ÖZET

Bu çalışmada, hassas tarımda kullanmak amacıyla tasarlanan 21 metre iş genişliğine sahip bir tarla pülverizatör buminun sonlu elemanlar yöntemiyle yapısal analizleri gerçekleştirilmiştir. Bum kollarına gelen farklı kuvvet değerlerinin ölçümü için G-programı, bir adet veri algılama kartı ve indüktif bir kuvvet algılayıcısı kullanılmıştır. Laboratuvar ortamında geliştirilen deney düzeneği farklı kuvvet büyüklüklerini 5 milisaniye aralıklarla ölçerek kaydedebilecek şekilde hazırlanmıştır. Deneysel olarak elde edilen kuvvet verilerinin analizi için ANSYS ortamında bir model geliştirilmiştir. Analizlerde bum malzemesi olarak çelik (SAE/AISI St 42) ve alüminyum (ISO AIMg2.5) kullanılmıştır. Yapılan denemelerde elde edilen sonuçlara göre, aktif bum dengelemesi için alüminyum malzemenin yapısal olarak çeliğe göre daha iyi olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Süreksiz analiz; Alüminyum; Çelik; Grafıksel programlama; Sonlu elemanlar; ANSYS



## 1. Introduction

Most of the machines used for agricultural operations are subjected to vibrations and oscillations due to irregular field conditions. Due to their large working widths, field sprayers are usually exposed to extreme vibrations and oscillations during field operations (Koc & Keskin 2011). Among the main reasons for non-uniform distributions of pesticides applied by field sprayers are the presence of oscillations and vibrations on the boom (Hedden 1961). The large working width and the vibration of field sprayer booms result in uneven clearance heights between the field sprayer boom and the field surface (Çilingir & Çelen 1995). To maintain a uniform pesticide distribution, an appropriate distance between the sprayer boom and the agricultural field surface must be maintained throughout the spraying operation (Pochi & Vannucci 2001; Jeon et al 2004).

In order to reduce fuel consumption and time during spraying, field sprayers with large working widths are used. However, as the width of the sprayer increases, the weight of the boom also increases. A 36 meters wide sprayer boom made of steel weighs approximately 1155 kilograms, whereas a similar boom with the same width made of aluminum weighs 770 kilograms. As in aviation industry, carbon fiber is becoming a more preferred material than steel or aluminum because of weight saving. A 36 meters wide sprayer boom made of carbon fiber weighs only 385 kilograms (Anonymous 2015a). However, the high cost of carbon fiber is the major factor limiting its use in field sprayers and other agricultural equipment.

Steel and aluminum are the most commonly used structural materials in agricultural machinery. Due to the higher resistance and relatively lower costs, steel materials are widely used in the sector. In the meantime, aluminum is also becoming a commonly used material in agricultural machinery because of its weight (Herrington & Latorre 1998; Lamb et al 2011).

When aluminum and steel are compared, aluminum is lighter, and has higher corrosion and temperature resistances than steel. However,

aluminum is more expensive, has lower yield and tensile strengths, and lower fracture resistance than steel. The cost of steel, on the other hand, is inexpensive and has better welding and machining properties than aluminum (Kasten 2010).

Finite element analysis can be implemented to analyze the strength of a structure. Using finite element analysis, a structure's vibration response and factor of safety can be obtained very close to the real values in a short time (Moaveni 2007; Chu & Lei 2014) identified that the theoretical finite element analysis results of a boom structures were close to the results of experimental analysis.

In this study, the sprayer boom characteristics were determined with mechanical analysis. A model of the boom was then manufactured based on the results of mechanical analysis. The critical points of failure of the boom system were identified by using an electro-hydraulic simulation in laboratory conditions. The critical values were measured with sensors and a data acquisition system. A computer program was developed in Labview G-programming language for this research. Finite element analysis was carried out in ANSYS software based on the data collected with laboratory experiments. The model then was used for testing different materials for the sprayer boom structure. Two boom materials, steel SAE/AISI St 42, and aluminum ISO AlMg2.5 were tested with the developed model. Finally, the outputs of the model were compared with the results obtained from laboratory experiments.

## 2. Material and Methods

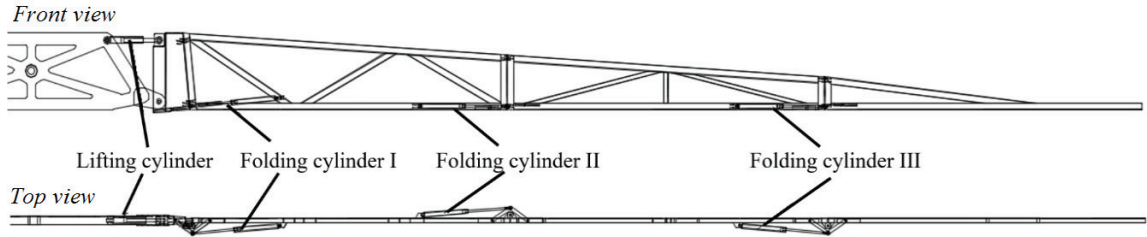
### 2.1. Materials

#### 2.1.1. Field sprayer boom for laboratory experiments

A prototype field sprayer boom was manufactured for this research. The working width of the field sprayer was 21 meters with three major sections. Left and right sides of the boom were 9 meters long and the center was 3 meters wide. The boom was designed in a way that the left and right sections of the boom could move independently. The boom

sections were manufactured from 3 meters long and 40x60x3 millimeters in cross-sectional dimensions of aluminum profile. The different motions of the boom sections were controlled using hydraulic cylinders. Two lifting cylinders were used to

maintain the distance between the ground surface and each side of the boom, and one hydraulic cylinder was used to move the whole structure vertically (Figure 1).



