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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Ayçiçeği Küspesinin Katalizörlü ve Katalizörsüz Koşullarda Hızlı Pirolizinin Ürün Verimleri ve Özelliklerine Etkisi

The Effect of Fast Pyrolysis of Sunflower Oil Cake on Product Yields and Properties under Catalyst and Non-Catalyst Conditions

Sabriye SARIOĞLU[1], Türkan AKTAŞ[2*]

Öz

Bu çalışma kapsamında ayçiçeği küspesinin hızlı pirolizinin katalizörsüz koşulda ve klinoptilolit katalizörü kullanılarak yapılmasının, elde edilen ürün verimleri ve ürün özellikleri üzerine etkileri araştırılmıştır. Araştırma kapsamında hammadde olarak kullanılan ayçiçeği küspesinin nem içeriği %5.92, kül içeriği %6.08, uçucu madde yüzdesi %71.30 ve sabit karbon yüzdesi %16.70 olarak belirlenmiştir. Elemental analiz sonuçlarına göre C, H, N ve S yüzdeleri sırasıyla %42.06, %6.26, %6.93, %0.00 olarak saptanmıştır. Isıl değeri ise 17.13 MJ/kg olarak hesaplanmıştır. Araştırma kapsamında ilk olarak, ısıtma hızı (100, 200, 300 ℃/dk), piroliz sıcaklığı (400, 500, 600℃) ve katalizör yüzdelerinin (%5, %10, %15) ürün verimleri (biyokömür, biyoyağ ve piroliz gazı) üzerine etkileri incelenmiştir. Ayçiçeği küspesinin hızlı piroliz işleminde klinoptilolit katalizörü eklenmesiyle yapılan denemelerde, katalizörün katı ürün verimini önemli düzeyde değiştirmediği, sıvı ürün verimini oldukça düşürdüğü ve gaz ürün verimini artırdığı belirlenmiştir. Biyokömür örneklerinde en yüksek üst ısıl değer, katalizörsüz koşulda 500 ℃ sıcaklık ve 300 ℃/dk ısınma hızında 22.95 MJ/kg olarak elde edilmiştir. Biyokömür örneklerinin mikroskobik yapısı incelendiğinde (SEM analizi) hammaddeye göre gözenekliliğin arttığı anlaşılmıştır. Katalizörsüz koşulda elde edilmiş olan piroliz gazı örneklerinin alt ısıl değerlerinin katalizörlü koşulda elde edilen örneklerinkine göre daha yüksek olduğu belirlenmiştir. Elde edilen gaz örneklerinde en yüksek alt ısıl değer 400 ℃ sıcaklıkta, 100 ℃/dk ısınma hızında 29.05 MJ/Nm³ olarak elde edilmiştir. Ayçiçeği küspesinin farklı sıcaklıklarda, farklı ısıtma hızlarında katalizörlü ve katalizörsüz koşulda pirolizi ile elde edilmiş olan biyoyağ örnekleri GC-MS yöntemiyle incelenmiş, özellikle fenol bileşiklerinin (Phenol, Phenol, 2-methoxy-) hemen her numunede olduğu saptanmıştır. Ayrıca Pyrazine-methyl, Pyrazine 2,6-dimethyl- (CAS) 2,6-Dimethylpyrazine gibi aromatik bileşiklerde tespit edilmiştir.

Anahtar Kelimeler: Ayçiçeği küspesi, Piroliz, Biyokömür, Biyoyağ, Piroliz gazı, Katalizörlü piroliz

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Abstract

Within the scope of this study, the effects of fast pyrolysis of sunflower oil cake on obtained product yields and product properties were investigated under non-catalytic condition and using clinoptilolite catalyst. The percentages of moisture, ash, volatile matter and fixed carbon content of sunflower oil cake used as raw material within the scope of the research were determined as 5.92%, 6.08%, 71.30% and 16.70%, respectively. According to the its elemental analysis results, the percentages of C, H, N and S were determined as 42.06%, 6.26%, 6.93%, 0.00%, respectively. The heating value was calculated as 17.13 MJ/kg. For this aim firstly, the effects of heating rate (100, 200, 300℃/min), pyrolysis temperature (400, 500, 600℃) and catalyst percentages (5%, 10%, 15%) on product yields (biochar, biooil and pyrolysis gas) were investigated. In the experiments conducted with the addition of clinoptilolite catalyst during fast pyrolysis of sunflower oil cake, it was determined that the catalyst did not change the solid product yield much, it considerably reduced the liquid product yield and increased the gas product yield. In the biochar samples, the highest heating value was obtained as 22.95 MJ/kg at 300°C/min heating rate and 500℃ pyrolysis temperature conditions without catalyst. When the microscopic structure (SEM analysis) of the biochar samples was examined, it was understood that the porosity increased compared to the raw material. It was determined that the heating values of the pyrolysis gas samples obtained under the non catalyst-free condition were higher than those of the samples obtained under the non-catalytic condition. The highest lower heating value for obtained gas samples was obtained as 29.05 MJ/Nm³ at 400°C temperature and 100°C/min heating rate conditions Bio oil samples obtained by pyrolysis of sunflower oil cake at different temperatures and different heating rate with and without catalyst were examined by GC-MS method, and it was determined that especially phenol compounds (Phenol, Phenol, 2-methoxy-) were found in almost every sample. In addition, aromatic compounds such as Pyrazine-methyl, Pyrazine 2,6-dimethyl-(CAS) 2,6-Dimethylpyrazine have also been detected.

Keywords: Sunflower oil cake, Pyrolysis, Biochar, Biooil, Pyrolysis gas, Catalyzed Pyrolysis

1. Giriş

Dünya genelinde artan nüfus ve gelişen teknoloji enerji tüketimindeki artışı tetiklemekte ve enerji giderek daha önemli bir konu haline gelmektedir. Bu durumu fosil yakıtların hızla tükeniyor olması ve çevreye verdiği yıkıcı zarar daha da kötü yönde etkilemektedir. Son yıllarda bu gibi durumlar gündeme gelmiş ve yenilenebilir enerji kaynaklarının kullanımı büyük önem kazanmıştır (Kapluhan, 2014).

Dünyada ve Türkiye'de en önemli yenilenebilir enerji kaynaklarından biri olan biyokütle kaynaklarının kolay erişilebilir olmaları ve ekonomik potansiyelleri nedeniyle kullanımları gittikçe daha büyük önem kazanmaktadır. Piroliz yöntemi biyokütlenin dönüştürülmesi için kullanılan en uygun yöntemlerden birisidir. Oksijensiz ortamda ve yüksek sıcaklıklarda organik maddenin termokimyasal bozunması olarak da tanımlanabilecek piroliz işlemi sırasında kimyasal kompozisyon ve fiziksel faz aynı anda ve geri dönülmez şekilde değişmektedir. Diken ve Kayişoğlu (2020) çeşitli biyokütle örnekleri için piroliz sıcaklığının 250-350 °C sıcaklık aralığında olduğunu bildirmişlerdir. Piroliz yöntemi, biyokütle hammaddelerinden çok çeşitli yakıtlar, çözücüler, kimyasallar ve diğer ürünlerin ticari üretimi için kullanılmaktadır (Yaman, 2004).

Hızlı piroliz işlemi ile elde edilen ürünler, katalizör kullanılarak ikincil ürünlere daha rahat dönüştürülebilmektedir. Katkı maddeleri veya doğal kül içeriği olan inorganik maddelerin varlığı biyokütlenin pirolizini oldukça etkilemektedir; alkali bileşikler ve asitli reaktifler ile etkisi daha fazladır (Öztürk Tophanecioğlu, 2009). Doğal katalizörler kullanılarak yüksek verimde kimyasal ürünler elde edilmiştir. Doğal katalizörlerin kullanılmasıyla elde edilen yüksek verimin yanı sıra ortamdan uzaklaştırılması ürün verimini ve bileşimi olumsuz olarak etkilemiştir. Katalizör olarak genelde zeolit katalizörler kullanılmaktadır (analcite, halloysit, natrolit, klinoptiloit, ZSM-5, şabazit gibi katalizörler). Bu katalizörlerin varlığında piroliz buharlarının katalitik olarak parçalanmasıyla, benzin ve dizel yakıt kaynama aralığında aromatik ve diğer hidrokarbon ürünleri elde edilebilmiştir (Bridgwater, 1996, aktaran Güzelçiftçi, 2016).

Ayçiçeği küspesi farklı pres yöntemleri ile (hidrolik pres, devamlı pres vs.) veya solvent ekstraksiyon yöntemiyle bütün ayçiçeği tohumlarından yağın çıkarılmasından sonra geriye kalan ürünün öğütülmesiyle elde edilen yağ fabrikası artığıdır. Günümüzde yem sanayinin en önemli ürününü ayçiçeği küspesi oluşturmaktadır. 2018 Yılı Ayçiçeği Raporu'na göre, bitkisel yağ üretimimizin %69'u, toplam sıvı yağ tüketimimizin yaklaşık %84'ü, toplam yağ kullanımının ise %32'si ayçiçeğinden karşılanmaktadır (Anonim, 2022a). Özellikle Trakya Bölgesi'ni oluşturan beş il (Edirne, Kırklareli, Tekirdağ, Çanakkale ve İstanbul illeri) Türkiye'de ayçiçeği ekim alanlarının %59,51'ini, üretim miktarının ise %62,04'ünü oluşturmaktadır (Semerci, 2012). Ayçiçeğinin yağa işlenmesi sonucunda yaklaşık %40-45 oranında küspe elde edilmesi bu fabrika artığının oldukça büyük bir potansiyele sahip olduğunu ve yem sanayinde kullanımının yanı sıra sürdürülebilir bir enerji kaynağı olarak değerlendirilebileceğini de göstermektedir.

Bu çalışmanın temel amacı ülkemizde ayçiçeği yağı üretim tesislerinin yan ürünü olan ayçiçeği posasının yaygın termokimyasal dönüşüm yöntemlerinden birisi olan piroliz yöntemi ile değerlendirilmesidir. Bu amaçla; ilk olarak farklı sıcaklık ve ısıtma hızı koşullarında katalizör kullanılarak ve katalizör kullanılmadan yapılan piroliz işlemlerinde bu değişkenlerin ürün verimleri üzerine etkileri incelenmiştir. İkincil olarak; elde edilen hızlı piroliz ürünlerinin bazı kalite özelliklerinin belirlenmesi ve karşılaştırılması yapılmıştır. Bu kapsamda; kullanılan ayçiçeği posasının özelliklerinin yanı sıra piroliz işlemleri sonucunda elde edilen biyokömür örneklerinin kısa analizleri (proksimit) ve elemental analizleri gerçekleştirilerek, ısıl değerleri, kimyasal bileşenleri ve mikroskopik yapıları incelenmiştir. Biyoyağ örneklerinin ise kimyasal bileşenleri belirlenerek karşılaştırılmıştır. Piroliz gazı örneklerinin bileşimleri analiz edilerek ısıl değerleri hesaplanmıştır.

2. Materyal ve Metot

Piroliz denemelerinde biyokütle materyali olarak Trakya bölgesinde yetiştirilen ayçiçeğinin yağa işlenmesi sonucu elde edilmiş olan yağ fabrikası artığı olan ayçiçeği posası (küspesi) kullanılmıştır. Katalizör olarak, doğal zeolit olan klinoptilolit kullanılmıştır.

Katalizörlü ve katalizörsüz koşullarda piroliz denemelerinin yapılması için kullanılan ince borusal sabit yataklı hızlı piroliz sisteminin şematik görünümü ve sistemi oluşturan parçalar *Şekil 1*' de görülmektedir. Hızlı piroliz reaktörü, 800 °C sıcaklıkta ve 50 bar maksimum çalışma basıncında çalışabilmektedir. Ünitede ısıtma hızı

dakikada 800 °C sıcaklık artışı düzeyine kadar ayarlanabilmektedir. Süpürücü gaz olarak azot gazı kullanılmıştır ve kullanılan gazın debisi azot tüpüne bağlanan bir akış ölçer kullanılarak ayarlanmıştır.

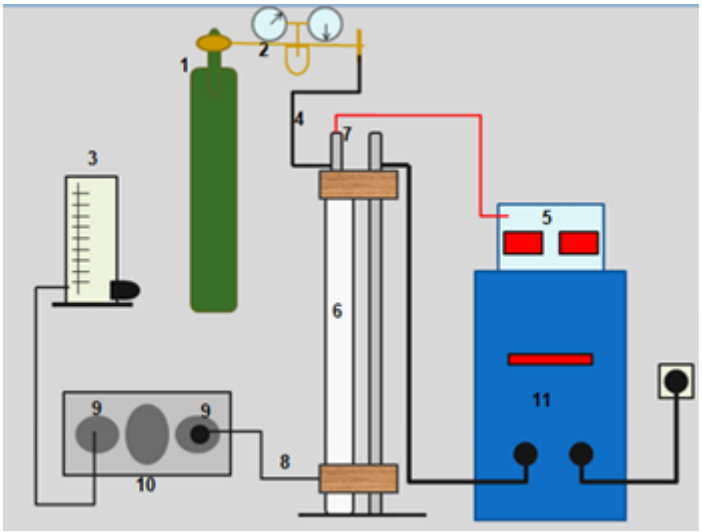

Figure 1. Fast pyrolysis system and its components (1: Nitrogen tube, 2 and 4: Nitrogen gas flow line, 3: Flowmeter, 5: Control unit, 6: Reactor, 7: Termocouple, 8: Pyrolysis gas flow line, 9: Liquid collection containers, 10: Condensing (cooling) unit, 11: Power supply)

Şekil 1. Hızlı piroliz ünitesi ve parçaları (1: Azot tüpü, 2 ve 4: Azot gaz akış hattı, 3: Akışölçer, 5: Kontrol ünitesi, 6: Reaktör, 7: Termokupl, 8: Piroliz gazı akış hattı, 9: Sıvı toplama kapları, 10: Yoğunlaştırma (soğutma) ünitesi, 11: Güç kaynağı)

Piroliz denemeleri hem katalizörlü hemde katalizörsüz olarak 3 farklı piroliz sıcaklığında (400, 500 ve 600 °C) ve 3 farklı ısıtma hızında (200, 400 ve 600°C/dk) gerçekleştirilmiştir (*Tablo 1*). Katalizör kullanılan piroliz denemelerinde 3 farklı katalizör yüzdesi denenmiştir (%5, %10 ve %15). Sürükleyici gaz olarak azot gazının debisi tüm deneme koşullarında 100 cm^3/dk olarak ayarlanmıştır. Yapılan ön denemelerde gaz çıkış süresinin 10 dakika süresince devam ettiği gözlenmiş ve bu sebeple piroliz sıcaklığında bekletme süresi 10 dakika olarak belirlenmiştir.

Tablo 1. Piroliz denemelerinde uygulanan değişkenler

Table 1. Variables applied in pyrolysis experiments.

	Sıcaklık (°C)- Isınma Hızı (°C/dk)	Deneme Kodları	Katalizör oranı (%)
Katalizörsüz Koşul	400-100	NK 1.1	0
	400-200	NK 1.2	0
	400-300	NK 1.3	0
	500-100	NK 1.4	0
	500-200	NK 1.5	0
	500-300	NK 1.6	0
	600-100	NK 1.7	0
	600-200	NK 1.8	0
	600-300	NK 1.9	0
Katalizörlü Koşul	400-100	K 2.1	5
	400-200	K 2.2	10
	400-300	K 2.3	15
	500-100	K 2.4	5
	500-200	K 2.5	10
	500-300	K 2.6	15
	600-100	K 2.7	5
	600-200	K 2.8	10
	600-300	K 2.9	15

Piroliz deneylerinde katı, sıvı ve gaz ürün verimleri aşağıda verilmiş olan eşitlikler (Eş. 1, 2 ve 3) kullanılarak hesaplanmıştır (Erdoğdu, 2018).

$$\text{Katı Ürün Verimi} = \frac{\text{Katı Ürün Miktarı (g)}}{\text{Beslenen Biyokütle (g)}} \times 100 \quad \text{(Eş.1)}$$

$$\text{Sıvı Ürün Verimi (\%)} = \frac{\text{Sıvı Ürün Miktarı (g)}}{\text{Beslenen Biyokütle (g)}} \times 100 \quad \text{(Eş.2)}$$

$$\text{Piroliz Gazı Verimi}(\%) = 100 - (\text{Katı ürün Verimi} + \text{Sıvı ürün Verimi} \quad \text{(Eş.3)}$$

Kısa analizler kapsamında hammadde ve biyokömür örneklerinin nem, kül, sabit karbon ve uçucu madde yüzdeleri belirlenmiştir. Nem içeriğinin saptanması için yaklaşık 5g örnek 3 tekerrürlü olarak tartılmıştır. Örnek kapları tartılarak, daraları kaydedilmiştir. Örnekler sabit ağırlığa gelinceye kadar (yaklaşık 24 h) 105±2 °C sıcaklıkta kurutma fırınında kurutulmuş, daha sonra kurutma fırınından alınan örnekler desikatörde bekletilip tekrar tartılarak ağırlıkları kaydedilmiş ve eşitlik 4 yardımı ile örneklerin nem içerikleri (% y.b.) hesaplanmıştır. Nem içeriği kurutmadan önceki ve kurutmadan sonraki örnek ağırlığına bağlı olarak aşağıdaki bağıntı ile hesaplanmıştır (Öztop ve Aktaş, 2012).

$$N_i = \frac{M_{top} - M_{kuru}}{M_{top}} x100 \quad \text{(Eş.4)}$$

Burada; N_i: Posa örneğinin nem İçeriği (%y.b.), M_{top}: Posa örneğinin başlangıçtaki kütlesi (su+kuru madde, g), M_{kuru}: Posa örneğinin kuru kütlesidir (g).

Kül içerikleri, EN14775 standardına uygun şekilde belirlenmiştir. Porselen krozeler 575±25 °C' de kül fırınında 4 saat bekletilmiştir. Daha sonra desikatöre alınmış, soğutulmuş ve tartım yapılmıştır. Tekrar kül fırınına yerleştirilip, sabit ağırlığa gelmesi beklenmiştir. Porselen krozeler sabit ağırlığa ulaşınca 0,5-2 g örnek (etüvde kurutulmuş) tartılmış ve fırına yerleştirilmiştir. Fırın sıcaklığı standarda uygun şekilde aşağıda belirtilen artış aralığı ile yükseltilmiştir ve eşitlik 5 yardımıyla kül içeriği hesaplanmıştır.

$$\%\text{Kül} = \frac{\text{Ağırlık}_{\text{Kroze+Kül}} - \text{Ağırlık}_{\text{Kroze}}}{\text{Ağırlık}_{\text{Kuru örnek}}} X100 \quad \text{(Eş.5)}$$

Posa ve biyokömür örneklerinin uçucu madde ve sabit karbon yüzdelerini saptamak amacıyla örnekler 24 saat 100 °C' de kurutulmuştur. 0.8-1g ağırlığında hazırlanmış olan örnekler seramik kaplara koyularak 600±50 °C' de 6 dakika ve hemen arkasından 950±20 °C'de 6 dakika kül fırınında tutulmuştur. Bu ısıl uygulamalardan sonra elde edilen örnekler tartılmış ve uçucu madde miktarı ilk ve son örnek ağırlıklarının farkından hesaplanmıştır (Akçay ve Aktaş, 2014). Sabit karbon yüzdesi (%SK) ise kül (%K) ve uçucu madde yüzdesine (%UM) bağlı olarak aşağıda verilen eşitlik 6 ile hesaplanmıştır (Akçay ve Aktaş, 2014).

$$\%\text{SK} = 100 - (\%\text{K} + \%\text{UM}) \quad \text{(Eş.6)}$$

Oksijen içeriği ise karbon, hidrojen, azot, sülfür ve kül yüzdelerine bağlı olarak aşağıdaki eşitlik 7'den yararlanılarak hesaplanmıştır.

$$\%\text{O} = 100 - \%\text{C} - \%\text{H} - \%\text{N} - \%\text{S} - \%\text{K} \quad \text{(Eş.7)}$$

Burada; O: Oksijen içeriği (%), C: Karbon içeriği (%), H: Hidrojen içeriği (%), N: Azot içeriği (%), S: Sülfür içeriği (%), K: Kül içeriğidir (%).

Biyokütle kaynağı olarak kullanılan ayçiçeği posasının ve elde edilen biyokömürlerin üst ısıl değerleri (ÜID) elemental içeriklerine bağlı olarak aşağıda belirtilen eşitlik 8 kullanılarak, modifiye edilmiş Dulong formülüne göre hesaplanmıştır (Mohanty ve ark., 2013).

$$\text{Üst Isıl Değer } (\text{MJ/kg}^{-1}) = (0{,}335 * \%\text{C}) + (1{,}423 * \%\text{H}) - (0{,}154 * \%\text{O}) \quad \text{(Eş.8)}$$

Her denemede piroliz sıcaklığına çıkıldıktan hemen sonra yoğunlaştırma ünitesinden sonra eklenmiş olan gaz çıkış kısmından en az 3 tekerrürlü olarak gaz örnekleri alınmıştır. Analizler gerçekleşinceye kadarki süreçte gazın sızmasını önlemek için gaz örneğinin çekildiği şırıngalara streç film sarılmıştır. Piroliz gazının bileşiminde bulunan CO, H_2, CH_4, CO_2 ve N_2 gazlarının hacimsel yüzdeleri AGILANT 7890B marka gaz kromotografi cihazı (GC) kullanılarak analiz edilmiştir.

Elde edilen gazın alt ısıl değeri (LHV_g) üretilen gazın birim kütlesindeki H_2, CO ve CH_4 bileşenlerinin oranları ve enerji içerikleri dikkate alınarak aşağıda verilen eşitlik 9 ile hesaplanmıştır (Waldheim ve Nilsson 2001).

$$LHVg = 10{,}8 \cdot \%H_2 + 12{,}63 \cdot \%CO + 35{,}8 \cdot \%CH_4 \quad \text{(Eş.9)}$$

Burada; LHVg (MJ.Nm-3) sentez gazın alt ısıl değeridir. Bileşenlerin enerji değerleri 1 atm basınç ve 0°C sıcaklık için aşağıda verilmiştir (Waldheim ve Nilsson 2001).

H_2:10,8 MJ·Nm^{-3}, CH_4: 35,8 MJ·Nm^{-3}, CO: 12,63 MJ·Nm^{-3}

3. Araştırma Sonuçları ve Tartışma

3.1. Ürün verimlerine ilişkin sonuçlar

Piroliz sıcaklığının ve ısıtma hızının ürün verimine etkisini incelemek amacıyla hazırlanan posa örneklerinin 400, 500, 600 °C piroliz sıcaklığı ve 100, 200, 300 °C/dk ısıtma hızı koşullarında hızlı pirolizleri gerçekleştirilmiştir. Katalizör kullanılmadan yapılan piroliz işlemlerinden sonra elde edilmiş olan ortalama ürün verimleri *Şekil 2*'de verilmiştir. %5, %10 ve %15 oranlarında katalizör kullanılarak yapılan piroliz işlemlerinden sonra elde edilmiş olan ortalama ürün verimleri de *Şekil 3*'te verilmiştir.

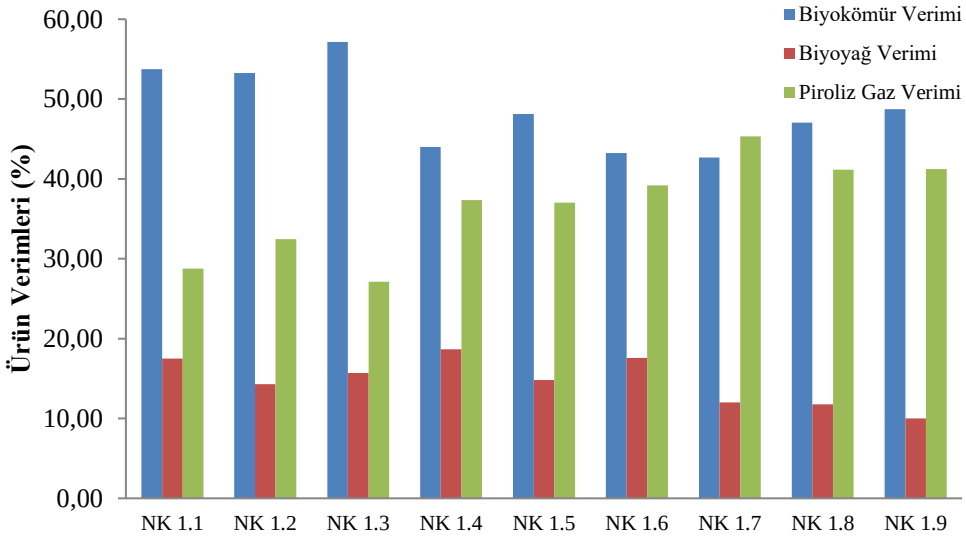

Figure 2. Pyrolysis product yields obtained in the non-catalyst condition

Şekil 2. Katalizörsüz koşulda elde edilen piroliz ürün verimleri

Şekil 2 incelendiğinde piroliz ürün verimlerinin piroliz sıcaklığından önemli ölçüde etkilendiği ve genel olarak, belli bir sıcaklığa kadar sıcaklık artışıyla sıvı verimlerinin arttığı fakat belli bir sıcaklıktan sonra sıvı veriminin düştüğü anlaşılmaktadır. Yorgun ve ark. (2001), farklı piroliz sıcaklıklarında azot atmosferi altında boru tip bir reaktör kullanarak ayçiçeği pres yağı posasının flaş piroliz deneylerini gerçekleştirmişler ve benzer sonuçlar elde etmişlerdir. Katalizörlü piroliz denemelerinde de katalizörsüz koşulla aynı sıcaklık ve ısınma hızları uygulanmıştır. Isınma hızlarına ek olarak katalizör yüzdeleri %5, %10, %15 olarak seçilmiştir. Farklı katalizör oranlarında elde edilen piroliz ürün verimleri *Şekil 3*'te verilmiştir.

Katalizörlü ve katalizörsüz piroliz işlemleri sonucunda elde edilen ürün verimleri karşılaştırıldığında elde edilen katı ürün yüzdelerinin her iki koşulda da sıcaklık artışı ile azaldığı fakat katalizör kullanımının az da olsa tüm koşullarda katı ürün verimini arttırdığı belirlenmiştir. En yüksek katı ürün verimi 400 °C sıcaklıkta, 100 °C/dk ısınma hızında ve %5 oranında katalizörle gerçekleştirilen işlem sonucunda %60 olarak elde edilirken, en düşük katı verimi 600 °C sıcaklıkta, 100 °C/dk ısınma hızında %42.67 olarak belirlenmiştir. Sıvı ürün yüzdeleri karşılaştırıldığında katalizör kullanımının her koşulda biyoyağ verimini azalttığı hem katalizörlü hem de katalizörsüz olarak yapılan denemeler sonucunda en yüksek sıvı verimlerinin 500 °C'de gerçekleştirilmiş olan piroliz işlemleri sonucunda, en düşük sıvı verimlerinin ise 600 °C'de gerçekleştirilmiş olan piroliz işlemleri sonucunda elde edildiği belirlenmiştir. İki koşul karşılaştırıldığında gaz verimlerinin katalizör kullanımı ile nispeten düştüğü saptanmıştır. Ürün verimleri açısından incelendiğinde genel olarak katalizör kullanımının büyük fark oluşturmadığı anlaşılmıştır. Pütün (2010) tarafından pamuk tohumunun pirolizine ilişkin çalışmada da farklı

piroliz sıcaklıklarında ve boru şeklindeki sabit yataklı bir reaktörde yapılan çalışmada katalizör ilavesinin biyo-yağ miktarının azalttığı ve katalizör miktarının arttırılması ile gaz ve kömür verimlerinin arttırdığı saptanmıştır.

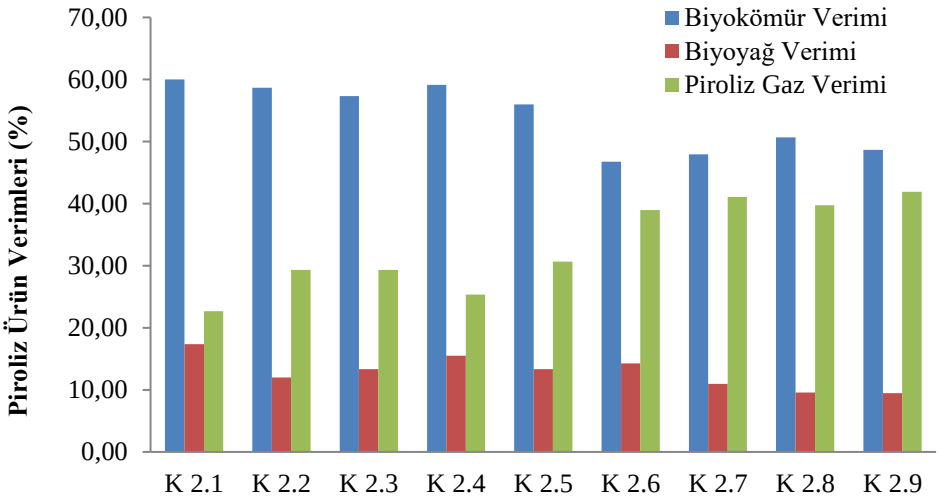

Figure 3. Pyrolysis product yields obtained under the catalyst condition

Şekil 3. Katalizörlü koşulda elde edilen piroliz ürün verimleri

3.2. Biyokömür örneklerinin kısa analizlerine ilişkin sonuçlar

Biyokömür örneklerinin nem değerleri incelendiğinde genel olarak piroliz edilmiş olan örneklerin yani biyokömürlerin (%1-2.5), hammadde nemine (%5.9) kıyasla çok daha düşük bir nem içeriğine sahip olduğu anlaşılmaktadır. Düşük sıcaklıkta (400 °C) katalizörsüz olarak gerçekleştirilen piroliz işlemi sonucunda elde edilmiş olan biyokömürlerin nem içeriklerinin daha yüksek olması genel olarak biyokütlelerde düşük sıcaklıklarda yapılan piroliz işleminde (500 °C'nin altında), biyokütlenin bileşenlerinden birisi olan ligninin hidrofobik bir polisiklik aromatik hidrokarbona (PAH) dönüşmemesi ve biyokömürün daha hidrofilik (suyu seven moleküler özellik) hale gelmesi ile açıklanabilir (Ghani ve ark., 2013). Öte yandan yüksek sıcaklıklarda elde edilen biyokömürler termal olarak daha kararlıdırlar ve daha hidrofobik (sudan kaça moleküler özellik) hale gelmektedirler. Bu açıdan incelendiğinde 400 °C piroliz sıcaklığında katalizörlü koşulda elde edilmiş olan biyokömürlerin 400 °C'de katalizörsüz koşulda elde edilen örneklerinkine göre daha kararlı olduğu söylenebilir.

Uçucu madde içeriği, yanma sürecini önemli ölçüde etkileyen önemli bir parametredir. Literatür verileri, biyokütlenin kömüre göre 2.5 kat daha fazla uçucu madde içerdiğini ve bunun ateşleme ve yanma koşulları üzerinde büyük bir etkiye sahip olduğunu göstermektedir (Mierzwa-Hersztek ve ark., 2019). Posanın ve farklı şartlarda piroliz işlemleri sonucunda elde edilmiş olan biyokömürlerin uçucu madde içerikleri, literatür verileriyle uyumlu olarak, araştırmamız sonucunda elde ettiğimiz tüm biyokömür örneklerinde uçucu madde içeriklerinin hammaddenin uçucu madde içeriğine kıyasla (%71.3), piroliz işlemi sonrasında oldukça azaldığı görülmektedir. Angın (2013), Palniandy ve ark. (2019) ve diğer pekçok araştırıcının sonuçlarına benzer şekilde, piroliz sıcaklığının ve ısıtma oranının artışı ile genel olarak katalizör kullanılmadan yapılan piroliz işlemleri sonucunda elde edilmiş olan biyokömürlerin uçucu madde içeriğinde hafif bir azalma olduğu görülmektedir. Katalizör kullanılarak elde edilmiş biyokömür örneklerinin uçucu madde yüzdelerinin ise biraz daha yüksek olduğu ve sıcaklık artışı ile çok değişmediği saptanmıştır.

Sabit karbon içeriği yanma sırasında kok oluşturacak madde miktarını göstermektedir. Sadiku ve ark. (2016) herhangi bir malzemenin sabit karbonun içeriğinin bir yakıtın ısıtma değerinin kabaca bir tahminini verdiğini ve sabit karbonun yanma sırasında ana ısı üreticisi olarak görev yaptığını belirtmişlerdir. Elde edilmiş olan biyokömürlerin sabit karbon içerikleri, %61.78 ile %73.15 arasında değişmiştir. Posa ve biyokömür örneklerinin sabit karbon içerikleri incelendiğinde piroliz işleminin tüm piroliz koşulları için sabit karbon içeriğinde yükselmeye sebep olduğu anlaşılmaktadır. Mierzwa-Hersztek ve ark. (2019)'da çalışmalarında benzer yönelimi

saptamışlardır. Sabit karbon içeriğinin genel olarak piroliz sıcaklığının ve ısıtma hızının artışıyla arttığı ve katalizör kullanımının sabit karbon içeriğinde nispeten düşüşe sebep olduğu görülmektedir.

3.3. Posa ve biyokömür örneklerinin ısıl değerlerine ilişkin sonuçlar

Ayçiçeği posasının ısıl değeri 17.13 MJ/kg iken ikincil ürün olarak elde edilmiş olan ürünlerden, katalizörsüz koşullarda elde edilmiş olan örnekler için bu değer 21.12-22.95 MJ/kg arasında değişmiştir. Bu sebeple elde edilen bu biyokömürlerin katı yakıt olarak kullanım potansiyellerinin de olduğu sonucuna varılabilir. Mohanty ve ark. (2013)' de biyokömürlerin ısıl değerinin kullanılan hammaddelerin ısıl değerlerinden yüksek olduğunu belirtmişlerdir. Öte yandan katalizör kullanımının ve kullanılan katalizör yüzdesinin artmasının biyokömürlerin ısıl değerleri üzerinde olumsuz etki yaptığı anlaşılmıştır. %15 oranında katalizör kullanımı en düşük ısıl değerlere sebep olmuştur. 400 °C ve 500 °C sıcaklıklarda %15 katalizör kullanım durumunda elde edilmiş olan biyokömürlerin ısıl değerleri hammaddenin ısıl değerinden daha düşük olmuştur (sırasıyla 14.37 ve 16.34 MJ/kg).

3.4. Posa ve biyokömür örneklerinin elemental analizlerine ilişkin sonuçlar

Örneklerin elemental bileşimleri incelendiğinde özellikle katalizör kullanılmadan gerçekleştirilen piroliz işleminin karbon oranını oldukça yükselttiği anlaşılmaktadır. Piroliz edilmemiş posanın karbon değeri %42.06 iken katalizör kullanılmadan elde edilmiş olan biyokömür örneklerinde en düşük karbon yüzdesi %56,05 (500°C-200°C/dk koşulunda) olarak ve en yüksek karbon yüzdesi ise %63.01 (600°C-300°C/dk örneğinde) olarak saptanmıştır. Katalizör kullanılarak elde edilmiş örneklerde ise karbon yüzdesi daha düşük olmak üzere %47.16-%58.28 arasında değişmiştir. Katalizör yüzdesinin %15'e yükseltilmesiyle bu düşüş daha fazla olmuştur. Avrupa Biyokömür Vakfı tarafından hazırlanmış olan (European Biochar Foundation (EBC)) Avrupa Biyokömür Sertifikasyonu, Sürdürülebilir Biyokömür Üretimi için Rehber başlıklı yayında, piroliz sonucunda elde edilecek biyokömürün karbon içeriğinin kuru kütlenin %50' sinden yüksek olmasının gerekliliği belirtilmiştir (European Biochar Certificate [EBC], 2012). Çalışmamız sonucunda katalizörsüz koşullarda elde edilen bütün biyokömür örneklerinin, karbon içeriği açısından bu gerekliliğe uygun olduğu görülmektedir.

Örneklerin oksijen içerikleri incelendiğinde piroliz işlemi ile elde edilen tüm biyokömür örneklerinde oksijen içeriğinin düştüğü belirlenmiştir. Piroliz edilmemiş ayçiçeği posasının oksijen içeriği %38,67 iken katalizör kullanılarak yapılan piroliz işleminden sonra elde edilmiş olan biyokömür örneklerinde daha yüksek oksijen içerikleri saptanmıştır. Oksijen yakıtlarda ısıl değeri düşürmekte ve bu durum bunların geleneksel yakıtlar gibi kullanılmasını engellemektedir (Özçiftçi ve Özbay, 2013). Bu sebeple katalizör kullanımının (özellikle yüksek oranda yani %15) elde edilen biyokömürlerin yakıt olarak kullanılabilme potansiyelini düşürebileceği söylenebilir. Katalizör kullanılarak elde edilmiş olan örneklerin ısıl değerlerinin oldukça düşük olması bu sonucu desteklemektedir.

Örneklerin hidrojen içerikleri incelendiğinde piroliz işlemi ile tüm biyokömürlerde hidrojen içeriğinin ayçiçeği posasının hidrojen içeriğine kıyasla düştüğü belirlenmiştir. Yakıtların yanması sırasında azot, N_2 ve NO_X gazlarına dönüşmekte ve çevreye zararlı olmaktadır. Çok az düzeyde azot küle dönüşmektedir. Sülfür içeriği de aynı şekilde SO_2 formuna dönüşmekte ve ısı değiştirici yüzeylerinde yoğuşmakta veya kül oluşturmaktadır. Bundan dolayı yakıtlarda düşük sülfür ve azot içeriği istenmektedir. Gerek katalizörsüz koşulda gerekse katalizör kullanılması durumunda elde edilmiş olan tüm örneklerin azot içeriklerinin hammaddenin azot içeriğine (%6.93) oldukça yakın bir değerde ve hatta birçok örnekte bu değerden yüksek olmakla beraber (%4.97-%7.20 aralığında) sülfür içeriklerinin sıfır ve sıfıra yakın değerde olması bu ürünler için olumlu bir durumdur.

3.5. Posa ve Biyokömür Örneklerinin SEM Görüntülerine İlişkin Sonuçlar

Şekil 4 ve *5*' de ayçiçeği posasına ve katalizörsüz/ katalizörlü koşullarda farklı sıcaklık ve ısınma hızlarında elde edilmiş olan biyokömür örneklerine ait 4.000 kez büyütme oranında elde edilmiş taramalı elektron mikroskobu (SEM) görüntüleri verilmiştir. Şekillerden de anlaşılacağı üzere piroliz edilmemiş hammaddenin yani posanın SEM görüntüsü ile biyokömür örneklerinin SEM görüntüleri karşılaştırıldığında, piroliz işlemi yapılmış tüm örneklerde gözenekliliğin başladığı ve sıcaklığın ve ısınma oranının artışıyla gözenek sayısında ve gözenek büyüklüğünde genel olarak bir artış olduğu görülmektedir. Benzer sonuçlar Shaaban ve ark. (2013) tarafından kauçuk odununun talaşından elde edilmiş olan biyokömür için de saptanmıştır. Gözenek oluşumu, piroliz sıcaklığına bağlı olarak biyokütledeki organik bileşiklerin ve uçucu bileşiklerin aşamalı olarak biyokütleden uzaklaşması yani buharlaşması ile açıklanabilir. Karbonca zenginleştirilmiş biyokömür örnekleri, karbonizasyon

sırasında uçucu maddenin evrimi nedeniyle oluşan çeşitli çatlak ve delikler içermektedir. Bilindiği gibi, daha yüksek uçucu madde salınımı, daha düşük yoğunluklu, daha yüksek gözenekli biyokömürler oluşturmaktadır (Özçimen ve Meriçboyu 2009). SEM görüntülerinden piroliz sıcaklığına bağlı olarak biyokömürün parçacık boyutlarının ve şeklinin etkilendiği anlaşılmaktadır. Ertaş (2010) bazı biyokütle artıklarının yavaş pirolizinden elde edilen ürünlerin karakterizasyonuna yönelik çalışmasında sıcaklık artışına bağlı olarak genelde boşlukların boyutunda ve oranında artış olurken hücre duvarı kalınlığında azalma meydana geldiğini, ayrıca sıcaklığın etkisiyle katı ürünlerin hücre duvarlarının çok ince ve kırılgan bir hal aldığını belirtmiştir. Genel olarak sıcaklığın artışı karbon yoğunluğunu artırmış, oksijen ise dehidrasyon ve buharlaşma yoluyla uzaklaştırılmıştır. Piroliz sıcaklığının artışı ile elemental analiz sonuçlarından olan karbon yüzdesinin de artması bu sonucu desteklemektedir. Bizim örneklerimizde de elde edilen SEM sonuçlarına göre sıcaklık arttıkça poroz yapının daha belirgin bir hal aldığı anlaşılmaktadır. Katalizörlü yapılan piroliz işlemleri sonunda elde edilen biyokömürlerde ise piroliz sıcaklığının artışıyla aynı şekilde gözenekliliğin arttığı fakat gözenek yapısının daha küçük kaldığı görülmektedir.

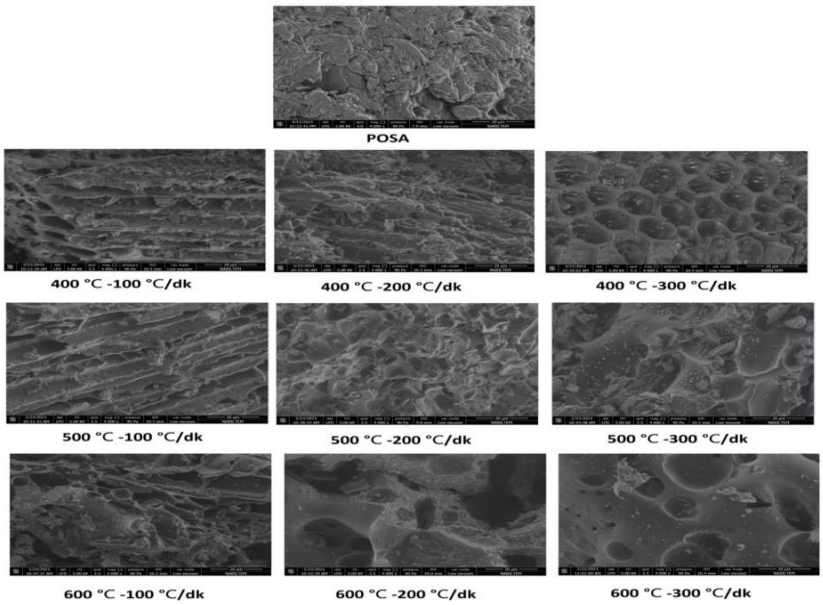

Figure 4. SEM images of biochars obtained at different temperatures and heating rates under catalyst-free pyrolysis conditions

Şekil 4. Katalizörsüz piroliz koşullarında farklı sıcaklık ve ısınma hızlarında elde edilen biyokömürlerin SEM görüntüleri

3.6. Piroliz Sıvı Örneklerinin Bileşimlerine İlişkin Sonuçlar

Ayçiçeği küspesinin farklı sıcaklıklarda katalizörsüz ve katalizörlü olarak hızlı piroliz işleminden elde edilmiş olan biyoyağ örneklerinin yapılarını detaylı aydınlatmak amacı ile bu ürünlerin içerdiği bileşikler GC/MS yardımıyla incelenmiştir. Kromatografik pikler WILEY kütle spektra veri kütüphanesi yardımıyla karakterize edilmiş olup, pik yüzdeleri ise TIC (toplam iyon kromatogram) pik alanlarından hesaplanmış ve piroliz sıvı örneklerinin en fazla içerdiği bileşiklerin yüzdeleri *Tablo 2*'de sunulmuştur. *Tablo 2* incelendiğinde ayçiçeği posasının hızlı piroliz yöntemiyle pirolizi sonucunda elde edilmiş olan biyoyağ örneklerinde kıymetli kimyasallardan olan 2-Furanmetanolün (Furfuril alkol) ve fenol bileşiklerinin (Phenol, Phenol 2-methoxy- gibi) yüzdelerinin diğer bileşenlere göre oldukça yüksek olduğu belirlenmiştir. 600°C-100°C/dk katalizörsüz koşulda ve 600°C-100°C/dk katalizörlü koşulda elde edilmiş olan örneklerde furfural bileşiklerinin (2-Furanmethanol yani Furfural alkol) yüksek oranda oluştuğu görülmektedir. Fenol ve furfural bileşikleri önemli bileşiklerdir ve yüksek ticari değere sahiptirler. Furfural, yenilenebilir, petrol esaslı olmayan önemli bir kimyasal hammaddedir. Bir dizi katalitik indirgeme ile çeşitli çözücülere, polimerlere, yakıtlara ve diğer yararlı kimyasallara dönüştürülebilir (Anonim, 2021a). Bununla birlikte 5-metil furfural, diğer daha kullanışlı bileşiklere dönüştürülebilir. Piroliz sonucunda elde edilmiş olan sıvıların özellikle fenolik yüzdelerinin yüksek olması literatürdeki sonuçlar ile de uyum sağlamaktadır (Ateş ve Işıkdağ, 2008, Öztürk Tophanecioğlu 2009).

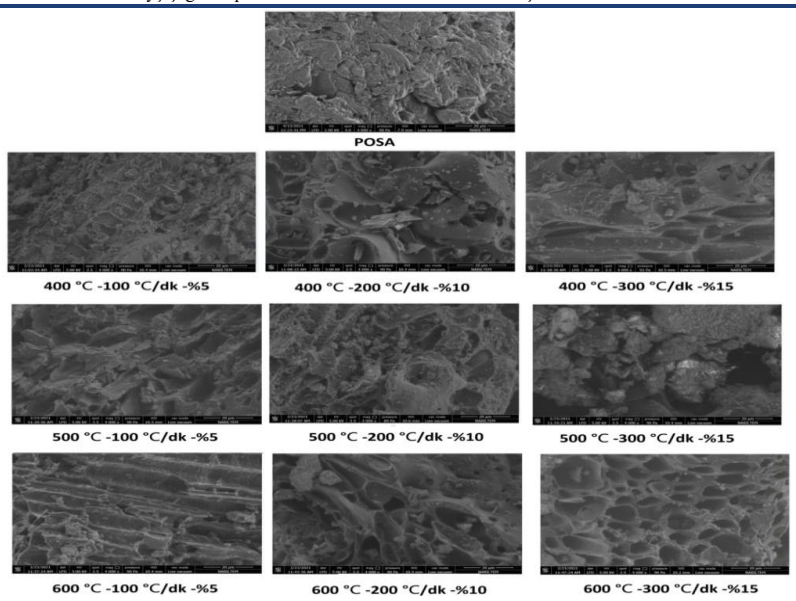

Figure 5. SEM images of biochars obtained at different temperatures, heating rates and catalyst ratios under catalyst pyrolysis conditions

Şekil 5. Katalizörlü piroliz koşullarında farklı sıcaklık, ısınma hızı ve katalizör oranlarında elde edilen biyokömürlerin SEM görüntüleri

Tablo 2. Ayçiçeği küspesinden farklı sıcaklıklarda elde edilen sıvı ürünlerde en fazla görülen (%) bileşenlerin piroliz koşullarına göre değişimi

Table 2. Variation of the most common (%) components in liquid products obtained from sunflower meal at different temperatures according to pyrolysis conditions.

Bileşikler	**NK 1.1**	**NK 1.2**	**NK 1.4**	**NK 1.5**	**NK 1.6**	**NK 1.7**	**NK 1.8**	**K 2.1**	**K 2.6**	**K 2.8**
2-Furanmethanol	12.88	5.88	-	14.25	15.15	-	11.45	-	8.97	13.32
Pyrazine, 2,5-dimethyl- (CAS) 2,5-Dimethylpyrazine	5.04	0.39	-	-	6.81	4.13	-	-	6.28	6.47
Phenol	5.80	5.90	-	7.36	8.20	-	-	1.75	4.96	8.25
Phenol, 2-methoxy-	8.04	12.56	-	6.87	5.12	8.35	8.35	-	7.32	8.72
Pyrazine, methyl-	3.79	4.36	-	9.64	4.84	1.80	7.8	-	5.41	4.06
Pyrazine, 2,6-dimethyl- (CAS) 2,6-Dimethylpyrazine	-	4.92	-	-	-	-	5.96	-	-	-
13-Docosenamide, (Z)-	-	-	11.34	-	13.50	8.24	-	41.44	5.36	8.52
2-Furanmethanol (CAS) Furfurylalcohol	-	-	-	-	-	8.35	-	9.54	-	-
2-Propanone, 1-(acetyloxy)-	-	-	-	-	-	-	-	4.86	5.98	-
cis-11-Eicosenamide	8.41	8.05	-	9.45	-	-	8.01	-	-	-

3.7. Piroliz gaz örneklerinin bileşimi ve ısıl değerlerine ilişkin sonuçlar

Şekil 6' da katalizörlü ve katalizörsüz koşulda hızlı piroliz işlemi sonucunda elde edilmiş olan piroliz gaz örneklerinin bileşimlerinin ve ısıl değerlerinin piroliz sıcaklığı ve ısınma hızına bağlı olarak değişimleri verilmiştir. Gaz bileşimleri incelendiğinde ısıl değeri artıran CH_4 ve CO gazının yüzdelerinin oldukça yüksek olduğu anlaşılmıştır. Yine ısıl değer üzerine etkili olan H_2 gazı içeriği ise yüksek sıcaklıklarda yapılan piroliz işlemlerinde daha yüksek olmuştur. Sonuçlar incelendiğinde piroliz sıcaklığının ve ısınma oranının artışıyla genel olarak gazın ısıl değerinde düşüş olduğu belirlenmiştir. Gerek katalizör kullanarak gerekse katalizörsüz olarak 600°C piroliz sıcaklığında elde edilen piroliz gaz örneklerinin ısıl değerinin genel olarak düşük olduğu saptanmıştır. Yine 600°C piroliz sıcaklığında %15 oranında katalizör kullanımı elde edilen gazın ısıl değerini oldukça düşürmüştür (6.9 MJ/Nm^3). Biyokütlelerden elde edilen piroliz gazının ısıl değerinin ortalama 17.10 MJ/Nm3 olduğu ve gaz bileşenleri açısından en fazla bulunan gazların CO ve CH_4 olduğu bilinmektedir (Anonim, 2021 b). Elde edilen piroliz gaz örneklerinin ısıl değerinin genelde bu ortalama değerden daha yüksek olduğu ve bu yüksek ısıl değerinin yüksek CH_4 içeriğinden kaynaklandığı söylenebilir.

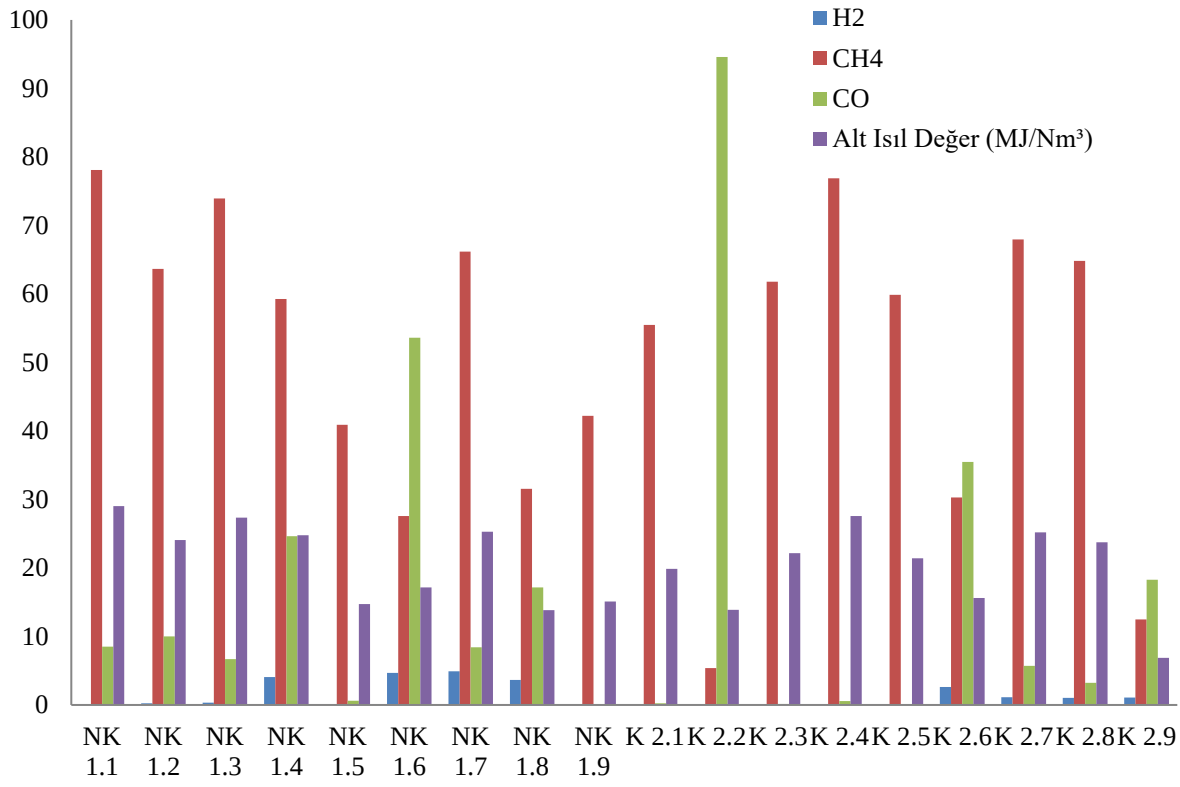

Figure 6. Gas components and calorific values that affect the calorific values of the pyrolysis gas

Şekil 6. Piroliz gazının ısıl değerlerini etkileyen gaz bileşenleri ve ısıl değerleri

4. Sonuç

Gerçekleştirilen bu çalışmada da biyokütleden alınan verimin artırılması hedeflenmiştir. Ayçiçeği küspesi hammadde olarak seçilmiş ve katalizörsüz koşullarda ve klinoptilolit katalizörü kullanılarak yapılan piroliz işlemleri sonucunda elde edilen ürün verimleri incelenmiştir. Ayçiçeği küspesi %5.92 nem, %6.08 kül, %71.30 uçucu madde ve %16.70 sabit karbon içeriğine sahip olup elemental analiz sonuçları ise %42.06 C, %6.26 H, %6.93 N, %0.00 S olarak belirlenmiştir. Ayçiçeği küspesinin ısıl değeri de 17.13 MJ/kg olarak hesaplanmıştır. SEM görüntüsü incelendiğinde, ayçiçeği küspesinin gözenekli bir yapıya sahip olduğu tespit edilmiştir.

Piroliz sıcaklığı ve ısıtma hızının etkilerini incelemek amacıyla 400, 500, 600 °C sıcaklıklarda, 100, 200, 300 °C/dk ısınma hızında ayçiçeği küspesi piroliz işlemine tabi tutulmuştur. Sıcaklık artışı sıvı verimini önce artırmış, sonra düşürmüştür. Katı verimi, sıcaklık artışı ile bir miktar düşmüş ancak sıvı verimine nispeten daha kararlı bir davranış göstermiştir. Sıcaklık artışı gaz ürün verimini artırmıştır. Genel olarak 400 °C ve 500 °C

sıcaklıklarda daha yüksek verimler alınmıştır. Katalizörlü koşulda, katalizör yüzdesinin artması ısınma hızını da göz önüne alarak %5'te artırmış, %10 ve %15 katalizör eklendiğinde bu verim azalmıştır. Katalizörlü koşulda katı verimi katalizör yüzdesi artışı ile azalmıştır. Aynı koşulda gaz verimi, katalizör yüzdesinin artmasıyla artış göstermiştir.

Katalizörlü ve katalizörsüz koşullarda piroliz sıcaklığı, ısınma hızı ve katalizör yüzdeleri gibi parametrelerle, ürün verimi ile ilgili kıyaslamalar yapıldığında katalizörün katı ürün verimini bir miktar artırdığı, sıvı ürün verimini nispeten değiştirmediği ve gaz ürün verimini bir miktar azalttığı sonucuna varılmıştır. Sayısal veriler arasında yapılan kıyaslamalar yine göstermiştir ki katalizör kullanımı ürün verimlerinde bariz bir değişiklik yaratmamıştır. Yine iki koşul kıyaslandığında amaç en yüksek ürün verimi elde etmek olarak belirlenirse, katalizörsüz koşullarda yapılan piroliz işlemlerinin daha olumlu sonuçlar verdiğini söylemek mümkündür.

Elde edilen biyokömürlerin kül analizleri incelendiğinde %5-10 arasında değerler almış ve düşük kaliteli kömür sınıfına girmektedir (Anonim, 2022b). Elemental analiz sonuçlarına bakılarak H/C oranları incelendiğinde, ayçiçeği küspesinde bu oran 0.15 iken, biyokömür örneklerinde 0.05 ile 0.09 aralığında hesaplanmıştır. Piroliz sıcaklığının artışı H/C oranını azaltmıştır. Biyokömür örneklerinde en yüksek ısıl değer, katalizörsüz koşulda 500 °Csıcaklıkta, 300°C/dk ısınma hızında 22.95 MJ/kg olarak elde edilmiştir. Elde edilen kalorifik değerler, hammadde ile karşılaştırıldığında ısıl değerin arttığı anlaşılmaktadır.

Biyokömürlerin SEM görüntüleri incelendiğinde katalizörsüz koşulda hammaddeye göre gözeneklilikte artış gözlenmiştir. Katalizörsüz piroliz koşullarında SEM görüntüleri incelendiğinde gözenekli yapı mevcuttur fakat katalizörsüz koşul ile karşılaştırıldığında gözeneklerin daha küçük olduğu söylenebilir.

Örneklerin GC analiz sonuçları incelendiğinde genel olarak katalizörsüz koşulun katalizörlü koşula göre daha yüksek ısıl değere sahip olduğu görülmüştür. En yüksek alt ısıl değer 400 °C sıcaklıkta, 100°C/dk ısınma hızında 29.05 MJ/Nm³ olarak elde edilmiştir. Sonuçlar doğalgazın alt ısıl değerinin (34.54 MJ/Nm³) altında kalmıştır (Anonim, 2021b).

Ayçiçeği küspesinin farklı sıcaklıklarda katalizörlü ve katalizörsüz koşulda elde edilen biyoyağ örnekleri incelendiğinde özellikle fenol bileşiklerinin (Phenol, Phenol, 2-methoxy-) hemen her numunede görüldüğü anlaşılmıştır. Aynı zamanda sıvı numunelerde Pyrazine-methyl, Pyrazine 2,6-dimethyl- (CAS) 2 6-Dimethylpyrazine gibi aromatik bileşiklerde tespit edilmiştir.

Araştırmaya ilişkin tüm sonuçlar değerlendirildiğinde, ayçiçeği küspesinin farklı koşullarda pirolizi sonucunda elde edilen biyokömürün katı yakıt potansiyelinin olduğu, biyoyağın ise sentetik sıvı yakıt olarak veya kimyasal hammadde olarak değerlendirilebilme potansiyeli olduğu belirlenmiştir. Bu sebeple ayçiçeği küspesinin pirolizi sonucu elde edilecek ürünlerin kullanılabilirliği üzerine daha ileri çalışmalar yapılması gerekli ve önemlidir.

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Bu çalışma için etik kuruldan izin alınmasına gerek yoktur.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

The Effects of Silver Nitrate and Pre-Cold Treatments on Callus Formation in Strawberry Anther Culture

Gümüş Nitrat ve Ön Soğuk Uygulamalarının Çilek Anter Kültüründe Kallus Oluşumuna Etkileri

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Abstract

Strawberry (*Fragaria* x *ananassa* Duch.) which is one of the widely grown berry species in the world has economic and commercial importance. In commercial strawberry varieties, in order to increase yield and quality, it is necessary to obtain starting materials that are resistant/tolerant to biotic and abiotic stress factors. Biotechnological methods have an important place in strawberry breeding studies due to the long and costly process of classical breeding methods, the genetic expansion of seed production, high ploidy level and strong heterozygosity. Haploid plant production is an efficient breeding method that has been successfully applied to most plant species. However, due to the lack of sufficient haploid studies on strawberry and the fact that a specific protocol for this species has not yet been developed the necessary progress has not been made in this regard. In this study, the effectiveness of some factors determine the success in anther culture which has a significant place in obtaining haploid strawberries was investigated. For this reason, first, different sodium hypochlorite doses (NaOCl; 1%, 2%, 3%) and application durations (10, 15, 20 min) were used to determine the appropriate method for sterilisation, then cold pre-treatments (24, 36, 48, 72 hours at +4 °C) and different silver nitrate doses ($AgNO_3$; 10, 20, 30, 40 mg l-1) were employed for callus induction in Festival strawberry variety. At the conclusion of the study it was observed that the lowest contamination rate (1%) was obtained by soaking in 1% sodium hypochlorite solution for 10 minutes. Cold pre-treatment of flower buds at +4 °C for 36 hours produced the highest callus induction rate (96%). The evaluation of the effect of $AgNO_3$ application at different doses on the callus induction rate revealed that the highest callus induction (82%) was obtained from 20 mg l-1 $AgNO_3$ dosage. This study showed that anther culture practices in strawberry can be improved by using cold pre-treatment, appropriate sterilization method and silver nitrate addition to the medium.

Keywords: Anther, Callogenesis, Silver nitrate, Micropropagation, Haploid

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Öz

Çilek (*Fragaria* x *ananassa* Duch.), dünyada yaygın olarak yetiştiriciliği yapılan, ekonomik ve ticari öneme sahip olan önemli üzümsü meyve türlerinden birisidir. Ticari çilek çeşitlerinde, verim ve kalitenin artırılması için biotik ve abiotik stress faktörlerine karşı dayanıklı veya tolerant başlangıç materyallerinin elde edilmesi gerekmektedir. Çilekte, klasik ıslah çalışmaları süreçlerinin uzun ve masraflı olması, tohumdan üretiminin genetik açılım göstermesi, yüksek ploidi seviyesi ve güçlü heterozigotluk sebebiyle, biyoteknolojik yöntemler ıslah çalışmalarında önemli bir yer tutmaktadır. Haploid bitki üretimi çoğu bitki türünde başarı ile uygulanan etkili bir ıslah yöntemidir. Ancak çilekte haploid bitki üretimi ile ilgili yeterli sayıda çalışmanın olmaması ve bu türe özgü bir protokolün hala geliştirilememesi gibi nedenlerle bu konuda gerekli ilerlemeler sağlanamamıştır. Bu çalışmada, haploid çilek eldesinde önemli bir yer tutan anter kültüründe başarıyı etkileyen bazı faktörlerin etkinliğinin araştırılması amaçlanmıştır. Bu nedenle, Festival çilek çeşidinde kallus indüksiyonu için öncelikle sterilizasyon için uygun yöntemi belirlemek amacıyla farklı sodyum hipoklorit dozları (NaOCl; %1, %2, %3) ve uygulama süreleri (10, 15, 20 dk) ardından farklı soğuk ön uygulamaları (+4 °C'de 24, 36, 48, 72 saat) ve gümüş nitrat dozları ($AgNO_3$; 10, 20, 30, 40 mg l-1) kullanılmıştır. Araştırmadan elde edilen verilerin değerlendirilmesi sonucunda en düşük kontaminasyon oranının (%1) %1'lik sodyum hipoklorit çözeltisinde 10 dakika bekletilerek elde edildiği belirlenmiştir. Çiçek tomurcuklarının 36 saat boyunca +4 °C 'de soğuk ön muameleye tabi tutulması ile en yüksek kallus indüksiyon oranı (%96) sağlanmıştır. Farklı dozlarda $AgNO_3$ uygulamasının kallus indüksiyonu üzerine etkisinin değerlendirilmesi ise en yüksek oranın (%82) 20 mg l-1 $AgNO_3$ dozundan elde edildiğini göstermiştir. Bu çalışma, çilekte anter kültürü çalışmalarının soğuk ön uygulaması, uygun sterilizasyon yöntemi ve besi ortamına gümüş nitrat ilavesi ile geliştirilebileceğini göstermiştir.

Anahtar Kelimeler: Anter, Kallogenesis, Gümüş nitrat, Mikroçoğaltım, Haploid

1. Introduction

Strawberry (*Fragaria* x *ananassa*), which is a commercially important crop, is one of the most produced and consumed berry fruit throughout the world. Its suitability for early harvest and its high export opportunities have increased its importance day by day, and gave rise to the strawberry breeding studies (Lahiri et al., 2022; Simpson, 2018; Bayram et al., 2016). Unlike many agricultural products strawberry can provide high value in fresh and processed markets. In addition to fresh consumption, it can also be consumed by freezing or drying, or as fruit juice, jam, marmalade, dessert, cake, ice cream and liquor (Chandler et al., 2012; Witter et al., 2012). The fact that the strawberry fruit can be consumed in different ways allows it to be grown in different ecologies throughout the year (Davis et al., 2007). It is also a beneficial food source for human health, as it is rich in antioxidants, vitamin C, fiber, polyphenols and potassium (Aaby et al., 2018; Andrianjaka-Camps et al., 2017; Michalska et al., 2017). Turkey ranks 4th in the world with a production value of 486 705 tons in 8.8 million tons of strawberry production (Anonymous, 2021).

Although the yield, fruit size and quality characteristics of strawberry genotypes have been improved with classical breeding methods, the increase in yield and quality and tolerance to stress factors have still not been fully achieved in commercial strawberry cultivars (Hummer et al., 2022; Mezzetti., 2013). The reasons for this situation are the time-consuming and costly process of classical breeding in strawberry, genetic expansion in production from seed, the high ploidy level and the difficulties caused by strong heterozygosity. Haploid technology, which is one of the biotechnological methods and a useful tool for plant breeding, allows the rapid development of productive, high quality, disease and pest resistant varieties (Nguyen et al., 2012). Successful results have been obtained by culturing anthers which have not reached their full maturation stage and contain mononuclear microspores that have reached the first pollen mitosis stage, under in vitro condition in approximately 250 different plant species until today (Irikova et al., 2011). Since strawberry is a herbaceous plant, it is a much more advantageous species in terms of hapliod technique compared to other woody fruit species. Obtaining haploid plants from anthers by callus induction in vitro is a very effective method for strawberry breeding studies. After callus formation, haploid plant production occurs by indirect embryogenesis or indirect organogenesis (Na et al., 2019; Niazian et al., 2017). As with other tissue culture techniques, there are many internal and external factors that affect the embryonic response in androgenesis (Pehlivan et al., 2017; Dunwell, 2010). The success of anther culture is related to factors such as genotype, donor plant physiology, microspore/pollen developmental stage, pre-treatments (temperature shock) and culture conditions (plant growth regulator and carbon source) (Na et al., 2019). The effects of culture medium type, myo-inositol, auxin and cytokinin combination treatment, silver nitrate ($AgNO_3$) and ferric ethylenediaminetetraacetic acid (Fe-EDTA) to callus induction in strawberry were determined from anther culture (Na et al. 2019). In addition to these factors, the effectiveness of heat shock application was investigated in a study by Kim et al 2020. Na et al. (2011) also investigated the effects of cold pre-treatment and medium content on anther culture of strawberry.

Although there are studies on strawberry anther culture around the world, there is no study on this subject in Turkey. Since there are not enough androgenesis studies in strawberry and a specific protocol for this species has not yet been developed, it has become inevitable to identify and develop effective protocols. In order to develop an effective protocol, it is necessary to first determine the factors affecting callus induction in anther culture. In this study, it was aimed to establish an effective protocol for callus induction from anther culture in Festival strawberry cultivar. In this context, the effects of sodium hypochlorite (NaOCl), pre-cold treatment and silver nitrate ($AgNO_3$) applications on callus formation were investigated.

2. Materials and Methods

2.1. Plant material

The 'Festival' strawberry cultivar used as plant material in the research was obtained from a commercial company. This cultivar is a hybrid of Oso Grande and Rosa Linda and is a short-day variety. Measurements and analyses were carried out in Akdeniz University Vocational School of Technical Sciences Laboratories.

2.2. Method

2.2.1. Growing donor plants

The plants of cv. Festival were grown in the greenhouses of Akdeniz University Research and Application Farm in the 2021-2022 growing season. Fresh tube seedlings were planted in the greenhouse soil in the autumn planting period. In order to meet the nutrient requirements, fertilization and cultural treatments were carried out before and after planting.

2.2.2. Removal of buds

Anthers taken from buds of different sizes were stained with acetocarmine and examined under a microscope in order to determine the appropriate bud size. In the cytological examination, buds (1-5 mm) containing anthers with microspores in the middle or late mononuclear period were taken. The development period of the buds (in terms of bud size) containing the anthers at the appropriate period for both cultivars was determined and the anthers in the buds were used as explants. Buds containing microspores at the appropriate stage from donor plants were collected in falcon tubes between 06:30 and 07:30 in the morning and brought to the tissue culture laboratory in an ice box.

2.2.3. Pre-cold application

In order to determine the effectiveness of the pre-cold application and increase the efficiency of callus induction from anther culture in strawberry, flower buds are exposed to +4 °C for different lengths of time. Buds at the appropriate pollen stage were collected from the plant and then were placed in the refrigerator in 90 mm diameter plastic petri dishes with moistened filter papers to be subjected to cold application at +4 °C for 24, 48, 36, 72 hours.

2.2.4. Surface sterilization of buds

Different sodium hypochlorite (NaOCl) doses (1%, 2%, 3%) and application durations (10, 15, 20 min) were tried for surface sterilization. Buds were kept in 70% ethanol solution for 30 seconds and then in different concentrations of NaOCl solutions containing 1-2 drops of Tween-20 for different durations. Sterilization was completed by passing buds through sterile distilled water three times.

2.2.5. Anther culture

After collecting from the buds with scalpel and forceps in a sterile cabinet, anthers were cultured on MS medium containing 0,4 mg l−1 BA+0,1 mg l−1 IAA+2,0 mg l−1 2,4-D and 30 g l−1 sucrose (*Figure 1*). In order to determine the effects of $AgNO_3$ on callus formation, different doses of $AgNO_3$ (10, 20, 30, 40 mg l −1) were also added to the MS medium. Experiments were conducted in petri plates measuring 90 mm in length x 20 mm in height and 10 anthers were placed in each petri dish. Cultures were maintained at 32°C for 48 hours then at 25°C in the dark for 8 weeks. During the study observations were made twice a week and the number of callus forming explants were recorded.

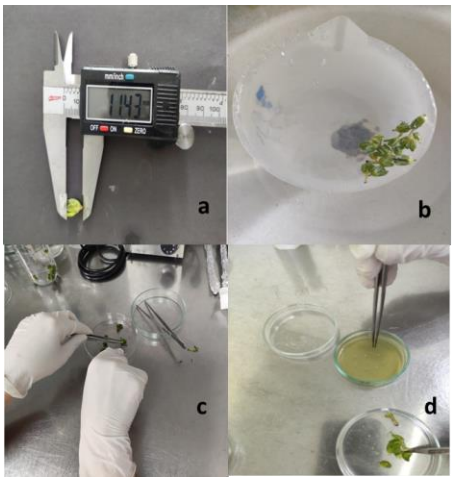

Figure 1. Explant preparation steps for anther culture a. Selection of appropriately sized flower buds b. Selected closed flower buds c. Removal of anthers d. Anther explants placed on petri plates containing MS medium

3. Results and Discussion

3.1. Surface sterilization of the buds

Microbial contamination is one of the most important threats in in vitro tissue culture (Daud et al., 2012). Virus, bacteria or fungal pathogens cause losses in plant tissue culture resulting in tissue necrosis, reduced shoot growth and reduced rooting. It is desirable that the sterilization measures are included into the process of culturing plant materials, ensuring only the microbial contaminants are destroyed and their biological activities maintain without being damaged. For this reason, it is necessary to carefully select the type and application periods of the disinfectants to be used in the sterilization process. Although ethanol is a powerful sterilizer, it is a highly toxic chemical for the explants. It is therefore recommended to keep the application time short. Among the chemicals used for surface sterilization, sodium hypochlorite is the most commonly preferred because it is easily available and can be diluted to appropriate concentrations (Tyagi et al., 2011). Therefore, in this study, different concentrations of NaOCl and different application times were tried for surface sterilization (*Table 1*). Among the different NaOCl doses (1%, 2%, 3%) and application durations (10, 15, 20 min) tried in the study, the best surface sterilization procedure with 1% infection rate and 95% anther survival rate involved keeping the explants in 70% ethanol for 30 seconds and then in 1% sodium hypochlorite for 15 minutes. Although no infection was observed in 3% NaOCl, the survival rate of anthers decreased to 65%.

Table 1. Infection and anther survival rate in surface sterilization for buds

Applications	Anther survival rate (%)	Infection rate (%)
1% NaOCl 10 minutes	92	2
1% NaOCl 15 minutes	95	1
1% NaOCl 20 minutes	90	1
2% NaOCl 10 minutes	90	1
2% NaOCl 15 minutes	85	1
2% NaOCl 20 minutes	85	0
3% NaOCl 10 minutes	70	0
3% NaOCl 15 minutes	70	0
3% NaOCl 20 minutes	65	0

3.2. Pre-cold application

Temperature shocks induce androgenetic response in different plants (Kiviharju and Pehu, 1998). The available stresses can be heat shock treatment (Kim et al., 2020) as well as cold pre-applications (Na et al., 2011). Cold pre-treatment is widely used to promote androgenesis in many other plants. It has been determined that the cold pre-treatment also provides an effective anther culture of strawberry (Shahvali-Kohshour et al. 2013).

The most effective cold pre-application for callus induction from anther culture of strawberry Seolhyang cv. was obtained by keeping it at 4°C for 72 hours (Na et al., 2011). In Camarosa, Selva and Paros strawberry cultivars at 4°C for two days and in Pajaro cv. at 4°C for three days cold pre-application were the best for promoting anther callogenesis (Shahvali-Kohshour et al., 2013).

In this study, four different cold pre-applications (24, 36, 48 and 72 hours) were performed. From the results it was determined that, among different pre-cold applications (24, 36, 48, 72 hours at +4°C), the highest callus induction rate (85%) was obtained from 36 hours application at +4°C (*Figure 2*). Although this rate is higher than the callus induction rate obtained from the Seolhyang cv. (Na et al., 2011), the effect of the temperature as pre-treatment on callus induction varies depending on the genotype (Kiviharju and Pehu, 1998).

Cold pre-treatment in strawberry protects microspores by preventing the decay of anther tissues and the release of toxic compounds from rotting anthers. The increase in free amino acid content in microspores caused by cold pre-treatment is thought to mediate the induction of embryogenesis. It also leads to greater survival of embryogenic pollen grains (Shahvali-Kohshour et al., 2013; Shariatpanahi et al., 2006).

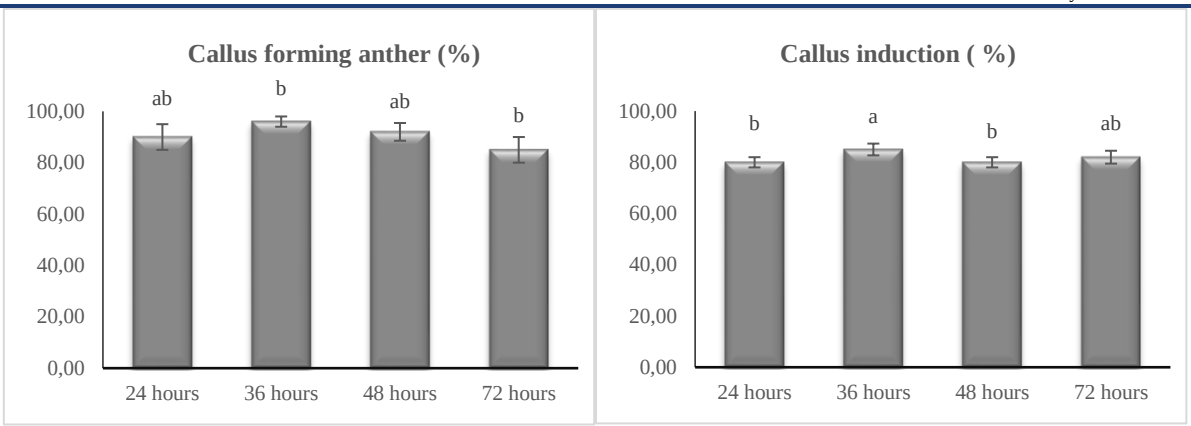

Figure 2. The effects of pre-cold applications on anther development and callus formation of Festival cv.

3.3. The effects of different silver nitrate concentrations on callus formation

Determining the medium and optimizing the most appropriate dose of each chemical component added to the medium is a prerequisite for success in haploid plant production in vitro (Hassan and Islam, 2021). Silver nitrate is one of the components added to the culture medium before the anthers are cultured or during the culture to increase the success of anther culture (Shahvali-Kohshour et al., 2013). Murashige and Skoog (MS) medium, Gamborg B5 medium and Lichter medium were used for callus formation from the anther for haploid plant production in Seolhyang cultivar, and the best results were obtained in MS medium. The effects of $AgNO_3$ on callus formation were also investigated in the same study, and the highest callus formation rate was determined as 41.4% in the MS medium with 25% silver nitrate (Na et al., 2019). Therefore, MS medium was used for the formation of callus from anther culture in the Festival cv. In terms of promoting anther development (anther response rate) and callus induction in different concentrations of $AgNO_3$, the best dose was determined as MS medium containing 20 mg l-1 $AgNO_3$ (*Figure 3*). In the presence of 20 mg l-1 silver nitrate in the MS medium, the highest callus formation rate was 82% (*Figure 4*), which is twice that of the callus formation rate obtained in Seolhyang cultivar (Na et al., 2019). In Camorasa strawberry cultivar, the highest callus formation was obtained in the presence of 15 mg l−1 $AgNO_3$ added to the culture medium (Shahvali-Kohshour et al., 2013).

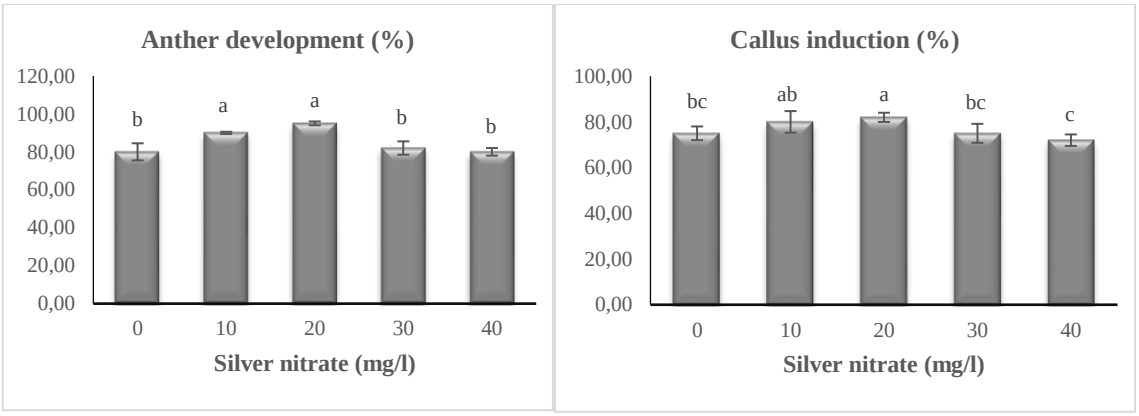

Figure 3. The effects of different concentrations of $AgNO_3$ applications on anther development and callus formation of Festival cv.

Another factor affecting anther culture is plant growth regulators added to the medium. In a study carried out by the Kim et al. (2019) investigating the effectiveness of different hormones for the propagation of Goha and Seolhyang cultivars in in vitro culture medium, it was determined that BA application was the most effective plant growth regulator for proliferation. Kim et al. (2020) used BAP, IAA, and 2.4-D as plant growth regulators in their study investigating the effects of various factors, including $AgNO_3$, on callus formation and plant regeneration from anther culture in strawberry. The highest callus formation rate of 71.4% was obtained at 25 mg l-1 $AgNO_3$ concentration.

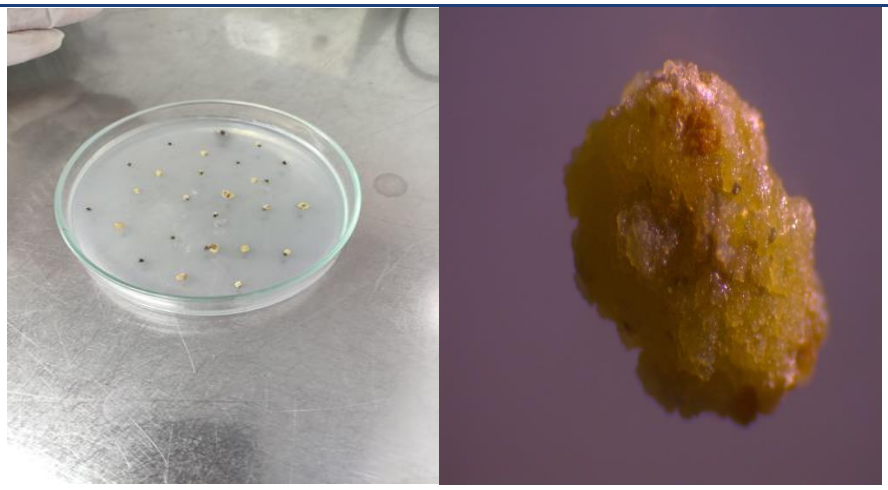

Figure 4. Callus formation from anther explant of Festival cv. on MS medium containing 20 mg l^{-1} $AgNO_3$

Indolacetic acid (IAA), benzyl amino purine (BAP), 2,4-Dichlorophenoxyacetic acid (2,4 D), of which the efficacy has been investigated previously for anther culture (Kim et al., 2020) were added in MS medium for all trials. Since the effects of cold pre-treatment and $AgNO_3$ on callus formation from the anther culture of the Festival cv. were investigated in this study, the hormones added to the nutrient medium were kept at same doses for all applications.

4. Conclusions

Haploid plant production is a very effective method for strawberry breeding. Callus formation is required for haploid plant regeneration through anther. Therefore, in this study we aimed to investigate the cold pre-treatment period, sterilization method and $AgNO_3$ concentrations on callus formation from anther explant using cv. Festival. Our findings suggest that cold pre-treatment of anthers for a period of 36 hours at +4ºC and adding of 20 mg l-1 $AgNO_3$ to the medium were best practices for promoting callus formation. Also, this study showed that strawberry anther culture can be improved by using cold pre-treatment, sterilization method and silver nitrate addition to the medium. We can speculate that our results provide the basis for further research to develop effective protocols for anther culture, a method used in the production of haploid strawberries.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Sevinç Şener and Ayşe Gül Nasırcılar; Design: Sevinç Şener, Ayşe Gül Nasırcılar and Ahmet Karaçan; Data Collection or Processing: Sevinç Şener, Ayşe Gül Nasırcılar and Ahmet Karaçan; Statistical Analyses: Sevinç Şener; Literature Search: Sevinç Şener and Ayşe Gül Nasırcılar; Writing Sevinç Şener and Ayşe Gül Nasırcılar.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of NaCl-induced Salt Stress on Germination and Initial Seedling Growth of *Lotus corniculatus* L. cv. 'Leo'

NaCl Kaynaklı Tuz Stresinin *Lotus corniculatus* L. cv. 'Leo'nun Çimlenmesi ve İlk Fide Büyümesi Üzerindeki Etkisi

Ramazan BEYAZ[1*], Ahmet KAZANKAYA[2]

Abstract

Lotus corniculatus L. is one of the agronomically and economically important perennial legume forage species with moderately salt-tolerant. It is well known that even the cultivars of the same species in plants have different responses in salinity. However, studies on the salt response of *L. corniculatus* and its cultivars, which are more advantageous than other forage crops such as white clover (*Trifolium repens*) and alfalfa (*Medicago sativa* L.), in the use of marginal agricultural lands affected by abiotic stress factors such as salinity, are limited. Under salt stress, the most crucial phases of the plant life cycle that are directly related to the survival of the plant are seed germination, growth, and vigour. Therefore, this study was carried out to determine the germination and growth responses of *L. corniculatus* cultivar 'Leo', which is known to have higher tannin content than other cultivars, under NaCl-derived salt stress in vitro. For this purpose, *L. corniculatus* seeds were cultured in MS (Murashige and Skoog/Gamborg) medium containing 0, 40, and 80 mM NaCl for 14 days. Seed germination percentage, mean germination time, germination rate index, shoot-root length, root to shoot length ratio, shoot-root fresh dry weight, shoot-root dry matter, the ratio of root to shoot dry matter, shoot-root water content and seedling vigour index parameters were measured. According to the results of the research, the germination percentage did not change in the applied NaCl treatments, but the germination rate decreased. However, shoot length decreased and root length increased. Although there was no statistically significant change in shoot and root fresh-dry weight, both decreased in 80 mM NaCl treatment. The shoot and root dry matter increased and the water content decreased. Also, the seedling viability index decreased. In 40 mM NaCl treatment, on the other hand, there was an increase in shoot fresh-dry weight, dry matter ratio and seedling viability index with the positive effect of low dose. Within the scope of this study, comprehensive information was presented for *L. corniculatus* (cultivar 'Leo'), an important forage plant, in terms of germination and seedling growth under salt stress.

Keywords: *Lotus corniculatus* L., Salinity, Germination, Initial seedling growth stage, *In vitro*

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Öz

Lotus corniculatus L. orta derecede tuza toleranslı, tarımsal ve ekonomik açıdan önemli çok yıllık baklagil yem türlerinden biridir. Bitkilerde aynı türün çeşitlerinin bile tuzluluğa farklı tepkiler verdiği iyi bilinmektedir. Ancak tuzluluk gibi abiyotik stres faktörlerinden etkilenen marjinal tarım arazilerinin kullanımında ak üçgül (*Trifolium repens*) ve yonca (*Medicago sativa* L.) gibi diğer yem bitkilerine göre daha avantajlı olan *L. corniculatus* ve çeşitlerinin tuz tepkisi üzerine yapılan çalışmalar sınırlıdır. Tuz stresi altında, bitkinin hayatta kalmasıyla doğrudan ilgili olan bitki yaşam döngüsünün en önemli aşamaları tohumların çimlenmesi, büyümesi ve canlılığıdır. Bu nedenle bu çalışma, diğer çeşitlere göre daha yüksek tanen içeriğine sahip olduğu bilinen *L. corniculatus* çeşidi 'Leo'nun NaCl türevli tuz stresi altındaki in vitro çimlenme ve büyüme tepkilerini belirlemek amacıyla yapılmıştır. Bu amaçla *L. corniculatus* tohumları 0, 40 ve 80 mM NaCl içeren MS (Murashige ve Skoog/Gamborg) besiyerinde 14 gün kültüre edilmiştir. Tohum çimlenme yüzdesi, ortalama çimlenme süresi, çimlenme oranı indeksi, sürgün-kök uzunluğu, kök-sürgün uzunluğu oranı, sürgün-kök yaş-kuru ağırlığı, sürgün-kök kuru maddesi, kök-sürgün kuru madde oranı, sürgün- kök su içeriği ve fide canlılık indeksi parametreleri ölçülmüştür. Araştırma sonuçlarına göre, uygulanan NaCl uygulamalarında çimlenme yüzdesi değişmemiş ancak çimlenme hızı azalmıştır. Bununla birlikte sürgün uzunluğu azalmış, kök uzunluğu artmıştır. İstatisitiki açıdan sürgün ve kök yaş-kuru ağırlığında önemli bir değişme olmamasına rağmen, 80 mM NaCl uygulamasında her ikisinde de azalış olmuştur. Sürgün ve kök kuru madde oranı artmış su içeriği azalmıştır. Ayrıca, fide canlılık indeksi de azalmıştır. 40 mM NaCl uygulamasında ise düşük doz olumlu etkisi ile sürgün yaş-kuru ağırlık, kuru madde oranı ve fide canlılık indeksinde artış olmuştur. Bu çalışma kapsamında önemli bir yem bitkisi olan *L. corniculatus* (kültivar 'Leo') için tuz stresi altında çimlenme ve fide gelişimi bakımından kapsamlı bilgi sunulmuştur.

Anahtar Kelimeler: *Lotus corniculatus* L., Tuzluluk, Çimlenme, İlk fide büyüme aşaması, In vitro

1. Introduction

Worldwide, millions of hectares of land are too saline to support commercial crop yields, and more land is rendered unproductive every year due to salt buildup. Problems with salinity in agriculture are often limited to dry and semiarid areas when rainfall is insufficient to move salts out of the root zone of the plant (Carter, 1975). When there is an excess of sodium chloride, sodium carbonate, sodium sulfate, or salts of magnesium, it is considered to be a salinity problem since the effect gets more pronounced the more excess there is (Chapman, 1975). Salts like chloride and sulphate make up the majority of soils and groundwater in nature (Tarchoune et al., 2010). The primary salt that causes salt stress is typically NaCl. A wide variety of wild plants are poisoned by sodium ions, whereas certain plants are harmed by high chloride concentrations. Because the low potassium level induced by absorbing more sodium hinders plant growth, plants engage their high affinity system for uptaking potassium when sodium enters the cytoplasm in order to absorb enough of this ion (Azarafshan and Abbaspour, 2014). Saline soil is any soil with water-soluble salt concentrations more than 4 dS m^{-1} (Shokat and Großkinsky, 2019). However, the saline area is divided into four main types based on its salinity, namely very severely salinized (ECe> 16 dS m^{-1}), severely salinized (ECe 8-16 dS m^{-1}), moderately salinized (ECe 4-8 dS m^{-1}), and mildly salinized (ECe 2-4 dS m^{-1}) (Dornburg et al., 2011). Salt harms plants by creating osmotic stress, ion imbalance, and oxidative damage. Plants have developed highly complex processes, including osmotic stress resistance, ion exclusion, and tissue tolerance, in order to survive salt stress (Wang et al., 2021). However, salt stress has an impact on plants by limiting their ability to absorb water, rupturing their biological membranes, causing ionic imbalance, oxidative damage, and nutritional imbalance, slowing down cell division and growth, reducing the rate of photosynthesis, altering their lipid metabolism, and affecting their yield characteristics. Salinity has a significant impact on seed germination, one of the most important stages of plant development (Jaleel et al., 2007). However, the most delicate and crucial stages of most plants' establishment in saline settings are during their seedling establishment (Çakmakçı and Dallar, 2019; Wang et al., 2019; Altuner et al., 2022).

Like many other cultivated plants and/or crops, legume family members, including *Lotus corniculatus* L. (also known Bird's-foot trefoil, BFT), are adversely affected by salinity. *L. corniculatus* a perennial legume forage is regarded as one of the most important forage plants in terms of agriculture due to its many benefits, including its ability to grow in low fertile, acidic, and its anti-bloating properties due to its tannin content, and it is also frequently used to stop roadside erosion (Wang et al., 2021). In temperate places of the world, *L. corniculatus* may eventually take the place of white clover and alfalfa (Savić et al., 2019). Despite all these important features, most *L. corniculatus* are sensitive to salinity (Bao et al., 2014). Under conditions of salt stress, seed germination behavior varies from plant species to species and also significantly from cultivar to cultivar (Munns and Tester, 2008). 'Leo' has a very good seedling vigour and high yielding cultivar (Undersander et al., 1993), but the comprehensive responses to salt stress are not reported yet about this cultivar. There is also a gap in our knowledge in germination and early seedling growth. Therefore, the primary goal of this study was to investigate the effects of salt stress on seed germination and early seedling growth in *L. corniculatus* cultivar 'Leo' under in vitro conditions.

2. Materials and Methods

2.1. Plant Material

In this study, the seeds of the *Lotus corniculatus* L. cv. 'Leo', harvested in 2022 and supplied by Utah State University, Plants, Soils and Climate Department, were used as plant material.

2.2. Plant Tissue Culture and SaltTreatments

The *L. corniculatus* seeds were surface sterilized in 50% commercial bleach (Clorox-USA, containing 8.25% sodium hypochlorite) in which 1 drop of Tween-20 (Acros Organics) was added for 25 minutes and then rinsed 3 times with distilled water. Sterilized seeds were sown on standard MS (Murashige and Skoog/Gamborg) (Gamborg et al., 1968), Plant Media, USA) medium containing 3% sucrose (Research Product International RPI, USA) and 7% agar (Plant Media, USA). For salt stress, seeds were sown in standard MS/Gamborg medium with 40 and 80 mM NaCl. Before autoclaving at 121°C, 7.25 psia for 20 minutes, the pH of the medium was adjusted to 5.7 with 1 M NaOH or HCl. Germination of seeds and subsequent seedling development were carried out at 25±1°C under white fluorescent lamps at an intensity of 30 μmol m^{-2} s^{-1} (PAR) in a photoperiod of 16h light and 8h dark.

2.2. Germination and Growth

When the growing radicle elongated to 0.2 cm, the seed was considered germinated. For 14 days, the proportion of seeds that germinated was recorded every 24 hours (ISTA, 2003). Mean germination time (MGT) was calculated according to Ellis and Roberts (1980). $MGT = \sum Dn/\sum D$, where n is the number of freshly germinated seeds on day D, and D is the number of days since the start of the experiment. The percentage of seeds that germinated after being exposed to salt stress were estimated using the equation:

$$\text{Germination percentage (GP)} = (\text{Number of germinating seeds}/\text{ Total number of seeds}) \times 100$$

(Al-Enezi et al., 2012) (Eq.1).

Growth parameters (length (cm) of shoot and root; fresh and dry weight (mg) of shoot and root) were measured in 14-day-old seedlings (*Figure 1.*). Dry weights were calculated after samples were dried in an oven (VWR Scientific Inc., USA) at 70°C for 48 hours (Beyaz et al., 2011). Water content (WC), dry matter (%) (DM) and vigor index (VI) were calculated according to the following formulas, respectively:

$$\text{Water content (WC)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100$$ (Zheng et al., 2008) (Eq.2).

$$\text{Dry matter (DM)} = (\text{dry weight}/\text{fresh weight}) \times 100$$ (Bres et al., 2022) (Eq.3).

$$\text{Vigor index (VI)} = (\text{average root length} + \text{average hypocotyl length}) \text{ x germination percentage (GP)}$$

(Abdul-Baki and Anderson, 1973) (Eq.4).

Germination rate was expressed as the germination rate index (GRI) according to Maguire (1962):

$$GRI = \Sigma \text{ No of Germinated Seeds}/\Sigma \text{ No of Days}$$ (Eq.5).

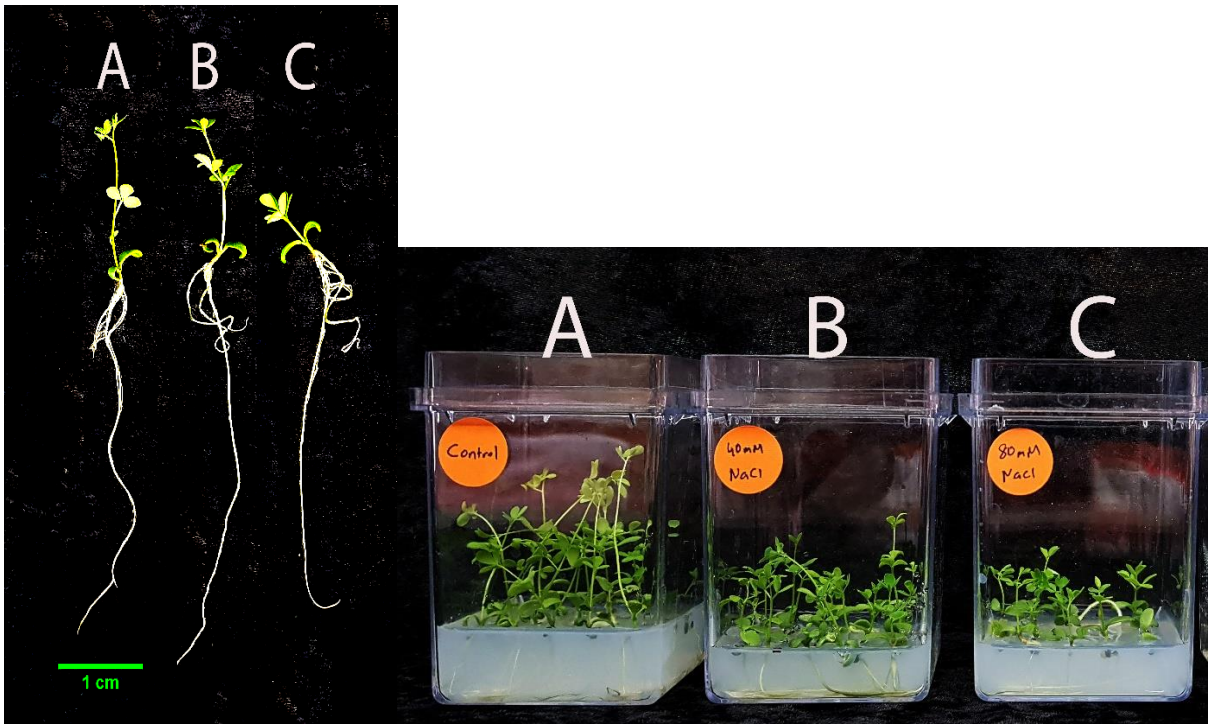


Figure 1. 14-days-old seedlings of Lotus corniculatus L. cultivar 'Leo' at different NaCl concentrations under in vitro conditions. A)0 mM NaCl-control- B) 40 mM NaCl C) 80 mM NaCl.

2.2. Statistical Analysis

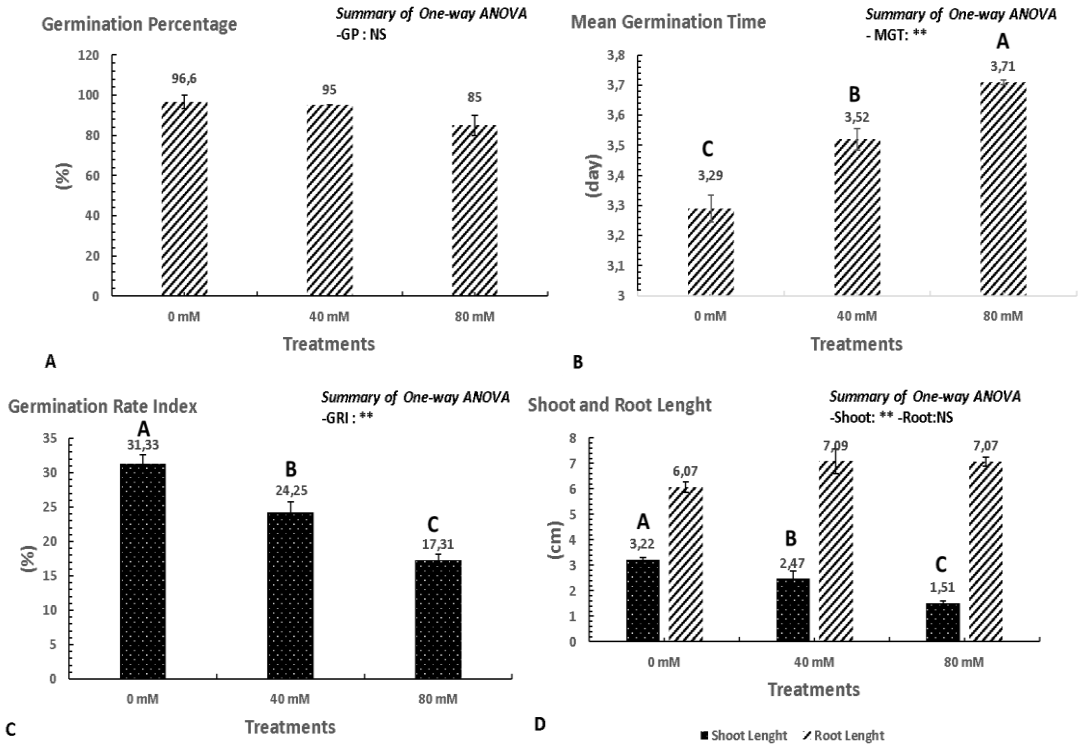
The study was carried out as a completely randomized design with 3 replications. For each treatment, a one-way ANOVA was done using the SPSS statistical program (Version 22). The means were compared using Duncan

multiple range test at $p<0.05$. Before statistical analysis, the data in percentages were transformed using Arcsine transformation (Snedecor and Cochran, 1967).