**Figure 1- The sprayer boom structure used for the experiments**

*Şekil 1- Denemelerde kullanılan pülverizatör buminun yapısı*

### 2.1.2. Load measurement set-up

An inductive force transducer and an NI 6009 data acquisition card were used for load measurements. A 30 millimeter diameter hydraulic cylinder was designed to mount and dismount the force transducer at the center of the field sprayer (Figure 2). The piston rod of the hydraulic cylinder was working as an inductive force measurement tool as well as a connecting part. The measurement set up was used to measure the upward and downward loads.



**Figure 2- Load measurement set-up**

*Şekil 2- Kuvvet ölçüm düzeneği*

### 2.1.3. Data acquisition

For data acquisition process, a graphical program was developed by using LABVIEW 8.2. The developed G-program facilitates the collection and recording of the inductive load data with the help of an NI 6009 data acquisition card to a personal computer. The inductive force measurements were displayed both in graphical and numeric indicators on the computer screen.

## 2.2. Method

### 2.2.1. ANSYS finite element formulation

#### 2.2.1.1. Static analysis

The static mathematical formulas in the ANSYS program which are valid for all degrees of freedom are given in Equation 1 and 2. The effects of inertia and damping effects were neglected except for static acceleration fields (Anonymous 2015b).

$$[K]\{u\} = \{F\} \quad (1)$$

$$[K]\{u\} = \{F^a\} + \{F^r\} \quad (2)$$

$$[K] = \text{Total stiffness matrix} = \sum_{m=1}^N [K_e]$$

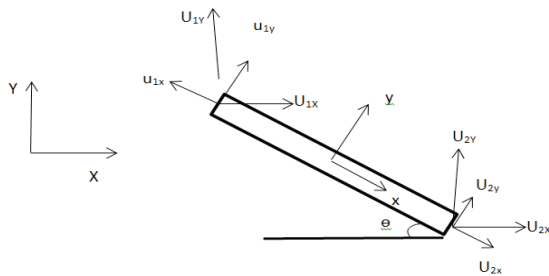
Where;  $\{u\}$ , nodal displacement vector;  $N$ , number of elements;  $[K_e]$ , elements stiffness matrix;  $\{F^r\}$ , reaction load vector;  $\{F^a\}$ , total applied vector.

Total applied vector defined in Equation 3.

$$\{F^a\} = \{F^{nd}\} + \{F^{ac}\} + \sum_{m=1}^N (\{F_e^{th}\} + \{F_e^{pr}\}) \quad (3)$$

Where;  $\{F^{nd}\}$ , applied nodal load vector;  $\{F^{ac}\} = - [M]\{a_c\}$ , acceleration load vector;  $[M]$ , total mass matrix;  $\{a_c\}$ , total acceleration vector;  $\{F_e^{th}\}$ , element thermal load vector;  $\{F_e^{pr}\}$ , element pressure load vector.

The nodal displacements for one member of the boom structure are obtained as shown in Figure 3.



**Figure 3- Static mode displacements of one member of the boom**

Şekil 3- Bum üzerindeki bir noktanın static mod yer değişimi

X-Y axis in Figure 4 indicates the global coordinate system, and x-y indicates the local coordinate system. The angle between the element member and the horizontal plane is shown with  $\theta$ . The displacements at two nodes (node 1 and node 2) are shown in both coordinate systems. The global displacements ( $U$ ) are related to the local displacements ( $u$ ) determined from the Equation 4-8.

$$U_{1X} = -u_{1x} \sin(\theta) + u_{1y} \cos(\theta) \quad (4)$$

$$U_{1Y} = u_{1x} \sin(\theta) + u_{1y} \cos(\theta) \quad (5)$$

$$U_{2X} = u_{2x} \cos(\theta) + u_{2y} \sin(\theta) \quad (6)$$

$$U_{2Y} = -u_{2x} \cos(\theta) + u_{2y} \sin(\theta) \quad (7)$$

$$\{U\} = [T]\{u\} \quad (8)$$

Where;  $T$ , transformation matrix.

Transformation matrix is shown in Equation 9.

$$[U] = \begin{bmatrix} U_{1x} \\ U_{1y} \\ U_{2x} \\ U_{2y} \end{bmatrix}, [T] = \begin{bmatrix} -\sin(\theta) & \cos(\theta) & 0 & 0 \\ \sin(\theta) & \cos(\theta) & 0 & 0 \\ 0 & 0 & \cos(\theta) & \sin(\theta) \\ 0 & 0 & -\cos(\theta) & \sin(\theta) \end{bmatrix}, [u] = \begin{bmatrix} u_{1x} \\ u_{1y} \\ u_{2x} \\ u_{2y} \end{bmatrix} \quad (9)$$

The reaction force values were calculated using the Equation 10-12.

$$\{F\} = [T]\{f\} \quad (10)$$

The relations between the node forces and the displacements are similar to a linear spring.

$$F = KX \quad (11)$$

$$\{f\} = K\{u\} \quad (12)$$

$K$  is related to the property of the material given by Equation 13.

$$K = A \cdot E \cdot L^{-1} \quad (13)$$

Where;  $A$ , cross sectional area;  $L$ , length and  $E$ , modulus of elasticity of the element member.

After substituting for  $\{f\}$  and  $\{u\}$  in terms of  $\{F\}$  and  $\{U\}$  and multiplying both sides with  $[T]$   $F$  can be written as Equation 14.

$$[F] = K[T]^{-1}[U][T] \quad (14)$$

The matrix of nodal forces were determined by substituting the values of the  $\{F\}$ ,  $[T]^{-1}$ ,  $[T]$ , and  $\{U\}$  matrices and multiplying (Equation 15).

$$\begin{bmatrix} F_{1x} \\ F_{1y} \\ F_{2x} \\ F_{2y} \end{bmatrix} = k \cdot \begin{bmatrix} -\sin(\theta) & \cos(\theta) & 0 & 0 \\ \sin(\theta) & \cos(\theta) & 0 & 0 \\ 0 & 0 & \cos(\theta) & \sin(\theta) \\ 0 & 0 & -\cos(\theta) & \sin(\theta) \end{bmatrix} \begin{bmatrix} -\sin(\theta) & \cos(\theta) & 0 & 0 \\ \sin(\theta) & \cos(\theta) & 0 & 0 \\ 0 & 0 & \cos(\theta) & -\cos(\theta) \\ 0 & 0 & \sin(\theta) & \sin(\theta) \end{bmatrix} \begin{bmatrix} u_{1x} \\ u_{1y} \\ u_{2x} \\ u_{2y} \end{bmatrix} \quad (15)$$

The same principle was applied for all of the members of the structure and finally the element stiffness matrices were obtained. The equation was then solved by applying boundary conditions and loads, which produced the displacement stresses.

### 2.2.1.2. Transient analysis

A transient dynamic analysis was used to determine the response of the boom structure under time-dependent loading with inertia and damping effects. The transient analysis solution method (ANTYPE, TRANS) was dependent on the degrees of freedom. The most widely used finite element discrete

equation of motion for dynamic structures were the virtual work method given in Equation 16.

$$[M]\{\ddot{u}(t)\} + [C]\{\dot{u}(t)\} + \{F^i(t)\} = \{F^a(t)\} \quad (16)$$

Where;  $[M]$ , structural mass matrix;  $[C]\{\ddot{u}(t)\}$ , structural damping matrix;  $\{\ddot{u}(t)\}$ , nodal acceleration vector;  $\{\dot{u}(t)\}$ , nodal velocity vector;  $\{u(t)\}$ , nodal displacement vector;  $\{F^i(t)\}$ , internal load vector;  $\{F^a(t)\}$ , applied load vector.

In linear structural dynamics systems, the internal load is linearly proportional to the displacement while the structural stiffness matrix remains constant. Therefore, Equation 16 was rewritten as Equation 17.

$$[M]\{\ddot{u}(t)\} + [C]\{\dot{u}(t)\} + [K]\{u(t)\} = \{F^a(t)\} \quad (17)$$

Where;  $[K]\{u(t)\}$ , structural stiffness matrix.

In addition to the above given direct time integration methods, other methods of solving transient problems were also included in ANSYS; such as the new method, generalized  $\alpha$  method, the Hilber Hughes-Taylor (HHT) method, and the Newmark family of time integration algorithms.

### 2.2.2. ANSYS graphical modeling

Modeling of the system in ANSYS was started by developing the mesh structure and assuming the boundary conditions. The boundary conditions used in the model are shown in Table 1.

**Table 1- Boundary conditions used for the ANSYS model**

*Çizelge 1- Kullanılan ANSYS modeli üzerindeki sınır koşulları*

Sizing		Statistics	
Use advanced size function	On: Curvature	Nodes	819202
Relevance center	Fine	Elements	518338
Curvature normal angle	12.0°	Aspect ratio	
Min size	5.0 mm	Min	1.16
Max face size	10.0 mm	Max	44.5
Max size	20.0 mm	Average	1.88
Growth rate	1.20		
Minimum edge length	1.7857e-002 mm		

### 2.2.3. Laboratory experiments

The loads on the boom joints were measured by using the inductive transducer. This transducer is attached at the center of 30 millimeters diameter piston with a specially constructed screw profile. The other end of the transducer was then attached to the boom elements. The measurements of the load were recorded with the NI 6009 data acquisition card on the computer. For the upward and downward movements of the boom structure, especially designed hydraulic cylinders were used. The cylinders were driven by the hydraulic output of the tractor. During the experiments, the tractor hydraulic system output pressure of 120 bars was used. To measure the static loads on the boom structure and the joints, calibrations were made by hanging known weights on three points of the 9 meters long boom structure. Following the calibration, static load data were collected. The data were then used as input for the ANSYS modeling.

Based on the inductive load data from the known masses a calibration equation was developed. The derived equation was then used in the Labview program. The derived equation with the coefficient of determination ( $R^2$ ) value of 0.99 is shown in Equation 18.

$$y = 11.304x + 0.8952 \quad (18)$$

Where;  $y$ , force (N) measured with the load sensor and  $x$ , known mass (kg).

Based on the calibration equation in the computer program, the unknown force values were obtained in 5 milliseconds intervals.

### 3. Results and Discussion

#### 3.1. Results

The results of the ANSYS structural analyses are given in Table 2 and Figures 4. Based on the loads on the boom structure, static and transient analyses were performed in ANSYS environment. Total deformation, equivalent stress and safety factor values on aluminum and steel boom structures were obtained from the analyses. Based on the static analysis that the total deformation on the aluminum structure was 27 mm, total stress was 189 MPa and the minimum critical safety factor was 0.45. On the other hand, the total deformation on the steel structure was 19 mm, total stress was 399.67 MPa and the minimum safety factor was 0.21.