3. Results

NaCl-induced salt stress had a significant ($p<0.01$) impact on mean germination time and germination rate index (speed of seed germination) but no noticeable effect on germination percentage (*Figure 2A-2B-2C*). The control had the highest percentage of seeds that germinated (96.6%), whereas the 80 mM NaCl treatment had the lowest percentage of seeds that germinated (85%). Under control, 40 mM NaCl, and 80 mM NaCl treatments, the mean germination time was measured at 3.29 days, 3.52 days, and 3.71 days, respectively. Germination rate index was recorded at 31.33%, 24.25% and 17.31% under control, 40 mM NaCl and 80 mM NaCl treatments, respectively. Overall, the germination percentage and germination rate index were reduced by 1.65% and 12.00%, 40% and 44.74%, under 40 mM NaCl and 80 mM NaCl treatments, respectively. On the orher hand, mean germination time was incresed by 6.99% and 12.76% under 40 mM NaCl and 80 mM NaCl treatments, respectively.

The shoot length was statistically significantly ($p<0.01$) affected by the NaCl induced salt stress, while the root length was not affected (*Figure 2D*). Maximum shoot lenght (3.22cm) was recorded in the control, while the lowest shoot lenght (1.51cm) was recorded for 80 mM NaCl treatment. However, root to shoot ratio was significantly ($p<0.01$) affected by NaCl treatments (*Figure 2E*). Maximum root to shoot ratio (1.88%) was recorded in the control, while the lowest shoot lenght (4.68%) was recorded for 80 mM NaCl treatment. Overall, as compared to control, shoot lenght was reduced by 23.29% under 40 mM NaCl, and 53.10% under 80 mM NaCl treatments. However, root to shoot ratio was increased by 52.65% under 40 mM NaCl treatment, and 148.93% under 80 mM NaCl treatment, in comparison with control. Nonsignificant differences were observed among NaCl treatments with respect to shoot and root fresh weight and shoot and root dry weight (*Figure 2F-2G*). However, a significant decrease was observed in total shoot and root fresh weights, depending on the levels of NaCl treatments. Total shoot and root wet weight were recorded as 0.130 mg per plant in the control group, 0.128 mg per plant in the 40 mM NaCl treatment, and 0.106 mg per plant in the 80 mM NaCl treatment. Likewise, a significant decrease was noted in total shoot and root dry weights (excluding 40 mM NaCl treatment) due to the levels of NaCl treatments.



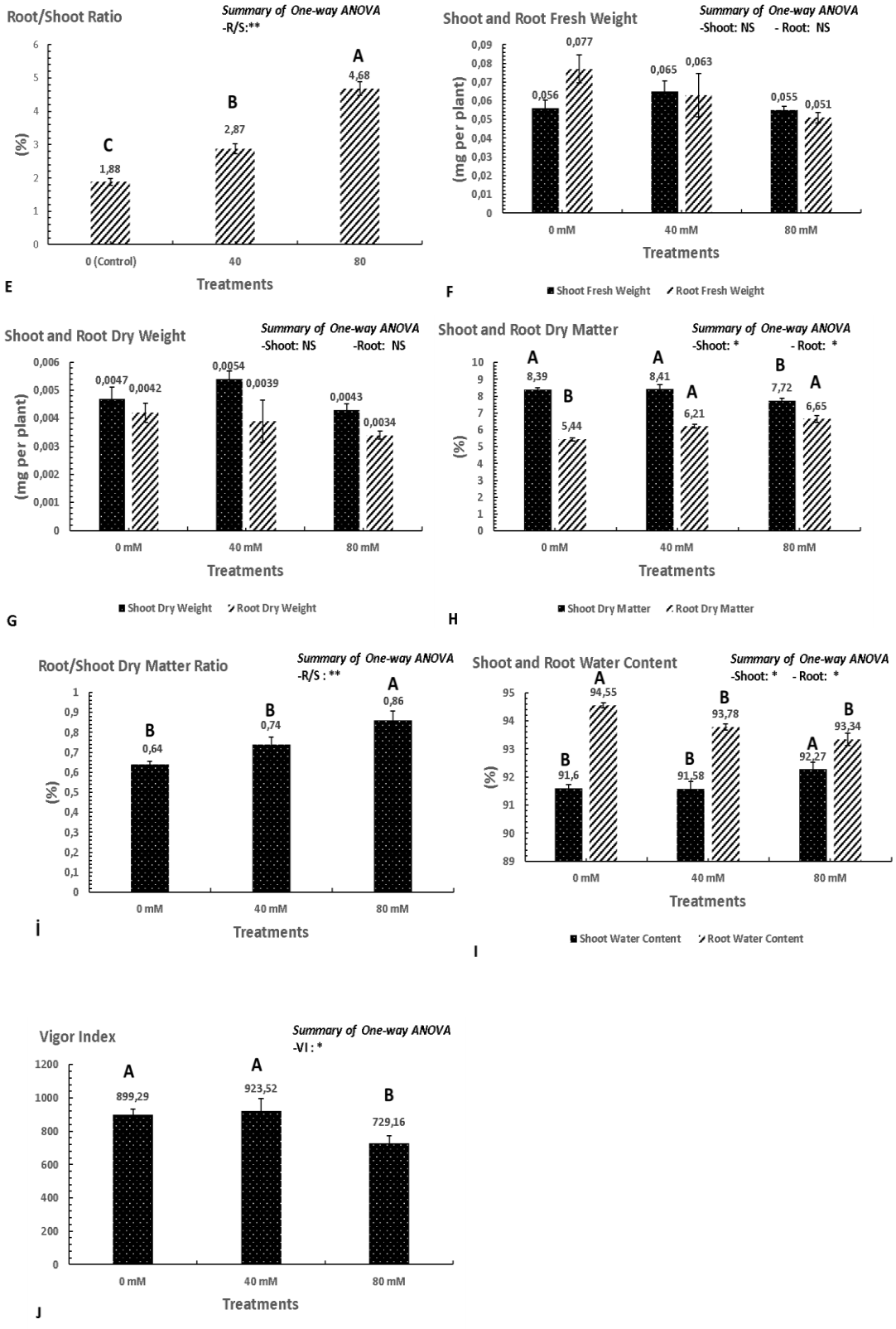


Figure 2. The effect of different levels (0, 40, and 80 mM NaCl) of the NaCl-induced salt stress on germination percentage, mean germination time, germination rate index, shoot and root length, root/shoot ratio, shoot and root fresh weight, shoot and root dry matter, the ratio of root to shoot dry matter, shoot and root water content, and vigor index of 14-days-old BFT seedling. **: p<0.01; *: p<0.05; NS: non-significant.

Total shoot and root wet weight were recorded as 0.0089 mg per plant in the control group, 0.0093 mg per plant in the 40 mM NaCl treatment, and 0.0077 mg per plant in the 80 mM NaCl treatment. Significant differences were found among NaCl treatments in terms of shoot and root dry matter, and also the ratio of root to shoot dry matter (*Figure 2H-2İ*). 8.39%, 8.41% and 7.72% values for shoot dry matter were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively. For root dry matter, the values of 5.44%, 6.21% and 6.65% were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively. The values of 13.83%, 14.62% and 14.37% were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively, when shoot and root dry matter were collected together. Shoot to root dry matter ratio and NaCl treatments showed significant (p<0.01) variations (*Figure 2İ*). Values of 0.64%, 0.74% and 0.86% were recorded in control, 40 mM NaCl treatment and 80 mM NaCl treatment, respectively. In comparison to the control, the ratio of root to shoot dry matter increased by 15.62% under 40 mM NaCl treatment and by 34.37% under 80 mM NaCl treatment. In terms of shoot and root water content, the NaCl treatments showed significant (p<0.05) variations (*Figure 2I*). The highest water content of shoot (92.27%) was obtained in 80 mM NaCl treatment while the lowest water content of shoot (91.60%) was measured under control. However, the highest water content of root (94.55%) was obtained in control while the lowest water content of shoot (93.34%) was measured under 80 mM NaCl treatment. Under 80 mM NaCl treatment, the shoot water content rose by 0.73% in comparison to control. The root water content decreased by 1.17% in the 80 mM NaCl treatment compared to the control.

4. Discussion

Due to the increase in salt of agricultural soils, forage legumes experience yield losses and low nutritional status (Savić et al., 2019). Although salt stress impacts a plant's growth at every stage, most plant species are known to be particularly vulnerable at the seed germination and seedling growth stages (Bybordi, 2010). The study's findings showed that the impacts of salinity levels were significant for mean germination time and germination rate index (germination speed) but not for germination percentage (*Figure 2A-2B-2C*). Specifically, 80 mM NaCl treatment prolonged the mean germination time by 12.7%, and slowed the germination speed by 44.74%. Ion imbalance, osmotic regulatory issues, and finally a decrease in seed water uptake are all caused by high salinity levels (Moss and Hoffman, 1977). Lotus corniculatus is also considered as a germplasm source for salt-tolerant plants (Ünlüsoy et al., 2023). However, the results of this study showed that seeds of *L. corniculatus* cultivar 'Leo' were significantly affected in terms of germination parameter (excluding germination percentage) performance starting from 80 mM NaCl treatment. It is crucial to test a plant's salt tolerance early on since seeds with a faster rate of germination in salty environments may be expected to establish themselves more quickly, producing larger yields (Petrovıć et al., 2016). Therefore, although there is no study on yield and yield components in this study, it can be interpreted that the negative effects of the germination parameters of the 'Leo' cultivar in 80 mM NaC treatment may also affect its yield. It is known that NaCl treatments between 100 mM (~10 dS/m) and 150 mM (~15 dS/m) of legume forage crops, including *Lotus corniculatus*, have negative effects on germination and seedling growth (Beyaz et al. 2011; Azarafshan and Abbaspour, 2014; Beyaz et al., 2018). However, it is well known that even cultivars within the same species can respond differently to many abiotic stress factors such as salinity due to their genotypic structure. Therefore, testing other cultivars of *Lotus corniculatus* for germination under salinity stress will contribute positively to the emergence of an average salt tolerance level. However, the results of this study confirmed the reports of Cokkızgın (2012), Topçu-Demiroğlu and Özkan (2016) and Bhattarai et al. (2020), that germination parameters such as germination percentage, mean germination time and germination rate index (speed of germination) reduced with the increasing salt stress levels in other legumes. According to Jaleel et al. (2007), at lower salt levels, germination was postponed, while at higher salinity regimes, it was suppressed. Therefore, the NaCl treatments applied in this study can be expressed as the low salinity level for the germination of *L. corniculatus* cultivar 'Leo'.

Salt stress negatively affects the growth parameters of plants depending on the applied level and time. In this study, the applied NaCl treatments had significant an impact on growth metrics such as shoot lenght, root to shoot ratio, shoot and root dry matter, the ratio of root to shoot dry matter, shoot and root water content and seedling vigour index (*Figure 2D-2E-2İ-2I-2H-2J*). On the other hand, applied NaCl treatments for parameters fresh and dry weights of shoots and roots had an effect, but this was not statistically significant (*Figure 2G-2F*). A significant decrease (55.10% at 80 mM NaCl) was observed in shoots and very little increase in roots, depending on the increased salt level. However, when looking at root to shoot ratio, there was a significant increase (148.93% ar 80

mM NaCl). The most common morphological sign of saline damage to a plant is slowed growth as a result of cell elongation inhibition (Nieman, 1965). However, under the salinity conditions, as the root to shoot ratio rises, the need to give components to the shoot decreases, which may boost the root's capacity to do so and provide an adaptation benefit (Cheeseman, 1988; Bernstein, 2013). Salt stress may have reduced shoot growth by impairing the efficiency of photosynthetic product transport and assimilation (Jaleel et al., 2007). According to Bernstein (2013), the reduced ability of the shoot to deliver nutrients to the root and growing tissues is one potential drawback of such a transformation, which is expected to have an impact on plant growth and survival, especially under long-term salinization. Therefore, the decrease in shoot length, increase in root length and root shoot ratio indicates that *L. corniculatus* tries to increase its adaptability at the salinity levels applied in the study. In supporting of our observation, Antonelli et al. (2021) reported that the root to shoot ratio of different *L. corniculatus* cultivars increased depending on the increasing salt level at greenhouse conditions. Similarly, Bandeoğlu et al. (2004) reported that salt stress caused a significant decrease in shoot length of lentil seedlings. Fresh and dry weight of shoot and root are important indicators of salinity tolerance of forage plants. NaCl caused an increase in shoot fresh and dry weight with low concentration and a decrease with the highest concentration (*Figure 2F-2G*). On the other hand, root fresh and dry weight decreased depending on the increasing salt level (*Figure 2F-2G*). It is seen that the root fresh and dry weight decreased more than the shoot. From the results of the research, it is understood that the 'Leo' cultivar shows low tolerance in terms of these parameters, even EC 4-8 dS m^{-1}, which are considered moderate salinity levels and applied. Similar to this research findings, Beyaz et al. (2011) reported that fresh-dry weight decreased due to increasing salinity in sainfoin, another important forage crops, in their study *in vitro* study. Uchiya et al. (2016) stated that root dry weight of *L. corniculatus* seedling decrease depend on increased salinity levels (from 0 to 150 mM). Moreover, Bao et al. (2014) noted that depend on increased salt stress (from 0 to 200 mM) shoot dry weight of *L. corniculatus* seedling decreased. The accumulation of dry matter determines the crop yield. Under stress conditions, changes may occur in the distribution of photosynthesis products in plant organs (such as leaf, stem and root) in order to adapt to the stress factor (Jiang et al., 2018). The accumulated shoot and root dry mass of the crops can also be used to study how different stress treatments affect their growth. The results of this study showed that with increasing salinity, the dry matter ratio in the shoot decreased (p<0.05), whereas the dry matter ratio in the root increased (p<0.05) (Figure 2H). However, the ratio of root to shoot dry matter increased with increasing salt levels (*Figure 2İ*). The increase in the dry matter ratio in the root under salt stress may be explained by the change in the allocation of biomass to increase the root resistance in stress tolerance. Similarly, Antonelli et al. (2021) reported that both of shoot and root dry matter of *L. corniculatus* cultivar San Gabriel seedlings decrease under salt stress (150 mM NaCl), but this decreasing is more in shoots. Beyaz et al. (2011) stated that dry matter of sainfoin seedlings increase depend on increasing salt stress (from 0 to 30 dS m^{-1}). Teakle et al. (2006) noted that shoots and roots dry matter of *L. corniculatus* cultivar San Gabriel decrase under salt stress (400 mM NaCl). The main effects of stress are a decrease in water content and damage to cellular membranes, which help the body deal with abiotic stress such as salt and drought (Zhou et al., 2012). The results of this study showed that the water content of the shoot increased, while the water content of the root decreased due to increasing salt levels (*Figure 2I*). The root-water-uptake of plants is constrained by salinity stress, which also affects plant growth (Wang et al., 2012). However, it was observed that the decrease in water content in the root was not very severe at the applied salt levels (40 and 80 mM). As one of the reasons, it can be speculated that the applied salt levels do not cause a severe negative effect on the root in terms of water uptake. Seedling vigor index is an important criterion to understand plant's response to salt stress. In this study, seedling vigour index increased at 40 mM NaCl and decreased at 80 mM NaCl (*Figure 2J*). Generally speaking, we may assume that when plants are exposed to low salt concentrations, the elongation of the stem may cause osmotic adjustment activity, which may enhance growth (Abdul-Qados, 2011). The seedling vigour index was calculated on the basis of seed germination and root and stem (shoot) growth. When shoot and root length are collected together, it is seen that there is an increase at 40 mM compared to the control group (*Figure 2D*). Therefore, it can be said that this increase in 40 mM NaCl is due to the low dose positive effect. In general, it has been reported in the literature (Khajeh-Hosseini et al., 2003; Cokkızgın, 2012; Dehnavi et al., 2020) that the seedling vigor index decreased in other crops due to increased salinity levels, similar to the results of this study.

5. Conclusions

In conclusion, this study demonstrated that applied NaCl treatments did not have a significant effect on the germination percentage of *L. corniculatus* cultivar 'Leo', however, highest NaCl treatment decreased the speed of germination (44.74%). However, 80 mM NaCl treatment resulted in a 53.10% decrease in shoot length and 16.47% increase in a seedling root length. In addition, 80 mM NaCl treatment decreased the total (shoot + root) fresh weight (20.30%), total dry weight (13.48%) and total water content (0.29%) and increased the total dry matter ratio (3.90%) in a seedling. However, it caused a decrease in the seedling survival index (17.15 %). Therefore, when the germination and growth parameters are evaluated cumulatively, it can be said that the cultivar 'Leo', which is a forage plant, suffered a significant loss of yield at the salinity level of 80 mM NaCl (~ 8 dS/m). However, it should also be tested at higher levels. In addition, it is suggested that in future studies, salt stress in nature should be tested for the drought cultivar 'Leo', which is known as the sibling stress factor.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Beyaz, R.; Design: Beyaz, R.; Data Collection or Processing: Beyaz, R.; Statistical Analyses: Kazankaya, A.; Literature Search: Beyaz, R.; Writing, Review and Editing: Beyaz, R., Kazankaya, A.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Influence of Carbon Source, Nitrogen and PEG on Caffeic Acid Derivatives Production in Callus Cultures of *Echinacea purpurea* L.

Echinacea purpurea L. Kallus Kültürlerinde Karbon Kaynağı, Nitrojen ve PEG'in Kafeik Asit Türevlerinin Üretimine Etkisi

Münüre TANUR ERKOYUNCU[1], Mustafa YORGANCILAR[2*]

Abstract

This study aimed to determine the effects of polyethylene glycol (PEG) as an abiotic elicitor and nutritional factors (different ammonium/nitrate ratios, carbon source and amount) in the culture medium on the production of Caffeic Acid Derivatives (CADs) in callus cultures of *Echinacea purpurea* L. Petiole and root explants were cultured on MS medium modified in terms of different types (sucrose and maltose) and amounts (sucrose 15, 45, 60 g l^{-1}, and maltose 15, 30, 45, 60 g l^{-1}) of carbon source, different concentrations (5, 10, 15 g l^{-1}) of PEG and ammonium nitrate ratios (0:35, 5:25, 15:15, 35:0 mM). The amounts of CADs in the callus obtained at the end of the 10-week culture period were analysed. In both explant types, the highest amount of CADs were obtained from the medium containing 15 g l^{-1} sucrose and 15 or 30 g l^{-1} maltose applications, while the highest amount of CADs was obtained in the medium containing 0:35 mM ammonium/nitrate in nitrogen applications. While the highest amount of CADs in root explant was obtained from the medium containing 10 g l^{-1} PEG applications, CADs content could not be obtained in petiole explant. As a result, the highest amounts of caftaric, chlorogenic, caffeic, and chicoric acids (respectively, 9.38, 0.71, 0.29, and 34.77 mg g^{-1}) were determined at callus obtained from root explant cultured on MS medium containing 30 g l^{-1} sucrose and 0:35 mM ammonium/nitrate. In conclusion, optimization of culture conditions and different elicitor applications were made to increase secondary metabolite content in *E. purpurea* L. under *in vitro* conditions and the results obtained were presented comparatively.

Keywords: Sucrose, Maltose, Nitrogen, PEG, Caffeic acid derivatives, *Echinacea purpurea* L., HPLC

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Öz

Bu çalışmada, abiyotik bir elisitör olarak polietilen glikolün (PEG) ve besin ortamındaki farklı beslenme faktörlerinin (farklı amonyum/nitrat oranları, karbon kaynağı ve miktarı) *Echinacea purpurea* L. kallus kültürlerinde kafeik asit türevlerinin miktarlarına etkisinin belirlenmesi amaçlanmıştır. Petiyol ve kök eksplantları karbon kaynağının farklı türleri (sakkaroz ve maltoz) ve miktarları (sakkaroz 15, 45, 60 g l^{-1} ve maltoz 15, 30, 45, 60 g l^{-1}), farklı amonyum nitrat oranları (0:35, 5:25, 15:15, 35:0 mM) ve farklı konsantrasyonlarda (5, 10, 15 g l^{-1}) PEG içeren MS besin ortamlarında 10 hafta boyunca kültür alınmış ve kültür süresi sonunda elde edilen kalluslarda kaftarik, klorojenik, kafeik ve kikorik asit miktarları tespit edilmiştir. Her iki eksplant türünde de en yüksek kafeik asit türevlerinin miktarları, karbon kaynağı uygulamalarında, 15 g l^{-1} sakkaroz ile 15 ve 30 g l^{-1} maltoz; nitrojen uygulamalarında ise 0:35 mM amonyum/nitrat içeren besin ortamlarında uyarılan kalluslarda tespit edilmiştir. PEG uygulamalarında ise, en yüksek miktarlarda kafeik asit türevleri, 10 g l^{-1} PEG içeren besin ortamından kök eksplantından uyarılan kalluslarda elde edilirken, petiyol eksplantından elde edilen kallus dokularında kafeik asit türevlerinden hiçbiri tespit edilememiştir. *E. purpurea* L kallus kültürlerinde, kafeik asit türevlerinin miktarını artırmak için, *in vitro* şartlar altında, abiyotik elisitör olarak PEG ve farklı beslenme faktörlerinin optimizasyonu araştırıldığı bu çalışmada, elde edilen sonuçlar karşılaştırmalı olarak sunulmuştur. Tüm uygulamalar değerlendirildiğinde; sonuç olarak, en yüksek kaftarik, klorojenik, kafeik ve kikorik asit miktarları (sırasıyla 9.38, 0.71, 0.29 ve 34.77 mg g^{-1}) 0:35 mM amonyum/nitrat ve 30 g l^{-1} sakkaroz içeren MS ortamında kültüre alınan kök eksplantından elde edilen kallus dokularında tespit edilmiştir.

Anahtar Kelimeler: Sakkaroz, Maltoz, Azot, PEG, Kafeik asit türevleri, *Echinacea purpurea* L., HPLC

1. Introduction

Echinacea purpurea L. is a medicinal herb mostly used to reduce the symptoms and duration of colds, influenza, and upper respiratory tract infections (Rajasekaran et al., 2013; Shahrajabian et al., 2020). It is rich in medicinally important secondary metabolites, such as alkamides (alkylamides), phenolic compounds (caffeic acid derivatives-CADs), glycoproteins and polysaccharides (Bruni et al., 2018). In particular, CADs namely cichoric acid, chlorogenic acid, caftaric acid, caffeic acid and echinacoside are characteristic phenolic compounds of *Echinacea* spp. and has important pharmacological properties (Murthy et al., 2014; Rady et al., 2018). Cichoric acid, caftaric acid and chlorogenic acid are the most studied CADs due to their anticancer, antiviral, anti-inflammatory, and antioxidant effects (Lin et al., 1999; Thygesen et al., 2007; Pleschka et al., 2009; Aarland et al., 2017; Chiou et al., 2017; Sharif et al., 2021)

Plant cell/tissue culture is a promising alternative technique to produce plant secondary metabolites. With cell/tissue culture techniques, secondary metabolites are produced in a short time independent of environmental factors (i.e. climate, geographical difficulties, seasonal restrictions, diseases and pests). At the same time, it is possible to increase the production of secondary metabolites with plant cell/tissue culture techniques, by optimizing the culture conditions (medium content, carbon source, growth regulators etc.) and elicitor sources (Rao and Ravishankar, 2002; Sökmen and Gürel, 2002; Tanur Erkoyuncu and Yorgancılar, 2015).

Secondary metabolites can be produced by using different biotechnological approaches, such as cell/callus culture techniques (Ram et al., 2013; Royandazagh and Pehlivan, 2016). Callus cultures are usually effected by various *in vitro* conditions including explant type, plant growth regulators, nutrient supply, carbohydrate source and other environmental conditions (Pehlivanet al., 2017; Khan et al., 2018). Optimization of callus cultures and different elicitor sources should be investigated in order to both increase biomass and increase the production of secondary metabolites. There are various studies on the optimization of *in vitro* culture conditions (different plant growth regulators types and concentrations) and different elicitor applications (methyl jasmonate, silver nanoparticle, yeast extract and fungal) to increase secondary metabolite production in *E. purpurea* L. (Ramezannezhad et al., 2019; Erkoyuncu and Yorgancilar, 2021; Demirci, 2022; Elshahawy et al., 2022), However, in different *in vitro* cultures (callus, shoot, cell suspension cultures) of *E. purpurea* L. research could be extended to increase both biomass production and the amount of CADs.

This study aims to investigate the effects of polyethylene glycol (PEG) as an abiotic elicitor and nutritional factors (different ammonium/nitrate ratios, carbon source and amount) in the culture medium on the production of CADs in callus cultures of *E. purpurea* L.

2. Materials and Methods

2.1. Sterilization and culture of seeds

Echinacea purpurea seeds obtained from plants cultivated in Selçuk University, Konya, Türkiye were used as starting material and sterilization procedure were applied as specified in Erkoyuncu and Yorgancilar (2021). Sterile seeds were cultured on MS (Murashige and Skoog, 1962) medium without plant growth regulators and 8-week-old sterile seedlings were used as explant sources throughout the study. All cultures were incubated in a growth cabinet (Sanyo: MLR-351H) at 24±2 ºC, 65 % humidity, 5 LS light intensity, and a photoperiod of 16/8 hours.

2.2. Preparation of different carbon sources and amount, ammonium/nitrate ratio and PEG containing medium

As a result of a previous study by Erkoyuncu and Yorgancılar, 2021, the highest CADs amounts in callus cultures of *E. purpurea* were obtained from callus induction from petiole and root explants. In this study, for optimum callus induction from petiole and root explant, the best combination of growth regulators (1.0 mg l^{-1} naphthalene acetic acid (NAA)+0.5 mg l^{-1} thidiazuron (TDZ); 0.5 mg l^{-1} NAA+0.5 mg l^{-1} benzylaminopurine (BAP), respectively), culture time (10 weeks), solid MS medium containing 30 g l^{-1} sucrose and 8 g l^{-1} agar were determined.

Both types of explants (root and petiole) were cultured on MS media containing the determined growth regulators combinations, modified in terms of carbon source and amounts, ammonium/nitrate ratios and PEG applications at different concentrations. Experiments were set up with 20 replications, 10 explants per petri dish, in order to obtain

sufficient material for CADs analysis. Cultures were maintained for a 10-week culture period under controlled conditions at 24±2 ºC, 65 % humidity, 5 LS light intensity, 16/8 photoperiod *(Figure 1).*

Different carbon sources and amount: Petiole and root explants were cultured on MS media containing suitable growth regulators combinations (1.0 mg l^{-1} NAA+0.5 mg l^{-1}TDZ; 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP, respectively) and different types and amount of carbon sources (sucrose 15, 45, 60 g l^{-1}, and maltose 15, 30, 45, 60 g l^{-1}). Since 30 g l^{-1} sucrose was used as a standard in previous studies, it was excluded in this study.

Different ammonium/nitrate ratios: Petiole and root explants were cultured on MS medium was modified in terms of ammonium/nitrate ratio (0:35, 5:25, 15:15, 35:0 mM) containing suitable growth regulators combinations (1.0 mg l^{-1} NAA+0.5 mg l^{-1} TDZ; 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP, respectively).

Polyethylene glycol (PEG): Petiole and root explants were cultured in MS media containing suitable growth regulators combinations (1.0 mg l^{-1} NAA+0.5 mg l^{-1} TDZ; 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP, respectively) and different concentrations (5, 10, 15 g l^{-1}) PEG.

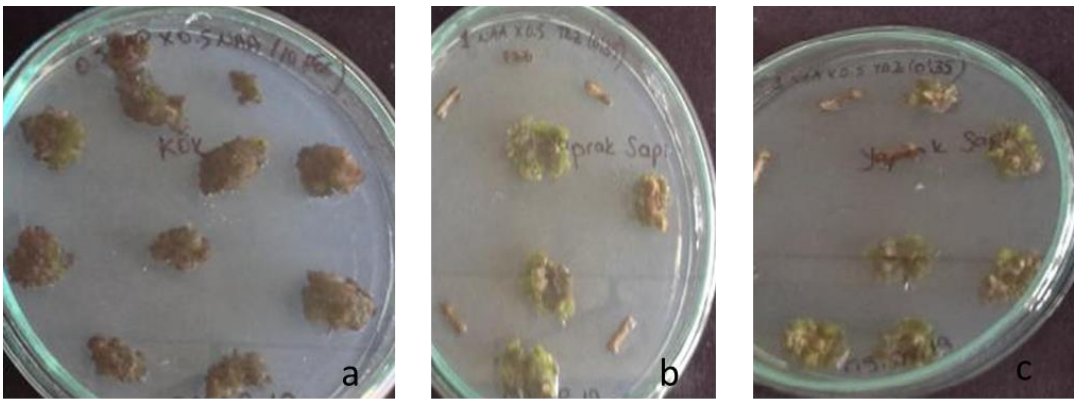

Figure 1. Callus tissues of different applications a. callus from root explant b. callus from petiole explant c. callus from petiole explant

2.3. Quantification of caffeic acid derivates

Cichoric, chlorogenic, caftaric and caffeic acid contents of 10 weeks old calli from all treatments were analysed. Following oven-drying for 1 d at 37 ºC, plant materials ground into fine powders (0.2 g) were extracted in an ultrasonic bath with 8 ml of methanol 70 % (v/v) for 15 min. The extracts were diluted with methanol 70 % (v/v) to make up the volume to 10 ml, filtered through a 0.45µm membrane filter and transferred to vials (Taha et al., 2009).

Standard stock solutions related CADs were prepared at 1, 5, 10, 25, 50 and 100 ppm, and were run at HPLC, the retention times of the standards were identified, and the calibration graphic was created with the absorption values read against the concentration values. According to these graphics, R2 ~ 0.99, and the results were evaluated according to the formulas in the graphics (*Figure 2*).

The HPLC–DAD method was employed for the analyses of caffeic acid derivates in the extracts. All the specifics of HPLC–DAD analyses, equipment's, and conditions were according to Erkoyuncu and Yorgancilar (2021).

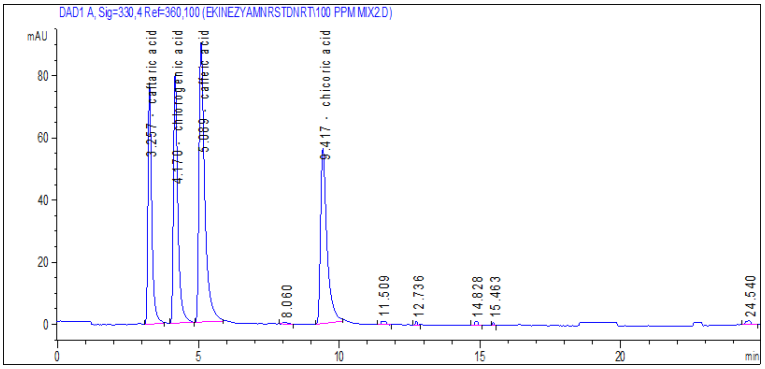

Figure 2. Chromatogram showing the separation times of the mixture of caffeic acid derivatives

3. Results and Discussion

3.1. The effect of carbon sources and amounts on the accumulation of CADs.

In order to determine the effect of the different carbon source on the amount of secondary metabolites, MS medium was modified in terms of sucrose (15, 45, 60 g l^{-1}) and maltose (15, 30, 45, 60 g l^{-1}) containing the growth regulator with the best callus formation (1.0 mg l^{-1} NAA+0.5 mg l^{-1} TDZ for petiole, and 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP for root explants) was reported (Erkoyuncu and Yorgancilar, 2021). The amounts of caffeic acid derivatives were analysed at the callus tissues obtained at the end of the 10-week culture period in both explant types. Dry weights (mg/calli) and content of CADs of calli developed in the applications are given in *Table 1* and *2*.

Table 1. Quantities of CADs in calli obtained from different sucrose levels (mg g^{-1})

Explant sources	Sucrose (g l^{-1})	Callus dry weight (mg/calli)	Caffeic acid derivatives (mg g^{-1})			
			Caftaric	Chlorogenic	Caffeic	Chicoric
Root derived	**15**	6.9	**0.83±0.02**	**0.16±0.05**	-	**2.57±0.24**
	45	7.1	0.14±0.03	-	-	0.40±0.01
	60	8.6	-	-	-	-
Petiole derived	**15**	4.6	**0.54±0.02**	**0.21±0.01**	-	**1.27±0.04**
	45	6.9	-	-	-	-
	60	5.4	-	-	-	0.09

When *Table 1* is examined, the highest amounts of caftaric (0.83 mg g^{-1}), chlorogenic (0.16 mg g^{-1}), and chicoric (2.57 mg g^{-1}) acids were detected in callus tissues obtained on MS medium containing 15 g l^{-1} sucrose from root explant. Similarly, the highest amounts of caftaric (0.54 mg g^{-1}), chlorogenic (0.21 mg g^{-1}), and chicoric (1.27 mg g^{-1}) acids were detected 15 g l^{-1} sucrose from petiole explants.

According to *Table 2*, the highest amounts of caftaric (1.28 mg g^{-1}), chlorogenic (0.37 mg g^{-1}), caffeic (0.10 mg g^{-1}), and chicoric (3.76 mg g^{-1}) acids were detected in callus tissues obtained after 10 weeks of culture MS medium containing 1.0 mg l^{-1} NAA+0.5 mg l^{-1} TDZ and 15 or 30 g l^{-1} maltose from root explants. Similarly, the highest amounts of caftaric (0.96 mg g^{-1}), chlorogenic (0.59 mg g^{-1}), caffeic (0.12 mg g^{-1}), and chicoric (3.75 mg g^{-1}) acids were detected 15 g l^{-1} maltose from petiole explants.

Table 2. Quantities of CADs in calli obtained from different maltose levels (mg g^{-1})

Explant sources	Maltose (g l^{-1})	Callus dry weight (mg/calli)	Caffeic acid derivatives (mg g^{-1})			
			Caftaric	Chlorogenic	Caffeic	Chicoric
Root derived	**15**	16.9	1.24±0.01	0.37±0.25	0.11±0.08	2.69±0.05
	30	12.3	**1.28±0.05**	**0.20±0.08**	**0.10±0.10**	**3.76±0.02**
	45	9.4	0.30±0.07	0.06±0.01	0.06±0.14	0.33±0.03
	60	29.2	0.30±0.01	-	-	0.33±0.04
Petiole derived	**15**	20.4	**0.96±0.05**	**0.59±0.20**	**0.12±0.01**	**3.75±0.05**
	30	31.5	0.57±0.08	0.18±0.01	0.06±0.01	1.16±0.04
	45	27.4	0.85±0.09	0.15±0.08	0.06±0.02	2.30±0.10
	60	20.0	0.90±0.10	0.55±0.06	0.09±0.03	1.73±0.05

Caffeic acid derivatives could not be detected in callus tissues developed from 45 and 60 mg l^{-1} sucrose applications in almost both explant types. For both explant types, the efficiency of using maltose was lower than sucrose in terms of amounts of caffeic acid derivatives. As can be understood from here, 15 or 30 g l^{-1} sucrose or maltose can be recommended for further studies for the production of CADs in callus culture of *E. purpurea* L. Erkoyuncu and Yorgancilar (2021) reported that they used 30 g l^{-1} sucrose when determining the best culture medium for callus development and production of caffeic acid derivatives.

Different carbon sources such as sucrose, maltose, fructose and glucose are used separately or in combination to support the growth of cell and tissue cultures (Kretzschmar et al., 2007). In addition to their effects on growth, carbon

sources play an active role in the biosynthetic pathways of many compounds by regulating the expression of a significant number of genes. (Ali et al., 2016). Moreover, in *in vitro* cultures, carbon sources are also commonly used to generate osmotic stress factors, so it is very difficult to separate the osmotic stress effect and nutritional role of carbon sources (Liu and Cheng, 2008). Although the osmotic stress applied by different carbon sources varies depending on the plant species and culture system, it has been reported in various studies that it can lead to the production of biomass and secondary metabolites (Suan et al., 2011). In our study, it was determined that high concentrations of sucrose and maltose applications in the medium did not have a positive effect on the amounts of caffeic acid derivatives in both explant types. Among the carbon sources, the highest amounts of caftaric, chlorogenic, caffeic and chicoric acids were obtained in both explant types in medium containing 30 g l^{-1} sucrose.

Similar to our results, Liu et al. (2006) investigated the capacity of *E. purpurea* L. to produce caffeic acid derivatives in hairy root cultures, the highest biomass and amounts of chicoric acid (19.21 mg g^{-1}), caftaric acid (3.56 mg g^{-1}) and chlorogenic acid (0.93 mg g^{-1}) were obtained in MS medium containing 30 g l^{-1} sucrose.

Romero et al. (2009), in their study investigating alkamide production from *in vitro* cultures of three different *Echinacea* species, were reported ½ B5 medium containing 3% (30 g l^{-1}) sucrose was twice as effective as medium containing 1%, 2%, 4%, 5% (respectively, 10, 20, 40, and 50 g l^{-1}). The effectiveness of carbon source type and concentration in the medium varies depending on the plant species, but is also affected by other chemical compounds of the medium.

Wu et al. (2006), in a study investigating the effects of different auxin types (IAA, IBA, NAA) and concentrations (1.0, 2.0, 4.0, 6.0 mg l^{-1}) and different sucrose (1%, 3%, 5%, 7%, 9%), ammonium/nitrate ratio (0:40, 0:35, 0:30, 5:25, 10:20, 15:15, 20:10, 25:5, 30:0), medium strength (1/4, 1/2, 3/4, 1/1, 3/2, 2/2) and pH (4, 5, 6, 7, 8, 9) levelson biomass increase, total phenol and flavonoid accumulation in adventitious root culture of *E. angustifolia*, the highest biomass increase and total phenol and flavonoid content, obtained from 1/2 MS medium containing 5% sucrose, 5:25 (mM) ammonium/nitrate, pH:6.0., 2 mg l^{-1} IBA.

Cui et al. (2013) in a similar study investigating the effects of different sucrose concentrations (0 %, 1 %, 3 %, 5 %, 7 %, 9 %) on the biomass increase and accumulation of caffeic acid derivatives in adventive root cultures of *E. angustifolia*, the highest biomass and amounts of chlorogenic acid (2.26 mg g^{-1}), echinacoside (4.66 mg g^{-1}), cynarine (1.57 mg g^{-1}) and chicoric acid (1.57 mg g^{-1}) were determined in ¼ MS medium containing 5% sucrose.

These findings reveal that many factors such as the chemical composition of the medium, the type and concentrations of growth regulators used, culture type, plant species and explant type should be evaluated together in order to obtain and increase secondary metabolite production *in vitro*. Therefore, optimization of *in vitro* culture conditions is very important. In addition, studies on different medicinal plant species carried out for similar purposes also support our findings. On the other hand, Khan et al. (2018) investigated the effects of carbon sources of different types (sucrose, maltose, glucose and fructose) and concentrations (1 %, 3 % and 5 %) on biomass and secondary metabolite production in Fagonia indica callus cultures, with the highest biomass increase. Total phenol and flavonoid contents were determined in calli obtained from medium containing 3 % sucrose and 3 % maltose, respectively.

3.2. The effect of different nitrogen (ammonium/nitrate ratios) applications on the accumulation of CADs

In order to determine the effect of the different nitrogen source on the amount of caffeic acid derivatives, root and petiole explants of *E. purpurea* were cultured on MS medium with modified 0:35, 5:25, 15:15, 35:0 mM ammonium/nitrate ratio. In both explant types, callus stimulation did not occur in medium containing 35:0 mM ammonium/nitrate ratio, while healthy calli were obtained in other applications. The amounts of caffeic acid derivatives in the callus tissues obtained at the end of the 10-week culture period were analysed and the values obtained are given in the *Table 3*.

The highest amounts of caftaric, chlorogenic, caffeic and chicoric acids (respectively, 9.39, 0.71, 0.29, and 34.77 mg g^{-1}) were determined in callus tissues obtained from root explant cultured in MS medium containing 0:35 mM ammonium/nitrate. The highest amounts of caffeic acid derivatives in the petiole explant were also obtained from the 0:35 mM ammonium/nitrate (*Figure 3*).

As a result, the highest amounts of caftaric, chlorogenic, caffeic and chicoric acids were determined in callus tissues obtained from root explant after 10 weeks of culture in MS medium containing 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP and 0:35 mM ammonium/nitrate ratio.

Table 3. Quantities of CADs in calli obtained from different ammonium/nitrate ratios (mg g^{-1})

Explant sources	Ammonium/Nitrate rate (mM)	Callus dry weight (mg/calli)	Caffeic acid derivatives (mg g^{-1})			
			Caftaric	Chlorogenic	Caffeic	Chicoric
Root derived	00:35	10.4	**9.38±0.10**	**0.71±0.04**	**0.29±0.30**	**34.77±0.09**
	15:15	4.6	0.09±0.01	0.09±0.09	0.08±0.25	0.22±0.01
	05:25	9.5	1.04±0.02	-	0.08±0.12	1.61±0.03
Petiole derived	00:35	61.4	**4.51±0.05**	**0.67±0.01**	**0.25±0.04**	**7.93±0.78**
	15:15	8.1	-	0.08±0.08	-	-
	05:25	11.5	3.37±0.09	0.39±0.09	0.07±0.15	6.78±0.05

Nitrogen sources are very important in the medium for the synthesis of secondary metabolites *in vitro*. In particular, the ratio of NH4+ to NO3- in the medium affects not only the growth of plant cell cultures (Veliky and Rose, 1973), but also the production of secondary metabolites (Smetanska, 2008). In our study, the highest amounts of caffeic acid derivatives were obtained in the two different explant types of *E. purpurea*, growth regulator combinations with the best callus development and 0:35 mM ammonium/nitrate ratio.

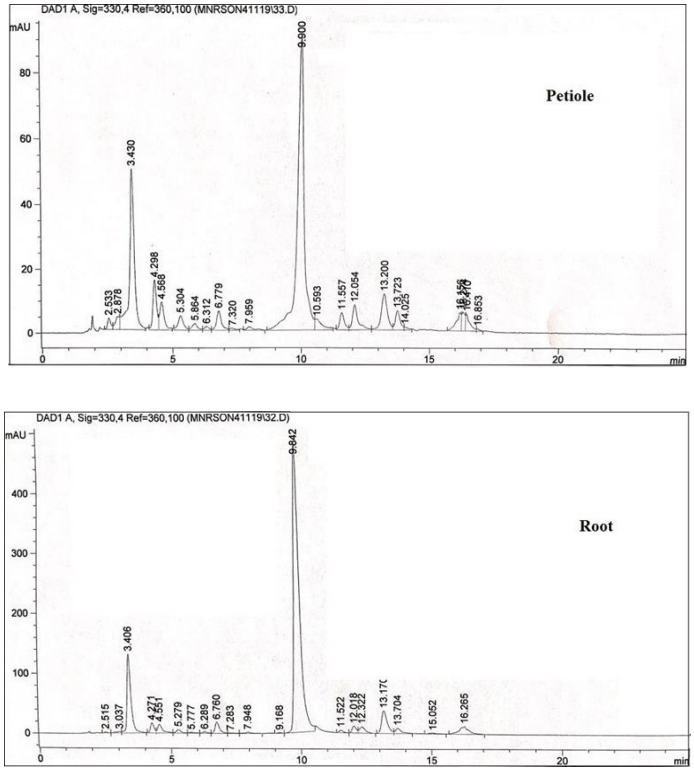

Figure 3. HPLC chromatogram of callus tissues obtained in 0:35 amonium/nitrate

Wu et al. (2006) obtained the highest total phenol and flavonoid content in adventive root culture of *E. angustifolia* in ½ MS medium containing 5:25 mM ammonium/nitrate, 2.0 mg l^{-1} IBA. For a similar purpose, Lee et al. (2011) examined the amount of secondary metabolites in MS medium containing different ammonium/nitrate ratios and in mulberry callus cultures, they found the highest amount of rutin in the medium containing 34/66 ammonium/nitrate and 5.0 mg l^{-1} IAA. Cui et al. (2010), obtained the highest hypersin content of 0:30 mM ammonium/nitrate, and total phenol and flavonoid content in ½ MS medium containing 10:20 mM

ammonium/nitrate in St. John's Wort adventitious root cultures. In our results, the highest amounts of caftaric, chlorogenic, caffeic, and chicoric acids were found in callus tissues obtained from root explant after 10 weeks of culture period on MS medium containing 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP and 0:35 mM ammonium/nitrate ratio. The difference between these studies is due to the genotype, which clearly shows that each genotype has its own specific response. The findings revealed that medium optimization is very important in secondary metabolite production *in vitro* and optimization conditions specific to each genotype should be determined.

3.3. The effect of different Polyethylene glycol (PEG) applications on the accumulation of CADs

To determine the effect of drought stress on the amount of caffeic acid derivatives, root and petiole explants of *E. purpurea* L. were cultured on MS medium containing the growth regulator with the best callus formation and different polyethylene glycol (PEG) concentrations (5, 10, 15 g l^{-1}). The amounts of caffeic acid derivatives were analysed at the callus tissues obtained at the end of the 10-week culture period in both explant types, but none of the caffeic acid derivatives could be detected in any of the callus tissues obtained from the petiole explant. For this reason, caffeic acid derivatives determined only at calli obtained from root explants are given in *Table 4*.

Table 4. Quantities of CADs in calli obtained from different PEG concentrations (mg g^{-1})

PEG (g l^{-1})	Callus dry weight (mg/calli)	Caffeic acid derivatives (mg g^{-1})			
		Caftaric	Chlorogenic	Caffeic	Chicoric
5	14.4	0.34±0.01	0.05±0.01	-	0.60±0.17
10	**27.3**	**1.88±0.12**	**0.58±0.05**	**0.07±0.01**	**6.07±0.08**
15	26.3	0.10±0.01	-	-	0.13±0.05

The highest amounts of caftaric (1.88 mg g^{-1}), chlorogenic (0.58 mg g^{-1}), caffeic (0.07 mg g^{-1}) and chicoric (6.07 mg g^{-1}) acids were detected at the callus tissues obtained from the root explant after 10 weeks of culture on MS medium containing 0.5 mg l^{-1} NAA+0.5 mg l^{-1} BAP, 30 g l^{-1} sucrose and 10 g l^{-1} PEG.

Water stress is one of the most important environmental stresses that can regulate the growth and development of plants, limit plant production, and change the physiological and biochemical properties of plants. It is known that water stress increases the production of secondary metabolites as an abiotic elicitor in plants (Zobayed et al., 2007). PEG is an osmotic agent that is used in many plants to induce water stress and cannot be taken up by the plant (Lemcoff et al., 2006). According to study was conducted on *Hypericum perforatum* by Pavlik et al. (2007) researches were studied the effect of PEG (1.25, 2.5, 5, 10, 15 g l^{-1}) and sucrose (10, 20, 30 g l^{-1}) on secondary metabolite amount. At the mentioned study it was found that mostly low concentrations of PEG (1.25 and 5 g l^{-1}) have increased the production of hypericin and hyperforin. In our study, unlike this, 10 g l^{-1} PEG application was more effective. Similarly, Yamaner and Erdag (2013) were reported that *Hypericum adenotrichum in vitro* shoot cultures obtained on modified MS medium containing different concentrations of PEG (2.5, 10, 15 g l^{-1}) during different culture periods, in 10 g l^{-1} PEG application for 15 days. They reported that the amounts of hypericin (2.1 times) and pseudohypericin (2.3 times) increased at the end of the culture period. Osmotic stress caused by PEG application increased secondary metabolite production in many plants; Ex. iridoid glycosides (catalpol, harpagoside, aucubin and harpagide) in the roots of *Scrophularia ningpoensis* Wang et al. (2010), production of paclitaxel in *Taxus chinensis* cell suspensions Kim et al. (2001) and phenethanoid glycosylates in *Cistanche deserticola* cell cultures Liu and Cheng (2008) were increased by PEG applications. However, in order to increase secondary metabolite production by PEG application *in vitro*, it is necessary to determine the optimum PEG concentration for each plant species and explant type.

4. Conclusion

Optimization of culture conditions and different elicitor applications were made to increase secondary metabolite content in *E. purpurea* L. under *in vitro* conditions and the results obtained were presented comparatively.

The effect of the changes in the nutritional factors (nitrogen amount, type and amount of carbon source) in the medium and the abiotic elicitor (drought) formed in the medium on the amounts of caffeic acid derivatives were determined.

Among these applications, the highest amounts of caftaric acid (9.38 mg g^{-1}), chlorogenic acid (0.71 mg g^{-1}), caffeic acid (0.29 mg g^{-1}) and chicoric acid (34.77 mg g^{-1}), it was reached in callus tissues obtained from root explant at the end of 10-week culture period in medium containing 0:35 mM ammonium/nitrate and 30 g l^{-1} sucrose.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Tanur Erkoyuncu M., Yorgancılar M.; Design Tanur Erkoyuncu M., Yorgancılar M.; Data Collection or Processing: Tanur Erkoyuncu M.; Statistical Analyses: Tanur Erkoyuncu M., Yorgancılar M.; Literature Search: Tanur Erkoyuncu M.; Writing, Review and Editing: Tanur Erkoyuncu M., Yorgancılar M.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Efficiency of GNSS-based Tractor Auto Steering for the Uniformity of Pass-to-Pass Plant Inter-Row Spacing*

GNSS Esaslı Traktör Otomatik Dümenleme Sistemlerinin Ekim İşleminde Paralel Geçişlerde Sıra Arası Mesafe Düzgünlüğüne Etkisi

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Abstract

Precision agriculture (PA) includes advanced technologies to increase efficiency and profitability of agricultural operations from tillage to harvest and offers sustainability of the natural resources and the environment. Automatic steering (AS) is the mostly-used PA technology in the world and in Türkiye providing many benefits. It has potential for efficient and sustainable agronomic practices including soil ridge tillage and sowing. Adequate spacing is needed to provide equal living area for each plant in sowing. Thus, in mechanized planting, pass-to-pass plant inter-row spacing (PIRS) should be equal in parallel passes. Research on the benefits of the AS for providing uniform PIRS in sowing is very limited. This work aimed to appraise the pass-to-pass PIRS deviations in planting with GNSS-based AS with three signal correction sources (RTK, CORS, SBAS) and without AS (manual steering) for comparison. The data were obtained from 24 farmer fields (cotton and corn) with PIRS set values of 70-75 cm located in the Cukurova region of Türkiye. Pass-to-pass PIRS values were manually measured and the deviations from the set value were analyzed in terms of root mean square error (RMSE). The mean PIRS variations in sowing by manual steering (7.4 cm) were found as significantly higher than the AS based soil ridge tillage and / or sowing (CORS: 5.0 cm, SBAS: 5.9 cm, RTK: 6.7 cm) ($p<0.05$). In sum, it was found that the AS technology offers benefits in lowering the pass-to pass PIRS variations but the level of benefit changes from farmer to farmer; hence, the AS should be used cautiously with proper settings for greater benefits.

Keywords: Precision agriculture, Auto steering, GNSS, Sowing, Plant inter-row spacing

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*This paper was prepared from the MS study of Mr. Mustafa Topcueri under the supervision of Dr. Muharrem Keskin.

Öz

Hassas tarım (HT), toprak işlemeden hasada kadar tarımsal faaliyetlerin verimliliğini ve kârlılığını artırmayı, doğal kaynakların ve çevrenin korunmasını ve böylelikle sürdürülebilirliği hedefleyen ileri teknolojileri içerir. Otomatik dümenleme (OD), dünyada ve Türkiye'de en yaygın kullanılan ve birçok fayda sağlayan HT teknolojisidir. OD; sırta toprak işleme ve ekim dâhil olmak üzere tarımsal işlemlerde verimli ve sürdürülebilir uygulamalara imkân sağlar. Makinalı hassas ekimde, her bitkiye eşit yaşam alanı sağlamak için bitki sıra aralarında yeterli boşluk gereklidir. Bu nedenle makinalı ekimde paralel yan yana geçişlerde bitki sıra arası mesafesi (BSAM) eşit olmalıdır. Ekimde eşit BSAM sağlamak için OD'nin faydalarına ilişkin yapılan araştırmalar oldukça sınırlıdır. Bu çalışma, üç farklı sinyal düzeltme kaynağı (RTK, CORS, SBAS) kullanan GNSS esaslı OD ile ekimde yan yana paralel geçişlerdeki PIRS sapmalarını karşılaştırmak amacıyla yapılmıştır. Çalışmada OD kullanılmayan (manuel yönlendirmeli) tarlalardaki BSAM değerleri de karşılaştırma amacıyla incelenmiştir. Veriler, Çukurova bölgesinde, sıra arası mesafe değerleri 70-75 cm olan 24 çiftçi tarlasından (pamuk ve mısır) elde edilmiştir. Paralel yan yana geçişlerdeki BSAM değerleri manuel olarak ölçülmüş ve ayarlanan değerden olan sapmalar hata kareler ortalamasının karekökü (RMSE) değeri ile analiz edilmiştir. Manuel dümenlemeli ekimde ortalama BSAM sapmaları (7.4 cm), OD ile yapılan sırta toprak işleme ve/veya ekime göre daha yüksek bulunmuştur (CORS: 5.0 cm, SBAS: 5.9 cm, RTK: 6.7 cm) ($p<0.05$). Özetle, OD teknolojisinin yan yana paralel geçişlerde BSAM değişimlerini azaltmada fayda sağladığı ancak fayda düzeyinin çiftçiden çiftçiye değiştiği belirlenmiştir. Bu nedenle, ileri düzeyde faydalar elde edebilmek için OD sistemleri uygun ayarlarla dikkatli bir şekilde kullanmalıdır.

Anahtar Kelimeler: Hassas tarım, Otomatik dümenleme, GNSS, Ekim, Bitki sıra arası mesafe

1. Introduction

Precision Agriculture (PA) also known as smart agriculture, digital agriculture and agriculture 4.0 includes advanced technologies to increase efficiency and profitability of agricultural practices from tillage to harvest and provide sustainability of the natural resources and the environment (Bora et al., 2012; DeLay et al., 2022; Mizik 2022; Vrchota et al., 2022). Along with developed countries, PA technologies are being adopted in some developing nations such as Türkiye especially in the last decade (Ozguven and Turker, 2010; Tekin, 2011; Akdemir, 2016; Keskin and Sekerli, 2016; Yaghoubi and Niknami, 2022).

Automatic guidance or automatic steering (AS) enables a tractor to move on a desired predetermined route. It is one of the most widely adopted PA tools (Say et al., 2017) and has been used in developed countries as early as by the end of 1990s while farmers started to use them in Türkiye after 2009. The adoption level of the AS systems has increased to 70-90% in some regions of some developed countries (Say et al., 2017; McFadden et al., 2023). Leonard (2014) stated that 80% of the grain growers in Australia use AS. In the USA, the GPS-based AS (83%) was the most popular PA system (Erickson and Widmar, 2015). Verma (2015) stated that AS is the mostly accepted technology in the Heilongjiang region of China while Silva et al. (2011) reported that the most preferred PA systems by sugar and ethanol producers were AS (39%) and satellite imaging (76%) in the Sao Paulo state of Brazil. AS systems are also widely used by Turkish farmers (Keskin et al., 2018; Topcueri and Keskin, 2019).

Various methods are employed for auto steering including mechanical, electrical, geomagnetic, image processing, ultrasonic and satellite-based techniques (GNSS) (Keskin et al., 2018; Juostas and Jotautiene, 2021). In the image based technique, tractor is guided by referencing the crop rows or soil ridge furrows detected by a digital camera (Garcia-Santillan et al., 2017; Yun et al., 2021; Vrochidou et al., 2022). In the ultrasonic method, ultrasonic sensors measure the distance to the plant row or soil ridge while in the mechanical contact method, an elastic touch sensor detects the plant row (Reichhardt, 2012). But the most common AS method is the GNSS-based technique (GNSS: global navigation satellite system). In addition, in greenhouses and orchards, guidance can be accomplished by detecting the distance to plant rows or trees with LASER, LIDAR, RADAR or ultrasonic sensors (Li et al., 2009; Unal and Topakci 2012; Mousazadeh, 2013; Bayar et al., 2015). Furthermore, driverless autonomous tractors and field robots are subjected to ongoing field trials and will be available soon. Steering is also used for the equipment attached to the rear of the tractor called “implement guidance” by using active or passive guidance methods which is mostly beneficial on sloped terrains and turns (Oksanen and Backman, 2016).

AS systems have two types: a) In the semi-automatic type, the operator steers the vehicle by following an indicator (display or lightbar), b) In the full automatic system, steering is done by means of an electric motor on the steering wheel or a hydraulic actuator mounted on the wheel steering system. In both methods, when needed, the operator can take the control of the tractor by using the steering wheel (Scarfone et al., 2021).

A GNSS-based AS system has four basic parts; a GNSS antenna, electric steering motor or hydraulic control unit, computer and a terminal (display). Steering angle sensors are also used usually on large tractors to increase steering accuracy. In the operation of an AS system, after a starting pass (A-B line) is created between two starting points (A and B), passes (swaths) parallel to the starting A-B line are established based on the working width of the equipment. When the machine comes to the end of the row, the tractor is turned to the side row manually but updates are on the way to do the end-of-row turnings automatically.

When properly used, the fully automated AS systems provide many benefits that can be summarized in four groups (Grisso et al. 2009; Ashworth et al., 2018; Baillie et al., 2018; Keskin et al., 2018; Kharel et al., 2020; Jotautiene et al., 2021; Burgers and Vanderwerf, 2022; Keskin and Sekerli, 2022): a) Efficiency: better work quality; equidistant parallel passes; parallel and straight soil ridges in tillage; eliminating markers in sowing; working at night; working at higher speeds; reduced overlaps and gaps in pesticide, fertilizer and lime applications without foam or flags; savings of fuel, pesticide, water, fertilizer and labor; maximized working width in harvest and spraying, b) Agronomic: better crop growth and yield; reduced plant damage and soil compaction when same tramlines are used, c) Ergonomic: easy to learn and operate encouraging young operators to work on farms; working in adverse weather conditions (fog, dust, sun glare); reduced driver workload and fatigue; safer work conditions, d) Sustainability: protection of environment, natural resources, health of humans, animals and other plants by reducing chemical inputs, irrigation water, fossil fuel and greenhouse gas emissions.

Sowing is one of the most crucial processes affecting crop yield. For better germination, plant growth and yield, a sufficient living space is needed for each plant to acquire sufficient light, heat, oxygen, water and nutrients (Blessing et al., 2020; Tilley et al., 2021). In modern farming, sowing must provide an equal amount of living space for each plant. Thus, the plant inter row spacing (PIRS) in parallel passes should be equal to the spacing of the other rows. If the PIRS is less than the set value, sufficient living space cannot be provided for the plants while if this distance is greater, the field area cannot be efficiently utilized and yield would be lower. AS systems can precisely adjust the PIRS in parallel passes if used properly.

There have been numerous studies on the benefits of AS systems especially in spraying (Batte and Ehsani, 2006; Hudson et al., 2007; Topcueri and Keskin, 2019). However, studies in planting are very limited. Baio and Moratelli (2011) found a parallelism error of 3.3 cm using AS with RTK which is five times better than the manual steering in sugarcane planting. Similarly, Voltarelli et al. (2013) determined errors up to 4.9 cm by using AS with RTK in sugarcane planting with 1.5 m row spacing. Santos et al. (2017) reported positioning errors lower than the manufacturer's specified value (3.8 cm) using an AS system with RTX (Real Time eXtended) signal correction in peanut sowing but field slope increased the error. In a similar work, a mean error less than 2 cm was reported in parallel passes in peanut sowing with AS using RTX (Santos et al., 2018). AS was effective reported by Zerbato et al. (2019) for improving the accuracy and quality of peanut sowing. Scarfone et al. (2021) reported that semi-auto guidance allowed to sow 1.2 extra ha per day lowering the planting cost by 2.4%.

AS systems are usually tested under controlled conditions on concrete or asphalt surfaces (Easterly et al., 2010; ASABE 2015); however, working conditions significantly differ in real field studies. Although some studies were conducted on the use of AS systems in planting, no study was found on the benefit of the AS in creating uniform plant inter-row spacing (PIRS) in parallel passes in real farmer field conditions. Thus, the goal of this work was to assess the performance of GNSS-based AS systems for uniform PIRS in parallel passes in planting in real farmer field conditions and compare it with the case in manual steering.

2. Materials and Methods

2.1. GNSS-based automatic steering (AS) systems

The accuracy of the GNSS-based AS systems depends on the signal correction method. The AS systems require precise error correction with high accuracy (up to 2 cm). In the study area (Adana and Mersin provinces), farmers usually use one of the three GNSS correction services in AS systems (Keskin et al., 2018):

a) Satellite-based augmentation system (SBAS): In this method, the correction signal is sent from SBAS satellites to the receivers. An annual subscription fee is paid by the farmers for this service. For example: Trimble RTX.

b) Continuously operating reference stations (CORS): Correction signal is sent over GSM mobile phone internet. Farmers in the study area use this correction source developed by the Turkish government (TUSAGA-AKTIF). An annual subscription fee and monthly mobile phone fee is paid by the farmers.

c) Real time kinematic (RTK): Farmers use an additional receiver to obtain correction signal which is stationary located on a nearby building or near the field (~5 km). Subscription fee is not needed but it is more expensive since an extra receiver must be purchased by the farmer.

The most common GNSS signal augmentation method in the study region was previously reported as CORS (49.1%) followed by SBAS (29.1%) and RTK (21.8%) (Keskin et al., 2018). RTK method is considered as the most accurate correction source usually in centimeter level (Jotautiene et al. 2021).

2.2. Study location and field data

The study was conducted in the Cukurova region (near: 36.971°N; 35.475°E) of Türkiye in which agricultural production is intensively carried out and many farmers utilize AS systems. Field data were obtained from farmer fields in different locations in Adana province (districts of Ceyhan, Saricam, and Yuregir) and Mersin province (district of Tarsus).

Farmers plant cotton and corn on soil ridges in the region. Some farmers use the AS only for ridge tillage and do the planting by referencing the ridges; so they do not utilize AS in sowing. The main cause for this is that

farmers cannot afford to purchase multiple AS systems due to their high costs (Civelek, 2022, Masi et al., 2023). However, some farmers although their number is relatively lower, use the AS systems for both ridge tillage and planting. Tillage and sowing by manual steering is common mainly on small farms due to economic constraints.

The study data were taken from the fields belonging to different farmers in the spring months of 2018. A total of 24 fields growing cotton and corn were examined; thus, the benefit of AS systems was studied under real farmer conditions. All fields had flat ground surface without sloping. Straight parallel passes (swaths) were utilized in all fields. The variations among the fields were also compared.

2.3. Plant inter-row spacing (PIRS) data

The PIRS created in the parallel passes may be smaller than the desired value leading to a narrower row spacing (*Figure 1a*) or it may be larger than the desired value resulting in wider row spacing (*Figure 1b*). Both situations are undesirable because ideally, the PIRS should be the same as the set row spacing.

The PIRS values were measured on the adjacent rows of the parallel passes. The measurements were conducted manually using a tape measure from 27 different locations per row on the beginning, middle and end of each pass-to-pass row (*Figure 2*).

a) Measurement near the beginning of the row (BoR) (nine data points)

b) Measurement near the middle of the row (MoR) (nine data points)

c) Measurement near the end of the row (EoR) (nine data points)

The means of the nine measurements were then averaged to obtain mean PIRS data for BoR, MoR and EoR locations (*Figure 2*). Average of these three means was also calculated to represent one PIRS value for each of the pass-to-pass adjacent plant rows. Measurements were taken from 10 to12 rows (27 data points per row) per field yielding data points of 270 to 324 for each of the 24 fields.

2.4. Deviations in PIRS values

The PIRS deviations from the set plant row spacing were calculated as the root mean square error (RMSE) values as in the Equation 1 presented below (Gisgeography, 2018):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(PIRS_m - PIRS_s)^2}{N}} \qquad \text{(Eq.1)}$$

where; RMSE=Mean deviation (cm), PIRSm=Measured plant inter-row space (cm), PIRSs=Set (desired) inter-plant row space (cm) and N=the number of data.

Also, percent deviation values (%) were computed as the mean deviation value divided by the set row spacing and multiplied by 100.

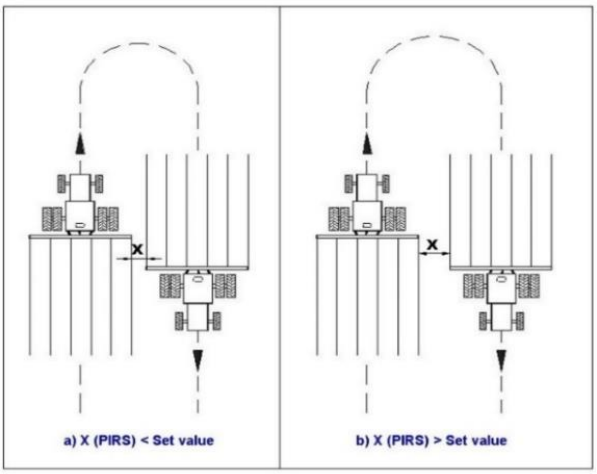

Figure 1. Plant inter row spacing (PIRS) in parallel passes in planting process with a six-row planter; a) row spacing is narrower than the set value b) row spacing is wider than the set value (not to scale)

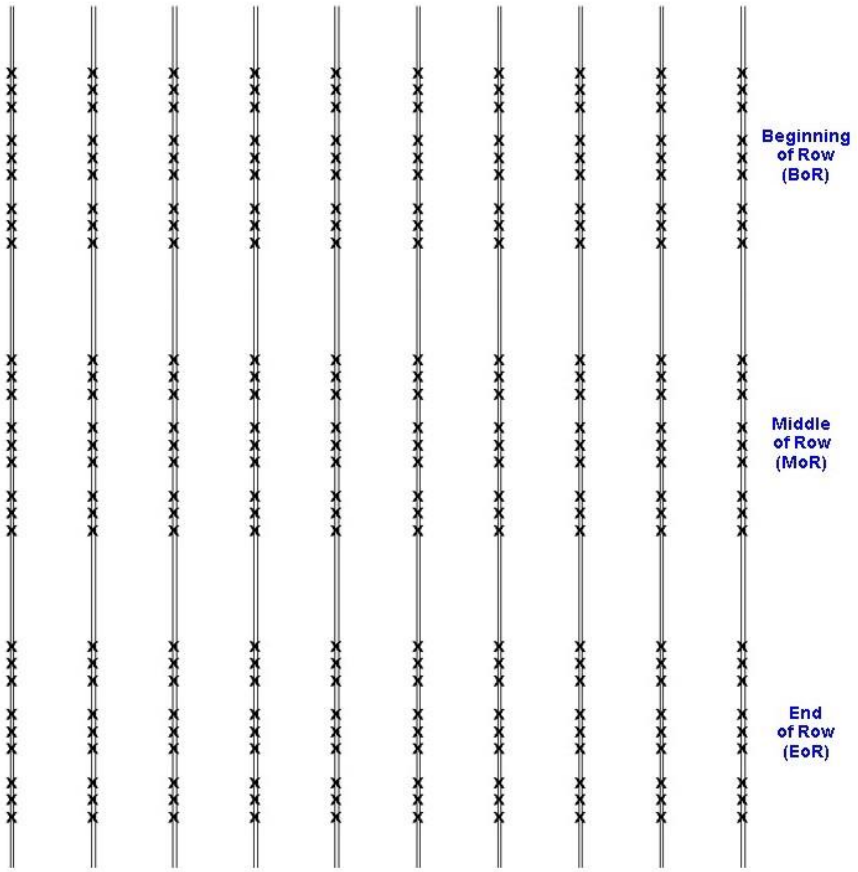

Figure 2. Measurement locations of the plant inter row spacing (PIRS) in parallel passes (double vertical lines represent pass-to-pass adjacent plant rows while crosses indicate individual measurement locations; 27 measurements per row)

2.5. Data analysis

The mean and standard deviation values for the PIRS deviations in parallel passes were calculated in MS Excel software (MS Office 2016). Statistical comparisons were made between AS signal correction methods, between fields and between the measurement locations (BoR, MoR, EoR) by using the analysis of variance (ANOVA) and Duncan's test for comparison in SPSS program (version: 17.0; IBM, NY, USA).

3. Results and Discussion

The effectiveness of the GNSS-based tractor automatic steering (AS) on the uniformity of plant inter-row spacing (PIRS) in parallel adjacent passes in sowing was examined in the study. For comparison, the case in fields ridge-tilled and planted by manual steering was also investigated. For this purpose, the PIRS values in parallel passes were measured and investigated in 24 farmer fields in different locations on which cotton or corn was planted (AS with different correction services in 18 fields and manual steering in 6 fields).

The pass-to-pass PIRS data in five farmer fields on which both ridge-tillage and planting was conducted with AS systems using RTK correction signal are presented in *Table 1*. In all five fields, both ridge tillage and sowing were conducted by using AS with RTK service and corn was planted with a row spacing of 70 cm. It was observed that the pass-to-pass PIRS values varied between the minimum of 55.0 cm and the maximum of 96.6 cm despite the set PIRS value of 70 cm in all fields (*Table 1*). The mean deviation (RMSE) values varied between the lowest 4.8 cm and highest 9.2 cm while the percent deviations were between 7.1 and 11.9%. Differences were observed between the fields in terms of mean PIRS deviations; the lowest mean percent deviation was in Field3 (7.1%) while the Field1 had the highest mean percent deviation (11.9%) (*Table 1*). The mean PIRS values on one of these fields (Field 3) are shown in *Figure 3* as an example.

Table 1. Plant inter-row spacing (PIRS) deviations (RMSE) for the fields both tilled and sowed by the GNSS-based auto steering (AS) with RTK signal augmentation

Field and location	Use of AS	Crop, PIRS[1]	PIRS Min, Max	Tractor power[2]	Data location[3]	Deviation mean±SD (cm)
Field 1 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	55.2 cm 96.6 cm	HP1: 130 HP2: 120	BoR MoR EoR **Mean**	7.0 ± 1.53 8.9 ± 0.35 9.2 ± 1.29 **8.3 ± 1.40 (11.9%)**
Field 2 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	57.9 cm 84.0 cm	HP1: 130 HP2: 120	BoR MoR EoR **Mean**	6.4 ± 0.24 6.8 ± 0.22 6.6 ± 0.53 **6.6 ± 0.38 (9.4%)**
Field 3 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	55.0 cm 82.0 cm	HP1: 130 HP2: 120	BoR MoR EoR **Mean**	4.8 ± 1.29 5.0 ± 0.93 5.3 ± 1.02 **5.0 ± 0.96 (7.1%)**
Field 4 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	59.7 cm 87.6 cm	HP1: 240 HP2: 130	BoR MoR EoR **Mean**	7.0 ± 0.70 6.3 ± 0.45 7.3 ± 0.71 **6.9 ± 0.69 (9.9%)**
Field 5 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	57.4 cm 85.3 cm	HP1: 240 HP2: 130	BoR MoR EoR **Mean**	6.2 ± 0.19 6.2 ± 0.46 7.1 ± 0.79 **6.5 ± 0.66 (9.3%)**

"+" sign: operation done with the use of AS
[1] PIRS: Plant inter-row spacing set value
[2] HP1: Power of the tractor used in soil ridge tillage (HP), HP2: Power of the tractor used in sowing (HP)
[3] BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row

Table 2 shows the PIRS values in parallel passes in seven farmer fields in which ridge tilled and/or planted with AS system using the SBAS correction. Corn was planted with 72.5 cm set row spacing in the first two fields while the spacing was 70 cm in the third and fourth fields with corn and in the last three fields, cotton was planted with 75 cm spacing (*Table 2*). In the first two fields, the lowest and highest pass-to-pass PIRS values were 54.3 and 97.0 cm, respectively despite the set value of 72.5 cm. The PIRS values varied from 65.1 to 77.4 cm even if the set value was 70 cm in the 3rd and 4th fields while in the last three fields, the set value was 75 cm but PIRS changed from the lowest 60.9 to the highest 84.2 cm. The mean deviation (RMSE) value was observed as varied between 2.3 and 13.3 cm while the percent deviations were between 3.5 and 17.7% in all seven fields (*Table 2*). It was observed that the variations in PIRS in the first two fields were much higher (14.6 and 17.7%) more than doubling the other five fields (from 3.5 to 7.2%). In both of these fields the ridge-tillage was done by using AS and the seeding was conducted by manual steering by referencing the ridges. In the interview with the farmer of the first two fields, no satisfactory answer was obtained as to the reason for this highest variations. The cause for this higher deviation in these two fields could be from the operator and/or the equipment settings such as the driver not showing enough care during the machinery operations in ridge tillage with AS and sowing with manual steering, for instance, when the GNSS augmentation signal was not suitable or interrupted, he/she continued to work manually and slippage occurred due to the settings of the planter not being attached properly. Also, the operator may not have precisely followed the ridges while sowing by manual steering (without AS). Significant differences were observed between the fields in terms of mean PIRS deviations of the minimum 3.5% and maximum 17.7% (*Table 2*). The mean PIRS measurements on one of these fields (Field 2) are shown in *Figure 4* as an example. In *Table 2*, Field 1 and 2, owned by the same farmer, were ridged-tilled by AS while planted by manual steering by referencing the ridges showing a mean PIRS deviation of 14.6% and 17.7%, respectively. However, Field 5, 6 and 7, owned by another farmer and were tilled and sown in same manner as in Field 1 and 2 reflected much lower PIRS deviations of 3.5%, 4.6% and 7.2%. This shows that both farmers used same method of tillage and sowing but got very distinct PIRS deviations in parallel passes which could be tied to the operator care for decent operation of the AS system and sowing machinery settings (Altinkaradag et al., 2017). Thus, these findings show that the farmers must be careful about the operation of the AS systems with proper tillage equipment and planter settings to get higher benefits from the AS systems in sowing.

Table 2. Plant inter-row spacing (PIRS) deviations (RMSE) for the fields tilled and/or sowed by the GNSS-based auto steering (AS) with SBAS signal augmentation

Field and location	Use of AS	Crop, PIRS[1]	PIRS Min, Max	Tractor power[2]	Data location[3]	Deviation mean±SD (cm)
Field 1 (Ceyhan, Adana)	Tillage:+ Sowing:−	Corn 72.5 cm	57.7 cm 92.1 cm	HP1: 95 HP2: 90	BoR	10.2 ± 1.63
					MoR	11.5 ± 0.50
					EoR	10.1 ± 2.05
					Mean	**10.6 ± 1.51 (14.6%)**
Field 2 (Ceyhan, Adana)	Tillage:+ Sowing:−	Corn 72.5 cm	54.3 cm 97.0 cm	HP1: 95 HP2: 90	BoR	11.9 ± 1.03
					MoR	13.2 ± 1.17
					EoR	13.3 ± 2.26
					Mean	**12.8 ± 1.54 (17.7%)**
Field 3 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	65.2 cm 77.4 cm	HP1: 115 HP2: 115	BoR	3.6 ± 0.53
					MoR	3.5 ± 0.25
					EoR	3.1 ± 0.53
					Mean	**3.4 ± 0.45 (4.9%)**
Field 4 (Yuregir, Adana)	Tillage:+ Sowing:+	Corn 70 cm	65.1 cm 76.3 cm	HP1: 115 HP2: 115	BoR	3.1 ± 0.26
					MoR	3.6 ± 0.45
					EoR	3.0 ± 0.26
					Mean	**3.2 ± 0.38 (4.6%)**
Field 5 (Saricam, Adana)	Tillage:+ Sowing:−	Cotton 75 cm	64.6 cm 82.3 cm	HP1: 110 HP2: 110	BoR	2.3 ± 0.65
					MoR	2.7 ± 0.70
					EoR	2.8 ± 0.85
					Mean	**2.6 ± 0.66 (3.5%)**
Field 6 (Saricam, Adana)	Tillage:+ Sowing:−	Cotton 75 cm	60.9 cm 84.2 cm	HP1: 110 HP2: 110	BoR	5.1 ± 1.35
					MoR	5.0 ± 0.04
					EoR	6.2 ± 1.19
					Mean	**5.4 ± 1.09 (7.2%)**
Field 7 (Saricam, Adana)	Tillage:+ Sowing:−	Cotton 75 cm	61.8 cm 80.6 cm	HP1: 130 HP2: 110	BoR	2.7 ± 0.10
					MoR	3.6 ± 1.35
					EoR	2.9 ± 0.48
					Mean	**3.1 ± 0.85 (4.1%)**

"+" sign: operation done with the use of AS; "−" sign: operation done without the use of AS
[1] PIRS: Plant inter-row spacing set value
[2] HP1: Power of the tractor used in soil ridge tillage (HP), HP2: Power of the tractor used in sowing (HP)
[3] BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row

The PIRS values in parallel passes in six farmer fields on which ridge tillage was done with the AS system using CORS correction and the sowing was carried out with manual steering (without AS) are presented in *Table 3*. Corn and cotton were planted with a set row spacing of 70 cm in all six fields. While the PIRS value was set to 70 cm, it varied between the lowest 52.8 cm and the highest 88.6 cm and the mean deviations (RMSE) were observed between 1.9 and 8.7 cm and the percent deviations were between 2.7% and 9.6% in all six fields (*Table 3*). The PIRS measurements of the first field are shown in *Figure 5* as an example.

Table 4 shows the PIRS values in parallel passes in six farmer fields which were both ridge tilled and planted with manual steering (without AS). Corn was planted with 75 cm set row spacing in the first three fields while in the remaining three fields, cotton was planted with 72.5 cm row spacing. It was found that even if the PIRS value was set to 75 cm in the first three fields, the lowest PIRS was 52.6 cm and the highest was 94.1 cm (*Table 4*). In the last three fields, the PIRS value varied from the lowest 51.7 cm to the highest 85.2 cm despite the set value of 72.5 cm. The mean deviations (RMSE) varied between 5.2 and 9.4 cm while the mean percent deviations were from 7.1 to 12.3%. The PIRS measurement values of the fifth field is displayed in *Figure 6* as an example.

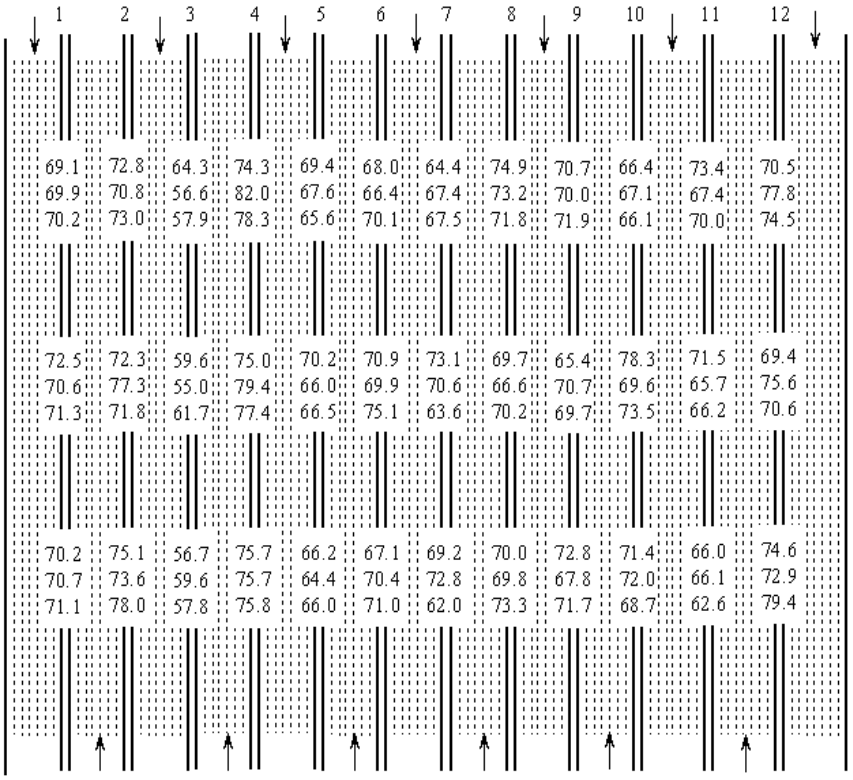

Figure 3. Mean PIRS values (n=3) in parallel passes in the field number 3 both ridge-tilled and sown with the GNSS-based AS with RTK augmentation (eight-row planter, set row spacing: 70 cm)

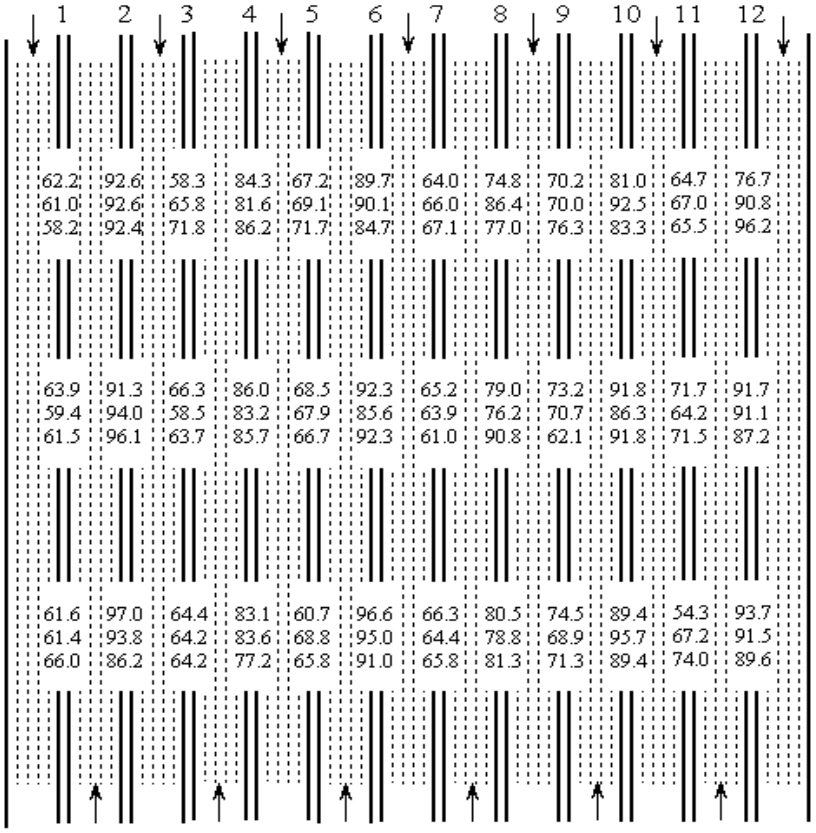

Figure 4. Mean PIRS values (n=3) in parallel passes in field number 2 ridge-tilled with the GNSS-based AS with SBAS service but planted by manual steering (six-row planter, set row spacing: 72.5 cm)

Table 3. Plant inter-row spacing (PIRS) deviations (RMSE) for the fields tilled by the GNSS-based auto steering (AS) with CORS signal augmentation

Field and location	Use of AS	Crop, PIRS[1]	PIRS Min, Max	Tractor power[2]	Data location[3]	Deviation mean±SD (cm)
Field 1 (Yuregir, Adana)	Tillage:+ Sowing:−	Corn 70 cm	60.2 cm 81.6 cm	HP1: 165 HP2: 110	BoR	4.4 ± 0.64
					MoR	3.3 ± 0.65
					EoR	4.9 ± 0.12
					Mean	**4.2 ± 0.82 (6.0%)**
Field 2 (Yuregir, Adana)	Tillage:+ Sowing:−	Corn 70 cm	59.2 cm 88.6 cm	HP1: 165 HP2: 110	BoR	7.1 ± 0.21
					MoR	7.0 ± 0.91
					EoR	6.1 ± 0.39
					Mean	**6.7 ± 0.68 (9.6%)**
Field 3 (Yuregir, Adana)	Tillage:+ Sowing:−	Corn 70 cm	52.8 cm 87.0 cm	HP1: 165 HP2: 110	BoR	8.7 ± 0.63
					MoR	8.5 ± 0.21
					EoR	8.4 ± 0.50
					Mean	**8.5 ± 0.41 (12.1%)**
Field 4 (Ceyhan, Adana)	Tillage:+ Sowing:−	Cotton 70 cm	65.3 cm 73.9 cm	HP1: 110 HP2: 110	BoR	2.0 ± 0.15
					MoR	1.9 ± 0.18
					EoR	1.9 ± 0.56
					Mean	**1.9 ± 0.33 (2.7%)**
Field 5 (Ceyhan, Adana)	Tillage:+ Sowing:−	Cotton 70 cm	67.9 cm 76.2 cm	HP1: 110 HP2: 110	BoR	2.1 ± 0.21
					MoR	2.2 ± 0.05
					EoR	2.2 ± 0.19
					Mean	**2.2 ± 0.16 (3.1%)**
Field 6 (Ceyhan, Adana)	Tillage:+ Sowing:−	Cotton 70 cm	57.3 cm 83.2 cm	HP1: 110 HP2: 110	BoR	7.4 ± 0.35
					MoR	6.4 ± 0.53
					EoR	5.5 ± 0.33
					Mean	**6.4 ± 0.89 (9.1%)**

"+" sign: operation done with the use of AS; "−" sign: operation done without the use of AS
[1] PIRS: Plant inter-row spacing set value
[2] HP1: Power of the tractor used in soil ridge tillage (HP), HP2: Power of the tractor used in sowing (HP)
[3] BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row

From the *Tables 1* to *4*, it is possible to see differences in the variations of the mean PIRS values. The reasons for these differences among the fields even belonging to same farmer may be tied to some factors such as different operators, tractor sizes, equipment settings, different field locations and planting at different times. Locations of the fields and the time of the operation have a potential to affect the GNSS signal quality and thus the positioning and signal augmentation accuracy. Also, some farmers use the AS only for ridge tillage and do the planting manually without AS (*Table 3*) by simply referencing the ridges mainly due to the high equipment cost and this caused higher variations in some fields. Operator experience and operational care is very important in planting by manual steering and an important factor to affect the variations in PIRS.

Figures 3, *4*, *5* and *6* depict the mean PIRS values observed in the fields. As can be seen in these figures, it was found on a general trend that the PIRS values, which were smaller than set row spacing value in one pass were larger in the next (adjacent) pass. It is thought that the smaller sowing width in one pass is compensated with wider width in the next pass with both AS and manual steering. For example, this is easily visible in *Figure 4* in which the PIRS values are lower than the set value (72.5 cm) in the first, third, fifth passes, etc. while they are higher in the second, fourth, sixth passes, etc.

In the statistical data analysis, the deviations in the mean plant inter-row spacing (PIRS) in parallel passes were statistically compared between the manual steering vs. automatic steering (AS), among the 24 fields and among the measurement locations (BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row) using Duncan's multiple comparison test.

Table 4. Plant inter-row spacing (PIRS) deviations (RMSE) for the fields both tilled and sowed by manual steering without the use of auto steering (AS)

Field and location	Use of AS	Crop, PIRS[1]	PIRS Min, Max	Tractor power[2]	Data location[3]	Deviation mean±SD (cm)
Field 1 (Saricam, Adana)	Manual steering	Corn 75 cm	52.6 cm 94.1 cm	HP1: 110 HP2: 110	BoR	6.0 ± 0.30
					MoR	9.0 ± 1.38
					EoR	6.6 ± 0.58
					Mean	**7.2 ± 1.59 (9.6%)**
Field 2 (Saricam, Adana)	Manual steering	Corn 75 cm	61.1 cm 89.2 cm	HP1: 110 HP2: 110	BoR	7.5 ± 0.20
					MoR	6.0 ± 0.49
					EoR	6.4 ± 0.40
					Mean	**6.7 ± 0.67 (8.9%)**
Field 3 (Saricam, Adana)	Manual steering	Corn 75 cm	64.1 cm 84.6 cm	HP1: 110 HP2: 110	BoR	5.2 ± 0.20
					MoR	5.5 ± 0.23
					EoR	5.3 ± 0.56
					Mean	**5.3 ± 0.35 (7.1%)**
Field 4 (Ceyhan, Adana)	Manual steering	Cotton 72.5 cm	56.1 cm 85.2 cm	HP1: 110 HP2: 110	BoR	8.5 ± 0.41
					MoR	8.5 ± 0.32
					EoR	7.8 ± 0.52
					Mean	**8.2 ± 0.50 (11.3%)**
Field 5 (Ceyhan, Adana)	Manual steering	Cotton 72.5 cm	54.6 cm 82.1 cm	HP1: 110 HP2: 110	BoR	9.4 ± 0.31
					MoR	9.1 ± 0.36
					EoR	8.1 ± 0.05
					Mean	**8.9 ± 0.66 (12.3%)**
Field 6 (Ceyhan, Adana)	Manual steering	Cotton 72.5 cm	51.7 cm 78.9 cm	HP1: 110 HP2: 110	BoR	8.4 ± 0.08
					MoR	8.2 ± 0.03
					EoR	7.3 ± 0.14
					Mean	**8.0 ± 0.51 (11.0%)**

"–" sign: operation done without the use of AS
[1] PIRS: Plant inter-row spacing set value
[2] HP1: Power of the tractor used in soil ridge tillage (HP), HP2: Power of the tractor used in sowing (HP)
[3] BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row

Table 5 lists the mean PIRS deviation values compared according to the steering methods (manual vs. AS) including the AS used in ridge-tillage and planting or only in ridge-tillage but not planting. The mean PIRS deviation value in the manual steering (7.4 cm) was found to be significantly higher compared to the deviations obtained in AS method with different correction services (CORS: 5.0 cm, SBAS: 5.9 cm, RTK: 6.7 cm) ($p<0.05$) (*Table 5*). However, it was observed that the differences between AS with different correction signals (CORS, SBAS, RTK) were not significant ($p>0.05$).

Table 5. Mean PIRS deviation values in parallel passes according to the steering method

Method of steering	RMSE; Mean ± SD*
AS with CORS signal augmentation	5.0 ± 2.52 cm [a]
AS with SBAS signal augmentation	5.9 ± 3.98 cm [ab]
AS with RTK signal augmentation	6.7 ± 1.36 cm [bc]
Manual steering (no AS system)	7.4 ± 1.40 cm [c]

AS: Automatic steering
*Different letters over the numbers (a, b, c) indicate significant differences ($p<0.05$).

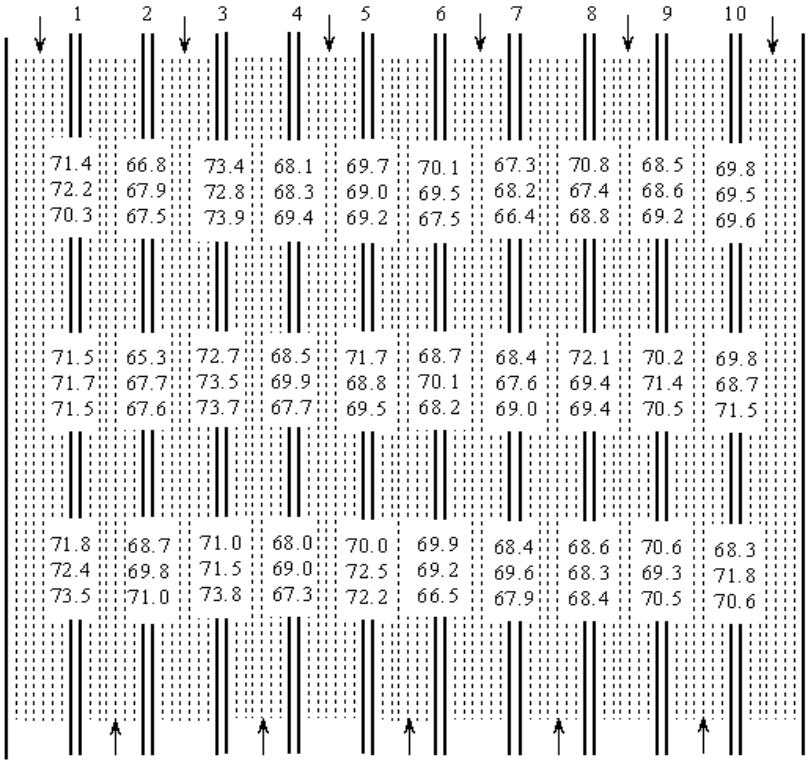

Figure 5. Mean PIRS values (n=3) in parallel passes in the field number 4 ridge-tilled with the GNSS-based AS systems with CORS service but planted by manual steering (eight-row planter, set row spacing: 70 cm)

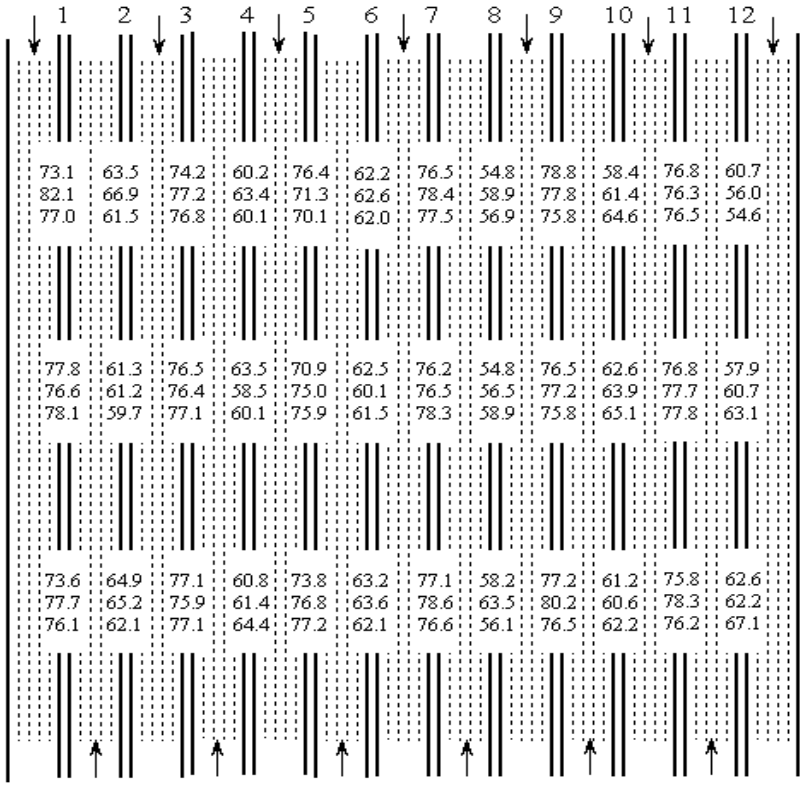

Figure 6. Mean PIRS values (n=3) in parallel passes in the field number 5 both ridge-tilled and sown with manual steering (six-row planter, set row spacing: 72.5 cm)

The mean deviation values in the PIRS in parallel passes taken from all 24 fields are presented in *Figure 7* and *Table 6*. It was observed that the mean PIRS deviation values were significantly different in most of the fields showing high variability among the fields ($p<0.05$). However, the differences within each steering method were relatively lower except two fields (F3 and F4) in which the ridge tillage was conducted with AS with SBAS correction service and the sowing carried out by manual steering (*Figure 7, Table 6*). The reasons for these differences among the fields may be tied to some factors such as different operators, tractor sizes, equipment settings, field locations and planting at different times (important for GNSS signal quality). It was also observed that some fields (Field 1 and Field 2) ridge-tilled by AS with SBAS correction service and planted by manual steering showed higher PIRS variations compared to the fields tilled and planted by manual steering (*Figure 7*). This is probably due to the low quality of work during the planting with manual steering. This means that the farmer did not get a good benefit from the AS to reduce the PIRS variations.

In field operations with machinery such as tillage and planting, higher deviations are usually expected after turning after finishing a row pass and beginning a new parallel pass in both automatic steering (AS) and manual steering during the system is adjusting to new row pass. Thus, the mean PIRS deviation values were compared according to the steering methods (manual vs. AS) used in ridge-tillage and/or planting as well as the PIRS measurement locations (BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row) (*Table 7*). It was observed that these differences were at very low levels according to the measurement locations (BoR, MoR and EoR) and can be considered as insignificant.

Sufficient spacing is required between plants and crop rows to provide enough living area, nutrients, water and light for each plant (Blessing et al., 2020). Crop inter row spacing is a crucial factor for plant growth, yield and farm's income (Tilley et al., 2021). Furthermore, equal plant inter-row spacing (PIRS) must be obtained in parallel adjacent passes in mechanized sowing with automatic steering (AS) or manual steering. If the PIRS value between the adjacent rows of the parallel passes is less than the desired set value, sufficient living space cannot be provided for the plants. Moreover, this may adversely affect the other future machinery operations such as hoeing machinery, fertilizer application machinery, sprayers and harvesters in row crops such as cotton, corn, soybean, etc. On the other hand, if this distance is greater than the desired set value, the area cannot be utilized sufficiently and yield would be lower (Tilley et al., 2021).

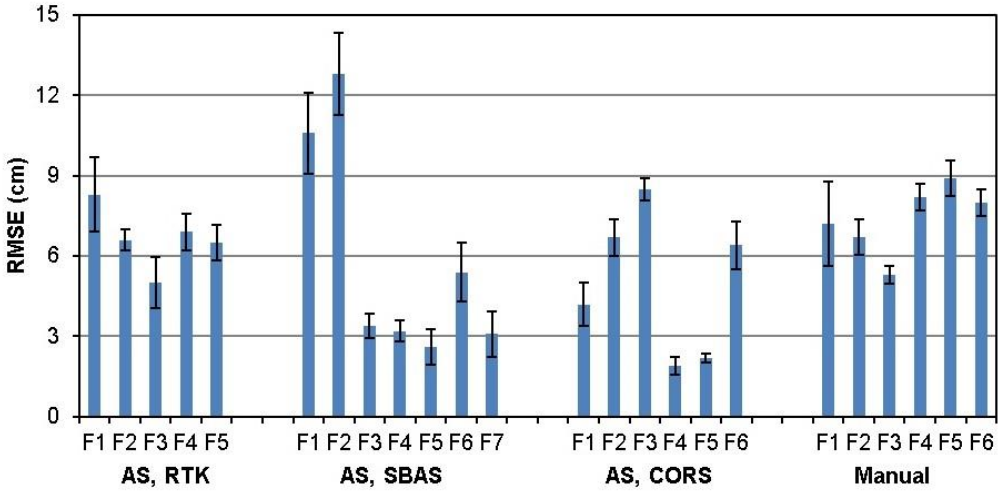

Figure 7. Mean PIRS deviations (RMSE) in parallel passes in all 24 fields according to the steering method (auto steering: AS and manual steering)

Table 6. Mean PIRS deviations in parallel passes according to the fields in each steering method

Method of steering	Fields	RMSE; Mean ± SD*
AS with RTK signal augmentation	F3	5.0 ± 0.96 cm [a]
	F5	6.5 ± 0.66 cm [b]
	F2	6.6 ± 0.38 cm [b]
	F4	6.9 ± 0.69 cm [b]
	F1	8.3 ± 1.40 cm [c]
AS with SBAS signal augmentation	F5	2.6 ± 0.66 cm [a]
	F7	3.1 ± 0.85 cm [a]
	F2	3.2 ± 0.38 cm [a]
	F1	3.4 ± 0.45 cm [a]
	F6	5.4 ± 1.09 cm [b]
	F3	10.6 ± 1.51 cm [c]
	F4	12.8 ± 1.54 cm [d]
AS with CORS signal augmentation	F4	1.9 ± 0.33 cm [a]
	F5	2.2 ± 0.16 cm [a]
	F1	4.2 ± 0.82 cm [b]
	F6	6.4 ± 0.89 cm [c]
	F2	6.7 ± 0.68 cm [c]
	F3	8.5 ± 0.41 cm [d]
Manual steering (without AS)	F3	5.3 ± 0.35 cm [a]
	F2	6.7 ± 0.67 cm [b]
	F1	7.2 ± 1.59 cm [b]
	F6	8.0 ± 0.51 cm [c]
	F4	8.2 ± 0.50 cm [cd]
	F5	8.9 ± 0.66 cm [d]

AS: Automatic steering
*Different letters over the numbers (a, b, c, d) indicate significant differences ($p<0.05$).

Table 7. Mean PIRS deviations in parallel passes according to the measurement locations

Method of steering	PIRS measurement location *	PIRS deviations RMSE ± SD **
AS with RTK service	BoR	6.3 ± 1.16 cm [a]
	MoR	6.7 ± 1.39 cm [ab]
	EoR	7.1 ± 1.49 cm [b]
AS with SBAS service	BoR	5.6 ± 3.79 cm [a]
	MoR	6.1 ± 4.15 cm [a]
	EoR	5.9 ± 4.17 cm [a]
AS with CORS service	BoR	5.3 ± 2.71 cm [ab]
	MoR	4.9 ± 2.62 cm [b]
	EoR	4.9 ± 2.34 cm [bc]
Manual steering (no AS)	BoR	7.5 ± 1.54 cm [a]
	MoR	7.8 ± 1.53 cm [b]
	EoR	6.9 ± 1.02 cm [b]

AS: Auto steering;
*BoR: Beginning of the row, MoR: Middle of the row, EoR: End of the row
**Different letters over the numbers (a, b, c) indicate significant differences ($p<0.05$).

AS systems are expected to adjust the PIRS value more precisely in parallel passes compared to manual steering if the equipment settings are properly set and the system is used suitably by the machinery operator. Various studies have been carried out on the utilization of AS systems that provide reductions in overlaps and gaps especially in spraying with significant savings in fuel, time, water, fertilizer, pesticide, labor and marking foam (Batte and Ehsani, 2006; Topcueri and Keskin, 2019; Kharel et al., 2020; Anastasiou et al., 2023; D'Antonio et al., 2023). However, studies on the effect of AS technologies in planting process on PIRS uniformity in parallel passes have been very limited. Baio and Moratelli (2011) found a parallelism error of 3.3 cm with AS and RTK service which is five times better than the manual steering in sugarcane planting. Similarly, Voltarelli et al. (2013) determined errors up to 4.9 cm by using AS with RTK signal augmentation service in sugarcane planting with row spacing of 1.5 m. Santos et al. (2017) reported that positioning errors were lower than the value specified by the machinery producer (3.8 cm) in AS system with RTX service in peanut sowing. In another study, the same authors determined an amount of mean error less than 2 cm in parallel passes in peanut sowing with AS using RTX service (Santos et al., 2018). In addition, Zerbato et al. (2019) stated that the AS system was more effective for improving the accuracy and quality of the peanut sowing compared to manual steering.

In this present work, comparable findings were obtained in regards to the findings of the previous studies. In the sowing process of cotton and corn on soil ridges under farmer conditions, the mean PIRS variations (RMSE) in parallel passes were from a minimum of 5.0 cm to a maximum of 6.7 cm for AS with different signal augmentation services while it was significantly higher (7.4 cm) with manual steering used both in ridge-tillage and sowing. Very low deviations in PIRS values in parallel passes as low as 2.7% were observed in some farmer fields while much higher values were also determined in other farmer fields as high as 17.7% with the usage of AS. Hence, it was found in this present study that AS systems reduce the PIRS variations in parallel passes in ridge tillage and/or sowing; however, the benefit level changes from farmer to farmer. The AS systems should be used with care with proper settings of the tillage and sowing equipment.

In regards to the three GNSS signal augmentation services used by the farmers in the study region, the mean RMSE variation in the pass-to-pass PIRS was slightly lower with CORS method (5.0 ± 2.52 cm) as compared to SBAS (5.9 ± 3.98 cm) and RTK methods (6.7 ± 1.36 cm) (*Table 5*). However, the differences between these signal correction methods (CORS, SBAS, RTK) were not significant pairwise ($p>0.05$) (*Table 5*). It should be noted that these values are average of five, seven and six fields for the RTK, SBAS, and CORS methods, respectively (*Figure 7*). In general, RTK and subscription-based precise SBAS services are considered to give better positioning accuracy with centimeter level than the CORS method; however, in the process of sowing, many factors affect the pass-to-pass PIRS including the care of the machine operator, machine settings, and the availability of the AS system in both ridge tillage and sowing, etc.

4. Conclusions

It was found that the mean pass-to-pass plant inter-row spacing (PIRS) deviations in manual sowing (7.4 cm) were higher than the AS based soil ridge tillage and / or sowing with three different GNSS augmentation methods (CORS: 5.0 cm, SBAS: 5.9 cm, RTK: 6.7 cm) ($p<0.05$). Hence, the AS technology was determined as favorable in mitigating the pass-to-pass PIRS variations compared to the manual steering along with other benefits such as easiness of usage, less operator fatigue, working at night, etc. But, some farmers employed AS only in ridge tillage but done the planting without AS by aligning the tractor according to the ridges created by AS. The use of AS both in ridge tillage and sowing reduced the deviations and increased the benefit of AS in some fields. It was observed that the degree of benefit obtained from the AS technology vary among the fields and farmers; hence, the AS should be employed with care and with proper tillage and planting equipment settings for greater degrees of benefits.

Limitation of the Study

The data of this study were collected from 24 fields belonging to different farmers in various locations in the Cukurova region of Türkiye. Factors such as different tractor operators, different models of tractors, different ridge tillage and planters, different geographical locations of the fields, and tillage and planting operations at different times (important for GNSS signal quality) can have influences on the data. However, this study is important since it reflects the real farmer conditions of the effect of AS on the deviations of pass-to-pass plant inter row spacing.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Keskin, M.; Design: Keskin, M., Topcueri, M.; Data Collection and Processing: Keskin, M., Topcueri, M., Sekerli, Y.E.; Statistical Analyses: Topcueri, M., Sekerli, Y.E.; Literature Search: Keskin, M., Topcueri, M.; Writing, Review and Editing: Keskin, M., Topcueri, M., Sekerli, Y.E.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Measuring the Progress of Organic Farming in Indian States: Are There any Agro-Climatic Implications?

Nilojyoti KONER[1*], Arindam LAHA[2]

Abstract

In the quest for a sustainable farming system around the world, organic farming has emerged as the most promising alternative system that can offer solutions to the growing sustainability issues associated with the current input-intensive agriculture system. Organic farming is also becoming increasingly important due to a significant rise in the demand for organic food across the world. Realizing the importance of organic agriculture in the current Indian agricultural scenario, the Government of India has taken several initiatives to promote organic farming across the country. As a result, there has been a considerable increase in different dimensions of organic farming especially in India over the last decade. However, studies have found that organic farming practices in India are more suited to a few specific regions (such as rain-fed zones, low-productivity areas, hills, and arid zones) as compared to the other parts of the country. In this backdrop, this paper attempts to evaluate the state-wise progress of organic farming in India. To measure the inter-state variation in the performance of organic farming, a composite index has been constructed by taking into account four different performance dimensions viz. area covered under organic cultivation, volume of organic production, volume of organic export, and number of producers practicing organic farming. The result suggests a wide disparity in performances in respect of organic farming among the states in India. Interestingly, the states that have performed well in organic farming are found to have a higher level of organic carbon stock in their soil. This shows the influence of agro-climatic conditions on the organic cultivation practices as popularly postulated in the literature.

Keywords: Agro-climatic condition, Organic carbon stock, Organic farming, Organic index, Indian states

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1. Introduction

In the quest for a sustainable agriculture system, organic farming has emerged as the most popular alternative farming system (Crowder and Reganold, 2015), as it promises to offer solutions to the problems relating to agricultural sustainability. "Organic agriculture is a production system that sustains the health of soils, ecosystems, and people. It relies on the ecological processes, biodiversity, and cycles adapted to local conditions rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation, and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved" (IFOAM, 2021). Considering its potential wide range of environmental, economic, and social benefits (Seufert et al., 2012), many countries across the globe have made significant efforts to promote organic farming. As a result, the organic farming movement is gaining gradual momentum worldwide. Organic farming is practiced in 187 countries (Willer et al., 2021). The global market for organic products is also increasing at a rapid pace fueled by the growing health awareness of consumers. Available data shows that the global organic market reached 106.4 billion US dollars in 2019 with the U.S.A, Germany, and France contributing nearly 64% of the total market (Willer et al., 2021). In India, the interest in organic farming is also growing. According to the latest available data, India ranks 8th in terms of the world's organic agricultural land and 1st in terms of total number of producers as per 2020 data (Willer et al., 2021).

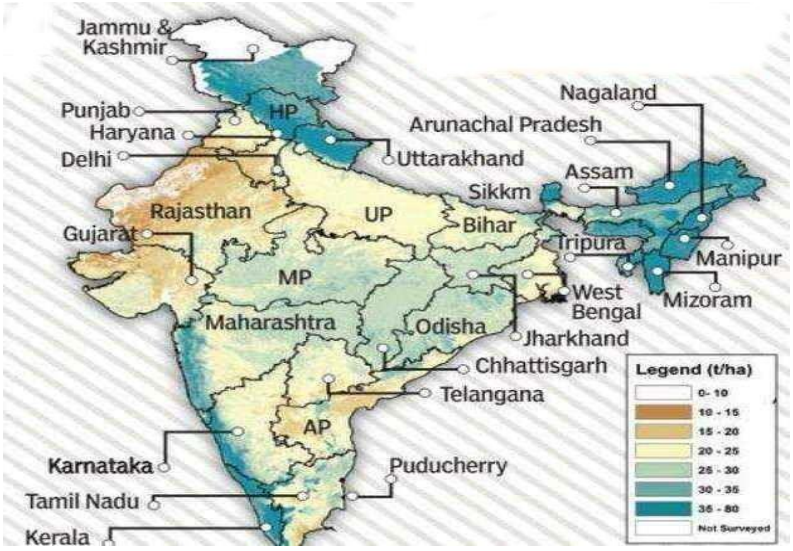
Before the introduction of the green revolution in the 1960s, the agricultural practices in the country were entirely based on organic farming techniques (Sruthy and Vibini, 2019). With the introduction of green revolution technologies, India has not only achieved food self-sufficiency but has also become a major exporter of foods. However, the extensive dependence on green revolution technologies (such as the use of synthetic inputs like chemical fertilizers and pesticides) has already started to show its ill effects on the environment, human health, and agriculture itself (Pingali, 2012; Turkboylari and Yuksel, 2021) and thus became a major cause of concern. Therefore, the time has come for the government to rethink its agricultural policy to make agriculture more sustainable. The introduction of the 'National Mission of Sustainable Agriculture' (NMSA) seems to be a step in this direction. Under the NMSA, the government of India has launched 'Paramparagat Krishi Vikash Yojana' (PKVY), a flagship programme designed to promote organic cultivation in the country. The scheme follows a cluster approach in promoting organic farming across the country. Since the implementation of this scheme in 2015, a total of 29,859 certified organic clusters have formed across Indian states covering about 0.59 million hectares of land and registering 13.9 million organic farmers (Reddy et al., 2022).

The potential of organic farming to provide improved livelihood opportunities and increased income for small-scale farmers with limited resources has made organic farming a popular strategy for economic development and poverty reduction (Qiao et al., 2016). Realizing the importance of organic farming in the Indian agricultural scenario, the government of India has taken several initiatives to promote organic farming. As a result, there has been a resurgence of interest in organic farming practices across the country. A considerable increase in different dimensions of organic farming (such as the size of the organic area, the volume of production, the quantity of exports, etc.) highlights the progression of the organic farming movement (see *Figure A.1- A.4*) in India. Therefore, the available data shows that India has recently made significant strides in different dimensions of organic farming. However, one pertinent question may arise in this context: has the advancement of the organic farming movement spread uniformly across the country? Or are there any particular regions (or states) that excelled more in organic farming than the other parts (or states) of the country? Is there any factor that can explain the variation in organic farming practices across India?

However, there is a lack of information on the state-wise progress of organic farming. Only a few studies in the existing literature shed some light on the status of organic farming in India (Deshmukh and Babar, 2015; Mitra and Devi, 2016; Yes Bank and ISCR, 2016; Wani et al, 2017; Babu and Karunakaran, 2021). To make a comparative assessment across Indian states, the performance in organic farming is evaluated based on one or two individual indicators (Deshmukh and Babar, 2015; Yes Bank and ISCR, 2016). However, such a comparison fails to depict an overall picture of the present state of organic farming in India. By addressing this gap of information in the existing literature, this paper objectively evaluates the state-wise progress of organic farming in India through a comprehensive organic index.

1.1. Agro-climatic region for organic farming:

Studies in the existing literature observed that organic farming is generally practiced in rain-fed zones, low-productivity areas, hills, and arid zones (Vaidya et al., 2007; Mitra and Devi, 2016; Wani et al., 2017; Babu and Karunakaran, 2021). In this context, ICAR-National Bureau of Soil Survey and Land Use Planning (NBSS and LUP), ICAR-Indian Institute of Social Science, Bhopal, and Food Agricultural Organizations jointly prepared a geospatial digital map identifying the regions holding high potential for organic farming based on the presence of organic carbon stock in soils. This map can be a guide to the government in targeting their organic farming policies (Behl, 2017).



Note: Figures signify contents of organic carbon in tone per hectare. More the organic carbon, the better the potential for organic farming

Figure 1: Organic carbon stock across states in India (Source: Behl, 2017)

This map highlighted strong potential for organic farming in the entire North- Eastern Region (including Sikkim, Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura) and parts of Kerala, Karnataka, Goa, and Maharashtra in Western Ghats (*Figure 1*). It also pointed out that among the northern Indian states Uttarakhand, Himachal Pradesh, and Jammu & Kashmir are the best zones to support organic farming, while states with black soils like Madhya Pradesh, Odisha, and Chhattisgarh have moderate potential for organic farming. However states like Punjab, Haryana, Uttar Pradesh, and West Bengal have low potential for organic farming as the organic carbon stock level of these states is depleted by their intensive cultivation practices.

The geospatial digital map has demarcated the regions (or states) with high potential for organic farming across the country. Therefore, it is important to know how these states have performed so far in different dimensions of organic farming. Further, it will be also interesting to know whether the recent progression of organic farming in the country is limited to these regions (or states) only. In this context, the following testable hypothesis can be drawn:

Hypothesis: Progress of organic farming in a state is contingent upon its agro-climatic condition (the level of organic carbon stock in the soil in particular).

2. Materials and Methods

2.1. Construction of organic index: Methodological issues

Progress of organic farming in a state can be measured by several dimensions like the size of the area covered under organic farming, the volume of organic production and exports, etc. The existing literature attempted to measure the performance of a state in organic farming by considering one or two individual

indicators (Deshmukh and Babar, 2015; Yes Bank and ISCR, 2016). Such analyses are solely based on an absolute performance (rather than relative performance) of a state in the individual indicators. However, this partial analysis fails to depict the overall position of a state in a national context. For instance, inter-state comparison based on the size of the organic area may provide misleading results as the larger states such as Rajasthan, Gujarat, and Madhya Pradesh are more likely to have a higher organic area in comparison to the smaller states like Sikkim, Meghalaya, and Goa. However, a comparative assessment of performance based on relative terms (e.g. the percentage of organic land to total cultivable land of the state) may bring out the overall picture. Therefore, this paper considers all performance dimensions in relative terms so that the inter-state comparison brings out the overall picture. To measure the progress of organic farming across the states of India, the present study has formulated a comprehensive index of organic farming (termed as Organic Index). This index is essentially built on four broad dimensions i.e., size of area, volume of production, volume of export, and number of producers. For each dimension, the performance of the state is evaluated in reference to the national average. For instance, to measure the performance concerning the organic area, first, the ratio of the organic area of a state to the total organic farming area of the country is computed. The dimension of the organic area is then worked out as a ratio between the share of a state regarding the organic area and the share of the total sown area of the state. A score higher than one indicates higher organic area vis-à-vis the proportion of total sown area in the state. Similarly, the performance metric for the other three dimensions is also measured (*Table 1*).

Table 1: Details of dimensions for measuring organic index

Dimension	Description	Proportional measure	Data sources
Size of Area (D_1)	Share of organic area as a proportion of the share of the total sown area of the state	D_1= A/B Where A indicates the ratio of the organic area of the state to the total organic area in India, and B indicates the ratio of the total sown area of the state to the total sown area in India	Agricultural Processed Foods and Export Development Authority (APEDA, 2019-20)and Department of Agriculture, Cooperation and Farmers' Welfare (as of 29.10.2020)
Volume of Production (D_2)	Share of the volume of organic production as a proportion of the share of the total volume of agricultural production of the state	D_2= C/D Where C indicates the ratio of the organic production of the state to the total organic production in India, and D indicates the ratio of the total agricultural production of the state to the total agricultural production in India	APEDA and Ministry of Agriculture and Farmers Welfare (2019-20)
Volume of Export (D_3)	Share of the volume of organic export as a proportion of the share of the total volume of agricultural export of the state	D_3= E/F Where E indicates the ratio of the volume of organic export of the state to the total volume of organic export in India, and F indicates the ratio of the total volume of agricultural export of the state to the total volume of agricultural export in India	APEDA and Ministry of Agriculture and Farmers Welfare (2019-20)
Number of Producers (D_4)	Share of the number of organic producers as a proportion of the share of the total number of cultivators of the state	D_4= G/H Where G indicates the ratio of the number of organic farmers in the state to the total number of organic farmers in India, and H indicates the ratio of the total number of cultivators in the state to the total number of cultivators in India	Lok Sabha Unstarred question no. 1496 dated 11 February 2020 and Agriculture Census Report, 2015-16

Source: Authors' own composition

Considering these three dimensions, a composite Organic Index (OI) is formed. It captures as much as information common to individual indicators. However, there is a certain drawback of this method: multicollinearity problems may arise if there is a high degree of correlation between the individual indicators (Tripathi and Seth, 2014). Factor analysis can be used to address this issue. Earlier studies have shown that factor analysis helps significantly reduce the dimension of data (when the original variables are highly correlated) so that the multicollinearity problem is eliminated (Brooks, 2014; Maji et al., 2020). Therefore, to construct a comprehensive index for organic farming data-driven weighting systems of Principal Component Analysis (PCA) have been used (following Research Centre-European Commission, 2008). In other words, the PCA method is used to derive the corresponding weights of several dimensions of the index. The weighting system under PCA intervenes to correct for overlapping information between two or more correlated indicators. The estimation process generally involves the following three steps. In the first step, the correlation matrix is

calculated to check the correlation structure of the data to know whether the individual indicators share common factors. If the correlation between individual indicators is small, then it is highly unlikely that they will share common factors. In the second step, two principal components (or factors) are identified for the analysis as they had maximum variances. Successive components explain progressively smaller portions of the variance and are all uncorrelated with each other. The third step deals with the construction of the weights from the factor loadings which involves rotating the number of factors chosen (from the earlier step) to enhance their interpretability. This is followed by the normalization of factor loadings by scaling to units sum.

The comprehensive index can be written as

$$OI = \frac{\sum_{i=1}^{4} W_i D_{is}}{\sum_{i=1}^{4} W_i} \quad \text{(Eq. 1)}$$

Where W_i(i=1, 2, 3, 4) are the corresponding weights of the dimensions. The weights (as per the PCA method) used to construct the organic index are 0.32 for dimension 1, 0.28 for dimension 2, 0.28 for dimension 3, and 0.12 for dimension 4 (see *Table A.1*). Finally, to make a comparison, states are classified into two categories depending on the values of OI. States with an organic index (OI) value below 1 (i.e., national average) have performed poorly, whereas, states having an organic index value above 1 have performed well in organic farming.

3. Results and Discussion

This paper aims to evaluate the state-wise progress of organic farming in India. For this purpose, the study has used four broad dimensions of organic farming i.e., size of area covered under this mode of cultivation, volume of organic production, volume of organic export, and number of producers practicing organic farming to measure a comprehensive organic index. Due to the non-availability of data relating to all four dimensions for all the states of India, the study is restricted to the 21 Indian states. For the inter-state analysis, a disaggregated analysis is carried out by taking each dimension of the index and then an overall analysis is conducted based on a comprehensive organic index.

3.1. *Current status of organic farming in India: a disaggregated analysis*

First, a state-level analysis for individual dimensions (i.e., size of area, volume of production, volume of export, and number of producers) of the index is carried out. Moreover, states' performances are compared with the national average to assess their relative progress in organic farming. The results are described as follows:

Spread of organic area across the Indian states (Dimension 1): One of the common measures of outreach of organic farming is the size of the area covered under this mode of cultivation. Therefore, area coverage is taken as one of the dimensions for measuring the state-wise performances of organic farming. The dimension size of the area (D_1) is worked out (shown in *Table 2*) as a ratio of two components: share of the organic area of a state to the total organic area in the country (denoted by A) and share of the sown area of the state to the total sown area in the country (indicated by B). Hence, it evaluates the performance of a state by comparing the share of the organic area of the state against its proportion of the total sown area. Therefore, if the share of the organic area of a state is higher vis-à-vis the proportion of total sown area (reflected by a D_1 value of more than 1), the state has recorded a better performance in organic farming and vice versa. So, the higher the value of D_1, the better the state's organic outreach.

Ranking of states based on dimension 1 (*Table 2*) shows that Sikkim (1st), Meghalaya (2nd), and Goa (3rd) hold the top positions while Punjab (19th), Haryana (20th), and West Bengal (21st) are at the bottom. Interestingly, Sikkim, Meghalaya, and Goa were also among the states with the highest share of the organic area to net sown area in the country in 2019, while the shares of the organic area to the net sown area of Punjab, Haryana, and West Bengal were among the lowest in the country (Khurana and Kumar, 2020). Moreover, a comparative analysis of performance between the states and the national average suggests that only eight states i.e., Sikkim (59.50), Meghalaya (11.21), Goa (5.79), Uttarakhand (3.65), Madhya Pradesh (3.57), Jammu & Kashmir (2.43), Himachal Pradesh (1.32), and Kerala (1.25) has performed better than the national average (1.00). It indicates that the proportion of organic area to total organic area in the country for each of these eight states is higher than their respective proportion of total sown area to the country's total sown area. However, all other states are lagging behind the national average. Khurana and Kumar (2020) also observed that a considerable portion of the

total organic area in India is concentrated only in a few states. The authors found that the top three states (i.e., Madhya Pradesh, Rajasthan, and Maharashtra) accounted for more than 40 percent of the total area under organic farming in 2019. However, most states had only a minor percentage of their net sown area under organic cultivation. The authors observed that despite having the largest area under organic farming, each of the top three states accounted for less than 5 percent of their net sown area under organic cultivation. The inter-state variation in dimension 1 is presented in *Figure 2*.

Table 2: Ranking of states on the basis of dimension 1

State	Organic area (in thousands Ha.)	Sown area (in thousands Ha.)	Share of each state to total organic area (A)	Share of each state to total sown area (B)	D_1 (A ÷ B)	Rank on the basis of D_1
Sikkim	75.72	77	0.0360	0.0006	59.5012	1
Meghalaya	45.38	245	0.0216	0.0019	11.2083	2
Goa	12.44	130	0.0059	0.0010	5.7886	3
Uttarakhand	42.05	698	0.0200	0.0055	3.6450	4
Madhya Pradesh	892.90	15149	0.4248	0.1191	3.5665	5
Jammu & Kashmir	30.28	754	0.0144	0.0059	2.4297	6
Himachal Pradesh	12.05	551	0.0057	0.0043	1.3237	7
Kerala	41.78	2023	0.0199	0.0159	1.2497	8
Maharashtra	282.50	17192	0.1344	0.1352	0.9943	9
Rajasthan	287.58	18024	0.1368	0.1417	0.9654	10
Assam	26.69	2801	0.0127	0.0220	0.5767	11
Gujarat	95.21	10302	0.0453	0.0810	0.5592	12
Karnataka	81.07	10006	0.0386	0.0787	0.4902	13
Andhra Pradesh	37.20	6209	0.0177	0.0488	0.3626	14
Tamil Nadu	26.01	4833	0.0124	0.0380	0.3256	15
Chhattisgarh	22.44	4651	0.0107	0.0366	0.2919	16
Uttar Pradesh	60.95	16469	0.0290	0.1295	0.2239	17
Telengana	8.74	4175	0.0042	0.0328	0.1267	18
Punjab	8.51	4137	0.0040	0.0325	0.1244	19
Haryana	6.16	3522	0.0029	0.0277	0.1058	20
West Bengal	6.39	5243	0.0030	0.0412	0.0738	21
India (Total)	2102.02	127191	1.000	1.0000	1.000	

Source: Author's own calculation

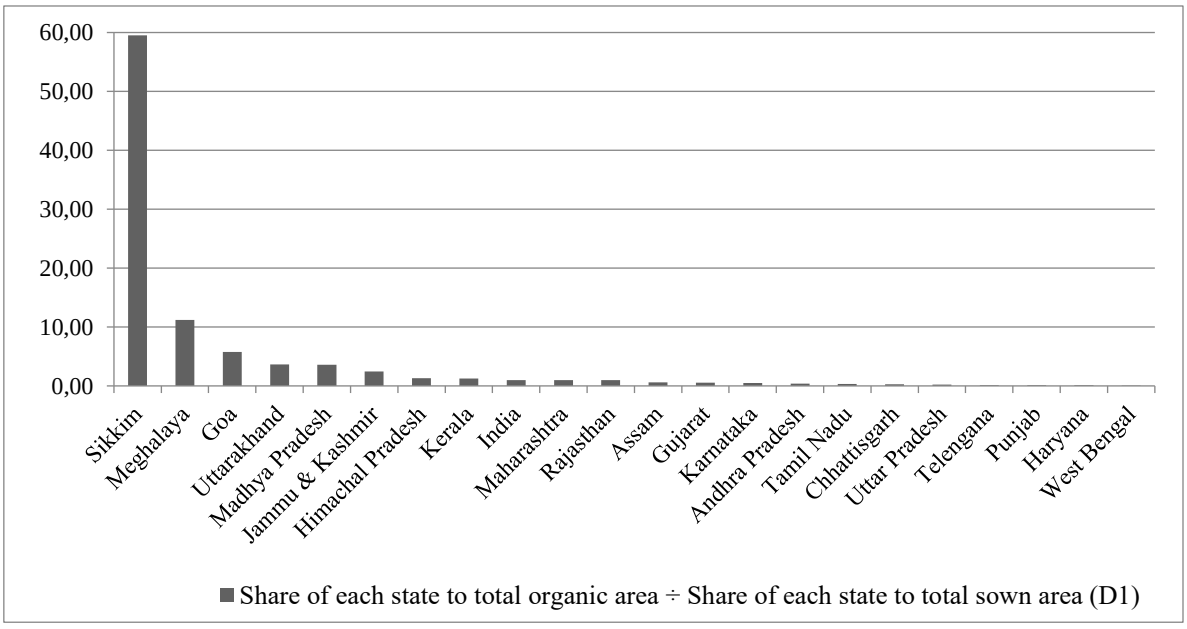


Figure 2: Spread of organic area across states in India

Volume of organic production across the states of India (Dimension 2): Volume of production can be considered an important measure of performance concerning organic farming. Hence, it is taken as the second dimension (D_2) of the inter-state performance evaluation of organic farming. As a relative measure, it compares the share of the volume of organic production of a state (C) against its share of the total volume of agricultural production (D) (shown in *Table 3*). Therefore, if the share of the volume of organic production of a state is higher (C) vis-à-vis the proportion of total volume agricultural production (D), the state has performed better in organic farming and vice versa.

Analysis based on dimension 2 (*Table 3*) indicates that among the states included in the study Kerala (1st), Goa (2nd), and Madhya Pradesh (3rd) are at the top, whereas Haryana (19th), Telangana (20th), and Punjab (21st) are the bottom. Moreover, analysis also reveals that states like Kerala (10.70), Goa (5.89), Madhya Pradesh (5.36), Jammu & Kashmir (4.55), Maharashtra (2.65), Sikkim (1.91), Rajasthan (1.36) and Uttarakhand (1.07) have obtained higher score on dimension 2 in comparison to all national average score (1.00). It indicates that the proportion of organic production to total organic production in India for each of these eight states is higher than their respective proportion of total agricultural production to the country's total agricultural production. Interestingly, a report published by Yes Bank and ISCR (2016) shows that six (i.e., Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Uttarakhand, and Jammu & Kashmir) out of these eight states have contributed 62 percent of the country's total organic production in the year 2014-15. However, the organic production of the rest of the states is lower than the national average. The inter-state variation in dimension 2 is given in *Figure 3*.

Table 3: Ranking of states on the basis of dimension 2

State	Organic production (in thousands tons)	Agricultural production (in thousands tons)	Share of each state to total organic production (C)	Share of each state to total agricultural production (D)	D_2 (C ÷ D)	Rank on the basis of D_2
Kerala	29.88	739.9	0.01157	0.00108	10.69807	1
Goa	2.50	112.68	0.00097	0.00016	5.88751	2
Madhya Pradesh	980.61	48447.1	0.37962	0.07080	5.36151	3
Jammu & Kashmir	28.01	1630.47	0.01084	0.00238	4.54996	4
Maharashtra	904.95	90414	0.35033	0.13214	2.65124	5
Sikkim	0.73	100.94	0.00028	0.00015	1.90991	6
Rajasthan	169.22	33078.4	0.06551	0.04834	1.35512	7
Uttarakhand	35.82	8847.3	0.01387	0.01293	1.07235	8
Himachal Pradesh	5.42	1542.1	0.00210	0.00225	0.93064	9
Assam	23.11	7468	0.00895	0.01091	0.81983	10
Karnataka	118.82	46942.8	0.04600	0.06861	0.67049	11
Gujarat	70.45	33670.5	0.02727	0.04921	0.55424	12
Meghalaya	1.87	1100.78	0.00072	0.00161	0.44958	13
Chhattisgarh	13.60	9186.5	0.00527	0.01343	0.39222	14
Andhra Pradesh	16.61	22693.6	0.00643	0.03317	0.19382	15
Tamil Nadu	17.82	25135.8	0.00690	0.03674	0.18780	16
West Bengal	18.56	29202.7	0.00718	0.04268	0.16832	17
Uttar Pradesh	138.36	234592.5	0.05356	0.34285	0.15623	18
Haryana	3.84	29315	0.00149	0.04284	0.03472	19
Telengana	2.29	20449.9	0.00089	0.02989	0.02972	20
Punjab	0.64	39562.2	0.00025	0.05782	0.00429	21
India(Total)	2583.11	684233	1.00000	1.00000	1.00000	

Source: Author's own calculation

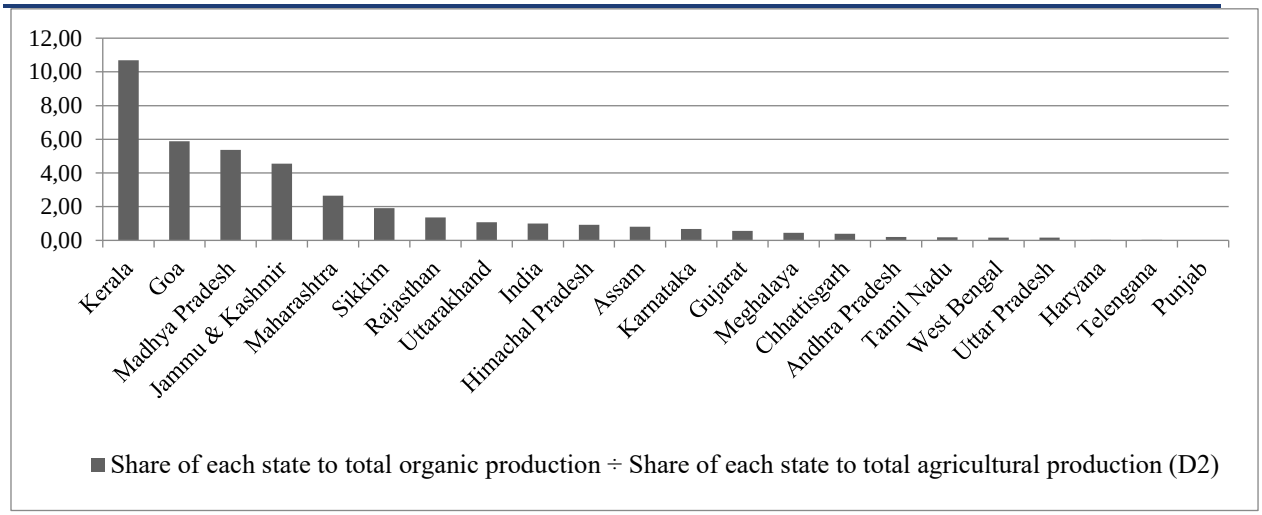


Figure 3: Volume of organic production across states in India

Volume of organic export across the states of India (Dimension 3): The size of the market is used as an indicator to measure the organic outreach of states. However, due to the lack of sufficient trade data on the domestic market, the study has focused solely on foreign markets (relying on international trade data) and the volume of organic export is used as a dimension (D_3) for the inter-state performance analysis. Like the other two dimensions, it is also computed (shown in *Table 4*) as a ratio of the share of the volume of organic export (E) and the share of the total volume of agricultural export (F) of the state. Therefore, if the value of D_3 is higher than 1 (indicates a higher share of the organic export vis-à-vis the proportion of the total agricultural export), it will reflect a better organic farming performance of the state.

Table 4: Ranking of states on the basis of dimension 3

State	Organic export (value in Crore)	Agricultural export (value in Crore)	Share of each state to total organic export (E)	Share of each state to total agricultural export (F)	D_3 (E ÷ F)	Rank on the basis of D_3
Madhya Pradesh	1670.20	5197.83	0.3864908	0.0352449	10.9658550	1
Goa	20.02	66.47	0.0046322	0.0004507	10.2773916	2
Jammu & Kashmir	14.45	89.53	0.0033440	0.0006071	5.5083863	3
Meghalaya	0.17	1.77	0.0000395	0.0000120	3.2871255	4
Sikkim	0.04	0.46	0.0000087	0.0000031	2.7776630	5
Kerala	310.34	7233.41	0.0718147	0.0490476	1.4641848	6
Telengana	112.89	2692.15	0.0261237	0.0182547	1.4310672	7
Karnataka	285.51	7010.25	0.0660683	0.0475344	1.3899052	8
West Bengal	270.82	6695.7	0.0626679	0.0454015	1.3803023	9
Rajasthan	107.13	3771.69	0.0247903	0.0255747	0.9693285	10
Uttarakhand	7.25	297.83	0.0016785	0.0020195	0.8311243	11
Maharashtra	471.44	21110.44	0.1090923	0.1431436	0.7621178	12
Gujarat	509.17	33589.97	0.1178244	0.2277636	0.5173101	13
Haryana	265.42	18301.5	0.0614197	0.1240970	0.4949328	14
Uttar Pradesh	100.71	9351.48	0.0233057	0.0634096	0.3675422	15
Tamil Nadu	79.60	7522.56	0.0184199	0.0510082	0.3611155	16
Himachal Pradesh	0.57	55.64	0.0001309	0.0003773	0.3469711	17
Andhra Pradesh	81.22	13154.99	0.0187937	0.0892001	0.2106918	18
Assam	6.99	2256.87	0.0016169	0.0153032	0.1056554	19
Chhattisgarh	4.82	1945.87	0.0011164	0.0131944	0.0846111	20
Punjab	2.69	7130.96	0.0006218	0.0483529	0.0128598	21
India(Total)	4321.45	147477	1.0000000	1.0000000	1.0000000	

Source: Author's own calculation

Ranking of states based on dimension 3 (*Table 4*) shows that Madhya Pradesh (1st), Goa (2nd), and Jammu & Kashmir (3rd) hold the top positions while Assam (19th), Chhattisgarh (20th), and Punjab (21st) are at the bottom. Moreover, a comparative analysis of performance between the states and the national average suggests that only nine states Madhya Pradesh (10.97), Goa (10.28), Jammu & Kashmir (5.51), Meghalaya (3.29), Sikkim (2.78), Kerala (1.46), Telangana (1.43), Karnataka (1.39) and West Bengal (1.38) has performed better compared to the national average (1.00). The inter-state variations in dimension 3 are presented in *Figure 4*.

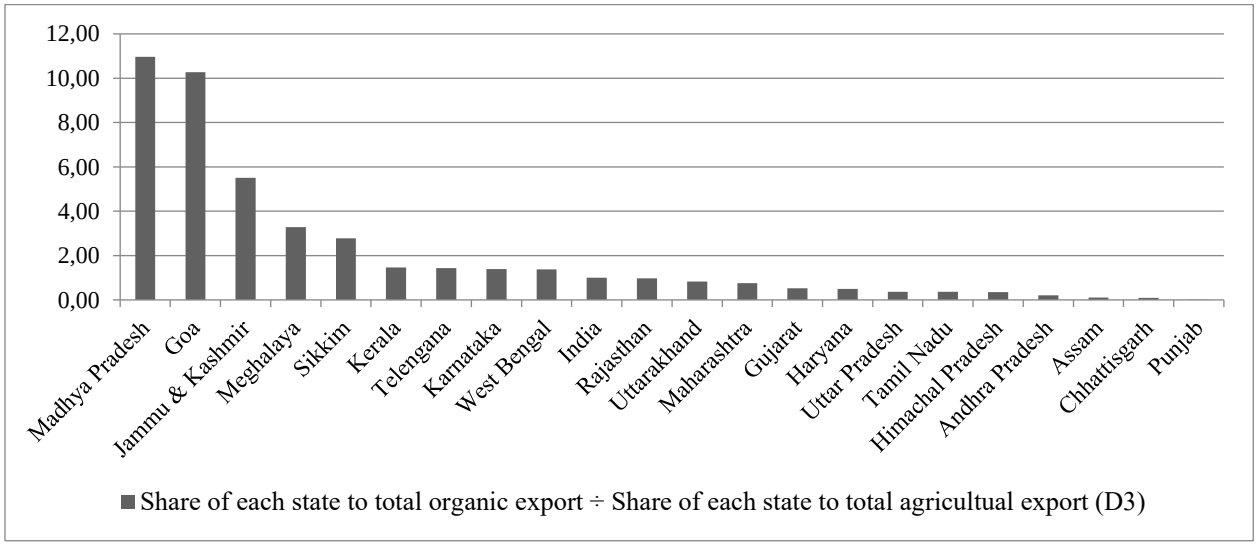


Figure 4: Volume of organic export across states in India

Table 5: Ranking of states on the basis of dimension 4

State	Organic farmers (in thousands)	Total farmers (in thousands)	Share of each state to total organic farmers (G)	Share of each state to total cultivators (H)	D_4 (G ÷ H)	Rank on the basis of D_4
Goa	25.2	74	0.01891	0.00059	31.81436	1
Uttarakhand	224.25	869	0.16827	0.00698	24.10833	2
Sikkim	7.5	71	0.00563	0.00057	9.86864	3
Rajasthan	307.5	7996	0.23073	0.06422	3.59275	4
Andhra Pradesh	265	8928	0.19884	0.07171	2.77298	5
Madhya Pradesh	191.4	10513	0.14362	0.08444	1.70086	6
Chhattisgarh	60	4124	0.04502	0.03312	1.35921	7
Punjab	12.5	1110	0.00938	0.00892	1.05206	8
Himachal Pradesh	10.5	1012	0.00788	0.00813	0.96931	9
Meghalaya	2.25	242	0.00169	0.00194	0.86860	10
Telengana	34.5	6117	0.02589	0.04913	0.52691	11
Assam	11	2751	0.00825	0.02210	0.37356	12
Maharashtra	62.9	15993	0.04720	0.12845	0.36743	13
Kerala	30.95	7917	0.02322	0.06359	0.36522	14
Karnataka	27.25	9057	0.02045	0.07274	0.28108	15
Tamil Nadu	15.6	7868	0.01171	0.06319	0.18523	16
Uttar Pradesh	31	24025	0.02326	0.19296	0.12055	17
Jammu & Kashmir	1.4	1404	0.00105	0.01128	0.09316	18
Gujarat	5	5510	0.00375	0.04426	0.08478	19
West Bengal	6	7292	0.00450	0.05857	0.07687	20
Haryana	1	1632	0.00075	0.01311	0.05724	21
India(Total)	1333	124505	1.00000	1.00000	1.00000	

Source: Author's own calculation

Number of organic Farmers across the states of India (Dimension 4): The number of organic farmers in a state is considered an individual indicator of organic farming outreach. The dimension number of producers (D_4) is computed (shown in *Table 5*) as a ratio of the share of the number of organic producers (G) and the share of the total number of cultivators (H) of the state. Therefore, if the share of the number of organic producers of a state (G) is higher than its share of the total number of producers (H), then the state has recorded a better performance in organic farming and vice versa.

Analysis based on dimension 4 (*Table 5*) indicates that among the states included in the study Goa (1st), Uttarakhand (2nd), and Sikkim (3rd) are the top-performing states. In addition, the share of organic farmers in Rajasthan (3.59), Andhra Pradesh (2.77), Madhya Pradesh (1.70), Chhattisgarh (1.36) and Punjab (1.05) are greater than the share of the state to total cultivators. These top eight states (i.e., Goa, Uttarakhand, Sikkim, Rajasthan, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, and Punjab) account for more than 71 percent of the country's organic farmers registered under the PKVY scheme (Khurana and Kumar, 2020). However, the rest of the states have fared poorly regarding their representation of organic farmers. The inter-state variations in dimension 4 are presented in *Figure 5*.

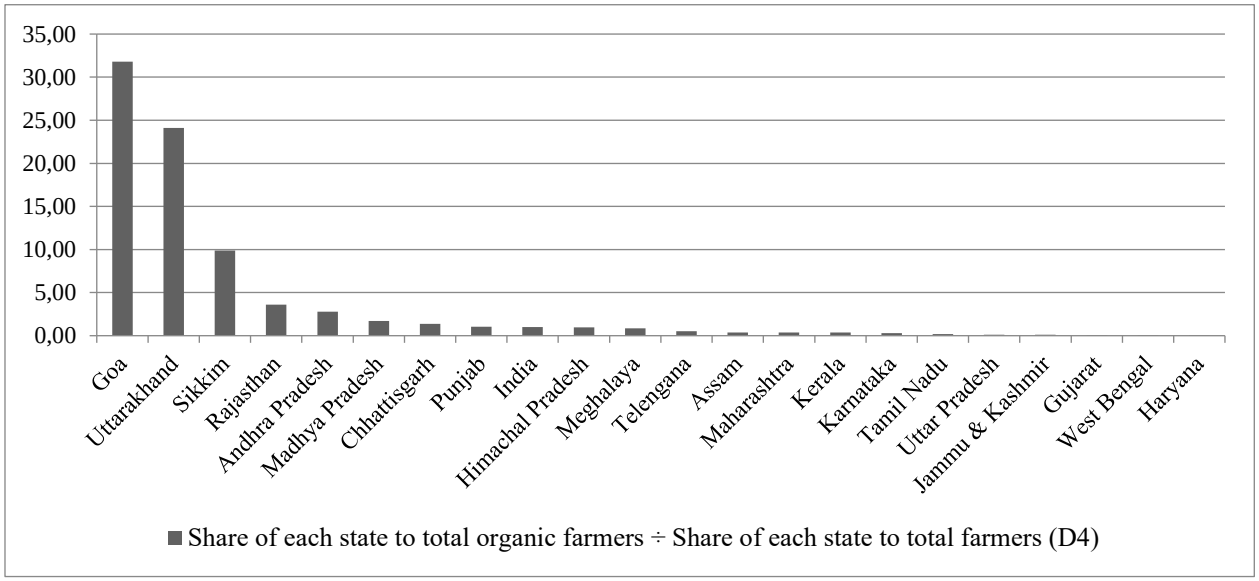


Figure5: Population of organic producers across states in India

3.2. *State of organic farming in India: Composite analysis based on organic index*

A disaggregated analysis of all four dimensions suggests that the performances of the states are not uniform in all the dimensions of organic farming. States such as Jammu & Kashmir and Kerala have performed poorly in terms of their representation of organic farmers, but the performance of these states regarding other dimensions of organic farming (i.e., volume of production and export) is found to be more than satisfactory. On the other hand, there is a stable representation of organic farmers in states like Andhra Pradesh, Chhattisgarh, and Punjab, however, the performance of these states regarding other dimensions of organic farming is found to be very poor. Similarly, Telangana, Karnataka, and West Bengal performed better in organic export, but the performance of these states regarding other dimensions of organic farming is found to be very poor. Thus a composite analysis considering all these dimensions is desirable to provide an overall picture of the state of organic farming in Indian States. To measure the inter-state variations in the practice of organic farming in a comprehensive way, the study has considered four dimensions of organic farming namely, size of the area (share of the organic area as a proportion of the share of the total sown area of the state), the volume of production (share of the volume of organic production as a proportion of the share of the total volume of agricultural production of the state), the volume of export (share of the volume of organic export as a proportion of the share of the total volume of agricultural export of the state) and the number of producers (share of the number of organic producers as a proportion of the share of the total number of producers of the state). Accordingly, the relative position of Indian states in the organic index is shown in *Table 6* and *Figure 6*.

Table 6: Organic index across states of India

State	Dimension 1 (D_1)	Dimension 2 (D_2)	Dimension 3 (D_3)	Dimension 4 (D_4)	Organic Index	Rank
Sikkim	59.501	1.910	2.778	9.869	21.537	1
Goa	5.789	5.888	10.277	31.814	10.196	2
Madhya Pradesh	3.566	5.362	10.966	1.701	5.917	3
Meghalaya	11.208	0.450	3.287	0.869	4.737	4
Uttarakhand	3.645	1.072	0.831	24.108	4.592	5
Kerala	1.250	10.698	1.464	0.365	3.849	6
Jammu & Kashmir	2.430	4.550	5.508	0.093	3.605	7
Rajasthan	0.965	1.355	0.969	3.593	1.391	8
Maharashtra	0.994	2.651	0.762	0.367	1.318	9
Himachal Pradesh	1.324	0.931	0.347	0.969	0.898	10
Karnataka	0.490	0.670	1.390	0.281	0.768	11
Andhra Pradesh	0.363	0.194	0.211	2.773	0.562	12
Telangana	0.127	0.030	1.431	0.527	0.513	13
Gujarat	0.559	0.554	0.517	0.085	0.489	14
Assam	0.577	0.820	0.106	0.374	0.488	15
West Bengal	0.074	0.168	1.380	0.077	0.466	16
Chhattisgarh	0.292	0.392	0.085	1.359	0.390	17
Tamil Nadu	0.326	0.188	0.361	0.185	0.280	18
Uttar Pradesh	0.224	0.156	0.368	0.121	0.233	19
Haryana	0.106	0.035	0.495	0.057	0.189	20
Punjab	0.124	0.004	0.013	1.052	0.171	21
India	1.000	1.000	1.000	1.000	1.000	

Source: Author's own calculation

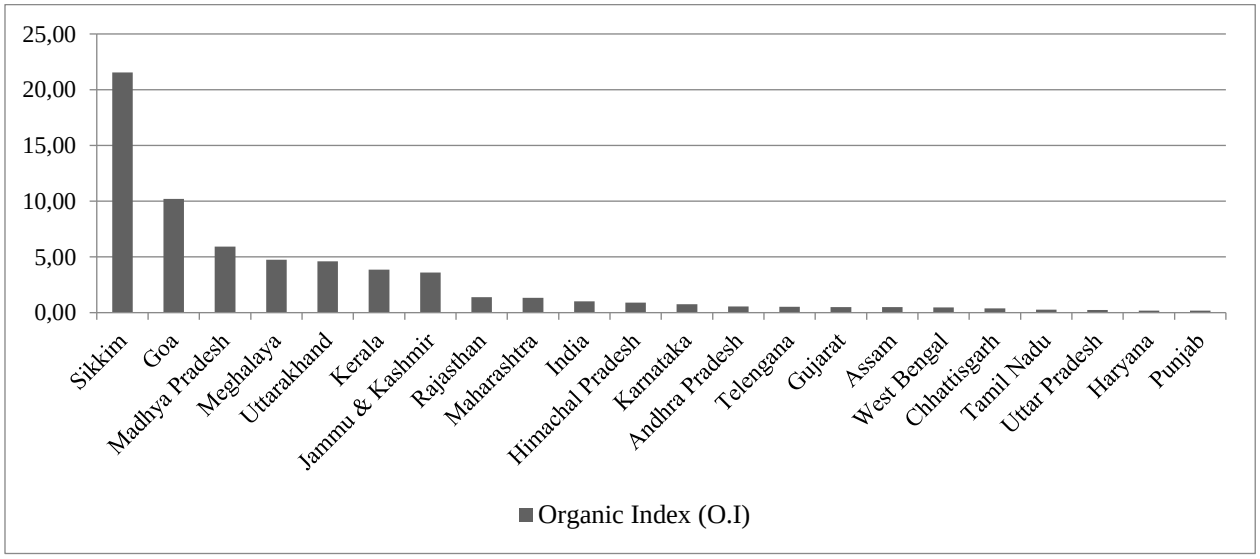


Figure 6: Positions of states across India as per the organic index

Table 6 indicates that Sikkim occupies the highest ranking in the OI with a value of 21.54 and Punjab is at the bottom position with a low OI value of 0.17. Incidentally, Sikkim which holds the top position as per our analysis became the first Indian state to be declared as a fully organic state in 2016 (Yadav, 2018). Among the 21 states included in the study, only nine states (i.e., Sikkim, Goa, Madhya Pradesh, Meghalaya, Uttarakhand, Kerala, Jammu & Kashmir, Rajasthan, and Maharashtra) performed well (i.e., above the national average) in organic farming outreach. The results also highlight that the majority of states (57 percent) bear low OI values (lying between 0.171 and 0.898) and thus show poor performance concerning organic farming. These include states like Himachal Pradesh (10^{th}), Karnataka (11^{th}), Andhra Pradesh (12^{th}), Telangana (13^{th}), Gujarat (14^{th}),

Assam (15th), West Bengal (16th), Chhattisgarh (17th), Tamil Nadu (18th), Uttar Pradesh (19th), Haryana (20th), and Punjab (21st).

The results of the study mostly correspond with the observations of the geospatial digital map. For instance, states such as Sikkim, Goa, Madhya Pradesh, Meghalaya, Uttarakhand, Kerala, Jammu & Kashmir, and Maharashtra which have performed well as per our analysis are also listed as the best regions in the geospatial digital map. Moreover, our analysis shows that states such as West Bengal, Uttar Pradesh, Haryana, and Punjab which are known for their achievements in conventional agricultural practices have performed very poorly in organic farming. Similar observations were also made by the map which suggested very little potential for organic cultivation in these four states due to their intensive cultivation practices. However, there are a few cases in which the findings of our study and the observations of the geospatial digital map differ. For instance, our analysis reveals that a few states such as Himachal Pradesh, Karnataka, Assam, and Chhattisgarh, despite their rich potential for organic cultivation practices have failed to register a noteworthy performance in organic farming. This may be due to the lack of initiatives by their state governments to promote organic farming in their states. On the other hand, the state of Rajasthan despite its limited potential has performed considerably well in the different dimensions of organic farming. The success of Rajasthan can be attributed to the efforts of its state government, which undertook a separate policy on organic farming in 2017 intending to promote an inclusive development of agriculture as organic, facilitating the environment of organic farming, and most importantly, making organic farming remunerative for farmers (GoR, 2017).

4. Conclusions and Policy Implications

In light of growing concern over the sustainability of the existing agriculture system, the quest for an alternative farming system has begun worldwide. Consequently, organic farming has emerged as an alternative farming system because it offers solutions to the problems of the agriculture sector in terms of environmental protection, conservation of non-renewable resources, and improved food quality. The organic farming movement has also gained considerable momentum worldwide due to a significant rise in the demand for organic food because of increasing consciousness about health problems arising from chemical pesticides and fertilizers-contaminated food. From the economic perspective, organic farming is becoming increasingly attractive to farmers as it not only involves lower costs but also generates higher returns as compared to conventional farming (Delate et al., 2003; Raj et al., 2005; Bektas and Miran, 2006; Gibbon and Bolwig, 2007; Singh and Grover, 2011). In the context of India, where the agriculture sector is currently in distress with reducing profitability due to the rising cost of inputs and stagnant output price, wider adoption of organic farming is considered to be a key strategy in effectively addressing these issues (Seufert et al., 2012; Reddy, 2017). Keeping these in focus, the government of India is promoting organic farming through the introduction of various initiatives. As a result, there has been a considerable improvement in different dimensions of organic farming (i.e., the size of the organic area, the volume of organic production, the volume of organic export, and the number of organic cultivators, etc.) in India over time. Each of these dimensions is analyzed individually to evaluate the state-wise practice of organic farming. However, a dimension-wise disaggregated analysis fails to depict the overall picture of organic farming. The reason is the lack of uniformity in dimension-wise performance across Indian states (for instance, some states are performing better in some dimensions but lagging in other dimensions of organic farming). Thus, a composite analysis based on all these dimensions is desirable to provide a composite picture of the present scenario of organic farming in India. The composite index of organic farming (referred to as the organic index) shows a wide disparity in performances concerning organic farming among the states in India. An inter-state analysis revealed that only nine states (Sikkim, Goa, Madhya Pradesh, Meghalaya, Uttarakhand, Kerala, Jammu & Kashmir, Rajasthan, and Maharashtra) have recorded a better performance in organic farming than the national average. However, more importantly, the analysis points out that the performance of the majority of states is far from satisfactory. Interestingly, it is observed that the states that are known for their achievements in conventional agriculture (such as West Bengal, Uttar Pradesh, Haryana, and Punjab) performed very poorly in terms of organic agriculture. More importantly, the result shows that the states that have performed well in organic farming also have a higher level of organic carbon stock in their soil. Therefore, the findings of this paper support our hypothesis that highlights the influence of agro-climatic conditions on the country's organic cultivation practices.

The results also point out that the progress of organic farming is mainly concentrated in certain parts of the country, and the majority of the states failed to register a noteworthy performance. This is evident from the fact that only 2 percent of the country's net sown area is farmed under the organic farming system, and just 1.3 percent of the Indian farmers are registered for organic farming (Khurana and Kumar, 2020). To address this issue, public policies on organic agriculture can be designed as follows:

First, to remove the regional disparity in organic farming performances, the government may encourage a larger number of farmers (in the states that are performing poorly) to adopt organic farming. For this purpose, awareness campaigns can be organized among the farmers to highlight the importance of organic agriculture and its potential benefits. There is also a need to study the perceptions of farmers in these states regarding organic farming as it can be helpful in the effective design of these awareness initiatives and improve their understanding of this alternative mode of cultivation.

Secondly, while designing the state's agricultural policies specific emphasis can be given to organic farming. It has been seen that states adopting separate organic agriculture policies made significant strides in different dimensions of organic farming. For instance, after the adoption of an exclusive policy on organic agriculture in 2003, Sikkim became a fully organic state in 2016. Few other states like Madhya Pradesh, Meghalaya, Uttarakhand, Kerala, and Rajasthan that have done well in organic farming have also adopted separate organic farming policies. However, at the policy level, there are apprehensions about the efficacy of organic farming in feeding the country's vast population (Reddy et al., 2022). The recent Sri Lankan experience shows that an unplanned nationwide shift from conventional to organic farming resulted in a substantial reduction in crop yield and created a food crisis in the country. Therefore, the central and the state governments need to adopt a well-calibrated approach, instead of an unplanned blanket adoption of organic farming. Considering the implications of agro-climatic factors on the performance of organic agriculture, a location-specific strategy would be more effective than the nation or state-wide blanket adoption. Many have advocated for prioritizing by default, rainfed, hilly, and tribal areas where the farmers use less chemical fertilizers for the conversion toward organic farming (Ramesh et al., 2005; Reddy et al., 2022). Apart from the agro-climatic factors, it has been observed that various agro-economic factors such as cost of cultivation, yield, prices of crops, access to markets, etc. play a significant role in organic conversion decisions. Therefore, it is also necessary to evaluate the prospect of organic farming from the agro-economical perspective. In this context, state-wise farm-level studies on organic farming can be undertaken to examine the economic feasibility of this alternative mode of cultivation and identify the challenges faced by organic farmers across the country.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Koner, N., Laha, A.; Design: Laha, A., Koner, A.; Data Collection or Processing: Koner, A.; Statistical Analyses: Koner, A.; Literature Search: Koner, A.; Writing, Review and Editing: Koner, A., Laha, A.

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Appendix

Figure A.1: Year-wise Progression of Organic Farming Area in India

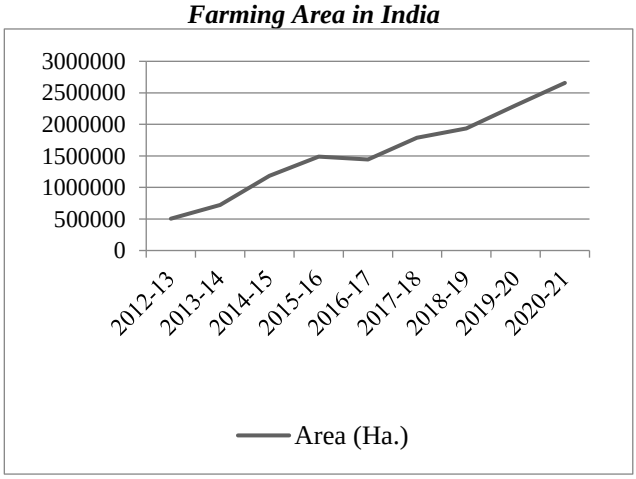


Figure A.2: Year-wise Progression of Organic Production in India

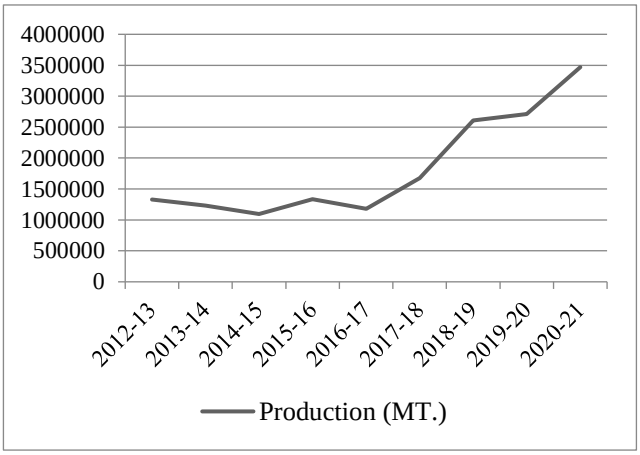


Figure A.3: Year-wise Progression of Organic Exports in India

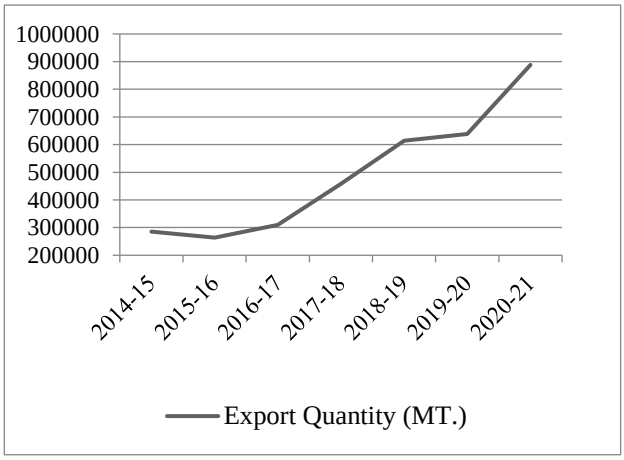
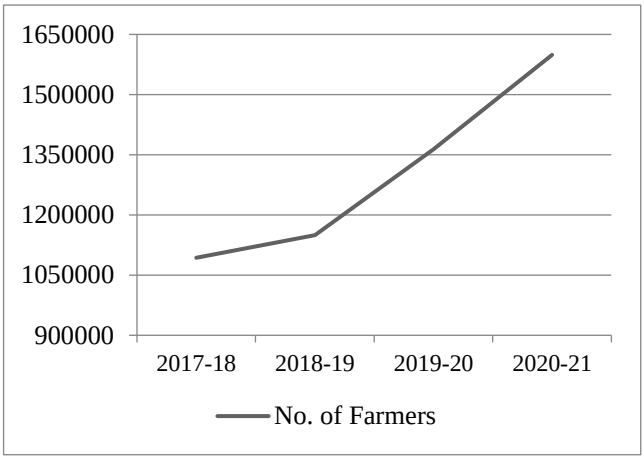


Figure A.4: Year-wise Progression of Organic Farmers in India



Source: Authors own calculation based on the data published by APEDA

Table A.1: Result of PCA and Determination of Final Weights

Component	Initial Eigen Values			Extraction Sums of Squared Loadings		
	Total	Percent of Variance	Cumulative Percent	Total	Percent of Variance	Cumulative Percent
D_1	1.916	47.89	47.89	1.916	47.89	47.89
D_2	1.01	25.251	73.141	1.01	25.251	73.141
D_3	0.686	17.154	90.294	KMO Measure of Sample Adequacy = 0.592		
D_4	0.388	9.706	100	χ^2=11.820 (p value = 0.066)		

Principal Component OI

Factors	Factor Loadings (A)	Factor Loadings (B)	Squared Factor Loadings (C)	Squared Factor Loadings (D)	Normalized by scaled to unity sum (E) = (C)/EV	Normalized by scaled to unity sum (F) = (D)/EV	(G) = Higher of (E) or (F)	Weight H = (G) × EV/TV	Normalized by scaled to unity sum (I) = H/∑H	Final Weight (J) = (I)
D_1	-0.039	0.913	0.001521	0.833569	0	0.7	0.7	0.29	0.32	0.32
D_2	0.856	-0.077	0.732736	0.005929	0.42	0	0.42	0.25	0.28	0.28
D_3	0.856	0.209	0.732736	0.043681	0.42	0.04	0.42	0.25	0.28	0.28
D_4	0.514	0.558	0.264196	0.311364	0.15	0.26	0.26	0.11	0.12	0.12
Total	2.19	1.6	1.73	1.19	1	1	1.8	0.89	1	1

Explained Variation (EV) = ∑Squared factor loadings
Total Variation (TV) = ∑Explained Variation 2.93

Source: Author's own composition

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Chemical Properties and Antioxidant Activity of Different Extracts from Purslane (*Portulaca Oleracea* L.)*

Semizotunun (*Portulaca Oleracea* L.) Farklı Ekstraktlarının Kimyasal Özellikleri ve Antioksidan Aktivitesi

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Abstract

Purslane (*Portulaca oleracea* L.) has been known as a medical herb with valuable nutritional and pharmacological properties. Herein, water, methanol, and acetone extracts were prepared from two morphologically different purslane samples, thereafter their content of β-carotene, chlorophyll a and b, total phenolic compounds (TPC), total flavonoids, and total flavonols was evaluated. Additionally, DPPH• scavenging activity and ferrous iron (Fe^{+2}) chelating activity of these extracts were also measured. The acetone extract had the highest amounts of β-carotene (14.16 mg g^{-1} DW), chlorophyll a (40.46 mg g^{-1} DW), chlorophyll b (9.94 mg g^{-1} DW), TPC (51.01 mg GAE g^{-1} DW), flavonoids (133.23 mg RE g^{-1} DW), and flavonols (46.94 mg RE g^{-1} DW). Whereas the lowest values were observed in the water extract, as 0.03 mg g^{-1} DW, 0.03 mg g^{-1} DW, 0.06 mg g^{-1} DW, (34.24 mg GAE g^{-1} DW, 10.98 mg RE g^{-1} DW, 3.28 mg RE g^{-1} DW, respectively. The acetone extract also showed higher DPPH• scavenging activity (IC_{50} = 57.23 µg mL^{-1}) compared to methanol (IC_{50} = 65.80 µg mL^{-1}) and water (IC_{50} = 71.47 µg mL^{-1}) extracts. Regarding the ferrous iron (Fe^{+2}) chelating activity, methanol extract exhibited the highest value followed by water and acetone extracts, being 12.78, 10.20 and 6.08%, respectively. A positive correlation was detected between the DPPH• scavenging activities of the purslane extracts and their content of TPC, flavonoid, flavonol, β-carotene, chlorophyll a, and chlorophyll b. On the other hand, there was no significant correlation between chelating activity of the extracts and their content of β-carotene, chlorophyll a, TPC, flavonoids, flavonols, and DPPH scavenging activity. Purslane extracts could be suggested as a natural antioxidant substance for many pharmaceutical and food applications.

Keywords: *Portulaca oleracea*, Purslane extract, DPPH• scavenging activity, Phenolic compound, Metal chelating

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Öz

Semizotu (*Portulaca oleracea* L.), besinsel değeri ve farmakolojik özellikleri ile tıbbi bir bitki olarak bilinmektedir. Çalışmada, morfolojik olarak farklı özelliklere sahip iki semizotu örneğinin su, metanol ve aseton kullanılarak üç farklı çözücü ile ekstraktları elde edilmiştir. Elde edilen ekstraktların β-karoten, klorofil a ve b, toplam fenolik madde (TFM), toplam flavonoid ve toplam flavonol içerikleri belirlenmişe olarak bu ekstraktların DPPH• radikal giderme aktivitesi ve Ferrous iron (Fe^{+2}) çelatlama aktivitesi de ölçülmüştür. Aseton ekstraktının en yüksek β-karoten (14.16 mg g^{-1} DW), klorofil a (40.46 mg g^{-1} DW), klorofil b (9.94 mg g^{-1} DW), total fenolik madde (51.01 mg GAE g^{-1} DW), flavonoid (133.23 mg RE g^{-1} DW) ve flavonol (46.94 mg RE g^{-1} DW) içeriğine sahip olduğu tespit edilmiştir. En düşük değerleri ise, sırasıyla 0.03 mg g^{-1} DW, 0.03 mg g^{-1} DW, 0.06 mg g^{-1} DW, 34.24 mg GAE g^{-1} DW, 10.98 mg REg^{-1} DW, 3.28 mg RE g^{-1} DW su ekstraktında bulunmuştur. Ayrıca aseton ekstraktının (IC_{50} = 57.23 µg mL^{-1}) metanol (IC_{50} = 65.80 µg mL^{-1}) ve su (IC_{50} = 71.47 µg mL^{-1}) ekstraktlarına kıyasla daha yüksek DPPH• giderme aktivitesi gösterdiği belirlenmiştir. Ferro demir (Fe^{+2}) çelatlama aktivitesi bakımından, metanol ekstraktı (%12.78) en yüksek ferro demir (Fe^{+2}) çelatlama aktivitesi göstermiş, bunu sırası ile su ekstraktı (%10.20) ve aseton ekstraktı (%6.08) takip etmiştir. Semizotu ekstraktlarının DPPH• giderme aktivitesi ile total fenolik madde, flavonoid, flavonol, β-karoten, klorofil a ve klorofil b içerikleri arasında pozitif bir korelasyon izlenmiştir. Öte yandan, ekstraktların ferro demir (Fe^{+2}) çelatlama aktivitesi ile β-karoten, klorofil a, TFM, flavonoid ve flavonol içerikleri ve DPPH• giderme aktivitesi arasında önemli bir korelasyon tespit edilmemiştir. Semizotu ekstraktlarının çeşitli farmasötik ve gıda sanayiinde doğal antioksidan madde olarak kullanılması önerilebilir.

Anahtar Kelimeler: *Portulaca oleracea*, Semizotu ekstraktı, DPPH• giderme aktivitesi, Fenolik bileşikler, Çelatlama aktivitesi

1. Introduction

Fruit, vegetables, and herbs are recognized as good sources of natural bioactive compounds which exhibit a wide range of nutritional and pharmacological properties. These compounds, particularly polyphenols, carotenoids, glucosinolates, and vitamins (vitamin C and tocopherol) have been demonstrated to represent high antioxidant activity and consequently protect and/or reduce the risk of many chronic diseases due to their ability to reduce oxidative stress and prevent oxidation of macromolecules (Barba et al., 2014; Shashirekha et al., 2015; Xu et al., 2017; Kök et al., 2018; Binici and Şat, 2021; Tahmaz et al., 2022). In response to the consumers' increased demand for more natural products, the natural antioxidants extracted from plants have been promoted as pharmaceuticals, cosmetic ingredients, and food additives, being safer, healthier, and causing fewer adverse responses compared to synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and TBHQ (tert-Butylhydroquinone) (Tadhani et al., 2007; Barba et al., 2014; Ünal, 2017; Xu et al., 2017).

Purslane (*Portulaca oleracea* L.), belonging to the family *Portulacaceae*, is an annual cosmopolitan herb. Because of its extreme adaptability and tolerance to severe conditions, purslane is widely spread all over the world, especially in tropical and subtropical areas (Uddin et al., 2012b; Alam et al., 2014b). Purslane leaves and stems are usually consumed fresh in various salads. However, fresh and dried purslane could be also added to various meals, soups, and even consumed as pickles. In addition, it could be used as a food additive (emulsifier or thickener) and as a natural ingredient in skincare products (Poeydomenge and Savage, 2007; Alam et al., 2014a; Binici et al., 2021).

Besides its usage in the human diet, purslane has been commonly used for medical purposes by various ancient cultures, being familiar as “a vegetable for long life” in Chinese culture. Recently, several health benefits have been confirmed by in vitro and in vivo research, experimental animals, and clinical studies. Due to its nutritional and pharmacological properties, World Health Organization (WHO) has stated common purslane as one of the most used medicinal plants and recognized it as a ‘‘global panacea”. Common purslane and its extracts are reported to exhibit antispasmodic, antiarthritic, antiseptic, antimicrobial, antifungal, anticancer, antidiabetic, and antimutagenic effects (Cho et al., 2008; YouGuo et al., 2009; Hasssan, 2014; Silva and Carvalho, 2014). They also play a valuable role in inhibiting some chronic diseases such as cardiovascular, and neurodegenerative (Dkhil et al., 2011).

A diverse group of valuable ingredients have been reported in purslane and/or its extracts including polyphenols, vitamins (particularly vitamins A and C), minerals (particularly potassium, calcium, and magnesium), and water-soluble polysaccharides. Several bioactive compounds principally glutathione, noradrenaline, dopamine β-carotene, and α-tocopherol were also documented (Uddin et al., 2014; Petropoulos et al., 2015; Binici et al., 2021). Various alkaloids such as nandigerine, angustureine, reticuline, itingensine, homolycorine were also identified in purslane (Aziz et al., 2016). Polyunsaturated fatty acids are the major fatty acid group in common purslane. Additionally, purslane is considered one of the richest vegetable sources of the essential fatty acid (alpha-linolenic acid (ALA), C18:3, n-3) accounting for about 60% of the total fatty acid content in leaves and 40% in seeds (Liu et al., 2000; Petropoulos et al., 2015). Furthermore, a remarkably higher level of melatonin (19000 pg g^{-1}) was detected in fresh purslane leaves compared to many other fruits and vegetables (Simopoulos et al., 2005). Other important chemical compounds found in significant amounts in purslane are flavonoids (particularly quercetin, rutin, and myricetin) and phenolic acids (particularly chlorogenic, rosmarinic, and E-vanillic acids) suggesting purslane and its extracts as an important source of antioxidants (Siriamornpun and Suttajit, 2010; Hassan, 2014; Sallam et al., 2017).

Many authors suggested methanol as the most suitable solvent for extracting phenolic compounds from purslane (Uddin et al., 2012a; Sallam et al., 2017). In contrast, Cai et al. (2004) stated higher levels of total phenolics when hot water was used as a solvent compared to methanol. In other study, acetone extracts were reported to possess higher levels of total phenolic content and antioxidant activity compared to methanol and water extracts (Güngören et al., 2017). The phenolic content of purslane and consequently its antioxidant activity is strongly affected by various factors including species origin, variety, plant maturity, and harvesting conditions (Alam et al., 2014a; Uddin et al., 2012a); as well as the extraction method and technique, used solvent, extraction temperature, and time (Alam et al., 2014a; Güngören et al., 2017; Sallam et al., 2017; Uddin et al., 2012a).

Therefore, a wide variation is seen in the literature related to the phenolic contents of purslane and its antioxidant activity.

There is limited data related to the chemical composition and antioxidant activity/capacity of purslane that is grown in Turkey. Moreover, there is inconsistency in the information about the most appropriate solvent for extracting a higher portion of phenolic compounds. Thus, the aim of this study was to identify the most effective solvent for the extraction of bioactive compounds from purslane. For this aim, the antioxidant activity and some chemical properties including the contents of fat-soluble pigments, total phenolic compounds, flavonoids, and flavonols of various purslane extracts (water, methanol, and acetone) were measured from two morphologically different samples.

2. Materials and Methods

2.1. Plant materials

Two morphologically different samples of purslane (*Portulaca oleracea* L.) were obtained from the domestic market of Erzurum-Turkey. The first sample (Sample-1) had large, green (dark), fleshy leaves, and thick stems, whereas the other sample (Sample-2) had small, yellow-green (chartreuse color), and thin leaves. The stems of this sample were thinner compared to Sample-1 and their lower bits were red in color. Each sample was cleaned thoroughly, cut into small pieces, and frozen at – 20°C. The frozen samples were freeze dried (lyophilized) at – 80°C for 36 h with Operon (FDU-8612, KR) lyophilizer. Then the dried samples were ground, homogenized with a high-speed blender (Waring, 8011ES), and kept in glass jars at 4°C until the extraction.

2.2. Purslane extracts preparation

Purslane extracts were prepared by maceration of each sample with distilled water, methanol, and acetone according to the procedures described by Alam et al. (2014a) with some changes. 10 g of lyophilized purslane powder was mixed with 150 mL of solvent (1:15, g mL^{-1}) in dim light at ambient temperature for about 14 h using an orbital shaker (Biocote-SSL1, UK). The mixture was then passed through filter paper (Whatman No:1) and the plant's remnant was reextracted with fresh solvent for 3 h following the same steps mentioned previously. A rotary evaporator (Heidolph laborata 4000 efficient- HB digital) was used to concentrate the supernatants and the final volumes were reduced to 70 mL. The concentrated supernatants were evaporated at 45°C using a vacuum oven (WiseVen, WOV-30). Obtained extracts were stored at – 20 °C in screw cap glass brown bottles until further analyses. The dried extracts were redissolved in methanol at 2000 µg mL^{-1} (stock solution) immediately prior to the analyses.

2.3. Fat-soluble pigments (β-carotene and chlorophyll a and b) contents

Estimation of β-carotene and chlorophylls a and b was carried out according to the procedures represented by Barros et al. (2011) as follows: 100 mg of the purslane dried extract was thoroughly mixed with 10 mL of the acetone-hexane mixture (4:6) for 5 min. The filtrates (obtained by filtration through Whatman, No:1) were then diluted with the acetone-hexane mixture and their absorbances were measured at three different wavelengths (453, 505, 645, and 663 nm) against blank. The amounts of the fat-soluble pigments were determined using the following formulas (Eq.1-Eq.3) and expressed as mg 100 g^{-1} DW.

$$Chlorophyll\ a = 0.999 \times A^{663} - 0.0989 \times A^{645} \quad \text{(Eq.1)}$$

$$Chlorophyll\ b = -0.328 \times A^{663} + 1.77 \times A^{645} \quad \text{(Eq.2)}$$

$$\beta - carotene = 0.216 \times A^{663} - 1.220 \times A^{645} - 0.304 \times A^{505} + 0.452 \times A^{453} \quad \text{(Eq.3)}$$

2.4. Total phenolic compounds (TPC) content

The content of total phenolic compounds was determined using the procedures reported by Barros et al. (2011) as follows: 0.5 mL of each purslane extract were thoroughly vortexed with Folin-Ciocalteu reagent (0.5 mL) and Na_2CO_3 solution (1.5 mL, 20%). After adjusting the final volume to 10 mL the tubes were let to stand for 30 min at ambient temperature under dark conditions. Thereafter the absorbance was read at 760 nm against blank. A standard curve was created using gallic acid and the obtained regression equation ($y = 0.0984x - 0.1909$, $R^2 = 0.9941$) was employed to calculate the total phenolic content in the extracts as mg GAE (gallic acid equivalent) g^{-1} DW.

2.5. Total flavonoids content

The extracts' content of total flavonoids was measured spectrophotometrically according to the method reported by Pękal and Pyrzynska (2014) with slight changes. Briefly, purslane extract (0.5 mL) was combined with distilled water (2 mL) and $NaNO_2$ (0.15 mL, 5%, w v^{-1}). After 5 min, $AlCl_3$ (0.15 mL, 10%, w v^{-1}) was added to the mixture and the tubes were left at ambient temperature for 6 min. After neutralizing the mixture with NaOH (2 mL, 4%, w v^{-1}), the final volume was adjusted to 10 mL and the tubes were left for 10 min. The absorbance was measured at 510 nm against a blank. Rutin was used for creating the standard curve and the obtained regression equation (y= 0.0114x – 0.0079, R^2 = 0.9941) was employed to calculate the total flavonoids content in the extracts as mg RE (rutin equivalent) g^{-1} DW.

2.6. Total flavonols content

The extracts' content of total flavonols was tested according to the procedures described by Almaraz-Abarca et al. (2007) and Al-Dabbas (2017) with slight changes as follows: Purslane extract solution was vortexed with 0.5 mL of aluminum trichloride (0.5 mL, 2%, w v^{-1}), sodium acetate (0.5 mL, 5%, w v^{-1}) and distilled water (8.5 mL). Thereafter, the tubes were left at ambient temperature for 10 min and the absorbance was read at 425 nm against blank. A standard curve was created using rutin and the obtained regression equation (y = 0.0194x + 0.0038, R^2 = 0.9937) was employed to calculate the total flavonols content in the extracts as mg RE g^{-1} DW.

2.7. DPPH• scavenging activity

DPPH• scavenging activity of purslane extracts was determined following the method reported by Brand-Williams et al. (1995) with slight changes. Succinctly, a methanolic solution of DPPH (1.01 mM) was thoroughly mixed with purslane extracts at several concentrations (140 – 180 µL of extracts) and the final volume was brought to 4 mL using methanol. The absorbance was read at 517 nm at the end of the incubation period (30 min in dark at ambient temperature). The DPPH• scavenging activity (%) of each extract was assessed using equation 4 (Eq.4):

$$\text{DPPH} \bullet \text{ scavenging activity } (\%) = [(A^{\text{DPPH}} - A^{\text{sample}}) \times A^{DPPH^{-1}}] \times 100 \quad \text{(Eq.4)}$$

where A_{sample} refers to the absorbance of DPPH methanolic solution mixed with the sample, and A_{DPPH} refers to the DPPH methanolic solution without sample. BHA and α-tocopherol were used as control. The concentration (IC_{50}) of the extract causing scavenging of 50% of DPPH• was reckoned from the graph of extract concentration versus scavenging activity (%), and the IC_{50} value was expressed as µg mL^{-1}.

2.8. Ferrous iron (Fe^{+2}) chelating activity

The ferrous iron (Fe^{+2}) chelating activity was measured by mixing the purslane extracts (0.5 mL) with $FeCl_2$ (0.1 mL, 2 mM) and ferrozine solutions (0.2 mL, 5 mM). The tubes were then allowed to stand for 10 min at ambient temperature, thereafter, the absorbance was read at 562 nm against blank. The control was prepared by mixing $FeCl_2$ and ferrozine solution and the percentage ferrous iron (Fe^{+2}) chelating activity of various purslane extracts was assessed using the equation 5 (Eq.5) (Kumar et al., 2008):

$$\text{Ferrous iron } (Fe^{+2}) \text{ chelating activity } (\%) = [(A^{\text{control}} - A^{\text{sample}}) \times A^{control^{-1}}] \times 100 \quad \text{(Eq.5)}$$

2.9. Statistical analysis

Completely randomized-factorial design (2 purslane samples × 3 solvents) was used in this research. The results were expressed as a mean ± standard deviation of triplicates. To determine the differences between means, variance analysis followed by Duncan Multiple Comparison Test was conducted using the SPSS program (IBM SPSS Inc., version 20).

3. Results and Discussion

3.1. Fat-soluble pigments (β-carotene and chlorophylls a and b) contents of purslane extracts

Chlorophylls and carotenoids are natural pigments found in numerous edible plants. Chlorophyll a and chlorophyll b are the most widespread forms of chlorophylls and are usually found in higher plants at a ratio of 3 to 1, respectively. β-carotene (a vitamin A precursor) is one of the prominent and widespread members of carotenoids. However, many studies reported the association between these pigments and several health benefits including prevention and/or

protection against diverse types of cancer, coronary artery diseases and infections along with their roles as an antioxidant and anti-inflammatory agents (Mishra et al., 2011; Ghosh et al., 2018; Pérez-Gálvez et al., 2020). The content of β-carotene, chlorophyll a, and chlorophyll b in different purslane extracts were displayed in *Table 1*. As noted in the table, the acetone extracts exhibited the highest contents of β-carotene (14.16 mg g^{-1} DW), chlorophyll a (40.46 mg g^{-1} DW), and chlorophyll b (9.94 mg g^{-1} DW). Whereas the water extracts had remarkably lower amounts of these pigments, being 0.03, 0.03, and 0.06 mg g^{-1} DW, respectively. This could be explained by the polarity of the used solvent. Since chlorophylls and carotenoids are fat-soluble compounds, so they are expected to be more soluble in acetone (organic solvent) than methanol and water. By contrast, no significant differences between extracts obtained from the morphologically different purslane samples were observed. Several studies revealed purslane as a key source of various biologically active compounds particularly vitamin C, vitamin A, β-carotene, and α-tocopherol (Siriamornpun and Suttajit, 2010; Uddin et al., 2012a; Alam et al., 2014b; Youssef and Mokhtar, 2014).

Table 1. Fat-soluble pigments (β-carotene, chlorophyll a and chlorophyll b) contents (mg g^{-1} DW) of various extracts from purslane plant.

	Sample-1			Sample-2			Avg.		
	β-carotene	**Chlorophyll a**	**Chlorophyll b**	**β-carotene**	**Chlorophyll a**	**Chlorophyll b**	**β-carotene**	**Chlorophyll a**	**Chlorophyll b**
Water	0.01 ± 0.00^{Ab}	0.03 ± 0.00^{Ab}	0.06 ± 0.01^{Ab}	0.05 ± 0.00^{Ab}	0.03 ± 0.00^{Ab}	0.06 ± 0.00^{Ab}	0.03 ± 0.02^{c}	0.03 ± 0.00^{b}	0.06 ± 0.01^{b}
Methanol	3.06 ± 0.55^{Ab}	10.29 ± 1.25^{Ab}	1.26 ± 0.34^{Ab}	4.10 ± 0.06^{Ab}	12.45 ± 2.25^{Ab}	0.87 ± 0.17^{Ab}	3.58 ± 0.67^{b}	11.37 ± 2.01^{b}	1.07 ± 0.32^{b}
Acetone	12.15 ± 5.57^{Aa}	40.55 ± 22.94^{Aa}	13.22 ± 9.46^{Aa}	16.18 ± 2.89^{Aa}	40.36 ± 7.02^{Aa}	6.66 ± 0.99^{Aa}	14.16 ± 4.54^{a}	40.46 ± 15.17^{a}	9.94 ± 7.01^{a}

SD: standard deviation; a-c: means with different (small) letters within the same column are significantly different ($p < 0.05$); A-B: means with different (capital) letters within the same line and same compound group are significantly different ($p < 0.05$). Results were presented on dry weight basis.

3.2. Total phenolic, flavonoids, and flavonols contents of purslane extracts

Phenolic substances are secondary metabolites synthesized by plants and play critical roles in various functions at plants and are associated with numerous benefits for human health (Kumar and Pandey, 2013; Ávila-Román et al., 2021;). Purslane and its extracts are well known for their abundance of polyphenolics and strong antioxidant properties (Alam et al., 2014b; Hussien, 2016). The total phenolic, flavonoids, and flavonols contents of purslane extracts were showed in *Table 2*. Regarding the total phenolic content, purslane extracts varied significantly ($p < 0.01$) depending on the polarity of the used solvent. The highest amount of total phenolic compounds was detected in the acetone extracts (51.01 mg GAE g^{-1} DW) followed by methanol (45.90 mg GAE g^{-1} DW) and water (34.24 mg GAE g^{-1} DW) extracts in decreasing order. The solubility of phenolic substances is highly associated with their chemical structures, nature, and polymerization degree. In addition, phenolics are willing to interact with other plant components, including carbohydrates and proteins resulting in the formation of complexes with different solubility features. Moreover, the polarity of the solvent used in the extraction also influences the solubility and consequently the extractability of phenolic substances. Therefore, different amounts of various kinds of phenolic substances could be obtained from the same plant using different solvent systems (Naczk and Shahidi, 2006). The efficiency of acetone to extract higher levels of phenolic substances could be attributed to its capability to suppress the formation of protein-polyphenol complex throughout the extraction process or break down hydrogen bonds that formed between the carboxyl group of protein and phenolic group (Wang et al., 2009). Our findings were in accordance with those observed by Güngören (2016) who reported a higher amount of total phenolic compounds in purslane acetone extract compared to methanolic and aqueous extracts. Likewise, our findings were aligned with Uddin et al. (2012a) and Sallam et al. (2017) who reported higher values of total phenolic contents in purslane methanolic extracts than those in aqueous extracts.

The flavonoids content significantly varied from 7.65 mg RE g^{-1} DW in water extract of Sample-1 to 167.19 mg RE g^{-1} DW in acetone extract of Sample-1. In parallel with TPC results, acetone was also found to be more efficient to extract flavonoids from purslane samples with content of 133.23 mg RE g^{-1} DW followed by methanol and water being 99.47 and 10.98 mg RE g^{-1} DW, respectively (*Table 2*). On the other perspective, extracts obtained from two

morphologically different samples were found to be significantly ($p < 0.01$) different in terms of flavonoids content. Similarly, Uddin et al. (2012a) and Sallam et al. (2017) stated that methanol was more effective for flavonoid extraction compared to other solvents (ethanol and water). Regarding the flavonoids levels, our results showed that methanolic extract had higher flavonoids levels than what was reported previously by Uddin et al. (2012a) and Alam et al. (2014a) who reported content of 0.13 – 1.44 and 28.7 – 49.18 mg RE g^{-1}, respectively. The high values observed in the current study could be attributed to variations in various factors such as subspecies, varieties, growing conditions and extraction process.

Table 2. Total phenolic (mg GAE g^{-1} DW), flavonoids (mg RE g^{-1} DW) and flavonols (mg RE g^{-1} DW) contents of various extracts from purslane (mean ± SD).

	Sample-1			Sample-2			Avg.		
	Phenolics	**flavonoids**	**Flavonols**	**Phenolics**	**flavonoids**	**Flavonols**	**Phenolics**	**flavonoids**	**Flavonols**
Water	29.18±0.47^{Bb}	7.65±0.43^{Ac}	0.80±1.28^{Ac}	39.31±4.42^{Ab}	14.30±0.75^{Ac}	5.76 ±1.16^{Ab}	34.24±6.22^{c}	10.98±3.68^{c}	3.28±2.93^{c}
Methanol	47.03±3.57^{Aa}	128.18±12.18^{Ab}	18.41±5.63^{Bb}	44.78±5.03^{Ab}	70.76±3.06^{Bb}	46.19±4.03^{Aa}	45.90±4.09^{b}	99.47±32.44^{b}	32.30±15.84^{b}
Acetone	48.29±1.81^{Aa}	167.19±19.81^{Aa}	42.51±10.20^{Aa}	53.72±0.79^{Aa}	99.27±11.85^{Ba}	51.36±8.23^{Aa}	51.01±3.23^{a}	133.23±39.96^{a}	46.94±9.60^{a}

SD: standard deviation; a-c: means with different (small) letters within the same column are significantly different ($p < 0.05$); A-B: means with different (capital) letters within the same line and same compound group are significantly different ($p < 0.05$). Results were presented on dry weight basis.

In terms of flavonols content, a significant ($p < 0.01$) difference was spotted between extracts obtained from two morphologically different purslane samples (*Table 2*). The acetone extract had the highest flavonols content among the studied extracts, at 46.94 mg RE g^{-1}, succeeded by methanol (32.30 mg RE g^{-1}) and water (3.28 mg RE g^{-1}) extracts. Furthermore, the flavonols content of the water extract was found to be remarkably lower than that of other extracts (*Table 2*). Thus, the most effective solvent for flavonols extraction from purslane was also found to be acetone. It is difficult to compare the obtained results with other works because none have been published concerning total flavonols content in purslane extracts. However, Binici et al. (2021) stated that the total flavonols amount in fresh purslane varied between 3.48–17.27 mg RE g^{-1}. In addition, several studies indicated the presence of various substances that belong to the flavonol group such as rutin, myricetin, quercetin, and campherol in several parts of purslane plants (Siriamornpun and Suttajit, 2010; Erkan, 2012; Hassan, 2014). Furthermore, rutin was reported as the dominant flavonoid in purslane leaves and the major flavonoid in stems and flowers is myricetin (Siriamornpun and Suttajit, 2010).

3.3. DPPH• scavenging activity of purslane extracts

DPPH• was used to estimate the capability of various purslane extracts to scavenge free radicals. DPPH• scavenging activities of water, methanol, and acetone extracts obtained from purslane samples were displayed in *Figure 1*. As seen in the figure the DPPH• scavenging activities of the purslane extracts were increased as the dose increased. The purslane extracts exhibited antioxidant activity varied between 3.74% (water extract of Sample-2) and 28.29% (acetone extract of Sample-2). However, the two morphologically different purslane samples almost showed similar antioxidant activities. Whereas different extracts significantly ($p < 0.01$) varied in their antioxidant activities from 3.74 to 25.35%. In general, the minimum antioxidant activity was observed in water extracts, while the other extracts (methanol and acetone) revealed higher antioxidant activities. Furthermore, these two extracts (methanol and acetone) almost showed similar antioxidant activities with no significant ($p > 0.05$) differences between them. Similarly, Güngören et al. (2017) reported a higher DPPH• scavenging activity of the acetone extract (3.28 – 16.1%) obtained from different purslane samples compared to aqueous extract (1.64 – 16.77%). Another study revealed a higher antioxidant activity of the purslane methanolic extract (32.70%) compared to the aqueous extract (20.20%) (Sallam et al., 2017). However, the high antioxidant activity of acetone and methanol extracts in our study could be explained by their high content of TPC, flavonoids, and flavonols. It was pointed out that solvent polarity is strongly impacting the extractability of various antioxidant substances and therefore the antioxidant capacity of the extracts (Wang et al., 2009).

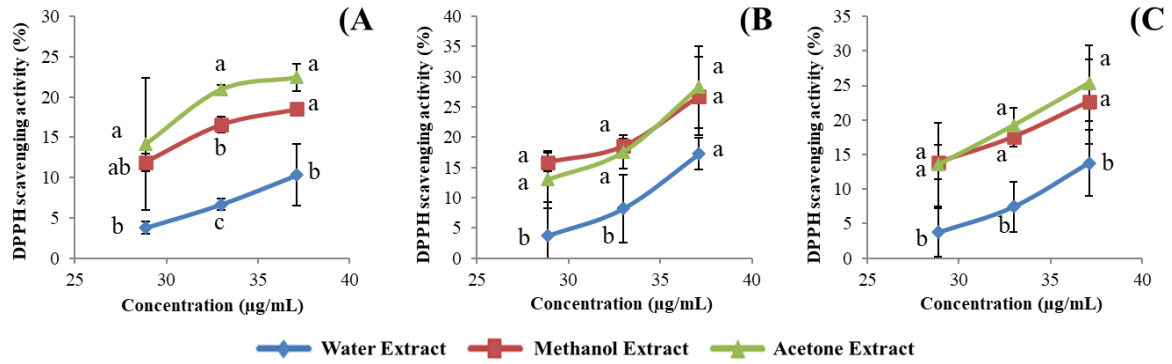

Figure 1. DPPH radical scavenging activity (%) of various extracts from purslane (A) Sample-1, B) Sample-2 and C) Average of both samples)

a-c: data points with different letters (within the same concentration) are significantly different ($p < 0.05$).

Regarding the IC_{50} value (half maximal inhibitory concentration) of purslane extracts, it was found to be between 50.65 µg mL^{-1} and 101.89 µg mL^{-1} (*Figure 2*). However, all extracts exhibited remarkably higher IC_{50} values than those of reference antioxidant substances (α-tocopherol: IC_{50} = 4.116 µg mL^{-1} and BHA: IC_{50} = 2.04 µg mL^{-1}). It is well known that the IC_{50} value is inversely proportional to the radical scavenging activity, i.e., a decrease in the IC_{50} value corresponds with an increase in radical scavenging activity. In the present study, the IC_{50} values were in parallel with the results of DPPH scavenging activity (%). Even though, no significant differences ($p > 0.05$) were identified between IC_{50} values of purslane extracts; in general acetone extract tends to have lower IC_{50} value compared to methanol and water extracts (*Figure 2*). Purslane is widely known as a rich source of various antioxidant compounds. The IC_{50} values of purslane methanolic extracts have been estimated to be between 2.50 – 3.29 mg mL^{-1} and 1.30 – 1.71 mg mL^{-1} according to Alam et al. (2014a) and Uddin et al. (2012a) respectively. Erkan (2012) reported the IC_{50} value of the methanolic crude extract of wild purslane as 511.8 µg mL^{-1}. In another study, the IC_{50} value of water extract obtained from purslane leaf was found to be 2.80 mg mL^{-1} (Siriamornpun and Suttajit, 2010). Our findings revealed stronger DPPH radical scavenging capacity with remarkably lower IC_{50} values than values obtained in the previous studies. However, several factors such as extraction technique, temperature, and time, as well as the size of the plant pieces and the used solvent (particularly solvent polarity) can affect the efficiency of the extraction process to extract certain groups of antioxidant substances and consequently the antioxidant features of the obtained extract (Naczk and Shahidi, 2006; Wang et al., 2009). According to the presented results, purslane extracts may consider as a promising antioxidant source.

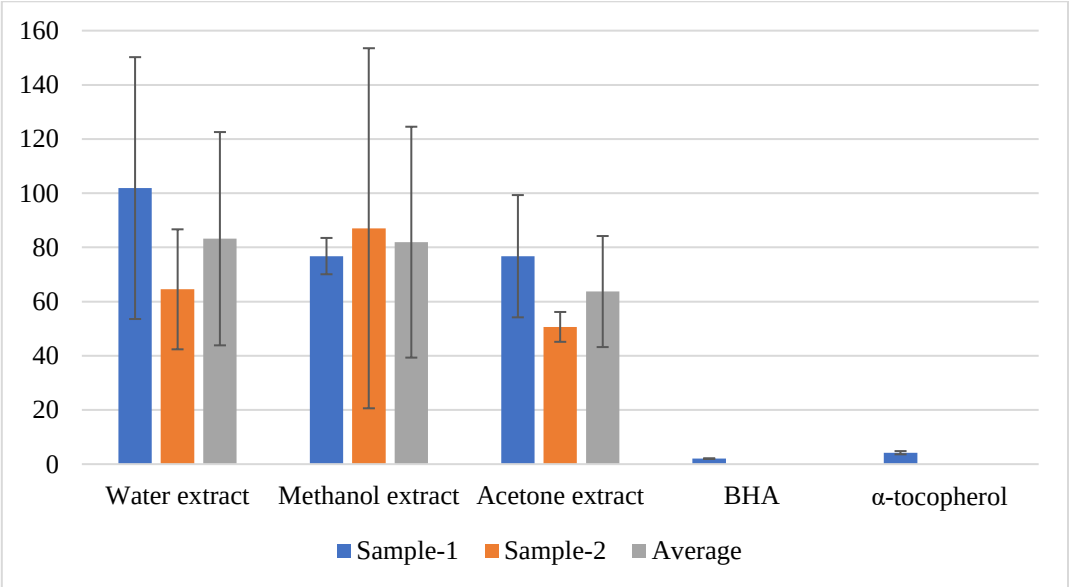

Figure 2. IC_{50} values of various extracts from purslane (µg ml^{-1}).

3.4. Ferrous iron (Fe^{+2}) chelating activity of purslane extracts

Transition metals such as iron, copper, and chromium have been suggested as catalysts in the formation of free radicals that can damage living cells. Substances that possess chelating activity serve as secondary antioxidants by binding metal ions and stabilizing them in the oxidized form, consequently inhibiting oxyradical formation and oxidative damage (Al-Dabbas, 2017). In the current study, the ability of various purslane extracts to capture ferrous iron (Fe^{+2}) ions was determined based on their capability to restrict the development of the ferrozine-Fe^{+2} complex. Obtained results revealed significant ($p < 0.01$) differences between extracts obtained from the two morphologically different purslane samples (*Table 3*). On the contrary, methanol extract showed higher metal chelating activity compared to the other extracts. Whereas acetone extract had the lowest chelating activity. Our findings were in accordance with Güngören et al. (2017) who stated higher ferrous iron (Fe^{+2}) chelating activity of aqueous purslane extracts (3.96 – 42.57%, at a concentration of 0.2 mg mL^{-1}) compared to acetone extracts (0.61 – 6.21%). Similarly, acetone extracts exhibited lower chelating activity than water extracts obtained from seaweeds according to Wang et al. (2009).

Table 3. Fe^{+2} chelating activity (%) of various extracts from purslane (mean±SD).

	Sample-1	**Sample-2**	**Avg.**
Water	5.82 ± 3.30^{Ba}	14.57 ± 3.05^{Aa}	10.20 ± 5.57^{b}
Methanol	8.69 ± 1.27^{Ba}	16.87 ± 1.15^{Aa}	12.78 ± 4.61^{a}
Acetone	1.50 ± 0.68^{Bb}	10.66 ± 0.64^{Ab}	6.08 ± 5.05^{c}

SD: standard deviation; a-c: means with different (small) letters within the same column are significantly different ($p < 0.05$); A-B: means with different (capital) letters within the same line are significantly different ($p < 0.05$). Extract concentration = 100 µg DW mL^{-1}.

As seen in *Table 3* various purslane extracts at a concentration of 100 µg mL^{-1} had ferrous iron (Fe^{+2}) chelating activity varied between 1.50 – 16.87%. Considering the extract concentration, values obtained in the current study were comparable to the values demonstrated by Güngören et al. (2017), contrarily it was remarkably higher than those stated by Peksel et al. (2006). The observed variation could be due to the differences in cultivars, growing conditions or extraction methods. It can be thought that the compounds showing metal chelating activity in plants were preserved better using our extraction method.