In addition to the structural analysis, a transient analysis was also conducted. The critical values of

the transient analysis are tabulated in Table 3. The transient analyses showed that the total deformation, equivalent stress and safety factor values for both aluminum and steel were obtained at 0.0025 s, 0.5 s and 1.0 s, respectively. For both materials, the critical safety factor values were obtained at 1.0 s. Transient analyses also showed that for aluminum structure, the total deformation was 29.33 mm, total stress was 220 MPa and the critical minimum safety factor was 0.39. For steel structure, these values were as 31.14 mm, 664 MPa and 0.12, respectively.

#### 3.2. Discussion

According to the structural and transient analyses, aluminum boom material produced greater factor of safety than steel, which is the better material of the two for field sprayer boom construction. This is due to the fact that aluminium is lighter in weight because of the internal molecular and mechanical properties (Kuziak et al 2008).

The critical points of failure observed during laboratory experiments overlapped with the critical

**Table 2- Static analysis results**

*Çizelge 2- Statik analiz sonuçları*

Parameters	Steel (SAE/AISI St 42)		Aluminum (ISO AlMg2.5)	
	Min	Max	Min	Max
Total deformation (mm)	0	19	0	27
Equivalent stress (MPa)	0.017	399.67	0.0031	189
Safety factor	0.21	15	0.45	15

**Table 3- Transient analysis results**

*Çizelge 3- Zamana bağlı analiz sonuçları*

Parameter	Steel (SAE/AISI St 42)						Aluminum (ISO AlMg2.5)					
	0.0025 s		0.5 s		1 s		0.0025 s		0.5 s		1 s	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Total deformation (mm)	0	1.69	0	14.64	0	31.14	0	1.70	0	13.59	0	29.4
Equivalent stress (MPa)	0	136	0	359.97	0	664	2.4	42.28	0	112.56	0	220
Safety factor	0.6	15	0.234	15	0.12	15	2.03	15	0.76	15	0.39	15



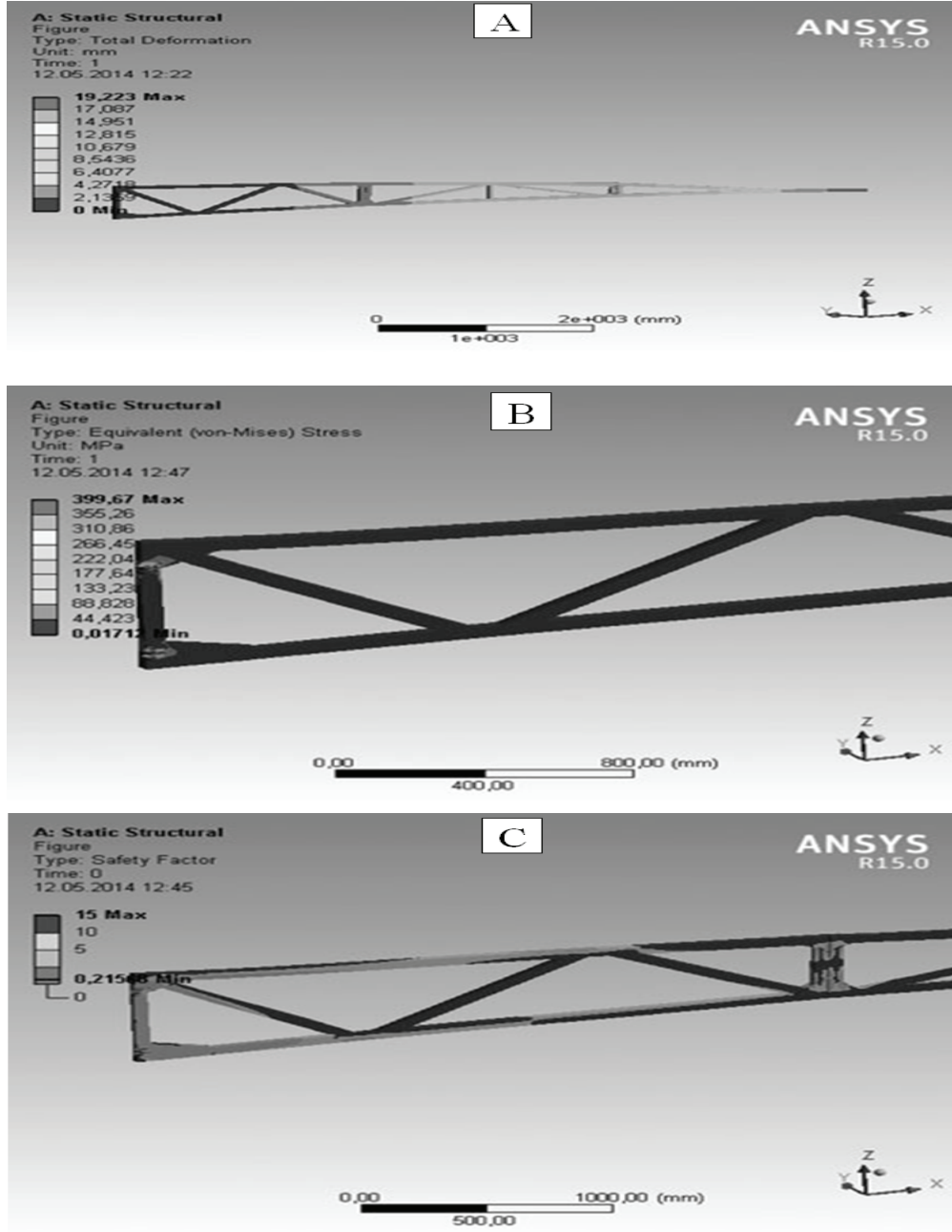
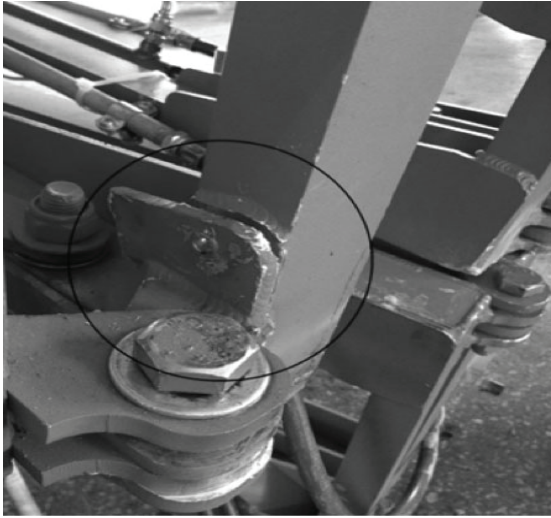