3.5. Correlation analysis

The correlation between the results of different assays is displayed in *Figure 3*. Correlation results revealed a strong positive relation between the extracts content of TPC with flavonoid (0.737, $p < 0.01$) and flavonol (0.756, $p < 0.01$) substances, proving the efficiency of acetone solvent in extracting polyphenolic compounds from purslane plant. Furthermore, TPC was also significantly correlated with β-carotene (0.708, $p < 0.01$) and chlorophyll a (0.664, $p < 0.01$) (*Figure 3*). On the other hand, DPPH• scavenging activity of purslane extracts was positively correlated with TPC (0.736, $p < 0.01$), flavonoid (0.823, $p < 0.01$) and flavonol (0.828, $p < 0.01$) compounds, indicating that polyphenolic substances could be the main contributors to the radical scavenging activity of purslane extracts. Moreover, a positive correlation was also observed between the scavenging activity of the extracts and their content of pigments including β-carotene (0.648, $p < 0.01$), chlorophyll a (0.678, $p < 0.01$), and chlorophyll b (0.544, $p < 0.05$). Several compounds including phenolics, flavonoids, flavonols, and various pigments are reported to exhibit a free radical scavenging activity (Miliauskas et al., 2004; Seyoum et al., 2006; Kumar and Pandey, 2013; Pérez-Gálvez et al., 2020;). In the purslane case, a good correlation between antioxidant activity including free radical scavenging activity with TPC and flavonoids contents was demonstrated by Youssef and Mokhtar (2014), Habibian et al. (2020) and Saffaryazdi et al. (2020), and A good correlation between DPPH• scavenging activity of purslane and its pigments content (chlorophyll a, chlorophyll b, carotenoids, and β-carotene) was also reported by Habibian et al. (2020) and Youssef and Mokhtar (2014). Regarding ferrous iron (Fe^{+2}) chelating activity, our findings showed that no significant ($p > 0.05$) correlation between chelating activity with β-carotene, chlorophyll a, TPC, flavonoids, flavonols, and DPPH scavenging activity. Wang et al. (2009) reported similar results in their study on seaweeds and suggested that the

chelating activity of the extracts was more effectively accomplished by other components such as polysaccharides, proteins, or peptides than phenolic compounds.

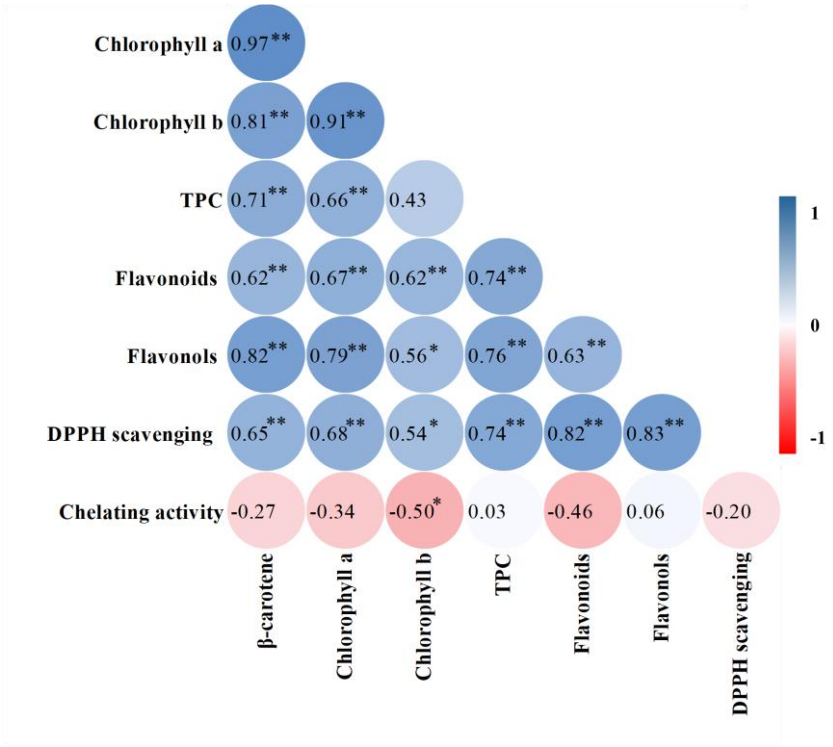

Figure 3. Pearson's correlation coefficient among different chemical attributes of purslane extracts. **: p < 0.01; *: p < 0.05

4. Conclusions

Investigating the chemical composition and antioxidant activity of various purslane extracts showed that the antioxidant activity was significantly affected by the solvent polarity, and the radical scavenging activity was mainly associated with the extract's content of phenolic compound. However, the acetone extract yielded the highest levels of the most studied parameters, while the lowest levels were found in the water extract. This is thought to be due to the capability of acetone to restrict the side interaction and/or the complexes formation throughout the extraction process. Unlike water and methanol, acetone has the advantage of evaporating quickly and easily at lower temperatures during the concentration of the extracts. In contrast, the acetone extract exhibited the lowest chelating activity, whereas the highest value was detected in the methanol extract. However, purslane extracts could be employed to naturally enhance the antioxidant properties of many pharmaceutical and food products.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study

Conflict of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Şat İ.; Supervision: Şat İ.; Methodology: Şat İ., Aoudeh E.; Formal analysis and investigation: Aoudeh E., Binici H.; Statistical Analyses: Aoudeh E., Binici H.; Writing - original draft preparation: Aoudeh E.; Writing - review and editing: Şat İ.; All the authors have read and agreed to the published version of the manuscript.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

The Relationship between Agriculture and Carbon Dioxide Emission in Türkiye: A Non-Linear Evidence

Türkiye'de Tarım Sektörü ve Karbondioksit Emisyonu Arasındaki İlişki: Doğrusal Olmayan Bir Kanıt

İbrahim ÜRKMEZ[1*], Ahmet SEVİM[2], Abdurrahman Nazif ÇATIK[3]

Abstract

Agricultural production has both increased and become more efficient with the development of technology. However, greenhouse gases such as CO_2 released into the air during production cause climate change. This situation also affects agricultural productivity. Therefore, the main objective of this paper is the examine the interaction between agricultural sector activity and CO_2 emissions in Türkiye in a non-linear framework. For this purpose, the Maki cointegration test and the Single Fourier frequency Toda & Yamamoto causality test were used to investigate the interplay between agricultural value added and CO2 using time series data covering the period from 1968 to 2018. In addition to the empirical analysis developed in the paper, our study adds to the literature by studying the relationship between CO_2 and energy consumption in the agricultural sector, as opposed to studies that use aggregate CO_2 emissions as an indicator of climate change. In addition, the short- and long-run interactions between CO_2 and agricultural productivity were investigated by estimating two separate equations where agricultural productivity and CO_2 emissions are used as dependent variables. The Maki cointegration test cointegration test shows the existence of a long-run relationship between agricultural value added and CO_2 emissions under structural breaks. The detected significant breaks are associated with significant events affecting the Türkiye economy. For instance, when agricultural value added is the dependent variable, the break dates of 1971 and 1974 coincide with the oil crisis, while the breaking dates of 2002 and 2008 coincide with Türkiye's 2001 financial crisis and the 2008 global financial crisis. Similarly, the break dates of 1973 and 1977 obtained in the CO_2 equation are associated with the 1970s' global oil crisis. Long-run parameter estimates derived from FMOLS and CCR estimators indicated that CO_2 emissions have a long-run, positive and significant impact on agricultural productivity. In addition, the long-run results support the existence of a positive and significant impact of agricultural productivity on environmental degradation. The gradual shift causality test also supports the presence of one-way causality, running from agriculture output to CO_2.

Keywords: Agriculture sector, Carbon dioxide emissions, Structural break analysis, Nonlinear analysis

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Öz

Tarımsal üretim, teknolojinin gelişmesi ile hem artmıştır hem de daha verimli hale geldiği gerçeği yadsınamaz. Ancak üretim esnasında havaya salınan CO_2 gibi sera gazları iklim değişikliğine neden olmaktadır. Bu durum da tarımsal verimliliği etkilemektedir. Dolayısıyla bu çalışmada Türkiye'de tarım sektörü aktivitesi ile CO_2 emisyonları arasındaki karşılıklı etkileşimi doğrusal olmayan bir çerçevede incelemeyi amaçlamaktadır. Bu amaçla, Maki eşbütünleşme testi ve Tek Fourier frekansı Toda & Yamamoto nedensellik testi, 1968'den 2018'e kadar olan dönemi kapsayan zaman serileri kullanarak tarımsal katma değer ile CO_2 arasındaki etkileşimi araştırmak için kullanılmıştır. Makalede yapılan ampirik analize ek olarak, çalışmamız, toplam CO_2 emisyonlarını iklim değişikliğinin bir göstergesi olarak kullanan çalışmaların aksine, tarım sektöründe CO_2 ve enerji tüketimi arasındaki ilişkiyi inceleyerek literatüre katkıda bulunmaktadır. Ayrıca, CO_2 ve tarımsal verimlilik arasındaki kısa ve uzun vadeli etkileşimler, tarımsal verimlilik ve CO_2 emisyonlarının bağımlı değişkenler olarak kullanıldığı iki ayrı denklemin tahmini ile araştırılmaktadır. Maki eşbütünleşme testi, tarımsal katma değer ile yapısal kırılmalar altındaki CO_2 emisyonları arasında uzun dönemli bir ilişkinin varlığını göstermektedir. Tespit edilen önemli kırılmalar, Türkiye ekonomisini etkileyen önemli olaylarla ilişkilidir. Örneğin, tarımsal katma değerin bağımlı değişken olduğu modelde, 1971 ve 1974 yıllarının kırılma tarihleri petrol kriziyle çakışırken, 2002 ve 2008 yıllarının kırılma tarihleri Türkiye'nin 2001 mali krizi ve 2008 küresel mali krizi ile çakışmaktadır. Benzer şekilde, CO_2 denkleminde elde edilen 1973 ve 1977'nin kırılma tarihleri, 1970'lerin küresel petrol krizi ile ilişkilidir. FMOLS ve CCR tahmincilerinden türetilen uzun dönemli parametre tahminleri, CO_2 emisyonlarının tarımsal verimlilik üzerinde uzun vadeli, olumlu ve önemli bir etkiye sahip olduğunu göstermektedir. Bunun yanında, uzun dönemli sonuçlar, tarımsal verimliliğin çevresel bozulma üzerinde olumlu ve önemli bir etkisinin varlığını desteklemektedir. Kademeli kayma nedensellik testi ise, tarımsal üretimden CO_2'ye kadar uzanan tek yönlü nedenselliğin varlığını desteklemektedir. Bu bulgular Türkiye'de tarımsal verimlilik ve CO_2'nin birbirini desteklediğini göstermektedir. Her ne kadar CO_2'nin tarımsal verimliliği pozitif etkilemesi olumlu görünse de çevreci olmayan bir tarıma işaret etmektedir.

Anahtar Kelimeler: Tarım sektörü, Karbondioksit emisyonları, Yapısal kırılma analizi, Doğrusal olmayan analiz

1. Introduction

Despite recent advances in production technology, agricultural output is adversely affected by a range of variables that reduce yields. These are mostly related to climate change, such as water scarcity, drought, plant pests and diseases, and altered vegetation seasons. Because of its effects on agricultural productivity, global climate change has major implications for food security and international trade. Climate change can adversely affect agriculture including loss of cultivated areas, shifting precipitation patterns, reduced irrigation water, and drought (Adams et al., 1998). Because of agriculture's importance, any climate-change-induced output reductions significantly affect the economy's macroeconomic fundamentals. For example, it may increase consumer prices for domestic produced and imported foods (Dellal, 2011).

At the same time as being affected by climate change, agriculture itself also significantly affects the environment. In particular, it contributes significantly to greenhouse gas emissions due to increasing mechanization, livestock production, soil tillage, and excessive nitrogen fertilizer usage. This suggests that countries must abandon the use of fossil fuels and increase the use of renewable energy in agricultural production (Ben Jebli and Ben Yousef, 2017).

The main objective of this study is to reveal the interaction between agricultural activity and CO_2 in Türkiye. For this purpose, the research question of the study can be stated as follows: Is there a statistically significant relationship between agricultural productivity and CO_2 in Türkiye? Türkiye presents an interesting case to analyze how environmental pollution affects agriculture. Firstly, according to the greenhouse gas inventory, agriculture's CO2 emissions in Türkiye have increased dramatically during the previous three decades. Türkiye's total per-capita greenhouse gas emissions are projected to grow to 6.3 tons of CO2 equivalent in 2020 from 4 tons in 1990. As measured by CO_2 equivalent, energy-related emissions accounted for 72 percent of Türkiye's emissions in 2020, followed by agriculture at 14 percent, industrial processes and product consumption at 12.7 percent, and the waste sector at 3.1 percent. Agriculture was projected to emit 73.2 Mt CO2 equivalent in 2020, a 58.8 percent increase from 1990 and a 7.5 percent increase from 2019 (TURKSTAT, 2021). The second reason Türkiye is an interesting case is that its arable land area has shrunk significantly over the last three decades, from approximately 32% of the total agricultural land area in 1990 to approximately 25% in 2020 (World Bank, 2022). There has been a similar climate-change-induced rise in temperature from an annual average of 12.7 °C in 1991 to 14.5 °C in 2020, according to the Turkish State Meteorological Service (TSMS, 2022). Climate change is universally recognized as having a substantial influence on diverse sectors, with the agricultural industry being arguably more susceptible to its effects compared to other industries. Therefore, in order to effectively address the impact of climate change on the agricultural sector, it is imperative to conduct comprehensive studies at both the national and international levels (Konukcu et al., 2020).

This study provides a novel contribution to the literature in this area. First, it offers a more specific analysis than previous literature on Türkiye, which has primarily concentrated on pollution's impact on overall economic activity, with few studies focusing on the agriculture sector (Bayraç and Doğan, 2016; Pakdemirli, 2020; and Çetin et al., 2020). In contrast, instead of examining CO_2 emissions at an aggregate level, our study examines the relationship between agricultural activities and CO_2 emissions due to the agriculture sector. Similarly, we also consider agriculture's energy consumption as a major determinant of agricultural activity. Second, this study considers the long-run environmentally degrading impact of agricultural activity. Third, our literature review suggests that research examining agricultural activity and pollution has mostly used linear estimation methodologies. However, some researchers have argued that linear models may be inappropriate for analyzing the relationship between emissions and agricultural activity because changes in the economic environment and abnormal climate conditions could create serious parameter instabilities, leading to biased empirical results. Our study therefore addresses these gaps in the literature by using Maki (2012) cointegration and Toda and Yamamoto (2016) causality tests to investigate the nonlinear impacts of local and global economic events on the relationship between agricultural activity and climate change.

The rest of the article is structured as follows. The following section briefly reviews the literature on climate-change-induced effects on agricultural productivity. We then present the study's methods and variables before summarizing the results of the cointegration and causality tests. We conclude with policy suggestions predicated on the estimation results.

2. Literature Review

Table 1 and *Table 2* presents the main findings from the literature analyzing the agriculture-CO_2 emission nexus. Some studies have employed temperature and precipitation data as indicators of climate change. Rosenzweig and Parry (1994) find that changing climate conditions reduce agricultural yields. Using the Ricardian model, Liu et al. (2004) measured how climate change has affected Chinese agriculture economically. They found that temperature increments have led to increasing average net agricultural income. Brown et al. (2010) estimated a panel data model for 133 countries covering 1961 to 2003. They found that increasing precipitation raises agriculture's share in GDP, rising temperatures have the opposite effect. Also, using the Ricardian model, Masud et al. (2014) showed that temperature, precipitation, farm size, educational information, land area, and labor input value all affect rice production in Malaysia.

Table 1. Time series studies.

Study	Country	Period	Variables	Methodology	Findings
Jebli and Youssef (2017)	Tunisia	1980-2011	GDP, CO2, AGR, trade openness, REN and NONREN	VECM causality	There is a bidirectional relationship between agriculture and CO_2.
Zafeiriou and Azam (2017)	France, Portugal and Spain	1992-2014	CO2 and AGR	ARDL, VECM causality	There is unidirectional causality from CO_2 to agriculture variable.
Waheed et al. (2018)	Pakistan	1990-2014	CO2, REN, AGR and forest area	ARDL cointegration, VECM causality	There is unidirectional causality from agriculture to CO2.
Jebli and Youssef (2019)	Brazil	1980-2013	CO2, GDP, CRW and AGR	ARDL, VECM causality	There is no short-run causality relationship. However, there is a bidirectional, long-run relationship between agriculture and CO2.
Ngarava et al. (2019)	South Africa	1990-2012	CO2, AGR, coal and electricity energy	ARDL, Granger causality	There is unidirectional causality from agriculture to CO2.
Pakdemirli (2020)	Türkiye	1961-2018	AGR and CO2	ARDL, VAR	There is a strong relationship between agriculture and CO2.
Çetin et al. (2020)	Türkiye	1968-2016	CO2, GDP, AGR, REN and LAND	Toda-Yamamoto	There is unidirectional causality from agriculture to CO2.
Wang (2022)	China	1985 -2019	AGR, CO2, LAND, Harvested, GDP, AGR export and NONREN	ARDL, Johansen cointegration	CO2 has a positive effect on agriculture.

Notes: AGR (Agricultural Value Added), REN (Renewable Energy Source), AGRE (State's Agricultural Expenditures), AGRPI (Agricultural Production Index), CRW (combustible renewables and waste consumption), EFP (Ecological Footprints), RQ (regulatory quality)

Regarding Türkiye, three studies (Dellal et al., 2011; Başoğlu and Telatar, 2013; Dumrul and Kilicarslan, 2017), have examined whether CO_2 emissions affect agriculture. Based on temperature and precipitation forecasts for seven geographical regions in Türkiye in 2050, Dellal et al. (2011) used both biophysiological and economic models to investigate whether climate change will affect Turkish agriculture. The results suggest that the crop yield will decline significantly in all regions. Shrinking agricultural land and climate change will reduce production by 2.2-12.9%. Başoğlu and Telatar (2013) applied multiple regression analysis to analyze how climate change affected Turkish agriculture from 1972 to 2011. They found that increasing precipitation increased agriculture's share in GDP, whereas rising temperature reduced it. Furthermore, Dumrul and Kilicarslan (2017) reported that temperature increases agricultural GDP over the long run while precipitation reduces it.

The present study aims to address two research gaps in the literature reviewed above. First, previous studies used total CO_2 emissions rather than those of the agricultural sector specifically while examining the relationship between climate change and agriculture. Second, previous studies assumed a time-invariant relationship between agricultural activity and climate change. Our study therefore considers nonlinearity in the relationship in both the long and short run by employing the Maki (2012) cointegration and the single Fourier-frequency Toda and Yamamoto causality tests.

Table 2. Panel Data studies.

Study	Country	Period	Variables	Methodology	Findings
Islam et al. (2014)	Southeast asian countries	1975-2011	CO2, AGR, fertilizer, capital and population	Panel ARDL and PMG	CO_2 has a positive effect on agriculture.
Khalid et al. (2016)	10 countries	1990-2014	AGR, GDP, CO2, capital, AGRE, LAND, AGRPİ, fertilizer	Panel OLS	CO_2 has a positive effect on agriculture.
Hayaloğlu (2018)	10 countries	1990-2016	GDP, AGR, CO2, LAND, population, capital and schooling	Panel OLS	CO_2 has a negative effect on agriculture.
Olanipekun et al. (2019)	African countries	1996-2015	EFP, GDP, REN, population and AGR and RQ	Emirmahmutoglu and Kose Granger causality	There is a bidirectional relationship between ecological footprint and CO2.
Qiao et al. (2019)	G20 countries	1990-2014	CO2, GDP, AGR and REN	Panel VECM causality	There is unidirectional causality from agriculture to CO2.

Notes: AGR (Agricultural Value Added), REN (Renewable Energy Source), AGRE (State's Agricultural Expenditures), AGRPI (Agricultural Production Index), CRW (combustible renewables and waste consumption), EFP (Ecological Footprints), RQ (regulatory quality)

3. Materials and Methods

This study employed annual data from 1968 to 2018. The availability of the data on agriculture-sector CO_2 emissions determined the estimation sample. These were retrieved from the International Energy Agency (IEA) as an indicator of climate change. Real agricultural value added was used to measure agricultural sector activity. In line with previous studies (Wang, 2022), agricultural land, agricultural energy consumption, and fixed capital formations were also included in the model as control variables.[1] Given that the present study aimed to quantify both the short- and long-run interactions between CO_2 and agricultural productivity, two separate equations were estimated, which treated agricultural output and CO_2 emissions as dependent variables. The linear forms of the estimated equations are formulated as follows:

Model 1: $$\boldsymbol{lnAGR_t = \beta_0 + \beta_1 lnCO2_t + \beta_2 LAND_t + \beta_3 lnENG_t + \beta_4 GFCF_t + \varepsilon_t} \quad \text{(Eq.1)}$$

Model 2: $$\boldsymbol{lnCO2_t = \beta_0 + \beta_1 lnAGR_t + \beta_2 LAND_t + \beta_3 lnENG_t + \beta_4 GFCF_t + \varepsilon_t} \quad \text{(Eq.2)}$$

Where $\boldsymbol{lnAGR_t}$ represents the natural log of real agricultural value added; $\boldsymbol{lnCO2_t}$ represents the natural log of agriculture-sector CO_2 emissions in tonnes (Mt); $\boldsymbol{lnENG_t}$ is the natural log of the energy consumption in the agricultural sector. Agricultural land ($\boldsymbol{LAND_t}$) and gross fixed capital formation ($\boldsymbol{GFCF_t}$) are included in their level form as they are defined in percentage terms. Agricultural land is defined as the proportion of total land that

[1] Appendix Table A1 provides detailed descriptions of the variables.

is cultivated. Gross fixed capital formation as a percentage of GDP is included as a control variable to quantify the effect of a change in total value added, i.e., investment on agricultural productivity and environmental quality.[2]

3.1. Maki (2012) Cointegration Test with Multiple Structural Breaks

The Johansen and Juselius (1990) cointegration test were used first to analyze the long-run relationship between agricultural activity and CO2 emissions. The Maki (2012) cointegration test was then used to investigate the long-run relationship between agricultural activity and CO2 emissions given unknown multiple structural breaks. Using Monte Carlo simulations, Maki (2012) demonstrated that the proposed test had better effect size and power than other cointegration with structural break tests (Gregory and Hansen, 1996; Hatemi J., 2008). The Maki (2012) cointegration test is based on the following four specifications:

Model 0: Level shift

$$\boldsymbol{y_t = \mu + \sum_{i=1}^{k} \mu_i D_{i,t}\ \beta' x_t + u_t,} \quad \text{(Eq.3)}$$

Model 1: Level shift with trend

$$\boldsymbol{y_t = \mu + \sum_{i=1}^{k} \mu_i D_{i,t} + \beta' x_t + \sum_{i=1}^{k} \beta'_i x_t D_{i,t} + u_t,} \quad \text{(Eq.4)}$$

Model 2: Regime shift and trend

$$\boldsymbol{y_t = \mu + \sum_{i=1}^{k} \mu_i D_{i,t} + \gamma t + \beta' x_t + \sum_{i=1}^{k} \beta'_i x_t D_{i,t} + u_t,} \quad \text{(Eq.5)}$$

Model 3: Regime shift with trend

$$\boldsymbol{y_t = \mu + \sum_{i=1}^{k} \mu_i D_{i,t} + \gamma t + \sum_{i=1}^{k} \gamma_i t D_{i,t} + \beta' x_t + \sum_{i=1}^{k} \beta'_i x_t D_{i,t} + u_t,} \quad \text{(Eq.6)}$$

Where $\boldsymbol{y_t}$ represents the dependent variables in Equations 1 and 2, i.e., the natural log of agricultural value added, $\boldsymbol{lnAGR_t}$, and the natural log of carbon emissions, $\boldsymbol{lnCO2_t}$. $\boldsymbol{x_t}$ represents the m-dimension vector of the explanatory variables. $\boldsymbol{x_t' = [lnCO2_t, LAND_t, lnENG_t, GFCF_t]}$ and $\boldsymbol{x_t' = [lnAGR_t, LAND_t, lnENG_t, GFCF_t]}$ are used in Equations 1 and 2, respectively. $\boldsymbol{u_t}$ is the white noise error term while $\boldsymbol{D_{i,t}}$ is a dummy variable taking the value 1 if $\boldsymbol{\tau > T_{Bi}}$ (i=1,.., k) and 0 otherwise, where k is the maximum number of breaks. $\boldsymbol{T_{Bi}}$ represents the time period of the break in the intercept, $\boldsymbol{\mu}$, and the vector slope coefficients, $\boldsymbol{\beta}$.

Based on these settings, the Maki (2012) cointegration test can be applied using the following steps if Model 1 is taken as the benchmark model. First, the maximum number of breaks (k) is determined and the model is estimated:

$$\boldsymbol{y_t = \mu + \mu_1 D_{1,t} + \beta' x_t + u_t.} \quad \text{(Eq.7)}$$

The null hypothesis with $\boldsymbol{\rho}$ = 0 is then tested against the alternative hypothesis with $\boldsymbol{\rho}$< 0 using the equation below:

$$\boldsymbol{\Delta \hat{u}_t = \rho \hat{u}_{t-1} + \sum_{j=1}^{p} \alpha_j \Delta \hat{u}_{t-j} + \varepsilon_t,} \quad \text{(Eq.8)}$$

Where $\boldsymbol{\varepsilon_t}$ $(0, \sigma^2)$ and $\boldsymbol{\hat{u}_t}$ are the OLS residuals from the model 1. Based on the recursive estimation of the model above, a single break is searched, and t-statistics are computed to test for ρ=0 for all possible periods of the break. The set of all possible partitions and the t-statistics are denoted as $\boldsymbol{T_1^q}$ and τ_1, respectively. In the case of k=1, the minimum t-statistic in τ_1 is used as the test statistic.

In the second step, the first breakpoint is selected by minimizing the sum of the squares (SSR) as follows:

$$\boldsymbol{SSR_1 = \Sigma_{t=1}^{T} (y_t - \hat{\mu} - \hat{\mu}_1 D_{1-t} - \hat{\beta}' x_t)^2,} \quad \text{(Eq.9)}$$

Where $\boldsymbol{\hat{\mu}}$, $\boldsymbol{\hat{\mu}_1}$, and β are the OLS estimates. Then the first breakpoint is denoted as $\boldsymbol{\hat{bp}_1 = arg\ min\ SSR_1}$

In the third step, the estimated breakpoint $\boldsymbol{bp_1}$ is included to the regression model. Based on the similar procedure the second breakpoint is searched before t-statistics are used to test for ρ=0 for all possible periods of the second break using the regressions given by

[2] *Figure A1* shows the visual inspection of the series. *Table A2* contains the desctiptive statistics of the variables without natural log form for AGR_t, $CO2_t$ and ENG_t.

$$\boldsymbol{y_t = \mu + \mu_1 D_{1,t} + \mu_1 D_{2,t} + \widehat{\beta}' x_t + u_t,} \quad \text{(Eq.10)}$$

$$\boldsymbol{\Delta \hat{u}_t = \rho \hat{u}_{t-1} + \sum_{j=1}^{p} \alpha_j \Delta \hat{u}_{t-j} + \varepsilon_t} \quad \text{(Eq.11)}$$

The set of all possible partitions and the statistics of $\boldsymbol{\rho}$ are denoted as $\boldsymbol{T_2^a}$ and τ_2, respectively. Furthermore, $\boldsymbol{\tau_p^2 = \tau_1 \cup \tau_2}$

In step four, as with estimation of the first breakpoint, the second breakpoint, bp_2, is estimated by minimizing $\boldsymbol{SSR_2}$ in the following equation:

$$\boldsymbol{SSR_2 = \Sigma_{t=1}^{T} (y_t - \hat{\mu} - \hat{\mu}_1 D_{1-t} - \widehat{\beta}' x_t)^2} \quad \text{(Eq.12)}$$

In the last step, the estimated first and second breakpoints are introduced into the model. Finally, steps 3 and 4 are repeated until the maximum number of breaks, k, is achieved. Finally, $\boldsymbol{\tau_{min}^k}$ is adopted as the t test statistic over the set $\boldsymbol{\tau_p^k = \tau_1 \cup \tau_2 \ldots \cup \tau_k^2}$.

3.2. Single Fourier-frequency Toda and Yamamoto (2016) causality test

We first examined the agricultural activity–CO_2 emissions relationship using the Toda and Yamamoto (1995) Granger causality test, which estimates the VAR model with (p + d) order, where p is the lag length and d is the maximum integration degree of the variables. It thereby aims to eliminate the constraint of the same degree of integration of the series in the Granger causality test, as in the following VAR (p + d) model:

$$y_t = \alpha + \beta_1 y_{t-1} + \cdots + \beta_{p+d} y_{t-(p+d)} + \epsilon_t \quad \text{(Eq.13)}$$

Where y_t represents the matrix of *k* endogenous variables $y_t' = [lnAGR_t, lnCO2_t, LAND_t, lnENG_t, GFCF_t]$. a and β represent the vector of the intercept and parameter matrices, respectively, while ϵ_t is the vector containing white noise error terms. Wald statistics with $\chi 2(p)$ degrees of freedom are used, where the null hypothesis of Granger non-causality is that the first *p* parameters are jointly equal to zero i.e., $H_0 : \beta_1 = \ldots = \beta_p = 0$.

Some authors (e.g., Enders and Jones (2015), Ventosa-Santaulària and Vera-Valdés (2008)) have argued that if the data-generating process is subject to structural changes, the null hypothesis of noncausality can be rejected despite the lack of a significant causal relationship between the two variables. Hence, if breaks are not accounted for, inferences about the significance of the Granger causality analysis may be incorrect. To address these issues, Nazlioglu et al., (2016) suggested a Granger causality test based on Fourier approximation. This is superior to the alternative nonlinear causality test because the Fourier approximation avoids the need to know the number, dates, and types of breaks. Instead, it uses a few low-frequency elements to represent the structural transitions as a smooth process.

To accommodate structural changes, Nazlioglu et al., (2016) modify the assumption that the intercept terms are constant, such that the VAR model in Equation (13) is redefined as follows:

$$y_t = \alpha(t) + \beta_1 y_{t-1} + \cdots + \beta_{p+d} y_{t-(p+d)} + \epsilon_t \quad \text{(Eq.14)}$$

Here, rather than being time variant, the intercept terms, α(t), are functions of time to model smooth structural shifts in y_t. To capture gradual, smooth shifts with an unknown date, number, and form of breaks, α(t) is modeled through one Fourier expansion as follows:

$$\alpha(t) = \alpha_0 + \sum_{k=1}^{n} \gamma_{1k} \sin\left(\frac{2\pi kt}{T}\right) + \sum_{k=1}^{n} \gamma_{2k} \cos\left(\frac{2\pi kt}{T}\right), \quad \text{(Eq.15)}$$

where k stands for the frequency for the approximation and n denotes the number of frequencies and γ_{1k} and γ_{2k} denote the frequency's amplitude and displacement, respectively. Instead of a higher frequency version of the intercept term, Nazlioglu et al., (2016) use a single frequency component because the number of frequencies is most usually related with stochastic parameter change, which leads to over-fitting by reducing the degrees of freedom. Accordingly, the single frequency component α(t) is redefined as follows:

$$\alpha(t) = \alpha_0 + y_1 \sin\left(\frac{2\pi kt}{T}\right) + y_2 \cos\left(\frac{2\pi kt}{T}\right) \quad \text{(Eq.16)}$$

Hence, the final form of the estimated equation for the gradual-shift causality test is as follows:

$$y_t = \alpha_0 + y_1 \sin\left(\frac{2\pi kt}{T}\right) + y_2 \cos\left(\frac{2\pi kt}{T}\right) + \beta_1 Y_{t-1} + \cdots + \beta_{p+d} y_{t-(p+d)} + \epsilon_t. \quad \text{(Eq.17)}$$

Here, the null hypothesis of Granger non-causality is tested in the same way as in the linear Toda and Yamamoto (1996) test presented in Equation (13), i.e. $H_0 : \beta_1 = ... = \beta_p = 0$. In order to increase the power of the test statistic in small samples while maintaining its robustness from the unit root and cointegration properties of the data, Nazlioglu et al., (2016) used the residual sampling bootstrap approach introduced by Efron (1979). The lag length of the VAR model *p* and frequency k are selected based on the minimum value of the information criterion.

4. Results and Discussion

4.1. Unit root tests

This section first considers the variables' unit root properties as shown in the Augmented Dickey and Fuller (1981), Phillips and Perron (1988), and Zivot and Andrews (1992) unit root test results, presented in *Table 3*. The linear unit root tests indicate that the unit root null hypothesis cannot be rejected for the levels of all series. However, it can be rejected after the first difference transformation of the variables. That is, all series can be treated as first difference stationary, i.e., I(1). In addition to the linear unit root tests, *Table 3* also shows the Zivot and Andrews (1992) unit root test results, which account for endogenous structural breaks. The results confirm the I (1) properties of the variables, as evidenced by the ADF and PP test results. This further indicates that the level of the variables contains a unit root with a significant structural break. The significant breaking dates obtained for some of the variables, namely 1982, 2001, 2007, and 2008, are associated with crisis periods.

Table 3. Unit Root Results

Variable	ADF		PP		Zivot and Andrews	
	I	I/T	I	I/T	Model A	Model C
$lnAGR_t$	1.21119 (1)	-0.87015 (1)	1.80089	-2.39450	-2.41100 (3) (2010)	-4.19287 (3) (2001)
$LnCO2_t$	-2.17432 (0)	-3.27227 (0)	-2.48737	-3.27529	-4.14442 (0) (2001)	-4.01409 (0) (2001)
$LAND_t$	-1.73336 (0)	-1.31479 (0)	-1.83769	-1.44427	-3.16379 (0) (2007)	-3.31020 (0) (1998)
$lnENG_t$	-1.44788(0)	-2.21067(0)	-1.47910	-2.25222	-3.04293 (0) (1982)	-4.06777 (0) (2008)
$GFCF_t$	-1.53217(0)	-2.98077(0)	-1.48054	-3.14246	-4.38833 (1) (1998)	-4.33980 (1) (1987)
$\Delta lnAGR_t$	-12.1163***(0)	-12.3363***(0)	-12.2735***	-27.0988***	-7.3797*** (4) (2005)	-7.2848*** (4) (2005)
$\Delta lnCO2_t$	-7.09263*** (0)	-7.40330*** (0)	-7.09324***	-7.45905***	-8.0732*** (0) (1978)	-8.2745*** (0) (1982)
$\Delta LAND_t$	-6.54591***(0)	-6.54384***(0)	-6.55681***	-6.54839***	-7.7085*** (0) (1984)	-7.6153*** (0) (1984)
$\Delta lnENG_t$	-7.08946***(0)	-7.15296***(0)	-7.08968***	-7.19996***	-7.5304*** (0) (1980)	-7.9341*** (0) (1980)
$\Delta GFCF_t$	-6.60696***(0)	-6.54070***(0)	-6.80615***	-6.71654***	-6.8008*** (0) (1989)	-6.7661*** (0) (1989)

Notes: *** denotes the series is stationary at the 1% significance level; I=intercept; T=trend. In the ADF tests, the SIC (Schwarz Information Criterion) is used to determine the optimum number of lags to a maximum of 10 lags. The bandwidth of the PP test is determined using the Newey-West method using the Bartlett kernel. Likewise, in the Zivot and Andrews test, which takes structural breaks into account, the maximum lag was searched up to 4 lags based on the minimum value of the t statistics. The values in parentheses indicate the optimum number of lags of the augmented part for the ADF and Zivot and Andrews tests.

4.2. Cointegration and causality test results

After applying the unit root tests to demonstrate the variables' first-difference stationarity, cointegration tests were applied to the long-run associations in Equation (1). More specifically, the Johansen and Juselius (1990) cointegration test was implemented to analyze the linear long-run relationship (see *Table 4*). The Maki (2012) cointegration test was used to investigate the variables' nonlinear long-run relationship. The test findings are shown in *Table 5*, panels (a) and (b), respectively.

Table 4. Johansen cointegration results

Hypothesized	Trace result			Max Eigenvalue result		
	Eigenvalue	Statistic	Critical Value (0.05)	Eigenvalue	Statistic	Critical Value (0.05)
None *	0.618756	101.0401	76.97277	0.618756	47.25142	34.80587
At most 1	0.343437	53.78869	54.07904	0.343437	20.61609	28.58808
At most 2	0.283467	33.17260	35.19275	0.283467	16.33319	22.29962
At most 3	0.201516	16.83941	20.26184	0.201516	11.02696	15.89210
At most 4	0.111856	5.812456	9.164546	0.111856	5.812456	9.164546

* Denotes rejection of the hypothesis at the 0.05 level.

Table 5. Maki Cointegration Test Results

Panel a. Dependent Variable $lnAGR_t$					
Model	**Test Statistics**	**Critical Value**			**Break Date**
		1%	**5%**	**10%**	
0	-5.4538155	−6.856	−6.306	−6.039	1975/1985/1998/2008/2012
1	-8.9773321***	−7.053	−6.494	−6.220	1971/1974/1998/2002/2008
2	-7.6119198	−8.336	−7.803	−7.481	1982/1990/1995/2007/2012
3	-8.0555682	−10.08	−9.482	−9.151	1975/1986/1995/2004/2012
Panel b. Dependent Variable $lnCO2_t$					
Model	**Test Statistics**	**Critical Value**			**Break Date**
		1%	**5%**	**10%**	
0	-6.3435031**	−6.856	−6.306	−6.039	1973/1977/1995/2011/2015
1	-5.4904613	−7.053	−6.494	−6.220	1973/1976/1997/2000/2007
2	-7.0583022	−8.336	−7.803	−7.481	1981/1988/1993/1999/2006
3	-6.4519885	−10.08	−9.482	−9.151	1978/1987/1997/2003/2009

Note: **, *** denote significant at the at 5% and 1%, respectively. The maximum lag length is taken as 3. 1000 bootstrap was used.

The Johansen cointegration results indicate that the null hypothesis of no cointegration can be rejected because the trace and max eigenvalue test statistics show that the variables include at least one cointegrating vector. Given that the statistical significance of long-run relationships indicated by linear cointegration tests may be misleading, the Maki cointegration was also implemented based on Equations 1 and 2. Here, agricultural value-added, and CO_2 emissions were employed as the dependent variables (see *Table 5*), thereby enabling up to five structural breaks to be tested for cointegration.

The test results corroborated the results of the Johansen test by rejecting the null hypothesis of no cointegration. However, *Table 5* indicates that the cointegrating relationships are subject to structural shifts in both models. As already noted, the breaking dates coincide with significant local and global economic events that affected Turkish's economy. When agricultural value added is the dependent variable, the breaking dates of 1971 and 1974 both coincide with the oil crisis, while the breaking dates of 2002 and 2008 coincide with Türkiye's 2001 financial crisis and the 2008 global financial crisis. Similarly, when CO2 emissions are the dependent variable, the breaking dates of 1973 and 1977 coincide with the 1970s' global oil crisis.

Table 6. Long-Run Coefficient Estimates: $lnAGR_t$ dependent variable

	FMOLS		CCR	
Variable	**Coefficient**	**Std. Error**	**Coefficient**	**Std. Error**
$lnCO2_t$	0.367689***	0.028980	0.373403***	0.033002
$LAND_t$	-0.010984***	0.003390	-0.010830**	0.004044
$lnENG_t$	-0.164949***	0.021127	-0.168943***	0.022531
$GFCF_t$	0.007562***	0.001441	0.007421***	0.001713
C	21.65796***	0.249539	21.61835***	0.292924
DUM1971	-0.080809***	0.016012	-0.082545***	0.016799
DUM1974	0.027658*	0.014544	0.027487*	0.015030
DUM1998	0.071604***	0.016758	0.069931***	0.019502
DUM2002	-0.007469	0.014192	-0.006647	0.014432
DUM2008	0.129407***	0.014632	0.129774***	0.017666

Note: *, **, *** denote significant at the at 10%, 5% and 1% respectively.

Table 7. Long-Run Coefficient Estimates: $lnCO2_t$ dependent variable

	FMOLS		CCR	
Variable	**Coefficient**	**Std. Error**	**Coefficient**	**Std. Error**
$lnAGR_t$	0.554916***	0.171254	0.578763**	0.218420
$LAND_t$	0.027145***	0.007672	0.028575***	0.008256
$lnENG_t$	0.535268***	0.047548	0.530739***	0.054319
$GFCF_t$	0.006681*	0.003436	0.006547*	0.003736
C	-7.605921*	4.040726	-8.218490	5.110718
DUM1973	0.106868***	0.037011	0.107688***	0.037351
DUM1977	0.164025***	0.032521	0.161703***	0.033721
DUM1995	0.100781***	0.036002	0.097140**	0.039411
DUM2011	0.122884**	0.047880	0.122268**	0.059637
DUM2015	0.156903***	0.042196	0.152421***	0.046492[3]

Note: *, **, *** denote significant at the at 10%, 5% and 1% respectively.

After confirming cointegration under multiple structural breaks, the long-run coefficients were estimated with full modified ordinary least squares (FMOLS) and canonical cointegrating regression (CCR) estimators (see *Tables 6* and *7*). For the estimation of the long-run parameters, following Ike et al. (2020) and Khan et al. (2020), dummy variables were constructed to determine the impact of structural breaks on the long-run parameters. As *Tables 6* and *7* indicated, these parameters were both statistically significant and yielded qualitatively the same results. Therefore, we interpreted the FMOLS parameter estimates for both equations. It is worth noting that all other structural break dates, with the exception of 2002, were significant at the 1% significance level. This suggests that the break dates from the Maki cointegration test are significant and ought to be included in the long-run parameter estimates.

Regarding the agricultural productivity model results, CO_2 emissions significantly increased agricultural productivity, in line with Wang's (2022) findings for China. All other variables were statistically significant at the one-percent level. An increase in land area devoted to agriculture significantly reduced agricultural productivity ($lnAGR_t$). This suggests that expanding arable farming may not improve agricultural sector efficiency, which may instead depend on rising capital formation.

[3] Diagnostic tests were conducted on the estimated models and are not presented in the text to save space in the paper. For example, the correlogram of residuals for the long-run estimation of model 2 is presented in the appendix.

Regarding the long-run coefficients when the model's dependent variable was CO_2 emissions, agricultural productivity significantly increased CO_2 emissions. This indicates that, in Türkiye, rising agricultural activity reduces environmental quality. The control variables also significantly increased CO_2 emissions. According to the FMOLS estimator, the LAND, ENG, and GFCF parameter coefficients were 0.027, 0.535, and 0.006, respectively, while similar coefficients were obtained from the CCR estimator.

Table 8. Error Correction Model Results

Panel (a): Dependent Variable $lnAGR_t$				
Variable	**Coefficient**	**Std. Error**	**t-Statistic**	**Probability**
$\Delta \boldsymbol{ln} CO2_t$	0.178403*	0.099716	1.789109	0.0805
$\Delta LAND_t$	-0.014309**	0.006772	-2.112893	0.0403
ΔENG_t	-0.100472**	0.041635	-2.413167	0.0200
$\Delta GFCF_t$	0.004677*	0.002343	1.996752	0.0521
ECM_{t-1}	-1.130477***	0.163053	-6.933187	0.0000
C	0.009941	0.006164	1.612650	0.1140
Panel (b): Dependent Variable $lnCO2_t$				
Variable	**Coefficient**	**Std. Error**	**t-Statistic**	**Probability**
$\Delta \boldsymbol{ln} AGR_t$	-0.036636	0.157673	-0.232357	0.8173
$\Delta LAND_t$	-0.011212	0.010221	-1.097022	0.2786
$\Delta \boldsymbol{ln} ENG_t$	0.127391*	0.065043	1.958554	0.0565
$\Delta GFCF_t$	0.009664***	0.003395	2.846465	0.0067
ECM_{t-1}	-0.338889**	0.127656	-2.654700	0.0110
C	0.041540***	0.007316	5.677594	0.0000

Note: *, **, and *** denotes significant at the at 10%, 5%, and 1% level, respectively.

Having estimated the long-run parameters, we then estimated the error correction models for the agricultural productivity and CO_2 emissions equations to analyze the dynamics of the short-run relationship. Based on Granger's representation theorem, the error correction term indicates how rapidly a dependent variable returns to equilibrium following a change in the other variables (Engle and Granger, 1987). *Table 8*, Panel (a), shows that the error correction term was negative and statistically significant for the agricultural productivity equation. That is, short-run imbalances in the system are corrected in the long run. Regarding the short-run coefficients, the short-run impact of CO_2 emissions is positive and statistically significant, as with the long-run estimates. The remaining variables have similar parameter estimates for both the long-run and short-run coefficients. *Table 8*, Panel (b), shows the error correction results for the CO_2 model. As with the agricultural productivity equation, there is a statistically significant negative error correction coefficient. This indicates that deviations from the long-run equilibrium are eliminated in the short run. Furthermore, increases in energy consumption and gross fixed capital formation have statistically significant positive impacts, similar to the long-run estimates in *Table 7*. However, the agricultural productivity parameter is statistically insignificant in the short run. This indicates that the error correction mechanism is functioning properly in both models.

Following the time series analysis of the agricultural productivity and CO_2 equations, the linear and regime-shifting Toda-Yamamoto causality tests were performed to identify any interactions between the variables (Table 9). Based on the linear causality test results, there is a one-way causal relationship, significant at the 5% level, from agricultural value added to the CO_2 emissions, and a one-way causal relationship, significant at the 10% level, from CO_2 emissions to agricultural land area. Given that the Maki's (2012) cointegration test indicated structural breaks in the data, a gradual-shift causality test was conducted. The results confirmed the linear Toda-Yamamoto causality test findings, i.e. the causal relationship runs from agricultural value added to CO_2 emissions.

Table 9. Causality Tests Results

	Toda & Yamamoto					Single Fourier-frequency Toda & Yamamoto				
Null hypothesis	**Wald st.**	**Bootstrap p-value.**	**d**	**p**	**f**	**Wald ist.**	**Bootstrap p-value**	**d**	**p**	**f**
$lnCO2_t \neq > lnAGR_t$	0.97586	0.64500	1	2	0	0.08880	0.94900	1	2	1
$LAND_t \neq > lnAGR_t$	0.73955	0.71200	1	2	0	0.27930	0.86600	1	2	1
$GFCF_t \neq > AGR_t$	0.00328	0.99700	1	2	0	0.41929	0.81400	1	2	1
$ENG_t \neq > lnAGR_t$	1.02208	0.60900	1	2	0	0.23805	0.88600	1	2	1
$lnAGR_t \neq > lnCO2_t$	9.15924**	0.01700	1	2	0	9.73685**	0.01200	1	2	1
$LAND_t \neq > lnCO2_t$	1.70389	0.44500	1	2	0	0.95943	0.63400	1	2	1
$GFCF_t \neq > lnCO2_t$	3.62570	0.16900	1	2	0	3.77221	0.17100	1	2	1
$lnENG_t \neq > lnCO2_t$	4.21913	0.13200	1	2	0	4.47276	0.13300	1	2	1
$lnAGR_t \neq > LAND_t$	3.63190	0.18000	1	2	0	1.62905	0.43500	1	2	1
$lnCO2_t \neq > LAND_t$	5.61466***	0.07600	1	2	0	3.30327	0.20600	1	2	1
$GFCF_t \neq > LAND_t$	1.42637	0.51300	1	2	0	0.74254	0.70600	1	2	1
$lnENG_t \neq > LAND_t$	1.65944	0.44100	1	2	0	2.68300	0.28100	1	2	1
$lnAGR_t \neq > GFCF_t$	2.40039	0.30900	1	2	0	2.84314	0.22400	1	2	1
$lnCO2_t \neq > GFCF_t$	2.21921	0.35700	1	2	0	2.55863	0.29900	1	2	1
$LAND_t \neq > GFCF_t$	0.29445	0.86500	1	2	0	3.16510	0.21100	1	2	1
$lnENG_t \neq > GFCF_t$	0.91726	0.60000	1	2	0	0.22564	0.90300	1	2	1
$lnAGR_t \neq > lnENG_t$	0.27264	0.88200	1	2	0	0.14066	0.91600	1	2	1
$lnCO2_t \neq > lnENG_t$	2.47495	0.28300	1	2	0	1.77270	0.39700	1	2	1
$LAND_t \neq > lnENG_t$	0.47725	0.76500	1	2	0	1.51865	0.48400	1	2	1
$GFCF_t \neq > lnENG_t$	1.34487	0.48300	1	2	0	0.22335	0.88300	1	2	1

Notes: ** and * denote significance at 5% and 10% level, respectively. The maximum lag length p was selected as 3 for both tests. d=dmax, p= lag length, f= frequency, 1000 bootstrap was used.

5. Conclusions

This study analyzed both the long-run and short-run relationships between CO_2 emissions and agricultural productivity in Türkiye. Our research differed from the literature on several aspects. First, we adopted novel methodologies in which the interactions between CO_2 and agricultural production were presumed to be nonlinear. To this end, we applied both the Maki (2012) cointegration and gradual-shift causality tests developed by Nazlioglu et al., (2016). Second, in contrast with prior studies, CO_2 emissions due to agriculture have been employed instead of total CO_2 in the regression analysis.

The cointegration analysis confirmed the long-run association between CO_2 and agricultural productivity. However, the Maki (2012) cointegration test results indicated that this relationship is subject to structural changes due to local and global events affecting Türkiye's economy. Therefore, a mutually positive relationship between agricultural productivity and CO_2 emissions has been confirmed by the long run parameter estimates. The positive and statistically significant association between CO_2 emissions and the agriculture industry is aligned with the results of some prior studies, e.g. Islam et al. (2014), Khalid et al. (2016) and Wang (2022). The adverse impact of agricultural activity on environmental quality can be linked to important climate change drivers, such as livestock activities, fertilizer use, land use, and soil cultivation methods, which deserve further investigation. The long-run coefficient estimates imply that an expansion in farmland reduces agricultural productivity while raising CO_2 emissions. That is, expanding the arable land area in Türkiye may not be a viable solution for increasing agricultural efficiency. In accordance with the findings of Wang (2022), the adverse environmental effects resulting from the escalation of agricultural activities in Türkiye can be ascribed to the growing reliance on fossil fuels within the agricultural sector, thereby leading to an elevation in greenhouse gas emissions. The aforementioned findings underscore the imperative

of transitioning Türkiye's agricultural technologies and energy consumption towards environmentally sustainable practices. Moreover, the outcomes of both the linear and gradual shift causality tests demonstrated a unidirectional causal association running from agricultural productivity to CO_2 emissions. This evidence is consistent with the findings reported by Qiao et al. (2019), Waheed et al. (2018), Jebli and Youssef (2019), Ngarava et al. (2019), and Çetin et al. (2020). This finding suggests that there is a need to enhance the environmental sustainability of agricultural production tools in Türkiye.

In light of our research, it is evident that proactive measures ought to be implemented in order to mitigate the factors that contribute to climate change. Several potential strategies could be contemplated for adoption, encompassing enhanced management practices pertaining to agricultural land utilization, regulation of water supply, collection and reutilization of wastewater, prevention of deforestation, and the cultivation of plant species that exhibit resistance to drought conditions. The implementation of these measures is expected to yield positive outcomes for the advancement of the agricultural sector and the mitigation of climate change. As additional data becomes accessible, forthcoming investigations may incorporate a broader time span to examine the aforementioned variables that influence agricultural productivity.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: İbrahim ÜRKMEZ, Ahmet SEVİM Design: İbrahim ÜRKMEZ, Abdurrahman Nazif ÇATIK.; Data Collection or Processing: İbrahim ÜRKMEZ, Abdurrahman Nazif ÇATIK.; Statistical Analyses: İbrahim ÜRKMEZ, Abdurrahman Nazif ÇATIK.; Literature Search İbrahim ÜRKMEZ, Ahmet SEVİM; Writing, Review and Editing: İbrahim ÜRKMEZ, Ahmet SEVİM, Abdurrahman Nazif ÇATIK.

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Table A1. Description of the variables

Notation	Definition	Unit	Source
AGR_t	Agriculture, forestry, and fishing (Value added)	2015 US$	WB (WDI)
CO_{2t}	Agricultural sector CO_2 emissions	Tonnes	IEA
$LAND_t$	Agricultural land	Percentage of total land area	WB (WDI)
ENGt	Agricultural energy consumption	Kilotonnes of oil equivalent (ktoe)	IEA
$GFCF_t$	Gross fixed capital formation	Percentage of GDP	WB (WDI)

Table A2. Descriptive statistics of the variables

	AGR_t	CO_{2t}	$LAND_t$	*ENGt*	$GFCF_t$
Mean	38500	160890.7	50.71169	2419.262	21.43254
Median	36000	140333.1	50.44762	2409.989	22.80306
Maximum	62000	378628.2	53.5621	5342.634	29.85714
Minimum	26100	31890.07	47.70864	583.4623	11.87302
Std. Dev.	975000	97376.58	1.40838	1404.081	5.550205
Skewness	0.8881	0.5564	0.3291	0.3782	-0.1318
Kurtosis	2.8476	2.2326	2.4418	1.9538	1.6608
Jarque-Bera	6.7537	3.8827	1.5827	3.5416	3.9586
J-B Probability	0.0342	0.1435	0.4532	0.1702	0.1382

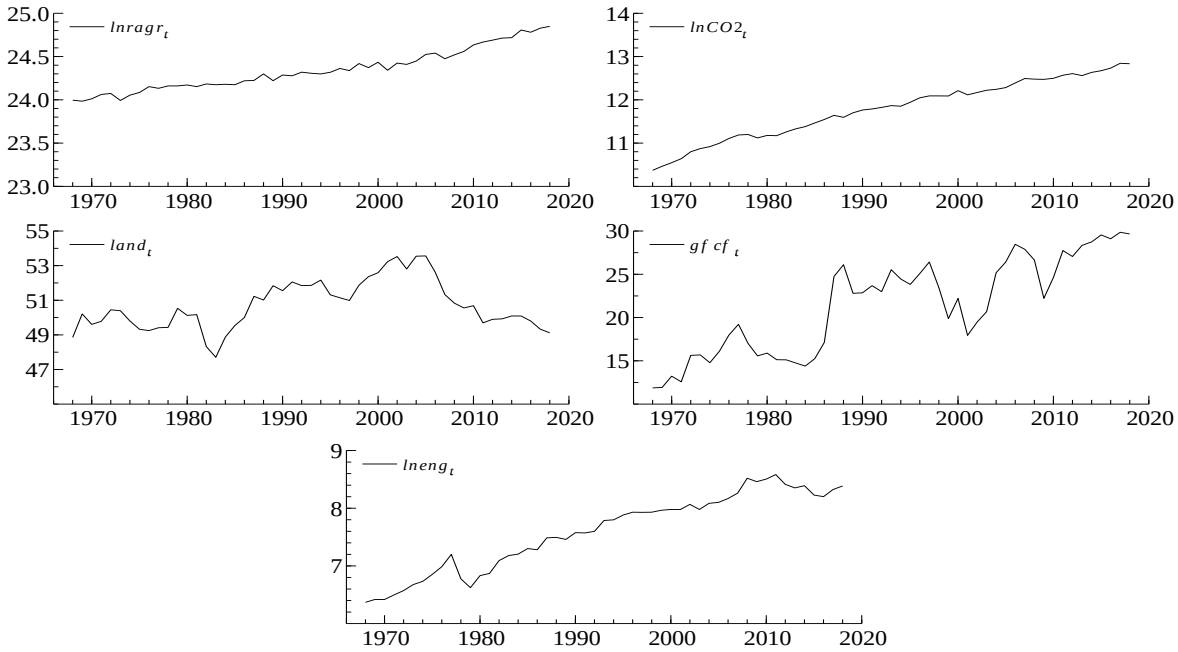
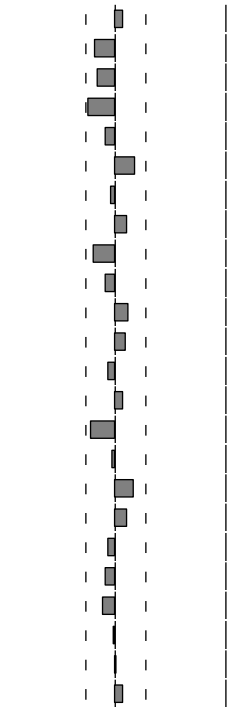


Figure A1. Time series plot of data

Sample (adjusted): 1969 2018
Included observations: 50 after adjustments



Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob*
		1	0.078	0.078	0.3215	0.571
		2	-0.187	-0.194	2.2189	0.330
		3	-0.161	-0.134	3.6495	0.302
		4	-0.249	-0.279	7.1533	0.128
		5	-0.097	-0.146	7.6940	0.174
		6	0.192	0.076	9.8615	0.131
		7	-0.035	-0.197	9.9365	0.192
		8	0.116	0.094	10.766	0.215
		9	-0.198	-0.342	13.247	0.152
		10	-0.089	0.004	13.762	0.184
		11	0.126	0.008	14.820	0.191
		12	0.101	-0.001	15.518	0.214
		13	-0.063	-0.125	15.801	0.260
		14	0.076	-0.014	16.217	0.300
		15	-0.221	-0.190	19.857	0.177
		16	-0.032	-0.036	19.935	0.223
		17	0.180	0.124	22.489	0.167
		18	0.120	-0.011	23.661	0.166
		19	-0.062	-0.114	23.986	0.197
		20	-0.083	-0.129	24.582	0.218
		21	-0.113	0.062	25.722	0.217
		22	-0.008	-0.132	25.728	0.264
		23	-0.005	-0.048	25.730	0.314
		24	0.078	-0.118	26.342	0.336

Figure A2. Model 2 correlogram of residuals

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Carbon and Nitrogen Stocks of Olive Orchard Soils in Izmir Province

İzmir Yöresi Zeytin Bahçe Topraklarının Karbon ve Azot Stokları

Aise DELİBORAN[1]*

Abstract

Soil organic carbon (SOC) and total nitrogen (TN) have a very important role in sustainable soil quality, crop production, and environmental impacts, and determining of carbon nitrogen ratio (C: N ratio) is very important for creating data banks in terms of ecosystem functions. Plants influence the interaction of SOC and TN, as well as ecosystem yield and the continental carbon cycle. Climate, atmosphere, and land-use change are all included in numerical models of the carbon (C) and nitrogen (N) cycles. This study was conducted to determine the SOC and TN stocks, the C: N ratio and their relationships with the soil properties of olive orchards in Aliaga, Bayindir, Bergama, Dikili, Foca, Karaburun, Kemalpasa, Menderes, Menemen, Odemis, Seferihisar, Selcuk, Tire, Torbali and Urla provinces of Izmir in Turkey. For this purpose, 129 soil samples were taken from 0-30 cm depth. The texture, pH, EC, lime, OM, SOC and TN content and stocks, Bulk density (Db) was determined. Db and C: N ratio varied between 0.84-1.31 g cm^{-3}, 5.17-80.50, and SOC density and stocks changed between 4.00-53.00 mg cm^{-3}, 1.25-1.59 kg m^{-2}, N density and stocks between 0.09-2.66 mg cm^{-3}, 0.03-0.80 kg m^{-2}, respectively. The highest BD was obtained from Tire, the highest SOC stocks from Karaburun, the highest TN from Seferihisar and Karaburun. The very small bulk density which is negatively associated with OM and clay is an important feature. The SOC contents were higher in relatively heavy rainfall regions. SOC and soil texture have a strong relationship. As a result, texture, precipitation, temperature, soil depths, and regeneration of soil affect the SOC and TN stocks. The results may be effective in terms of sustainable soil quality and ecosystem functions for olive cultivation.

Keywords: Total nitrogen stocks, Soil organic carbon stocks, Bulk density, Olive orchards

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Öz

Toprak organik karbonu (SOC) ve toplam azotu (TN) sürdürülebilir toprak kalitesi, bitkisel üretim ve çevresel etkilerde çok önemli bir role sahiptir, Karbon: Azot (C: N) oranının belirlenmesi ise ekosistem fonksiyonları açısından veri bankalarının oluşturulması için oldukça önemlidir. Bitkiler, ekosistem verimi ve kıtasal karbon döngüsünün yanı sıra SOC ve TN etkileşimini de etkiler. İklim, atmosfer ve arazi kullanımındaki değişikliklerin tümü, karbon (C) ve nitrojen (N) döngülerinin sayısal modellerine dahil edilir. Bu çalışma, Türkiye de İzmir ili Aliağa, Bayındır, Bergama, Dikili, Foça, Karaburun, Kemalpaşa, Menderes, Menemen, Ödemiş, Seferihisar, Selçuk, Tire ve Torbalı ilçelerindeki zeytin bahçelerinin SOC ve TN stokları, C: N oranı ve bunların toprak özellikleri ile ilişkilerini belirlemek amacıyla yürütülmüştür. Bu amaçla 0-30 cm derinlikten 129 adet toprak örneği alınmıştır. Toprak tekstürü, toprak reaksiyonu (pH), elektriksel iletkenlik (EC), kireç, organik madde (OM), SOC ve TN içeriği ve stoklar, hacim ağırlığı (Db) değerleri belirlenmiştir. Sırasıyla, Db ve C: N oranı 0.84-1.31 g cm^{-3} ve 5.17-80.50 arasında, SOC yoğunluğu ve stokları 4.00-53.00 mg cm^{-3}, 1.25-1.59 kg m^{-2} arasında, N yoğunluğu ve stokları ise 0,09-2,66 mg cm^{-3} ve 0.03-0.80 kg m^{-2} arasında değişmiştir. En yüksek Db Tire'den, en yüksek SOC stokları Karaburun'dan, en yüksek TN ise Seferihisar ve Karaburun'dan elde edilmiştir. OM ve kil ile negatif ilişkili olan çok küçük kütle yoğunluğu önemli bir özelliktir. SOC içerikleri, nispeten yoğun yağış alan bölgelerde daha yüksekti. SOC ve toprak dokusu arasında güçlü bir ilişki vardır. Sonuç olarak, toprak tekstürü, yağış, sıcaklık, toprak derinlikleri ve toprağın yenilenmesi SOC ve TN stoklarını etkiler. Sonuçlar, zeytin yetiştiriciliği için sürdürülebilir toprak kalitesi ve ekosistem fonksiyonları açısından etkili olabilir.

Anahtar Kelimeler: Toplam azot stokları, Toprak organik karbon stokları, Hacim ağırlığı, Zeytin bahçeleri

1. Introduction

Carbon and nitrogen are very important due to their oxidation into the atmosphere in the global warming pursuance. Especially, the biogeochemical cycles of carbon and nitrogen are remarkable and the importance of this has increased in terrestrial ecosystems around the world. The largest factor of terrestrial carbon is soil organic carbon (SOC). The carbon amount that consists of SOC is bigger than the carbon current in live vegetation (Post and Kwon, 2000). Furthermore, the diversification of SOC pools has an additive effect on the carbon dioxide (CO_2) concentrations in the atmosphere (Smith, 2008). For these reasons, appreciation of SOC latent and improving efficient processes to reduce the atmospheric CO_2 concentration are critically significant (Fu et al., 2010).

The land-use alteration, growth, and other factors affect SOC stocks in different ways in distinct ecosystem and districts (Yimer et al., 2007). In the carbon and nitrogen cycle, soils play a critical act. According to Schlesinger (1997), soil includes approximately 75% SOC and 95% total nitrogen (TN). SOC and TN have a very important act in sustainable soil quality, crop production, and ecological impacts (Bauer and Black, 1994; Doran and Parkin, 1994). The TN content of soils has a significant impact on the fertility of the earth's soil. SOC sequestration is winning global care due to the expanding requirement to balance the quickly growing atmospheric level of CO_2. The fortification of CO_2 is connected with an increase in universal heating possible and alters in the quantity and effectuality of rainfall (Lal and Follet, 2009). The increase of nitrous oxide (N_2O) levels in the atmosphere is directly related to widespread human intervention in the nitrogen cycle, which is mostly influenced by agricultural activities (Prather et al., 1995). As tropical habitats are converted to agriculture, grazing, or silviculture, there is an increasing back demand for tropical soil N_2O emission to become extra crucial (Duxbury, 1994). For this reason, comprehension of soil carbon and N storage back demand and growing efficient procedures to reduce the CO_2 and N_2O levels of the atmosphere are crucial (Fu et al., 2010).

Plants influence the interaction of SOC and TN, as well as ecosystem yield and the continental carbon cycle. Climate, atmosphere, and land-use change are all included in numerical models of the carbon (C) and nitrogen (N) cycles (Pepper et al., 2005). The C: N ratio of soil provides information about the soil's degree of degradation and humus structure (Brady and Weil, 2008). While the C: N ratio is high, the decomposition of organic matter (OM) is slow, and while the C: N ratio is small, the decomposition takes place quickly. In short, the C: N ratios of soils are important in terms of the mineralization of OM (Sakin and Sakin, 2014). If the C: N ratio of the organic substance is more than 30, nitrogen immobilization occurs at the beginning of decomposition. If the C: N ratio of organic material has greater than 30, nitrogen immobilization occurs at the start of decomposition. If the C: N ratio is between 20-30, mineralization and immobilization are in balance. If this ratio is lower than 20, mineral nitrogen is released at the start of decomposition (Tisdale and Nelson, 1985).

In the soil, irrigation and fertilization parameters affect the C and N amounts and percentage ratios. According to studies, these two elements raised the amounts and proportion of C and N, and the C:N ratios (6.5:1-25.0:1) were substantially different (Kelliher et al., 2012; Schipper et al., 2012). Also, it is known that it is very difficult to realize that the bonding and storage times of C and N are long in a short time (Condron et al., 2012). The C: N ratio in soils ranges from 8 to 17 (Alistair Pitty, 1979), and it is a critical pointer to soil quality (Zhang et al., 2011), and it also affects pH, nutrient accumulation, and humic substance content in the soil (Yano et al., 2000). According to Berg and McClaugherty (2003) the density of humus levels and the C: N ratio have a significant correlation. Tillage degrades OM, and because of this, the C: N ratio is narrower in cultivated soils (Seeber and Seeber, 2005). Because of this, forest soils have a higher C: N ratio than agricultural soils (John et al., 2005; Puget and Lal, 2005). Also, OM regulates soil aggregation, thus increasing soil porosity, increasing porosity then increasing micropore, and resultantly decreases in soil bulk density (Erhart and Hartl, 2010; Aktas and Yuksel, 2020).

The soil C: N ratio is a soil fertility marker, due to the strong relationship between soil OC and N. Various factors change the soil C: N ratio, including climate (Miller et al., 2004), characteristics of soil (Ouedraogo et al., 2006), vegetation type (Diekow et al., 2005; Puget and Lal, 2005) or agricultural methodologies (Zhang et al., 2009). Mechanical, chemical, and biological reactions could not decompose soil OM because of the particle size distribution (Krull et al., 2003). The concentration of clay affects OC accumulation, and OC increased with increasing clay content (Burke et al., 1989). Compared to loamy soils, sandy soils have low OC and N content, and a higher C: N ratio. Similar results were taken for the central United States (Franzmeier et al., 1985); (Sims and Nielsen, 1986; Burke et al., 1989), as well as in other parts of the country (Grigal and Ohmann, 1992); (Conant

et al., 1998; Homann et al., 2004) and some countries of the World (Paruelo, 1998; Hontoria et al., 1999). These findings revealed a variety of trends. SOC which was expected to increase with a decrease in temperature, decreased in southern Oregon (Homann et al., 1995), and Finland forests (Liski and Westman, 1997). Climatic conditions, seasonal weather diversity, altitude differences, and many factors may cause se variety of trends (Homann et al., 2007).

It is considered that determining of C: N ratio is very important for creating data banks in terms of ecosystem functions. In the literature review, it was determined that olive orchard soils were not examined in terms of C and N stocks, C: N ratio, and their relations with soil properties. Also, Izmir province and its districts, which have an important share in Turkey's olive cultivation, are considered to be important in this regard. The target of this study was to determine the amount of SOC and TN in olive orchard soils, as well as their stocks, C: N ratio, and relationships with other soil properties of Aliaga, Bayindir, Bergama, Dikili, Foca, Karaburun, Kemalpasa, Menderes, Menemen, Odemis, Seferihisar, Selcuk, Tire, Torbali and Urla provinces in Izmir.

2. Material and Method

2.1. Research area

The research was conducted in olive orchard fields of Izmir province in Turkey's Aegean Region (*Figure 1*). The effect of the Mediterranean climate is observed in that the summers are dry and hot, while the winters are mild and rainy. July and August are the hottest, while January and February are the coldest. The air temperature, precipitation, and sunshine duration for the mean yearly are 17.5 ^{0}C, 713.8 mm, and 8.1 hours, respectively, even though the number of days below zero does not reach ten (MGM, 2022).

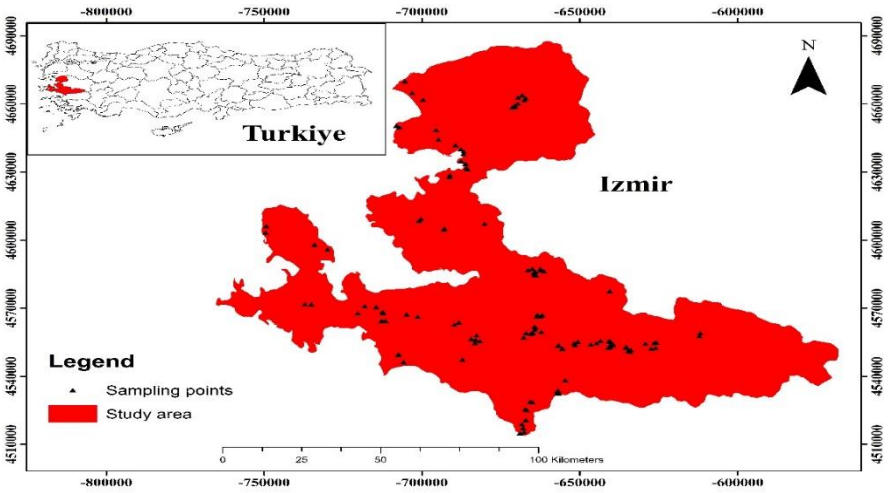

Figure 1. Izmir province and research region

2.2. Sampling method and soil analysis

In this study, samples of soil were taken consecutively from 0-30 cm depth with GPS coordinates in November-December 2015. These samples were analyzed after they were dried by air and sieved from 2 mm sieves. The texture was determined by the hydrometer method, and soil reaction (pH) by a pH meter from a soil + pure water mixture (1:2.5 ratio) (Tuzuner, 1990). While electrical conductivity (EC) was evaluated using an electrical permeability device from a 1:5 soil+distelled water mixture, lime ($CaCO_3$, %) as volumetric by Scheibler calcimetry, OM (%) by the Walkey-Black method (Tuzuner, 1990). The total C and N content was calculated by dumas method dry consumption with a LECO CNS-2000 analyzer (McGeehan and Naylor, 1988). Also, bulk density (Db) was made by Black (1965).

The soil C and N stocks were determined according to the following Equation (1):

$$E = d \times h \times (TOC\ or\ TN) \div 10 \quad \text{(Eq.1)}$$

E: The TOC or TN stock (mg ha^{-1}),
d: The soil bulk density (mg m^{-3}),
h: The sampled layer (cm),

TOC and TN are the total soil levels of organic C and N (g kg^{-1}), respectively, and 10 is the unit converter. OC density and N density was calculated according to the following Equation (2) and Equation (3):

$$OC\ dencity = \text{OC content}\ (\%) \times bulk\ density\ (g\ /\ cm) \times 10 \quad \text{(Eq.2)}$$

$$N\ density = N\ content\ (\%) \times bulk\ density\ (g\ /\ cm) \times 10 \quad \text{(Eq.3)}$$

Official classification set of soil mapping direction of the Geological State Offices of the Federal States of Germany (*Table 1*) were used for determined BD and OC contents (Arbeitsgruppe Boden, 2005).

Table 1. Official classification systems of soil mapping instruction of the Geological State Offices of the Federal States of Germany (in 30 cm depth of OC and N of soils of the allotment gardens of the northwestern Ruhr area) (Arbeitsgruppe Boden, 2005)

	Bulk density[a] g cm^{-3}	**OC content[a] %**	**OC density[b] mg cm^{-3}**	**OC stock kg m^{-2}**	**N content[c] %**	**N density[d] mg cm^{-3}**	**N stock kg m^{-2}**
Very low	<1.2	0.6	<9	<2	<0.04	<0.6	<0.2
Low	1.2-1.4	0.6-1.2	9-17	2-4	0.04-0.07	0.6-1.0	0.2-0.3
Modarate	1.4-1.6	1.2-2.4	17-32	4-8	0.07-0.13	1,0-1.7	0.3-0.5
High	1.6-1.8	2.4-4.8	32-56	8-16	0.13-0.24	1.7-2.8	0.5-0.8
Very high	1.8	4.8-8.7	56-80	16-24	0.24-0.42	2.8-3.9	0.8-1.2
Extreme high	-	>8.7	>80	>24	>0.42	>3.9	>1.2

[a] Arbeitsgruppe Boden (2005)
[b] Calculated from the regression equation: Bulk density = −0.065 × OC + 1.49; R2 = 0.49; n = 83;
[c] Calculated from the regression equation: N = 0.047 × OC + 0.0114; R2 = 0.81; n = 83
[d] Calculated from the regression equation: Bulk density = −1.33 × N + 1.49; R2 = 0.57; n = 83

3. Results and Discussion

3.1. Soil Properties of the Olive Orchard

Olive orchard soil properties of Izmir province were presented in *Table 2*. The sand, clay, and silt content of soils ranged from 22.95% to 83.68%; from 0 to 48.32%, and from 2.72% to 65.78%, respectively in General. While the soil reaction and lime ranged from 5.36 to 8.12, and from 1.16% to 73.22%; OM and EC ranged from 9.7 g kg^{-1} to 37.8 g kg^{-1}, and from 0.08 dS m^{-1} to 0.90 dS m^{-1} (*Table 2*). In General, according to Anonymous (1951), the majority of olive orchard soils (51.16%) were sandy loamy (SL), 17.05% of soils were clayed loam (CL), and 15.50% of soils were sandy clayed loam (SCL), 8.53% of soils clayey (C), 3.88% of loamy (L) and 3.10% of sandy (S) textured. Also, the vast majority of soils were slightly alkaline, neutral, and moderately acidic. All soils are characterized by low OM levels or in the low and poor humus class. This can be detailed with the soil textures. The SOM generation and mineral aggregation decline in the surface range of sandy soils and this decrease can be explained by the deeper level of highly transformed OM (Gonzalez Parra and Candas, 2004). For example in soils in the south of Europe, it is emphasized that low OM levels may be partially related to the semiarid Mediterranean condition (Gallardo et al., 2000). Also, studies have shown that most of the lands belonging to the olive orchards of the region are SL, CL, SCL, and a few soils is C texture, slightly alkaline and moderately acidic, salt-free, and poor in humus (Aydogdu, 2011; Turan et al., 2013; Deliboran et al., 2020).

Table 2. Descriptive statistics parameters of olive orchards soils in Izmir province

Province	N		Texture (%)			pH	EC (dS m^{-1})	Lime (%)	OM (g kg^{-1})
			Sand	Clay	Silt				
Aliaga	9	Mean	47.14	31.74	21.12	6.95	0.53	16.85	20.9
Bayindir	23	Mean	66.35	13.58	20.07	6.19	0.22	3.033	18.7
Bergama	12	Mean	42.02	38.87	19.11	7.73	0.61	17.42	18.4
Dikili	8	Mean	55.94	26.11	17.95	7.19	0.50	12.69	19.2
Foca	2	Mean	44.68	35.60	19.72	7.71	0.71	10.67	16.7
Karaburun	4	Mean	53.74	22.04	24.22	7.11	0.41	10.85	28.2
Kemalpasa	10	Mean	42.18	32.30	25.52	7.48	0.53	19.74	24.6
Menderes	9	Mean	45.43	27.88	26.69	7.54	0.41	26.73	25.0
Menemen	3	Mean	65.01	15.60	19.39	7.48	0.42	2.78	17.4
Odemis	8	Mean	71.74	14.29	13.97	6.41	0.19	1.59	13.9
Seferihisar	7	Mean	61.58	19.03	19.39	6.71	0.26	5.76	22.2
Selcuk	10	Mean	62.13	17.29	20.58	7.21	0.20	3.30	19.5
Tire	5	Mean	69.84	9.44	20.72	7.63	0.15	17.68	15.9
Torbalı	12	Mean	55.53	10.23	34.24	7.44	0.35	11.86	20.8
Urla	7	Mean	43.67	31.87	24.46	7.30	0.40	28.68	19.6
		Minimum	22.95	8.32	2.72	5.36	0.08	1.16	9.7
		Maximum	83.65	48.32	65.76	8.12	0.90	73.22	37.8
		Mean	55.66	22.15	22.09	7.10	0.368	12.14	20.09
General	129	Standart Devision	14.04452	12.3159	9.02838	0.68	0.20319	15.6274	5.72040
(Izmir)		Coefficient variation	0.26	0.56	0.41	0.10	0.55	1.28	0.28
		Variance	208.663	151.822	81.512	0.463	0.041	244.22	32.723
		Skewness	-0.318	0.275	1.345	-0.809	0.483	1.900	0.718
		Kurtosis	-0.933	-0.898	4.383	-0.498	-0.716	3.320	0.329

3.2. Bulk Density of the Olive Orchard Soils

In General, Db values ranged from 0.84 to 1.31 g cm^{-3}. The mean highest Db was obtained from Tire province. Tire was followed by Bayindir = Bergama = Menemen = Odemis = Torbali = Urla > Kemalpasa > Dikili = Selcuk > Menderes > Foca > Seferihisar > Aliaga > Karaburun (*Table 3*). The very low bulk density seen in loamy sandy and sandy loam soils is one of the most important features of garden soils (Burghardt and Schneider, 2018). In our study, these results were obtained from olive orchard soils, and Db has a negative correlation with OM (r=-0.189) and clay (r=-0.148) (P <0.05) (*Table 4*). Also, the majority of olive orchard soils (51.16%) were SL, 17.05% of

Tablo 3. Describe statistics parameters of Izmir province soils

Province	N		*Bulk density (Db, g cm^{-3})*	*Soil organic carbon (SOC, g kg^{-1})*	*SOC density (mg cm^{-3})*	*SOC stocks (kg m^{-2})*	*Total nitrogen (N, g kg^{-1})*	*N density (mg cm^{-3})*	*N stocks (kg m^{-2})*	*C: N ratio*
Aliaga	9	Mean	1.01	18.8	20.30	6.09	1.0	1.05	0.31	20.91
Bayindir	23	Mean	1.11	12.9	15.39	4.62	0.8	0.95	0.28	16.09
Bergama	12	Mean	1.11	18.5	20.78	6.23	0.8	0.90	0.27	22.53
Dikili	8	Mean	1.08	18.5	20.28	6.08	1.2	1.28	0.38	15.54
Foca	2	Mean	1.06	18.4	19.79	5.94	1.0	1.08	0.32	20.69
Karaburun	4	Mean	0.98	24.7	26.47	7.94	1.6	1.60	0.48	16.05
Kemalpasa	10	Mean	1.09	19.0	20.19	6.06	1.0	1.10	0.33	24.71
Menderes	9	Mean	1.07	23.4	25.44	7.63	1.3	1.41	0.42	19.77
Menemen	3	Mean	1.11	11.3	12.80	3.84	1.2	1.32	0.40	9.60
Odemis	8	Mean	1.11	8.0	8.92	2.68	0.8	0.83	0.25	10.80
Seferihisar	7	Mean	1.03	18.3	20.96	6.29	1.6	1.73	0.52	12.68
Selcuk	10	Mean	1.08	14.4	15.58	4.67	1.1	1.19	0.36	13.12
Tire	5	Mean	1.14	19.7	21.96	6.59	0.9	1.09	0.33	27.13
Torbalı	12	Mean	1.11	17.6	19.45	5.84	1.1	1.22	0.37	16.50
Urla	7	Mean	1.11	19.3	23.99	7.20	1.1	1.24	0.37	15.81
		Min.	0.84	4.3	4.00	1.25	0.1	0.09	0.03	5.17
		Max.	1.31	43.1	53.00	15.93	2.4	2.66	0.80	80.50
		Mean	1.09	17.0	18.49	5.55	1.1	1.13	0.34	17.52
General	129	Standart Devision	0.09176	9.16671	10.16513	3.05902	0.47253	0.50875	0.15259	9.42483
(Izmir)		Coefficient variation	0.08	0.59	0.55	0.55	0.45	0.51	0.45	0.54
		Variance	0.008	84.029	103.330	9.358	0.223	0.259	0.023	89.426
		Skewness	0.039	0.953	1.127	1.134	0.492	0.541	0.544	3.181
		Kurtosis	0.078	0.285	1.100	1.120	-0.341	-0.069	-0.072	16.006

soils were CL, and 15.50% of soils were SCL textured. Increased SOC with the addition of compost reduces the Db of garden soils (Maynard, 2000), and there is a negative correlation between Db and OC content (Burghardt and Schneider, 2018). According to Kashi et al. (2016); the Db of walnut garden soils was 1.74 mg m^{-3} at 25 cm depth. Db of soils with different land use investigated, the lowest Db was obtained from irrigated farmland (1.01 g cm^{-3}), and the highest Db was determined from the orchard (1.52 g cm^{-3}) ($P < 0.05$) (Ozturkmen et al., 2021). It is known that intensive use of animal manure increases the bulk weight of soils (Adeyemo et al., 2019). According to Brye et al. (2005), increasing doses of farm manure gradually reduces the Db and soil prevents jamming. Like results were found in other studies (Ozturkmen et al., 2020; Ozturkmen and Ramazanoglu, 2020).

3.3. Soil Organic Carbon (SOC), Total Nitrogen (TN), and C: N Ratio

In General, SOC, TN content and C: N ratio varied between 4.3-43.1 g kg^{-1}; 0.1-2.4 g kg^{-1} and 5.17-80.50, respectively. While the mean SOC values of all province were ordered as follow: Karaburun > Menderes > Tire > Urla > Kemalpasa > Aliaga > Bergama = Dikili > Foca > Seferihisar > Torbali > Selcuk > Bayindir > Menemen > Odemis, the mean TN content of all province as follow: Karaburun = Seferihisar > Menderes > Dikili = Menemen > Selcuk = Torbali = Urla > Aliaga = Foca = Kemalpasa > Tire > Odemis = Bergama = Bayindir (*Table 3*). According to the classification of Arbeitsgruppe Boden (2005) (*Table 1*), while Karaburun (24.7g kg^{-1}=2.47%) was high, and Odemis (8.0 g kg^{-1}=0.8%) was low; the other province moderate in terms of SOC content; Karaburun (1.6 g kg^{-1}=0.16%), and Seferihisar (1.6 g kg^{-1}=0.16%) were high, the other provinces were moderate in terms of TN (*Table 3*). The mean C: N values of all province were ordered as follow: Tire > Dikili > Kemalpasa > Bergama > Aliaga > Foca > Menderes > Torbali > Bayindir > Karaburun > Urla > Selcuk > Seferihisar > Odemis > Menemen (*Table 3*). Clay percent showed a positive relation with SOC content (r=0.146), ratio of C: N (r=0.187) and with OM (r=0.209) ($P<0.05$). Content of SOC may increase by increasing soil clay content (Nichols, 1984; Burke, 1989), but this should not be generalized, other parameters such as the aluminum level of the soil or particular surface area may also change the SOC level (Percival et al., 2000; Krull et al., 2003). Our study, especially when compared to soil organic matter (OM) models such as Century (Parton et al., 1987) and RothC (Jenkinson, 1990), which suggest that OM solubility decreases as clay concentration increases, it is thought that the correlation of clay concentration and SOC content is important. According to Hernanz et al. (2009), semiarid regions soils of the Mediterranean have a low SOC content because of strong OM mineralization and the lack of harvest wastes following drought periods on rainfed crops. Some researchers reported that soils by tree cover exhibit an increase at C and N which is similar to our findings (Albretch and Kandji, 2003; Parras-Alcantara et al., 2013).

As a result of our study, the great majority of olive orchard soils were SL, CL, and SCL texture, and as a result of these properties, the SOC of soils ranged from 8 g kg^{-1} (Odemis) to 24.7 g kg^{-1} (Karaburun). Also, SOC content showed a positive significant correlation between TN (r=0.684) and the ratio of C: N (r=0.331) while TN showed a negative relation to C: N ratio (r=-0.297) ($p<0.01$). OM level showed a positive significant relationship by SOC (r=0.223) and TN content (r=0.284) ($p<0.01$) (*Table 4*). Parras-Alcantara et al. (2013) claimed that SOC content is 10.1 g kg^{-1} and TN values are 1.07 g kg^{-1} in olive orchard soils at 27 cm depth, and also TN content and C: N ratio decrease at 27cm< depth, and that this is related to an increase in soil clay content by depth (between 27-176 cm). Greater clay content is often connected with high decomposed OM and a lower C: N ratio (Puget and Lal, 2005; Yamashita et al., 2006; Kashi et al., 2016). At 25 cm depth of walnut gardens soils, SOC, TN, and C: N was 3 g kg^{-1}, 0.33 g kg^{-1}, and 31.2, respectively (Kashi et al., 2016). C: N ratios range from 8:1 to 15:1 in the Mississippi River Delta region of eastern Arkansas (Brady and Weil, 2008) and between 4.32:1 and 6.04:1 in Harran plain soils (Sakin et al., 2011b). It is known that C: N ratio was induced by low rainwater, full resolution, extraction rates, or extreme cultivation techniques. The C: N increases by precipitation, while it reduces by increased temperatures (Miller et al., 2004). According to Callesen (2007), there is a positive correlation between C: N ratios, rainwater, and temperature. Also, it is argued that although the cultivation methods and farming activities utilized 10 years ago had not affect C: N ratios (Sainju et al., 2008; Fu et al., 2010), techniques of modern farming and agriculture do (Puget and Lal, 2005; Yimer et al., 2007).

Table 4. The relationship between measured soil parameters, BD, SOC, TN stock and C: N

	SOC	**TN**	**C/N**	**pH**	**EC**	**Lime**	**OM**	**Sand**	**Clay**	**Silt**	**BD**	**SOC Density**	**TN Density**	**SOC Stocks**	**TN Stocks**
TN	0,684**	1													
C/N	0,331**	-0,297**	1												
pH	0,328**	0,090	0,330**	1											
EC	0,148*	-0,022	0,175*	0,470**	1										
Lime	0,291**	-0,015	0,445**	0,501**	0,380**	1									
OM	0,223**	0,284**	0,017	0,153*	0,291**	0,374**	1								
Sand	-0,271**	-0,053	-0,247**	-0,619**	-0,694**	-0,632**	-0,381**	1							
Clay	0,146*	-0,031	0,187*	0,475**	0,652**	0,402**	0,209*	-0,781**	1						
Silt	0,236**	0,130	0,142	0,332**	0,220**	0,458**	0,323**	-0,523**	-0,120	1					
Db	0,001	-0,109	0,055	0,045	-0,210**	-0,030	-0,189*	0,092	-0,148*	0,045	1				
SOC Density	0,987**	0,651**	0,341**	0,325**	0,120	0,291**	0,194*	-0,260**	0,129	0,241**	0,144	1			
TN Density	0,691**	0,979**	-0,282**	0,100	-0,044	-0,006	0,254**	-0,051	-0,044	0,143	0,076	0,689**	1		
SOC Stocks	0,987**	0,651**	0,341**	0,325**	0,120	0,291**	0,194*	-0,260**	0,130	0,241**	0,143	1,000**	0,689**	1	
TN Stocks	0,691**	0,979**	-0,279**	0,103	-0,045	-0,004	0,253**	-0,052	-0,044	0,145	0,076	0,689**	1,000**	0,689**	1

3.4. Soil Organic Carbon (SOC) and Total Nitrogen (TN) Stocks

In general, while SOC density and SOC stocks changed between 4.00-53.00 mg cm^{-3}, and 1.25-1.59 kg m^{-2}, N density and N stocks varied between 0.09-2.66 mg cm^{-3}, and 0.03-0.80 kg m^{-2}, respectively. According to the mean values of the province, the highest mean SOC density (26.47 mg cm^{-3}), and mean SOC stocks (7.94 kg m^{-2} / 79.4 mg ha^{-1}) were obtained from Karaburun. Karaburun was followed by Menderes > Urla > Tire > Seferihisar > Bergama > Aliaga > Dikili > Kemalpasa > Foca > Torbali > Selcuk > Bayindir > Menemen > Odemis (*Table 3*). The density of SOC expresses the percentage of SOC in a unit of soil volume. According to the classification of Arbeitsgruppe Boden (2005) (*Table 1*), Karaburun, Menderes, Urla, Tire, Seferihisar, Bergama, Aliaga, Dikili, Kemalpasa, Foca, Torbali, and Selcuk were in moderate class, Bayindir, Menemen, and Selcuk were low, Odemis was a very low class in terms of SOC density and SOC stocks. As a result, SOC density and stocks were higher in Karaburun, Menderes, Urla, Tire, and Seferihisar than in the other province, respectively. The climatic condition has an impact on stocks of SOC and TN (Post et al., 1982; Sakin et al., 2011b), and the SOC content reduces by increases in the average temperature (Post et al., 1982; Tremblay et al., 2002; Ganuza and Almendros, 2003; Lemenih and Itanna, 2004; Wang et al., 2004; Sakin et al., 2010). The SOC contents were higher in regions with relatively heavy rainfall in our study. While the precipitation amount of Karaburun, Menderes, Tire, and Seferihisar was 800 mm<, the precipitation amount of Aliaga, Foca, Dikili, Menemen, and the other province were changed between 650-720 mm. In this case, it is clear that as precipitation increases, temperature decreases. High temperatures generally stimulate the decomposition of OM; resulting in SOC decreases. Also, it is known that SOC stock is caused by the texture. For example, at 27 cm depth of soils in Spain, SOC stocks of olive orchards were 34.1 mg ha^{-1} where soils were less clayey and sandier (Parras-Alcantara et al., 2013). SOC stock value was higher than the researchers and its results that the vast majority of olive orchard soils were SL, CL, and SCL textures, in our study. Sand percent had a substantial negative connection with clay (r=-0.781), silt (r=-0.523), and SOC content (r=-0.271), C: N ratio (r=-0.247), SOC stocks (r=-0.260) ($p<0.01$). Silt percent showed the highest positive significant correlation on SOC stock (r=0.241) ($p<0.01$). These findings confirm the previous studies that indicated the role of soil texture in SOC, TN, and C: N ratios. Soil parameters such as SOC and TN were impacted by the mineral composition and textural level of the soil. They are associated with SOC aggregate stability as the clay level rises (Jiao et al., 2012).

This condition affects soil aggregation and clay content, as well as indirectly affects SOC storage by absorbing organic matter from the soil. As a result, soil texture plays a role in chemical and physical preservation mechanisms, either directly or indirectly (Plante et al., 2006). Shortly, clayey soils have high SOC stock values due to the stability mechanisms of the clays in the soil (Burke et al., 1989; Leifeld et al., 2005). Parras-Alcantara et al. (2013) claimed that can be observed this effect in olive orchard soils. The highest mean N density (1.73 mg cm^{-3}) and mean N stocks (0.52 kg m^{-2}) were obtained from Seferihisar. It was followed by Karaburun > Menderes > Menemen > Dikili > Urla > Torbali > Selcuk > Tire > Foca > Aliaga > Bayindir > Bergama > Odemis (*Table 3*). According to the classification of Arbeitsgruppe Boden (2005) (*Table 1*) Seferihisar was high, Karaburun, Menderes, Menemen, Dikili, Urla, Torbali, Selcuk, Tire, Foca, Aliaga, and Odemis were moderate, Bayindir, Bergama was low class. The nitrogen stock level, like the carbon stock content, was higher in Seferihisar, Karaburun, Menderes, and Menemen than in other provinces. It may be caused by high rainfall. Furthermore, temperament and moisture circumstances have little impact on carbon and nitrogen stores. But estimates are done to deal with their effects on carbon for the statement of their relationship with nitrogen (Sakin, 2012). Furthermore, in our investigation, TN concentrations were comparatively high in locations with high SOC, indicating a favorable C: N relationship (*Table 4*). Clay may reduce SOC oxidation, and clay and nitrogen have a beneficial association (Sakin et al., 2011a; 2011b). A study reported that nitrogen mineralization decreases, as the amount of clay in the soil increases (Cote et al., 2000). Clay concentration was directly proportional to aggregate size and accumulation, and the potential N mineralization decreased (McLauchlan, 2006).

4. Conclusions

The very low bulk density is one important qualification of olive orchard soils. These soils have SL, CL, and SCL textures. Bulk density was negatively related to OM and clay level. SOC and soil texture has a very strong correlation, SOC content is increased with increased clay content, and SOC and organic matter content were positively correlated with clay. The sand was negatively correlated by SOC content and SOC stocks, silt showed the highest positive

significant correlation with SOC stock. TN concentrations were relatively high in locations with high SOC, indicating a favorable C: N relationship. Rainfall, warmth, soil depths, material migration from high locations to the plains, and soil regeneration are all contributors to the high C and N content. The close C: N ratios are dependent on high resolution and separation levels due to high temperatures, oxidation, and fertilizer application by farmers (which include high levels of nitrogen).

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Deliboran, A.; Design: Deliboran, A.; Data Collection or Processing: Deliboran, A.; Statistical Analyses: Deliboran, A.; Literature Search: Deliboran, A.; Writing, Review and Editing: Deliboran, A.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Analysis of Factors Affecting Corn Seed Preferences of Producers: Case of Sakarya Province, Turkey*

Üreticilerin Mısır Tohumu Tercihlerini Etkileyen Faktörlerin Analizi: Sakarya İli Örneği

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Abstract

This research was carried out in 8 districts representing approximately 91.92% of corn production in Sakarya province. In the sampling of the study, the formula developed for limited populations of the simple random sampling method was used. The main material of the research is the data obtained from the questionnaires made with 261 corn producers. Determining the factors affecting the corn seed preferences of the producers is extremely important in terms of establishing the targets of the corn breeders and the marketing strategies of the seed suppliers in the market. In order to measure the attitudes of corn producers towards corn seed preferences, factor analysis was conducted to test 88 judgments were gathered under more specific factors. It was determined that the producers in the Söğütlü district of Sakarya found the factors of consumption expectations 2.11 times more important and the factors of vegetative characteristics of the variety 10.5 times more important than the producers of other districts. According to the producers in Ferizli district, it was determined that vegetative characteristics were 16.2 times more important factors and expectations of consumption were 5.14 times more important factors compared to other district producers. The 29 propositions in the first grouping that named "The Importance Level of the Producers to Corn Breeding, Education and Corn" were collected in 9 different factor groups. The 21 propositions in the second grouping that named "The Importance Level of Environmental and Vegetal Characteristics in Variety Preference" were collected in 7 different factor groups. The 18 propositions in the third grouping that named “The Importance Level of Expectations of the Manufacturers from the Product and the Firms” were collected in 6 different factor groups. And the 20 propositions in the last grouping that named "The Corn Seed Packaging Technical Specifications and Technical Information Content Significance Level" were collected in 5 different factor groups. For manufacturers, to give a company's guarantee for seeds, to take care of producers after sales, to set up demonstrations in the target areas, to make inspections in field after planting, etc. issues are more important than to distribute promotions, to organize fairs travels and to use eye-catching packaging etc. issues.

Keywords: Seed market, Seed marketing, Market share, Purchase behavior, Producer tendencies

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Öz

Bu araştırma, Sakarya ili mısır üretiminin yaklaşık %91.92'sini temsil eden 8 ilçede gerçekleştirilmiştir. Araştırmanın örneklemesinde, sınırlı popülasyonlar için geliştirilmiş olan basit tesadüfi örnekleme yöntemi kullanılmıştır. Araştırmanın ana materyalini 261 mısır üreticisi ile yapılan anketlerden elde edilen veriler oluşturmaktadır. Üreticilerin mısır tohumu tercihlerini etkileyen faktörlerin belirlenmesi, mısır yetiştiricilerinin hedeflerinin ve tohum tedarikçilerinin pazardaki pazarlama stratejilerinin oluşturulması açısından son derece önemlidir. Mısır üreticilerinin mısır tohumu tercihlerine yönelik tutumlarını ölçmek amacıyla faktör analizi yapılarak daha spesifik faktörler altında toplanan 88 yargı test edilmiştir. Sakarya ili Söğütlü ilçesindeki üreticilerin diğer ilçelerdeki üreticilere göre tüketim beklentileri faktörlerini 2.11 kat, vejetatif özellik faktörlerini ise 10.5 kat daha önemli buldukları belirlenmiştir. Ferizli ilçesindeki üreticiler için ise diğer ilçe üreticilerine göre bitkisel özelliklerin 16.2 kat, tüketim beklentilerinin ise 5.14 kat daha önemli faktörler olduğu belirlenmiştir. Üreticilerin, mısır yetiştiriciliğine, eğitimine ve mısıra verdikleri önem düzeyi adlı birinci gruplandırmadaki 29 önerme burada 9 ayrı faktör grubunda, çevresel ve bitkisel özelliklerin çeşit tercihindeki önem düzeyi adlı ikinci gruplandırmadaki 21 önerme 7 ayrı faktör grubunda, üreticilerin üründen ve firmalardan beklentilerinin önem düzeyi adlı üçüncü gruplandırmadaki 18 önerme 6 ayrı faktör grubunda ve mısır tohumluk ambalajı teknik özellikleri ve teknik bilgi içeriği önem düzeyi adlı son gruplandırmada ise 20 önerme 5 ayrı faktör grubunda toplanmıştır. Firmalar için uygulaması kolay olduğundan sık sık yapılmakta olan promosyonlar dağıtmak, fuar ve gezi organizasyonları düzenlemek, göz alıcı ambalaj kullanmak vb. gibi hususların esasen üretici nazarında firmanın sattığı tohumluğa garanti vermesi, satış sonrası da üreticiyle ilgilenmesi, hedef bölgede demonstrasyonlar kurması, ekim sonrası tarla kontrolleri yapması vb. gibi hususlar kadar önem arz etmediği belirlenmiştir.

Anahtar Kelimeler: Tohum pazarı, Tohum pazarlaması, Pazar payı, Satın alma davranışı, Üretici eğilimleri

1. Introduction

Wild form of maize originated approximately 7000 years ago in what is now Mexico and through selections, it has become a cultivated crop. Maize, regardless of its origin, spread across the world shortly after the discovery of the American continent, is a crop with high adaptability capacity, it has biotypes with adaptation capability ranging from tropical to temperate regions and with growth periods ranging from 6 weeks to 13 months (Brown et al., 1986). Maize is a warm-weather cereal grown in almost all parts of the world, between the 58th and 40th parallels of latitude, except Antarctica, from sea level up to an altitude of 4000 m, in areas with abundant sunlight. Its entry into our country was through North Africa. The fact that this plant is called maize in our country is a sign that it entered through Egypt and Syria (Anonymous, 2021). The most suitable temperature range for maize growth is between 25-30°C, the minimum temperature requirement is 15°C and the maximum average temperature requirement is 35°C (Emeklier, 2002).

World corn production is estimated to be 1.198 million tons in 2021/2022, making it the most widely produced grain. The main maize producing countries are the United States, China, Ukraine and Brazil, in that order (USDA, 2021). In our country, maize production in the 2019/2020 marketing year was 6.5 million tons. It is estimated that consumption is around 8 million tons. Despite the very high production, the supply deficit resulting from consumption is met with high quantities of imports, while biosecurity measures and trade facilitation have changed the countries from which imports are made (Taşdan, 2020). In Turkey, corn sown area increased from 5.9 million hectares in 2010 to 6.9 million hectares in 2020, while corn production increased from 4.4 million tons in 2010 to 6.5 million tons in 2020, an increase of 47% (TUIK, 2021). As can be seen, corn production and use are very wide both in the world and in our country. This reveals the need for a detailed examination of such matters as the production and marketing of corn seed, the needs of the main and secondary sectors and the development status.