Figure 4- A, steel material static analysis total deformation; B, steel material static analysis equivalent stress; C, steel material static analysis safety factor

Şekil 4- A, çelik malzeme static analiz güvenlik faktörü; B, çelik malzeme statik analiz toplam yer deęişimi; C, çelik malzeme static analiz eş deęer gerilme

points identified by the ANSYS analysis. The failure points of the boom structure observed during experimental activities as shown in Figure 5. The point of failure was also identified during designing the boom.



**Figure 5- Sprayer boom failure point during experiments**

*Şekil 5- Denemeler sırasında pülverizatör bumu üzerinde görülen kopma*

The same points of failures were also identified by the modeling and analysis using ANSYS prior to experimental failures for both steel and aluminum boom materials. Thus, instead of materials (aluminum or steel), boom design should be renewed. For this purpose, dismountable joint aluminum alloy material can be used.

Due to the higher strength of aluminum, one can say that aluminum is better than steel for boom construction. Moreover, the lighter weight of aluminum has an advantage to save fuel consumption of tractors due to the lower loads on the tractor carrying the field sprayer.

Zhou et al (2011) reported that vehicles made of aluminum were lighter than the ones made of steel hence improving the fuel efficiency. One of the problems for field sprayer booms is the collision with

the ground and the breakdown of boom structures during field operations. Materials with higher spring properties are desired to reduce the breakdown due to collisions with the ground. The solution used by the automobile industry to solve this problem, which is using aluminum and Advanced High Strength Steels (AHSS) can be adapted for boom manufacturing too. The workability of aluminum is also better than that of steel. Nowadays, lighter weight with high strength like the AHSS can be used as an alternative to aluminum for boom manufacturing (Anonymous 2015c). Although the price of aluminum is expensive than that of steel by at least 30%, aluminum has many other advantages than steel that it is preferred as a boom material.

#### 4. Conclusions

According to this research, it is concluded that field sprayer booms constructed from aluminum are better than steel constructed booms. During the experiments, it was observed that the critical point of failure of the booms is at the universal joint connection of the boom wings to each other. The critical point of failure of the booms found during the experimental analysis was the same point found by using the structural and transient ANSYS analysis of the system. From this research, it can be concluded that maximum care must be given to the critical point of failures mentioned above during the design of field sprayer booms.

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## TARIM BİLİMLERİ DERGİSİ-JOURNAL OF AGRICULTURAL SCIENCES

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#### Derginin Kapsamı

Tarım Bilimleri Dergisi, tarım bilimleri alanında yapılan özgün araştırmaları ve yeni bulguları içeren makaleleri yayımlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayanan araştırmalar derginin kapsamı dışındadır. Derleme makaleler, yayım komisyonunun çağrısı üzerine hazırlanmışsa normal inceleme ve değerlendirme sürecinden geçirilerek yayımlanır.

#### Makale Hazırlama

Makaleler, A4 boyutundaki kâğıdın tek yüzüne 12 punto Times New Roman yazı tipinde ve çift satır aralıklı yazılmalıdır. Sayfanın sağında, solunda, altında ve üstünde 3'er cm boşluk bırakılmalıdır. Makalenin her sayfası ve satırları numaralandırılmalıdır. Yazar ad(lar)ı açık olarak yazılmalı ve herhangi bir akademik unvan belirtilmemelidir. Editörler kurulu, anlatım dili yeterli olmayan makaleleri değerlendirme dışı tutabilir. Yazar(lar)ın makale göndermeden önce eseri dil yönünden bir dil bilimciye incelettirmesi tavsiye olunur. Sıralama olarak, İngilizce özet ve peşinden Türkçe özet verilir. Bu durum şekil ve çizelge başlıkları için de geçerlidir.

Makale; Türkçe Başlık, Türkçe Özet, Anahtar Kelimeler, İngilizce Başlık, İngilizce Özet, Keywords, 1.Giriş, 2.Materyal ve Yöntem, 3.Bulgular ve Tartışma, 4.Sonuçlar, Teşekkür (varsa), Kısaltmalar ve/veya Semboller (varsa), Kaynaklar bölümleri ile Şekil ve Çizelgelerden oluşmalıdır. Bölüm adları koyu yazılmalıdır.

Makale, “Kaynaklar” bölümü dâhil 16 sayfayı geçmemelidir. Yazar(lar), bu kısımların oluşturulmasında derginin web sayfasındaki **Makale Hazırlama Şablonunu** kullanmalıdır.

**Başlık:** Kısa ve açıklayıcı olmalı, 14 punto ve koyu, kelimelerin ilk harfi büyük olmalı, ortalanarak yazılmalı ve 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 13 punto ve koyu yazılmalıdır.