Seeds are among the most important inputs of agricultural production and their use of quality seeds is important for increasing yield and production, as well as for obtaining more resilient, less costly and more competitive products. The seed sector in our country has made great progress in the last 25 years and both the increase in domestic seed production and the development of local seed production have been addressed by the private sector through significant efforts. The share of the private sector in seed production has increased over the years. The first scientific studies on seed quality control were initiated in 1869 by Friedrick Nobbe in a laboratory established in Tharand, Germany. In our country, the first scientific studies in the field of variety development and seed production were started in the late 1920s with the establishment of the Adapazarı, Adana, Eskişehir, Samsun Seed Improvement Stations and Ankara High School of Agriculture in 1933 (Anonymous, 2017).

Sakarya Province, which was selected as the research area, was the fourth province in Turkey with the highest corn production (328 thousand tons and 7.1% share) in the year the research was conducted and the first in the Marmara Region, while in 2020 it was in the eighth place in the country with 267.886,0 hectares and 267.280,0 tons production and first in the Marmara Region (TÜİK, 2021). It is known by researchers that producers are influenced by many factors when making their choice of corn seed. Among these factors are the resistance of the variety to diseases, pests, high temperatures and drought, as well as the low grain harvesting moisture in terms of pricing, the high capacity of yield, the grain yield as well as the silage and total yield which must be satisfactory to respond to a possible change of harvest time or purpose and the high quality of all kinds. In addition to these, many environmental factors such as dealer or expert advice, neighbor or friend choices and advice and producers' socio-economic status can be counted. Karadavut and Taşkın (2014) determined that speculative conversations on the product in the written and visual media directly affect the consumers. The aim of this study is to analyze what these factors are and how effective they are in seed selection by producers, which are more important and which are less important for producers and to bring the results to the literature by also revealing any regional differences.

2. Materials and Methods

2.1. Materials

The main material of the research is composed of the data collected from the corn producers in Sakarya province. These data have been obtained from the surveys conducted between the end of 2014 and the beginning of 2015 with the corn producers in Sakarya province. It has been understood that the corn production of 8 out of 16 districts of Sakarya province constitutes 91.92% of the provincial production and thus the other 8 districts have

been excluded from the scope of the study. In the agricultural economics researches, the populations under study are generally limited populations (Çiçek and Erkan, 1996). Therefore, the formula developed for limited populations, namely the simple random sampling method, has been used in the sampling phase of the research and 247 figure has been calculated, however, surveys have been conducted for more reliability and valid 261 samples have been included in the study.

2.2. Methods

In the sampling phase of the research, the corn production areas of the 8 districts in Sakarya province and the number of corn producers in the relevant districts have been taken as the basis from the 2012 Farmer Registration System (ÇKS) records of Sakarya Province Agriculture and Forestry Directorate.

$$n = N\partial 2t2 \div (N - 1)\, d2 + \partial 2t2 \quad \text{(Eq. 1).}$$

The formula is as follows;
N: Population Size - Number of Corn Producers in 8 Districts (7.672,0)
n: Sample Size (247)
∂: Standard Deviation (22.92)
x̄: Arithmetic Mean (28.10)
t: Table Value for Confidence Level (1.96)
d: Represents the acceptable error as a percentage of the mean (28.10 * 0.10 = 2.81).

The sample size was calculated with a 95% confidence limit and 10% error margin of the population average and a 5-point Likert scale was used in the survey questions comprising evaluations of "Not at all important", "Not important", "Neither important nor unimportant", "Important" and "Very important". Data obtained from the surveys were subjected to Factor Analysis that forms the purpose of this study. A general rule stating that the number of variables to be analyzed must be one fourth or fifth of the number of observations is among the considered criteria (Atalay Oral and Akpınar, 2015). Factor Analysis is a statistical technique that brings together variables that are related to one another over a large number of data, attempting to obtain a few unrelated variables. In Factor Analysis, correlations between the observed variables are taken into consideration as the numerous variables are sought to be explained by fewer factors. Factor Analysis aims to bring together related variables in a p-variable event and to find a few new (common) unrelated variables (Tatlıdil, 2002). The validity and reliability of the data were tested by Cronbach's Alpha analysis, the suitability for Factor Analysis and the adequacy of the sample by the Kaiser-Meyer-Olkin (KMO) test. A Bartlett's test of sphericity was also applied to determine the suitability of factor analysis. Yüzbaşı (2022), in his study, performed factor analysis to determine the reasons for individuals' "zara honey" preference and used Correlation Matrix, Bartlett Test and Kaiser-Meyer-Olkin (KMO) tests to investigate the suitability of the data for factor analysis. Logistic Regression analysis was conducted to determine to what extent the factors affecting the preferences affect the preferences (Karadavut and Taşkın, 2014). Also in this study, logistic regression analysis was applied to the obtained factor scores and effort was made to identify which factors were more or less important for which producers. Since correct modelling is important in logistic regression analysis, the suitability of the model was examined to see if the Nagelkerke and Hosmer & Lemeshow values were meaningful.

3. Results and Discussion

3.1. Determinations about producers

The average age of the farmers is 55.5 (*Table 1*). In the research area, 69 producers, or approximately 26% are over the age of 60 and 16 producers, or approximately 6% are over the age of 70. When evaluated according to the requirement of "being 18 or over at the time of publication of the Regulation and not having reached the age of 41", which is one of the conditions of the application to the project called "Young Farmer Project" implemented by the Ministry of Agriculture and Forestry (Anonymous, 2019); only 9.19% of the producers in the research area are in the Young Farmer condition. Mixed farms are farms that produce in multiple directions. In mixed farms, labor has the highest marginal value among the production factors (Cinemre and Ceyhan, 1998). However, it is not possible to speak of a marginal value from a producer population with an average age of 55.5 and with a young population that is constantly migrating.

According to the World Health Organisation's (WHO) revised age range list, which updates age ranges according to changing and developing technologies, 66-79 is considered middle age while 80-100 is considered elderly (Çataloğlu, 2018). WHO's newly determined age ranges in 2018 are 0-17 Teenager, 18-65 Young, 66-79 Middle Age, 80-99 Elderly. Accordingly, 86.64% of the surveyed businesses are aged 18-65, that is young. Nonetheless, 55.5 is a relatively high average age in terms of agricultural employment.

Table 1. Age groups of the farmers

Age group	Count	%
21 to 30	1	0.38
31 to 40	23	8.81
41 to 50	64	24.52
51 to 60	89	34.1
61 to 70	69	26.44
71 and upper	15	5.75
Total	261	100

The 5488 numbered Agriculture Law dated April 18th 2006 defines agricultural production as "production of plants, animals, aquaculture products, microorganisms and energy using land, water and biological resources along with agricultural inputs" (Anonymous, 2019a). Therefore, when the income generated from production in compliance with this definition is considered Agricultural Activity Income and the remaining income is evaluated as Non-Agricultural Activity Income, approximately 97.32% of the producers residing in the research region have 56.71% of Non-Agricultural Activity income. 80.46% of the producers have social security from Bağ-Kur, 14.54% have it from SSK and 3% have no social security.

According to the statistics, 20.3% of the businesses had a gross annual income below 25.000,0 Turkish Liras and 11.49% had a gross annual income above 60.001,0 Turkish Liras in 2015. 54.79% considered themselves to be of a middle-income status, 14.56% were of low-income and 27.59% were of good standing. A statistically significant relationship was also found between the status of the businesses with and without agricultural income in the society. Examining the *Table 2*, it was found that 83% of the producers were primary school graduates and 11% were secondary school graduates. There were no higher education graduates, mainly due to the fact that young population in the region had distanced themselves from farming for both economic and socio-cultural reasons and had chosen to reside in towns or cities and work in something other than farming.

Table 2. Distribution of educational level

Education	Count	%
Literate	5	1.92
Elementary	217	83.14
Mid collage	29	11.11
High collage	10	3.83
Total	261	100

3.2. Determinations about the production area where the research was applied

Sample size of this study is the total agricultural land possession of Sakarya according to 2016 TurkStat data, which is 1.692.549,0 decares. Of this, 732.648,0 decares is cultivated with Field Crops and 1.692,0 decares is left uncultivated. The total of cultivated and unsown land is 734.340,0 decares, which is 43.4% of total agricultural land. Vegetable land is 80.637,0 decares, which is 4.8% of total. Fruit land is 866.882,0 decares, which is 51.2% of total. Ornamental land is 10.690,0 decares, which is 0.6% of total (Dellal et al., 2018). The agricultural land possession of 261 businesses in 8 districts, which have been identified as survey area, is 11903 decares in total. However, 261 businesses, which were surveyed in 2014, had produced plant products in 12.702,0 decares in 2013, including first and second products.

In this production pattern, corn holds a share of approximately 67% with 8.467,0, wheat 1.367,0 with 11%, barley 455 with 3.6%, sunflower 386 with 3% and other products 2.027,0 with about 16%; the other group is dominated by hazelnut and vetch production.

3.3. Factor analysis applied to the obtained data

Factor analysis is one of the widely used multivariate statistical techniques which reduces a large number of variables that are interrelated into a smaller set of more meaningful, easily interpretable and independent factors (Cengiz and Kılınç, 2007). Before starting factor analysis, reliability analysis is used to determine whether the data is internally consistent. Cronbach's alpha coefficient is a weighted standardized mean calculated by taking the ratio of the sum of the variances of the k items in the scale to the total variance and takes values between 0 and 1. The range of possible values of alpha coefficient and the corresponding reliability of the scale are $0.00 \leq \alpha < 0.40$ indicates that the scale is unreliable, $0.40 \leq \alpha < 0.60$ indicates that the scale is of low reliability, $0.60 \leq \alpha < 0.80$ indicates that the scale is quite reliable, $0.80 \leq \alpha < 1.00$ indicates that the scale is highly reliable (Azabağaoğlu et al., 2015). Cronbach's alpha coefficient, used to explain or question the homogeneous structure of the items in the scale, is a measure of the internal consistency of the items. Cronbach's alpha is expressed as follows; if $0 < R2 < 0.40$, it is not reliable; if $0.40 < R2 < 0.60$, it is of low reliability; if $0.60 < R2 < 0.80$, it is fairly reliable; and if $0.80 < R2 < 1.00$, it is highly reliable (Alpar, 2013).

In the factor analysis presented in *Table 3*, a high correlation is sought between the variables and the confidence in the results of the factor analysis decreases as the correlation decreases. The standard Cronbach's alpha statistic, which shows the reliability of the variables, was calculated as 0.871. This value indicates that the questionnaire items using the Likert type scale have high reliability and that the variables consist of items that are consistent with each other and measure the same characteristics.

Table 3. Reliability test

Cronbach's Alpha	Standard Cronbach's Aplha	Variables
0.841	0.871	88

At first, the suitability of the data for factor analysis and the adequacy of the sample were analyzed using the Kaiser-Meyer-Olkin (KMO) test. This test is used to measure the adequacy of the sample and concerns the size of the sample. To this end, it compares the magnitude of the observed correlation coefficients with the magnitude of the partial correlation coefficients (Nakip, 2003). In general, a KMO value greater than 0.50 indicates that the variables are suitable for factor analysis and the number is sufficient. The Kaiser-Meyer-Olkin (KMO) Test is computed by comparing the calculated simple correlation coefficients with the partial correlation coefficients. The value of the test varies between 0 and 1 (Norusis, 1994). KMO values higher than 0.90 indicate that the sample is sufficient, KMO values between 0.89 and 0.80 indicate that the sample is valuable and KMO values between 0.79 and 0.60 indicate that the sample is of moderate value. KMO values below 0.6 indicate that the sample is not sufficient (Sharma, 1996).

Another method for determining the suitability of factor analysis is to examine the entire correlation matrix. The Bartlett's Test of Sphericity is a statistical test that assesses the presence of correlations amongst the variables. It provides the statistical significance of the correlation matrix, indicating that at least some of the variables have a significant correlation. However, the researcher should note that increasing the sample size has resulted in Bartlett Test becoming more sensitive in detecting the relationships between the variables. The researcher can then assess the factorability of the general variables and individual variables by using the overall significance of the correlation matrix and the measure of adequacy of the sample with Bartlett Test. The purpose of factor analysis is always to obtain factors, hence the aim is to provide a basal statistical correlation level within the set of variables for a fundamental basis (Hair et al., 1998).

Table 4 shows that the Kaiser-Meyer-Olkin (KMO) value for the group of factors of producers' preferences for variety attributes of environmental and plant characteristics was 0.711, with a significance value of 0.000. In other words, the Kaiser-Meyer-Olkin (KMO) test was 71.1%. It can be said that the data set is suitable for factor analysis, given that the value> 0.50 and the sampling is adequate and of medium value. Bartlett test also shows significance when the significance value is examined. From this, it can be seen that there are high correlations among the variables and that the data set is suitable for factor analysis.

Table 4. KMO and Barlett's test (The level of importance of environmental and plant characteristics in variety preference)

Kaiser-Meyer-Olkin	Measure of Sampling Adequacy	0.711
	Approx. Chi-Square	1737.91
Bartlett's Test of Sphericity	Df	210
	Sig.	0.000

Table 5. Factor analysis of importance level of environmental and plant characteristics in variety preference

Factors and Variables	Factor loadings	Deviation (%)	Eigen value
Factor 1 (Vegetative Characteristics in Variety Selection)		21.735	4.564
High Temperature Tolerance	0.809		
Drought Tolerance	0.780		
Low Harvest Moisture Rate in Grain	0.575		
The Ability to Give the Same Yield in Every Soil	0.500		
Factor 2 (Purchasing Behaviors in Variety Preference)		11.588	2.433
Variety Seeking Purpose (Grain, silage, etc.)	0.725		
Opportunity to Deferred Payment	0.720		
Tried Before The Local Farmers	0.571		
Cheaper	0.540		
Convenient supply	0.497		
Factor 3 (Environmental Factors in Variety Preference)		8.260	1.735
Suitability of the Variety for Second Crop Cultivation	0.772		
Effective Advertising Campaign for Variety	0.754		
Factor 4 (Recommendations for Variety Preference)		7.053	1.481
Neighbor & Seed Dealer Recommendations	0.848		
Agriculture Province-District Professionals and Agricultural Consultant Recommendations	0.797		
Factor 5 (Usage Expectations in Variety Preference)		6.251	1.313
It it's for Fresh Consumption, Sugar Content Should be High	0.864		
Suitable for Frequent Sowing	0.644		
It it's for Silage, Animals like to Consume	0.511		
Factor 6 (Expectations of consumption in variety selection)		5.557	1.167
Be Resistant to Lying Down	0.780		
It it's for Silage, Lower Leaves couldn't Burned or Dried	0.736		
Early Vegetative Variety	0.424		
Factor 7 (Hereditary Features in Variety Preference)		5.078	1.066
High Yielding of the Variety	0.764		
High Quality Values (Protein, Starch etc.)	0.459		

The eigenvalue is a coefficient taken into account both in calculating the variance explained by factors and in determining the number of significant factors. According to this criterion, initially, factors with an eigenvalue of 1 or greater are assumed to be significant and those with an eigenvalue less than 1 are considered to be insignificant. Thus, an eigenvalue of 0.999 would lead to the factor being considered insignificant, while an eigenvalue of 1 would require the factor to be considered significant. This, however, can be seen as a drawback for Variance Contribution Criterion when sensitivity is taken into account. As can be seen in all the tables, the eigenvalues of the factors are greater than 1, thus being significant. Another criterion that can be used to determine the number of factors is Total Variance Percentage Criterion, where when the contribution of each additional factor to the total variance is less than 5%, the maximum number of factors has been reached (Cengiz and Kılınç, 2007).

Interpretation and naming of factors are done taking into account the factor loads. According to this, if the correlation of the variable with the common factor is greater than ± 0.30, it is accepted that the variable has a

meaningful relationship with the said common factor. Therefore, it can be said that the variables in the tables have a meaningful relationship with the positive factor weights of the common factors since their factor loads are greater than ± 0.30. Some researchers claim that this rate should be ± 0.40. From this point of view, again the variables in the tables have a meaningful relationship with the positive factor weights of the common factors since their factor loads are greater than ± 0.40.

In this study, the number of factors to be derived from the features grouped under headings is determined by using Variance Contribution (Eigenvalue) Criterion. In this case, factor analysis was performed on the data set grouped under the heading of the importance levels of environmental and plant characteristics in the variety preference and a total of 7 factors containing 21 recommendations were formed according to the results of the said analysis, consisting of the components seen in *Table 5*. According to the variance contribution criterion, the eigenvalue statistic greater than 1 indicates the total number of factors and there are 7 factors with eigenvalues greater than 1, which explain 65.522% of the total variance.

As seen in *Table 6*, the Kaiser-Meyer-Olkin (KMO) value for the "Level of Importance of Expectations from Products and Companies" group of factors of the producers was 0.792 (79.2%) and the significance value was 0.000. Since the value is> 0.50, the data set is suitable for factor analysis and it is seen to be significant when the Bartlett test significance value is examined. There is a high correlation between the variables.

Table 6. KMO and Barlett's test (The importance level of the expectations of farmers from product and companies)

Kaiser-Meyer-Olkin	Measure of Sampling Adequacy	0.792
	Approx. Chi-Square	4112.533
Bartlett's Test of Sphericity	df	153
	Sig.	0.000

The factor analysis of the data set collected and analyzed in the section of "Level of Importance of Producers' Expectations from Products and Companies" yields a total of eighteen recommendations made up of six factors presented in *Table 7* according to the results of the analysis. According to the variance participation criterion, the principal value statistic indicating the total number of factors greater than one, there are six factors with principal values greater than one and these factors explain 75.647% of the total variance.

The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy for the "Importance Level of Technical Characteristics and Technical Information Content of Maize Seed Packaging" group of factors was 0.816 (81.6%), with a significance level of 0.000. This value also indicates that the data set is suitable for factor analysis; when the Bartlett's test of significance was examined, it was also found to be significant. There is also a high correlation between the variables (*Table 8*).

Table 9 has been analyzed in the section "Level of Importance of Seed Packaging Technical Specifications and Technical Information Content" and the factor analysis of the data set, collected in this section, has been conducted. According to the criterion of participation in variance, there are 5 factors with eigenvalues greater than 1 and these factors explain 73.215% of the total variance.

3.4. Applying logistic regression analysis to the factor scores obtained

In this phase of the study, logistic regression analysis was applied to the factor scores obtained and the dependent variable was taken as the districts. Independent variables can be age, gender and education, etc. The logistic model can be derived from a theoretical basis using index functions or random utility models (Anderson et al., 2005). As previously mentioned, it was understood that the maize production of 8 districts out of 16 districts in Sakarya province constituted 91.92% of the total provincial production, so the other 8 districts were excluded from the scope of the research. The variable "gender" was added as a dummy variable in the analysis and each district tested was accepted as 1 and the others as 0 in the test. Correct modelling is important in logistic regression analysis. Nagelkerke and Hosmer&Lemeshow values are an important analysis for the appropriateness of the model. The Nagelkerke R square value used to explain the model was found to be significant for Söğütlü and Ferizli districts *(Table 10)*.

Table 7. Factor analysis of the importance level of the expectations of farmers from product and companies

Factors and Variables	Factor loadings	Deviation (%)	Eigen value
Factor 1 (Expectations from the Product-Seed)		29.929	5.387
Grains are not Undersized and Broken	0.974		
Not Different Sizes of Grains (Large and Small)	0.971		
Seed Cleanliness (Foreign Matter)	0.970		
The Production Date of the Seed Should be New	0.955		
Medication of Seed for Subterranean Pests	0.947		
Seed Has All Quality Certificates	0.677		
Factor 2 (Expectations from Seed Company Officers)		15.532	2.796
Company Guarantees Seed	0.832		
Company Establishes Demonstrations in the Local Areas	0.715		
Companies Support Applications After Sales	0.634		
Companies Performing Field Controls After Sowing	0.601		
Factor 3 (Promotional Expectations from the Company)		9.848	1.773
Promotional Products (Hat, Bag, Pen, etc.)	0.888		
The company's Fair, Meeting, etc. Arrangement and Taking Away to Farmers	0.886		
Factor 4 (Product Origin Expectation)		7.082	1.275
Imported Seed	0.846		
Domestically Produced Seed	0.710		
Factor 5 (Suggestion-Supply Expectations from the Company)		6.985	1.257
Company's Proposal for Varieties According to Soil and Climate	0.864		
Company Delivering the Seed at the Appropriate Time	0.856		
Factor 6 (Training-Promotion Expectations from the Company)		6.271	1.129
Visual Instructions of the Companies in Village Cafe (Video, slide, etc.)	0.760		
Company's Village Cafe Meetings	0.679		

Table 8. KMO and Barlett's test (The importance level of the content of the technical characteristics and information of the corn seed packaging)

Kaiser-Meyer-Olkin	Measure of Sampling Adequacy	0.816
Bartlett's Test of Sphericity	Approx. Chi-Square	4241.141
	df	190
	Sig.	0.000

Analysis of the data obtained from Söğütlü district revealed that the scores of the 3rd, 4th, 5th and 6th factor groups were found to be significant. The 3rd factor group, "Environmental factors in variety preference" was found to be 2.65 times (1/0.377) less important than the scores from other districts, likewise the 4th factor group, "Recommendations for variety choice" was calculated to be 2.05 times (1/0.488) less important. Factor group 5, " Usage Expectations in Variety Preference" was found to be 2.11 times and factor group 6, "Vegetative features in variety preference" 10.5 times more important than the other districts *(Table 12)*.

Table 9. Factor analysis of importance level of the content of the technical characteristics and information of the corn seed packaging

Factors and Variables	Factor loadings	Deviation (%)	Eigen value
Factor 1 (Expectations from Packaging)		28.056	5.611
Place and Date of Production Should Be Written on Seed Packaging	0.861		
Company Name and Variety Name Should Be Written	0.823		
Seed Packaging Must Be Resistant to Impact and Stacking	0.802		
The packaging must be able to maintain the germination strength of the seed for a long time	0.798		
Packaging, Water, Air and Light Proof	0.784		
Storage Conditions and Minimum Durability Period Should Be Written on the Package	0.770		
Factor 2 (Packaging Information Content Expectations)		23.845	4.769
Foreign Substance Content Information Should Be Written on the Packaging	0.850		
Yield Capacity Under Optimum Conditions Should Be Written	0.846		
Germination Power and Rate Should Write on Package	0.840		
Information on Planting Techniques Should Write on Package	0.773		
Soil and Fertilizer Requirements of the Variety Should Write on Package	0.744		
Drought Resiliency Status Information Should Write on Package	0.712		
The Frequency of Water Demand of the Variety Should Be Written	0.703		
Factor 3 (Packaging Design Expectations)		10.407	2.081
The Packaging Must Be Of Weight That Is Held By Hand Easily Transportable	0.860		
The Packaging Must Be Able to Be Opened and Closed Again in a Practical Way	0.791		
Factor 4 (Packaging Environmental Expectations)		5.492	1.098
The raw material of the packaging must be suitable for recycling	0.836		
The design quality of the packaging must be high	0.788		
Raw Material Must Be Environmentally Friendly	0.748		
Factor 5 (Packaging Quality Expectations)		5.415	1.083
Packaging Must Have All Required Quality Certificates	0.775		
The Writing on It Should Be Understandable Shape and Size	0.759		

Table 10. Model significance

	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
Söğütlü District	85.563[a]	0.310	0.615
Ferizli District	29.979[a]	0.322	0.813

In the Hosmer-Lemeshow test, it is desired that the significance value is greater than .05. The result was high in both cases *(Table11)*.

Test results from Ferizli district showed that the 1st, 2nd, 3rd, 5th, and 6th factor groups were significant. The first factor group, "Vegetative characteristics in variety selection" was calculated to be 16.2 times more important than scores obtained from other districts. Similarly, the sixth factor group, "Expectations of consumption in variety selection," was calculated to be 5.14 times and the third factor group, "Environmental Factors in Variety Preference" was calculated to be 18.8 times more important. The fifth factor group, "Usage Expectations in Variety Preference" was determined to be 32.25 times (1/0.031) less important and the second factor group, "Purchase behavior in variety preference" was 8.54 times (1/0.117) less important *(Table 13)*.

Table 11. Hosmer and Lemeshow test

	Chi-square	Df	Sig.
Söğütlü District	5.216	8	0.734
Ferizli District	0.116	8	0.998

Table 12. Model results for Söğütlü district

Söğütlü District	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I.for EXP (B)	
							Lower	Upper
Factor 1 (Vegetative characteristics in variety selection)	0.091	0.321	0.081	1	0.776	1.096	0.584	2.057
Factor 2 (Purchasing Behaviors in Variety Preference)	-0.332	0.321	1.065	1	0.302	0.718	0.382	1.347
Factor 3 (Environmental Factors in Variety Preference)	-0.975	0.349	7.794	1	0.005***	0.377	0.190	0.748
Factor 4 (Recommendations for Variety Preference)	-0.718	0.308	5.431	1	0.020**	0.488	0.267	0.892
Factor 5 (Usage Expectations in Variety Preference)	0.748	0.313	5.696	1	0.017**	2.112	1.143	3.902
Factor 6 (Expectations of consumption in variety selection)	2.353	0.457	26.456	1	0.000***	10.513	4.289	25.768
Factor 7 (Hereditary Features in Variety Preference)	0.257	0.329	0.611	1	0.435	1.293	0.678	2.466
Cinsiyet/Gender	-22.238	40192.933	0.000	1	1.000	0.000	0.000	.
Sabit/Constant	18.198	40192.933	0.000	1	1.000	79999103.267		

%95, *%99 significance level

Table 13. Model results for Ferizli district

Ferizli District	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I.for EXP (B)	
							Lower	Upper
Factor 1 (Vegetative characteristics in variety selection)	2.788	1.241	5.043	1	0.025**	16.240	1.426	185.015
Factor 2 (Purchasing Behaviors in Variety Preference)	-2.147	1.112	3.728	1	0.053*	0.117	0.013	1.033
Factor 3 (Environmental Factors in Variety Preference)	2.934	1.530	3.676	1	0.055*	18.797	0.937	377.125
Factor 4 (Recommendations for Variety Preference)	-1.112	1.055	1.111	1	0.292	0.329	0.042	2.602
Factor 5 (Usage Expectations in Variety Preference)	-3.469	1.100	9.941	1	0.002***	0.031	0.004	0.269
Factor 6 (Expectations of consumption in variety selection)	1.637	0.747	4.806	1	0.028**	5.140	1.189	22.210
Factor 7 (Hereditary Features in Variety Preference)	0.660	0.630	1.097	1	0.295	1.935	0.563	6.652
Gender	15.267	40192.962	0.000	1	1.000	4268926.264	0.000	.
Constant	-25.239	40192.962	0.000	1	0.999	0.000		

*%90, **%95, ***%99 significance level

According to the factor analysis results of the importance levels of environmental and vegetative characteristics on the variety choice of producers, it can be said that this variability between these factor groups is caused by the socio-economic structure differences which are widely felt in the region. With a similar study (Gedikli et al., 2015), factor analysis was used to determine the producers' problems related to corn and regression analysis was used to identify the factors effective on the amount of drug use and 27 factors expressing the problems encountered by

farmers in corn production were reduced to 7 main factors with factor analysis. The factor scores obtained by factor analysis were interpreted by regression analysis taking into account demographic and social variables.

In his (Kutlu, 2017) study, factor analysis and multiple linear regression analysis were used to identify the factors affecting the sustainability of local seeds. The researcher stated that 49 local varieties were identified in the research field, logistic regression analysis was used to analyze the factors affecting the sustainability of production with local seeds from the producers' point of view and the factors affecting the sustainability of local seeds were found to be awareness, marketing and pricing, gene resource and organic farming at the level of 1%, and from the consumers' point of view, consumer awareness, price, promotion and information were found to be important at the level of 1%. In their (İkikat Tümer et al., 2018) research, probit regression analysis was used to analyze the factors affecting producer satisfaction and it was found that the age of the producer, experience and producer income had a positive effect on satisfaction and that the number of family members, broiler breed, distance to the supplier company and mortality rate had a negative effect on producer satisfaction.

4. Conclusions

Producers' preferences for corn seed varieties and the extent to which these preferences are affected by various factors are highly important in terms of breeders' variety development stages and firms' marketing and post-sales strategies. It is known that the seed companies in Turkey have a very high proportion of domestic capital, which is about 90%. The markets in which these predominantly local companies operate are generally regional markets. The sector, which is mostly early life stage, also has a large number of employees with inexperience, with it being reported that about 60% of them have worked at the same firm for below 5 years. Therefore, it is inevitable to support the seed sector, which can be termed local and national, in order to be able to compete with international companies. These seed producers and seed marketers should identify other marketing channels other than their own institutions and use these channels effectively. In addition, it is of great importance that the existing public agricultural R&D resources are put into the service of the domestic and national seed sector in the form of cooperation in accordance with the legislation. Although it seems like a radical decision, it has been determined that the producers are constantly in search of new and better seeds. This situation is a great advantage for companies and creates a suitable environment for new varieties to enter the market and spread. There are some plant-based negativities related to corn varieties that negatively affect the purchase. Corn breeders and indirectly marketers should find solutions to these problems.

The average age of producers in the research area was found to be 55.5 and approximately 85% of them were found to be within the age range of 41-70. Consequently, it can be understood that the young population is distancing itself from agriculture and continuing to do so. Even though the majority of the producers are perceived to be young according to the World Health Organization age criteria, they are quite old in terms of agricultural employment. It should be facilitated for them to closely follow and access technological developments and solution programs should be implemented to ensure that the educated young human resources needed by the sector remain in this sector, policies should be implemented that make the agricultural sector attractive to this educated young human resource.

A statistically significant difference has been observed between the annual gross incomes of high school graduates and literate producers. The annual gross incomes of high school graduates are statistically significantly higher than those of literate producers. Despite the high level of education of the population in the research area, there are no university graduates among the surveyed Corn producers, the main reason for this being the economic and social reasons for the young educated population to move away from farming and the countryside and to prefer to work in a salaried job in the cities. At this point, the National Young Farmer Project implemented and concluded by the state and the subsequent Expert Hands project implemented are of great importance in keeping the educated young population in agriculture or returning them to agriculture and the countryside.

When considering the factors influencing farmers' preferences for maize seed, it appears that practices such as distributing promotions that are easy to implement and frequently used by companies, organizing fairs and trips, using attractive packaging, etc. do not have the same importance as guaranteeing the seed sold by the company, taking care of farmers after the sale, setting up demonstrations in the target area and making field checks after sowing, etc.. Therefore, it may be beneficial for companies to take this into account when determining their

marketing and post-sales strategies and tactics and combining as many of these practices as possible may lead to more satisfactory results.

In the choice of variety among producers, some plant characteristics such as plant height, stem thickness, weight, cob size and number, as well as advice from acquaintances, village headman, leading farmers, dealers and expectations in terms of consumption such as harvesting. As grain to sell, as silage to use as animal feed or to sell and vegetative characteristics suitable for either consumption or selling purposes, as well as some plant hereditary characteristics are found to cause differences in purchase behavior even if production is done in the same province. And these subjects have a determining effect in decision making concerning seed selection. For example, in Ferizli district, "plant characteristics" such as tolerance to high temperatures which may cause the lower leaves to burn, and tolerance to drought, as well as the price difference due to lower harvest moisture resulting from grain sowing, are 16.2 times more important than for other district producers. And "vegetative characteristics" such as lodging resistance, prevention of lower leaves from burning and drying early for silage varieties and early variety are 5.14 times more important. The reason for this is that in Ferizli district, producers not only produce maize but also have a significant large-scale livestock production and in addition to maize production, they have to produce silage and yield maize.

In addition to the producers in Söğütlü district being suitable for frequent planting, the "Consumption Expectations" with factors such as silage that the animals like to eat is 2.11 times, similarly with lodging resistance, silage varieties of lower leaves not burning early and early variety have 10.5 times more importance compared to other district producers which is due to their production of professional large-scale livestock.

This is because lodging, lower leaves burning, high temperature and drought can cause negative outcomes such as crumbling in the corn stem before the harvest date and the animals not being fond of consuming the silage or the harvest, or even not consuming it at all. Therefore, it is important for the private or public employed breeders and seed marketing companies conducting breeding work to consider these matters. As a result of the study, it is seen that many matters are important for the corn producers of the same province in different regions. Consequently, these matters can provide guidance to breeders and seed marketing companies in the breeding and marketing efforts they will carry out in the future.

In light of the ever-changing global and regional economic and associated sociological conditions, national and regional research on factors that influence producers' preferences should be conducted at certain intervals in order to assist breeders and marketing firms in setting their short and long-term goals.

Ethical Statement

Since it is an article produced from a master's degree study that used research data before 2020, there is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Authors contribution rates to the study are equal.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Important Agricultural Traits of Some Soybean (*Glycine max* (L.) Merr.) Genotypes and Adaptation in the Eastern Mediterranean Transition Zone

Doğu Akdeniz Geçit Kuşağında Bazı Soya (*Glycine max.* (L.) Merr.) Genotiplerinin Önemli Tarımsal Özellikleri ve Adaptasyonunun Belirlenmesi

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Abstract

This research was carried out to determine the yield and agronomic characteristics of some soybean genotypes under main crop conditions in the Eastern Mediterranean Transition Zone in the years 2021-2022. In the research, 13 varieties (Adel, AP-3773, Arısoy, Asya, Atakişi, Atlas-3616, J-112, Lider, Planet, Racer, Sonya, Winchester, Yemsoy) 1 genotype (Yeniköy-9) were used. The experiment was conducted in a randomized block design with three replications. It was determined that the average plant height varied between 64.05 cm (J-112) and 114.25 cm (Winchester). The lowest average first pods height (1.74 cm) was obtained from J-112 variety while the highest value (13.69 cm) was obtained from Yemsoy variety. The lowest number of branches per plant values i.e. 1.83 no. plant^{-1} and 2.13 no. plant^{-1} were recorded from J-112 and Planet variety respectively. In the experiment, the number of pods per plant varied between 72.20 pods plant^{-1} (Arısoy) to 105.61 pods plant^{-1} (AP-3773). However, the average weight of 100 seeds varied between 15.07 g (Asya) to 22.10 g (Lider). The lowest average protein content (33.58%) was obtained from Yemsoy variety while the highest value (41.50%) was obtained from J-112 variety. It was determined that the average seed yield varied between 379.74 kg da^{-1} (Arısoy) and 580.73 kg da^{-1} (Lider). According to the data obtained as a result of the experiment, Lider variety was come front with the highest seed yield (580.73 kg da^{-1}) and, J-112 variety had the high protein content with 41.50%, Planet (21.96 g) and Yeniköy-9 (20.87 g) varieties had the highest 100 seed weight. According to production goals, one of this superior varieties may be prefer to grow in the Eastern Mediterranean Transition Zone.

Keywords: Soybean (*Glycine max.* L.), Seed yield, Protein content, Agricultural traits, Adaptation

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Öz

Bu araştırma, 2021-2022 yıllarında Doğu Akdeniz Geçit Kuşağında bazı soya çeşitlerinin ana ürün koşullarında verim ve agronomik özelliklerinin belirlenmesi amacıyla yapılmıştır. Araştırmada 13 çeşit (Adel, AP-3773, Arısoy, Asya, Atakişi, Atlas-3616, J-112, Lider, Planet, Racer, Sonya, Winchester, Yemsoy) ve 1 genotip (Yeniköy-9) kullanılmıştır. Deneme tesadüf blokları deneme desenine göre 3 tekerrürlü olacak şekilde dizayn edilmiştir. Denemde ortalama bitki boyu 64.05 cm (J-112) ile 114.25 cm (Winchester) arasında değiştiği belirlenmiştir. En düşük ortalama ilk bakla yüksekliği 1.74 cm ile J-112 çeşidinden elde edilirken, en yüksek ortalama ilk bakla yüksekliği ise 13.69 cm ile Yemsoy çeşidinden elde edilmiştir. Bitki başına en düşük ortalama dal sayısı J-112 (1.83 adet/bitki) çeşidinden elde edilirken en fazla ise bitki başına ortalama dal sayısı Planet (2.13 adet/bitki) çeşidinden elde edilmiştir. Denemede ortalama bitki başına bakla sayısı 72.20 adet/bitki (Arısoy) ile 105.61 adet/bitki (AP-3773) arasında değiştiği belirlenmiştir. 100 tohum ağırlığı ise 15.07 g (Asya) ile 22.10 g (Lider) arasında değiştiği bulunmuştur. Ortalama protein oranı bakımından ise en düşük değeri %33.58 ile Yemsoy çeşidinden elde edilirken, en yüksek ortalama protein oranını %41.50 ile J-112 çeşidinden elde edilmiştir. Ortalama soya tohum verimi 379.74 kg da^{-1} (Arısoy) ile 580.73 kg da^{-1} (Lider) arasında değiştiği saptanmıştır. Deneme sonucunda elde edilen verilere göre en yüksek ortalama soya tohum verimine sahip olan Lider (580.73 kg da^{-1}) çeşidi, en yüksek ortalama protein oranı bakımından J-112 (%41.50) çeşidi ve en yüksek ortalama 100 tohum özellikleri bakımından Planet (21.96 g) çeşidi ve Yeniköy-9 (20.87 g) genotipinin öne çıktığı saptanmıştır. Üretim amaçlarına göre bu üstün çeşitlerden biri Doğu Akdeniz Geçiş Kuşağında yetiştirilmek üzere tercih edilebileceği saptanmıştır.

Anahtar Kelimeler: Soya (*Glycine max.* L.), Tohum verimi, Protein içeriği, Tarımsal özellikler, Adaptasyon

1. Introduction

Soybean (*Glycine max.* L.), a member of legume family, is one of the most valuable oil seed crop produced in the world as a source of protein for both human and animal as well as raw material for biodiesel production (Arıoğlu, 2014; Basal and Szabó, 2020; Deines et al., 2022; Şahin and İşler, 2022).

Soybean, known as yellow gold in Asia, is a very valuable industrial plant because of its high minerals and vitamin contents and is used in the production of more than 400 industrial products (Yıldırım, 2017). Furthermore, soybean contains an average of 36-45% high protein content, as well as 18-24% oil, 26% carbohydrates, and 8% mineral substances (Arslan and Arıoğlu, 2003; Arıoğlu, 2014; İlker et al., 2018; Şahin and İşler, 2021).

The remaining pulp of soybean seeds after oil extraction in the oil industry is very rich in crude protein (Arıoğlu, 2014). The digestion rate of amino acids in soybean meal is 97% and therefore it has a great value in animal nutrition (Arıoğlu, 2014; Carciochi et al., 2019). Soybean meal added to the ration mixtures of poultry increases the egg yield by 40% as well as meat yield by approximately 52% (Arıoğlu, 2014).

Although the country of origin of the soybean plant is Far East countries, soybean is mostly grown in USA and Latin American countries. According to the world production data of 2020, 34% of world soybean production is provided by Brazil, 31% by USA and 13% by Argentina. The statistics of 2020 show that 353 million tons of soybeans were produced in 126 million hectares of land in the world. Brazil is the leading soybean producing country with 121 million tons of production. After Brazil, the United States of America with 112 million tons and Argentina with a production of 48 million tons are the main soybean producer and exporter countries (Anonymous, 2023a). According to the statistics of 2020, the world average soybean yield is 278 kg da^{-1} whereas the average yield of soybean in Türkiye is 441 kg da^{-1}. 93% of Türkiye's soybean production is obtained from the Mediterranean Region and 3% from the Black Sea Region (Anonymous, 2023b).

This research was carried out to determine the agronomic and quality characteristics of some soybean genotypes as the main crop in the Eastern Mediterranean Transition Zone. This study was carried out to identify desirable genotypes in the Eastern Mediterranean Transition Zone of Türkiye.

2. Materials and Methods

2.1. Materials

In the research, 13 varieties (Adel, AP-3773, Arısoy, Asya, Atakişi, Atlas-3616, J-112, Lider, Planet, Racer, Sonya, Winchester, Yemsoy) and, 1 genotype (Yeniköy-9) were used. The Research was conducted at the Oil Seed Research Institute Application and Research location (37º07'38.87"N; 36º11'59.16"E, 65 m). The experimental area has clay texture and consists of soils with alkali (8.19) reaction and low organic matter ratio (1.67%). Some climate data for the cultivation period of 2021 and 2022 for Osmaniye province are given in *Table 1*.

Table 1. Climate parameters of the research field as of 2021, 2022 and long-year average

Months	Precipitation (mm)			Temperature (°C)			Relative humidity (%)		
	LY	2021	2022	LY	2021	2022	LY	2021	2022
May	39.1	6.4	89.8	25.2	25.1	25.4	62.2	66.5	72.6
June	18.3	0.8	0.0	28.0	29.1	28.2	65.5	65.1	66.1
July	10.7	11	0.0	28.6	29.4	28.9	64.3	62.8	69.6
August	33.6	9.6	72.4	25.7	25.7	26.1	59.8	60.7	65.6
September	68.7	9.2	12.4	21.1	21.5	21.9	58.4	49.1	62.2
October	91.5	24.2	33	14.6	16.5	16.5	61.7	60.4	66.1
Total/Av.	261.9	61.2	207.6	23.9	24.6	24.5	61.9	60.8	67.0

Av.: Average; LY: Long Year.

2.2. Methods

The field experiment was designed in a randomized block design with 3 replications and 4 rows in each plot. The plots were sown in rows with 70 cm row spacing, 5 cm plant to plant distance and 5 m long plots. Each plot area consists of 14 cm^2. The first year sowing of the experiment was done on May 30, 2021, and the second year was done

by hand on May 31, 2022. The experiment was harvested by hand on October 30, 2021, and in the second year on October 31, 2022. The experimental land was processed with a cultivator and the seed bed was made ready for planting. Before planting, 20 kg of Diammonium Phosphate (DAP 18-46-0) fertilizer was applied per decare and then 20 kg da^{-1} 33% ammonium sulfate fertilizer was applied before the first water. Weeds were removed from the plots by hoeing twice, by hoeing machine between the rows and manually over the rows. After the experimental land was prepared, irrigation, hoeing, disease and pest control operations were carried out in a suitable manner.

Plant height, first pods height, number of branches per plant, number of pods per plant, 100 seed weight and protein content were calculated based on 20 plants from the middle two rows of soybeans that reached harvest maturity. On the other hand, seed yield was calculated by manually harvesting the remaining two rows in the middle after the rows on the sides of each plot consisting of four rows were discarded as the edge effect. The yield per decare was found with the seeds obtained from the harvest.

2.3. Statistical Analysis

The data obtained in the study was subjected to analysis of variance (ANOVA) according to the randomized block experimental design, using the JMP statistical package program, and the parameters that were found to be statistically significant in the analysis were subjected to the LSD multiple comparison test.

3. Results and Discussion

The differences between soybean genotypes in terms of plant height according to the two-year average values were found to be statistically significant ($P < 0.05$) (*Table 2*). The plant height value for 2021 varies between 51.77 cm and 113.87 cm. In 2022, it was found to be between 53.29 cm and 114.63 cm. The highest plant height was obtained from Winchester (114.25 cm) varieties, while the lowest plant height value was obtained from J-112 (64.05 cm) variety in the mean of two years (*Table 3*). The plant height was recorded in this study was similar values obtained by Sevilmiş and Arıoğlu (2019), Çubukcu et al. (2020), Kulan et al. (2017), while it was found to be lower according to by Onat (2018), Bakal et al. (2016). The difference in plant height of varieties might be sourced of genetic, environmental differences.

Table 2. Results of the analysis of variance for characteristics studied in the present experiment

SV	df	PH	FPH	BP	PN	100-SW	SY	PC
Block	2	ns	ns	ns	ns	ns	ns	ns
Year	1	ns	ns	**	ns	ns	**	ns
Cultivars	13	**	**	**	**	**	**	**
Y × V	13	ns	ns	**	ns	ns	**	ns

SV: source of variation, df: degree of freedom, PH: Plant height, FPH: First pods height, BP: Branches per plant, PN: Pods number, 100-SW: 100-seed weight, SY: Seed yield, PC: Protein content, **: $P < 0.05$

According to the results of analysis of variance, the first pods height difference between genotypes was found to be statistically significant ($P < 0.05$) (*Table 2*). The average first pods height varied between 1.70 cm and 13.85 cm in 2021, whereas it was found to vary between 1.77 cm and 13.53 cm in the year of 2022. The maximum first pods height was obtained from Yemsoy (13.69 cm) and Planet (12.62 cm) genotypes, while the minimum first pods height was obtained from J-112 (1.74 cm) cultivar (*Table 3*). In soybean breeding programmes, varieties with high first pods height important in order to prevent the harvest losses during mechanical harvesting (Arıoğlu, 2014; Bakal et al., 2016). The first pods height in this study was found to be similar to Sevilmiş and Arıoğlu (2019), Çubukcu et al. (2020) but it was found to be lower than the first pods height reported by Kulan et al. (2017) and Aşık and Yıldız (2018). The difference in the height of the first pods may be due to the difference in genetical and, ecological conditions (Kınacı, 2011).

According to the analysis of variance, differences in the number of branches per plant among soybean genotypes were found to be statistically significant ($P < 0.05$) (*Table 2*). According to the average of the two years; highest number of branches per plant value was 4.60 no. plant^{-1} in Atakişi , whereas the lowest number of branches per plant values was 1.83 in J-112 (*Table 4*). On the other hand, Bakal et al. (2016), observed the number of branches per plant value between 1.63 no. plant^{-1} and 3.47 no. plant^{-1}, Kulan et al. (2017) obtained between 1.70 no. plant^{-1} and 5.50 no. plant^{-1}, Sevilmiş and Arıoğlu (2019) obtained between 2.80 and 5.30 no. plant^{-1}. The

differences in terms of the number of branches per plant might be sources of genetic characteristics of cultivars and climatic conditions are also considered to be effective.

Table 3. The plant height and, the first pods height data of the soybean varieties grown in the Eastern Mediterranean Transition Zone in 2021, 2022

Cultivars	Plant height (cm)			First pods height (cm)		
	2021	2022	Average	2021	2022	Average
Adel	90.59±3.82 bcd	86.67±3.99 bcd	88.63±1.96 c	6.96±0.38 c	7.60±0.18 de	7.28±0.53 e
AP-3773	95.16±2.92 bc	71.60±5.77 d	83.38±2.10 cde	9.60±0.33 b	10.87±0.97 bc	10.24±0.27 bc
Arısoy	69.70±2.95 g	72.80±2.81 d	71.25±2.86 f	8.88±0.81 b	8.95±0.83 cde	8.92±0.40 d
Asya	84.89±1.62 cdef	83.70±3.40 cd	84.30±0.97 cde	6.95±0.83 c	7.16±0.46 e	7.06±0.43 e
Atakişi	76.70±1.88 defg	85.47±2.47 cd	81.09±2.17 de	9.38±0.82 b	9.27±0.31 cd	9.33±0.86 cd
Atlas-3616	82.14±5.64 cdefg	80.40±4.69 d	81.27±1.24 de	9.30±0.95 b	8.93±0.93 cde	9.12±0.96 cd
J-112	51.77±3.02 h	76.33±2.02 d	64.05±1.69 g	1.70±0.25 e	1.77±0.62 g	1.74±0.25 g
Lider	88.07±3.04 bcd	83.27±3.24 cd	85.67±3.12 cd	4.63±0.11 d	4.65±0.58 f	4.64±0.10 f
Planet	86.59±3.00 bcde	88.03±1.50 bcd	87.31±1.16 c	12.23±0.18 a	13.00±0.97 a	12.62±0.18 a
Racer	81.89±2.11 cdefg	53.29±2.18 e	67.59±1.13 fg	9.15±0.98 b	10.69±0.70 bc	9.92±0.26 bcd
Sonya	71.46±3.27 fg	88.13±2.52 bcd	79.80±2.33 e	10.48±0.05 b	9.73±0.69 c	10.11±0.27 bcd
Winchester	113.87±0.97 a	114.63±0.87 a	114.25±0.90 a	10.03±0.40 b	10.43±0.61 bc	10.23±0.24 bc
Yemsoy	101.07±3.22 ab	102.74±1.40 ab	101.91±1.48 b	13.85±0.52 a	13.53±0.25 a	13.69±0.17 a
Yeniköy-9	72.67±4.63 efg	99.29±1.42 abc	85.98±2.94 cd	9.40±0.45 b	11.88±0.20 ab	10.64±0.31 b
Average	83.33±2.38	84.73±2.33	84.03±1.98	8.75±0.47	9.18±0.50	8.97±0.47
$LSD_{(0.05)}$	9.37	8.23	5.64	1.65	1.94	1.26
CV	6.70	5.79	4.00	11.20	12.64	8.36

a,b,c Values within a row with different superscripts differ significantly at $P < 0.05$

In this study, the analysis of variance of the number of pods per plant of soybean genotypes was given in *Table 2*. In 2021, the number of pods per plant of the genotypes varied between 71.95 pods plant^{-1} and 105.74 pods plant^{-1}, while in 2022 the number of pods per plant of the genotypes varied between 72.44 pods plant^{-1} and 105.48 pods plant^{-1}. Considering the average of the two the genotype AP-3773 had the highest number of pods per plant (105.61 pods plant^{-1}), while the Arısoy had the lowest number of pods per plant (72.20 pods plant^{-1}) (*Table 4*). Although the increase in the number of pods per plant affects the yield, it is not a unique criteria determining of the yield (Bakal et al. 2016). Aremu and Ojo (2005) stated that there is a significant relationship between environmental factors and the number of pods per plant. The number of pods per plant in our study was higher than the results of Onat (2018) and Sevilmiş and Arıoğlu (2019), whereas in this results were found to be similar with Kulan et al. (2017), Yıldırım (2017) and Gümüş and Beyyavaş (2020). The difference in the number of pods per plant in the studies may be due to the different genotypes and the differences of ecological factors.

According to the analysis of variance results, differences in the number of 100-seed weights among soybean genotypes were found to be statistically significant ($P < 0.05$) (*Table 2*). When the 100-seed weight data of soybean genotypes in 2021 was examined, it ranged between 15.11 g and 22.01 g, while the 100-seed weight data in 2022 changed between 15.02 g and 22.24 g. According to the average results of two years, minimum 100-seed weight value of 15.07 g was obtained from the Asya variety. The highest 100-seed weight value was obtained from the genotype Lider i.e. 22.10 g, followed by Planet with 21.96 g and Yeniköy-9 genotypes with 20.87 g, respectively (*Table 5*). 100 seed weight in soybean is affected by various factors such as sowing time, genotype difference, environmental conditions, cultural processes. 100 seed weight, which is also important in determining the amount of seed to be used per decare, is a factor that has a significant effect on grain yield per decare. The factors affecting yield in soybean vary according to the number of pods per plant, the number of plants per unit area, the weight of 100 seeds and the number of seeds per pod (Onat et al., 2017; Gümüş and Beyyavaş, 2020). In this findings of 100

seed weights were find to similar to the findings of Onat et al. (2017), Güllüoğlu et al. (2016), Choi et al. (2016), Dağtekin and Bilgili (2020).

Table 4. The branches per plant and, the pods number data of the soybean varieties grown in the Eastern Mediterranean Transition Zone in 2021, 2022

Cultivars	Branches per plant (no. plant^{-1})			Pods number (pods plant^{-1})		
	2021	2022	Average	2021	2022	Average
Adel	2.73±0.15 cd	3.63±0.13 b	3.18±0.07 bc	73.59±2.97 ef	75.83±2.20 gh	74.71±2.35 gh
AP-3773	2.31±0.10 de	2.67±0.18 de	2.49±0.09 de	105.74±3.01 a	105.48±1.54 a	105.61±2.64 a
Arısoy	2.35±0.13 de	2.48±0.08 ef	2.42±0.03 e	71.95±0.76 f	72.44±0.99 h	72.20±0.87 h
Asya	2.40±0.23 cde	2.07±0.10 fg	2.24±0.11 e	91.54±1.06 bc	93.33±1.28 bc	92.44±1.29 bc
Atakişi	4.80±0.09 a	4.40±0.07 a	4.60±0.12 a	96.27±1.53 b	91.78±1.54 bcd	94.03±0.38 b
Atlas-3616	2.50±0.14 cd	2.31±0.23 ef	2.41±0.02 e	90.30±2.06 bc	92.83±1.49 bc	91.57±2.15 bc
J-112	1.93±0.34 ef	1.73±0.19 g	1.83±0.23 f	92.97±1.43 b	96.44±2.02 b	94.71±0.99 b
Lider	4.54±0.13 a	4.27±0.10 a	4.41±0.03 a	85.70±1.40 cd	85.69±3.80 def	85.70±0.45 de
Planet	1.65±0.05 f	2.60±0.17 de	2.13±0.05 ef	85.83±1.45 cd	89.04±2.34 cde	87.44±1.29 cde
Racer	2.37±0.13 cde	3.32±0.20 bc	2.85±0.15 cd	79.87±3.87 de	84.04±4.48 ef	81.96±1.00 ef
Sonya	2.50±0.18 cd	3.19±0.09 bc	2.85±0.08 cd	89.90±3.74 bc	92.19±1.84 bc	91.05±3.72 bcd
Winchester	2.87±0.26 bc	3.04±0.32 cd	2.96±0.29 c	91.41±0.68 bc	94.86±1.59 bc	93.14±0.94 b
Yemsoy	3.32±0.15 b	3.39±0.12 bc	3.36±0.17 b	77.57±2.54 ef	80.50±0.59 fg	79.04±3.42 fg
Yeniköy-9	2.36±0.18 de	2.46±0.16 ef	2.41±0.14 e	76.72±3.81 ef	76.44±2.29 gh	76.58±2.67 fgh
Average	2.76±0.14	2.97±0.12	2.87±0.13	86.38±1.53	87.92±1.48	87.15±1.47
$LSD_{(0.05)}$	0.51	0.48	0.39	6.94	6.33	5.64
CV	10.87	9.43	8.01	4.79	4.29	3.86

[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$

According to the results of analysis of variance, the differences between seed yields of soybean genotypes were found to be statistically significant ($P < 0.05$) (*Table 2*). When the seed data of soybean genotypes in 2021 was examined, the seed yield changed between 360.35 kg da^{-1} and 590.90 kg da^{-1}, while the seed yield value in 2022 varied between 399.13 kg da^{-1} and 570.55 kg da^{-1}. According to the average of two years, the highest seed yield was obtained from Lider variety i.e. 580.73 kg da^{-1}, and the lowest seed yield was obtained from Arısoy variety i.e. 379.74 kg da^{-1} (*Table 5*). Arıoğlu et al. (2015), showed that the seed yield varied between 428.80 kg da^{-1} and 537.70 kg da^{-1}, Likewise Bakal et al. (2016) found this value between 305.00 kg da^{-1} and 467.20 kg da^{-1}, Yıldırım (2017) recorded this value between 272.81 kg da^{-1} and 399.83 kg da^{-1}, Onat (2018) observed this value between 368.29 kg da^{-1} and 433.43 kg da^{-1} and Gümüş and Beyyavaş (2020) between 166.60 kg da^{-1} and 332.14 kg da^{-1}. The changes in the seed yield values of the different studies may be due to the different genetic structures and ecological conditions of the cultivars included in the trials.

According to the analysis of variance, the protein content differences of soybean genotypes were found to be statistically significant ($P < 0.05$) (*Table 2*). According to the years 2021, 2022 and the average of these two years, the highest protein content value was obtained from J-112 variety i.e. 41.50%, and the lowest protein content value was obtained from Yemsoy variety i.e. 33.58% (*Table 6*). The protein content value of our study is higher than the findings of Altınyüzük and Öztürk (2017), Bakal et al. (2016), Onat (2018) and Sevilmiş and Arıoğlu (2019) findings, but lower than the value recorded by Çubukcu et al. (2019).

Table 5. The 100-seed weight and, the seed yield data of the soybean varieties grown in the Eastern Mediterranean Transition Zone in 2021, 2022

Cultivars	100-seed weight (g)			Seed yield (kg da^{-1})		
	2021	2022	Average	2021	2022	Average
Adel	16.55±0.17 gh	16.71±0.21 de	16.63±0.19 ghı	554.14±28.95 ab	565.57±24.85 a	559.86±26.83 ab
AP-3773	18.41±0.19 def	19.14±0.06 c	18.78±0.08 ef	536.69±31.82 abc	537.77±26.64 a	537.23±27.57 abc
Arısoy	20.24±0.07 bc	20.90±0.33 ab	20.57±0.17 bc	360.35±31.68 f	399.13±30.67 d	379.74±30.76 h
Asya	15.11±0.27 ı	15.02±0.28 f	15.07±0.27 j	547.85±24.57 abc	552.68±24.29 a	550.27±18.17 ab
Atakişi	15.83±0.88 hı	16.11±1.02 ef	15.97±0.92 ıj	517.67±21.68 abc	523.62±27.39 ab	520.65±23.15 abcd
Atlas-3616	18.05±0.36 ef	18.06±0.21 cd	18.06±0.24 ef	471.97±24.47 cde	456.09±34.24 bcd	464.03±28.96 defg
J-112	17.67±0.17 fg	17.81±0.17 cd	17.74±0.17 efg	430.18±26.68 def	457.14±24.36 bcd	443.66±17.34 efgh
Lider	22.01±0.62 a	22.19±0.36 ab	22.10±0.49 a	590.90±25.59 a	570.55±24.88 a	580.73±22.30 a
Planet	21.67±0.57 a	22.24±0.46 a	21.96±0.48 ab	503.30±28.99 bcd	499.04±25.93 abc	501.17±27.46 bcdef
Racer	19.74±0.76 bcd	20.84±0.81 ab	20.29±0.78 cd	397.70±26.84 ef	447.50±23.65 bcd	422.60±17.43 gh
Sonya	16.39±0.64 ghı	16.05±1.12 ef	16.22±0.87 hij	488.79±24.54 bcd	457.43±30.91 bcd	473.11±26.92 cdefg
Winchester	19.12±0.18 cde	19.01±0.11 c	19.07±0.13 de	526.34±22.06 abc	514.22±29.20 abc	520.28±24.23 abcd
Yemsoy	17.39±0.27 fg	17.71±0.33 cd	17.55±0.30 fgh	433.52±25.51 def	439.58±26.57 cd	436.55±25.90 fgh
Yeniköy-9	21.02±0.61 ab	20.71±0.43 b	20.87±0.52 abc	502.51±30.66 bcd	513.86±26.61 abc	508.19±18.26 bcde
Average	18.51±0.35	18.75±0.37	18.63±0.36	490.14±11.43	495.30±9.98	492.72±10.26
$LSD_{(0.05)}$	1.42	1.52	1.41	79.59	70.73	70.78
CV	4.54	4.85	4.51	9.67	9.49	8.56

a,b,c Values within a row with different superscripts differ significantly at P < 0.05

Table 6. The protein content data of the soybean varieties grown in the Eastern Mediterranean Transition Zone in 2021, 2022

Cultivars	Protein content (%)		
	2021	2022	Average
Adel	37.02±0.32 def	37.31±0.24 efg	37.17±0.20 ef
Ap-3773	36.26±0.41 efg	36.16±0.93 g	36.21±0.24 f
Arısoy	38.25±0.59 cde	38.90±0.41 bcd	38.58±0.48 cd
Asya	34.50±0.53 gh	33.44±0.22 h	33.97±0.47 g
Atakişi	38.60±0.60 bcd	39.32±0.58 bcd	38.96±0.29 bc
Atlas-3616	35.42±0.56 fgh	36.46±0.46 fg	35.94±0.49 f
J-112	40.82±0.67 a	42.18±0.50 a	41.50±0.44 a
Lider	37.36±1.05 def	37.94±0.34 def	37.65±0.64 de
Planet	38.12±0.89 cde	39.85±0.55 bc	38.99±0.54 bc
Racer	39.01±0.80 abcd	39.33±0.44 bcd	39.17±0.30 bc
Sonya	38.67±1.15 bcd	39.53±0.45 bc	39.10±0.70 bc
Winchester	40.46±0.40 ab	39.98±0.44 b	40.22±0.34 b
Yemsoy	33.96±0.49 h	33.20±0.55 h	33.58±0.10 g
Yeniköy-9	39.78±0.19 abc	38.32±0.85 cde	39.05±0.38 bc
Average	37.73±0.35	37.99±0.40	37.86±0.35
$LSD_{(0.05)}$	2.01	1.58	1.29
CV	3.17	2.47	2.03

a,b,c Values within a row with different superscripts differ significantly at P < 0.05

4. Conclusions

The importance of soybean is increasing day by day, and intensive breeding studies have been conducted the development of new varieties. The important features in soybean breeding are the determination of varieties suitability for machine harvesting and, the selection of varieties that are resistant to shedding and high seed yield. This study was conducted to determine of yield and important agricultural characteristic of some soybean varieties and genotypes grown in Eastern Mediterranean Transition Zone. The Winchester (114.25 cm) and Yemsoy (101.91 cm) genotypes were found good in terms of plant height, similarly AP-3373 (105.61 pods plant^{-1}) in terms of pods number, Lider (22.10 g), Planet (21.96 g), and Yeniköy-9 in terms of 100 seed weight (20.87 g) were found good. In terms of seed yield, Lider and Asya varieties produced the highest yield. As a result of the experiment, it was determined that the varieties i.e. Lider, Adel, Asya and Yeniköy-9 provided more yield than the other varieties and J-112 variety was found to be important in terms of protein content.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The author declares that they have no conflict of interest.

Authorship Contribution Statement

Concept; Design; Data Collection or Processing; Statistical Analyses; Literature Search; Review and Editing: Yılmaz, M.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Vegetable Losses and Waste Along the Supply Chain and Farmers' Willingness to Pay for Recycling: Towards to Green Supply Chain*

Tedarik Zinciri Boyunca Sebze Kayıpları ve Atıkları, Geri Dönüşüm için Çiftçinin Ödeme İstekliliği: Yeşil Tedarik Zincirine Doğru

Selime CANAN[1*], Ebru Nur ULUIŞIK[2]

Abstract

The aims of the study were (i) to determine amount of the vegetable losses and waste generated along the supply chain by production system such as under greenhouse and conventional system in open area, (ii) to explore the amount of willingness to pay for recycling vegetable losses and waste by composting and its determinants, (iii) to reveal the economic feasibility of composting vegetable losses and waste at district level and (iv) to calculate the individual and social cost of vegetable losses and waste along the supply chain by production system in Turkey. The farm level research data were collected from 81 conventional farms and 45 greenhouse farms in the Samsun province of Turkey by using questionnaires. In addition, 50 traders and 17 greengrocers, 13 supermarkets and 9 local marketers were interviewed. When quantifying vegetable losses and waste in mass, vegetable supply chain was examined in five different stages such as production, postharvest handling and storage, processing and packaging, distribution and retail. Contingent valuation method was used to asses willingness to pay of farmers, traders/merchants in wholesale market hall, greengrocer and super markets for composting of vegetable losses and waste. The economic feasibility of recycling of waste was revealed by using the net present value, cost-benefit analysis and internal rate of return. According to the research findings, the loss rates of vegetables produced per hectare in the greenhouse at the farm, wholesaler and retailer levels were respectively 2.2%; 1% and 20.3%. The loss rates of vegetables produced per hectare in the conventional farm at the farm, wholesaler and retailer levels were 3%, respectively; 0.9% and 16.8%. The individual loss of producers in the examined area was 0.67 thousand US $/year. The rates of individual loss of farms to annual agricultural income were 1.7% and 2.4% in greenhouse and conventional farms, respectively. Other individual losses in wholesales, retailer and consumers were 0.25, 4.89 and 1.02 thousand US $, respectively. The total social loss in the examined area was 6.83 thousand US $. When an assessment was made at the national level, the social loss was about 4% of the annual agricultural income. According to the research results, it has been concluded that depending on the amount of losses, the compost production facility to be established in the district can economically recycle vegetable losses and wastes.

Keywords: Vegetable losses and waste, Vegetable production system, Vegetable Supply chain, Willingness to pay, feasibility of waste recycling

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*This study was summarized from the Ebru Nur Özmen's MSc. thesis.

ÖZ

Çalışmanın amaçları (i) örtü altı ve konvansiyonel sistem gibi açık alanda üretim sistemi ile tedarik zinciri boyunca oluşan bitkisel kayıpların ve atıkların miktarını belirlemek, (ii) bitkisel kayıplar ve atıkların geri dönüşümü için çiftçinin ödeme istekliliği miktarını araştırmak, (iii) sebze kayıplarını ve atıklarını kompostlaştırmanın bölge düzeyinde ekonomik fizibilitesini ortaya koymak ve (iv) Türkiye'de tedarik zinciri boyunca sebze kayıpları ve atıklarının bireysel ve sosyal maliyetini üretim sistemine göre hesaplamaktır. İşletme düzeyindeki araştırma verileri, Samsun ilindeki 81 konvansiyonel işletmeden ve 45 sera işletmesinden anketler kullanılarak toplanmıştır. Ayrıca 50 tüccar ve 17 manav, 13 süpermarket ve 9 yerel pazarlamacı ile görüşme yapılmıştır. Sebze kayıplarını ve atıklarını kütlesel olarak ölçerken sebze tedarik zinciri; üretim, hasat sonrası işleme ve depolama, paketleme, dağıtım ve perakende satış olmak üzere beş farklı aşamada incelenmiştir. Çiftçilerin, tüccarların/tüccarların hallerde, manavlarda ve süper marketlerde sebze kayıpları ve atıklarının kompostlanması için ödeme yapma istekliliğini değerlendirmek için koşullu değerleme yöntemi kullanılmıştır. Atıkların geri dönüştürülmesinin ekonomik fizibilitesi net bugünkü değer, maliyet-fayda analizi ve iç karlılık oranı kullanılarak ortaya koyulmuştur. Araştırma bulgularına göre serada üretilen sebzelerin hektar başına işletme, toptancı ve perakendeci düzeyindeki kayıp oranları sırasıyla %2,2; %1 ve %20,3'dür. Konvansiyonel işletmelerde hektar başına üretilen sebzelerin işletme, toptancı ve perakendeci düzeyindeki kayıp oranları sırasıyla %3; %0,9 ve %16,8'dir. Araştırma alanındaki üreticilerin bireysel kaybı ise 0,67 bin ABD Doları/yıl olarak gerçekleşmiştir. İşletmelerin bireysel kaybının yıllık tarımsal gelire oranı seralarda %1,7, konvansiyonel çiftliklerde ise %2,4'dür. Diğer bireysel kayıplar toptancı, perakendeci ve tüketicide sırasıyla 0,25, 4,89 ve 1,02 bin ABD Dolarıdır. Araştırma alanındaki toplam sosyal kayıp ise 6,83 bin ABD Dolarıdır. Ulusal düzeyde bir değerlendirme yapıldığında sosyal kaybın yıllık tarımsal gelirin yüzde 4'ü civarında olduğu görülmektedir. Araştırma sonuçlarına göre ilçede kurulacak kompost üretim tesisinin kayıp miktarına bağlı olarak bitkisel kayıp ve atıkların ekonomik olarak geri dönüştürülebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Sebze kayıpları ve atıkları, Sebze üretim sistemi, Sebze tedarik zinciri, Ödeme istekliliği, Atık geri dönüşüm fizibilitesi

1. Introduction

For several decades, rapid increase in the world population has caused to intensive use of resources due to pressure of food demand. Nations have exhibited tremendous effort to ensure food security and food safety. Despite all remedies that put into practice for balancing food supply and demand, response of food production to the increasing food demand has become more complex issue. Most nations have focused on alternative ways to reduce the pressure of food demand on resources to provide more sustainable food production. Therefore, food losses have come into the agenda worldwide. The issue of reducing the food losses along the food supply chain have increased their importance more and more in agriculture and food industry. Especially, the product losses along the fresh vegetable supply chain (VSC) had priority due to they are perishable. However, stakeholders of fresh VSC have ignored the product loss. Unfortunately, product wastes have been disposed by randomly landfilling and it hindered the switching to green supply chain. Lipinski et al. (2013) suggested that food losses at the levels of production, retail and consumer in developed country were 10%, 5% and 28%, respectively, while that of developing countries were 14%, 7% and 7%, respectively. Although product losses may vary depending on plant type, varieties and production system, the amount of food loss in Turkey was 26 million tons/year (Salihoğlu et al., 2018). Reducing the food losses along the supply chain was complex issue having multiple level and actors and it was very difficult to reduce food losses with current management practices. There has been in need of changing production and marketing structure and habits of actors take place along the supply chain such as producer, traders, merchants, consumer etc. This situation forces the actors take place along the supply chain and policy makers to reduce food losses. In Turkey, vegetables have produced both in open area and under greenhouse and high level of food loss and waste along the VSC. However, quality data about food losses along the supply chain associated with farm type in vegetable production were required by farmers, other actors in VSC and policy makers to develop action plan for reducing vegetable losses and waste (VLW). The issue of reducing food losses has been required urgent solutions and good quality of data related to amounts of food losses along the supply chain, and opportunity cost of recycling it. Up to now, lots of studies have been conducted on food losses and reduction of food losses. While studies on food losses were quite high in developed countries, there has been very limited study on food losses in developing countries, as well as Turkey. Some previous studies examined the amount of food loss and reasons behind the food losses focusing on specific product, or product groups (Hazarika, 2006; Gangwar et al., 2007; Hazarika, 2008; Khan et al., 2008; Murthy et al., 2009; Sharma and Singh, 2011; Bahattarai et al., 2013; Abass et al., 2014; John, 2014; Kalidas and Akila, 2014; Arah et al., 2015a; Rehman et al., 2015; Jha et al., 2016; Kirigia et al. al., 2017; Bantayehu et al., 2018; Chegere, 2018; Verma et al., 2019). On the other dimension, some researchers focused on the issues of reducing and recycling of food waste (Jeger and Plumbley, 1988; Basavaraja, 2007; Gajanana et al., 2011; Begum et al., 2012; Ku et al., 2013; Kannan et al., 2013; Adepoju, 2014; Kiaya, 2014; Arah et al., 2015b; Kumari and Pankaj, 2015; Kumar and Kalita, 2017; Rahiel et al., 2018; Tadesse et al., 2018; Krijger et al., 2020). Some studies compared the reasons of product losses in developed countries with those of developing countries (Hodge et al., 2011; Prusky, 2011). However, there has been a limited number of studies related to exploring the relationships between pesticide use and food losses (Harris and Lindblad, 1978; Cappellini and Ceponis, 1984; World Resources, 1998). There have been also a few studies focusing on food losses and recycling of food wastes in Turkey and these studies examined the food losses in terms of technical aspects and oulined the general situation by using macro level data (Sessiz and Özdemir, 2007; Baran et al., 2012; Demirbaş et al., 2017; Tatlıdil et al., 2013; Demirbaş and Gölge, 2018; Salihoğlu et al., 2018; Demirbaş, 2018; PHP, 2019; Çiftçi and Demirbaş, 2020; Alabouıd and Bayhan, 2022). When glancing at the economic aspect of the food losses, it has been clear that the study focusing on VLW along the supply chain and economic feasibility of recycling VLW by production system such as under greenhouse and conventional methods in open area was scarce worldwide. Also, there has been less or no study related to VLW along the supply chain and the feasibility of recycling product losses in Turkey. Despite the previous study conducted by Erden et al. (2017), Elik et al. (2019) and Bayramoğlu et al. (2020) examined the food losses along the supply chain and designing the strategy for reducing food waste in Turkey, they ignored the differences arising by production system. This research gap has motivated the research. Therefore, the study intended to fill information gap existing in literature due to there has been no information about the amount of VLW along the supply chain by production system such as under greenhouses and conventional system in open area. The study tested the prior hypothesis of whether the amount of VLW has changed associated with production system, or not at first. Following the study focused on the hypothesis of whether VLW was more in retail level comparing to the harvest and post-harvest losses. The study

also tested the hypothesis of whether switching to green supply chain by composting the VLW at district level was economically feasible, or not. The objectives of the study were (i) to determine amount of the VLW generated along the supply chain by production system such as under greenhouse and conventional system in open area, (ii) to explore the amount of willingness to pay for recycling VLW by composting and its determinants, (iii) to reveal the economic feasibility of composting VLW at district level and (iv) to calculate the individual and social cost of VLW along the supply chain by production system in Turkey.

2. Materials and Methods

2.1. Research coverage and data

Research focused on the VLW along the VSC, reasons behind the losses and feasibility of composting food waste. VSC was examined in five different stages, which were production, postharvest handling and storage, processing and packaging, distribution and retail. The research covered the active stakeholders along the VSC such as vegetable farmers, wholesale level traders and merchants, retail level actors such as greengrocers, supermarkets and seller at local bazaar. Since it was an appropriate environment that allows comparison due to the summer and winter vegetable have been produced both under greenhouses and in open area together, Çarşamba district of the Samsun Province of Turkey was selected as a case and identified as a research area. Carşamba District, which forms one of the fertile delta plains of the Yeşilırmak river, has an area of 69129 ha (Anonymus, 2021a). The average altitude above sea level was 128 meters. 72% of the villages of the district were settled on the plain. The district has a humid and temperate climate. The annual average precipitation was 936,9 mm and the annual average temperature was 15,1 °C. (Anonymus, 2021). There was a total of 57 thousand hectares of agricultural land in the district, 4.68% of it was vegetables. There was also 911 hectares of meadow pasture and 6 thousand hectares of forest in the research area (Anonymous, 2021a; Anonymus, 2021b). The most common vegetables are beans, tomatoes, peppers, eggplants, cucumber, lettuce, cabbage, parsley. Lettuce, cucumber, tomato, pepper and eggplant were the main vegetables under greenhouse production (TUIK, 2020). The average farm size was 2,73 hectares. Map of the research area was depicted in *Figure 1*.

Farm level research data were collected from randomly selected 27 greenhouse farms and 81 conventional farms produced vegetable in open area in Çarşamba district of Samsun province, Turkey. Questionnaires were administered to the operators of sample farmers in 2020 considering the production year of 2019-2020. Regarding the wholesale actors of supply chain, wholesale research data were gathered from 50 traders/merchants, which was all active traders/merchants in Çarşamba wholesale market hall by using semi structured interviews. For the retail level data, well designed questionnaires were administered to randomly selected 17 greengrocers, 9 sellers from district bazaar and managers of 13 supermarkets.

Figure 1. Map of the research area

2.2. Determining amount of the VLW and its cause along the supply chain

VLW was explored along the VSC with the case of Çarşamba district of Samsun province in Turkey. VSC included the farm level such as greenhouses and conventional production in open area, wholesale level such as traders and merchants, and retailer level actors such as greengrocers, super markets and sellers in local bazaar. After removing non-human use of vegetable and reducing losses such as harvest losses, packaging and transportation, edible vegetable produced for human consumption started to travel from both greenhouse and

conventional farms to traders/merchants that took place in wholesale market hall. Post-harvest losses such as handling, storage etc. was occurred in this stage. Then edible vegetables reached to greengrocers, sellers in local bazaar, or supermarkets. Finally, edible vegetables were eaten by consumers (*Figure 2*).

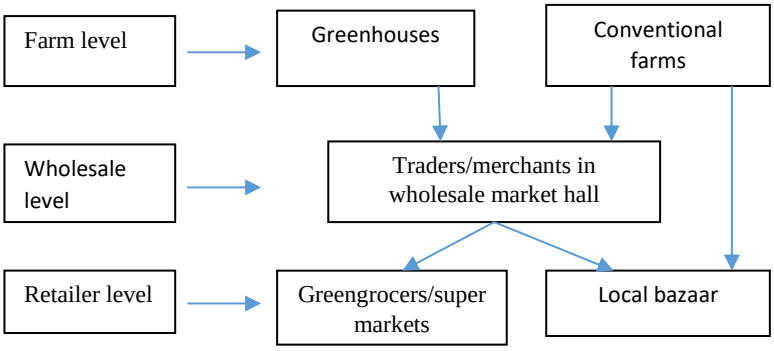

Figure 2. Vegetable supply chain

In the study, VLW was defined as decrease in the amount of edible vegetable in mass produced for consumers along the VSC from harvest to consumption. VLW were quantified by using the framework suggested by HLPE (2014) due to being easy to apply and easy to relate to specific data. Based on the FAO (1981), Stuart (2009), Parfitt et al. (2010), FAO (2011), Gunders (2012), Gustavsson et al. (2011) and HLPE (2014), FW occurred at consumer level and FL occurred any stage before the consumer level, regardless of the real underlying explanatory cause, and regardless of its behavioural character or not, or of its voluntary character or not. Non-avoidable waste was not considered as a VLW in the study. When quantifying VLW in mass, VSC was examined in five different stages such as production, postharvest handling and storage, processing and packaging, distribution and retail. Monetary losses in mass (ML) were also calculated in the study. HLPE (2014) suggested that loss of value added linked to the degradation of the food quality or to food loss or waste could take place at every step of the food chains. Time could be an important determinant of ML. When calculating the ML, prices of good quality vegetable, prices of lower quality vegetable, discounts applied in greengrocers and super markets and transaction costs of supply chain agents such as merchants, retailers and market operators etc. Since the vegetable was composite variable including many species, weighted average value of prices was considered by using the share of species in total vegetable mass. Same approach was also adopted for calculating transaction costs.

The causes of VLW along the supply chain were explored at micro, meso and macro level. The definitions of micro, meso and macro level causes at the behind of food loss and waste suggested by HLPE (2014) were used in the study. Micro-level cause was defined as the causes of food loss and waste at each particular stage of the food chain from production to consumption. Meso-level cause dealt with organization of different actors, relationships along the food chain and state of infrastructures etc. and it could be found at another stage of the supply chain. Macro-level cause was related the factors explained by more systemic issues such as a malfunctioning food system, the lack of institutional or policy conditions to facilitate the coordination of actors (including securing contractual relations), to enable investments and the adoption of good practices (HLPE, 2014).

2.3. Exploring the amount of willingness to pay for composting VLW and its determinants

Contingent valuation method (CVM) was used to asses willingness to pay (WTP) of farmers, traders/merchants in wholesale market hall, greengrocer and super markets for composting of VLW. The specific question used in the analysis was a hypothetical scenario-based question that asked respondents about their willingness to pay for the composting of VLW. The exact wording of the question typically presented a situation where the respondents are asked how much they would be willing to pay for a certain environmental service, in this case, the composting of VLW. This hypothetical scenario question is chosen because it allows researchers to gauge the monetary value individuals place on the given service, even though it might not currently have a market price.

According to the question type feature, the maximum amount of expressed willingness to pay was determined. The reason for choosing this method and question type lies in the nature of the service being assessed – composting of VLW. Since this service might not have an established market value, it is necessary to elicit individuals'

preferences and their monetary valuations through a structured question. The hypothetical scenario question helps simulate a market-like situation where respondents express their stated willingness to pay, which can then be analysed to understand the potential economic value of the composting service to different stakeholders. CVM has been commonly used method, when an individual was asked to important to understand what is the individual's WTP to composting of VLW in mass generated along the VSC (Hanemann, 1984, Johansson et al., 1985, Mitchell and Carson, 1989, Akgüngör, et al., 1999; Winpenny, 1991). For farmers, bazaar sellers, traders/merchants in wholesale market hall, greengrocer and super markets, to explore information on WTP in a detail, we need a statistical model that relates individuals' responses to monetary amounts. Multiple regression model was used to explain variations in their willingness to pay for composting vegetable waste. While analysing the model, business data was divided into three groups as farmers, wholesalers and retailers. Generally, the WTP was a function of socio-economic variables (Hanemann, 1984; Johansson et al., 1985; Danso et al., 2002; Abdullahi et al., 2023; Yelboğa et al., 2023). The general form of multiple regression model constructed for determining the determinants of WTP is depicted below.

$$WTP = \alpha + \beta X + e \quad \text{(Eq.1)}$$

where α is constant, X is the explanatory variables, β is an unknown parameter, and e is the disturbance term, which is normally distributed.

The explanatory variables of the WTP model were income (1000 US $/year), age (year), gender, education, experience of operators (year), labour (MLU) and operating capital (1000 US $/year) Gender was a dummy variable and 1 was assigned for male and 0 was assigned for female in the model. The variable of education was proxy variable. Primary school was included into the model by using 1, while that of secondary school, high school and university were 2,3 and 4, respectively. VSC actors were included into the model as dummy. When the respondent was producer (conventional or greenhouse), in first dummy (D1) variable producers equalled, 1 while the rest were 0. In second dummy (D2), wholesalers equalled 1, while that of other VSC actors were 0. In third dummy variable (D3), all retailers were assigned 1, while that of others were 0 (*Table 1*).

Table 1. Description of explanatory for linear regression model

Variable name	Description of variable	Greenhouse[1]	Conventional farm[1]	Retailer[1]	Wholesale[1]
		Mean	Mean	Mean	Mean
WTP	US $/year	750.00 (137.78)	1722.22 (512.16)	14222.31 (5501.30)	18144.10 (929.80)
Gender of operators	male = 1. female = 0	0.93 (0.01)	0.91(0.03)	0.95 (0.02)	0.90 (0.03)
Age of operators	year	51.48 (2.30)	52.61 (2.49)	49.44 (13.20)	54.00 (12.34)
Education level of operators	primary school = 1. secondary school = 2. high school = 3 and university = 4	2.48 (0.20)	2.36 (0.24)	2.64 (0.82)	2.60 (0.77)
Experience	year	31.35 (3.76)	30.18 (8.37)	18.13(5.26)	24.74 (2.83)
Labour	MLU	8.58 (0.28)	6,73 (0.26)	1.62 (0.63)	6.04 (2.02)
Operating capital	1000 US $/year	1048.31 (866.78)	1361.14 (735.99)	6203.13 (2743.17)	8816.59 (1398.01)
Income	1000 US $/year	116.91 (1.24)	106.07 (0.98)	4346.79 (1433.10)	7902.37 (994.11)
Dummy	D1: farmers=1, others=0, D2: wholesalers=1, others=0, D3: retailers=1, others=0	0,04 (0,20)	0,59 (0,49)	0,14(0,35)	0,23(0,42)

1The numbers in parentheses indicate the standard error.

In order to determine the most appropriate regression model, the linear model with the highest coefficient of determination and the smallest standard error among the tried models was preferred. While applying the linear regression models, care was taken to provide the necessary assumptions such as the conformity of the data to the

normal distribution, the constant variance of the error terms, and the absence of autocorrelation problem between the error terms.

2.4. Examining the economic feasibility of composting VLW at district level

When economically analysing the alternative of switching to composting, the wet weight of the VLW generated in Çarşamba district was considered 34378 tons, on average. Dry weight of VLW was 20% of its wet weight. The amount of produced compost was equal to 50% of dry VLW. The average dry weight of VLW in Çarşamba was 6876 tons, resulting in 3438 tons of compost in a year. The price of compost per ton was 409.36 US $. If VLW generated along the VSC in Çarşamba were composted, it would be annually gained extra revenue by approximately 1.46 million US $. Based on the quantity of VLW along the VSC in Çarşamba, for constructing district level compost plant with 5 tons of capacity per day, 3500 m^2 of land and investment by 541.435.44 US $ were required to compost generated VLW along the VSC. It was assumed that district level compost plant was actively worked along with the 10 months in a year for producing compost. Economic life of the district level compost plant was 10 years. Required investment per ton for composting was 15.79 US $. The cost of raw material was also included by 66.128.69 US $ in order to consider the opportunity cost in the feasibility analysis. The cost of cleaning raw materials from inorganic materials in compost plant was 19.838.60 US $. And the cost of raw material purchasing/collecting was 66.128.69 US $.

A technician, 3 workers and an office staff work at the compost plant. In addition, 2 workers who will collect the VLW from the lost collection centres and deliver them to the facility work with a mini garbage truck. Farms sell their VLW to this facility for 2.92 US $/per tonne, both recycling the VLW and covering the transportation costs. Others in the VSC leave the VLW to the VLW bins, where the compost plant collect the VLW and transport it to the plant. on the other hand, the compost plant has the opportunity to earn economic profit by selling the compost it produces directly to farmers or fertilizer distributor. Thus, VLW will be offered to farms again as compost (*Figure 3*).

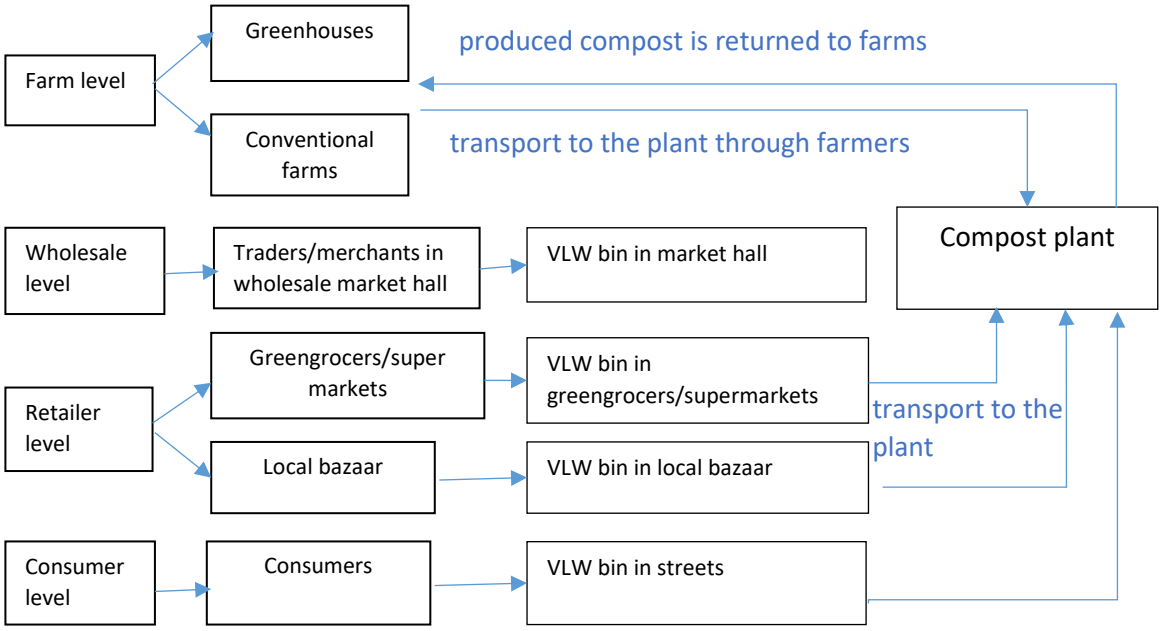

Figure 3. Compost production process

The analyses used to test the economic feasibility of recycling VLW in the compost plant in the district are given below:

(Net Present Value): $NPV = \sum_{t=0}^{n} CFt \div (1 + r)^t$ (Eq.2)

where:

CFt=net cash flow during a single period t

r=discount rate(0,12)

t=time period cash flow

n=number of periods (10 year)

(Cost Benefit Analysis): $NPV = \sum_t^n \frac{Bt-Ct}{(1+r)^t} = \sum_t^n \frac{Nt}{(1+r)^t}$ (Eq.3)

where:

B=benefits

C=costs

N=net results

(Internal Rate of Return): $IRR = r_a + \frac{NPVa}{NPVa-NPVb}(r_b - r_a)$ (Eq.4)

where:

ra=lower discount rate chosen (0,05)

rb=higher discount rate chosen (0,20)

Na=NPV at ra

Nb=NPV at rb

2.5. Calculating the individual and social cost of VLW along the supply chain

The social cost calculation aimed to provide a comprehensive understanding of the economic implications of VLW on a larger scale, beyond the individual or farm level (Buzby et al., 2014; ReFED, 2016; Stenmark et al., 2016). By quantifying the total economic losses associated with wasted vegetables, the study was highlighting the significance of reducing waste and improving waste management practices within the vegetable production and distribution system (Buzby et al., 2014; ReFED, 2016; Stenmark et al., 2016; Campoy-Muñoz et al., 2017). The results of the social cost calculation were used in various ways to guide policy and decision-making processes in conclusions.

Individual costs were calculated for each level of VSC. While calculating individual costs, the loss amount of each vegetable variety was multiplied by its price. Çarşamba district and national losses were calculated by area rate based on the areas of the sample farms. The social cost of VLW was calculated by adding up the individual losses at the national level.

2.5. Statistical analysis

Parametric methods (t-test and analysis of variance) for continuous and normally distributed variables in the comparison of waste types and amounts in tomato production, alternative recycling methods, and loss amounts caused by using inappropriate waste recycling method by management types, greenhouse types and provinces. Non-parametric methods (Chi-square, Friedman, Kruskall Wallis, Mann Whitney U, Wilcoxon, Duncan multiple comparison test etc.) were used when the measurement levels of data were nominal and ordinal level.

3. Results and Discussion

3.1. Farm attributes

In the research area, greenhouses and conventional farms produce vegetables on 0.614 and 1.687 ha, respectively. Pepper (35%), tomato (26%), eggplant (17%), cucumber (17%), bean (4%), lettuce and parsley (0.1%) were produced in greenhouse lands. Bean (53%), pepper (22%), tomato (13%), eggplant (9%), zucchini (2%), cucumber (1%), lettuce, spinach, onion and pea (0,1%) production were found in the supply of conventional farms.

Operators of greenhouses were younger. The family labour force and the number of households in operators of conventional farms was higher. The operating capital, total capital, farm income per labour force, farm income, gross income, net return on total capital, rate of return on total capital and rate of return on operator's capital of

greenhouses were higher than conventional farms. According to the results of comparative socio-economic analysis, there was a statistical difference between the farm size, farm income, gross income of conventional farms and greenhouses (*Table 2*).

Table 2. Socio-economic characteristics of vegetable farms

Variables	Greenhouses[1]	Conventional farms[1]
Age of the farm operator (year)	51.48 (2.30)	52.61(2.49)
Labour (MLU)	1.33 (0.24)	1.40 (0.23)
Family size (person)	4.00 (0.28)	4.00 (0.26)
Farm size (ha)*	0.614 (0.68)	1.687 (5.79)
Operating capital (1000 US $/ha)	249.61(186.36)	117.96 (185.88)
Total capital (1000 US $/ha)	2330.81(33.34)	1860.26(46.43)
Farm income (1000 US $/ha)**	278.37 (9.08)	91.93(1.73)
Farm income (1000 US $/MLU)	128.51(38.39)	110.77(47.27)
Gross income (1000 US $/ha)**	217.61(19.30)	109.34 (15.98)
Net return on total capital (1000 US $/ha)	13.67 (0.78)	3,41 (1.84)
Rate of return on total capital	5.45(2.48)	4.80 (1.46)
Rate of return on operator's capital	11.49(2.64)	9.48(2.79)

1The numbers in parentheses indicate the standard error.
* The difference between farms protected the environment and conventional is statistically significant at 1% probability level.
**The difference between farms protected the environment and conventional is statistically significant at 10% probability level.

3.2. Losses along the VSC by production system

824 kg of vegetables produced in one-hectare greenhouse and 502 kg of conventionally produced vegetables were lost during the production. Disease and pest control in the examined farms was carried out entirely by chemical means. During this application, the farms generally stated that they paid attention to the recommended dose. However, they added that when they could not reach a solution, they could use more pest than necessary or turn to a different pest. 6.66% of greenhouses lose vegetables due to wrong spraying. Conventional farms, there was no farm that lost product due to wrong spraying. While 57% of greenhouses bore diseases, 73% of conventional farms bore diseases. In addition, 9% of greenhouses bore pest, while 8% of conventional farms bore pest. The difference in disease and pest bearing rates between the two farm types reveals that conventional farms were more at risk for vegetable loss.

Vegetable farming requires intensive workforce. Seedling planting, hoeing and harvesting require a lot of labour. Therefore, the ability and knowledge of temporary and permanent workers was very important in terms of reducing VLW. In the research area, labour deficiencies that will result in VLW during the production process were not encountered. However, 3.70% of conventional farms experience VLW due to labour during the hoeing. Farm was not found in the greenhouses that experienced VLW due to hoeing.

In the research area, disasters such as floods, hail, and frost were not observed during the 2019-2020 production period. However, in the past years, most of the vegetables have been lost due to such disasters. Compared to greenhouses in conventional farms, more product loss occurs due to climate. Despite this, the rate of vegetable farms that have insurance against VLW caused by hail, storm, tornado, fire, landslide, earthquake, flood and flood risks was 4.4% in greenhouses and 2.5% in conventional farms.

The most critical process in terms of VLW was harvest. The losses that occur during the harvest phase directly cause a decrease in the income of the farms. The rates of losses at the harvesting of vegetables produced in a hectare greenhouse and conventional farm were 1.3% and 2.3%, respectively. While the rates of greenhouses experiencing losses during the harvesting due to temporary workers and other reasons (weight loss, rash appearance, shrinkage, etc.) were respectively,27.93% and 91.11%, the rates of conventional farms were 25.92% and 93.82%, respectively (*Figure 4*). The vegetable that temporary workers cause the most loss was tomato in greenhouses and conventional farms. Due to the soft and delicate structure of tomatoes, there was a possibility of more crushing and damage. For this reason, it was the product that needs more care during harvest. Adepoju, in his study in Osun State of Nigeria in 2014, found that Ogbomosho farmers lost 95.5% of their tomato income after harvest. Arah et al. (2015a) emphasized that postharvest losses in tomatoes were partially affected by preharvest practices (fertilization, pruning, variety selection, irrigation, etc.). Vegetables, most of which are harvested by

hand, must be plucked from the branch without damage by the harvesting workers. Freshness was one of the most important features sought in the products to be taken to the trader. For this reason, harvests were divided into time and made at frequent intervals. In this process, the availability, experience and skills of temporary and permanent workers were very important. In order to reduce losses, the rates of managers of greenhouse and conventional farms who are willing to attend the farms upon the proposal to participate in the harvest-related course were 44.4% and 54.2%, respectively.

Post-harvest spoiling causes significant economic losses. The reasons such as lack of knowledge about post-harvest physiology, lack of post-harvest storage or unsuitable storage conditions cause increased losses. Post-harvest losses in agricultural products may continue during packaging, transportation and storage of products. The loss rates of vegetables produced in a hectare greenhouse during packaging and selling to the trader were 0.05% and 0.6%, respectively. The loss rates of vegetables produced on a hectare conventional farm during packaging and selling to the trader were 0.1% and 0.59%, respectively. Jeger and Plumbley (1988) stated that spoilage in tropical fruits and vegetables can be managed with an integrated approach that includes biological and environmental control before and after harvest. In the farms examined, vegetables were not stored after harvest and the waiting period of the vegetables that will go to sale after harvest was at most 1 day. Farms sold vegetables immediately after harvesting piecemeal, so that farm-level harvested vegetables did not spoil. During sale of the product to the trader; the packed vegetables were loaded into the farm manager's vehicle and transported to the trader. VLW after the transport belonged to the trader.

Farmers sold vegetables to the fresh vegetable and fruit wholesale hall or directly to the local market (*Figure 3*). All of the greenhouses sold their products to the wholesale hall. Conventional farms sold their vegetables to wholesale hall (89%) and local market (11%). Some of the conventional farms sold their vegetables in the local market by packaging. Vegetables brought to the local market could become unusable due to spills on the ground during sales, as well as losses due to the inability to reach the seller of all the vegetables offered in the local market. Unsold vegetables could spoil prematurely during hot periods when they were put on hold.

The vegetable transferred from the greenhouses was lost to the traders, during the unloading of the product from the operator's vehicle (0.4%), packaging (0.03%), storage (0.5%) and transportation to the retailer (0.1%). The vegetable transferred from the conventional farms was lost to the traders, during the unloading of the product from the operator's vehicle (0.4%), packaging (0.1%), storage (0.3%) and transportation to the retailer (0.9%).

The average amount of vegetables traded by a trader in the fresh vegetable and fruit wholesale hall was 27918.16 kg/year. Traders were buying and selling more than one type of vegetable at the same time. All of the vegetables were loaded into vehicles not as a single type, but as multiple vegetable types. Stabbing the stems of vegetables onto other vegetables during transportation was an important factor affecting the losses. The most used vehicle for transporting products was a pickup truck. This vehicle was widely used because it was fast and convenient for product transportation. The average transportation distance of the traders interviewed was found to be 500 km. The traders were trying to stack them in accordance with the characteristics of the vegetables. Stacking types were crate, sack, bagging, tie method and stacking directly into the frame of the vehicle to be transported. Critical and soft vegetables such as tomatoes were placed in crates. The rate of traders who store different type of vegetables in the same place was 51%. The average storage period was 15 days. The storage capacity at the traders was on average 25 tons. The rate of traders with cold storage was 90%. The ambient temperature of vegetables was 8.41 degrees on average.

Part of the vegetables that reach the wholesale hall from the producer were shipped to the provinces of Ordu, Giresun, Trabzon and Rize provinces to be delivered to the consumer. The other part of the vegetables in the wholesale hall were bought by the greengrocers and supermarkets in the district. Buying periods of vegetables for greengrocers and supermarkets vary. Since there was no storage or cooling process in the examined greengrocers, the loss rates experienced in hot summer periods were high. In supermarkets, the careless choice of consumers when buying vegetables caused crushing of vegetables. Since a different consumer would not want to buy the damaged vegetable, the sale of vegetables became difficult and they were subject to decay over time. The loss rates at retailer level of vegetables produced in a hectare greenhouse and conventional farms were 20.3% and 16.8%.

As a result, the ratio of vegetables reaching the consumer from one-hectare greenhouse and conventional farms was 72.8% and 75.3%, respectively (*Table 3*). There was a statistical difference between the amount of VLW during the pre and post-harvest in greenhouses and conventional farms (t= 6.41, p=0.002).

Doğan (2014) stated that VLW was approximately 8 times higher in developing countries than in developed countries. Kirigia et al. (2017) examined the losses in the VSC from the farm to the consumer in Africa and determined the loss rate as 50%. Rahiel et al. (2018) determined that VLW rate of potatoes and other vegetables pre and post-harvest was between 30% and 50% in the Tigray province of Ethiopia.

Table 3. WLW along the fresh VSC by farm type

	Greenhouse farms			**Conventional farms**		
	kg	**%**	**%**	**kg**	**%**	**%**
Production losses (a)	824,3			501,7		
Raw production (total harvest)	87564,5			32719,7		
Non-human uses	5253,9			1963,2		
Farm level edible vegetable weight (kg/ha) b	82310,6	100,0		30756,5	100,0	
Losses from farm to traders/merchants (kg) (c)=d+e+f	1772,0	2,2	7,9	929,8	3,0	12,2
Harvest losses (d)	1087,1	1,3	4,9	693,7	2,3	9,1
Farm level packaging (e)	42,8	0,0	0,1	41,7	0,1	1,5
Transportation from farm to trader/merchant (f)	642,1	0,9	2,9	194 ,4	0,6	2,6
Wholesale level weight (kg)	80538,6	97,8		29826,7	97,0	
Post-harvest losses (kg) (g)=h+i+j+k	794,0	1,0	3,5	288,8	0,9	3,8
Handling (h)	301,4	0,4	1,3	131,5	0,4	1,7
Packaging (i)	28,4	0,0	0,1	10,5	0,1	0,1
Storage (j)	355,2	0,5	1,6	104,1	0,3	1,4
Transportation from trader/merchant to retailer (k)	109,0	0,1	0,5	42,7	0,1	0,6
Retail weight (kg)	79744,6	96,8		29537,9	96,1	
Losses in retailer (l)	16691,9	20,3	74,5	5158,5	16,8	67,9
Consumer weight (m)=b-c-g	63052,7	76,5		24379,4	79,3	
Consumer waste(kg)	3152,6	3,8	14,1	1218,9	4,0	16,1
Consumed vegetable (eaten) kg	59900,1	72,8		23160,5	75,3	
Total losses along the supply chain (kg) (n)=c+g+l	22410,5	27,2	100,0	7596,0	24,7	100,0
Total losses along the supply chain including production losses (kg) (o)=a+c+g+l	23234,8			7380,5		

*Consumer waste was considered 5% of the consumer weight based on the results of the studies conducted by Bayramoğlu et al. (2020) and Elik et al., (2019).