**Özet ve Anahtar Kelimeler:** Türkçe ve İngilizce özetlerin her biri 300 kelimeyi geçmemelidir. Türkçe ve İngilizce özetlerde sırasıyla “Özet” ve “Abstract” kelimeleri kullanılmalıdır. Özet, çalışmanın amacını, nasıl yapıldığını, sonuçları ve sonuçlar üzerine yazar(lar)ın yaptığı değerlendirmeleri içermelidir. Özetlerin 1 satır altına, her anahtar kelimenin ilk harfi büyük diğerleri küçük harflerle, mümkünse başlıkta kullanılmayan, çalışmayı en iyi biçimde tanımlayacak ve aralarında noktalı virgül (;) olacak şekilde en fazla 6 anahtar kelime yazılmalıdır.

**1. Giriş:** Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilir.

**2. Materyal ve Yöntem:** Kullanılan materyal ve yöntem aynı başlıkta verilmelidir. Alt başlık verilecekse bölüm numarası ile birlikte numaralandırılmalı (2.1. gibi) ve italik yazılmalıdır. Yeni veya değiştirilmiş yöntemler, aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte açıklanmalıdır.

**3. Bulgular ve Tartışma:** Elde edilen bulgular verilmeli, gerekirse çizelge, şekil ve grafiklerle desteklenerek bulgular açıklanmalıdır. Elde edilen bulgular tekrardan kaçınılması amacıyla ya çizelge ya da grafik olarak verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. İstatistikî analiz yönteminin doğru seçilmediği ve/ya analizin gereği gibi yapılmadığı durumlarda editörler kurulu makaleyi değerlendirme dışında tutabilir. Bulgular tartışılmalı ancak gereksiz tekrarlardan kaçınılmalıdır. Bulguların başka araştırmalarla benzerlik ve farklılıkları verilmeli, nedenleri açıklanmalıdır.

**4. Sonuçlar:** Elde edilen sonuçlar, bilime ve uygulamaya katkısıyla birlikte kısa ve öz olarak verilmelidir. Giriş ile Bulgular ve Tartışma bölümünde verilen ifadeler bu kısımda aynı şekilde tekrar edilmemelidir.

**Teşekkür:** Gerekli ise mümkün olduğunca kısa olmalı ve yapılan katkı ifade edilerek verilmelidir.

**Kısaltmalar ve/veya Semboller:** Makalede kısaltmalardan mümkün olduğunca kaçınılmalıdır. Semboller Makale Hazırlama Şablonunda belirtildiği gibi verilmelidir. Kısaltma ve semboller metin içinde ilk kez kullanıldığı yerde açıklanmalıdır. Uluslararası geçerliliği olan ve yerleşik kısaltmalar tercih edilmelidir. Kısaltmalar makalenin başlığında kullanılmamalıdır. Semboller SI sistemine göre verilmelidir.

**Kaynaklar:** Eserde yararlanılan kaynaklara ilişkin atıf metin içinde “(Yazarın soyadı yılı)” yöntemine göre yapılmalıdır. Örnek: (Doymaz 2003), (Basunia & Abe 2001). Yazara atıf yapılırsa sadece yayının yılı parantez içine alınmalıdır. Örnek: Doymaz (2003)’e göre ya da Basunia & Abe (2001). Üç ya da daha fazla yazar için makale içindeki atıfta “et al” kullanılmalıdır. Örnek: (Lawrence et al 2001) veya Lawrence et al (2001)’e göre. Aynı yazarın aynı yıl içinde 1’den fazla yayını varsa, yıldan sonra küçük harfler verilmelidir. Örnek: (Akpınar et al 2003a). Aynı yazarın birden fazla yayınına atıf yapılacaksa yıldan sonra noktalı virgül (;) işareti ile ayırt edilmelidir. Örnek: (Akpınar 2007; 2009; 2013). Birden fazla atıf yapılırsa atıflar arasında noktalı virgül (;) kullanılmalı ve eskiden yeniye doğru yıl sırasına göre verilmelidir. Örnek: (Perl et al 1987; Bailly et al 1996; Copeland & McDonald 2001; Goel & Sheoran 2003). Eğer bilginin, kaynağın belirli bir sayfasından ya da sayfalarından alındığı belirtilmek istenirse (Hardeman & Jochemsen 2012, s 657-674; Naess 1991, s 34) biçiminde gösterilmelidir. Kaynaklarda Anonim ya da Anonymous şeklinde gösterim yapılmamalıdır.

Kaynaklar bölümünde metin içinde atıf yapılan tüm kaynaklar alfabetik olarak (yazarların soyadlarına göre) ve orijinal dilinde verilir. Aynı yazara birden çok atıf yapılıyorsa önce tek isim, sonra iki isim ve sonra da üç ve daha fazla yazarlı kaynak sırasına göre hepsi kendi içinde eskiden yeniye yıl sırasına göre verilmelidir. İki veya daha fazla yazarlı eserlerin bildiriminde son yazardan önce “&” kullanılmalıdır. Örnek: Lawrence K C, Funk D B & Windham W R (2001). Dergi isimleri kısaltma yapılmadan tam adı ile ve italik yazılmalıdır. Kongre kitaplarında Türkçe ya da yabancı dilde özeti yayınlanmış çalışmalara atıf yapılamaz. Makaledeki yanlış atıf ve kaynak gösterimlerine ait sorumluluk yazar(lar)a aittir. Kaynaklar bölümündeki her bir kaynağın sonuna nokta (.) konmamalıdır.