The rates of farms that do not recycle VLW in greenhouses and conventional farms was 26% and 57%, respectively. The ratio of farms that used the remaining domes in the greenhouses as tomato paste and offered them for sale was 28.3%. Additionally, the rate of farms that used peppers as paste was 45.7%. While the rate of farms that fed the eggplants remaining in the farms without being sold to the animals was 7%, the rate of the farms that fed the cucumbers to the animals was 4.5%. The ratio of farms that used the remaining domes in the conventional farms as tomato paste and offered them for sale was 27.8%. Additionally, the rate of farms that used peppers as paste was 35.8%. The remaining VLW were not recycled and were thrown away or left in the field.

The traders and retailers did not attempt to recycle VLW. The rate of traders and retailers who throw away their vegetable losses was 77%. The rate of those who sold their vegetable losses at very low prices was 22.45%.

3.3. Willingness to pay for composting of VLW and factors affected the amount of willingness to pay

While the rate of greenhouses that want to recycle VLW was 75%, the rate of conventional farms was 63%. The amounts of WTP were 109.65 US $ for greenhouses and 25.17 US $ for conventional farms. While the rate of retailers that want to recycle VLW was 80%, the rate of wholesalers was 85%. The amounts of WTP were 2079.29 US $ for retailers and 2652.65 US $ for wholesalers.

The rate at which VSC managers explained the total change in the "amount of WTP" was 98% of the variables "gender, age, education, experience, labour, income, and operating capital". The variables of education, income and operating capital of VSC had an impact on the amount of WTP for the expense incurred in recycle of VLW. However, in VSC, the variables of gender, age and experience did not have an effect on the amount of WTP. The increase in the education of VSV, income, and operating capital positively affected the amount of WTP. When the education of the managers increased by 1 level, the amount of WTP increased by 12.30 US $. When income increased by 1 US $, the amount of WTP increased by 0,002 US $. When operating capital increased by 1 US $, the amount of WTP increased by 0,001 US $. There is a positive relationship among WTP and all types of VSC (*Table 4*).

Table 4. Factors affecting willingness to pay

	Coefficients	Standard Error	T value	P value
Experience	0,535	1,705	0,314	0,754
Gender	0,032	0,057	0,568	0,571
Age	-0,953	1,433	-0,665	0,507
Education	12,298	21,520	5,715	0,019*
Labour	1,087	2,255	0,482	0,630
Income	0,002	0,001	5,913	0,001*
Operating capital	0,001	0,001	5,891	0,021*
D1	235,323	93,684	2,512	0,033*
D2	774,969	118,647	6,532	0,001*
D3	397,968	177,821	2,238	0,026*
R^2	0.982	1513.77	-	-
F	1094.38	-	-	0,001*

* Significant at 5% probability level.

3.4. Economic feasibility of composting VLW at district level

Several alternatives were considered when determining VLW management strategies for farms in the VSC. There was a tomato paste plant in Bafra where another district of Samsun Province. Tomato and pepper paste were produced in the plant. The vegetable farms in Çarşamba District did not sell their tomatoes and peppers to this plant due to the length of the road and because they did not have any contracts. Selling the tomatoes and peppers directly to the tomato paste plant from the farms secured by the contract would have been one of the best alternatives to reduce the loss of these products. However, the vegetable farms in Çarşamba district were not only producing tomatoes and peppers. The VLW recycling method, which would include other vegetables, would be more inclusive.

When farms bought a forage machine to convert VLW into animal feed or a drying oven to dry, the return on capital did not exceed the opportunity cost. Since Samsun Province had humid weather conditions, it was not possible to dry vegetables by using the sun. In addition, dried vegetables were turned into food and legal permission was required during the packaging or processing stages of the food. There were separate costs for all these. When the farms wanted to take VLW to the biogas plant in Samsun, they would cover the transportation costs themselves and would not earn any income. However, if the farms gave VLW to the compost plant in the district for a certain fee, they would have covered the costs of transportation. If the VLW from other stakeholders of the VSC were also collected from VLW collection centres, the plant could produce more compost and the farmers could benefit from more compost.

The compost production process was planned according to the attitudes and behaviours of all stakeholders in the VSC (*Figure 3*). In the compost production process, farms would carry VLW to the compost plant with trailers for a certain fee. For other stakeholders in the VSC, compost production from VLW would be sustainable if local government distributed VLW bins such as garbage container to collect VLW in fresh fruit and vegetable wholesale hall, local markets, supermarkets/grocers and settlements and if the compost plant collects VLW itself.

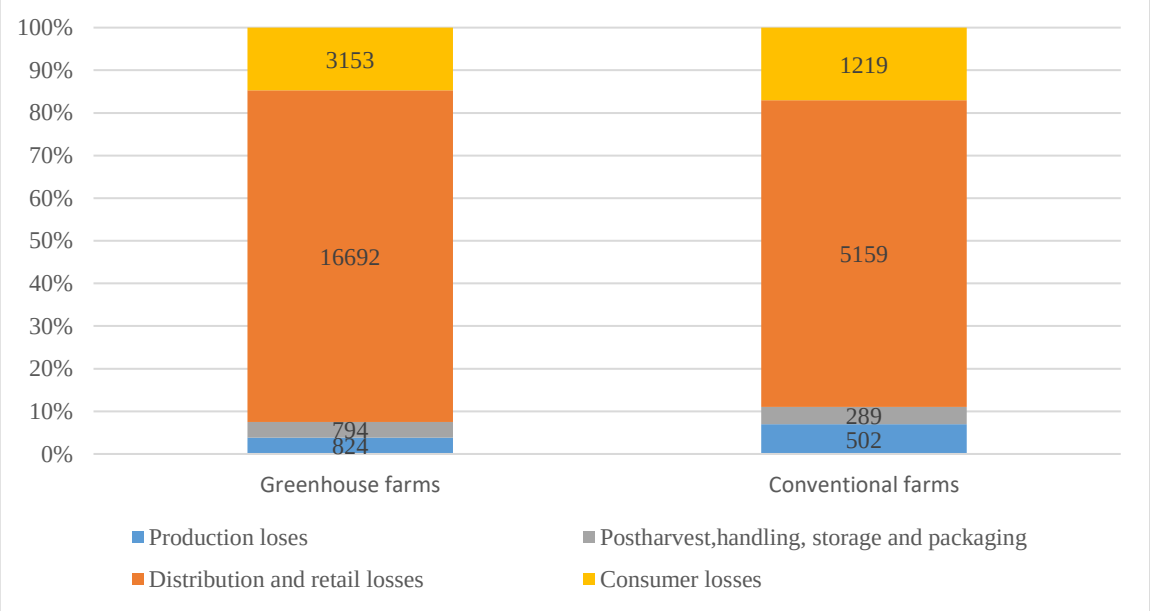

Figure 4. Distribution of total product loss along the VSC by farm type (kg/ha)

The initial capital needed for the establishment of the compost plant in the district was 540 thousand US $, which consisted of amount of fixed investment (66.34%) and working capital (33.68%.) The fixed investment amount of the compost production facility was 360 thousand US $, which consisted of machinery (54.84%) and equipment costs, land, construction and VLW collection area costs (41%). The working capital of the facility was 180 thousand US $, which consisted of fixed costs (49%) and variable costs (51%).

Table 5. Feasibility of compost plant

Amount of VLW (tonne/year)	34,378.00
Amount of compost (tonne/year)	3,437.80
Compost price (US $/tonne)	409.36
Compost income (US $/yıl)	1,407,286.55
Machine/equipment cost	196,975.27
Land supply cost	33,260.23
Construction project cost	17,019.59
Construction cost	94,502.92
Fixed investment capital (US $/year)	66,810.80
Fixed costs	88,890.55
Variable costs	93,336.44
Working capital (US $/year)	182,226.99
Initial investment (US $/year)	541,435.44
Net present value (US $/10years)	4,351,498.15
Internal rate of return	0.41
Benefit/cost ratio	2.21
Payback period	less than 1 year

As a result of the 540 thousand US $ investment made in the compost plant today, a net profit of 4.35 million US $ would be obtained with the present value during the 10 years, which is the economic life of the investment. This showed that the project could be done economically. The internal rate of return for the compost plant was calculated as 41%. This value indicated that the investment was profitable, as it was greater than the 12%

opportunity cost of capital. The benefit-cost ratio above 1 means that the present value of the cash inflows to be provided during the useful life of the investment is higher than the present value of the expenses incurred for this investment. In addition, the investment cost of the facility could be paid with cash flow within the first year (*Table 5*).

3.5. Social cost of VLW along the supply chain

The individual cost of farmers in the examined area was 0.67 thousand US $. The individual cost of greenhouses was 1.7% of their annual agricultural income. In conventional farms, this rate was 2.4%. Individual costs of traders were 0.25 thousand US $. The stakeholders of VSC with the highest individual cost were groceries/supermarkets. Individual costs of groceries/supermarkets were 4.89 thousand US $. And individual cost of consumers was 1.02 thousand US $. The total social cost in the examined area was 6.83 thousand US $ (*Table 6*).

When the calculation was made for the whole of Çarşamba district, the individual costs were 796.88 thousand US $ for the farmers. Individual costs of traders were 275.22 thousand US $. Individual costs of groceries/supermarkets were 5201.29 thousand US $. Individual cost of consumers was 1139 thousand US $. And the total social cost was 7412.39 thousand US $ (*Table 6*).

When individual costs were calculated at the national level, they were 216785.58 thousand US $ for farmers. Individual costs of traders were 72067.20 thousand US $. Individual costs of groceries/supermarkets were 133599.64 thousand US $. Individual cost of consumers was 300307.63 thousand US $. And the total social cost was 1925167.95 thousand US $ (Table 6). This value was approximately 4% of the annual agricultural income of 49.27 billion US $.

Buzby et al. (2014) assessed the total value of Food Loss and Waste (FLW) in the United States at $161.6 billion, with meat, poultry, fish, vegetables, and dairy contributing the most. Expanding to the entire food lifecycle, a 2016 report estimated a $218 billion value of FLW in the US, distributed across on-farm, processing, consumer-facing businesses, and households (ReFED, 2016). In the European Union, Stenmark et al., (2016) estimated the value of FLW at approximately 143 billion euros, with households accounting for two-thirds due to higher food value as it moves through the supply chain. Campoy-Muñoz et al., (2017) have directly incorporated FLW into economic models for comprehensive assessment. This study used models based on social accounting matrices to evaluate the impact of reducing avoidable FLW in various sectors across Spain, Germany, and Poland. Findings indicated potential economic output reductions ranging from -1.21% to -2.15%, emphasizing the intricate relationship between FLW and the broader economy.

Table 6. Monetary value of VLW

			Vegetable land (ha)	Farmers cost (Thousand US $)	Trader's cost (Thousand US $)	Cost of groceries/supermarkets (Thousand US $)	Consumer cost (Thousand US $)	Social cost (Thousand US $)
Individual costs	Greenhouses	Sample farms	0,61	0,30	0,13	2,81	0,53	3,77
		Çarşamba	415,00	201,51	90,29	1898,16	358,51	2548,47
		Türkiye	70897,50	34425,03	15425,21	324277,18	61246,25	435373,67
	Convantional farms	Sample farms	1,69	0,37	0,12	2,08	0,49	3,06
		Çarşamba	2682,90	595,38	184,93	3303,12	780,49	4863,92
		Türkiye	821758,60	182360,55	56641,99	1011730,36	239061,38	1489794,28
	Total	Sample farms	2,30	0,67	0,25	4,89	1,02	6,83
		Çarşamba	3097,90	796,88	275,22	5201,29	1139,00	7412,39
		Türkiye	892656,10	216785,58	72067,20	1336007,54	300307,63	1925167,95

4. Conclusions

The study delved into the examination and recycling of Vegetable Losses and Wastes (VLW) within the Vegetable Supply Chain (VSC) in the Çarşamba District of Samsun Province. The research findings revealed important insights into the stages and factors contributing to VLW, highlighting critical areas for intervention and improvement. Harvest emerged as a pivotal phase causing significant VLW due to manual labor, mishandling, and

inadequate training. The research emphasized the necessity of tailored training programs for workers and farm managers to enhance skills, reduce losses, and improve practices during harvesting and packaging. Although VLW-related challenges were shared between greenhouse and conventional farms, the actual quantities of VLW differed, with conventional farms experiencing higher losses. Insurance against VLW was relatively low, especially among conventional farms. Encouraging both farm types, particularly those more vulnerable to natural conditions, to invest in agricultural insurance could play a crucial role in mitigating VLW-associated losses. Recycling of VLW through composting emerged as an economically feasible solution, with a proposed compost plant exhibiting favorable profitability. The research underscored the importance of incentivizing greenhouse and conventional farms to participate in VLW recycling, suggesting the need to focus on enhancing income and working capital for these entities.

The research quantified individual and social losses across various stakeholders within the VSC. These findings further emphasized the economic and environmental significance of reducing VLW throughout the supply chain. It is clear that collaborative efforts involving governments, private sectors, local authorities, NGOs, and educational institutions are essential to effectively monitor and manage VLW, mitigating both individual and societal losses. Future research focusing on geographical variations among greenhouse and conventional farms could enhance the effectiveness of VLW monitoring and recycling initiatives. By systematically addressing the identified challenges and gaps, the vegetable supply chain could become more sustainable, economically resilient, and environmentally conscious, ultimately contributing to the betterment of the entire agricultural sector.

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Ethical Statement

This study was prepared under the permission numbered 2020/758, dated 27.11.2020, from the Ethics Committee of the Social and Human Sciences Ethics Committee of Ondokuz Mayıs University.

Conflicts of Interest

The authors declare no competing interests.

Authorship Contribution Statement

Selime Canan contributed to the project idea, design and execution of the study. Ebru Nur Özmen completed the survey work. Ebru Nur Özmen and Selime Canan analyzed and wrote the manuscript.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Hazro İlçesi (Diyarbakır) Bağ Alanlarında Salkım Güvesi [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)]' nin Ergin Popülasyon Değişimi ve Bulaşma Oranının Belirlenmesi*

Determination of Adult Population Fluctuation, Infection Rate of European Grapevine Moth [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)] in the Vineyards of Hazro District (Diyarbakır)

Ramazan SERMEN[1*], Mehmet KAPLAN[2]

Öz

Üzüm hem taze hem de kuru olarak insan beslenmesinde tüketilen önemli bir meyvedir. Bununla birlikte Türkiye ekonomisi için üzüm önemli bir ihraç ürünüdür. Bağlarda bitki koruma açısından birçok zararlı böcek ve hastalık etmeni bulunmaktadır. Bağ alanlarında çiçek ve salkımda beslenerek verim ve kaliteyi olumsuz etkileyen ana zararlı Salkım güvesi *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae)'dir. Bu çalışma, mazruni üzüm çeşidinin yoğun olarak yetiştirildiği Diyarbakır ili Hazro ilçesinde bağlarında 2021-2022 yılları arasında her biri 10'ar dekar olan iki bağda eşeysel çekici tuzaklar kullanılarak yürütülmüştür. Bağlarda ana zararlı olan Salkım güvesi'nin erginlerinin doğaya ilk çıkış zamanı, ergin popülasyon değişimi, aktif olarak doğada bulunduğu süre, döl sayısı ile bağlardaki salkımların bulaşıklık oranının belirlenmesi amaçlanmıştır. Çalışma sonucunda *L. botrana* ergin eşeysel çekici tuzaklarda ilk olarak 18 Nisan tarihinde ve erginlerin son olarak ta 14 Kasım tarihinde tespit edilmiştir. *L. botrana* ergin uçuşlarının bağ alanlarında yaklaşık 8 ay süreyle doğada aktif oldukları saptanmıştır. Zararlının 2021 yılında Hürriyet mahallesinde toplam 296 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 21 Ekim (21 ergin/tuzak), Yazgı mahallesinde ise toplam 1844 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 8 Temmuz (198 ergin/tuzak) tarihinde tespit edilmiştir. 2022 yılında ise, Hürriyet mahallesinde toplam 663 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 27 Haziran (80 ergin/tuzak), Yazgı mahallesinde ise toplam 2055 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 20 Haziran (233 ergin/tuzak) tarihinde tespit edilmiştir. Popülasyon yoğunluğuna bağlı olarak zararlının bölgede hasattan önce 3, hasat sonrası ise 1 olmak üzere yılda 4 tepe noktasını oluşturduğu ve doğa şartlarında tahmini olarak 4 döl verebildiği belirlenmiştir. Hürriyet mahallesindeki bağda salkımların zararlı ile bulaşık oranının yıllara göre sırasıyla %5 ve %23, Yazgı mahallesindeki bağda ise salkımların zararlı ile bulaşık oranının yıllara göre sırasıyla %25 ve %45 oranında olduğu tespit edilmiştir.

Anahtar Kelimeler: Salkım güvesi, Bulaşma oranı, Popülasyon değişimi, Bağ

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* Bu Çalışma Ramazan Sermen'in Yüksek Lisans tezinden üretilmiştir.

Abstract

Grape is an important fruit consumed both fresh and dried in human nutrition. However, grapes are an important export product for the Turkish economy. There are many harmful insect and disease factors in terms of plant protection in vineyards. The main harmful insect species that affects the yield and quality negatively by feeding on flowers and clusters in vineyards is European Grapevine Moth [*Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae)]. This study was carried out by using sexual attractive traps in two vineyards of 10 decares each between the years 2021-2022 in Diyarbakir Province Hazro District, where the mazruni grape variety is grown intensively. It was aimed to determine the first emergence time, adult population change, the period of active presence in nature, the number of progeny and the infestation rate of the clusters in the vineyards of the cluster moth, which is the main pest in the vineyards. As a result of the study, it was determined that the adult flights of *L. botrana* were first detected in the traps on 18 April, ended on 21 November, and they were active in nature for about 8 months. A total of 296 adults/traps were counted in the Hürriyet neighborhood in 2021, the maximum weekly population density was 21 October (21 adults/traps), and a total of 1844 adults/traps were counted in Yazgı neighborhood, and the maximum weekly population density was 8 July (198 adults/traps).) was detected on in 2022, a total of 663 adults/traps were counted in Hürriyet neighborhood, the maximum weekly population density was 27 June (80 adults/trap), and a total of 2055 adults/traps were counted in Yazgı neighborhood, and the maximum weekly population density was 20 June (233 adults/traps). trap) was detected. Depending on the population density, it has been determined that the pest creates 4 peaks per year, three before harvest and one after harvest, and can give an estimated 4 generations under natural conditions. In the vineyard in Hürriyet neighborhood, the rate of pest and infestation of the clusters was determined to be 5% and 23%, respectively, and in the vineyard in Yazgı neighborhood, the ratio of *L. botrana* to the pest and infestation was 25% and 45%, respectively.

Keywords: European Grapevine Moth, Infestation rate, Population fluctation, Vineyard

1. Giriş

İnsan beslenmesinde büyük bir öneme sahip olan üzüm, yaş ve kuru olarak tüketilen bir meyve türüdür. Ayrıca ülkemiz ekonomisi için önemli bir ihraç ürünüdür. Dünya Gıda ve Tarım Örgütü (FAO)' ya göre, 2020 yılı itibariyle dünyada 6.9 milyon ha alanda bağcılık yapılmaktadır. Bu üretim alanının %13.4'ü ile İspanya'da ilk sırada yer almaktadır. İspanya'yı sırasıyla Çin, Fransa, İtalya ve Türkiye izlemektedir. Türkiye'de 2020 üretim döneminde 4.2 milyon dekar alanda üzüm üretimi gerçekleşmiş olup, üzüm üretim miktarı ise 4.1 milyon tondur (Anonim, 2021a). Güneydoğu Anadolu Bölgesi'nde Diyarbakır İli bağcılık açısından önemli bir yere sahip olup, 2021 yılı Türkiye İstatistik Kurumu (TÜİK) verilerine göre, toplam 171.675 da alanda bağcılık yapılmaktadır. İldeki üzüm üretimi toplamı 100.727 ton, ortalama verim ise 630 kg da^{-1}'dır. Üzümler genellikle sofralık, pekmez, kurutmalık ve şaraplık olarak değerlendirilmektedir (Anonim, 2022).

Diyarbakır İli Hazro ilçesinde 9.144 da alanda bağcılık yapılmakta olup toplam üzüm üretimi ise 3.320 ton'dur. ÇKS sistemine kayıtlı ise toplam 4.432 da alanda bağcılık tarımsal faaliyeti gerçekleştirilmekte olup tamamı sofralık üzüm üretiminden oluşmaktadır. Hazro İlçesinde toplam 12 köyde bağcılık yapılmaktadır. Toplam işletme sayısı 959, toplam arazi sayısı ise 1.083'dür. Yörede yaygın olarak 11 tane yöresel çeşit yetiştirilmektedir. Sofralık üzüm çeşitlerinden, Diyarbakır il genelinde yaygın olan ve bölgede sevilerek tüketilen yöresel "Şire (Sin. Mazrumi)" üzüm çeşidinin yetiştiriciliği yaygındır. Bu çeşitlerden 10 tanesi eski dönemlerden günümüze ulaşan genotipler olup üreticiler tarafından farklı isimlendirilmiş ve özellikle yaşlı bağlarda kaybolmakta olan gen kaynaklarımızdır (Karataş ve ark., 2015). İlçede üzüm geleneksel yöntemlerle pekmez, pestil ve cevizli sucuk şeklinde değerlendirilmekte ve önemli bir geçim kaynağını teşkil etmektedir. Ayrıca, organik üzüm üreticiliği de yapılmaktadır (Anonim, 2021b).

Türkiye üzüm üretiminde verimi ve kaliteyi olumsuz olarak etkilemekte olan çok sayıda yabancı ot, hastalık etmeni ve zararlı böcek türleri vardır. Bağlarda bulunan yabancı ot türlerinin besin elementi, su, ışık ve yer konusunda asmalarla rekabet ederek ürününü verim ve kalitesini düşürmelerinin yanı sıra, birçok hastalık ve zararlıya konukçuluk ettiklerini bildirmişlerdir (Kara ve Ata, 2021). Bağlarda gelişmeyi ve verimi önemli oranda olumsuz etkileyen zararlı böcek türlerinden Mardin İlindeki bağ üreticilerin birçoğunun habersiz olduğunu bildirmişlerdir (Kaplan ve Bayhan, 2017). Zararlı böcek türlerinden Salkım güvesi (*Lobesia botrana* Den. & Schiff.) doğrudan üründe zarar verdiği için bağların ana zararlısı olarak kabul edilir. Söz konusu zararlının larvaları omcalarda tomurcuk, çiçek, koruk ve olgun tanelerde beslenmesiyle zarar vermektedir. Erken dönemde yaptıkları zarar sonucu tomurcuk ve çiçek dökülmelerine neden olarak seyrek taneli salkım oluşumuna sebep olmaktadır. Koruk ve olgun dane döneminde ise larvalar tanenin içerisinde beslenir ve bir tane ile yetinmeyip birden fazla tanede yer değiştirmesiyle zarar verir. Olgun tanede beslenme daha fazla olmakta ve tanede beslenme sonucu doğrudan yaptığı zarar yanında akan şekerli su sonucu saprofit fungusların çoğalmasına neden olarak dolaylı zarara sebep olmaktadır (Anonim, 2008). Mardin ili bağlarının ortalama %10 ile %18 oranında Salkım güvesi (*Lobesia botrana* Den. & Schiff.) ile bulaşık olduğunu, zararlının doğrudan tomurcuk, çiçek, koruk ve olgun tanelerde beslenerek zarar verdiğini, üründe verim ve kalite kaybına neden olduğunu bildirmiştir (Kaplan, 2020).

Çalışmada, Salkım güvesi'nin mücadelesine yönelik bazı biyoekolojik kriterlerden, doğada ilk ergin görülme zamanı, popülasyon değişimi, doğada aktif olarak bulunduğu süre, doğal koşullarda döl sayısı ile bağlardaki bulaşıklık oranının belirlenmesi amaçlanmıştır.

2. Materyal ve Metot

Çalışmanın ana materyalini, ilaçlama yapılmayan her biri 30 yaşındaki, 10'ar dekarlık telli terbiye sistemi ile kurulu ve mazruni üzüm çeşidinin üretildiği iki bağ, Salkım güvesinin biyolojik evreleri (yumurta, larva, prepupa, pupa ve ergin), delta tipi eşeysel çekici tuzaklar (Trece® incorporated Pherocon® CAP),ve çeşitli laboratuvar malzemeleri oluşturmuştur. Meteorolojik veriler ise Diyarbakır 15. Meteoroloji Bölge Müdürlüğünden alınmıştır.

2.1. Salkım güvesinin ergin popülasyon değişiminin belirlenmesi

Salkım güvesinin ergin popülasyon değişiminin belirlenmesi amacıyla 2021-2022 yılları arasında Diyarbakır İli Hazro İlçesi Yazgı ve Hürriyet mahallelerinde mazruni üzüm çeşidinin üretildiği iki bağda çalışma yürütülmüştür. Hürriyet mahallesindeki deneme alanına ilk feromon tuzaklar ortalama nispi nem %55.3 ve sıcaklık 7.9 °C olduğu 12 Nisan tarihinde asılmıştır. Yazgı mahallesindeki deneme alanına ilk feromon tuzaklar ortalama nispi nem %55.3 ve ortalama sıcaklık 7.9 °C olduğu 12 Nisan tarihinde asılmıştır. Her iki bağa 3 adet olacak

şekilde omcaların güney yönüne salkım seviyesinde ve hakim rüzgar yönünde asılmıştır (Altındişli ve Kısmalı, 1996; Anonim, 2008). Haftalık olarak yapılan tuzak kontrollerinde yapışkan tuzaklarda yakalanan kelebekler sayılarak kaydedilmiştir. Tuzakların feromon içeren kapsülleri, üretici firma beyanına uygun olarak 4-6 haftada bir ve diğer kısımları ise kirlendikçe değiştirilmiştir.

2.2. Salkım güvesinin üzüm salkımlarındaki bulaşıklık oranı belirlenmesi

Salkım güvesinin bulaşıklık oranını belirlemek için, Hürriyet ve Yazgı mahallelerindeki iki bağda hasat döneminde köşegenler yönünden gidilerek her birinde bağı homojen olarak temsil edecek şekilde rasgele seçilen 20 omcanın 4 farklı yönünden her birinin değişik kısımlarından olmak şartıyla beşer adet salkım olmak üzere toplamda 100 salkım kontrol edilmiştir (Kısakürek, 1972). Salkımlar üzerinde zararlının herhangi bir biyolojik döneminin veya zararının görülmesi durumunda salkım bulaşık kabul edilmiştir. Zararlı ile bulaşık salkım sayısının toplam salkım sayısına oranlanmasıyla da her bağdaki yüzde (%) bulaşıklık oranı belirlenmiştir.

3. Araştırma Sonuçları ve Tartışma

3.1. Salkım güvesinin popülasyon değişimi

Diyarbakır ili Hazro ilçesi Hürriyet ve Yazgı mahallelerindeki 2021 ve 2022 yıllarına ait Salkım güvesi'nin erginlerinin eşeysel çekici tuzaklarda yakalanma sayılarına göre popülasyon değişimleri *Şekil 1, 2, 3* ve *4*'te verilmiştir.

3.1.1. Hürriyet mahallesi bağ alanına ait 2021 yılındaki Salkım güvesinin popülasyon değişimi

Diyarbakır ili Hazro ilçesi Hürriyet mahallesi bağlarındaki Salkım güvesi'nin erginlerinin eşeysel çekici tuzaklarda yakalanma sayılarına göre 2021 yılına ait popülasyon değişimi *Şekil 1*'de verilmiştir.

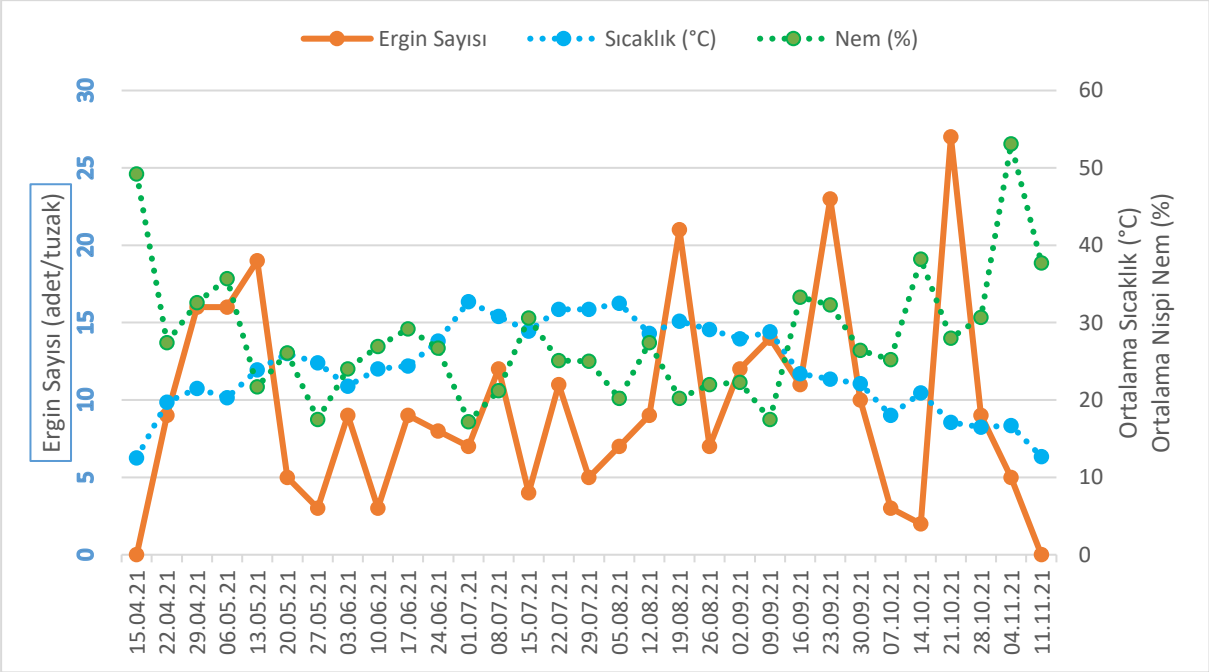

Figure 1. Climate data of the Hürriyet neighborhood vineyard area for 2021 and the adult population change of the cluster moth in the pheromone trap

Şekil 1. Hürriyet mahallesi bağ alanına ait 2021 yılı iklim verileri ve salkım güvesinin feromon tuzaktaki ergin popülasyon değişimi

Şekil 1. İncelendiğinde, Hürriyet mahallesindeki deneme alanına ilk feromon tuzak ortalama nispi nem %55.3 ve ortalama sıcaklık 7.9 °C olduğu 12 Nisan tarihinde asılmıştır. Feromon tuzaklar ilk ergin yakalanana kadar haftada iki kez, ilk ergin tespitinden sonra haftalık olarak düzenli bir şekilde kontrol edilmiştir. Yapılan kontrollerde ilk ergin (9 ergin/tuzak) 22 Nisan tarihinde ortalama nispi nem %27.4 ile ortalama sıcaklık değerinin 19.7 °C olduğu ve bitki sürgünlerinin 3-4 cm olduğu dönemde yakalanmıştır. Yıl içerisinde yapılan kontroller

sonucunda salkım güvesinin popülasyon yoğunluğunun en yüksek olduğu tarihler, 13 Mayıs (19 ergin/tuzak), 19 Ağustos (21 ergin/tuzak), 23 Eylül (23 ergin/tuzak) ve hasat sonrası 21 Ekim (21 ergin/tuzak) olarak tespit edilmiştir. Hürriyet mahallesindeki bağda 2021 yılında yapılan tuzak kontrollerinde yakalanan salkım güvesi ergin sayısının toplamı 296 olarak belirlenmiştir. Salkım güvesinin hasat dönemine kadar ergin popülasyon değişimine bağlı olarak üç, hasattan sonra ise bir olmak üzere toplam dört tepe noktası oluşturduğu tespit edilmiştir. Tepe noktalarının 13 Mayıs, 19 Ağustos, 23 Eylül ile 21 Ekim tarihlerinde oluştuğu ve buna bağlı olarak yılda 4 döl verdiği görülmüştür. Ayrıca tepe noktaları asmanın çiçeklenme dönemi (1. döl), ben düşme dönemi (2. döl), olgunlaşma dönemine (3. döl) ve hasat sonrası döneme denk geldiği tespit edilmiştir. Salkım güvesi ergin uçuşlarının 22 Nisan tarihinde başlayıp 4 Kasım tarihinde son bulunduğu ve yaklaşık 7 ay süreyle doğada aktif oldukları gözlemlenmiştir.

3.1.2. Yazgı mahallesi bağ alanına ait 2021 yılındaki Salkım güvesinin popülasyon değişimi

Diyarbakır ili Hazro ilçesi Hürriyet mahallesi bağlarındaki Salkım güvesi'nin erginlerinin eşeysel çekici tuzaklarda yakalanma sayılarına göre 2021 yılına ait popülasyon değişimi *Şekil 2*'de verilmiştir.

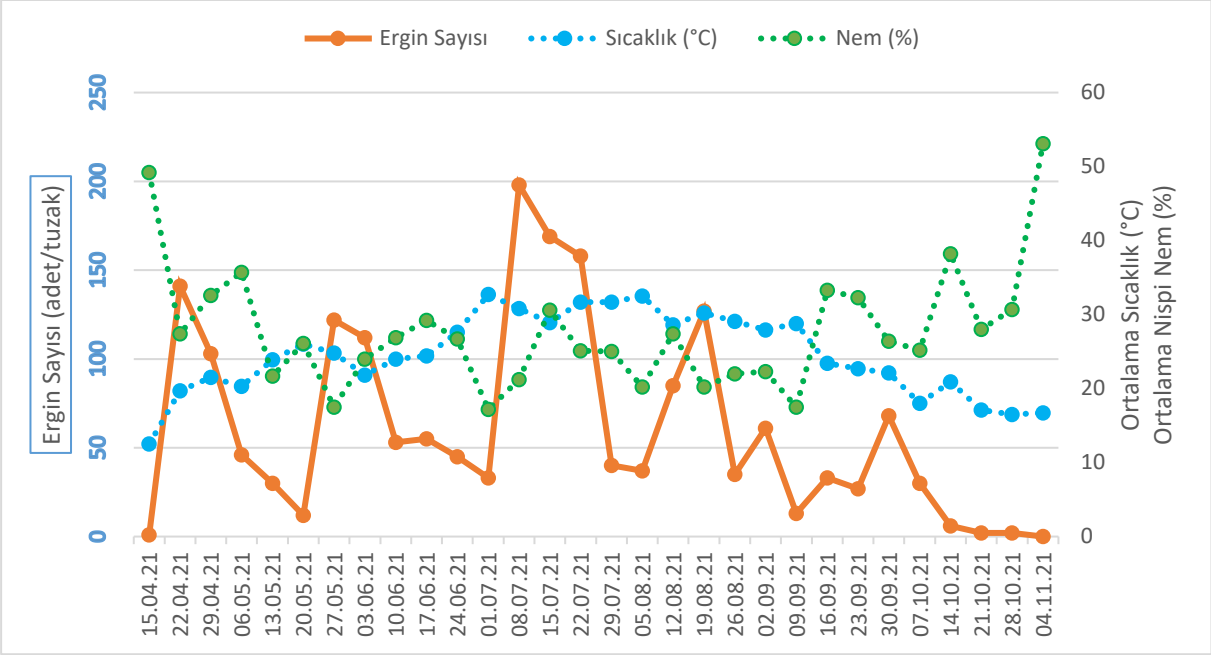

Figure 2. Climate data of Yazgı district vineyard area for 2021 and adult population change of cluster moth in pheromone trap

Şekil 2. Yazgı mahallesi bağ alanına ait 2021 yılı iklim verileri ve salkım güvesinin feromon tuzaktaki ergin popülasyon değişimi

Şekil 2 incelendiğinde, Yazgı mahallesindeki deneme alanına ilk feromon tuzak ortalama nispi nem %55.3 ve ortalama sıcaklık 7.9 °C olduğu 12 Nisan tarihinde asılmıştır. Feromon tuzaklar ilk ergin yakalanana kadar haftada iki kez, ilk ergin tespitinden sonra haftalık olarak düzenli bir şekilde kontrol edilmiştir. Yapılan kontrollerde ilk ergin (1 ergin/tuzak) 15 Nisan tarihinde ortalama nispi nem %49.2 ile ortalama sıcaklık değerinin 12.5 °C olduğu ve bitki sürgünlerinin 3-4 cm olduğu dönemde yakalanmıştır. Yıl içerisinde yapılan kontroller sonucunda salkım güvesinin popülasyon yoğunluğunun en yüksek olduğu tarihler, 22 Nisan (141 ergin/tuzak), 27 Mayıs (122 ergin/tuzak), 8 Temmuz (198 ergin/tuzak) ve 19 Ağustos (127 ergin/tuzak) olarak tespit edilmiştir. Yazgı mahallesindeki bağda 2021 yılında yapılan tuzak kontrollerinde yakalanan salkım güvesi ergin sayısının toplamı 1844 olarak tespit edilmiştir. Salkım güvesinin hasat dönemine kadar ergin popülasyon değişimine bağlı olarak dört tepe noktası oluşturduğu tespit edilmiştir. Tepe noktalarının 22 Nisan, 27 Mayıs, 8 Temmuz ile 19 Ağustos tarihlerinde oluştuğu ve buna bağlı olarak yılda 4 döl verdiği görülmüştür. Ayrıca tepe noktaları asmanın sürgün boyu 15-20 cm olduğu dönem (1. döl), çiçeklenme dönemi (2. döl), koruk dönemi (3. döl) ve ben düşme dönemine (4. döl) denk geldiği tespit edilmiştir. Salkım güvesi ergin uçuşlarının 15 Nisan tarihinde başlayıp 28 Ekim

tarihinde son bulunduğu ve yaklaşık 7 ay süreyle doğada aktif oldukları gözlemlenmiştir.

3.1.3. Hürriyet mahallesi bağ alanına ait 2022 yılındaki Salkım güvesinin popülasyon değişimi

Diyarbakır ili Hazro ilçesi Hürriyet mahallesi bağlarındaki Salkım güvesi'nin erginlerinin eşeysel çekici tuzaklarda yakalanma sayılarına göre 2022 yılına ait popülasyon değişimi *Şekil 3*'te verilmiştir.

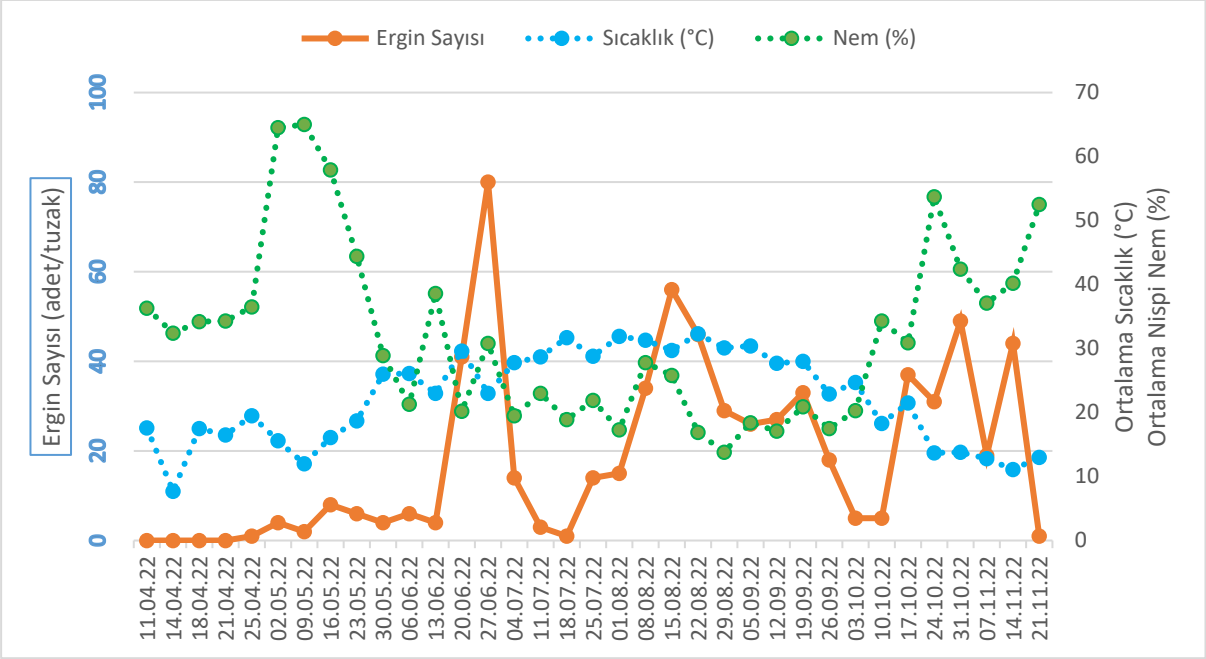

Figure 3. Climate data of the Hürriyet neighborhood vineyard area in 2022 and the adult population change of the cluster moth in the pheromone trap

Şekil 3. Hürriyet mahallesi bağ alanına ait 2022 yılı iklim verileri ve salkım güvesinin feromon tuzaktaki ergin popülasyon değişimi

Şekil 3 incelendiğinde, Hürriyet mahallesindeki deneme alanına ilk feromon tuzak ortalama nispi nem %40.3 ve ortalama sıcaklık 17 °C olduğu 7 Nisan tarihinde asılmıştır. Feromon tuzaklar ilk ergin yakalanana kadar haftada iki kez, ilk ergin tespitinden sonra haftalık olarak düzenli bir şekilde kontrol edilmiştir. Yapılan kontrollerde ilk ergin (1 ergin/tuzak) 25 Nisan tarihinde ortalama nispi nem %36.5 ile ortalama sıcaklık değerinin 19.5 °C ve bitki sürgünlerinin 0-5 cm olduğu dönemde yakalanmıştır. Yıl içerisinde yapılan kontroller sonucunda salkım güvesinin popülasyon yoğunluğunun en yüksek olduğu tarihler, 27 Haziran (80 ergin/tuzak), 15 Ağustos (56 ergin/tuzak)ve hasat sonrası 31 Kasım (49 ergin/tuzak) olarak tespit edilmiştir. Hürriyet mahallesindeki bağda 2022 yılında yapılan tuzak kontrollerinde yakalanan salkım güvesi ergin sayısının toplamı 663 olarak belirlenmiştir. Salkım güvesinin hasat dönemine kadar ergin popülasyon değişimine bağlı olarak iki, hasattan sonra ise bir olmak üzere toplam üç tepe noktası oluşturduğu tespit edilmiştir. Tepe noktalarının 27 Haziran, 15 Ağustos ile 31 Kasım tarihlerinde oluştuğu ve buna bağlı olarak yılda 3 döl verdiği görülmüştür. Ayrıca tepe noktaları asmanın koruk dönemi (1. döl), ben düşme dönemi (2. döl) ve hasat sonrası döneme denk geldiği tespit edilmiştir. Salkım güvesi ergin uçuşlarının 25 Nisan tarihinde başlayıp 21 Kasım tarihinde son bulunduğu ve yaklaşık 8 ay süreyle doğada aktif oldukları gözlemlenmiştir.

3.1.4. Yazgı mahallesi bağ alanına ait 2022 yılındaki Salkım güvesinin popülasyon değişimi

Diyarbakır ili Hazro ilçesi Yazgı mahallesi bağlarındaki Salkım güvesi'nin erginlerinin eşeysel çekici tuzaklarda yakalanma sayılarına göre 2022 yılına ait popülasyon değişimi *Şekil 4*'te verilmiştir.

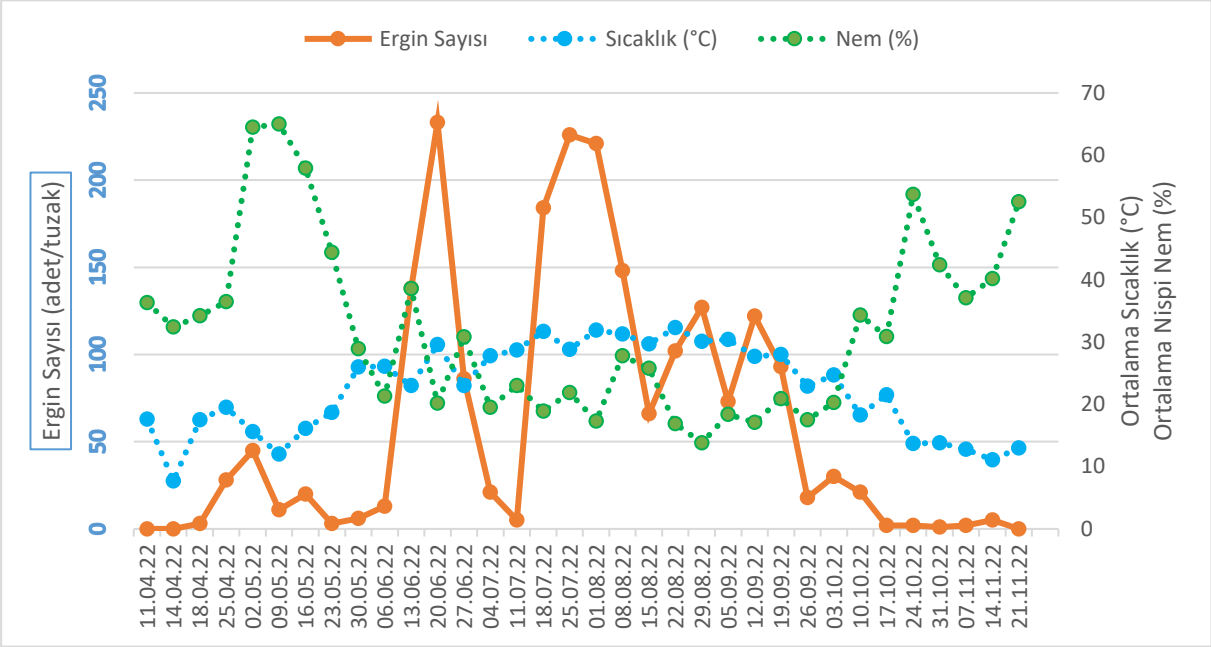

Figure 4. Climate data of Yazgı district vineyard area in 2022 and adult population change of cluster moth in pheromone trap

Şekil 4. Yazgı mahallesi bağ alanına ait 2022 yılı iklim verileri ve salkım güvesinin feromon tuzaktaki ergin popülasyon değişimi

Şekil 4 incelendiğinde, Yazgı mahallesindeki deneme alanına ilk feromon tuzak ortalama nispi nem %40.3 ve ortalama sıcaklık 17 °C olduğu 7 Nisan tarihinde asılmıştır. Feromon tuzaklar ilk ergin yakalanana kadar haftada iki kez, ilk ergin tespitinden sonra haftalık olarak düzenli bir şekilde kontrol edilmiştir. Yapılan kontrollerde ilk ergin (3 ergin/tuzak) 18 Nisan tarihinde ortalama nispi nem %34.2 ile ortalama sıcaklık değerinin 17.5 °C olduğu ve bitki gözlerinin yeni patlamaya başladığı dönemde yakalanmıştır. Yıl içerisinde yapılan kontroller sonucunda salkım güvesinin popülasyon yoğunluğunun en yüksek olduğu tarihler, 20 Haziran (233 ergin/tuzak), 25 Temmuz (226 ergin/tuzak), 29 Ağustos (127 ergin/tuzak) ve 12 Eylül (122 ergin/tuzak) olarak tespit edilmiştir. Yazgı mahallesindeki bağda 2022 yılında yapılan tuzak kontrollerinde yakalanan salkım güvesi ergin sayısının toplamı 2055 olarak tespit edilmiştir. Salkım güvesinin hasat dönemine kadar ergin popülasyon değişimine bağlı olarak dört tepe noktası oluşturduğu gözlemlenmiştir. Tepe noktalarının 20 Haziran, 25 Temmuz, 29 Ağustos ile 12 Eylül tarihlerinde oluştuğu ve buna bağlı olarak yılda 4 döl verdiği görülmüştür. Ayrıca tepe noktaları asmanın koruk dönemi (1. döl), koruk dönemi (2. döl), ben düşme dönemine (3. döl) ve hasat dönemine (4. döl) denk geldiği tespit edilmiştir. Salkım güvesi ergin uçuşlarının 18 Nisan tarihinde başlayıp 14 Kasım tarihinde son bulunduğu ve yaklaşık 8 ay süreyle doğada aktif oldukları gözlemlenmiştir.

Diyarbakır İli Hazro İlçesinde denemenin yapıldığı Hürriyet ve Yazgı bağ alanlarında Salkım güvesi ergininin kurulan eşeysel çekici tuzaklar sayesinde her iki yılda da doğaya ilk çıkışının nisan ayının ortasında başladığı, kasım ayının sonlarına kadar devam ettiği ve doğada yaklaşık 8 ay aktif oldukları gözlemlenmiştir. Yapılan çalışmaya benzer olarak (Şekerden-Çağlar, 2009), Hatay ilinde Salkım güvesi erginlerinin doğaya ilk çıkışlarının mart ayında olduğunu ve asmanın vejetasyon süresi boyunca aktif olduklarını; (Mamay ve Çakır, 2014), Şanlıurfa ilinde *L. botrana* erginleri doğaya ilk çıkışlarının nisan ayı sonundan itibaren başlandığı, doğada aktif olarak ekim ayının ilk yarısına kadar devam ettiklerini; (Kaplan ve ark., 2016), Mardin İli Mazıdağı ilçesinde *L. botrana* erginlerinin doğaya ilk olarak 10-18 Nisan tarihlerinde çıkış yaptıkları, yaklaşık olarak doğada 7 ay süreyle aktif kaldıklarını; (Aslan ve Candan, 2018), Gaziantep ilinde yaptıkları çalışmada Salkım güvesi erginlerinin doğaya ilk çıkışlarının mayıs ayının ilk haftasında başlayıp popülasyon yoğunluğunun ağustos ayının ilk haftasına kadar devam ettiklerini; (Çakmak, 2019), Adıyaman İli Besni ilçesinde *L. botrana*'nın doğaya ilk ergin çıkışının 12 Nisan'da başlayıp 9 Kasım'a kadar sürdüğünü ve erginlerin doğada 7 ay süre ile aktif olduğunu; (Özdem ve ark., 2022), tarafından Ankara ilinde yürütülen bir çalışmada, *L. botrana*'nın ilk ergin çıkışı nisan ayında gerçekleştiği,

yaklaşık altı ay boyunca doğada aktif olduğu ve yetişkinlerin uçuşunun eylül ayında sona erdiği bildirmişlerdir.

Çalışmanın yürütüldüğü 2021-2022 yıllarında ilk ergin çıkışları sırasıyla 15 Nisan tarihinde ortalama nispi nem %49.2, ortalama sıcaklık değerinin 12.5 °C ve 18 Nisan tarihinde ortalama nispi nem %34.2, ortalama sıcaklık değerinin 17.5 °C olduğu Yazgı mahallesinde tespit edilmiştir. Buna karşın Öztürk ve Canıhoş (2002), ilk ergin uçuşları orantılı nem değerlerinin %40-70 arasında olduğunu bildirmişler. Ayrıca Anonim (2008), ergin uçuşlarının akşamüzeri sıcaklık değerinin 10 °C üzerinde olduğunda gerçekleştiği belirtilmiş ve çalışma sonucundaki verilerle benzerlik göstermektedir. Salkım güvesinin 2021-2022 yıllarında yapılan popülasyon takibi süresince tepe noktalarının Hürriyet mahallesinde mayıs, haziran, ağustos, eylül ile ekim, kasım, Yazgı mahallesinde ise nisan, mayıs, haziran, temmuz ile eylül aylarına denk geldiği ve bu farklılığın nedeninin iklimsel değişikliklerden dolayı oluştuğu kanaatine varılmıştır. Popülasyon yoğunluğuna bağlı olarak zararlının bölgede hasattan önce üç, hasattan sonra ise bir olmak üzere yılda 4 tepe noktasını oluşturduğu ve dolayısıyla doğal şartlarda 4 döl verdiği anlaşılmaktadır. Hürriyet mahallesinde hasattan önce üç hasattan sonra ise bir olmak üzere dört tepe noktası oluştuğu, Yazgı mahallesinde ise hasattan önce dört tepe noktası oluştuğu ve hasattan sonra tepe noktasının oluşmadığı gözlemlenmiştir. Çalışma yapılan yıllarda zararlının oluşturduğu tepe noktaları ile bitkinin fenolojisini ilişkilendirdiğimizde, ilk tepe noktasının asmanın sürgün boyu 15-20 cm olduğu döneme (1. döl), ikinci tepe noktasının çiçeklenme dönemine (2. döl), üçüncü tepe noktasının koruk- ben düşme dönemine (3. döl) ve dördüncü tepe noktası ise hasat sonrası dönem ile olgunlaşma dönemine (4. döl) denk geldiği tespit edilmiştir. Yapılan benzer çalışmalar; (Şekerden-Çağlar, 2009), Hatay ilinde Salkım güvesi erginlerinin popülasyon yoğunluğunun asmanın çiçeklenme, koruk ve olgunlaşma dönemlerinde daha yüksek olduğu; (Saeidi ve Kavoosi, 2011), 2008-2009 yıllarında Sisakht (İran) bölgesinde *L. botrana*'nın tamamlanmamış dördüncü döl ile üç döl verdiğini; (Karnıbüyükler, 2014), *L. botrana*'nın Meram (Konya) ilçesinde üç döl verdiğini ve bunun mayıs, temmuz ile ağustos aylarına denk geldiğini; (Mamay ve Çakır, 2014), Şanlıurfa merkez ilçeye bağlı Ulubağ ve Öğütçü köyü bağ alanlarında *L. botrana*'nın doğal şartlarda 3-4 döl verdiklerini; (Kaplan ve ark., 2016), 2012 ve 2013 yıllarında Mardin ili Mazıdağı ilçesinde *L. botrana*'nın mayıs, temmuz ve ağustos olmak üzere üç tepe noktası oluşturduğu, bu tepe noktaları asmanın çiçeklenme dönemi (1. döl), koruk dönemi (2. döl) ve ben düşme dönemine (3. döl) denk geldiğini; (Ünlü ve Güleç, 2018), Manisa ili Ahmetli ve Turgutlu ilçelerinde Salkım güvesi ergin sayıları bakımından hasat tarihine kadar üç, hasattan sonra da bir olmak üzere toplam dört tepe noktası oluştuğunu; (Özdem ve ark., 2022), Ankara ilinde Salkım güvesinin her iki yılda da nisan ve haziran ayı olmak üzere iki tepe noktası oluşturduğu ve yılda iki döl verdiğini bildirmişlerdir.

Denemenin yapıldığı bağ alanlarında salkım güvesine karşı kurulan eşeysel çekici feromon tuzaklarda yapılan ergin sayım sonucuna göre; 2021 yılında Hürriyet mahallesinde toplam 296 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 21 Ekim (21 ergin/tuzak), Yazgı mahallesinde ise toplam 1844 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 8 Temmuz (198 ergin/tuzak) tarihinde tespit edilmiştir. 2022 yılında ise, Hürriyet mahallesinde toplam 663 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 27 Haziran (80 ergin/tuzak), Yazgı mahallesinde ise toplam 2055 ergin/tuzak sayılmış olup, en fazla haftalık popülasyon yoğunluğu 20 Haziran (233 ergin/tuzak) tarihinde tespit edilmiştir. Yazgı mahallesinde kurulan deneme alanının ovada, Hürriyet mahallesinde ise dağlık alanda olması ve yükseklik farkından dolayı oluşan ekolojik koşullardan kaynaklı iki lokasyon arasında bazı farklar oluşmakta ve bu nedenle Yazgı mahallesindeki asma gözlerinin bir hafta önce sürdüğü tespit edilmiştir. Bu iklimsel farklılıktan dolayı her iki yılda da ilk ergin çıkışı da Yazgı mahallesinde görülmüştür. Ayrıca Yazgı mahallesindeki bağların daha bakımlı olmasından dolayı iki deneme alanlarında kurulan tuzaklarda yakalanan toplam ergin popülasyon yoğunluğunun farklı olmasına neden olduğu kanaatine varılmıştır. Nitekim böcekler faaliyetlerini devam ettirmeleri, gelişme ve çoğalmayı sürdürebilmeleri için uygun ekolojik koşullara sahip, besin çeşidi ve miktarınca zengin olduğu yerleri tercih etmektedirler. Dolaysıyla böyle yerlerde böcekleri popülasyon yoğunluğuda artmaktadır. Yapılan çalışmaya benzer olarak (Güleç, 2014), Manisa ili Turgutlu ve Ahmetli ilçelerinde yaptığı çalışmada, Salkım güvesi ergin sayısı 2012 yılı hasat dönemine kadar en fazla Turgutlu ilçesi Derbent beldesi (439 adet/tuzak/hafta), Ahmetli ilçesi Karaköy (394 adet/tuzak/hafta)'ünde 23 Ağustos tarihinde, hasat döneminden sonra ise Turgutlu ilçesi Akçapınar (504 adet/tuzak/hafta) ve Ahmetli ilçesi Gökkaya beldesi (477 adet/tuzak/hafta)'nde 30 Ağustos tarihinde ve yine 2013 yılında hasat dönemine kadar en fazla Turgutlu ilçesi Sarıbey (345 adet/tuzak/hafta), Ahmetli ilçesi Ataköy (306 adet/tuzak/hafta)'ünde 22 Ağustos tarihinde, hasat döneminden sonra ise Turgutlu ilçesi Sarıbey (647 adet/tuzak/hafta) ve Ahmetli ilçesi Ataköy (426 adet/tuzak/hafta)'ünde 12 Eylül tarihinde yakaladığını belirtmiştir.

3.2. Salkım güvesinin üzüm salkımlarındaki bulaşıklık oranı

Diyarbakır İli Hazro İlçesi Hürriyet ve Yazgı mahallesinde belirlenen bağlarda, salkım güvesinin bulaşıklık oranını saptamak üzere 2021-2022 yıllarında söz konusu deneme alanlarının her birinde bağı homojen olarak temsil edecek şekilde rasgele seçilen 100 salkım kontrol edilmiştir. Yapılan Salkım kontrolleri sonucu elde edilen bulaşıklık oranı verileri *Tablo 1*'de verilmiştir.

Tablo 1. Hürriyet ve Yazgı mahalleleri bağlarında salkım güvesinin 2021-2022 yıllarındaki bulaşıklık oranı ile feromon tuzaklarda yakalanan toplam ergin sayısı

Table 1. Growth of cluster moth in the vineyards of Hürriyet and Yazgı neighborhoods in 2021-2022 infestation rate and total number of adults caught in pheromone traps

İlçe	Mahalle	Bulaşıklık Oranı (%)			Toplam Ergin Sayısı (adet/tuzak)	
		2021	2022	Ortalama	2021	2022
Hazro	Hürriyet	5	23	14	296	663
	Yazgı	25	45	35	1844	2055

Salkım güvesinin bulaşıklık oranını belirlemek için üzümlerin hasada yakın döneminde tespit çalışmaları yapılmıştır. Tablo 1. incelendiğinde, salkım güvesinin popülasyon yoğunluğunun artmasıyla salkımlardaki bulaşıklık oranınında paralel olarak arttığı görülmüştür. Hürriyet mahallesinde bulunan deneme alanındaki salkımlar ortalama %14, Yazgı mahallesinde bulunan deneme alanındaki salkımlar ise ortalama %35 oranında *L. botrana* ile bulaşık olduğu tespit edilmiştir. Manisa ilindeki bağlarda yapılan bir çalışmada, 2012-2013 yıllarında Turgutlu ilçesinde sırasıyla %41 ve %62, Ahmetli ilçesinde ise %46 ve %58 oranında salkım güvesi ile bulaşık olduğunu bildirmiştir (Güleç, 2014). (Mamay ve Çakır, 2014), Şanlıurfa'da yapılan çalışmada *L. botrana'*nın bulaşıklık oranının en yüksek 2011-2012 yıllarında Öğütçü köyünde sırasıyla %52 ve %34 olduğunu bildirmişlerdir. (Kaplan ve ark., 2016), salkım güvesinin bulaşıklık oranı 2012-2013 yıllarında Ömürlü köyündeki bağda sırasıyla %12 ile %15 iken, Evciler köyünde bu oran %10 ile %18 olduğunu bildirmişlerdir. Yine Adıyaman'da yapılan benzer bir çalışmada, zararlının çeşitlere göre bulaşıklık oranı Alphonse Lavallee, Serpenekıran ve Besni kurutmalık üzüm çeşitlerinde sırasıyla %41, %66 ve %44 oranında olduğu ve telli terbiye sistemi ile goble (kütük) sistemindeki bulaşıklık oranı bakımından ise sırasıyla %66 ile %73 oranında olduğunu bildirmiştir (Çakmak, 2019).

4. Sonuç

Diyarbakır ili Hazro bağ alanlarında Salkım güvesi popülasyonunun izlendiği her iki yılda da eşeysel çekici tuzaklarda kelebeklerin ilk olarak nisan ortasında doğada görülmüştür. Bağın fenolojisi boyunca mayıs, temmuz ve ağustos aylarında hasattan önce 3 tepe noktası ve eylül ayında 1 tepe noktası olmak üzere 4 tepe noktası oluşturduğu, ekim- kasım aylarında kışlamaya çekildiği ve dolayısıyla yaklaşık 7 ay doğada aktif kaldığı gözlenmiştir. Nitekim Salkım güvesinin bağlarda yılda 3-4 döl verdiği belirlenmiştir. İki yıllık veriler birlikte değerlendirildiğinde, bağların zararlı ile ortalama bulaşıklık oranı en düşük %5 ve en yüksek %45 oranında olduğu belirlenmiştir. Zararlı ile mücadelede yumurtadan larva çıkışının takip edilmesi koşulu ile mayıs-haziran (çiçeklenme) ve ağustos (ben düşme) aylarında yapılacak birer ilaçlama ile başarılı bir sonuç alınabileceği öngörülmektedir. Ayrıca, bağlarda uygun budamanın ve yabancıotların temizlenmesi vb. kültürel önlemlerin uygulanması zararlının faaliyetlerinin ve zararının azaltılması bakımından. önemlidir.

Etik Kurul Onayı

Bu çalışma için etik kuruldan izin alınmasına gerek yoktur.

Çıkar Çatışması Beyanı

Makale yazarları olarak aramızda herhangi bir çıkar çatışması olmadığını beyan ederiz.

Yazarlık Katkı Beyanı

Planlama: Sermen, R, Kaplan, M.; Materyal ve Metot: Sermen, R, Kaplan, M.; Veri toplama ve İşleme: Sermen, R, Literatür Tarama: Sermen, R,; Makale Yazımı, İnceleme ve Düzenleme: Sermen, R, Kaplan, M.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of Deficit Irrigation to Some Bio – Active Compounds, Quality Traits and Grain Yield in Maize

Kısıtlı Sulama Uygulamalarının Mısırda Bazı Biyo – Aktif Bileşenler, Kalite Özellikleri ve Tane Verimine Etkileri

Elif ÖZDEMİR[1*], Bayram SADE[2]

Abstract

As a result of environmental difficulties and water scarcity, maize production has been challenged in arid and semi – arid regions. A management strategy for stabilizing corn production under water shortage conditions involves the use of drought – tolerant hybrids and suitable irrigation regimes. The study was conducted in the Prof. Dr. Abdulkadir AKÇIN trial area of "Selcuk University, Agriculture Faculty, Crop Science Department, Konya, TÜRKİYE" during 2019 and 2020 growing seasons. The aim of the current work is determining the effects of deficit irrigation and grain colour factors on water shortage tolerance of maize in terms of bio – active compounds, quality traits and grain yield. The factors of the trial were irrigations [50% (I1), 75% (I2) and 100% (I3) of evaporation from Class A evaporation PAN] and genotypes [DKC 5783, red corn and Sakarya]. It was noted that DKC 5783 had the highest values at two (thousand grain weight, grain yield), Sakarya had at two (total phenolic compounds and grain crude oil) and red corn had at four (total anthocyanin content, total antioxidant activity, grain crude protein and starch) properties among eight under I1 during 2019 as well as red corn had at two (total anthocyanin content and total phenolic compounds), Sakarya had at two (grain crude protein and grain crude oil) and DKC 5783 had highest values at four (thousand grain weight, total antioxidant activity, starch and grain yield) features among eight under I1 during 2020. Thousand grain weight values of I3 were 6.71% and 0.57% more than I2 while starch was 9.19% and 3.96% more than I2 during 2019 – 2020. Red corn had better contents of bio – active compounds than other two varieties during both years of the trial. The mean yield of I3 was 6.87% more than I2 in 2019 and 9.05% more than I2 in 2020 which revealed that regulated deficit irrigation might help growers to cope with decline in water availability also 25% water restriction caused tolerable decreases in grain yield and some yield compounds of the current work.

Keywords: Water scarcity, Physiology, Anthocyanins, Antioxidants, Phenolics

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Öz

Mısır üretimi kurak ve yarı kurak bölgelerde çevresel problemler ve su kıtlığının bir sonucu olarak zorlaşmaktadır. Kuraklığa dayanıklı hibritlerin ve uygun sulama rejimlerinin belirlenmesi su kıtlığı koşullarında mısır üretimini stabilize etmeye yönelik stratejilerdir. Deneme mısırda kısıtlı sulama uygulamaları ve tane rengi faktörünün bazı kalite özellikleri üzerine etkilerini belirlemek amacıyla 2019 – 2020 yetiştirme sezonlarında "Selçuk Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Prof. Dr. Abdulkadir AKÇIN Deneme Alanı, Konya, TÜRKİYE" de yürütülmüştür. Denemede faktör olarak sulama [Class A evaporasyon kabından gerçekleşen buharlaşmanın %50 (I1)' si, %75 (I2)' i ve %100 (I3)' ü] ve genotipler [DKC 5783, kırmızı mısır ve Sakarya] yer almıştır. DKC 5783' ün 2019 yılı I1 uygulamasında sekiz özelliğin ikisinde (bin tane ağırlığı ve tane verimi), Sakarya' nın ikisinde (total fenolik bileşenler ve tane ham yağı), kırmızı mısırın dördünde (total antosiyanin içeriği, total antioksidan aktivitesi, tane ham protein ve nişasta) en yüksek değerlere sahip oldukları, bununla birlikte kırmızı mısırın 2020 yılı I1 uygulamasında sekiz özelliğin ikisinde (total antosiyanin içeriği ve total fenolik bileşenler), Sakarya' nın ikisinde (tane ham protein ve tane ham yağ), DKC 5783' ün ise dördünde (bin tane ağırlığı, total antioksidan aktivitesi, nişasta ve tane verimi) en yüksek değerlere sahip oldukları kaydedilmiştir. 2019 – 2020 yetiştirme sezonlarının I3 uygulamasında bin tane ağırlığının I2' ye göre sırasıyla %6.71 ve %0.57 yüksek olduğu, nişasta içeriğinin ise her iki yılda da I2' ye göre %9.19 ve %3.96 yüksek bulunduğu belirlenmiştir. Kırmızı renkli mısırın biyo – aktif bileşen içeriğinin denemenin her iki yılında da diğer varyetelerden daha iyi olduğu görülmüştür. 2019 yılında I3' teki ortalama verim I2' den %6.87, 2020 de ise %9.05 yüksek bulunmuş, bu sonuçlar kısıtlı sulama uygulamalarının su kullanım etkinliğini olumlu yönde etkilediğini göstermiştir. Ayrıca %25' lik su kısıntısının mevcut çalışmada bazı verim unsurlarında tolere edilebilir kayıplar oluşturduğu izlenmiştir.

Anahtar Kelimeler: Su kıtlığı, Fizyoloji, Antosiyaninler, Antioksidanlar, Fenolikler

1. Introduction

Increasing crop productivity is necessary because of climate, rainfall changes (Nasseri, 2021) as well as population but in many countries around the world, abiotic stressors as drought cause yield loses (Soltanbeigi, 2019) and adversely affect human and animal nutrition.

In plant production, the average product loss due to biotic and abiotic stress factors varies between 65% and 87% (Kacar et al., 2009). Water stress is one of the most well – known abiotic stress factors. When usable areas in the world are classified according to stressors, water stress, which is a natural stress factor, constitutes the largest slice with a share of 26% (Blum, 1986; Kalefetoğlu and Ekmekçi, 2005).

The world' s annual precipitation average is 1000 mm, Türkiye' s is 643 mm, and Konya' s is 322 mm. Accordingly, water scarcity is a characteristic feature of Türkiye, especially Konya (Sade, 2008). Because of previous reasons, water reserves of our country must be used without waste, precautions must be taken in terms of economic usage of water resources and studies must be supported to prevent waste of water.

Being many usage areas of water resources, supports researchers to develop methods for maximum yield with minimum water consumption. Limited irrigation treatments are one of the previous methods as well. The aim of limited irrigation practices is decreasing amount of irrigation water or decreasing irrigation frequency thus increasing water use efficiency of plants. Many studies were conducted to increase water use efficiency and grain yield in arid and semi – arid regions of developed and developing countries (Igbadun et al., 2008).

Limited irrigation treatments not being supposed to affect plant growth and development in negative way. Otherwise it may cause high yield loses. Pandey et al., (2000) stated that uncontrolled water shortage during vegetative development stage causes 7% ─ 11% while during reproductive stage 23% ─ 27% yield loses. Water scarcity changes hormone synthesis, which weakens the relationship between assimilate and grain, that results in ovule sterilization and decreases the number of grains (Moosavi, 2012). Çakır (2004) reported that the maize is more tolerant to water shortage in the vegetative development period compared to other development stages, and water scarcity stress during the flowering period causes significant yield losses. According to the previous knowledge, it is important to restrict the amount of water in such a way that water use efficiency is increased while irrigation water consumption is reduced. On the contrary negative effects of water scarcity, water restriction effects plant metabolism in positive ways. The effects of deficit irrigation treatments on bio – active compounds as soluble solids, polyphenols and anthocyanins were investigated, in terms of the results deficit irrigation increased the content of bio – active compounds (Yang et al., 2022). In this study, which was carried out for two years, the effects of deficit irrigation practices and grain colour on bio – active compounds, grain quality characteristics and grain yield in maize were examined.

2. Materials and Methods

2.1. Materials

A two – year field study was conducted during 2019 and 2020 in the "Experimental Farm of Selcuk University, Agriculture Faculty, Crop Science Department", Konya, Türkiye (32°31′N, 37°52′E). The typical soil type of this area has low organic matter and poor soil fertility. The soil had a clay – loamy texture, the pH was 7.80, and the organic matter was 1.07% (**P** = 5.33 mg kg^{-1}; **B** = 0.98 mg kg^{-1}; **Cu** = 0.97 mg kg^{-1}; **Fe** = 2.84 mg kg^{-1}; **Mn** = 5.42 mg kg^{-1}; **Zn** = 1.01 mg kg^{-1}).

The Turkish State Meteorological Service provided weather data for the growing seasons of 2019 – 2020. During the growing season (April - October) of both years, we also observed greater minimum and maximum temperatures than long years; nevertheless, rain totals during both years were lower than long years (*Table 1*).

Two hybrid corns, Sakarya (*Zea mays indentata* L., originated by Turkey) and DKC 5783, are high yielding middle maturity varieties. Red corn (RC) (*Zea mays indentata* L.) has red – coloured grains, also middle maturity, and a long – term, self – pollinated population. The seeds of Sakarya and RC (red corn) were provided from "The Ministry of Agriculture and Forestry Maize Research Institute, Republic of Turkey".

Experimental design, treatments, and crop management

A field study was designed with three irrigations using drip irrigation method composed of 50% (I1), 75% (I2) and 100% (I3) of evaporation from "Class A Evaporation PAN" was applied in a randomized complete block design (RCBD) with split arrangement along with three replications.

Three factors, irrigations [I_1 (50%), I_2 (75%) and I_3 (100%)] and genotypes (Sakarya, RC, DKC 5783), were used in experiment with the main plots irrigations and the subplots being different genotypes.

Maize seeds were manually sown. The fertilizers were applied at 200 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5, and 70 kg ha^{-1} pure K_2O respectively (Ayrancı and Sade, 2004; Karaşahin and Sade, 2012). The sources of fertilizers were urea (46%), diammonium phosphate (18 – 46 – 0) and potassium nitrate (13 – 0 – 46).

The P and K were applied at the time of sowing as basal fertilizer, but N was applied split in three doses. All agronomic management practices were the same during the experimental work.

Table 1. Monthly climate data during the growth period of corn in 2019 and 2020 in Konya, Turkey

Months	Temperature (°C)						Average relative humidity (%)		Total precipitation (mm)	
	Maximum		Minimum		Average					
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
April	17.00	18.10	6.40	7.80	11.10	12.60	58.50	53.30	26.40	36.40
May	26.20	24.70	1.50	11.80	19.70	18.00	39.80	46.60	5.40	36.00
June	28.90	28.20	17.60	15.90	23.00	22.00	47.20	44.40	32.60	32.80
July	30.50	33.00	18.40	20.60	24.30	26.90	39.70	35.70	9.20	-
August	30.50	31.50	19.40	19.10	24.80	25.50	40.70	33.20	2.00	7.80
September	27.40	30.70	15.10	17.70	21.00	23.90	41.30	42.60	10.40	10.40
October	23.40	25.70	12.00	12.40	17.30	18.40	49.60	47.80	6.60	12.20
Total	-	-	-	-	-	-	-	-	91.60	135.60
Mean	26.27	27.41	14.62	15.04	20.17	21.04	45.25	43.37	-	-
1929-2000	24.72		10.55		17.92		-		157.70	

The sowing pattern was 70 cm (between rows) × 20 cm (plant to plant in rows). The plants were irrigated with a drip irrigation method. Dripper (16 mm diameter) spacing of 20 cm was selected based on soil characteristics and dripper discharge. A drip irrigation system was installed in the field before sowing. Lateral pipes of 7 m long were placed in experimental plots within four plant rows and a lateral spacing of 70 cm.

As a part of the sowing process, the Class A Evaporation Pan was set up. 2019 sowing was conducted in the second week of May and 2020 sowing was conducted in the last week of April. Until the emergence in 2019, irrigation was provided (13 m^3 per subplot), and the emergence in 2020 was provided without irrigation (*Table 2*).

Table 2. Amount of irrigation water applied during all season (mm)

Irrigations	2019	2020
I1 (50%)	414.46	368.48
I2 (75%)	506.40	527.21
I3 (100%)	633.00	714.29

In order to compensate for the soil's moisture deficit, the main plots of the study were irrigated until emergence in 2019.

The amount of the irrigation water was calculated using the equation of Öktem et al., (2003):

$$I = A\ x\ Epan\ x\ Kcp \quad \text{(Eq.1)}$$

where I is the amount of irrigation water (mm), A is the plot area (m^2) and Epan (mm) is the cumulative water depth from Class A Evaporation Pan based on irrigation frequencies. Kcp is the crop pan coefficient; determined as 50% of total evaporation of Class A Evaporation Pan in the 7 – day irrigation frequency was Kcp_1 (I1), 75% was Kcp_2 (I2), and 100% was Kcp_3 (I3) (Demirok and Tuylu, 2019; Öktem et al., 2003; Şahin and Al-Bayati, 2018). The trial area was irrigated for ten times in both years of the trial (except the irrigations until emergence). The irrigation was started in 24.06.2019 and 28.05.2020 while it finished in 10.09.2019 and in 14.09.2020.

2.2. Methods

The following analysis were performed with the grain samples obtained from each sub – plot in both growing seasons.

Thousand grain weight: Thousand grain weight was determined according to Tezel (2007) by counting 400 grains three times and weighing them.

Total antioxidant activity: Radical supressing activities of antioxidants were measured from the bleaching rate of methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl**)** (Khampas et al., 2013). A 4.5 mL DPPH solution was added to the 0.5 mL phenolic extract. The mixture was mixed and left to stand for 30 min in unlit conditions. The absorbances of the samples were determined at 517 nm against solvent blank.

The rate of DPPH radical scavenging was calculated using the following formula:

$$Scavanging\ rate\ = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100 \quad \text{(Eq.2)}$$

where A_0 is the absorbance of the control (0.5 mL extraction solvent with 4.5 mL DPPH solution) and A_1 is the absorbance in the presence of phenolic extract solutions.

Total anthocyanin content: Total anthocyanin content of maize grains were determined according to Cervilla et al., (2012). 0.1 g of grain sample was homogenized with 5 ml of propanol and HCl solution. The homogenate was centrifuged at 5000 rpm, afterwards left at room conditions for 24 hours then centrifuged at 6500 rpm again. After the centrifuge, the absorbances of the samples were determined in the range of 535 – 650 nm. The obtained values were adapted to the following formula and recorded as the TAC of maize grains.

Finally, the results were calculated and the absorbances corrected at 535 – 650 nm using the formula below:

$$A = A_{535} - A_{650} \quad \text{(Eq. 3)}$$

Total phenolic compounds: Total phenolic compounds were determined according to Mohsen and Ammar (2009) with spectrophotometry by using gallic acid standard.

Starch: Grain starch content was detected according to Alan et al., (2014) by determining optical degree of rotation with polarimeter. Starch content was determined by the formula using previous values and recorded as % starch.

Crude grain protein: Crude grain protein content of the grains were determined according to Özdemir and Sade (2020a).

Crude grain oil: Crude grain oil content was determined according Özdemir and Sade (2019a) and recorded as % crude grain oil.

Grain yield: After the physiological maturity, all cobs of four rows were taken from each sub – plot to discover grain yield (kg da^{-1}) at 15% moisture content according to Özdemir and Sade (2019b).

All values in the tables are means. All data was analysed by MSTAT – C statistical analysis programme. Means were grouped in terms of LSD multiple range test ($P \leq 0.05$).

3. Results and Discussion

Variance analysis results, mean values and LSD groups of TGW that were detected in different genotypes under deficit irrigation conditions during 2019 and 2020 growing seasons were presented at *Table 3 – 4* and *Figure 1A – 2A.*

Table 3. Variance analysis results of TGW (thousand grain weight, g), TAC (total anthocyanin content, mg kg $^{-1}$ C3G), TAA (total antioxidant activity, %), TPC (total phenolic compounds, mg 100 g^{-1} GAE), GCO (grain crude oil, %), GCP (grain crude protein, %), starch (%) and grain yield (kg da^{-1}) properties in 2019

VS	DF	TGW	TAC	TAA	TPC	GCO	GCP	Starch	Grain Yield
Replication	2	444.11	0.11	0.12	17.24	3.89	0.46	45.54	14267.96
Irrigation (I)	2	24459.46**	23.94**	2.62	200.42**	4.69	11.00**	481.55**	211933.97**
Error	4	2747.45	0.22	1.33	26.81	2.05	0.11	57.90	13178.87
Genotype (G)	2	15994.24**	292.65**	153.59**	1811.67**	2.68	21.12**	160.25**	1615602.14**
I × G	4	2283.96	12.03**	21.77**	710.05**	3.66	1.95*	304.42**	24279.41
Error	12	5083.12	0.80	4.79	60.40	4.59	1.77	79.95	106809.134
Total	26	51012.36	329.78	184.25	2826.61	21.58	36.42	1129.63	1986071.49
CV (%)		7.20	4.63	4.31	3.50	16.75	6.00	3.78	10.91

VS (variation of source) *p≤0.05; **p≤0.01

While effects of irrigations on TGW were investigated it was observed that values increased in parallel water supply during both years of the experiment therewithal means in 2020 were higher than 2019. Tolerable decreases were observed under I2 in accordance I3, such that mean TGW under I3 during 2019 was 6.71% higher than I2. Similarly, TGW under I3 was 0.60% higher than I2 during 2020. Remarkable variations were observed in both years of the experiment according to genotypes as well, such that the highest TGW was obtained from DKC 5783, and it was followed by RC and Sakarya respectively. As a result, DKC 5783 maintained its superiority in TGW during both years of the experiment. Durmuş et al., (2015) stated that limited irrigation decreased TGW of maize and the means were obtained from the deficit irrigation treatments ranged between 286.40 g – 312.00 g while TGW of full irrigation ranged between 285.90 – 361.00 g. Similarly NeSmith and Ritchie (1992) reported that TGW of maize decreased in parallel water restriction. Karam et al., (2003) also stated that deficit irrigation decreased TGW nearby 18.00%. Variance analysis results, mean values and LSD groups of TAC that were detected in different genotypes under deficit irrigation conditions during 2019 and 2020 growing seasons were presented at *Table 3 – 4* and *Figure 1B – 2B*. While effects of limited irrigation on TAC were investigated it was observed that TAC increased in parallel water supply, in accordance previous detection the highest value of the group was obtained from I3. Unlike previous year in 2020, TAC values from I1 and I3 were close to each other whilst the highest value was obtained from I2. While means of TAC were investigated according to varieties it was observed that RC had highest TAC in both years of the experiment whilst TAC of other genotypes was close to each other. Statistically significant variations were observed in I × G interaction during both years of the experiment in terms of TAC and the highest value of I1 was obtained from RC. Žilić et al., (2012) determined TAC among 2.50 mg kg^{-1} C3G and 696.00 mg kg^{-1} C3G while Özdemir and Sade (2020b) analysed TAC among 1.19 mg kg^{-1} C3G – 306.90 mg kg^{-1} C3G in maize grains. Results of the current study are compatible with the previous detections as well.

Table 4. Variance analysis results of TGW (thousand grain weight, g), TAC (total anthocyanin content, mg kg $^{-1}$ C3G), TAA (total antioxidant activity, %), TPC (total phenolic compounds, mg 100 g^{-1} GAE), GCO (grain crude oil, %), GCP (grain crude protein, %), starch (%) and grain yield (kg da^{-1}) properties in 2020

VS	DF	TGW	TAC	TAA	TPC	GCO	GCP	Starch	Grain Yield
Replication	2	1473.23	0.60	16.33	277.31	0.21	0.02	10.95	9595.40
Irrigation (I)	2	28469.87**	1.98**	39.64	344.78	5.85	0.56**	343.49**	568314.18*
Error	4	2224.83	0.08	23.41	130.69	3.02	0.04	25.67	81880.91
Genotype (G)	2	9351.96*	203.35**	1.97	1478.98**	1.13	12.19**	85.94**	1051691.36**
I × G	4	2622.86	8.91**	131.39**	1142.82**	1.91	8.66**	325.77**	86129.02
Error	12	10537.96	1.06	61.41	302.07	3.29	0.59	41.23	205450.22
Total	26	54680.73	216.00	274.18	3676.67	15.44	22.08	833.07	2003061.11
CV (%)		9.23	7.85	12.75	7.66	11.96	4.12	2.69	14.44

VS (variation of source)
*p≤0.05; **p≤0.01

Variance analysis results, mean values and LSD groups of TAA that were detected in different genotypes under deficit irrigation conditions during 2019 and 2020 growing seasons were presented at *Table 3 – 4* and *Figure 1C*

– *2C*. Red corn was the genotype with the highest TAA in 2019 and followed by Sakarya and DKC 5783 respectively. In accordance previous detections RC was the genotype with the highest TAA under I1. Unlike 2019, the highest TAA was obtained from I1 × DKC 5783 in 2020 and significant variations were not observed among irrigations and genotypes. Oladeji et al., (2017) reported that TAA of maize grains ranged between 11.38% ─ 20.70%. Results of the current study are compatible with the previous knowledge. It was observed that RC -which is anthocyanins rich in genotype- is also rich in antioxidants as expected.

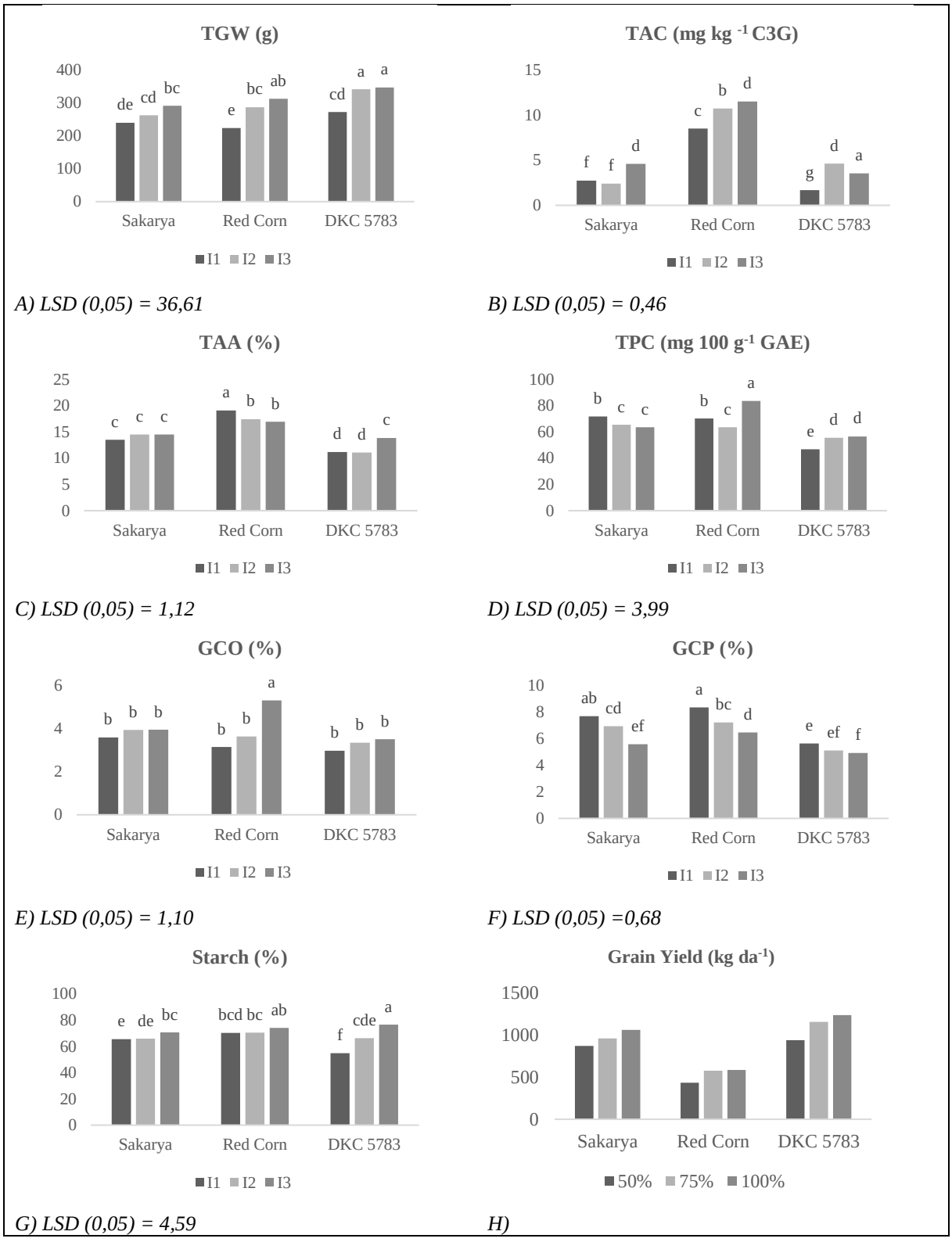
Variance analysis results, mean values and LSD groups of TPC that were detected in different genotypes under deficit irrigation conditions during 2019 and 2020 growing seasons were presented at *Table 3 – 4* and *Figure 1D – 2D*. While TPC values of maize grains were investigated it was observed that RC had higher TPC than other two genotypes during both years of the experiment. The highest TPC in 2019 was obtained from I3, on the contrary the highest TPC of 2020 was from I1. While means of S × G were investigated during 2019 it was observed that Sakarya and RC had higher TPC whilst RC and DKC 5783 had higher TPC than Sakarya during 2020. Bacchetti et al., (2013) stated that they determined TPC in maize flour between 115.40 mg 100 g^{-1} GAE ─ 175.50 mg 100 g^{-1} GAE, while Özdemir and Sade (2020b) between 30.35 mg 100 g^{-1} GAE – 47.76 mg 100 g^{-1} GAE. The results of the current study are also compatible with the previous knowledge as well.

Variance analysis results, mean values and LSD groups of GCP that were detected in different genotypes under deficit irrigation conditions during 2019 and 2020 growing seasons were presented at *Table 3 – 4* and *Figure 1F – 2F*. Grain crude protein content decreased because of increasing irrigation water and the highest GCP values were obtained from I1 treatment in both years of the trial. While means of GCP investigated in terms of genotypes, it was detected that RC had highest GCP in both years of the experiment. In parallel previous year, during 2019 the highest GCP was obtained from I1 × RC as well while the highest GCP was detected from I1 × Sakarya during 2020. In the literature, there are studies in which include findings that limited irrigation practices increase grain protein concentration (Anjum et al., 2017; Hafızoğlu, 2020).

Variance analysis results, mean values and LSD groups of starch content that were detected in different genotypes under deficit irrigation conditions during 2019 and 2020 growing seasons were presented at *Table 3 – 4* and *Figure 1G – 2G*. While effects of deficit irrigation on starch content was investigated it was observed that starch content induced in terms of increasing water supply. The highest starch content was reached in I3 in both years of the experiment and followed by I2 and I1 applications, respectively. While means of I × G interaction were investigated in 2019 it was seen that the highest starch values were obtained from the genotype RC whereas DKC 5783 had the highest starch content under I1 during 2020, thus RC could not maintain its superiority in the second growing season. In terms of the results of the current work water restriction decreased starch content of maize. Mohammedkhani and Heidari (2008) stated that water scarcity may decrease grain starch content of maize too.

The highest grain yield determined in I3 and I2 of DKC 5783, respectively; and I3 was 6.87% higher than I2 during 2019 (n = 3) (*Table 3 – 4*, *Figure 1H – 2H*). The grain yield from I3 and I2 of Sakarya were close to DKC 5783. Both, Sakarya and DKC 5783, had the lowest grain yield under I1 therewithal DKC 5783 had 7.77% more grain yield than Sakarya (n = 3). Similar yield changes were detected among the treatments of RC as other genotypes such that the highest value was from I3 and followed by I2 and I1 (n = 3). The highest grain yield was from I3 and followed by I2 and I1, respectively during 2020 (n = 9). The grain yield of I3 was 9.05% higher than I2, whereas 48.36% higher than I1. A remarkable difference was also observed among the genotypes (n = 9). The highest mean of the current property was from DKC 5783, followed by Sakarya and RC. The yield of DKC 5783 was 27.82% more than Sakarya and 72.45% greater than RC.

Some studies declared that corn tolerates deficit irrigation with no significant yield loss (Igbadun et al., 2008) nevertheless Desoky et al., (2021) reported that the yield contributing properties like PH, grain number per kernel, thousand – grain weight decrease due to water shortage. Moharramnejad et al., (2019) also postulated that water stress is harmful for crop growth and yield; it was opined that floret abortion because of water scarcity caused lower cob weight, lower grain weight, and yield as a result the crop did not produce more effective florets per flower (Halli et al., 2021). The yield loss effects of irrigation shortage were observed in the current study, like previous literature. DKC 5783 was the most notable genotype with its highest yield values in all treatments, followed by Sakarya and RC.



A) LSD (0,05) = 36,61

B) LSD (0,05) = 0,46

C) LSD (0,05) = 1,12

D) LSD (0,05) = 3,99

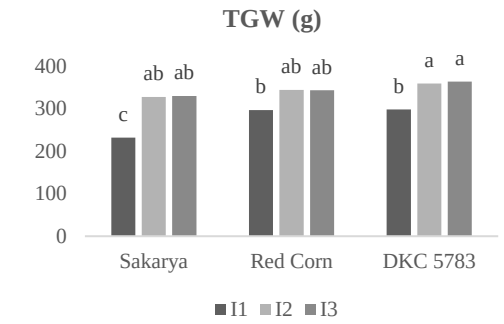
E) LSD (0,05) = 1,10

F) LSD (0,05) =0,68

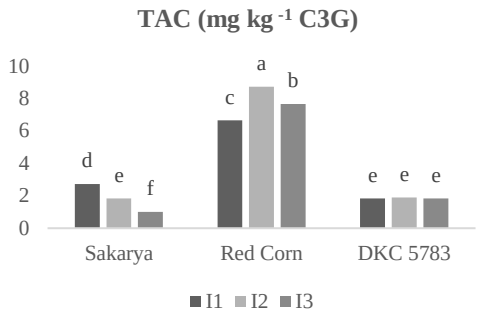
G) LSD (0,05) = 4,59

H)

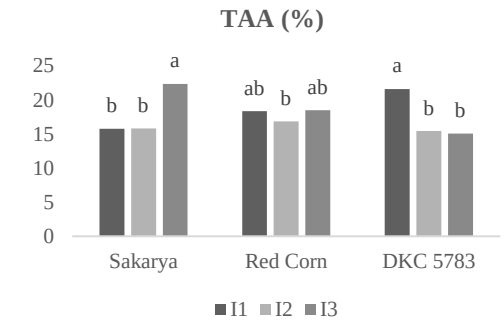
Figure 1. Means of TGW (thousand grain weight), TAC (total anthocyanin content), TAA (total antioxidant activity), TPC (total phenolic compounds), GCO (grain crude oil), GCP (grain crude protein), starch and grain yield properties in 2019; I1 is 50%, I2 is 75% and I3 is 100% of total evaporation of Class A Evaporation PAN.



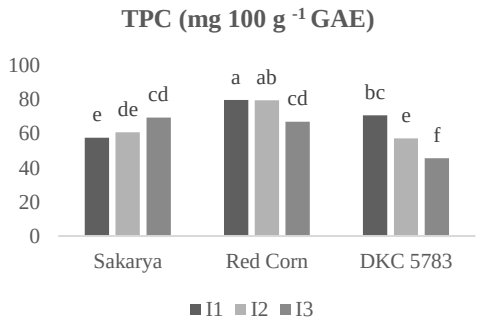
A) LSD (0,05) = 52,72



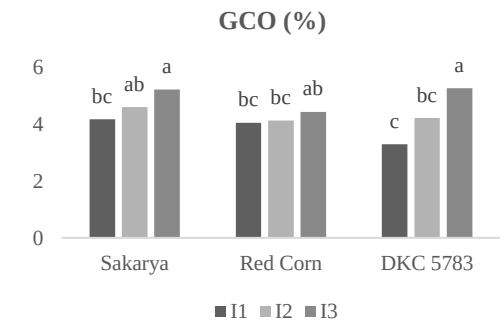
B) LSD (0,05) = 0,53



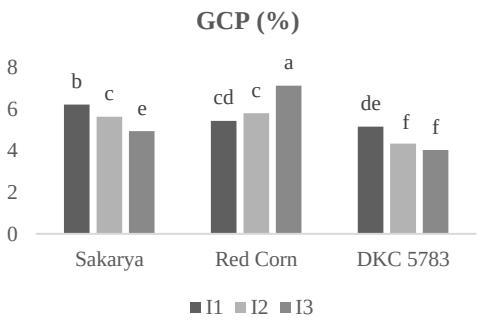
C) LSD (0,05) = 4,02



D) LSD (0,05) = 8,92

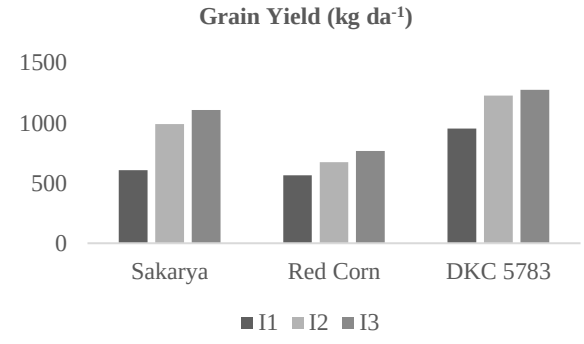


E) LSD (0,05) = 0,93



F) LSD (0,05) = 0,39

G) LSD (0,05) = 3,29



H)

Figure 2. Means of TGW (thousand grain weight), TAC (total anthocyanin content), TAA (total antioxidant activity), TPC (total phenolic compounds), GCO (grain crude oil), GCP (grain crude protein), starch and grain yield properties in 2020; I1 is 50%, I2 is 75% and I3 is 100% of total evaporation of Class A Evaporation PAN.

4. Conclusions

The aim of the current work was to determine effects of deficit irrigation and grain colour on quality traits of maize like TGW, TAA, TAC, TPC, starch, CGP and CGO. According to the results, water shortage -particularly I1- negatively affected quality traits while I2 caused tolerable declines. The results of the experiment showed that limited irrigation in Konya basin can significantly contribute to water saving with satisfactory grain yield in corn farming areas. DKC 5783 was ahead of the other two genotypes in terms of grain yield. Red corn ranked last in yield. It is opined that the most important reason of RC not to be reached high yield values is, it's being a population. Colour factor was also effective especially on bio – active compounds such that RC had more amount of bio – active content than other two varieties during both years of the trial thus may be an alternative genotype under limited irrigation conditions, also 25% water restriction caused tolerable decreases in some yield compounds of the current work.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

There is no conflict of interest between the article authors.

Authorship Contribution Statement

Concept: Özdemir, E., Sade, B.; Design: Özdemir, E., Sade, B.; Data Collection or Processing: Özdemir, E.; Statistical Analyses: Özdemir, E., Sade, B.; Literature Search: Özdemir, E.; Writing, Review and Editing: Özdemir, E., Sade, B.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Küçük Menderes Havzası'nda Meteorolojik Kuraklık Projeksiyonları: Havza Ölçeğinde Zamansal ve Mekânsal Bir Değerlendirme

Projected Changes in Meteorological Drought in Küçük Menderes River Basin: A Basin-Scale Spatio-Temporal Assessment

Yıldız GÜNEY[1*]

Öz

Kuraklık, Türkiye'nin bulunduğu coğrafyada geçmişten günümüze pek çok konuda önemli etkileri olan bir doğal afettir. Küçük Menderes Havzası gibi tarımsal açıdan önemli alanlarda su kaynaklarının korunması, havza yönetimi gibi konularda kuraklığın zamansal ve mekânsal değişiminin bilinmesi önemlidir. Nitekim havzada özellikle tarım sektöründe çalışanlar tarafından kuraklığın olumsuz etkileri kendini hissettirmeye başlamıştır. Bu çalışmanın temel amacı tarımsal açıdan önemli bir saha olan Küçük Menderes Havzası'nda iklim değişikliği senaryolarını kullanarak meteorolojik kuraklıkla ilgili gelecek yıllarda öngörülen değişimleri tespit etmektir. Bu amaçla Çeşme, Selçuk, Kuşadası, Ödemiş, İzmir (Bölge) meteoroloji istasyonlarının aylık ortalama sıcaklık ve aylık toplam yağış verileri kullanılmıştır. Ayrıca HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M küresel iklim modellerinin 1971-2000 referans dönemi ile 2016-2099 yılları arası iklim projeksiyonu iki farklı senaryo (RCP4.5 ve RCP8.5) verileri Küçük Menderes Havzası'nı kapsayan bölge için aylık ortalama sıcaklık ve aylık toplam yağış olarak Meteoroloji Genel Müdürlüğü'nden elde edilmiştir. Öncelikle modellerin referans dönem verileri ile araştırma alanındaki meteoroloji istasyonlarının rasat verileri kullanılarak hata değerleri hesaplanmıştır. Yapılan hata analizleri sonucu araştırma alanı için en uygun model olduğu tespit edilen HadGEM2-ES modelinin gelecek dönem RCP 4.5 ve RCP 8.5 senaryolarının kaba çözünürlük sonuçları 2016-2040, 2041-2070 ve 2071-2099 yıllarını kapsayan periyotlar halinde değerlendirilmiştir. Kuraklık analizleri için elimizdeki veri setine en uygun olan ve Akdeniz Havzası'nda pek çok çalışmada kullanılmış olan Bagnouls Gaussen kuraklık indeksi kullanılmıştır. Havzanın özellikle tarımsal açıdan en önemli kesimi olan doğu kesiminde her iki senaryoya göre de periyotlar arasında kuraklık değerlerinin artacağı tespit edilmiştir. Kuraklıktaki bu değişimlerin havza yönetiminden tarıma, su kaynaklarının korunmasından ekolojiye etkileri olacaktır. Havzada yeraltı suyunun bilinçsizce tüketimi gelecekte kuraklığın etkilerinin şiddetlenmesiyle daha büyük bir problem haline gelecektir. Havza kapsamında yapılacak bütün planlama çalışmalarında bu etkiler göz önünde bulundurulmalıdır.