#### **Dergi:**

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpınar E, Midilli A & Bicer Y (2003a). Single layer drying behaviour of potato slices in a convective cyclone dryer and mathematical modelling. *Energy Conversion and Management* **44**(10): 1689-1705



**Kitap:**

Yıldırım O (1996). Bahçe Bitkileri Sulama Tekniği. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1438, Ders Kitabı: 420, Ankara  
Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

**Kitapta Bölüm:**

Fıratlı Ç (1993). Arı yetiştirme. (Ed: M Ertuğrul), *Hayvan Yetiştirme*, Baran Ofset, Ankara, s. 30-34  
Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

**Yazarı Belirtilmeyen Kurum Yayınları:**

TÜİK (2012). Tarım İstatistikleri Özeti. Türkiye İstatistik Kurumu, Yayın No: 3877, Ankara  
ASAE (2002). Standards S352.2, 2002, Moisture measurement-unground grain and seeds. ASAE, St. Joseph, MI

**İnternette Alınan Bilgi:**

FAO (2013). Classifications and standards. <http://www.fao.org/economic/ess/ess-standards/en/> (Erişim tarihi:10.02.2013)

**Tez:**

Koyuncu T (1992). Tarım arabalarında kullanılan çarpma etkili frenlerin araştırılması. Yüksek lisans tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Ankara  
Berbert PA (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Cranfield University (Unpublished), UK

**Tam Metin Kongre/Sempozyum Kitabı:**

Yağcıoğlu A, Değirmencioğlu A & Çağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, Turkey, pp. 565–569  
Kara Z & Beyoğlu N (1995). Konya ili Beyşehir yöresinde yetiştirilen üzüm çeşitlerinin göz verimliliklerinin belirlenmesi üzerine bir araştırma. *Türkiye II. Ulusal Bahçe Bitkileri Kongresi. Bildiriler (II)*: 3-6 Ekim, Adana, s. 524-528

**Şekiller ve Çizelgeler:** Şekil, grafik, fotoğraf ve benzerleri “Şekil”, sayısal değerler ise “Çizelge” olarak belirtilmelidir. Tüm şekil ve çizelgeler makalenin sonuna yerleştirilmelidir. Şekil ve çizelgelerin boyu tek sayfa düzeninde en fazla 16x20 cm ve çift sütun düzeninde ise genişliği en fazla 8 cm olmalıdır. Şekil ve çizelgelerin boyutu baskıda çıkabilecek çözünürlükte olmalıdır. Araştırma sonuçlarını destekleyici nitelikteki resimler 600 dpi çözünürlüğünde ”jpg” formatında olmalıdır. Renkli resimler yerine gri ya da siyah tonlu resimler tercih edilmelidir. Çizelgelerde düşey çizgi kullanılmamalı ve makale hazırlama şablonunda belirtildiği gibi hazırlanmalıdır. Her çizelge ve şekle metin içerisinde atıf yapılmalıdır. Tüm çizelge ve şekiller makale boyunca sırayla numaralandırılmalıdır (Çizelge 1 ve Şekil 1). Çizelge ve şekil başlıkları ve açıklamaları kısa ve öz olmalıdır. Çizelge ve şekillerin İngilizce başlıkları, Türkçe başlığın hemen altına italik olarak yazılmalı, ilk yazılan Türkçe başlık yazısı koyu olmalıdır. Şekil ve çizelge başlık yazıları 9.5 punto, şekil ve çizelgelerin içindeki yazılar 9 punto, çizelge altı yazılar 8 punto Times New Roman yazı karakterinde olmalıdır. Şekillerde yatay ve düşey kılavuz çizgiler ve rakamlar bulunmamalıdır. Ancak istatistiksel karşılaştırmalar yapıyorsa küçük harfler bulunabilir. Çizelge ve şekillerde kısaltmalar kullanılmış ise hemen altına bu kısaltmalar açıklanmalıdır. Şekil ve çizelge başlıkları ile çizelge altı yazılarının sonuna nokta (.) konmamalıdır.

**Birimler:** Tüm makalelerde SI (Système International d’Units) ölçüm birimleri kullanılmalıdır. Ondalık kesir olarak nokta kullanılmalıdır ( 1,25 yerine 1.25 gibi). Birimlerde “/” kullanılmamalı ve birimler arasında bir boşluk verilmelidir (m/s yerine m s<sup>-1</sup>, J/s yerine J s<sup>-1</sup>, kg m/s<sup>2</sup> yerine kg m s<sup>-2</sup> gibi). Sayı ile sembol arasında bir boşluk bırakılmalıdır ( 4 kg N ha<sup>-1</sup>, 3 kg m<sup>-1</sup> s<sup>-2</sup>, 20 N m, , 1000 s<sup>-1</sup>, 100 kPa, 22 °C ve % 29 gibi). Bu kuralın istisnaları düzlemsel açılar için kullanılan derece, dakika ve saniye sembolleridir (°, ’ ve ”). Bunlar sayıdan hemen sonra konmalıdır (10°, 45’, 60”) gibi). Litrenin kısaltması “l” değil “L” olarak belirtilmelidir. Cümle sonunda değilse sembollerin sonuna nokta konulmamalıdır (kg. değil kg).

**Formüller ve Eşitlikler:** Formüller numaralandırılmalı ve formül numarası formülün yanına sağa dayalı olarak parantez içinde gösterilmelidir. Formüllerin yazılmasında Word programı matematik işlemcisi kullanılmalı, ana karakterler 12 punto, değişkenler italik, rakamlar ve matematiksel ifadeler düz olarak verilmelidir. Metin içerisinde atıf yapılacaksa “Eşitlik 1” biçiminde verilmelidir (...ilişkin model, Eşitlik 1’ de verilmiştir).