Anahtar Kelimeler: İklim değişikliği, Kuraklık, HadGEM2-ES küresel iklim modeli, Bagnouls Gaussen kuraklık indeksi, Küçük Menderes Havzası

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Abstract

Drought is a natural disaster that has significant effects on many issues from the past to the present in the geography where Turkey is located. It is important to know the temporal and spatial change of drought in issues such as the protection of water resources and watershed management in agriculturally important areas such as Küçük Menderes Basin. In fact, the negative effects of drought have started to be felt especially by those working in the agricultural sector in the basin. The main objective of this study is to determine the predicted changes related to meteorological drought in the future years by using climate change scenarios in the Küçük Menderes Basin which is an important agricultural area. For this purpose, monthly mean temperature and monthly total precipitation data of Çeşme, Selçuk, Kuşadası, Ödemiş, İzmir (Bölge) weather stations were used. Furthermore, HadGEM2-ES, MPI-ESM-MR and GFDL-ESM2M global climate models' 1971-2000 reference period and 2016-2099 climate projection two different scenarios (RCP4.5 and RCP8.5) data were obtained from the General Directorate of Meteorology as monthly mean temperature and monthly total precipitation for the region covering the Küçük Menderes Basin. First of all, error values were calculated by using the reference period data of the models and the observation data of the weather stations in the study area. As a result of the error analysis, rough resolution results of future period RCP 4.5 and RCP 8.5 scenarios of the HadGEM2-ES model, which was found to be the most suitable model for the study area, were evaluated in periods covering 2016-2040, 2041-2070 and 2071-2099. The Bagnouls Gaussen drought index, which is the most suitable for the data set we have and has been used in many studies in the Mediterranean Basin, was used for drought analysis. It was determined that drought values would increase between periods according to both scenarios, especially in the eastern part of the basin, the most important part in terms of agriculture. These changes in drought will have effects on agriculture, basin management, conservation of water resources and ecology. Unconscious consumption of groundwater in the basin will become a more significant problem in the future with the growing effects of drought. These effects should be taken into consideration in all planning efforts to be made related to the basin.

Keywords: Climate change, Drought, HadGEM2-ES global climate model, Bagnouls Gaussen drought index, Küçük Menderes Basin

1. Giriş

Doğal afetlerden biri olan kuraklığın geniş çevrelerce kabul edilmiş, evrensel tek bir tanımı yoktur (Wilhite, 2000). Kuraklık, genel itibariyle yüksek sıcaklık ve düşük yağış nedeniyle ortaya çıkan, olağan dışı ve uzun süreli bir su açığı eksikliğini ifade eder (Zargar ve ark., 2011; Sordo-Ward ve ark., 2017; Chen ve ark., 2019). Kuraklık olayı oluşum mekanizmaları ve kuraklık olayından etkilenen farklı bileşenlerine dayalı olarak meteorolojik, tarımsal, hidrolojik ve sosyo-ekonomik kuraklık olarak sınıflandırılabilir (Tate ve Gustard, 2000). Bu çalışmada ele alınan kuraklık türü olan meteorolojik kuraklık, yağışların normal seviyelerinin altında olduğu uzun bir dönemdir ve genellikle diğer kuraklık türlerinden önce ortaya çıkar (Chen ve ark., 2009).

Kuraklığın önemi yarattığı etkilerden kaynaklanır (Wilhite, 2000). Yavaş gelişen ve insan faaliyetlerini etkileyene kadar varlığı yeterince anlaşılamayan kuraklık; sel, taşkın, fırtına gibi afetlerden farkı olarak ani can kayıplarına yol açmaz. Fakat dünyanın belli bölgelerinde yetersiz beslenme hatta açlık şiddetli kuraklıkları takip eder (Maybank ve ark., 1995). Anadolu coğrafyasında kuraklık ve bunun sebep olduğu özellikle tarım sektöründeki ekonomik kayıplar, tüm bölgeyi etkileyen büyük sosyo-ekonomik patlamalara sebep olmuştur. Geçmişte kuraklıkların Osmanlı köylerine zarar vermiş olduğu muhakkaktır (Faroquhi, 2001). Kaynaklara göre XVI. yüzyılının sonlarından itibaren Osmanlı İmparatorluğu'nda ve hatta Batı Akdeniz'deki ülkelerde de kuraklığa bağlı kıtlık defalarca yaşanmıştır (Lu, 2018). Hatta Celali İsyanları gibi büyük toplumsal patlamalarla kuraklık arasında ilişki kuran pek çok çalışma da vardır (White, 2013; Karademir, 2014; Yılmaz ve Kadıoğlu, 2017). Dolayısıyla kuraklık bulunduğumuz coğrafyada birçok konuda etkileri olan önemli bir afettir.

Diğer doğal afetlerin çoğundan farklı olarak, kuraklığın başlangıcını belirlemek zordur. Kuraklık yavaş gelişir ve insan faaliyetleri devam eden yağış azalmasından etkilenmeye başlayana kadar varlığı tanınmaz (Maybank ve ark., 1995). Küçük Menderes Havzası'nda iklim değişikliğinin bazı olumsuz etkileri aktüel olarak kendini göstermeye başlamıştır ve özellikle bundan direkt olarak etkilenen sektörlerdeki insanların konuyla ilgili farkındalık ve endişeleri söz konusudur. Nitekim özellikle tarım sektöründeki insanların bu konudaki görüşleri bunu destekleyen sonuçlar ortaya koymaktadır. Akyüz (2019) tarafından Küçük Menderes Havzası'ndaki çiftçilere iklim değişikliği konusunda yapılması muhtemel yayın çalışmaları içerisinde en önemli ve en önemsiz olarak hangilerini gördükleri ile ilgili fikirleri sorulmuştur. İklim değişikliğinin tarıma etkileri konusu, çiftçilerin en önemli gördükleri konu olmuştur. Tarımsal deneyimi ortalama 25 yıl olan çiftçilere göre havzada tarımsal faaliyetlerini etkileyecek iklim değişikliğinden kaynaklanan en önemli tehlike ise kuraklık ve erozyondur (Akyüz, 2019). Dolayısıyla yavaş gelişen ve insan faaliyetlerini etkileyene kadar varlığı tam olarak anlaşılamayan kuraklık olgusu havzada kendini problem olarak kabul ettirecek kadar ciddi bir hal almıştır. Ayrıca özellikle son yıllarda bazı sivil toplum kuruluşları belediyeler ile birlikte havzada su kıtlığı, kuraklık, iklim değişikliği perspektifinde çalıştaylar, toplantılar düzenleyerek havzada bu konudaki sorunları tartışma platformları yaratma gayreti içerisinde girmişlerdir. KEÇİ Kültür Ekoloji Çevre ve İletişim Derneği liderliğinde düzenlenen "Kuyu Kurumadan" su çalıştayları bunun en güncel örneklerinden olup özellikle Ödemiş'teki toplantısına çiftçilerin yoğun katılımı söz konusu olmuştur (Beyaz Haber Ajansı, 2022). Geniş ve verimli tarım alanlarına sahip havzada son 20 yıla damgasını vuran sorunun kuraklık ve yeraltı sularının gelişi güzel kullanımı olduğuna Vardar (2010)' da dikkati çekmiştir. Alüvyal örtünün kalınlığının yer yer 200 metreyi geçtiği ovada yeraltı suyunun yoğun tüketimi sonucu son yıllarda 150 metrelerde dahi suya ulaşılamamaktadır. Küresel iklim değişikliği ile birlikte bu aşırı tüketimin gelecek yıllarda kaynakların sürdürülebilir kullanımıyla ilgili daha büyük problemlere yol açması beklenmektedir (Vardar, 2010).

Havzanın özellikle Bayındır, Beydağ, Kiraz, Ödemiş ve Tire ilçelerinde geniş sahalarda tarım yapılmakta, söz konusu alanlar dahilindeki nüfusun yaklaşık % 97'si tarımla uğraşmaktadır (Yoldaş, 2011). Tarımsal faaliyetlerin yoğun olduğu verimli topraklara ve ürün çeşitliğine sahip bu havzada yoğun tarımsal sulama, hayvancılık ve sanayileşme sebebiyle son 30 yıldır yeraltı su seviyelerinde ciddi bir düşüş gözlemlenmiştir. Havzanın sürdürülebilirliği için ciddi risk oluşturan bu düşüşlerin ileriki yıllarda daha kötü sonuçları ile karşılaşmamak için havzada pek çok baraj ve gölet gibi su yapısı projesi Devlet Su İşleri Genel Müdürlüğü tarafından planlanmış ve yapılmıştır (Şahin ve ark., 2018). Araştırma alanında halkın büyük bir bölümünün tarım ve hayvancılıkla geçimini sağlaması, yer yer yılda 3 kez hasat yapılan geniş ve verimli tarım alanlarına sahip olması, stratejik bazı tarım ürünlerinin yetiştirilmesi özellikle iklim değişikliği sürecinde artması öngörülen kuraklığın bölge için araştırmaya değer bir problem olduğunu göstermektedir.

Bu araştırma kapsamında Küçük Menderes Havzası'nda meteorolojik kuraklığın gelecekte zamansal ve mekânsal olarak nasıl değişeceği araştırılmıştır. IPCC'nin iklim senaryolarına göre gelecekte Küçük Menderes Havzası'nda kuraklığın mekânsal dağılışı haritalamak, Küçük Menderes Havzası'nda kuraklığın zamansal değişimiyle ilgili olumlu ve olumsuz iklim senaryolara göre öngörüleri ortaya koymak, çiftçilerin kendi kişisel gözlemlerinden yola çıkarak havzadaki kuraklıkla ilgili endişe etmelerinin bilimsel açıdan da anlamlı olup olmadığını tespit etmek çalışmanın temel amaçlarıdır. Böylece Küçük Menderes Havzasındaki çiftçilerin problem ve araştırılması en öncelikli konu olarak gördükleri kuraklıkla ilgili gelecek yıllarda havzadaki değişimin nasıl olacağı tespit edilecektir.

2. Materyal ve Yöntem

2.1. Araştırma Alanı ve Veri

Küçük Menderes Havzası Batı Anadolu'da 38°41'05" ve 37°24'08" kuzey enlemleri ile 28°24'36" ve 26°11'48" doğu boylamları arasında bulunmaktadır (Şekil 1). Yaklaşık 702.931 hektarlık alanıyla Türkiye'deki 25 büyük hidrolojik havzadan biridir (Orman ve Su İşleri Bakanlığı Su Yönetimi Genel Müdürlüğü, 2018).

Küçük Menderes Havzası'nın iklimi Köppen-Geiger iklim sınıflamasına göre kışları ılık, yazları çok sıcak tipik Akdeniz iklimi (Csa) tipidir (Öztürk ve ark., 2017). Yaz kuraklığının belirgin olduğu araştırma alanında yağışlar Kasım, Aralık, Ocak, Şubat aylarında yoğunluk gösterir. Yıllık yağış miktarı ise 700 mm civarındadır (Vardar, 2010). Kuraklık, dünyanın pek çok bölgesi için önemli bir doğal afettir. Yağış rejiminin mevsimsel olduğu veya yağış değişkenliğinin oldukça yüksek olduğu kurak, yarı kurak alanlar yeryüzünde kuraklığa en hassas alanlardır (Maybank ve ark., 1995). Araştırma alanının da içinde bulunduğu Akdeniz Havzası dünyada yağışın mevsimsel olması ve yağış değişkenliğinin yüksek olması özelliği ile karakterize olmuş bir bölgedir (Dünkeloh ve Jacobeit, 2003; Deitch ve ark., 2017). Dolayısıyla sahip olduğu iklim karakteriyle Küçük Menderes Havzası kuraklığa son derece hassas olan bir alandır.

Bu çalışmada IPCC'nin 5.değerlendirme raporundaki (AR5) iklim senaryolarından RCP 4.5 ve RCP 8.5 senaryosu ile üretilen HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M küresel iklim modelinin RegCM4 modeli ile ölçeği küçültülen çıktıları kullanılmıştır. 20 km çözünürlüklü olan veri seti 1971-2000 referans dönemi ve 2016-2099 yılları arasını kapsamaktadır. Çalışma alanını içine alan bölge için, aylık ve yıllık toplam yağış (mm) ile aylık ve yıllık ortalama sıcaklık (°C) değerleri analizlerde kullanılmıştır. Çalışma kapsamında kullanılan küresel iklim modeli çıktıları Meteoroloji Genel Müdürlüğü'nden araştırma amaçlı kullanılmak üzere ücretsiz olarak temin edilmiştir. Meteoroloji Genel Müdürlüğü 2011 yılından beri AR5 kapsamında geliştirilen RCP 4.5 ve RCP 8.5 senaryolarını ve HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M küresel modellerini tercih ederek, Türkiye ve çevresi için 20 km çözünürlüklü bölgesel iklim projeksiyonlarını üretmeye başlamıştır (MGM, 2015; MGM, 2023). Ayrıca Çeşme, Ödemiş, Selçuk, Kuşadası, İzmir (Bölge) meteoroloji istasyonlarının 1971-2000 yıllarına kapsayan yıllık toplam yağış ve yıllık ortalama sıcaklık verileri model doğrulaması amacıyla Meteoroloji Genel Müdürlüğü'nden temin edilmiştir (MGM, 2021). Meteoroloji istasyonu verileri küresel iklim modellerinin referans dönem verileriyle karşılaştırmak ve modellerin hata paylarını hesaplamak için kullanılmıştır. Meteoroloji istasyonları araştırma alanında 1970-2000 yılları arasında düzenli yağış ve sıcaklık ölçümü yapan istasyonlardan havzanın tamamını temsil edebilecek lokasyonlarda olmalarına dikkat edilerek seçilmiştir. Dolayısıyla kullanılan istasyonların seçiminde havzanın batısını, doğusunu, güneyini ve kuzeyini temsil edebilecek ve 20 km çözünürlüklü küresel iklim modeli verilerinin lokasyonlarına en yakın konumdaki istasyonlar seçilmiştir (*Şekil 1*). Çalışmadaki iklim verilerinin analizinde Microsoft Excel, haritaların üretilmesinde coğrafi bilgi teknolojileri yazılımlarından ArcGIS 10.2 programı kullanılmıştır.

MGM (2013) tarafından "kötümser" olarak nitelenen RCP 8.5 senaryosu, en yüksek ışınımsal zorlama (2100 yılında 8.5 w/m^2) ve konsantrasyon tahmini ile güncel şartlara göre gelecek yıllarda küresel anlamda öngörülen en kötümser senaryodur. "Mutedil" olarak nitelenen RCP 4.5 senaryosuna göre ise radyatif (ışınımsal) zorlamanın yüzyılın son çeyreğinde 4.5 w/m^2'ye çıkması öngörülmektedir. RCP 4.5 senaryosu nispeten daha iyimser bir senaryodur (MGM, 2013; Gürkan, 2015).

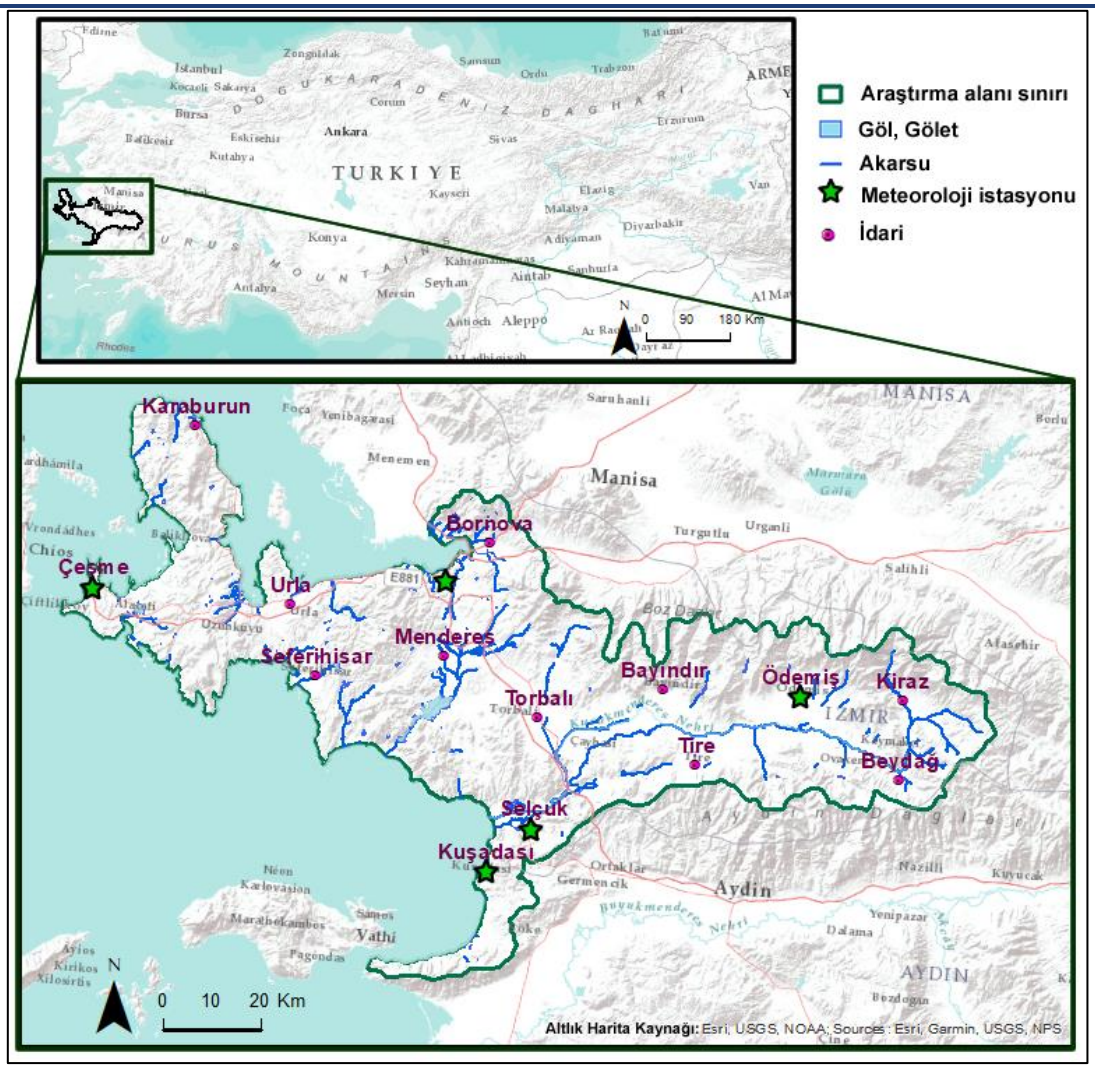

Figure 1. Location map of the study area

Şekil 1. Araştırma alanının lokasyon haritası

2.2. Yöntem

Belli sınırlar içerisinde hata payları bulundurması kaçınılmaz olan iklim modellerinin hata oranlarının hesaplanması için literatürde en çok kullanılan yöntemlerden olan ortalama hata değeri (ME) ve kök ortalama kare hata değeri (RMSE) uygulanmıştır (Şen, 2007; Güney, 2017; Eskioğlu ve ark., 2017; Gürkan ve ark., 2020). İklim modellerinin doğrulaması amacıyla 1971-2000 dönemine ait HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M küresel iklim modeli referans verisi ve Selçuk, Kuşadası, Çeşme, Ödemiş, İzmir Meteoroloji İstasyonlarının 1971-2000 dönemine ait yağış ve sıcaklık verilerinden yararlanılmıştır. Doğruluk analizi sonuçlarını sayısal bir şekilde ortaya koyabilmek amacıyla her yılın ortalama sıcaklık ve toplam yağış verileriyle ortalama hata (ME) ve kök hata kareler ortalaması (RMSE) değerleri hesaplanmış, ayrıca sonuçlar grafik olarak verilmiştir. Böylece HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M küresel iklim modellerinin hata payı sayısal olarak ortaya konulmuş, araştırma alanı için en uygun küresel iklim modeline karar verilmiştir.

Yapılan hata analizleri sonucu araştırma alanı için en uygun model olduğu tespit edilen HadGEM2-ES modelinin gelecek dönem RCP 4.5 ve RCP 8.5 senaryolarının kaba çözünürlük sonuçları 2016-2040, 2041-2070 ve 2070-2099 yıllarını kapsayan periyotlar halinde değerlendirilmiştir. Ayrıca 1971-2000 yıllarını kapsayan referans dönem ve 2016-2099 genel periyodu için de analizler yapılmıştır (*Şekil 2*).

Literatürde farklı boyutlarıyla ele alınan kuraklığı değerlendirmede pek çok farklı yöntem kullanılmıştır (Türkeş ve Demirörs, 2010; Konukçu ve ark., 2020; Sarış ve Gedik, 2021; Bakanoğulları ve ark., 2022). Bu

çalışmada kuraklığın ele alınan boyutu meteorolojik kuraklıktır ve araştırma alanının da içinde bulunduğu Akdeniz Havzasında pek çok çalışmada kullanılmış Bagnouls Gaussen Kuraklık İndisi (BGI) kullanılarak değerlendirilmiştir. Bagnouls ve Gaussen (1953) Akdeniz biyoikliminin analizi, bunun bitkilerin yaşamsal faaliyetleri ve tarım potansiyeliyle ilişkisi üzerinde duran araştırmacılardandır (Di Castri, 1973). BGI'nın seçilme sebebi araştırma alanının da içinde bulunduğu Akdeniz Havzası'nda bu yöntemin uygulandığı pek çok çalışmanın olması ve elimizdeki veri setinin bu formülde kullanılması gereken parametrelere uygun olmasıdır. Zira, Meteoroloji Genel Müdürlüğü'nden küresel iklim modeli verilerinin sadece aylık verileri temin edilebilmiştir.

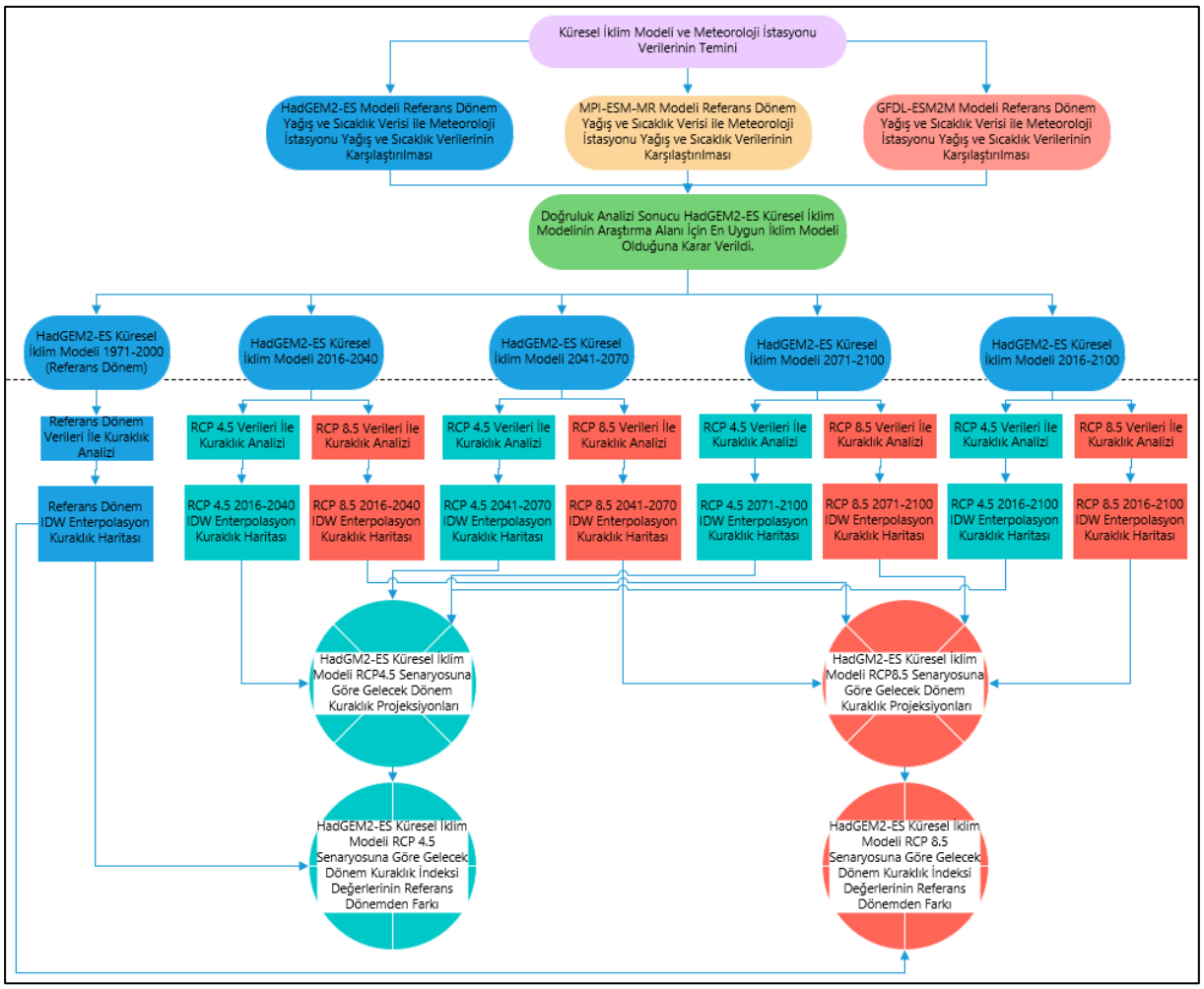

Figure 2. Flow chart of research method

Şekil 2. Araştırma yönteminin iş akış şeması

BGI, aylık ortalama sıcaklık ve yağış miktarı verileri gibi kolayca elde edilebilen meteorolojik verilerle evapotranspirasyon tahmin edilerek aylık nem dengesi bazında hesaplanır. Eşitlik (1)'de verildiği şekilde uygulanması kolay bir formülü vardır. BGI hesaplarken aylık ortalama sıcaklığın iki katının aynı aydaki yağış miktarından fazla olduğu aylar hesaba katılır. Bu şekilde hesaplanan Bagnouls Gaussen kuraklık indeksi değerleri *Tablo 1*'de sunulmuş olan değer aralıklarına göre değerlendirilip, hangi kuraklık sınıfına girdiği tanımlanır (Cebeci ve ark., 2013; Pogetti ve ark., 2019). BGI'nın normalde 4 sınıflı bir kuraklık tanımlaması vardır (*Tablo 1*). Ancak bu çalışmada kuraklık ile ilgili daha detaylı değerlendirme yapabilmek için haritalama aşamasında lejant 10 sınıflı olarak düzenlenmiştir. BGI analizi sonrası benzer yaklaşımla lejant düzenlemesi Güney (2017) tarafından da yapılmıştır. Bu çalışmada ise indeks değeri 0 olan alanlar "Nemli" ve 1-50 arasında olan alanlar "Yarı Nemli" olarak orijinal sınıflandırmalarındaki şekilde bırakılmıştır. 51-130 BGI değeriyle "Kurak" olarak tanımlanan alanlar ve >130 BGI değeriyle "Çok Kurak" olarak tanımlanan alanlar kendi içinde 20 sınıf aralığıyla bölünmüştür. Böylece 51-70 (Kurak 1), 71-90 (Kurak 2), 91-110 (Kurak 3), 111-130 (Kurak 4), 131-150 (Çok Kurak 1), 151-170 (Çok Kurak 2), 171-190 (Çok Kurak 3), 190'dan büyük (Çok Kurak 4) şeklinde düzenlenerek haritalama

esnasında lejant düzenlenmiştir (*Şekil 5-7*). Böylece araştırma alanında en geniş alanı kaplayan Kurak ve Çok Kurak sınıflarının alt derecelendirmeleri yapılarak daha detaylı değerlendirme yapabilmek mümkün olmuştur.

$$BGI = \sum_{i=0}^{12}(2ti - pi)ki \quad \text{(Eş.1)}$$

Eşitlikte;
ti: i ayındaki aylık ortalama sıcaklık (°C)
pi: i ayındaki aylık toplam yağış miktarı (mm)
ki: 2ti-pi>0 olduğu aylar.

Tablo 1. Bagnouls Gaussen Kuraklık İndeksi'nin sınıflaması ve sınıf tanımları (Cebeci ve ark., 2013)

Table 1. Classification and class definitions of Bagnouls Gaussen Drought Index

BGI Değeri	Sınıf	Tanım
0	1	Nemli
1-50	2	Yarı-nemli
51-130	3	Kurak
>130	4	Çok kurak

Referans dönemi (1971-2000) ile gelecek dönem (2016-2040, 2041-2070 ve 2071-2099 periyotları ve 2016-2099 genel periyodu) arasındaki farklar alınarak gelecek dönemde gerçekleşmesi öngörülen değişimler ortaya konulmuştur (Güney, 2017). Elde edilen BGI ve fark değerleri IDW enterpolasyonla haritalanıp, araştırma alanı kesilerek havzanın gelecek dönem kuraklık projeksiyonları ve fark haritaları elde edilmiştir (*Şekil 2*).

3. Araştırma Sonuçları ve Tartışma

3.1. Model Doğruluk Analizi

Araştırmada kullanılan meteoroloji istasyonlarının 1971-2000 dönemine ait sıcaklık ve yağış rasat verilerinin iklim modellerinin referans dönem değerleriyle birlikte analizi sonucu elde edilen ortalama hata (ME) ve kök hata kareler ortalaması (RMSE) değerleri sonucunda araştırma alanı için en uygun iklim modelinin HadGEM2-ES modeli olduğuna karar verilmiştir (*Tablo 2*).

Tablo 2. İklim modellerinin 1971-2000 referans periyodu yıllık ortalama sıcaklıklarının ve yıllık toplam yağış verilerinin meteoroloji istasyonlarının gözlem verileri ile karşılaştırarak hazırlanmış ortalama hata (ME) ve kök ortalama kare hata (RMSE) analizi sonuçları

Table 2. Mean error (ME) and root mean square error (RMSE) analysis results prepared by comparing the 1971-2000 reference period annual average temperatures and annual total precipitation data of climate models with the observation data of meteorology stations

METEOROLOJİ İSTASYONU	SICAKLIK						YAĞIŞ					
	HadGEM2-ES		MPI-ESM-MR		GFDL-ESM2M		HadGEM2-ES		MPI-ESM-MR		GFDL-ESM2M	
	ME	RMSE	ME	RMSE	ME	RMSE	ME	RMSE	ME	RMSE	ME	RMSE
Çeşme	0.3	0.6	0.1	0.6	-1.9	2.0	202.1	268.7	604.3	656.1	718.2	778.3
Ödemiş	-2.4	2.5	-2.5	2.6	-4.6	4.6	52.0	185.3	-206.9	265.2	-148.5	236.5
Selçuk	-1.0	1.1	-0.8	1.1	-3.0	3.1	644.9	708.3	-21.4	244.8	-104.6	305.9
Kuşadası	1.2	1.3	0.8	1.0	-1.3	1.4	386.3	464.0	717.2	764.0	793.5	881.8
İzmir (Bölge)	-3.0	3.0	-3.0	3.1	-5.1	5.2	267.2	316.5	-136.8	267.7	-102.7	245.7
ORTALAMA	0.98	1.7	-1.08	1.68	-3.18	3.26	310.5	388.56	191.28	439.56	231.18	489.64

Araştırmada kullanılan meteoroloji istasyonlarının 1971-2000 dönemine ait sıcaklık rasat verilerinin HadGEM2-ES modelinin referans periyodu sıcaklık verileriyle karşılaştırılmasında ortalama hata (ME) 0.3 (Çeşme) ile -3.0 (İzmir-Bölge) arasında değişen değerlerde çıkmıştır. En küçük kareler hata değeri (RMSE) ise 0.6 (Çeşme) ile 3.0 (İzmir-Bölge) değişen değerlerde hesaplanmıştır. Model gözlem değerlerine en yakın sonucu 0.3 °C fazla sıcaklık kestiriminde (sıcak bias) bulunarak Çeşme istasyonunda vermiştir. Model gözlem değerlerinden en uzak sonucu ise 3 °C daha düşük sıcaklık kestiriminde bulunarak (soğuk bias) İzmir-Bölge istasyonunda vermiştir. İstasyonların ortalama hata değerlerinin (ME) ortalaması 0.98 °C'dir. *Şekil 3*'deki yıllık ortalama sıcaklık değerlerinin grafik eğrilerinde genel itibariyle model ile gözlem verileri benzer paterne

sahiptirler. Diğer bir deyişle genel itibariyle gözlem ve model verilerinin birlikte artıp azalması söz konusuyken, bazı yıllar model ve gözlem çizgileri çakışacak kadar yakın değerlerdedir. Yıllık olarak model ile gözlem değerleri arasında en büyük fark İzmir Bölge istasyonunda -4.6 °C ile 1994 yılındayken, en düşük fark ise 0.0 °C (model ve istasyon ölçüm değeri aynı) Çeşme istasyonunda 1975 ve 1972 yıllarında olduğu tespit edilmiştir (*Şekil 3*).

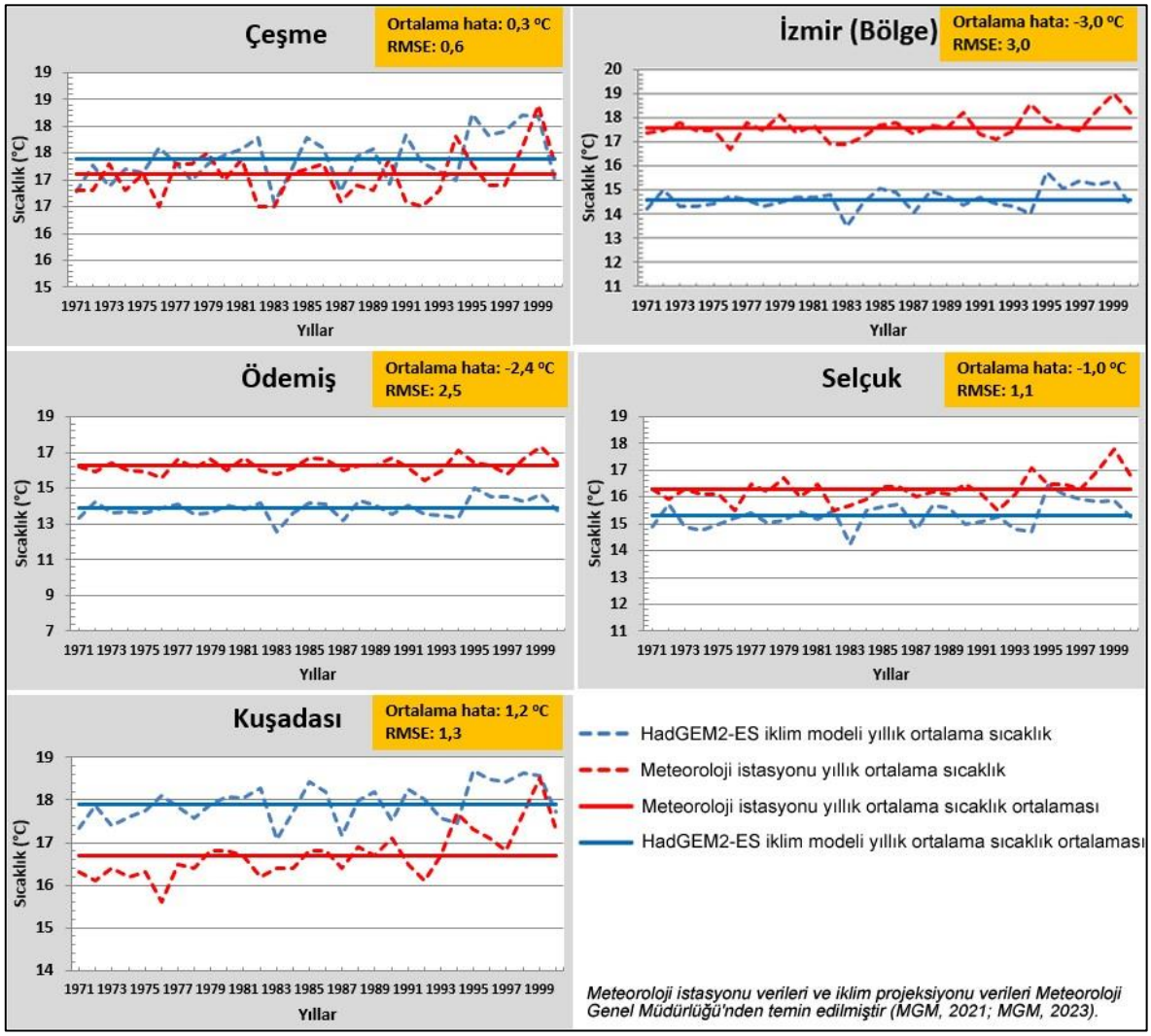

Figure 3. Comparison of annual average temperature data of Çeşme, İzmir (Bölge), Ödemiş, Selçuk, Kuşadası meteorology station and reference period of HadGEM2-ES climate model (1971-2000)

Şekil 3. Çeşme, İzmir (Bölge), Ödemiş, Selçuk, Kuşadası meteoroloji istasyonu ile HadGEM2-ES iklim modelinin referans döneminin (1971-2000) yıllık ortalama sıcaklık verilerinin karşılaştırması

Araştırmada kullanılan meteoroloji istasyonlarının 1971-2000 dönemine ait yağış rasat verilerinin HadGEM2-ES modelinin referans dönem yağış değerleriyle kıyaslanmasında ortalama hata değeri (ME) 52 mm (Ödemiş) ile -644.9 mm (Selçuk) arasında değişen değerlerde çıkmıştır. En küçük kareler hata değeri (RMSE) ise 185.3 (Ödemiş) ile 708.3 (Selçuk) değişen değerlerde hesaplanmıştır. Model gözlem değerlerine en yakın sonucu 52 mm fazla yağış kestiriminde (yüksek bias) bulunarak Ödemiş istasyonunda vermiştir. Model gözlem değerlerinden en uzak sonucu ise 644.9 mm daha fazla yağış kestiriminde bulunarak (yüksek bias) Selçuk istasyonunda vermiştir. İstasyonların ortalama hata değerlerinin (ME) ortalaması 310.5 mm'dir. Bu durumda modelin yağış verisinin hata payının sıcaklığa göre daha yüksek olduğunu söyleyebiliriz. Yıllık olarak modelle gözlem değerleri arasında en büyük fark Selçuk istasyonunda 1095.7 mm ile 1987 yılındayken, en düşük fark ise -0.5 mm Ödemiş istasyonunda 1979 yıllarında olduğu tespit edilmiştir (*Şekil 4*).

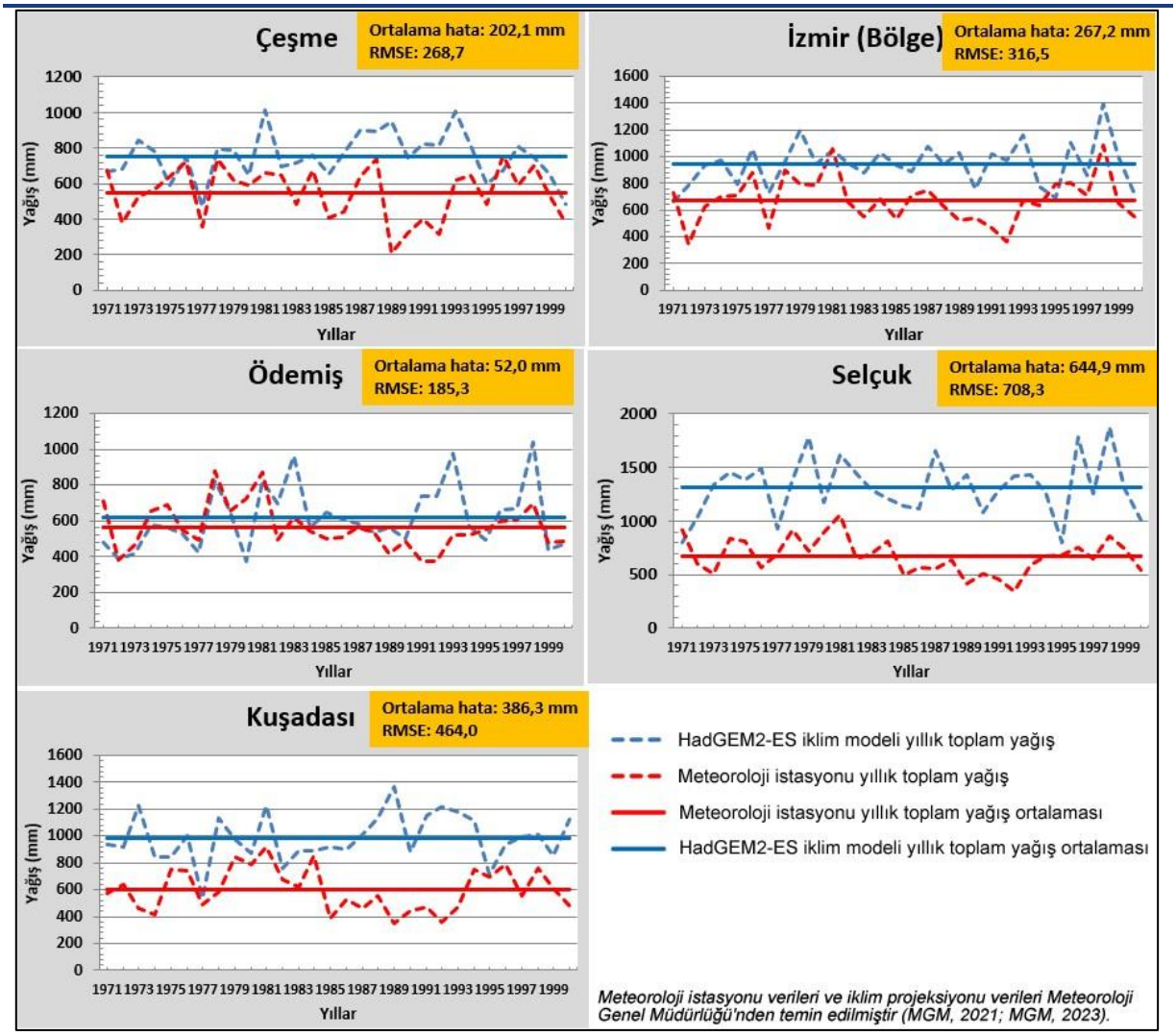

Figure 4. Comparison of annual total precipitation data of Çeşme, İzmir (Bölge), Ödemiş, Selçuk, Kuşadası meteorology station and reference period of HadGEM2-ES climate model (1971-2000)

Şekil 4. Çeşme, İzmir (Bölge), Ödemiş, Selçuk, Kuşadası meteoroloji istasyonu ile HadGEM2-ES iklim modelinin referans döneminin (1971-2000) yıllık toplam yağış verilerinin karşılaştırması

3.2. HadGEM2-ES Küresel İklim Modeli RCP 4.5 Senaryosuna Göre 1971-2000 Referans Dönemi ve Gelecek Dönem Kuraklık Projeksiyonları

Şekil 5'te 1970-2000 referans dönemi ve HadGEM2-ES küresel iklim modelinin RCP 4.5 senaryosu için Bagnouls-Gaussen kuraklık indeksi (BGI) dağılış haritaları verilmiştir. HadGEM2-ES küresel iklim modeli RCP 4.5 senaryosu verilerinin analizi ile elde edilen kuraklık projeksiyonlarına göre araştırma alanında 1970-2000 referans döneminde Bayındır'ın kuzeyinde küçük bir alanda yarı nemli BGI sınıfı, Bayındır ve çevresinde kurak 1 sınıfı, havzanın doğusu ve güneyinde kurak 2, havzanın orta ve batı kesiminde ağırlıklı olarak kurak 3 ve kurak 4 sınıfları, Menderes ilçe merkezinin olduğu kesimde çok kurak 1 BGI sınıflarına ait değerler bulunmaktadır. 1970-2000 referans döneminde araştırma alanında BGI minimum değeri 47.7 iken, maksimum değeri 142.0, havza ortalaması 91.8'dir. 2016-2099 genelinde ve daha detaylı incelemek için ayrılan alt dönemlerde ise kurak 2, 3, 4 ve çok kurak 1, 2, 3, 4 BGI sınıflarına ait değerler olduğunu görüyoruz. HadGEM2-ES küresel iklim modeli RCP 4.5 senaryosuna göre 2016-2099 genel döneminde havzada minimum BGI değeri 73.5, maksimum değer 258.6, havza ortalaması ise 117.1'dir. Dolayısıyla 2016-2099 genel gelecek dönem projeksiyonunda 1971-2000 referans dönemine göre daha yüksek maksimum, minimum ve ortalama BGI değerleri söz konusudur (Şekil 5).

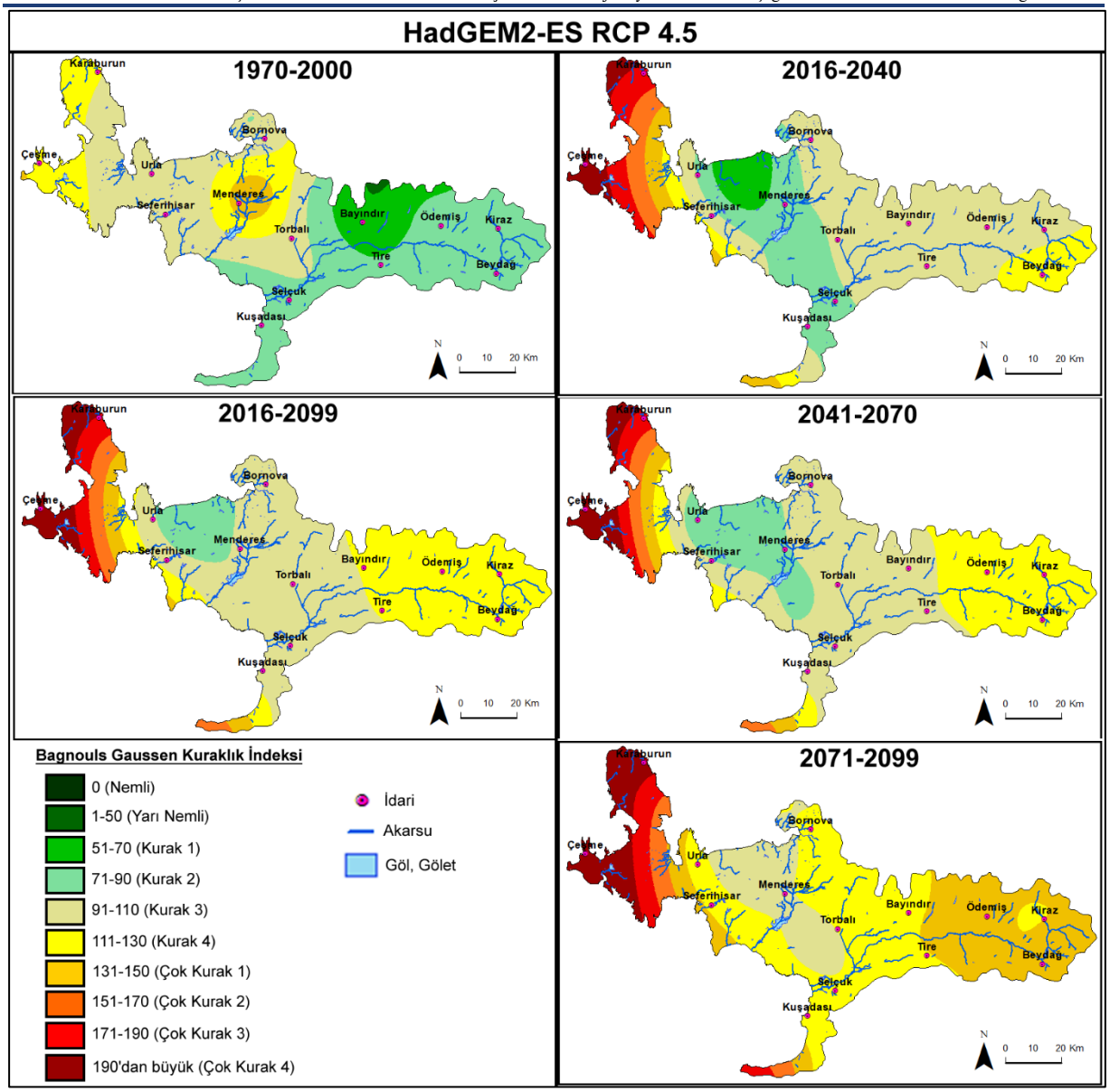

Figure 5. Reference period and future drought projections according to HadGEM2-ES global climate model RCP 4.5 scenario in the study area

Şekil 5. Araştırma alanında HadGEM2-ES küresel iklim modeli RCP 4.5 senaryosuna göre referans dönem ve gelecek dönem kuraklık projeksiyonları.

Havzadaki gelecek dönem kuraklığı ele aldığımızda 2016-2040 döneminde HadGEM2-ES RCP 4.5 senaryosuna göre minimum BGI değeri 53.8, maksimum değer 226.2, havza ortalaması 107.1; 2041-2070 periyodunda BGI değerleri artarak minimum 71.0, maksimum 277.1, havza ortalaması 113.6 olmuş iken; 2071-2099 döneminde ise minimum BGI değeri 93.1, maksimum BGI 255.1, ortalama BGI 132.5'tir. Dolayısıyla 2041-2070 ile 2071-2099 periyodundaki maksimum BGI değerini saymazsak havzanın minimum, maksimum ve ortalama BGI değerlerinde periyotlar arasında sürekli bir artış olduğunu söyleyebiliriz. Bunun sonucunda özellikle havzanın orta ve doğu kesiminde ilk gelecek dönem periyodundan son gelecek dönem periyoduna doğru kuraklık sınıflarının daha üst kademede daha geniş alanlar kaplamaya başladığı dikkati çekmektedir. Nitekim 2016-2040 periyodunda havzanın tarımsal açıdan en önemli bölgesi kabul edilen doğu kesiminde kurak 3 sınıfı geniş alan kaplarken, 2071-2099 gelecek dönem periyoduna geldiğimizde ise bu kesimde kurak 3 sınıfı ortadan kalkmış, onun yerine kurak 4 ve çok kurak 1 sınıfı geniş alanlarda yerini almıştır. İyimser senaryoya göre bile havzanın tarımsal açıdan en önemli bölgesindeki bu değişim endişe vericidir. Referans dönemde Bayındır'ın kuzeyinde zaten sınırlı bir alanda görülen yarı nemli sınıfı 2016-2040 döneminde ortadan kalkmıştır (*Şekil 5*).

İyimser senaryoya göre özellikle havzanın yoğun olarak tarım yapılan doğu ve kısmen orta kesiminde kuraklığın periyotlar arasında kademeli olarak artacağı 2071-2099 periyodunda ise söz konusu bölgelerde kuraktan çok kurak sınıfına geçiş olacağı tespit edilmiştir.

HadGEM2-ES küresel iklim modeli RCP 4.5 senaryosuna göre araştırma alanındaki BGI değerlerinin değişimini ortaya koyabilmek için gelecek dönem periyotlarından referans dönemin farkı alınarak haritalanmıştır (*Şekil 5*). Havzada kuraklıkla ilgili değişikliklerin yönünü ve şiddetini yansıtan BGI fark haritaları incelendiğinde BGI değerlerinin havzanın çok büyük bir kesiminde artma göstermiştir. Sadece havzanın orta kesiminde Menderes ve çevresinde kuraklık indeksi fark değerlerinin negatif değerler olduğu görülmektedir. Havzanın tarımsal açıdan en önemli kesimi olan doğu kesiminde özellikle kuraklık indeksi fark değerlerine baktığımızda önemli artış olacağı ön görülmektedir. Bu artışlar 2071-2099 periyodunda diğer periyotlara göre daha belirgindir (*Şekil 6*).

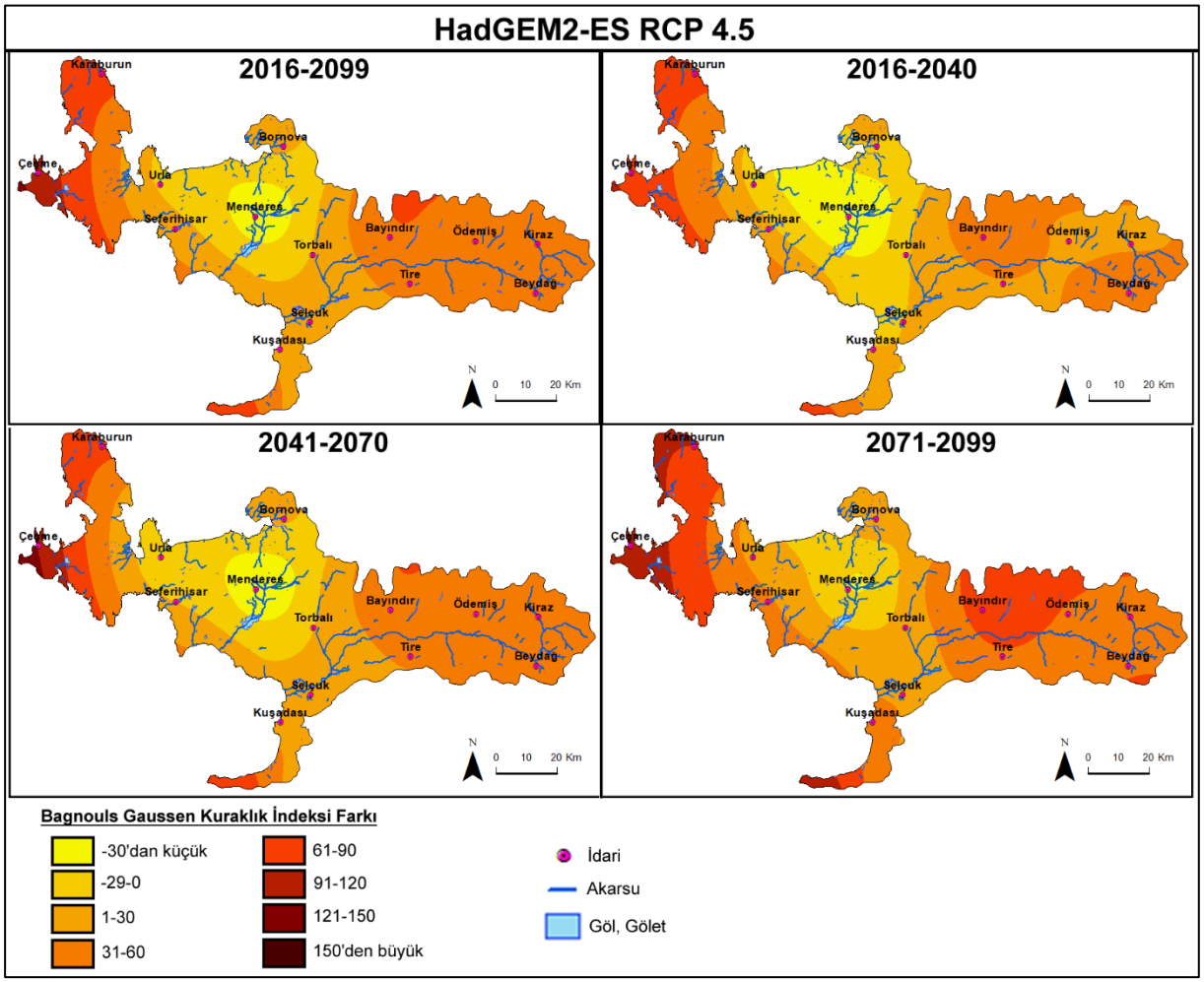

Figure 6. The differences of the future period drought index values from the reference period (1970-2000) according to the HadGEM2-ES global climate model RCP 4.5 scenario in the research area.

Şekil 6. Araştırma alanında HadGEM2-ES küresel iklim modeli RCP 4.5 senaryosuna göre gelecek dönem kuraklık indeksi değerlerinin referans dönemden (1970-2000) farkları.

3.3.HadGEM2-ES Küresel İklim Modeli RCP 8.5 Senaryosuna Göre 1971-2000 Referans Dönemi ve Gelecek Dönem Kuraklık Projeksiyonları

Şekil 7'de 1970-2000 referans dönemi ve HadGEM2-ES küresel iklim modelinin RCP 8.5 senaryosu için Bagnouls-Gaussen kuraklık indeksi (BGI) dağılış haritaları verilmiştir. HadGEM2-ES küresel iklim modeli RCP 8.5 senaryosu verilerinin analizi ile elde edilen kuraklık projeksiyonlarına göre araştırma alanında 1970-2000 referans döneminde Bayındır'ın kuzeyinde küçük bir alanda yarı nemli BGI sınıfı, Bayındır ve çevresinde kurak 1 sınıfı, havzanın doğusu ve güneyinde kurak 2, havzanın orta ve batı kesiminde ağırlıklı olarak kurak 3 ve kurak

4 sınıfları, Menderes ilçe merkezinin olduğu kesimde çok kurak 1 BGI sınıflarına ait değerler bulunmaktadır. 1970-2000 referans döneminde araştırma alanında minimum BGI değeri 47.7, maksimum değer 142.0, havzanın ortalama değeri ise 91.8'dir. 2016-2099 genelinde ve daha detaylı incelemek için ayrılan alt dönemlerde ise yarı nemli sınıflarına ait değerler ne genel periyotta ne alt dönemlerde bulunmamaktadır. Gelecek dönem projeksiyonlarında kurak 2, 3, 4 ve çok kurak 1, 2, 3, 4 BGI sınıflarına ait değerler olacak öngörülmektedir. 2016-2040 periyodunda Menderes'in kuzey ve güneyinde görülen kurak 2 sınıfı 2040-2070 döneminde yerini kurak 3'e 2071-2099 döneminde ise kurak 4'e bırakacağı ön görülmektedir (*Şekil 7*).

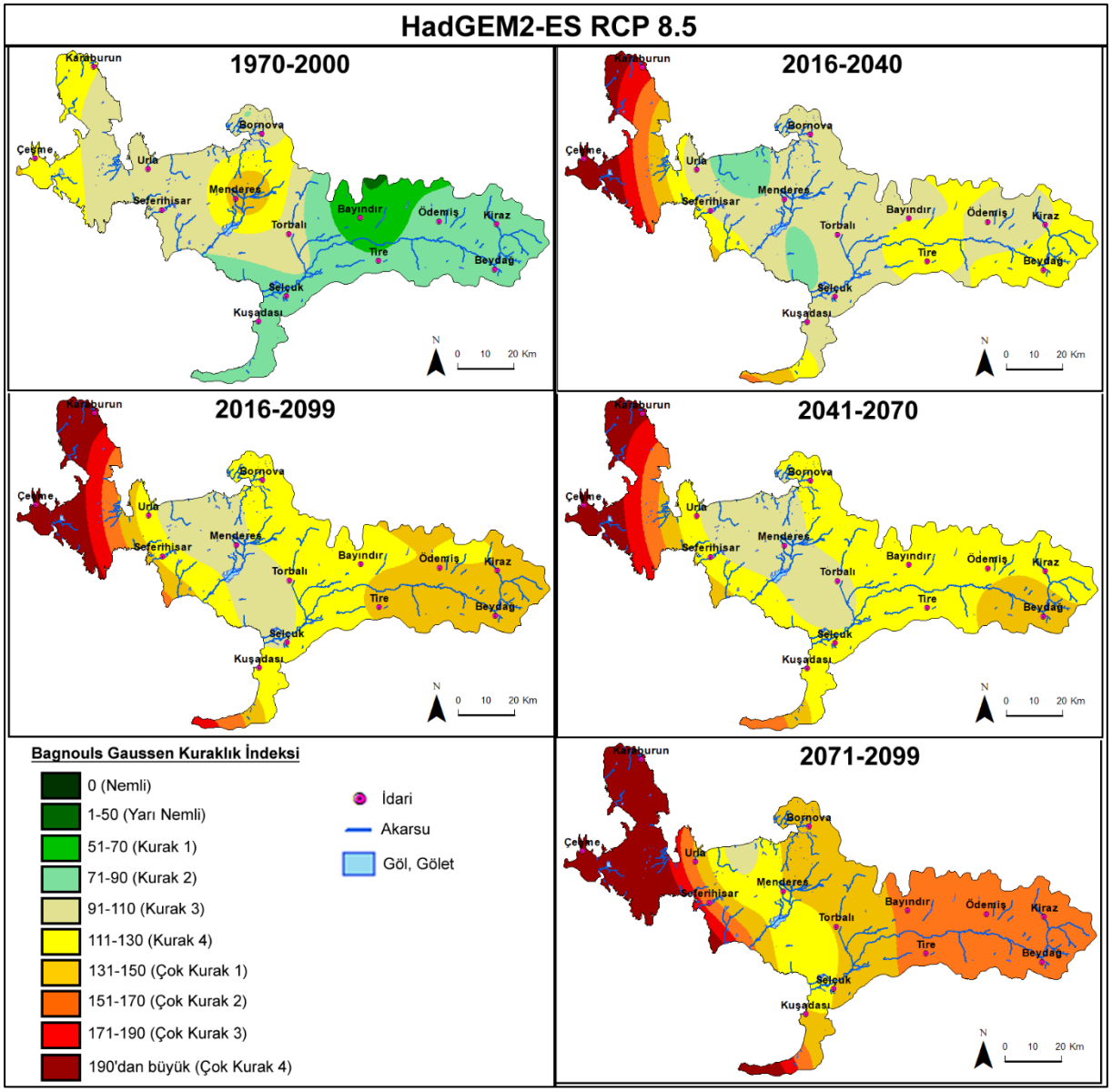

Figure 7. Reference period and future drought projections according to HadGEM2-ES global climate model RCP 8.5 scenario in the study area

Şekil 7. Araştırma alanında HadGEM2-ES küresel iklim modeli RCP 8.5 senaryosuna göre referans dönem ve gelecek dönem kuraklık projeksiyonları

HadGEM2-ES küresel iklim modeli RCP 8.5 senaryosu analiz sonuçlarına göre 2016-2099 genel döneminde araştırma alanında minimum BGI değeri 93.7, maksimum BGI 270.3, havza ortalaması 133.1'dir. Dolayısıyla 2016-2099 genel gelecek dönem projeksiyonunda 1971-2000 referans dönemine göre daha yüksek minimum, maksimum ve ortalama BGI değerleri söz konusudur. Aynı zamanda RCP 4.5 senaryosuna göre de daha yüksek minimum, maksimum ve ortalama BGI değerleri öngörülmektedir. Havzadaki gelecek dönem kuraklığı ele

aldığımızda 2016-2040 periyodunda minimum BGI değeri 82.2, maksimum BGI 261.0, havza ortalaması 115.5; 2041-2070 periyodunda minimum BGI 93.0, maksimum 254.1, havza ortalaması 128.3 iken; 2071-2099 döneminde minimum BGI 102.8, maksimum 294.0, ortalama değer ise 160.2'dir. Dolayısıyla 2016-2040 ile 2041-2070 periyodundaki maksimum BGI değerinin saymazsak havzanın minimum, maksimum ve ortalama BGI değerlerinde periyotlar arasında sürekli bir atış olduğunu söyleyebiliriz. Bunun sonucunda özellikle havzanın orta ve doğu kesiminde ilk gelecek dönem periyodundan son gelecek dönem periyoduna doğru kuraklık sınıflarının daha üst kademede daha geniş alanlar kaplamaya başladığı dikkati çekmektedir. Nitekim 2016-2040 periyodunda havzanın tarımsal açıdan en önemli bölgesi kabul edilen doğu kesiminde kurak 3 ve kurak 4 sınıfı geniş alan kaplarken, 2071-2099 gelecek dönem periyoduna geldiğimizde ise bu kesimde kurak 3 ve kurak 4 sınıfı ortadan kalkmış, onun yerine çok kurak 1 ve çok kurak 2 sınıfı geniş alanlarda yerini almıştır. Kötümser senaryoya göre havzanın tarımsal açıdan en önemli bölgesi olan ve halihazırda zaten yeraltı suyu ve kuraklık ile ilgili problemleri bulunan bu sahadaki kuraklığın gelecekte çok daha ciddi bir problem olacağı öngörülmektedir (*Şekil 7*).

Kötümser senaryoya göre de özellikle havzanın yoğun olarak tarım yapılan doğu ve kısmen orta kesiminde kuraklığın periyotlar arasında kademeli olarak artacağı 2071-2099 periyodunda ise söz konusu bölgelerde kuraktan çok kurak sınıfına geçiş olacağı tespit edilmiştir. Kötümser senaryoda iyimser senaryoya göre kuraklığın değerleri genel anlamda daha büyük elde edilmiştir.

HadGEM2-ES küresel iklim modeli RCP 8.5 senaryosuna göre araştırma alanındaki BGI değerlerinin değişimini ortaya koyabilmek için gelecek dönem periyotlarından referans dönemin farkı alınarak haritalanmıştır (*Şekil 8*). Havzada kuraklıkla ilgili değişikliklerin yönünü ve şiddetini yansıtan BGI fark haritaları incelendiğinde BGI değerlerinin havzanın çok büyük bir kesiminde artma göstermiştir. Sadece havzanın orta kesiminde Menderes ve çevresinde kuraklık indeksi fark değerlerinin negatif değerler olduğu görülmektedir. Kuraklık indeks değerlerindeki artışların 2071-2099 periyodunda diğer periyotlara göre çok daha fazla olacağı öngörülmektedir (*Şekil 8*). *Şekil 6*'da sunulan iyimser senaryoya göre kötümser senaryonun bütün periyotlardaki referans dönem farkları daha fazladır.

Muluk ve ark. (2013) Türkiye genelinde büyük havzalardaki kişi başına düşen su miktarını hesaplamış ve bu değerlendirme içerisinde Küçük Menderes Havzası Türkiye'deki 25 büyük havza içerisinde mutlak su kıtlığı yaşayan tek havza olması ile dikkati çekmiştir.

MGM (2014) tarafından yapılan projeksiyon çalışmalarında Küçük Menderes Havzası'nda gelecekte sıcaklıklarda 3 ila 5 °C'lik artışların yaşanacağı, yağışlarda ise 2011-2040 periyodunda artma eğilimi olsa da 2099'a kadar olan kalan dönemde % 10 ila % 20 arasında azalma olacağı öngörülmüştür. Dolayısıyla sıcaklardaki artışa karşın yağış miktarlarında azalış denklemi sonucunda havzada kuraklığın ilerleyen yıllarda daha da artacağı, halihazırda özellikle tarım sektöründe kendini hissettiren su probleminin önümüzdeki yıllarda daha da artacağı ortadadır. Kuraklığın şimdilik tarım sektöründekiler için endişe verici boyutu ilerleyen yıllarda etki alanını geliştirerek kuraklık problemini derinleştirecektir. Eskiden ovada yılda 3 kez hasat yapan çiftçiler sondaj yasaklarıyla birlikte ürün desenlerini ve bazı ziraat alışkanlıklarını değiştirmeye mecburdur.

Akyüz ve Atış (2018) Küçük Menderes Havzası'nda tarım sektöründeki üreticilere geçmiş 15 yıllık periyotta iklimde bir değişiklik gözleyip gözlemediklerini sormuş, çiftçilerin % 98 gibi çok büyük bir kısmı iklimde bir değişiklik yaşandığı şeklinde cevap vermiştir. Havzadaki çiftçilere göre sıcaklık ve kuraklık çok artmış, yağışlar azalmış, ekstrem olaylar ve nem ise geçtiğimiz yıllardan çok farklı olmamıştır (Akyüz ve Atış, 2018). Dolayısıyla projeksiyon sonuçları havzadaki çiftçilerin gözlemleri ile uygun sonuçlar vermiştir. Akyüz ve Atış (2018)'ın günümüzde hissedilmekte olan iklim değişikliği etkilerinin gelecekte hangi periyotta daha da yoğunlaşacağı sorusuna çiftçilerin yarısı "önümüzdeki 10 yıl içerisinde" cevabını vererek, iklim değişikliğinin yoğun etkilerinin kısa zamanda içerisinde göstermesini beklediklerini ortaya koymuşlardır. Çiftçilerin bu beklentisi bu çalışma kapsamında yapılan kuraklık projeksiyonu sonuçlarını ile pek örtüşmemektedir. Nitekim gerek iyimser senaryoda gerekse kötümser senaryoda 2071-2099 periyodu kuraklığın en şiddetli olduğu periyottur. Dolayısıyla çiftçilerin kişisel öngörülerinin aksine projeksiyon sonuçları iklim değişikliğinin etkilerinden kuraklığın uzun vadede daha yoğun hissedileceğini ortaya koymuştur.

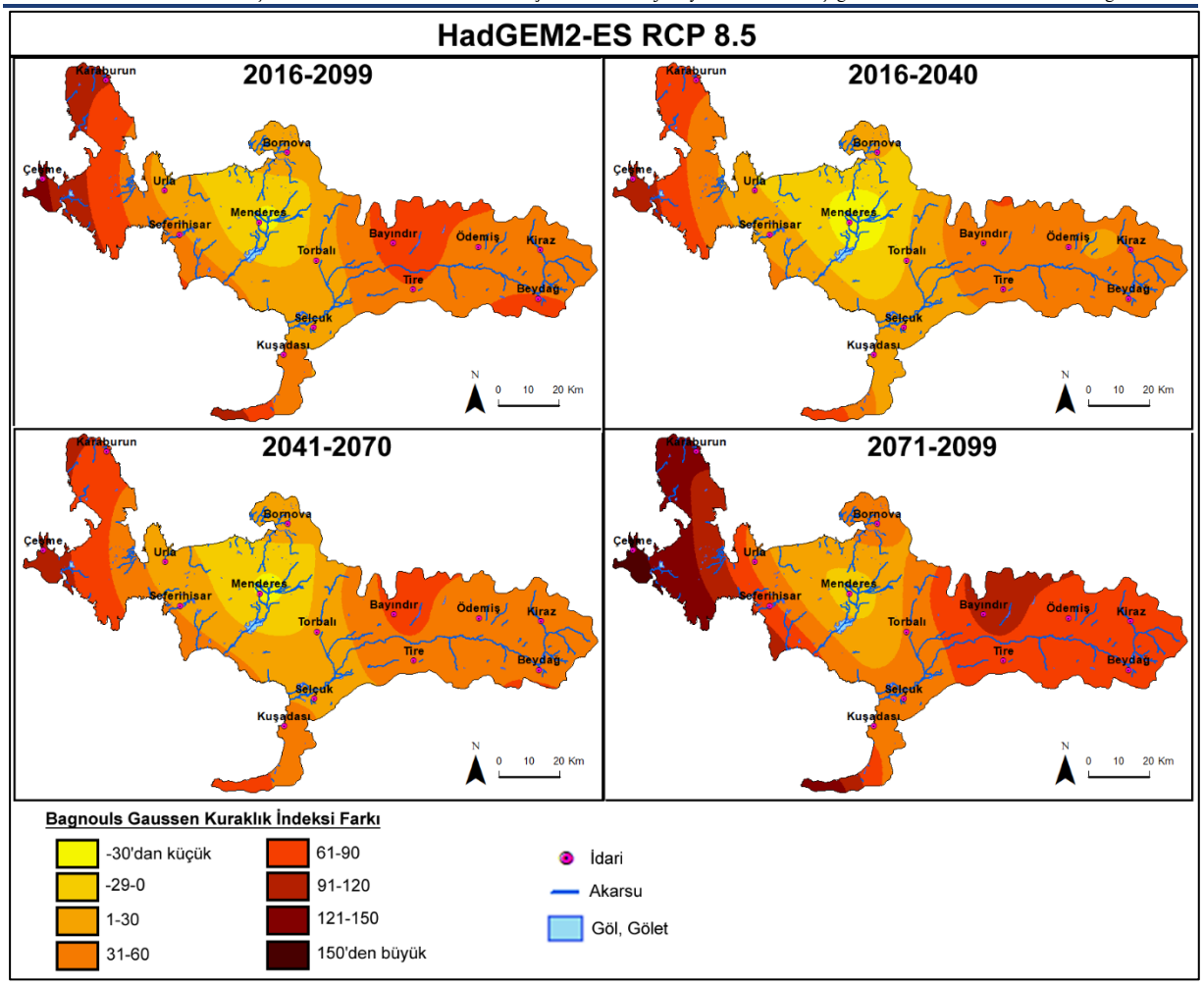

Figure 8. The differences of the future period drought index values from the reference period (1970-2000) according to the HadGEM2-ES global climate model RCP 8.5 scenario in the research area.

Şekil 8. Araştırma alanında HadGEM2-ES küresel iklim modeli RCP 8.5 senaryosuna göre gelecek dönem kuraklık indeksi değerlerinin referans dönemden (1970-2000) farkları

4. Sonuç

Analizler sonucu ortaya konulan projeksiyon sonuçlarına genel olarak bakıldığında hem iyimser (RCP 4.5) hem de kötümser (RCP 8.5) senaryosuna göre genel periyot (2016-2099) boyunca özellikle havzanın tarım açısından önemli doğu kesiminde kuraklıklarda artış beklenmektedir. RCP 8.5 senaryosuna göre öngörülen kuraklık artışı RCP 4.5 senaryosuna göre artıştan daha fazladır. Hem iyimser senaryoda hem de kötümser senaryoda 2071-2099 periyodu kuraklığın en fazla arttığı dönemdir.

Çalışmanın sonuçları açıkça gösteriyor ki havzada tarım sektöründe çalışanların kuraklıkla ilgili endişe duymaları ve tarım sektörünün gelecekteki birinci problemi olarak bunu görmeleri doğru bir endişedir. İklim modeli verileri gerek iyimser senaryoda gerekse kötümser senaryoda çiftçilerin kuraklıkla ilgili endişelerindeki haklılığını bilimsel olarak da desteklemektedir.

Küresel iklim değişikliğinin ve kuraklık riskinin farkında olan çiftçi kesimine özellikle yeraltı suyunun sürdürülebilir kullanımı ile ilgili bilimsel temelli yönlendirmeler yapılmalıdır. Zira küresel iklim değişikliklerinin olumsuz etkilerini minimuma indirme noktasında yapılabilecek çok şey vardır. Kuraklığın etkilerinin azaltılması hususunda tarımsal ürün deseninden su tüketimine kadar bilimsel temelli eğitim ve yönlendirmeler yapılmalıdır.

İklim değişikliğine karsı dünyada en hassas alanlardan birinde bulunan Küçük Menderes Havzasında kuraklık ile ilgili endişeler kendini somut olarak halihazırda hissettirmeye başlamıştır. Kuraklıkla ilgili problemlerin

önümüzdeki dönemde artması öngörülmektedir. Buna göre havza bazlı planlamalar yapılırken bu konunun göz önünde bulundurulması gerekmektedir.

Teşekkür

İklim projeksiyonu ve meteoroloji istasyonu verilerini araştırma amaçlı olarak ücretsiz temin etmemizi sağlayan Meteoroloji Genel Müdürlüğüne teşekkür ederim.

Etik Kurul Onayı

Bu çalışma için etik kuruldan izin alınmasına gerek yoktur.

Çıkar Çatışması Beyanı

Makale yazarları olarak aramızda herhangi bir çıkar çatışması olmadığını beyan ederiz.

Yazarlık Katkı Beyanı

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

First Report of Natural Infection of Watermelon Mosaic Virus (WMV) Infecting Bottle Gourd and Snake Melon

Su Kabağı ve Acur'da Doğal Enfeksiyona Neden Olan Karpuz Mozaik Virüsü (WMV)'nün İlk Raporu

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Abstract

Cucurbitaceous crops, one of the main crops of agriculture, are sensitive to many plant viruses. In August 2019, virus-like symptoms were observed on some cucurbit plants grown in private home gardens in Antalya and Denizli provinces (Turkey). A total of 53 leaf samples were sampled from plants with the most symptoms (melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* L.), bottle gourd (*Lagenaria siceraria* (Molina) Standl.), and snake melon (*Cucumis melo* var. *flexuosus*) and tested by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) against possible watermelon mosaic potyvirus (WMV) infection. The coat protein gene (CP) specific primer sets amplified a gene product of nearly 820 bp fragment from symptomatic plants. WMV infections were detected in 31 individual cucurbit plants, including 11 melons, 8 watermelons, 7 snake melons and 5 bottle gourds. The presence of viral infection was found only in ornamental squash plants in Antalya province and in all cucurbits sampled in Denizli province. To better comprehend the molecular characteristics of virus isolates, the amplified viral DNA fragments were cloned in a proper prokaryotic plasmid, sequenced by Next Generation Sequencing (NGS) and recorded to GenBank. Bioinformatic analyses using the Basic Local Alignment Search Tool (BLAST) showed that the identified CP gene sequences exhibited significant nucleotide homogeneity, supported by a high nucleotide similarity index with that of other isolates around the world. In addition, Turkish isolates isolated from Antalya and Denizli regions showed approximately 94% nucleotide similarity among themselves. For phylogenetic inference, WMV sequences were subjected to multiple alignments with isolates from different geographic origins of the same viruses. Molecular phylogeny showed that all WMV isolates are closely related to other world WMV isolates at variable rates. WMV is wide host range viruses in cucurbit crops, however, this work is the first scientific report of WMV isolates detected in bottle gourd and snake melon from the South and West Regions of Turkey all over the world.

Keywords: WMV, RT-PCR, Molecular Phylogeny, Bottle gourd, Snake melon

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Öz

Tarımın ana ürünlerinden biri olan kabakgiller, birçok bitki virüsüne karşı hassastır. Ağustos 2019'da Antalya ve Denizli ilinde (Türkiye) müstakil ev bahçelerinde yetiştirilen bazı kabakgil bitkilerinde virüs tipi semptomlar gözlenmiştir. Çoğu belirtili olan bitkilerden (kavun (*Cucumis melo* L.), karpuz (*Citrullus lanatus* L.), su kabağı (*Lagenaria siceraria* (Molina) Standl.) ve acur (*Cucumis melo* var. *flexuosus*) toplam 53 yaprak örneği toplanmış ve olası watermelon mosaic virus (WMV) enfeksiyonuna karşı Revers-Transkriptaz Polimeraz Zincir Reaksiyonu (RT-PCR) ile test edilmiştir. Kılıf protein genine (CP) özel primer setleri, simptomatik bitkilerden yaklaşık 820 bp'lik bir gen ürününü amplifiye etmiştir. WMV enfeksiyonları, 11 kavun, 8 karpuz, 7 acur ve 5 su kabağı dahil olmak üzere 31 farklı kabakgil bitkisinde tespit edilmiştir. Antalya ilinde sadece su kabağı bitkilerinde, Denizli'den toplanan tüm kabakgil örneklerinde viral enfeksiyon varlığı saptanmıştır. Virüs izolatlarının moleküler özelliklerinin daha iyi anlaşılması için amplifiye edilmiş viral DNA fragmanları uygun bir prokaryotik plazmide klonlanmış, Yeni Nesil Dizileme (NGS) ile dizilenmiş ve gen bankasına kaydı yapılmıştır. Basic Local Alignment Search Tool (BLAST) ile gerçekleştirilen biyoinformatik analizler, belirlenen WMV-CP gen dizilerinin dünyadaki diğer izolatlarınkiyle yüksek bir nükleotid benzerlik indeksi ile desteklenerek önemli bir nükleotit homojenitesi sergilediğini göstermiştir. Ayrıca, Antalya ve Denizli illerinden izole edilen Türk izolatları kendi aralarında yaklaşık %94 oranında nükleotit benzerliği göstermiştir. Filogenetik çıkarımlar için, WMV dizileri, aynı virüslerin farklı coğrafik kökenlerinden gelen 30 izolatla çoklu hizalamaya tabi tutulmuştur. Moleküler filogeni ise tüm WMV izolatlarının değişen oranlarda diğer dünya WMV izolatları ile yakından ilişkili olduğunu göstermiştir. WMV, kabakgil bitkilerinde geniş konukçu dizisine sahip virüsler arasında yer almakta ve bununla birlikte bu çalışma, Türkiye'nin Güney ve Batı Bölgeleri'nde su kabağında ve acurda belirlenen WMV izolatlarının tüm dünyadaki ilk bilimsel raporudur.

Anahtar Kelimeler: WMV, RT-PCR, Moleküler filogeni, Su kabağı, Acur

1. Introduction

Cucurbits are one of the important plant groups that support humans for their consumable products, fiber sources and other purposes. Family Cucurbitaceae contains nearly 1000 species from five subfamilies, including watermelon, melon, bowler, cucumber, fig-leaf gourd, porongo, winter squash, and pumpkin (Bisognin, 2002). Among these, snake melon (*Cucumis melo* var. *flexuosus*), which is considered to be native to Anatolia, Iran, Afghanistan, and Southwest Asia, is an open field vegetable consumed in the immature stage for table and pickle, with a production of around 22 000 tons in Turkey (Vural et al., 2000). It is a source of vitamins and minerals (potassium, phosphorus, magnesium, vitamin A) as well as functional bioactive compounds (secondary metabolites, polyphenols) that serve as curatives for human health (Ilahy et al., 2019). Originating from Africa, bottle gourd (*L. siceraria*) is a climbing perennial cucurbit vegetable grown in tropical regions, with 24 different shaped varieties (Stephens, 1994; Awala et al., 2019). Besides its decorative use, its fruit is widely used for medicinal purposes thanks to its different natural biological ingredients (Saeed et al., 2022).

Cucurbits are invaded by many pathogens, including viruses, bacteria, and fungi (Horuz and Aysan, 2018). Cucurbit viruses are the largest production-limiting pathogens causing product losses by up to 100% on sensitive varieties under the appropriate conditions (Coutts et al., 2011)). More than 50 viruses encompassing zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), squash mosaic virus (SqMV), and WMV, are the main hosts of cucurbits (Karanfil and Korkmaz, 2020). WMV, one of the first described pathogens of cucurbit mosaic infections, was first isolated in watermelon (*Citrullus lanatus*) Rio Grande Valley (Webb and Scoot, 1965). WMV, a member of the Potyvirus genus, is infectious to more than 170 plant species, including cucurbits and weeds, and causing rapid and severe outbreaks in cucurbit-grown fields worldwide. Severe WMV infection reduces the quality of cucurbit crops, rendering them unmarketable, resulting in crop losses of up to 100%, especially in early-season infections (Fletcher et al., 2000; Katis et al., 2006; Coutts et al., 2011). An insect-transmitting pathogen is transmitted by aphids in a non-persistent style (Brunt et al., 1996). Depending on the host and the time of infection, the virus causes mosaic, dark green blistering, mottling, deformity on leaves and knobbly, discoloration, and distortion in fruit (Delmiglio and Pearson, 2006). DNA-based molecular methods and protein-based serological methods were commonly employed to accurately diagnose the associated pathogen (Al-Ani et al., 2011; Khalifa et al., 2015; Kızmaz et al., 2016).

The occurrence of WMV has been reported almost worldwide as a prevalent virulent pathogen of cucurbit plants, chiefly in Mediterranean countries (Akbar et al., 2015; Chatzivassiliou et al., 2016; Niu et al., 2017; Qiu et al., 2018). In Turkey, WMV infection has mostly been reported in cucurbits such as cucumber, melon and watermelon (Sevik and Arli-Sokmen, 2003; Yeşil, 2018; 2019; 2020). However, no attempt has been made to detect WMV infection in bottle gourd and snake melon so far. In this study, we attempted to determine the causal viral pathogens in cucurbit showing viral-suspicion cultivated in Denizli and Antalya Provinces of Turkey. We analyzed their respective gene sequences and phylogenetic relationships.

2. Materials and Methods

2.1. Sample collection and virus source

In 2019, cucurbit leaves samples showing virus-like symptoms were observed in Denizli and Antalya provinces (Turkey). Samples were bulked from mostly suspicious plants in a home garden. The number of samples collected is listed in *Table 1*. Samples were transported to the laboratory by placing them in a styropor box containing an ice pack immediately and maintained at -80 ºC until analyzed.

Table 1. Number of cucurbit plants collected from the South and West region of Turkey

District	Number of samples				Total
	Melon	Watermelon	Snake melon	Bottle gourd	
Denizli	**15**	**10**	**10**	**5**	**40**
Antalya	**-**	**9**	**-**	**4**	**13**
Total	**15**	**19**	**10**	**9**	**53**

2.2. Total RNA extraction

The RNA extraction was accomplished by the silica-capture method with a minor difference according to Foissac et al. (2001). Frozen cucurbit tissues (100 mg) were grounded in grounding buffer added 1 µl of β-

mercaptoethanol and transferred to 1.5 ml microfuge tubes. The homogenates added 100 µl sarkosyl (10%) were incubated for 10 min at 70 °C and then on ice for 5 min and centrifuged for 10 min at 13.000 rpm. 300 µl of the liquid phase were carefully was poured into a new tube consisting of 150 µl EtOH, 300 µl NAI (6M), and 25 µl resuspended silica. The solution was vortexed thoroughly to attain a homogeneous suspension before incubation in intermittent shaking for 10 min. The tubes were centrifuged for 1 min at 6000 rpm. The upper phase was removed and the pellet was dissolved with 500 ml wash buffer (repeat 2 wash steps). The pellet containing RNAs was resuspended with 100 µl of RNA-free water. The tubes were centrifuged for 10 min at 13000 rpm and supernatant consisting of total RNAs was preserved at-80°C until the cDNA and RT-PCR process.

2.3. Amplifications of Coat Protein Gene (CP)

RNA-enriched solutions were used as a template for cDNA synthesis. The cDNA synthesis was fulfilled using a reverse primer with 2 µl of extracted RNA following the instruction from the RevertAid First Strand cDNA kit (Vilnius, Thermo-Fermentas, Lithuania). For WMV-specific detection, CP-specific primer pairs generating 822 bp amplicons were utilized based on the reference publications (Sharifi et al., 2008). A reverse transcription-polymerase chain reaction (RT-PCR) method was set up to detect the occurrence of each viral agent in infected cucurbit tissues. The 2 µl of synthesized cDNAs were submitted to PCR assays in a final volume of 25 µl containing 18.3 µl of nuclease-free water, 2.5 µl of 10× reaction buffer, 0.5 µl of dNTPs (20 mM), 1.5 µl of $MgCl_2$ (25 mM), 0.5 µL of each primer (100 pmol), 0.2 µl of Taq DNA polymerase. The primers and temperature cycles used in the PCR reaction are listed in *Table 2*.

Table 2. Primers and termocycling program used in PCR tests to detect watermelon mosaic potyvirus infections

	Forward primer	Reverse primer	Cycling program
WMV	5'-ATTCACGTCCCTTGCAGTGTG-3'	5'-GAATCAGTGTCTCTGCAATCAGG-3'	3m 94°C **1m 94°C** **1m 60°C** **1m 72°C** } **35 cycles** 10m 72°C

2.4. Molecular cloning and nucleotide sequencing

All PCR-amplified fragments are gel-purified using a gel extraction kit (Thermo Scientific) and introduced into a prokaryotic cloning vector (pGEM T-Easy vector system, Promega) using standard cloning techniques with some modifications, and afterward transferred separately in *E. coli* (JM109 strain). At least one independent isolate of each sample was grown in LB medium containing ampicillin (1%). The recombinant plasmids consisting of viral CP gene were purified from bacterial solution and sequenced by next-generation sequencing (Sentebiolab/Ankara/Turkey).

2.5. Phylogenetic inference and Multiple alignment

To determine the sequence similarity of the viral CP genes, the unpublished nucleotide sequences of cucurbit isolates were compared with the viral nucleotide sequences stored in NCBI (nucleotide BLAST, BLASTn). The definitively diagnosed viral sequences were recorded in GenBank. Phylogenetic relationship, multiple alignments, and nucleotide analysis of sequences detected were achieved using CLC Main Workbench (version 6.7.1), Sequence Demarcation Tool (Version 1.2), and Mega X program (Kumar et al., 2018). The evolutionary relationship was presumed using the Neighbor-Joining method and robustness was calculated by 1000 bootstraps search. Soybean mosaic virus isolate (FJ376388) was assigned as an outgroup virus isolate.

3. Results and Discussion

3.1. Viral detection

In the inspection of cucurbit plants carried out in Antalya and Denizli provinces, virus-like symptoms were observed in melon, watermelon, snake melon, and bottle gourd (*Figure 1*). WMV-related symptoms of cucurbit leaves were summarized in *Table 4*. The symptoms produced by plant viruses can be explained by a close relationship between the virus and the host. Plant viruses have developed various strategies that have a suppressive effect on plant physiology (Krajcsi and Wold, 1998). Although they have few genes compared to their hosts, they have the potential to turn the plant cell's reproductive mechanism in their favor by stopping the synthesis of various

macromolecules to capture the host cells. After viral infection, various symptoms occur, which are manifestations of the disease because of abnormal metabolic or morphological changes occurring in the host (Martelli and Russo, 1985).

Figure 1. Some signs induced by watermelon mosaic potyvirus infection in cucurbit specimens collected from surveyed areas. A-B, C, E-F, and G-H represent the snake melon, bottle gourd, watermelon, and melon samples from Denizli province, respectively, while D is the bottle gourd sample from Antalya province.

Gathered samples were tested by RT-PCR against the WMV infections. DNA fragments equivalent to 820 bp were obtained on agarose gel, corresponding to the CP gene of the causative agent in diseased cucurbits (*Figure 2*). RT-PCR assays amplified ~820 bp products in 31 out of 53 symptomatic and asymptomatic cucurbit samples. WMV amplicons were detected in 30 samples from Denizli and 1 from Antalya. No DNA band is amplified from negative control. Plant-based infection-related information based on RT-PCR tests is presented in *Table 3*.

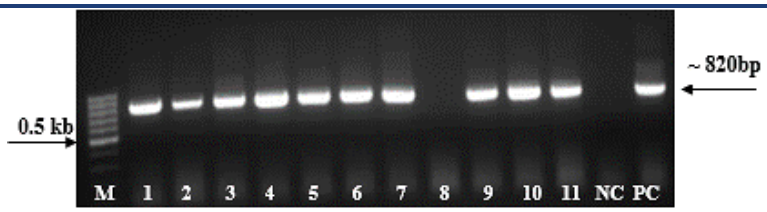

Figure 2. DNA fragments of watermelon mosaic potyvirus amplified using RT-PCR assay in infected cucurbits

WMV is a prevalent biotroph pathogen for cultivated plants and weeds (Mahgoub et al., 1997; Kheder et al., 2017). Common symptoms of cucurbit-related WMV infections were well-documented in many agroecosystems worldwide. Similar symptoms as in this study have also been reported in New Zealand from buttercup squash (Fletcher et al., 2000), watermelon in Saudi Arabia (Santosa et al., 2018), cucurbitaceous vegetables in the Czech Republic (Svoboda and Hale, 2011), cucurbit crops in USA and southern United States (Fernandes et al., 1991; Ali et al, 2012), watermelons and pumpkins in Uganda (Masika et al., 2017).

In this study, WMV infection was not detected in all cucurbits collected, probably due to different viral agents. However, WMV was detected in all symptomatic cucurbit species (melon, watermelon, snake melon, and bottle gourd) within the study. Although there have been many previous reports of WMV in melons and watermelons, according to the literature review, there are no reports of the natural presence of WMV in snake melons and bottle gourds. Based on our online surveys, the only record is that Cucumber green mottle mosaic virus has been determined in bottle gourd plants in Saudi Arabia biologically and molecularly (Amer, 2015). However, several cucurbit plant-associated WMV isolates, including snake melon and bottle gourd (Acc. no: AB127934 and AB218280), have been characterized experimentally (mechanical inoculation) in Pakistan in host range tests (Ali et al., 2006). In addition, in Argentina, WMV partial polyprotein gene sequences (825 bp) from bottle gourd have been directly submitted in the GenBank public database (MN006915.1 and MN006914.1), not published in any journal. Therefore, the above reports confirmed that natural WMV infections of snake melon and bottle gourd plants in this study are the first published report globally.

The presence of WMV infections in Turkey was well-determined using various methodologies, such as serological, molecular, and biological indexing in different localizations and plant sources. Randa-Zelyüt et al. (2022) reported natural infection of WMV in wild carrot (*Daucus carota*) in Çanakkale province of Turkey. It was also determined for the first time that WMV-2 naturally infects *S. angulatus* in the Black Sea Region of Turkey (Korkmaz et al., 2016). WMV infection of cucurbit plants has been extensively studied in distinct localities of Turkey. In Uşak province, viral agents were confirmed in 106 of 175 cucurbit samples from 5 species in cucurbit growing areas using DAS-ELISA and biological indexing. Following this study, signs such as yellowing, vein banding, blistering, curling, asymmetry in the leaves and stunting were observed in field observations (Dikici and Tarla, 2020). In Tekirdağ, Edirne, and Kırklareli provinces, 502 melon and watermelon samples were tested against 7 viruses, including WMV. Overall, the associated pathogen was confirmed in all survey areas (Köklü and Yilmaz, 2006). In Samsun province, WMV infection has been reported on cucumber, melon, pumpkin, squash and watermelon plants using serological methods by Sevik and Arli-Sokmen, (2003). In Turkey, on the other hand, WMV has been detected in different agricultural areas of Turkey (Konya, Karaman and Aksaray) (Yeşil, 2013; Yeşil and Ertunç, 2012; 2013), Diyarbakır and Mardin (Kızmaz et al., 2016; Korkmaz et al., 2021), Tokat (Korkmaz et al., 2018), Ankara and Antalya (Topkaya et al., 2019), Adana (Kamberoğlu et al., 2015), Van (Usta et al., 2018), Eastern Mediterranean Region (Adana, Osmaniye, Mersin) (Kamberoğlu and Keçe, 2016) and Bingöl (Güller and Usta, 2020). WMV infecting cucurbits is also associated with seeds of squash plants (*Cucurbita pepo*). In Turkey, WMV infection in squash seeds has been reported in Aksaray, Yozgat and Nevşehir provinces of Turkey (Yeşil, 2018; 2019; 2020).

Table 3. Watermelon mosaic potyvirus infection based on plant samples and numbers according to the provinces

District	TS				IS				HS			
	M	**WM**	**SM**	**BG**	**M**	**WM**	**SM**	**BG**	**M**	**WM**	**SM**	**BG**
Denizli	15	10	10	5	11	8	7	4	4	2	3	1
Antalya	-	9	-	4	-	-	-	1	-	9	-	3
Total	**15**	**19**	**10**	**9**	**11**	**8**	**7**	**5**	**4**	**11**	**3**	**4**
	53				**31**				**22**			

TS: Tested Samples, IS: Infected Samples, HS: Healthy Samples, M; melon, WM: watermelon, S: Snake melon, BG: Bottle gourd

3.2. Sequencing, Multiple Alignment, and Phylogenetic Relationship

The CP-DNA bands of some positive isolates (1 of bottle gourd, 2 of melon, 1 of watermelon, and 1 of snake melon from Denizli province, and 1 of bottle gourd from Antalya province) were successfully cloned and sequenced. The CP gene sequences of causative agents were further analyzed using BLAST analysis at the nucleotide level. Nucleotide BLAST analysis showed that sequences exhibited substantial nucleotide consensus with that of other isolates in the world, displaying a high nucleotide similarity score. After sequence validation, all six WMV sequences, 5 from Denizli and 1 from Antalya, were archived in the GenBank database under the accession numbers submitted in *Table 4*.

Table 4. Symptoms and distribution of watermelon mosaic potyvirus isolates related to various cucurbit crops in Turkey along with Genbank accession numbers

Cucurbit species	District	Isolate name	Acc. No	Symptoms
Melon	Denizli	Denizli 8	OM988079	mosaic, mottle, malformation, filiformis
Melon	Denizli	Denizli 32	OM988082	
Watermelon	Denizli	Denizli 16	OM988080	mosaic, mottle, little leaf, flecking
Snake melon	Denizli	Denizli 24	OM988081	mosaic, mottle, rosetting, bumps in fruit
Bottle gourd	Denizli	Denizli 5	OM988078	vein banding, crumpled leaves, mosaic in fruit
Bottle gourd	Antalya	Antalya 40	ON010742	

Multiple alignment analyses using 30 verified sequences showed that Denizli-WMV isolates have high nucleotide similarity among themselves (OM988078, 79, 80, 81, and 82), but not with Antalya isolate (ON010742). Approximately 94% nucleotide similarity was determined amongst Turkish isolates from Antalya and Denizli districts (*Figure 3*). In a comparison of the nucleotide sequences using the sequence demarcation tool, nucleotide variations were on the order of 3.28%, corresponding to 27 nucleotides, among isolates from different localities in this study. This data showed the genetic diversity of RNA-structured viruses in cucurbit plants.

3.3. Phylogenetic Relationship of Identified Isolates

The phylogenetic inference of WMV sequences was investigated along with 30 related sequences from distinct hosts and ecological origins. Consistent with the nucleotide similarity index, the phylogenetic dendrogram divided all WMV isolates into two dominant groups, possibly resulting from the presence of two distinct developmental pathways. The phylogenetic tree clustered all Denizli-WMV isolates in the same group, but not the Antalya isolate, probably because of similar genetic traits originating from the same region, the Turkish-Antalya isolate showed a close phylogenetic affinity with the isolates from France, Turkey, and China (*Figure 4*).

Different host populations of WMV have the potential to cause epidemics in agroecological areas worldwide. Therefore, it is essential to eliminate or control viruses from commercial areas growing cucurbit crops. Systematic monitoring and early detection of viral disease agents, together with ecological and quantitative epidemiological approaches, may facilitate their control (Jeger, 2020; McLeish et al., 2020). Different control approaches, such as prophylactic measures, cross-protection, and resistant cultivars, have been adopted for general plant viral diseases. In particular, prophylactic measures preventing or limiting the contact of virus-infected aphids and cucurbitaceous plants are important in protection against plant viruses. Considering that WMV is carried by over 35 species of aphids, insecticidal measures come to the fore (Lecoq and Desbiez, 2008). In addition, weed removal near planting areas and crop rotation in the same area can reduce the seasonal virus population. Plastic mulching also has a repellent effect on aphids and can significantly delay the viral spread.