## JOURNAL OF AGRICULTURAL SCIENCES

### Guide for Authors

**Journal of Agricultural Sciences is abstracted and/or indexed in:** Science Citation Index – Expanded, TUBITAK-ULAKBIM, CAB Abstracts, CAB International, FAO AGRIS/CARIS, and Directory of Open Access Journals (DOAJ).

*Journal of Agricultural Sciences (JAS)* is an international, double-blind peer-reviewed, open-access journal, published by the Faculty of Agriculture, Ankara University. The journal invites original research papers containing new insight into any aspect of Agricultural Sciences that are not published or not being considered for publication elsewhere. Preliminary, confirmatory or inconclusive research, review articles, case and local studies and works presenting taxonomy will not be published.

Before preparing papers for journal, authors should read through **Guide for Authors** and consult a current issue to make themselves familiar with general format.

The journal uses double-blind system for peer-review; both reviewers and authors' identities remain anonymous. The paper will be peer-reviewed by two reviewers from outside and one editor from the journal typically involve in reviewing a submission. Please note that authors are required to pay \$ 150 for each manuscript published.

### Manuscript Submission

Manuscripts should be submitted to journal's online submission system by the corresponding author. All submissions should include following documents:

- ✓ **Title page** with author names, titles, addresses and contact information (in Word format).
- ✓ **Manuscript** (in PDF and Word format version 2003 or above). All author names, titles and affiliations should be excluded.
- ✓ **Transfer of Copyright Form**. This form should be filled and signed by all authors and sent electronically as a scanned copy. Authors of the accepted papers should send the original version of this form.
- ✓ **Submission Check List** (in PDF format).
- ✓ **Ethics Committee Approval** (if needed).

Papers should be written with fluent English without any grammatical and typographical errors. Manuscripts with any of those errors will be rejected and sent to the authors for corrections before submission and review.

Manuscripts should be typed using Times New Roman font 12 pt. with numbered lines, in the left-hand margin and double spacing throughout, i.e. also for abstracts, footnotes and references. The pages of the manuscript, including the title page, abstract, references, tables, etc. should be numbered consecutively. Make the width at 3 cm for all margins. Place tables and figures with captions after the text. Each figure and table should be referred to in the text. Avoid excessive use of italics to emphasize part of the text.

Manuscripts should include the following sections; **Title** (short, specific and informative), **Keywords** (indexing terms, up to 6 items), **1. Introduction, 2. Material and Methods, 3. Results and Discussion, 4. Conclusions, Acknowledgements** (if needed), **Abbreviations and Symbols** (if needed), **References, Figures and Tables** with captions not exceeding 16 pages (with references). All headings and titles should be written in Bold.

### Acknowledgements

Acknowledgements should be a brief statement at the end of the text and may include source of financial support. The contract number should be provided.

### References

Cite references in the text as author's family name should be followed by the year of the publication in parentheses (Peter 2010; Basunia & Abe 2001). Use et al after the first author's family name for citations with three or more authors (Lawrence et al 2001). For citations of the same authors published on the same year, use letters after the year (Dawson 2009a).

References cited in the text should be arranged chronologically. The references should be listed alphabetically on author's surnames, and chronological per author. Names of journals should be in full titles rather than the abbreviations. Avoid using citations of abstract proceedings. The following examples are for guidance.

### Journal Articles

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* 44(6): 1691-1696

Akpinar E, Midilli A & Biçer Y (2003a). Single layer drying behavior of potato slices in a convective cyclone dryer and mathematical modeling. *Energy Conversion and Management* 44(10): 1689-1705

### **Books**

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

### **Book Chapter**

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds.), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

### **Publications of Institutions / Standard Books**

ASAE (2002). Standards S352.2, 2002, Moisture measurement - unground grain and seeds. ASAE, St. Joseph, MI

### **Internet Sources**

FAO (2013). Classifications and standards. Retrieved in April, 12, 2011 from <http://www.fao.org/economic/ess/ess-standards/en/>

### **Thesis and Dissertations**

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Cranfield University (Unpublished), UK

### **Conference Proceedings (Full papers)**

Yağcıoğlu A, Değirmencioğlu A & Çağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, pp. 565–569

### **Tables and Figures**

Tables and Figures should be numbered consecutively and accompanied by a title at the top. All tables and figures should not exceed 16x20 cm size. Figures should have high resolution, minimum 600dpi in jpg format. For publication purposes use grayscale images. Avoid using vertical lines in tables.

### **Illustrations**

Do not use figures that duplicate matter in tables. Figures can be supplied in digital format, or photographs and drawings, which can be suitable for reproduction. Label each figure with figure number consecutively.

### **Units**

Units of measurement should all be in SI units. Use a period in decimal fractions (1.24 rather than 1,24). Avoid using “/”. Include a space between the units (m s<sup>-1</sup> rather than m/s, J s<sup>-1</sup> rather than J/s, kg m s<sup>-2</sup> rather than kg m/s<sup>2</sup>). Units should have a single space between the number and the unit (4 kg N ha<sup>-1</sup>, 3 kg m<sup>-1</sup> s<sup>-2</sup>, 20 N m, 1000 s<sup>-1</sup>, 100 kPa, 22 °C). The only exceptions are for angular definitions, minutes, seconds and percentage; do not include a space (10°, 45», 60», 29%). The abbreviation of liter is “L”.

### **Formulas and Equations**

Number each formula with the reference number placed in parentheses at the end. Use Word mathematical processor for formulas with 12pt., variances in Italics, numbers and mathematical definitions in plain text. If needed, refer as “Equation 1” in the text (...the model, as given in Equation 1).

**Note: Title and Abstract, Tables and Figures Captions are translated into Turkish by editor for authors whose native language is not Turkish.**