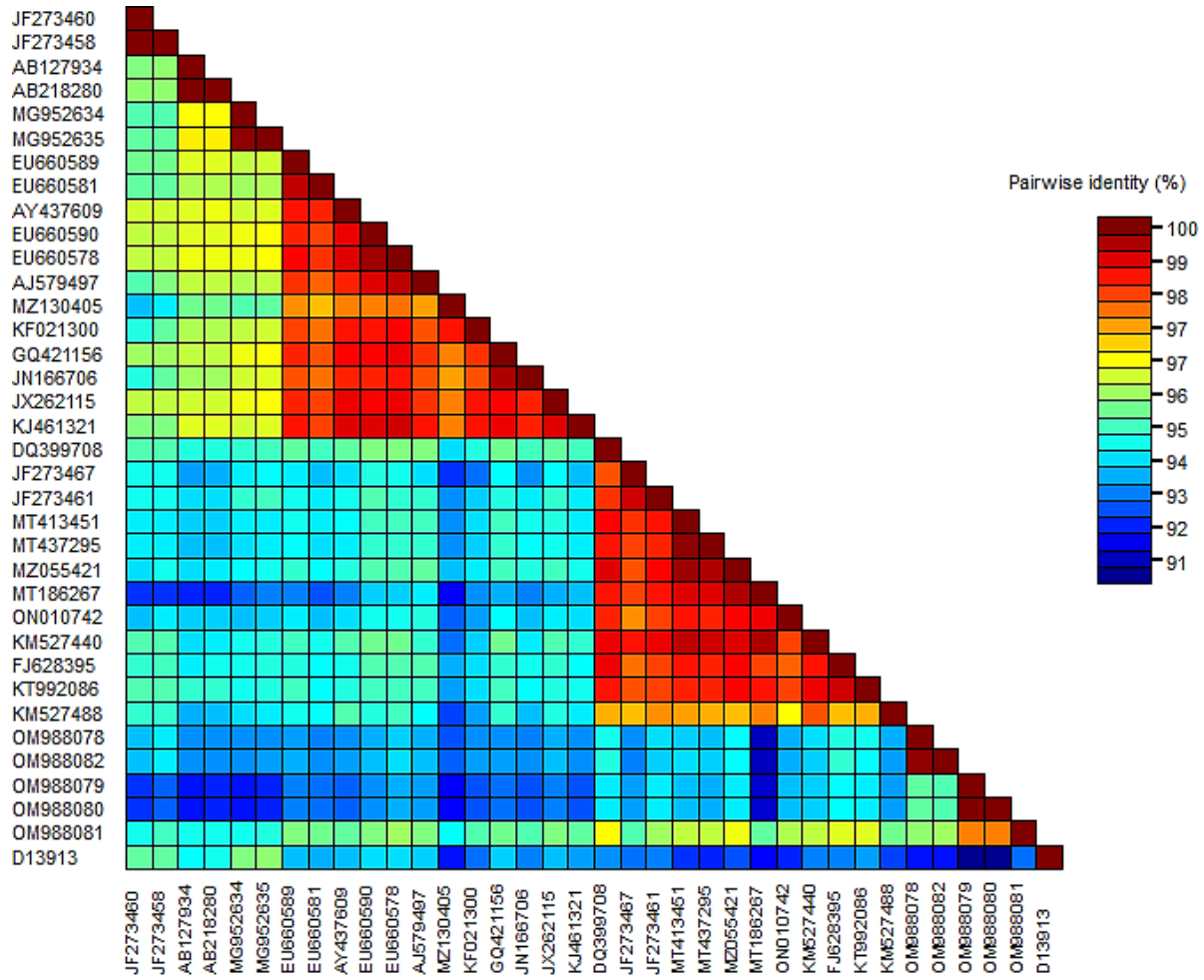

Figure 3. Nucleotide similarity rates of watermelon mosaic potyvirus sequences detected in diseased cucurbits worldwide, along with Antalya and Denizli isolates. The isolates belong to; France (JF273460, JF273461, JF273467, JF273458, EU660589, EU660581, AY437609, EU660578), Pakistan (AB127934, AB218280), Turkey (MG952634, MG952635, MZ130405, KF021300, MT413451, MT437295, MZ055421, MT186267), Italy (EU660590), Spain (AJ579497), Iran (GQ421156, JN166706), Serbia (JX262115), Ukraine (KJ461321), China (DQ399708, KM527440, KM527488), Poland (FJ628395), South Korea (KT992086) and, USA (D13913).

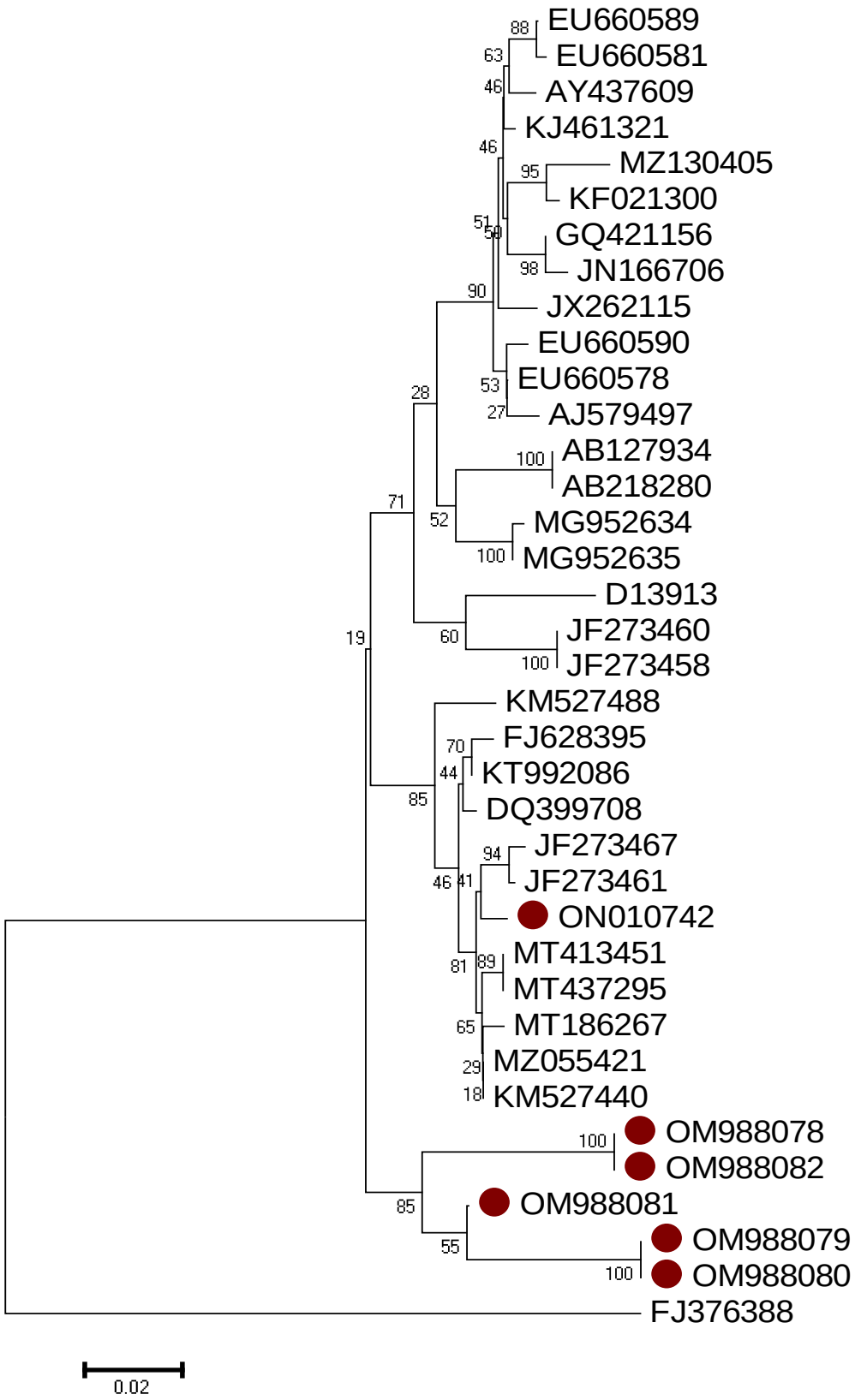

Figure 4. Phylogenetic dendrogram of watermelon mosaic potyvirus isolates. Soybean mosaic virus (FJ376388) was assigned as an outgroup. Bootstrap scores are demonstrated on each branch. The isolates from this study are marked with a red circle.

4. Conclusions

Turkish WMV isolates denominated Denizli 8, Denizli 32, Denizli 16, Denizli 24, Denizli 5 and Antalya 40 were obtained from symptomatic melon, watermelon, snake melon and bottle gourd plants. Two molecular phylogroups emerged in the phylogenetic relationships of the detected WMV isolates. In the dendrogram, the Denizli isolates formed an independent group, while the Antalya isolate showed a genetic affinity with isolates from different hosts from Asia and Europe. This is the first molecular detection and molecular characterization of WMV isolates from snake melon and bottle gourd in Turkey and the world.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Korkmaz, G., Dermirel, S.; Design: Dermirel, S., Usta, M.; Data Collection or Processing: Usta, M., Güller, A.; Literature Search: Güller, A., Korkmaz, G.; Writing, Review and Editing: Güller, A., Usta, M.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Hasat Sonrası Farklı Depolama Koşullarının Biyolojik Malzeme Üzerine Olası Etkilerin Belirlenmesi: Soya Örneği

Determining the Possible Effects of Different Storage Conditions on Biological Material After Harvesting: Soy Example

Yasemin VURARAK[1*]

Öz

Tropik ve yarı tropik iklimlerde yağlı tohumların muhafazası, çimlenme kabiliyetinin ve kalitenin korunması bakımından önemlidir. Yağlı tohumlar hasattan hemen sonra uygun depolama şartlarında muhafaza edilmediklerinde derhal bozulmaya ve kalitesini kaybetmeye başlar. Bu çalışma, farklı depolama koşullarının soya tohum kalitesi üzerine etkilerini belirlemek amacıyla yarı tropik iklim tipine sahip Adana ili koşullarında yürütülmüştür. Denemeler, depolama ve laboratuvar çalışmaları dikkate alınarak 2^5'lik faktöriyel deneme desenine göre 2 yıl ve 3 tekerrürlü olarak Faktöriyel Tesadüf Parsellerinde düzenlenmiş ve analiz edilmiştir. Çalışmada, depolama süresi (7 ve 9 ay), depolama koşulu (geleneksel ambar, soğuk hava deposu), paket malzemesi (PE çuval, vakumlu torba) ve farklı irilikte soya çeşidi (Yeşilsoy, Nazlıcan) faktörlerinin soya tohum stokları üzerinde yağ, protein ve çimlenme kabiliyetine etkileri belirlenmiştir. Çalışmanın sonunda, yağ, protein ve çimlenme oranları üzerinde depolama süresi ve çeşidin istatistiksel olarak $p \leq 0.01$ önem seviyesinde etkisinin olduğu tespit edilmiştir. Aynı zamanda çimlenme üzerinde depolama koşulunun $p \leq 0.01$, paket malzemesinin ise $p \leq 0.05$ önem seviyesinde etkili olduğu ancak bu iki faktörün yağ ve protein üzerinde istatistiki olarak bir etkisinin olmadığı tespit edilmiştir. Veriler değerlendirildiğinde soya depolamada her iki çeşit için de "Geleneksel ambar + PE çuval" ile yapılan depolamadan en kötü sonuçların alındığı belirlenmiştir. Bu depolamada, depolama başlangıcında Yeşilsoy (%93.5) ve Nazlıcan (%93) çeşitlerinin oldukça yüksek olan çimlenme oranlarının, 7 ay depolama sonunda sırasıyla %56, %45.83, 9 ay depolama sonunda ise sırasıyla % 50.33, %42.67 ye kadar azalmış olduğu belirlenmiştir. Sonuç olarak soyanın depolanmasında; küçük tohumlu soya çeşitlerinin tercih edilmesi, soğuk hava deposunda muhafaza edilmesi ve depolama süresinin 7 aydan fazla olmaması şartıyla çimlenme kabiliyetini diğer konulara göre daha fazla korunduğu belirlenmiştir. Ayrıca soğuk hava deposu şartlarında paket malzemesi olarak maliyet dikkate alındığında vakumlu yerine PE malzemenin de kullanılabileceği kanaati oluşmuştur. Yağlı tohum tür ve çeşitlerine göre depolama şartlarının değiştiği ve çeşitlere göre bu koşulların çevre şartları da dikkate alınarak belirlenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Biyolojik materyal, Çimlenme, *Glycine max* (L.) Merrill, Hasat sonrası işlemler, Muhafaza, Yağlı tohum

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Abstract

In tropical and semi-tropical climates, the preservation of oilseeds is important for the preservation of germination ability and quality. When the oilseeds are not kept under proper storage conditions immediately after harvest, they immediately begin to deteriorate and lose their quality. This study was conducted in Adana province conditions with a semi-tropical climate type to determine the effects of different storage conditions on soybean seed quality. Trials, storage and laboratory studies were organized and analyzed in factorial coincidence parcels with 2 years and 3 repetitions according to the factorial trial pattern of 2^5. In the study, factors such as storage time (7 and 9 months), storage condition (traditional warehouse, cold storage warehouse), packaging material (PE sack, vacuum bag) and different large soybean variety (Yeşilsoy, Nazlican) have been determined to affect oil, protein and germination ability on soy seed stocks. At the end of the study, it was determined that the storage time and the variety had a statistically significant level of $p \leq 0.01$ on fat, protein and germination rates. At the same time, it was determined that the storage condition $p \leq 0.01$ and the package material $p \leq 0.05$ were effective on germination, but these two factors had no statistical effect on fat and protein. When the data were evaluated, it was determined that the worst results were obtained from storage with "Traditional warehouse + PE sack" for both varieties in soy storage. In this storage, the very high germination rates of the Yeşilsoy (93.5%) and Nazlican (93%) varieties at the beginning of storage decreased by 56% and 45.83% respectively after 7 months of storage. At the end of 9 months of storage, it was determined that it decreased by 50.33% and 42.67%, respectively. As a result, in the storage of soy; it is determined that the ability to germinate is more protected than other issues provided that small seed soy varieties are preferred, stored in cold storage warehouse and storage time is not more than 7 months. In addition, when the cost as package material is taken into consideration in cold storage warehouse conditions, it is believed that PE material can be used instead of vacuum bag. It is thought that the storage conditions change according to the type and varieties of oilseed and these conditions should be determined by taking into account the environmental conditions according to the varieties.

Keywords: Biological material, Germination, *Glycine max* (L.) Merrill, Post-harvest operations, Storage, Oilseed

1. Giriş

Çin'den Dünyaya yayıldığı kabul edilen soya (*Glycine max* (L.) Merrill), *Leguminosae* ana familyası ve Papilionidea alt familyasına (2n=40) ait bir üyedir (Kakde ve Chavan, 2012; Zhou ve ark., 2013). Ortalama %40 protein, %20 yağ, %35 karbonhidrat ve %5 kül içeriği ile gıda, yem, ilaç, boya, tekstil gibi sektörlerde 400'den fazla kullanım alanı bulunmaktadır. Soya, diğer baklagillerden farklı olarak yüksek yağ oranı ve çoğu bitki proteinlerine göre tüm temel amino asitleri bir arada bünyesinde bulundurması nedeniyle endüstri bitkileri içinde "benzersiz bir bitki" olarak tanımlanmaktadır (Barrett, 2006; Şahin ve İşler, 2022). Tüm tahılların hasattan sonra, üretim sezonu dışındaki ay, mevsim ve yıllarda kullanılabilmeleri yanında savaş, doğal afetler, açlık, kıtlık, kuraklık gibi özel durumlar karşısında kalitelerini kaybetmeden uygun koşullarda depolanmaları zorunludur (İnan ve ark., 2006; Dizlek, 2012). İnsan gıdası ve hayvan yemi olarak kullanılan soya, baklagil olarak da iyi bir protein kaynağı olmasına rağmen depolanabilir olma kapasitesi diğer yağlı tohumlara göre oldukça düşüktür (Goswami ve ark., 2017). Ilıman iklimlerde hasattan hemen sonra uygun olmayan depolama şartlarında yağlı tohumların muhafaza edilmesi ile çok büyük ekonomik kayıplara neden olan kızışma olayı meydana gelir. Soya hasadından sonra da sıklıkla görülen kızışma, yüksek nem, sıcaklığı azaltılmamış ve havalandırılmamış tohum yığınlarının bir sonucu olarak oluşan küflenmedir (Dizlek, 2012). Depolanabilir olma kapasitesi, genetik olarak yönetilen bir faktör olsa da hasat öncesi uygulamalar, hasat yöntemi, çevresel faktörler, tohumun depolama nemi, depo bağıl nemi ve sıcaklığı, depolama süresi, paket/ambalaj malzemesi gibi pek çok faktörden de etkilenir (Shelar ve ark., 2008; Ghasemnezhad ve Honermeier, 2009; Balešević ve ark., 2010; Meena ve ark., 2018). Soya tohum kabuğu, genel olarak %9 selüloz, %9-10 oranında protein içermektedir. Hasat ve depolama sırasında tohum kabuğunda oluşan kırılma, çatlama, ezilme gibi deformasyonlar kabuktaki protein yapısının bozularak tohumun depolama ömrünün azalmasına neden olmaktadır (Motlagh ve Shaban, 2014). Buna ek olarak, depolama sırasında kabukta oluşan deformasyondan kaynaklı, çimlenme organı germ/hipokotilin içinde açığa çıkan yağların da bozulması çimlenme gücünü azaltır (Barnes, 1998). Soya tohumları, uygun koşullarda depolanmazlarsa 3-4 ay gibi bir kısa süre içinde canlılıklarını kaybedebilirler (Monira ve ark., 2012). Bu nedenle, tropik ya da yarı tropik bölgelerde hasattan kısa bir süre sonra başlayan bozulmaların, uygun depolama şartlarını oluşturarak önlenmesi, tohumun canlılık ve kalitesinin teminat altına alınması bakımından şarttır (Suriyong ve ark., 2015).

Türkiye'de soya üretimi 1930'lu yıllardan sonra başlamış ve günümüze kadar üretim alanında önemli ölçüde artışlar kaydedilmiştir. Türkiye'de 2021 yılı kayıtlarına göre soya üretim miktarı 155 bin 225 tondur. Bu miktarın 98 bin 596 tonu (%63.5) Adana ili ovalarından elde edilmekte ve üretim miktarı bakımından Türkiye'de 1. sırada yer almaktadır (TÜİK, 2023). Bölgede en önemli sorun yarı tropik iklime sahip olmasından dolayı depolanma koşullarının tohum kalitesini etkilemesidir. Türkiye'de üreticiler hasattan sonra soya tohumlarını yaygın olarak, polietilen (PE) çuvallar içinde dış etkenlere maruz kalan ambar koşullarında depolamaktadırlar. Soğuk hava depo miktarı sınırlıdır. Ayrıca, düşük oksijen içerikli paketleme (vakumlu), kağıt torba, jüt ya da kenevir gibi lifli çuval kullanımı pek yaygın değildir. Genellikle soya tarımı yapılan alanlarda nisan sonu-mayıs başında ana ürün, haziran sonu-temmuz başında ise II. ürün soya ekimi yapılmaktadır. Bu nedenle, ana ya da ikinci ürün için ekim sezonuna kadar soya tohumlarının uygun koşullarda depolanmış olması gerekmektedir. Çalışma, soya tarımında hasat ve seleksiyon işlemleri yapıldıktan sonra bir sonraki ekim sezonuna kadar depolanması gereken soya tohumları için en uygun depolama yönteminin belirlenmesi amacıyla 2017 ve 2018 üretim sezonlarında olmak üzere iki yıl süreyle yürütülmüştür. Çalışmanın ana amacı, Adana ili gibi yarı tropik iklime sahip bölgelerde hasattan sonra farklı depolama şartlarının soya tohumluğunun çimlenme kabiliyeti, protein ve yağ oranları üzerine olan etkilerini belirlemektir.

2. Materyal ve Metot

2.1. Materyal

Deneme alanı: Çalışmada, üretim, depolama ve laboratuvar faaliyetleri Adana ilinde bulunan Tarım ve Orman Bakanlığına bağlı Doğu Akdeniz Tarımsal Araştırma Enstitüsü'nde (DATAEM) yürütülmüştür. Adana ili, Türkiye'nin güneyinde Akdeniz İklimi etkisinin baskın olduğu yarı tropik bir bölge içinde yer almaktadır ve soya üretimine uygun 1. sınıf tarım alanlarından oluşan ovalara sahiptir. Uzun yıllar iklim verilerine göre ilde en yüksek sıcaklık Ağustos ayında 45.6 °C, en düşük sıcaklık ise Ocak ayında -8.1 °C dir. Yağışlar en çok Aralık, en az Temmuz ayında görülür. İkinci ürün yetiştirme döneminde aylık ortalama sıcaklık 27 °C, toplam yağış 48 mm ve aylık ortalama güneşlenme süresi 10.9 saat civarındadır.

Bitkisel materyal: Araştırmada bitkisel materyal olarak kullanılan Yeşilsoy ve Nazlıcan soya çeşidine ait bazı teknik özellikler *Tablo 1*'de verilmiştir ('DATAEM', 2023). Çeşitler arasında en önemli fark, yağ, protein oranları ve tohum iriliğidir. Ayrıca Yeşilsoy soya çeşidi silajlık, Nazlıcan soya çeşidi ise yağ sanayi için DATAEM tarafından geliştirilmiş yüksek verimli yerli çeşitlerdir.

Tablo 1. Çalışmada kullanılan Soya çeşitlerine ait bazı teknik özellikler

Table 1. Some technical characteristics of soy varieties used in the study

Parametreler	Yeşilsoy	Nazlıcan
Tohum verimi (t ha^{-1})	35-40	40-45
Yeşil ot verimi (t ha^{-1})	45-60	30-35
Bitki boyu (cm)	110-150	120-150
Yaprak formu	oval	parçalı
Çiçek rengi	mor	mor
Protein oranı (%)	32-33	33-35
Yağ oranı (%)	17-20	20-22
1000 tohum ağırlığı (g)	155-165	180-210
Tohum büyüklüğü	Küçük	iri
Uzunlukxgenişlik (mm)	7.12x5.73	7.34x5.91
Küresellik (%)	83.61	81.03
Kullanım amacı	silaj	dane

Depo özellikleri: Çalışmada, iki farklı depo kullanılmıştır. Bunlar: 1) Soğuk hava deposu: Kontrollü koşullarda deponun iç sıcaklığı 7±0.4 °C, bağıl nemi %60 olarak deneme süresince sabitlenmiştir. Depo raflı sistemlidir. Tohum stokları deneme süresince karanlık ortamda depolanmıştır. 2) Geleneksel ambar: Dört duvarlı, yüksek tavanlı ve çatılı bir bina şeklindedir. Depo raflı sistemlidir. Tohum stokları depolama süresi boyunca dış hava koşullarından etkilenmesini engelleyecek bir kontrol sistemi bulunmamaktadır.

Paketleme malzemesi özellikleri: Paketleme malzemesi olarak iki farklı paket malzemesi kullanılmıştır. Bunlar: 1) Polietilen (PE) malzemeden yapılmış 25 kg'lık çuval, 2) Vakumlanabilir 10 kg lık torba. Vakumlu torbalar -0.1 MPa basınç altında vakumlanmıştır.

2.2. Metot

Deneme planı: Çalışma, depolama ve laboratuvar çalışmaları kapsamında 2^5'lik Faktöriyel Deneme Desenine göre iki yıl ve üç tekerrürlü olarak "Faktöriyel Tesadüf Parselleri" planında düzenlenmiştir. *Tablo 2*' de bu 5 faktör kodlanmış ve açıklanmıştır.

Tablo 2. Deneme faktörleri

Table 2. Trial factors

No	Faktörler	Kod	Açıklamalar	
1	Yıl	A	A1: 2017	A2: 2018
2	Depolama süresi	B	B1: 7 ay	B2: 9 ay
3	Depolama koşulu	C	C1: Geleneksel ambar	C2: Soğuk hava deposu
4	Paket malzemesi	D	D1: PE çuval	D2: Vakumlu torba
5	Soya çeşidi	E	E1: Yeşilsoy	E2: Nazlıcan

İklim verileri: Depolamanın başlama ve bitiş tarihleri *Tablo 3*'de, depolama süresince kayıt altına alınan geleneksel ambar ortam sıcaklığı ve nemi ise *Tablo 4*'de verilmiştir.

Faaliyetler: Her iki yılda da Kasım ayı içinde hasat edilen soyalar, selektörden geçirilerek yabancı maddelerden, kırık, çatlak tohumlardan temizlenmiş ve bir gün sonra depolanmışlardır. Tohum stoklarının depolanma öncesi nem, yağ, protein ve çimlenme testleri yapılmıştır. Hasattan sonra, depolama süresini tamamlayan stoklar yağ, protein ve çimlenme oranlarının belirlenmesi için analize hazırlanmışlardır.

Tablo 3. Farklı depolama koşulları için depolama başlangıç ve bitiş tarihleri

Table 3. Start and end dates for different storage contidation

Yıllar	Depolama başlangıç	7 ay depolama bitiş	9 ay depolama bitiş
2016-2017	11 Kasım 2016	3 Mayıs 2017	3 Temmuz 2017
2017-2018	30 Kasım 2017	10 Mayıs 2018	7 Temmuz 2018

Tablo 4. Deneme süresince yıl ve aylara göre kaydedilen geleneksel ambar ortam sıcaklığı ve nemi

Table 4. Traditional warehouse ambient temperature and humidity, year by month, during the trial

Yıllar	Aylar	Maks. ve min. ortam sıcaklığı (°C)	Ort. Nem (%)
2016-2017 depolama sezonu	Kasım 2016	21.6-8.8	50.5
	Aralık 2016	13.7-3.8	65.2
	Ocak 2017	13.6-2.7	81.6
	Şubat 2017	18.6-4.3	71.8
	Mart 2017	19.2-11.5	58.0
	Nisan 2017	24.5-14.5	61.8
	Mayıs 2017	24.4-17.7	70.5
	Haziran 2017	28.3-21.8	73.2
	Temmuz 2017	32.8-27.9	69.8
2017-2018 depolama sezonu	Kasım 2017	20.1-9.5	64.5
	Aralık 2017	15.6-7.8	70.1
	Ocak 2018	12.8-7.6	73.1
	Şubat 2018	16.5-10.4	69.2
	Mart 2018	21.2-13.9	70.2
	Nisan 2018	25.2-14.6	61.5
	Mayıs 2018	28.9-18.5	63.5
	Haziran 2018	30.2-22.8	73.1
	Temmuz 2018	29.9-25.6	73.3

Analiz yöntemleri: Yağ oranı (%): Depolama öncesi ve sonrası alınan tohum örnekleri petrolyum benzin ile Soxhelet cihazı kullanılarak önce ekstrakte edilmiş ve elde edilen sonuçlar % olarak hesaplanarak yağ oranı belirlenmiştir (James, 1995). Protein oranı (%): Depolamaöncesi ve sonrası alınan örneklerin azot (N) içeriği Kjeldahl Metodu ile Protein oranı ise N x 5.71 formülü ile belirlenmiştir (AOAC, 1990). Depolama nemi (%): Depolama öncesi örneklerin nem oranları nem tayin cihazı (Wile 200, Finlandiya) kullanılarak ölçülmüştür (Suthar ve Das, 1996). Çimlenme oranı (%): Depolama öncesi ve sonrası çimlendirme oranının belirlenmesi için 9 cm çapında petri kaplarına yerleştirilmiş çimlendirme kağıdı üzerinde 25 adet tohum kullanılmıştır. Çimlendirme için 50 ml saf su uygulanmış ve petriler çimlenme dolabına yerleştirilmiştir. Çimlendirme dolabı 25 ± 2 ºC sıcaklıkta ayarlanmış ve karanlık ortam oluşturulmuştur. Çimlenme ile ilgili sayımlar sekizinci güne kadar, her gün aynı saatte yapılmıştır. Eşitlik (1) kullanılarak çimlenme yüzdesi hesaplanmıştır (Arif ve ark., 2000):

$$Çimlenme\ yüzdesi\ (\%) = \frac{Çimlenen\ tohum\ sayısı\ (adet)}{Toplam\ tohum\ sayısı\ (adet)} \qquad \text{(Eş. 1)}$$

İstatistiksel analizler: Analizler, 2^5'lik faktöriyel deneme deseni planında Faktöriyel Tesadüf Parsellerine göre yapılmıştır. Çalışmada, faktörler yıl (A), depolama süresi (B), depolama koşulu (C), paketleme (D), çeşit (E) olarak ele alınmıştır. Denemenin istatistiksel modeli (Eş. 2) aşağıda verilmiştir.

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_j + \zeta_j + (\alpha\beta)_{ij} + \cdots + (\alpha\beta\gamma\delta\zeta)_{ijklm} + e_{ijklmn} \qquad \text{(Eş. 2)}$$

$$i = 1,2; j = 1,2; k = 1,2; l = 1,2; m = 1,2; n = 1,2,3$$

Burada;

Y_{ijklmn}: A faktörünün i-inci seviyesi, B faktörünün j-inci seviyesi, C faktörünün k-ıncı seviyesi, D faktörünün l-inci seviyesi, E faktörünün m-inci seviyesinin birlikte uygulandığı n-inci tekerrürdeki gözlem değerini, μ = Genel populasyon ortalamasını, α_i : = A faktörünün i-inci seviyesinin etkisini, β_j : = B faktörünün j-inci seviyesinin etkisini, γ_k : = C faktörünün k-inci seviyesinin etkisini, δ_j : = D faktörünün l-inci seviyesinin etkisini, ζ_j : = E faktörünün m-inci seviyesinin etkisini göstermekte olup, ikili, üçlü, dörtlü ve beşli interaksiyonu etkilerini ise e_{ijklmn} : A faktörünün i-inci, B faktörünün j-inci, C faktörünün k-inci, D faktörünün l-inci, E faktörünün m-inci seviyesinin birlikte uygulandığı n-inci tekerrürdeki tesadüfi hatayı göstermektedir.

Depolama süresinin tamamlandığı 7 ve 9 ayın sonunda elde edilen tüm verilerin istatistiki değerlendirmeleri SPSS istatistik paket programının 25. versiyonu kullanılarak yapılmıştır (IBM, 2020). Önemli bulunan farklılıklar LSD çoklu karşılaştırma testine ($P<0.05$ veya $P<0.01$) tabi tutularak gruplandırılmıştır.

3. Araştırma Sonuçları ve Tartışma

Depolama öncesinde yapılan yağ ve protein analizine göre Yeşilsoy ve Nazlıcan soya çeşitlerinin sırasıyla iki yıl için ortalama yağ oranı %22.98, %21.72 ve protein oranı %38.07, %38.97 olarak belirlenmiştir. Çimlenme oranının ise ortalama %93.5, 93.0 olduğu kayıt altına alınmıştır. Çeşitlerin depolama nem değerlerinin ise sırasıyla ortalama %10.94, %9.26 olduğu ölçülmüştür. Depolama sonrası elde edilen verilerin karşılaştırılmasında bu veriler dikkate alınmıştır.

Çalışma sonunda, elde edilen iki yıllık veriler birleştirilip istatistiki olarak değerlendirilmiş ve varyasyon analiz tabloları oluşturulmuştur (*Tablo 5*). Faktörler ayrı ayrı incelendiğinde yağ, protein ve çimlenme oranları üzerinde yıl (A), depolama süresi (B) ve çeşit (E) faktörlerinin istatistiksel olarak önemli ($p\leq 0.01$) olduğu tespit edilmiştir. Yine istatistiki olarak çimlenme üzerinde, depolama koşulu (C) ($p\leq 0.01$) ve paket malzemesi (D) ($p\leq 0.05$) seçiminin önemli olduğu belirlenmiştir. Ancak, bu iki faktörün yağ ve protein üzerinde istatistiki olarak önemli olmadığı tespit edilmiştir. İkili kombinasyonlar değerlendirildiğinde, A x E interaksiyonunun yağ oranı ($p\leq 0.05$), protein ve çimlenme oranı ($p\leq 0.01$) üzerinde önemli olduğu belirlenmiştir. Bunun yanı sıra A x B interaksiyonunun protein ve çimlenme ($p\leq 0.01$), A x C interaksiyonunun protein ve çimlenme ($p\leq 0.05$), B x C interaksiyonunun protein ($p\leq 0.0$), C x E interaksiyonunun ise çimlenme ($p\leq 0.05$) oranları üzerine önem seviyesinde etkili olduğu tespit edilmiştir.

Üçlü kombinasyonlarda, A x B x C interaksiyonunun yağ ($p\leq 0.01$), B x C x D interaksiyonunun ise çimlenme oranları ($p\leq 0.05$) üzerinde önemli seviyesinde etkili olduğu görülmektedir. Dört ve beşli kombinasyonlar incelendiğinde, A x B x C x D interaksiyonunun yağ ($p\leq 0.01$), A x C x D x E interaksiyonunun ise çimlenme oranları ($p\leq 0.01$) üzerinde önem seviyesinde etkili olduğu tespit edilmiştir. Ek olarak, beşli kombinasyonun istatistiki olarak önemli olmadığı belirlenmiştir. Benzer sonuçlar elde eden Kandil ve ark. (2013) çalışmalarında, farklı depolama koşullarını incelemişlerdir. Araştırıcılar, ”depolama süresi x soya çeşidi”, “depolama süresi x depolama koşulu” ve “depolama koşulu x paket malzemesi” ikili interaksiyonların istatistiki anlamda çimlenme üzerine $p\leq 0.01$ önem seviyesinde önemli olduğunu belirlemişlerdir. Yine aynı çalışmada, “depolama süresi x soya çeşidi x paketleme malzemesi” üçlü interaksiyonun $p\leq 0.01$önem seviyesinde çimlenme üzerinde önemli bir etkisinin olduğunu tespit etmişlerdir.

Her bir faktörün yağ, protein ve çimlenme üzerine etkileri *Tablo 6*'da tanımlayıcı istatistikler ve özellik ortalamalarına ait karşılaştırmalar yapılarak verilmiştir. Analiz tablosu içindeki tüm veriler, yine tablo içinde verilen depolama öncesi verilerle karşılaştırılarak aşağıda maddeler halinde değerlendirilmiştir.

Tablo 5. Varyans analiz tablosu

Table 5. Analyses of variance

Kaynak	SD	Yağ		Protein		Çimlenme	
		Kareler ortalaması	P değeri	Kareler ortalaması	P değeri	Kareler ortalaması	P değeri
A	1	19.729	.000**	34.788	.000**	5781.510	.000**
B	1	5.920	.000**	24.251	.000**	3540.510	.000**
C	1	.476	.103ns	.371	.462ns	6817.510	.000**
D	1	.187	.304ns	1.688	.120ns	404.260	.022*
E	1	9.151	.000**	12.449	.000**	7579.260	.000**
A x B	1	.005	.872ns	31.430	.000**	1625.260	.000**
A x C	1	.000	.973ns	3.394	.029*	463.760	.014*
A x D	1	.246	.239ns	.737	.302ns	152.510	.153ns
A x E	1	1.545	.004**	13.017	.000**	4173.844	.000**
B x C	1	.119	.411ns	6.747	.002*	29.260	.528ns
B x D	1	.012	.796ns	.560	.367ns	25.010	.560ns
B x E	1	.017	.759ns	.042	.804ns	65.010	.348ns
C x D	1	.128	.395ns	.013	.891ns	270.010	.059ns
C x E	1	.155	.349ns	.133	.660ns	348.844	.032*
D x E	1	.005	.865ns	1.094	.209ns	86.260	.281ns
A x B x C	1	1.490	.005**	1.975	.093ns	152.510	.153ns
A x B x D	1	.634	.061ns	2.010	.090ns	14.260	.660ns
A x B x E	1	.091	.472ns	.030	.835ns	58.594	.373ns
A x C x D	1	.025	.704ns	.314	.499ns	.094	.971ns
A x C x E	1	.107	.437ns	.539	.377ns	86.260	.281ns
A x D x E	1	.001	.938ns	.489	.399ns	41.344	.454ns
B x C x D	1	.019	.740ns	5.573	.006ns	753.760	.002**
B x C x E	1	.050	.592ns	1.342	.165ns	472.594	.013*
B x D x E	1	.437	.118ns	.287	.518ns	128.344	.189ns
C x D x E	1	.028	.690ns	.911	.251ns	237.510	.076ns
A x B x C x D	1	.940	.023*	1.533	.138ns	55.510	.386ns
A x B x C x E	1	.020	.737ns	.037	.816ns	2.344	.858ns
A x B x D x E	1	.017	.759ns	.539	.377ns	225.094	.084ns
A x C x D x E	1	.116	.417ns	.239	.555ns	698.760	.003**
B x C x D x E	1	.624	.063ns	.958	.239ns	14.260	.660ns
A x B x C x D x E	1	.432	.120ns	.805	.280ns	52.510	.399ns
Hata	64	.174		.679		72.823	
Toplam	96						

A: Yıl; B: Depolama süresi; C: Depolama koşulu; D: Paketleme; E: Çeşit; ns, *, ** = önemsiz, p≤0.05 düzeyinde önemli, p≤ 0.01 düzeyinde önemli; SD = Serbestlik derecesi

Tablo 6. Tanımlayıcı istatistikler ve özellik ortalamalarının karşılaştırılması

Table 6. Comparing descriptive statistics and feature averages

Konu		Yağ oranı			Protein oranı			Çimlenme oranı		
		Ort. (%)	Std. Dev.	CV (%)	Ort. (%)	Std. Dev.	CV (%)	Ort. (%)	Std. Dev.	CV (%)
A	A1	22.02[b]	0.59	2.68	37.69[b]	1.52	4.03	53.10[a]	22.91	43.15
	A2	22.93[a]	0.62	2.70	38.90[a]	1.01	2.60	68.63[b]	13.51	19.69
B	B1	22.72[a]	0.75	3.30	38.80[a]	1.21	3.12	66.94[b]	16.96	25.34
	B2	22.23[b]	0.68	3.06	37.79[b]	1.45	3.84	54.79[a]	21.61	39.44
C	C1	22.55	0.76	3.37	38.36	1.58	4.12	52.44[b]	16.16	30.82
	C2	22.40	0.75	3.35	38.23	1.25	3.27	69.29[a]	20.59	29.72
D	D1	22.52	0.75	3.33	38.43	1.50	3.90	58.81[a]	21.32	36.25
	D2	22.43	0.77	3.43	38.16	1.34	3.51	62.92[b]	19.16	30.45
E	E1	22.78[a]	0.80	3.51	37.93[b]	1.26	3.32	69.75[b]	15.44	22.14
	E2	22.17[b]	0.56	2.53	38.65[a]	1.49	3.86	51.98[a]	20.75	39.92
Depolama öncesi çeşitlere göre yağ, protein ve çimlenme oranları (%)										
E1	A1	22.61			37.93			94		
	A2	23.35			38.22			93		
	Ort	22.98			38.07			93.50		
E2	A1	21.95			39.72			94		
	A2	21.50			38.22			92		
	Ort	21.72			38.97			93		
Genel ort.		22.35			38.52			93.25		

A: Yıl (A1: 2017, A2: 2018) ; B: Depolama Süresi (B1: 7 ay, B2: 9 ay); C: Depolama Koşulu (C1: Geleneksel ambar, C2: soğuk hava deposu; D: Paketleme (D1: PE çuval, D2: Vakumlu torba; E: Soya Çeşitleri (E1: Yeşilsoy, E2: Nazlıcan); Std. Dev: Standard Sapma; CV: Değişim katsayısı

3.1. Yılın etkileri (A)

Yağ, protein ve çimlenme oranları yıllara göre istatistiki olarak %1 önem seviyesinde farklılık göstermiştir. Ancak bu durumun depolama başlangıcındaki yağ ve protein oranları ve iklim verileri ile doğrudan ilgili olduğu düşünülmektedir.

3.2. Depolama süresinin etkileri (B)

Analizlere göre, depolama süresi arttıkça yağ, protein ve çimlenme oranlarının azaldığı ve istatistiksel olarak faklı gruplarda yer aldıkları belirlenmiştir. Depolama öncesi iki yılın genel ortalaması olarak stok tohumların yağ, protein ve çimlenme oranları sırasıyla %22.35, %38.52 ve %93.25 iken 7 aylık depolama sonunda yağ oranının %22.72, protein oranının %38.80'e çıktığı, çimlenme oranının ise %66.94'e kadar azaldığı belirlenmiştir. 9 aylık depolama süreci tamamlandığında ise bu verilerin %22.23, %37.79 ve %54,79'a kadar azaldığı belirlenmiştir. Depolama süresi arttıkça öncelikli olarak çimlenme kabiliyetinde ciddi anlamda ve olumsuz yönde bir etkinin olduğu, hatta depolama öncesine göre ilk 7 aylık dönmede %28.2 oranında, 9 aylık depolama sonunda ise %41.2 oranında çimlenme kabiliyetinin azaldığı belirlenmiştir.

3.3. Depolama koşulunun etkileri (C)

Geleneksel ambar şartlarının, soğuk hava deposu şartlarına göre yağ, protein oranları üzerinde istatistiki olarak bir etkisinin olmadığı, ancak bu koşulun çimlenme üzerinde %1 önem seviyesinde etkili olduğu belirlenmiştir. Soğuk hava deposunda saklanan tohumların genel olarak çimlenme oranlarının (%69.29) geleneksel ambar şartlarına göre (%52.44) daha yüksek olduğu tespit edilmiştir. Depolama öncesi çimlenme oranına göre bu veriler analiz edildiğinde, geleneksel ambarda depolanan tohumların %43.56, soğuk hava deposunda depolanan tohumların ise %25.69 oranında çimlenme kabiliyetini kaybettikleri belirlenmiştir.

3.4. Paket malzemesi seçiminin etkileri (D)

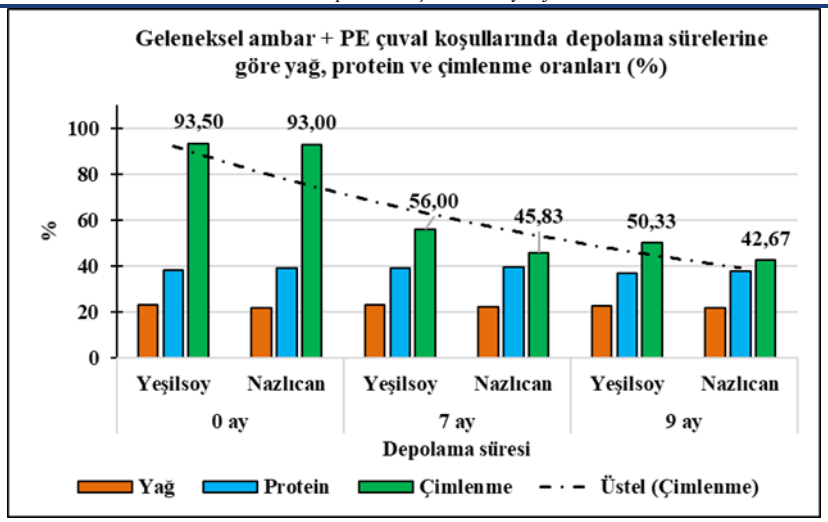
Paketleme malzemesinin yağ ve protein üzerine istatistiki olarak bir etkisinin olmadığı tespit edilmiştir. Ancak çimlenme kabiliyeti üzerinde %1 önem seviyesinde etkili olduğu belirlenmiştir. PE malzeme ile paketlenip depolanan tohumların çimlenme kabiliyetinin (%58.81), vakumlu torba ile paketlenerek depolanan tohumlara göre (%62.92) daha düşük olduğu tespit edilmiştir. Aynı zamanda depolama öncesi çimlenme oranına göre bir karşılaştırma yapıldığında, PE çuvalla paketlenip depolanan tohumların %36.93, vakumlu torba ile paketlenip depolanan tohumların ise %32.25 oranında çimlenme kabiliyetlerini kaybettikleri belirlenmiştir.

3.5. Soya çeşidinin etkisi (E)

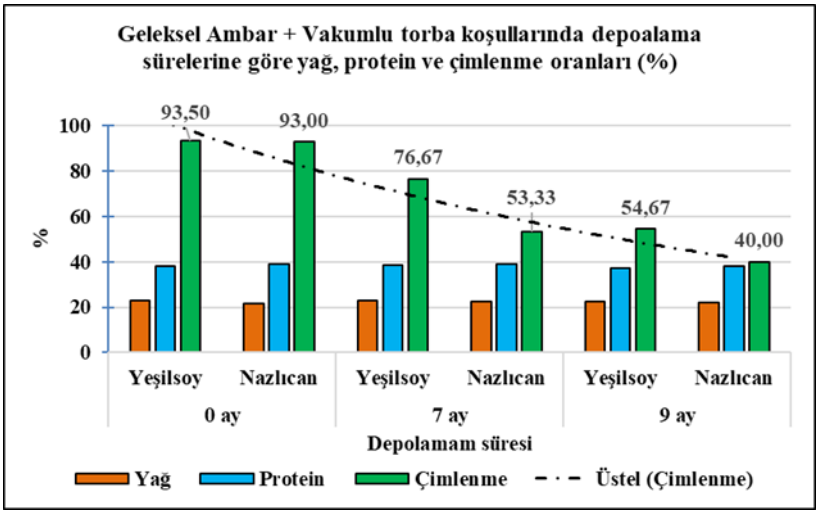
Çeşidin depolama sonrasında yağ, protein oranı ve çimlenme kabiliyeti üzerinde istatistiki olarak %1 önem seviyesinde farklıklar oluşturduğu tespit edilmiştir. Küçük tohumlu olan Yeşilsoy çeşidinin depolama öncesine göre yağ oranı bir miktar yükselirken, protein oranının düştüğü, iri daneli Nazlıcan çeşidinde ise tam tersi durumun oluştuğu tespit edilmiştir. Bu durumun doğrudan çimlenme üzerinde de etkisi olduğu düşünülmektedir. Depolama sonunda, iri tohumlu Nazlıcan çeşidinde çimlenme oranının (%51.98), küçük tohumlu Yeşilsoy çeşidine göre (%69.75) azaldığı belirlenmiştir. Depolama öncesi çimlenme oranına göre bu veriler karşılaştırıldığında, Yeşilsoy çeşidinde %25.20, Nazlıcan çeşidinde ise %44.25 oranında çimlenme kabiliyetini kaybettikleri tespit edilmiştir.

Çalışmada depolama süresi ilerledikçe yağ ve protein miktarlarının depolama öncesine göre azalmasına paralel olarak çimlenmenin de azalmış olabileceği söylenebilir. Benzer sonuçlar bildiren (Reinhold, 2000) tarafından yapılan çalışmada hasat ve depolama koşullarına bağlı olarak tohum, kabuk, kotileton ve çimlenme organında oluşan deformasyondan dolayı yağ ve protein yapısının bozuluma uğradığı ve çimlenme üzerinde doğrudan etkili olduğu bildirilmiştir. Singh ve Dadlani (2003) çalışmalarında bez ve PE çuvallarda farklı çeşit soyaları depolamışlar ve depolama sonunda çeşitlerin farklı çimlenme oranları olduğunu belirlemişlerdir. Ayrıca sürenin 8 aydan 14 aya kadar uzatıldığında %94-84 oranında olan çimlenme yüzdelerinin, bez torbalarda depolanması ile %3-1'e kadar dramatik bir şekilde azaldığını belirlenmiştir. Bir diğer çalışmada, soğuk hava deposunda depolanan soya tohumları (>%92) ve ılık depoda (>%78) orta düzeyde depolanan tohumlar için tohum canlılığı çalışma boyunca yüksek kalmıştır. Ancak geleneksel depoda saklanan soya tohumlarının 20 ay sonra neredeyse çimlenme oranının %0 'a kadar düştüğü belirlenmiştir (Mbofung ve ark., 2013). Kandil ve ark. (2013) çalışmalarında, 3, 6, 9, 12 aya kadar olan periyotlarda soğuk hava deposunda ve geleneksel depolarda saklanan soya tohumlarının çimlenme kabiliyeti üzerine etkilerini araştırmışlardır. Bu süre içinde soyalar farklı ambalajlarda depolanmışlardır. Sonuç olarak depolanma süresi arttıkça çimlenme kabiliyetinin azaldığı, en iyi sonuçların ise bez torbalarda 3 ay süre ile depolanan tohumlarda olduğu belirlenmiştir. Benzer bir çalışmada, soya tohumlarının soğuk hava deposunda 4 ay depolanması ile %40 çimlenme sağlanırken, PE çuval ve geleneksel ambar ortamında 2 ay canlılığını koruyabildiği bildirilmiştir. Çalışmada kağıt, alüminyum folyo ve PE lamine poşetlerde saklanan tohumların 10 aya kadar bez torbalarda saklanan tohumlardan daha yüksek çimlenme oranına sahip olduğu da tespit edilmiştir (Radhakrishna, 1982). Pek çok literatürde soya tohumunun yanlış depolama şartlarına maruz kalması ile içeriğindeki karbonhidrat, yağ ve protein yapılarının bozulmasına bağlı olarak çimlenme gücünün azaldığı bildirilmektedir (Bhattacharya ve Raha, 2002; Kakde ve Chavan, 2011). Monira ve ark. (2012) çalışmalarında, farklı saklama kaplarında aynı nemde depoladıkları soya tohumlarının depolama süresinin artmasıyla birlikte kalay kap (%87.3) ve polietilen torbada (%84.7) depolanan tohumların çimlenme oranının bez torbada (%68.5) depolanan tohumlara kıyasla daha iyi olduğu bildirilmiştir. Soya depolamada en yüksek çimlenme oranı ve en düşük küf popülasyonu miktarı için kalay kaplı depolama materyallerinin kullanımını önermişlerdir (Rahman ve Rahman, 1997). Diğer bir çalışmada, bez ve PE torbalarda saklanan soya tohumların çimlenme yüzdeleri değerlendirilmiştir. PE torbalarda muhafaza edilen soya tohumlardan JS-71-05 çeşidinde %94 ve PK-327 soya çeşidinde ise %84 gibi yüksek çimlenme yüzdesi ile depolanma süresini tamamladıkları belirlenmiştir. 8 aylık depolamadan sonra bez torba içinde paketlenmiş tohumlarda belirtilen çeşitlere göre sırasıyla çimlenme oranının %3 ve %1'e düşerek tohumluk vasıflarının tamamen kaybedildiği bildirilmiştir (Singh ve Dadlani, 2003).

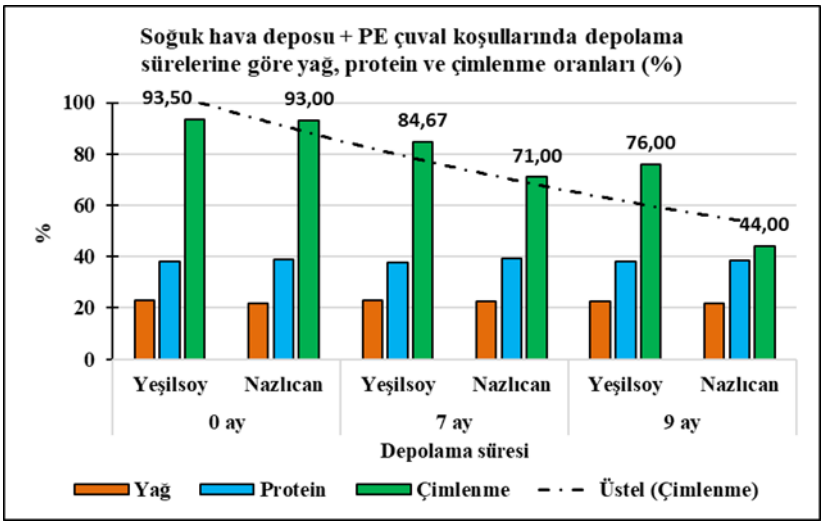
Geleneksel ambar ve soğuk hava deposunda depolanan soya tohum stoklarının paketleme malzemesine göre depolama süresi boyunca yağ, protein ve çimlenme oranlarındaki değişimleri *Şekil 1* de (a), (b), (c) ve (d) figürleri ile verilmiştir.


Geleneksel ambar + PE çuval koşullarında depolama sürelerine göre yağ, protein ve çimlenme oranları (%)
93,50
93,00
56,00
45,83
50,33
42,67
%
Yeşilsoy
Nazlıcan
0 ay
7 ay
9 ay
Depolama süresi
Yağ
Protein
Çimlenme
Üstel (Çimlenme)

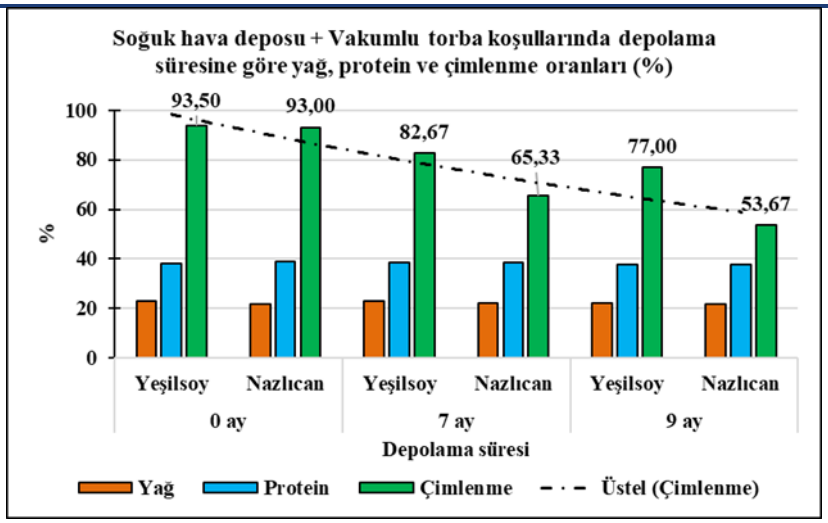
(a)


Geleksel Ambar + Vakumlu torba koşullarında depoalama sürelerine göre yağ, protein ve çimlenme oranları (%)
93,50
93,00
76,67
53,33
54,67
40,00
%
Yeşilsoy
Nazlıcan
0 ay
7 ay
9 ay
Depolamam süresi
Yağ
Protein
Çimlenme
Üstel (Çimlenme)

(b)


Soğuk hava deposu + PE çuval koşullarında depolama sürelerine göre yağ, protein ve çimlenme oranları (%)
93,50
93,00
84,67
71,00
76,00
44,00
%
Yeşilsoy
Nazlıcan
0 ay
7 ay
9 ay
Depolama süresi
Yağ
Protein
Çimlenme
Üstel (Çimlenme)

(c)



(d)

Figure 1. Changes in oil, protein and germination rates of soybean seeds stored under different conditions at the beginning of storage.

Şekil 1. Farklı koşullarda depolanan soyanın, başlangıç durumuna göre yağ, protein ve çimlenme oranlarındaki değişimleri (a), (b), (c), (d)

Şekil 1 değerlendirildiğinde soya depolamada her iki çeşit için de "Geleneksel ambar + PE çuval" ile yapılan depolama ile en kötü sonuçların alındığı belirlenmiştir. Bu depolamada, depolama başlangıcında Yeşilsoy (%93.5) ve Nazlıcan (%93) çeşitlerinin oldukça yüksek olan çimlenme oranlarının, 7 ay depolama sonunda sırasıyla %56, %45.83, 9 ay depolama sonunda ise sırasıyla % 50.33, %42.67'ye kadar azalmış olduğu belirlenmiştir. Soyanın en iyi koşullar oluşturularak depolanması bakımından bir değerlendirme yapılacak olursa "Soğuk hava deposu + PE çuval" ya da "Soğuk hava deposu + vakumlu torba" koşulları için benzer sonuçlar alındığı söylenebilir. Ancak bu iki koşul arasından Nazlıcan soya çeşidi için özellikle "Soğuk hava deposu + vakumlu torba" koşulunun daha avantajlı olduğu söylenebilir. Çimlenme oranına bağlı olarak verimin korunması bakımından 7 ay süre ile soğuk hava deposu ve PE malzeme (%84.67) ya da vakumlu torba (%82.67) ile depolanan Yeşilsoy soya çeşidinin çimlenme oranının diğer tüm alternatiflerden daha yüksek olduğu tespit edilmiştir.

4. Sonuç

Soya tohumunun depolanması sonrasında istatistiksel olarak çimlenme üzerine depolama süresi, depolama koşulu ve çeşidin $p \leq 0.01$, paket malzeme özelliklerinin ise $p \leq 0.05$ önem seviyesinde etkili olduğu belirlenmiştir. Yağ ve protein oranları üzerinde ise, depolama koşulu ve paket malzemesinin istatistiki olarak önemli olmadığı tespit edilmiştir. Ancak konular arasındaki farkların mutlak değerleri dikkate alındığında çimlenme üzerinde en etkili faktörlerin sırasıyla çeşit, depolama koşulu, depolama süresi ve son olarak da paket malzemesi seçimi olduğunu söyleyebiliriz. Depolamada yağ oranı üzerine en etkili faktörün ise çeşit, ikinci faktörün depolama süresi olduğunu, protein oranı üzerinde de bu iki faktörün sıralamasının yer değiştirdiğini söylemek mümkün görülmektedir. Sonuç olarak yağlı tohumlardan biri olan soyanın depolanmasında çimlenme kabiliyetinin korunması bakımından küçük tohumlu soya çeşitlerinin tercih edilmesi, tercihen vakumlu torba ya da maliyeti daha düşük olan PE çuval ile ambalajlanarak soğuk hava deposu koşullarında 7 ay süreyle depolanmasının verim potansiyelinin korunması bakımından önemli olduğu belirlenmiştir. Soya tohumlarının depolanmasında farklı çeşitlerin farklı tepki verdiği bu nedenle her soya çeşidine özgü depolama koşullarının belirlenmesi gerektiği sonucuna varılmıştır.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Defining of Some Morphological Characteristics in order to Determine the Infrastructure of Conservation Strategies of Indigenous Cattle for Designing Sustainable Methods and Conservation of Biodiversity: Eastern Anatolian Red Example Reared Under Ex-Situ Conditions in Türkiye

Sürdürülebilir Yöntemlerin Tasarlanması ve Biyoçeşitliliğin Muhafazasına Yönelik Yerli Sığırları Koruma Stratejilerinin Alt Yapısını Belirlemek için Bazı Morfolojik Özelliklerin Tanımlanması: Türkiye'de Ex-Situ Koşullarda Yetiştirilen Doğu Anadolu Kırmızısı Örneği

Sadrettin YÜKSEL[1]*, Alpay KARAÇUHALILAR[2]

Abstract

This study was designed to protect indigenous animal breeds as an infrastructure for the continuation of biodiversity and to be a link in the production chain. For this purpose, the interaction of the information on Eastern Anatolian Red (EAR) breed, which were evaluated or protected according to different methods, and some morphological characters of animals reared under ex-situ conditions, with the sources of variation were evaluated. It were used materials preserved according to different methods and 207 head calves obtained during four years in the study. The materials that consisted of 350 head ex-situ, 200 head in-situ, 50 piece in-vitro was conserved in original environment. The weights and measurements of calves were recorded at birth and weaning. Difference of CV was the highest that was seen between males and females in 2022 (%9.24, %19.23 respectively) in birth. It was observed a certain level of difference for CV for BL, WH, CD, CW, CG, RH and FWG both between sexes and between years in weaning, but these differences were not as large as at birth. BW, WH, CW, CG, RH and FWG differed between sexes in birth period ($p< 0.05$). In the same period, all body measurements were different between years ($p< 0.01$). In weaning period, difference of CV for WW was the highest that was seen by sex in 2019 (%9.64, %15.47 female, male, respectively). In the same period, it was close CV values of body measurements determined by both sexes and years. There was an interaction between sexes and WW, CW, FWG ($p< 0.05$), years and all body measurements ($p< 0.01$) in the weaning period. The increase in studies on EAR breed could result in a modulation of sustainable of animal biodiversity potentially impacting livestock control and efficiency.

Keywords: Eastern Anatolian Red, Ex-situ rearing, Conservation of biodiversity, Calf, Morphological Characteristic

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Öz

Bu çalışma, biyoçeşitliliğin devamı için, bir altyapı olarak yerli hayvan ırklarının korunması ve üretim zincirinin bir halkası olması amacıyla tasarlanmıştır. Bu maksatla farklı yöntemlere göre koruma altına alınmış veya değerlendirilmiş Doğu Anadolu Kırmızısı (DAK) ırkına ait bilgilerin ve ex-situ koşullarda yetiştirilen hayvanların bazı morfolojik karakterlerinin, varyasyon kaynakları ile etkileşimi ele alınmıştır. Çalışmada, farklı yöntemlerle korunan materyallerin durumu ve dört yıl boyunca elde edilen 213 baş buzağıya ait veriler kullanılmıştır. Koruma altındaki materyaller, 350 baş ex-situ, 200 baş in-situ, 50 adet in-vitrodan oluşmaktadır. Buzağıların doğum ve sütten kesim dönemlerine ait ağırlıkları ve vücut ölçüleri kayıt altına alınmıştır. Dişi ve erkekler arasında en yüksek varyasyon katsayısı (VK) farkı 2022 yılında doğum ağırlıklarında görülmüştür (sırasıyla %9.24, %19.23). Sütten kesim döneminde vücut uzunluğu (VU), cidago yüksekliği (CY), göğüs derinliği (GD), göğüs genişliği (GG), göğüs çevresi (GÇ), sağrı yüksekliği (SY) ve ön incik çevresi (ÖİÇ) için VK değerleri hem cinsiyetler arasında hem de yıllar arasında belirli bir düzeyde farklılık göstermiş, ancak bu farklar doğumdaki değerler kadar büyük olmamıştır. Doğum döneminde VU, CY, GG, GÇ, SY ve ÖİÇ ortalamaları cinsiyetler arasında farklılık göstermiştir ($p< 0.05$). Aynı dönemde yıllar arasındaki tüm vücut ölçüsü değerlerine ait farklılıklar önemli bulunmuştur ($p< 0.01$). Sütten kesim döneminde cinsiyetler arasında görülen en yüksek VK farkı 2019 yılında CY değerinde görülmüştür (dişi, erkek için sırasıyla %9.64, %15.47). Aynı dönemde hem cinsiyet, hem de yıllara göre belirlenen vücut ölçülerinin VK değerleri birbirine yakın bulunmuştur. Sütten kesim döneminde cinsiyet ile CY, GD, ÖNÇ ($p< 0.05$), yıl ile tüm vücut ölçüleri ($p< 0.01$) arasında anlamlı bir etkileşim görülmüştür. Nihayi olarak DAK ırkı üzerine yapılan araştırmalardaki artış, sürdürülebilir bir biyoçeşitlilik modülasyonuyla sonuçlanıp, potansiyel olarak çiftlik hayvanlarının kontrolünü ve verimliliğini etkileyebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Doğu Anadolu Kırmızısı, Ex-situ yetiştirme, Biyoçeşitliliğin korunması, Buzağı, Morfolojik özellik

1. Introduction

The livestock, particularly cattle and sheep, have played an important part in the achieving of food, scientific study, and employment overall world. Because humankind as a living creature have probably an the longest history of agriculture culture on the planet. These definitions can also bring up the morphological type scoring process (Soysal et al., 2016) in these animals in line with the estimation of climatic factors with some methods (Halimi et al., 2023). Today, many things in terms of animal husbandry may have changed or developed, but still, for the continuation of human life, you need to a detailed description of indigenous animals. Eastern Anatolian Red is one indigenous cattle breeds of Türkiye. The origin of this breed goes back to about 3500 years ago (Ozdemir and Dogru, 2009). Previous breeders raised these animals both food production and draft animal in agricultural activities (Üresin, 1936). However, since the beginning of the 1970s, farmers begun to used by choosing certain exotic breeds (Yüksel, 2019a). Desired characters included products such as more meat and milk. Thus, this breed have began to decline in numbers. Sustainable conservation of these cattle are very important due to their rooted past, which may have promoted the realization of traditional production and modern breeding studies. Nonetheless, determination of morphological characterization the breed is important for the conservation and biodiversity of cattle breeds (Alderson, 1992). By studying the phenotype of the breed, it is possible to improve diversity and reproductivity for profitable livestock and functionality (Eding and Laval, 2002). According to visual methods provide a painless mechanism to evaluate the EAR breed morphologically but is very laborious, and precision requires a high sampling rate (Garcia-Lamazares, 2008).

It were used materials preserved according to different methods and EAR calves obtained during four years in the study. The materials that consisted of 350 head ex-situ, 200 head in-situ, 50 piece in-vitro was conserved in original environment. Previous researchers described the morphology and some phenotypes of the EAR cattle breed. However, this study focused on measurements and evaluations in ex-situ conditions in terms of creating infrastructure for new research and strategies in cattle breed, bringing these strategies into production and protecting the breed.

2. Materials and Methods

In this study, morphological characteristics was conducted from January 2019 to December 2022 on purebred EAR cattle herd consisting 213 head in Eastern Anatolian Agricultural Research Institute. The weather of the area is consisted of by a continental climate and cold season. The region is classified as area having a little amount of rainfall throughout the summer located 1950 m above the sea level, 39°55′15.49″N, 41°17′12.90 E. The information about the practices consists of the projects carried out by the Ministry of Agriculture and Forestry, to which the institute is affiliated. Total 213 (106 females and 107 males) purebred EAR calves were used for the body measurements and weights. All calves received colostrum 2 liter approximate, within first 1-3 h of birth. Then, their mothers were suckled to them until weaning. Starter and grass hay were available *ad-libitum* starting on 5-6 day. It was used a precision bascule (10 g sensibility) for body weights and a measuring stick also for body measurements. These phenotypic characteristics were recorded for each animal individually. Measures are as following; body length (BL) the horizontal distance from the point of shoulder to pin bone, wither height (WH) distance (vertical) from the bottom of the front foot to the highest point over wither, chest depth (CD) distance from the shoulder to just behind the front legs, chest width (CW) distance between two scapula bones, heart girth (HG) by placing the measuring tape around the animal at the point of smallest circumference just behind the forelegs, rump height (RH) distance from the apex of the hipbone to the ground perpendicular to the ground and front wrist girth (FWG) circumference of the fore ankle.

In the analysis of the data were used the descriptive analysis method and was benefited from the SPSS package program (SPSS, 2004). On the other hand, were also statistically analyzed by the least squares techniques by using SPSS statistics software program (SPSS, 2004). A general linear model was carried out for fixed effect of sex and years the main sources of variation for studied traits in statistical analysis. The mathematical differences detected were grouped according to the Duncan multiple comparison test (Duncan, 1955).

3. Results

3.1. Evaluation of conservation practices

It should have stated that scientists and readers have been arguing for always that calves "needed a space to feed around with sufficient of feeds for profitability, a rearing programme for these animals and a variety of environmental regulations to keep them sustainable conserved". Although the studies we have carried out in this context have kept the EAR breed on the agenda to a certain extent, there is still more research to be done. Activities for the breeding and protection program made for the breed are given in *Table 1*.

Table 1. Local level of the Eastern Anatolian Red breed within the project

Rearing style	Conservation style	Location	*Condition	Frequency (number)
Semi-intensive	Ex situ-in vivo	EAAR	-	> 350
Extensive	In situ- in vivo	Erzurum/ Olur/Kekikli	+	> 100
Extensive	In situ- in vivo	Artvin/ Ardanuç/Güleş	+	> 100
Intensive	In vitro-in vivo	MAF/GDARP/ICLRT	-	≥ 50

EAAR: Eastern Anatolian Agricultural Research, Semi-intensive: a system between extensive and intensive, MAF: Ministry of Agriculture and Forestry, GDARP: General Directorate of Agricultural Research and Policies, ICLRT: International Center for Livestock Research and Training, *: It is supported in cash by the Ministry of Agriculture and Forestry.

Most of the EAR cattle breed are found in certain number in the hubbly and steep areas. The dominant issues to address therefore relate to reducing under-research, enhancing production with local EAR breed, combating uncontrolled crossbreeding, and achieving rates and research of biodiversity growth that would contribute to animal production development. Thus, it is important to make the EAR breed a link in the animal production chain. The studies carried out for this purpose are given in *Table 2*.

Table 2. Usage strategy of the Eastern Anatolian Red breed within the projects

Location	Issue	Reference
Erzurum/TÜRKİYE	Calves feeding and overall herd performance	Yüksel et al., 2021
Erzurum/TÜRKİYE	Relationship among housing, feeding, age and fattening performances, comfort, slaughterhouse characteristics	Yüksel, 2019b
Erzurum/TÜRKİYE	Relationship between rearing style and social awareness	Yüksel et al., 2019
Erzurum/TÜRKİYE	Relationship among housing condition, feeding style, age and meat quality	Yüksel et al., 2019
Erzurum/TÜRKİYE	Relationship between age and meat quality	Kopuzlu et al., 2018
Erzurum/TÜRKİYE	Relationship between finishing system and meat quality	Yüksel et al., 2012
Erzurum/TÜRKİYE	Reletionship between duration of finishing period and meat quality	Özlütürk et al., 2008
Erzurum/TÜRKİYE	Reletionship between finishing period and meat quality	Ünlü et al., 2008
Erzurum/TÜRKİYE	Fattening performance	Özlütürk et al., 2006
Erzurum/TÜRKİYE	Controlled crossbreeding, genotype environment interaction, meat and carcass quality	Özlütürk et al., 2004

3.2. Evaluation of some morphological characterization

The data obtained by descriptive analysis and the least squares techniques for birth and weaning periods are presented in *Table 3*, *Table 4*, *Table 5* and *Table 6*, respectively. The CV for the birth weight varied from 9.62% to 19.23% in female, from 8.49% to 9.24% in male for years and variation was hight in females. Birth weight for males and 2019 year were significantly (P=0.010) greater than females and another years. The highest CV for BL

in the males and females were in 2020 and 2022 years, respectively, with a highly significant (P=0.000) mean differences between the sexes. And, WH for this parameter was significantly (P=0.000) larger in males than females. Furthermore, the highest CV ratios of CD was in 2021 (7.93%) and 2022 (5.79%) years for females and males respectively, and the least squares means differences were no significant for sex and were highly significant (P=0.000) among years. Over years, CV belonging to CW differed significantly (P=0.000) with 5.65, 5.23, 8.06, 8.94 and 5.56, 4.96, 8.86, 7.94 for females and males, respectively.

The CV for the CG varied from 4.25% to 6.53% in female, from 3.36% to 4.95% in male for years and differences for the least square means was significantly (P=0.015, P=0.000) in terms of sex and years. Significant (P=0.033, P=0.000) difference was found in males and females for third the least square means. Similarly, the least square mean differences for FWG was found significantly (P=0.000) both males and females.

CV value of sex and years were calculated in weaning period. Sex had variation a different ratio on CV values of WW (15.47), BL (5.63), WH (4.65), CD (6.27), CW (6.90), CG (7.31) and RH (4.85) values, with male values having higher than that of female. Females had higher FWG' CV value. In 2022 year, CV ratio of WW, BL, WH was higher both male and female (18.87, 11.99, 4.44 and 17.76, 9.56, 3.98 respectively), than other year' values. The highest CV values were found in 2019 for CD (in both sex, in male and female, 6.27 and 4.96, respectively), in males (6.90) in 2019, in females (5.49) in 2020 for CW, in males (7.31) in 2019 and in females (7.76) in 2022 for CG. Analysis of variance showed effect of sex on some parameters while no effects the similar were found on some's. Sex had a significant effect on WW ($p = 0.020$), CW ($p = 0017$) and FWG ($p = 0.000$) values, with male values having higher than that of female. But, no effects the similar were found on BL, WH, CD, CG and RH ($p > 0.05$). On the other hand, there was effect of years on all parameters considered in the study (P=0.000). 2019 year value had the highest levels of WW in the current dataset (58.81) with 2020 and 2022 (47.16, 45.48 respectively), the lowest. BL was similar for both 2021 and 2022 years effects (67.63, 67.94 respectively), with 2019 having the highest value (73.18). WH and RH values tended to be higher in 2019 year (79.85, 85.64 respectively) than in other years; however, 2021 year had an similar trend in terms of CY. 2021 year values had the highest levels of CD, CW and CG in the current assessments (34.85, 24.39 and 101.61 respectively) with 2022 year' CG value, the lowest (90.47). In terms of FWG values, 2020 and 2021 have the lowest values (10.23 and 10.29 respectively), while 2019 is the highest (11.18)

4. Discussion

Although the Erzurum and surrounding provinces are gifted with a very high potential for livestock resources, the EAR breed's contribution to the region because of some reasons is much lower than in an ideal world. Whereas, EAR breed production has to potential a considerable contribution to the economy of the region (Yüksel et al., 2019) and plays a nonignorable role in providing commodities like meat and meat products, live animals, hides and skins, and leather products. Although there are opportunities and desire to rearing with this breed of rearers (Yüksel, 2019a), there is still the paradox of the ever-increasing enters of some breeds and animal products from outside the region, even if state of enterprise of the region are tending to inconvenience. Bujko et al. (2019) reported higher CV values than our findings for birth weights of different herds. CV values of defined BW in both male and female EAR calves for 2019 and 2020 years were lower than the values reported for the Gascon breed by Bures et al. (2008). However, the same researchers reported higher CV values than 2021 and 2022 years of male calves of the present study. Our findings for CV values in birth period were partially similar to some researchers reports (Putra et al., 2014). CV values defined for WH in both sex in the study were lower than the values for Gascon breed reported by Bures et al. (2008).

Some researchs have shown that when sex for diverse breeds are compared at the same period variation will be found in birth weight. The similarities found in the effect of sex on birth weight are in line with variations found by Nahar et al. (2016), Ulutaş et al. (1996) for the Red Chittagong and EAR breeds, respectively, ($p<0.05$). Higher BW than from different years and sexs of this study were reported for Braford (Vaz et al., 2020), male Sumbo Ongale (Said et al., 2016), Brown Swiss X EAR F_1 (Koçyiğit et al., 2015), Gascon calves (Bures et al., 2008). However, it was reported lower BW values in terms of both sources of variation for Yerli Kara (Sakar and Zülkadir, 2022), Red Chittagong (Nahar et al., 2016), female Sumbo Ongale (Said et al., 2016), Aceh (Putra et al., 2014), Assam (Kayastha et al., 2008), EAR (Yıldız et al., 2008), and EAR calves (Ulutaş et al., 2001).

Table 3. Descriptive Analysis values for the birth period of Eastern Anatolian Red calves reared under ex-situ conditions.

MC	S	2019 n=28 F, 30 M			2020 n=29 F, 27 M			2021 n=33 F, 27 M			2022 n=16 F, 17 M			Overall n=106 F, 107 M		
		(X̄)	SD	CV (%)	(X̄)	SD	CV (%)	(X̄)	SD	CV (%)	(X̄)	SD	CV (%)	(X̄)	SD	CV (%)
BW (kg)	F	26.23	2.52	9.62	18.81	2.26	12.02	20.51	3.51	17.15	19.13	3.68	19.23	22.01	4.17	18.94
	M	27.70	2.35	8.49	19.87	2.29	11.53	21.29	2.48	11.69	21.08	2.08	9.24			
BL (cm)	F	57.67	3.93	6.82	48.44	2.73	5.64	53.18	3.72	7.00	50.12	4.33	8.65	53.11	5.04	9.48
	M	58.63	3.43	5.87	48.88	3.44	7.05	53.96	3.48	6.45	51.82	3.02	5.84			
WH (cm)	F	68.96	2.57	3.73	62.51	1.88	3.01	63.72	1.94	3.05	62.00	2.82	4.56	64.93	3.79	5.83
	M	70.13	2.82	4.03	62.74	2.14	3.41	64.37	2.51	3.91	62.76	2.33	3.72			
CD (cm)	F	27.85	1.40	5.05	25.51	1.32	5.41	26.57	2.10	7.93	23.68	1.01	4.28	26.02	2.13	8.18
	M	28.06	1.43	5.12	24.55	1.01	4.12	26.74	1.53	5.74	24.52	1.41	5.79			
CW (cm)	F	17.60	0.99	5.65	16.58	0.86	5.23	16.36	1.31	8.06	16.06	1.43	8.94	16.90	1.28	7.57
	M	17.90	0.99	5.56	16.81	0.83	4.96	16.74	1.48	8.86	16.94	1.34	7.94			
HG (cm)	F	71.71	3.30	4.61	64.82	2.75	4.25	62.63	3.19	5.11	64.81	4.23	6.53	66.62	4.73	7.09
	M	72.40	3.50	4.85	65.11	2.18	3.36	64.51	3.26	5.06	66.58	3.29	4.95			
RH (cm)	F	74.00	2.47	3.35	66.62	2.07	3.12	68.03	2.37	3.50	66.56	3.09	4.65	69.44	3.97	5.71
	M	74.43	2.84	3.83	67.11	2.24	3.34	69.25	2.62	3.79	67.47	2.76	4.10			
WFG (cm)	F	10.53	0.50	4.82	9.01	0.50	5.64	9.00	0.71	7.98	9.43	0.54	5.76	9.69	0.87	8.97
	M	10.85	0.49	4.55	9.40	0.41	4.43	9.44	0.60	6.46	9.88	0.62	6.33			

Birth weight (BW), body length (BL), wither height (WH), chest depth (CD), chest width (CW), heart girth (HG), rump height (RH), front wrist girth (FWG), (X̄): mean, SD: Standard deviation, CV: Coefficient of variation, S: Sex, F: Female, M: Male, MC: Morphological characters

Table 4. Variance analysis and multiple comparison test results of Eastern Anatolian Red calves reared under ex-situ conditions in birth period.

MC	Overall	Sex ($\bar{X}\pm S_{\bar{x}}$)			Birth year ($\bar{X}\pm S_{\bar{x}}$)				
		Female	Male	p-value	2019	2020	2021	2022	p-value
BW (kg)	21.83±0.19	21.22±0.26	22.46±0.27	0.001	26.97^{a}±0.35	19.34^{c}±0.35	20.93^{b}±0.34	20.12^{bc}±0.46	0.000
BL (cm)	52.84±0.25	52.40±0.34	53.29±0.35	0.071	58.11^{a}±0.46	48.67^{d}±0.46	53.57^{b}±0.45	50.98^{c}±0.60	0.000
WH (cm)	64.64±0.17	64.30±0.23	65.00±0.23	0.035	69.55^{a}±0.31	62.63^{c}±0.31	64.05^{b}±030	62.38^{c}±0.41	0.000
CD (cm)	25.81±0.10	25.69±0.14	25.94±0.14	0.225	27.96^{a}±0.19	24.54^{c}±0.19	26.66^{b}±0.19	24.11^{c}±0.25	0.000
CW (cm)	16.87±0.08	16.68±0.11	17.07±0.11	0.015	17.75^{a}±0.15	16.70^{b}±0.15	16.55^{c}±0.14	16.50^{c}±0.20	0.000
HG (cm)	66.56±0.23	66.02±0.31	67.11±0.32	0.015	72.05^{a}±0.42	64.98^{b}±0.42	63.53^{c}±0.41	65.71^{b}±055	0.000
RH (cm)	69.17±0.18	68.0±0.25	69.55±0.25	0.033	74.21^{a}±0.33	66.87^{c}±0.33	68.62^{b}±0.32	67.01^{c}±0.43	0.000
FWG (cm)	9.69±0.04	9.49±0.05	9.89±0.05	0.000	10.69^{a}±0.07	9.21^{c}±0.07	9.22^{c}±0.07	9.66^{b}±0.09	0.000

Birth weight (BW), body length (BL), wither height (WH), chest depth (CD), chest width (CW), heart girth (HG), rump height (RH), front wrist girth (FWG), $\bar{X}$: mean, $S_{\bar{x}}$: Standart error, MC: Morphological character, a-d: values with different letters in the same column are statistically different

Table 5. Descriptive Analysis values for the weaning period of Eastern Anatolian Red calves reared under ex-situ conditions.

MC	S	2019 n=27 F, 30 M			2020 n=29 F, 27 M			2021 n=33 F, 27 M			2022 n=16 F, 17 M			Overall n=105 F, 101 M		
		(X̄)	SD	CV (%)	(X̄)	SD	CV (%)	(X̄)	SD	CV (%)	(X̄)	SD	CV (%)	(X̄)	SD	CV (%)
WW (kg)	F	59.03	5.69	9.64	44.90	6.30	14.02	50.13	6.85	13.66	45.98	8.16	17.76	51.56	9.02	17.49
	M	58.59	9.06	15.47	49.42	7.03	14.23	55.03	7.34	13.74	44.98	8.49	18.87			
BL (cm)	F	73.89	3.66	4.96	60.59	3.43	5.66	66.82	3.98	5.96	68.06	6.50	9.56	67.47	6.34	9.39
	M	72.47	4.08	5.63	62.15	3.48	5.60	68.44	4.52	6.60	67.82	8.13	11.99			
WH (cm)	F	79.63	2.22	2.79	73.31	2.35	3.20	75.45	2.65	3.51	72.94	2.90	3.98	75.93	3.94	5.18
	M	80.07	3.72	4.65	74.81	2.72	3.63	76.19	3.61	4.73	75.59	3.22	4.44			
CD (cm)	F	34.60	1.72	4.96	32.52	1.50	4.62	34.03	1.05	3.07	32.88	1.32	4.01	33.56	1.65	4.91
	M	34.10	2.14	6.27	33.07	1.30	3.93	34.07	1.07	3.14	32.53	1.62	4.99			
CW (cm)	F	22.37	1.15	5.13	22.59	1.24	5.49	24.15	0.97	4.03	22.82	0.88	3.87	23.15	1.41	6.09
	M	22.40	1.54	6.90	23.04	1.32	5.71	24.63	1.08	4.38	23.00	0.87	3.77			
HG (cm)	F	97.44	4.64	4.76	92.59	6.33	6.84	100.18	4.37	4.36	90.71	7.04	7.76	96.34	6.97	7.23
	M	96.37	7.04	7.31	95.30	6.13	6.43	103.04	4.77	4.63	90.24	6.12	6.78			
RH (cm)	F	85.41	2.55	2.98	78.76	2.63	3.34	81.27	2.71	3.33	79.76	3.49	4.38	81.91	4.15	5.06
	M	85.87	4.17	4.85	80.63	3.16	3.92	82.44	3.99	4.84	79.35	3.95	4.98			
WFG (cm)	F	11.04	0.44	3.96	10.07	0.42	4.14	10.00	0.33	3.31	10.35	0.55	5.34	10.53	0.63	5.98
	M	11.32	0.44	3.93	10.39	0.45	4.29	10.57	0.47	4.29	10.59	0.67	6.30			

Body length (BL), wither height (WH), chest depth (CD), chest width (CW), heart girth (HG), rump height (RH), front wrist girth (FWG), (X̄): mean, SD: Standard deviation, CV: Coefficient of variation, S: Sex, F: Female, M: Male, MC: Morphological characters

Table 6. Variance analysis and multiple comparison test results of Eastern Anatolian Red calves reared under ex-situ conditions in weaning period.

MC	Overall	Sex ($\bar{X}\pm S_{\bar{x}}$)			Birth year ($\bar{X}\pm S_{\bar{x}}$)				
		Female	Male	p-value	2019	2020	2021	2022	p-value
WW (kg)	50.92±0.53	49.06±0.69	51.32±0.67	0.020	58.81^{a}±1.00	47.16^{c}±1.01	52.58^{b}±0.98	45.48^{c}±1.30	0.000
BL (cm)	67.49±0.33	67.71±0.42	68.20±0.41	0.410	73.18^{a}±0.62	61.37^{c}±0.62	67.63^{b}±0.60	67.94^{b}±0.80	0.000
WH (cm)	75.61±0.21	75.02±0.33	75.64±0.32	0.176	79.85^{a}±0.47	74.06^{b}±0.48	75.82^{ab}±0.46	72.77^{c}±0.61	0.000
CD (cm)	33.46±0.10	33.42±0.17	33.40±0.17	0.922	34.35^{ab}±0.25	32.80^{b}±0.25	34.85^{a}±0.24	32.71^{b}±0.32	0.000
CW (cm)	23.11±0.08	22.74±0.12	23.13±0.11	0.017	22.39^{b}±0.17	22.81^{b}±0.17	24.39^{a}±0.17	22.91^{b}±0.22	0.000
HG (cm)	95.67±0.41	94.21±0.53	95.33±0.52	0.135	96.91^{b} ±0.78	93.94^{c} ±0.78	101.61^{a} ±0.76	90.47^{d}±1.00	0.000
RH (cm)	81.66±0.24	80.85±0.34	81.68±0.33	0.083	85.64^{a}±0.50	79.70^{c}±0.50	81.86^{b}±0.49	79.56^{c}±0.63	0.000
FWG (cm)	10.53±0.03	10.28±0.04	10.63±0.04	0.000	11.18^{a}±0.06	10.23^{c}±0.06	10.29^{c}±0.06	10.47^{b}±0.08	0.000

Weaning weight (WW), body length (BL), wither height (WH), chest depth (CD), chest width (CW), heart girth (HG), rump height (RH), front wrist girth (FWG), $\bar{X}$: mean, $S_{\bar{x}}$: Standart error, MC: Morphological character, a-d: values with different letters in the same column are statistically different

Effects of sex and birth season on body measurements of calves were evaluated, for example, Sakar and Zülkadir (2022), Said et al. (2016), Pudra et al. (2014), Bures et al. (2008), Ulutaş et al. (2001), and Ulutaş et al. (1996). The effect of sex in terms of BL in birth are in line with those observations found by Sakar and Zülkadir (2022), Ulutaş et al. (1996) and Said et al. (2016). The effect of sex in terms of BL in birth are in line with those observations found by Sakar and Zülkadir (2022), Ulutaş et al. (1996) and Said et al. (2016), but, our results are numerically higher than the observations of these researchers. Unlike our findings, Yerli Kara (Sakar and Zülkadir, 2022), Aceh (Putra et al., 2014), and EAR (Ulutaş et al., 1996) were reported that WH of EAR calves had lower numerical value compared to this study'. The WH values detected for the Gascon breed was reported higher than our findings (Bures et al., 2008). It was observed significant difference in CG values between calves of this study with different breed calves reared different farm conditions. Our results for CG were considerably higher compared to those reported by Sakar and Zülkadir (2022), Said et al. (2016), Putra et al. (2014), Bures et al. (2008), and Ulutaş et al. (2001). Higher values for FWG are those of EAR reported in this study when compared stating references (Sakar and Zülkadir, 2022).

Due to different the segmented nature of the animal production process, calves are at the most convenient time weaned and then transported directly to a feedlot or backgrounding facility. So weaning age and period in calves has a considerable contribution in the strategy of livestock and plays a vital role in providing activities like breeder selection, live animal, herd renewal to enhance animal production. The higher CV values for WW were those of 2022 year for both males and females (%18.87, %17.76 respectively) on the basis of discussed years. The lowest values were also sighted in 2019 year. While the CV ratio between sexes was higher in 2019 (%15.47 male, %9.64 female), this ratio was close to each other in other years. This result can also be reported for BL, WH, CD, CW, CG and RH. After all, it was reported that EAR calves showed a very differentness during the experimental period and speculated that this may have been a result of effect of years.

There were sex interactions ($p< 0.05$) for WW, CW and FWG in the study. This is in contrast to Sakar and Zülkadir, (2022) and Nahar et al. (2016) who reported that received calves that studed different local breeds had no interaction of sex compared to our result. However Bahashwan (2016) followed Dhofari calves' performance for the 105-d weaning period and noted effect of sex on calf WW. Our finding values were higher than those found in the literature Yerli Kara (Sakar and Zülkadir, 2022), Red Chittagong (Nahar et al., 2016), EAR (Ulutaş et al., 2001) breed calves but are lower to those found by Holstein heifer calves (Wickramasinghe et al., 2022), Braford (Vaz et al., 2020), Droughtmaster x local yellow (Tao et al., 2018), Dhofari (Bahashwan, 2016), Sistani (Bazzi and Ghazaghi, 2011).

The authors hypothesized that BL may be most beneficial longer calves in sustainable livestock. BL value in current study was lower than in Yerli Kara (Sakar and Zülkadir, 2022) and Holstein heifer calves (Wickramasinghe et al., 2022), but partly similar to Southern Chinese Cattle reported by Wang et al. (2020). Also, Tao et al. (2018) reported that was the Droughtmaster x local yellow breed was lower than our findings. The authors reported that WH reaches its maximum at 2019 year, stabile by decreasing after it until the study final. The results were lower than those reported by Sakar and Zülkadir (2022), Wang et al. (2020) and Tao et al. (2018) for different calve values. Sakar And Zülkadir (2022) reported that was consistent on chest girth the effect of sex with our findings for Yerli Kara breed ($p<0.01$). However, the numerical values of this studing were lower than our findings. Wang et al. (2020) reported higher results for Southern Chinese Cattle. Sakar and Zülkadir (2022) reported that noted no effect of sex on WH ($p> 0.05$) by being consistent with our findings. The values that reported high for Holstein heifer calves (Wickramasinghe et al., 2022) and low for Yerli Kara calves (Sakar and Zülkadir, 2022) were different by comparison our findings. Sex was effect on FWG ($p<0.01$), that was consistent with the report of Sakar and Zülkadir (2022). Wang et al. (2020) reported similar values.

5. Conclusion

The increase in studies on EAR breed could result in a modulation of sustainable of animal biodiversity potentially impacting livestock control and efficiency. Although current findings do at a certain level support our hypothesis, it was thought there be a wide interaction between formal protection programmes and status in animal production of EAR breed. Although the CV in the birth weight varied greatly between the years (except 2022), not much variation was observed between the sexes. This determination was also observed for the body measurements

that was studied in this research. In birth period, sex did significantly affect BW, WH, CW, CG, RH and FWG, but didn't BL and CD. All parameters were affected by years. The CV in the weaning weight showed proportionately variance between the years (except 2019), not much variation was observed between the sexes. Body measurements followed a similar course. In weaning, sex did significantly affect BW, WH, CH, CG, RH and FWG, but didn't BL and CD. All parameters were affected by years. In weaning, sex affected BL, WH, CG and RH, years did all parameters. This study showed that the region was the favorable for getting sustainable livestock for the EAR cattle breed. Thus, this breed is a potential resource in terms of both making profitable use of limited resources and fit material for scientific research in the future.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Yüksel, S., Karaçuhalılar, A.; Design: Yüksel, S.; Data Collection or Processing: Yüksel, S., Karaçuhalılar, A.; Statistical Analyses: Yüksel, S.; Literature Search: Yüksel, S., Karaçuhalılar, A.; Writing, Review and Editing: Yüksel, S.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

In vitro Micropropagation of Immortelle Grass (*Helichrysum italicum* (ROTH) G. DON)*

Ölmez Otu (*Helichrysum italicum* (ROTH) G. DON) Bitkisinin *in vitro* Mikroçoğaltımı

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Abstract

Immortelle grass (*Helichrysum italicum* (Roth) G. Don), which spreads in the Southern Marmara and Aegean regions, can be grown in arid and semi-arid regions. In addition, due to its rich essential oil and secondary metabolite content, it has an important place in modern medicine and cosmetics, including traditional treatment methods. Although the propagation of plants by shoot regeneration *in vitro* has been achieved in many plant species, studies on tissue culture in immortelle grass are limited. This study aims to optimize the tissue culture study in immortelle grass and provide a basis for the next *in vitro*, molecular, and secondary metabolite studies. In addition, it promotes the plant by optimizing the healthy and disease-free seedling production method for cultural agriculture in the region. Three different (15%, 25%, and 35%) NaOCl concentrations were tested for 10 and 20 minutes during the sterilization phase of the explants. The most successful result was obtained in the medium containing 35% NaOCl for 10 minutes. Sterilized explants were transferred to MS and Gamborg B5 nutrient media containing BAP, GA, and NAA plant growth regulators for shoot regeneration. The best regeneration in explants was obtained in MS medium containing 0.5 mg L^{-1} BAP, 1 mg L^{-1} GA, and 0.2 mg L^{-1} NAA. No growth was observed in trials containing Gamborg B5, and vitrification and darkening occurred in the explants. After four weeks, the shoots reaching a length of 3 cm were taken into MS and ½MS medium containing 0 MS, 0.5 mg L^{-1} IBA, 1 mg L^{-1} IBA, 1.5 mg L^{-1}, and 2 mg L^{-1} IBA as a rooting medium. 100% rooting was observed in all prepared media within four weeks. As a result of micropropagation studies, the rooted plants were transferred to the acclimatization stage within three months and then moved to the pots in the greenhouse and to the field one month later.

Keywords: *Helichrysum italicum*, Regeneration, Rooting, Acclimatization, MS, Gamborg B5

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Öz

Güney Marmara ve Ege bölgesinde yayılış gösteren ölmez otu bitkisi (*Helichrysum italicum* (Roth) G. Don) kurak ve yarı kurak bölgelerde yetiştirilebilmektedir. Ayrıca zengin uçucu yağ ve sekonder metabolit içeriğinden dolayı geleneksel tedavi yöntemleri dahil modern tıp ve kozmetik alanında önemli bir yere sahiptir. Bitkilerin *in vitro* koşullarda sürgün rejenerasyonu yoluyla çoğaltılması birçok bitki türünde elde edilmiş olmasına rağmen, ölmez otunda doku kültürü üzerine yapılan çalışmalar oldukça sınırlıdır. Bu çalışmanın amacı, ölmez otu bitkisinde doku kültürü çalışmasının optimizasyonunun yapılması ve bir sonraki *in vitro*, moleküler ve sekonder metabolit çalışmalarına zemin sağlamaktır. Bunun yanı sıra bölgede kültür tarımı için sağlıklı ve hastalıksız fide üretimi yönteminin optimize edilerek bitkiyi tanıtmaktır. Eksplantların sterilizasyon aşamasında üç farklı (%15, %25 ve %35) NaOCl konsantrasyonu 10 ve 20 dakika süre ile denenmiştir. En başarılı sonuç 10 dakika %35 NaOCl içeren ortamdan elde edilmiştir. Steril edilen eksplantlar sürgün rejenerasyonu için BAP, GA ve NAA bitki büyüme düzenleyicileri içeren MS ve Gamborg B5 besin ortamlarına aktarılmıştır. Eksplantlarda en iyi rejenerasyon 0.5 mg L^{-1} BAP, 1 mg L^{-1} GA ve 0.2 mg L^{-1} NAA içeren MS ortamında elde edilmiştir. Gamborg B5 içeren denemelerde herhangi gelişim gözlenememiş olup eksplantlarda vitrifikasyon ve kararma oluşmuştur. Dört hafta sonunda 3 cm uzunluğa ulaşan sürgünler köklendirme ortamı olarak 0 MS, 0.5 mg L^{-1} IBA, 1 mg IBA, 1.5 mg L^{-1} ve 2 mg L^{-1} IBA içeren MS ve ½MS ortamına alınmıştır. Hazırlanan tüm ortamlarda dört hafta içerisinde %100 köklenme görülmüştür. Mikroçoğaltım çalışmaları sonucu üç ay içerisinde köklenmiş bitkiler aklimatizasyon aşamasına alındıktan sonra seradaki saksılara ve bir ay sonra tarlaya aktarılmıştır.

Anahtar Kelimeler: *Helichrysum italicum*, Rejenerasyon, Köklendirme, Aklimatizasyon, ½ MS, Gamborg B5

1. Introduction

The immortelle grass, belonging to the Asteraceae family, is a shrubby and perennial plant. It has yellow flowers, furry leaves and stems and can grow up to 90 cm in height. Due to the long-lasting appearance of its showy flowers, it is known as the immortal flower, the unfading flower, and the everlasting flower in popular culture. Additionally, it has a sharp scent that can last for a long time. This genus has more than 600 species worldwide, with its native range including Africa (244 species in South Africa), Madagascar, Australia, Asia, and Eurasia. It also grows in the steppes of America, Scandinavia, the Atlantic, Europe, the Balkans, Russia, Siberia, the Caucasus, Central Asia, Mongolia, China, and Türkiye in sandy and semi-hard soils (Umaz and Umaz, 2020). In the Turkish flora, this genus is represented by 27 taxa, 15 of which are endemic and commonly found in Anatolia (Albayrak et al., 2010). *Helichrysum* species have ornamental value due to their unique morphological features, flower structure, and colors. Moreover, essential oils and other secondary metabolites obtained from the flowers and vegetative parts of the plant are known to have anti-inflammatory, anti-allergic, and antimicrobial effects (Dimitrova and Nacheva, 2018).

Medicinal aromatic plants are a group of plants rich in biochemicals called secondary metabolites. One of the most important functions of secondary metabolites is to play a role in the defense systems of the plant against biotic and abiotic stress conditions (Umarusman et al., 2019). The immortelle grass is a plant that is rich in biochemical compounds. The types and amounts of these compounds present in the plant vary depending on the plant part from which they are isolated, the season in which the plant is found, and the conditions under which the plant is grown. The main compounds found in the plant include α-pinene, 2-methylcyclo-hexyl pentanoate, neryl acetate, 1,7-di-epi-αcedrene, γ-curcumene, and thymol (Ninčević et al., 2019). Studies on the compounds found in the plant have shown that α-pinene in particular, has a cytotoxic effect on T leukemia cells, lung cancer cells, and cervical cancer cells, thereby preventing the cancer progression (Staver et al., 2018).

In vitro micropropagation provides the opportunity to produce a large number of clonal plants of a plant part under aseptic and controlled conditions, utilizing the totipotent ability of the plant. One of the most significant advantages of micropropagation is that the resulting plants are genetically identical and have similar forms. Due to its small seeds and low germination performance, *Helichrysum* species are challenging to propagate. Additionally, the limited number of cuttings that can be taken from each Helichrysum seedling makes it challenging to meet the high demand for seedlings. *In vitro*, micropropagation of Helichrysum provides a solution to overcome these challenges. Therefore, this study aims to optimize the *in vitro* micropropagation of *Helichrysum* to contribute to both its commercial production and future *in vitro* studies.

2. Materials and Methods

2.1. Plant Material

The starting material used in the study was obtained from the collection garden of the Tekirdag Namik Kemal University Faculty of Agriculture Research and Production Unit (ZIRAATBIYOTEK). Explants were taken from 1 year old *Helichrysum italicum* plants before flowering in spring.

2.2. In Vitro Culture Conditions

In the conducted study, MS (Murashige and Skoog, 1962) basic nutrient medium and Gamborg B5 (Gamborg et. al., 1976) nutrient medium were used as the nutrient media. 3% sucrose was used as the carbon source in these media. 6.5 g L^{-1} plant agar (Duchefa) was used as the gelling agent in the prepared media. Since shoot cultures released phenolic compounds, 0.06% activated charcoal was added to the media. Plant growth regulators (BAP, GA, NAA, and IBA) used in the experiments were dissolved in appropriate solvents. Then, stock solutions were prepared with distilled water and added to the media in the required amounts and concentrations. The pH of the nutrient media was adjusted to 5.6-5.8 using 1 N NaOH or HCl. The prepared nutrient media were sterilized at 121°C, 1.2 kg cm^2 (Tekbal-ST2) under pressure for 20 minutes. All cultures were incubated at 24±2°C with a 16-hour light 8-hour dark photoperiod under blue-red LED light.

2.3. Explant Preparation and Surface Sterilization

The fresh shoots of immortelle grass seedlings found in the collection garden were preferred as explants in the study. The collected shoots were washed with 2-3 drops of commercial detergent on a magnetic stirrer for 5

minutes as a pre-sterilization step and then rinsed under running tap water for approximately 1 hour. The pre-sterilized explants were placed in a sterile cabinet and soaked in a 70% ethanol solution for 10 seconds. Then, they were sterilized in a magnetic stirrer in a commercial laundry bleach solution diluted to 15%, 25%, and 35% for 10 and 20 minutes, respectively. Finally, they were rinsed thrice with autoclaved distilled water for 5 minutes each time (Daneshvar Royandazagh and Pehlivan, 2016). The obtained sterile explants were prepared for *in vitro* culture environments in different doses and combinations of plant growth regulators to improve shoot regeneration.

2.4. Regeneration Conditions of Explants

The sterilized explants were transferred to nutrient media containing different plant growth regulators to induce regeneration. In this stage, BAP, GA, and NAA were added to solid MS and Gamborg B5 nutrient media in various combinations. The dosage of plant growth regulators added to the nutrient media varied between 0.2-2 mg L^{-1} (*Table 1*). Control nutrient media without plant growth regulators were prepared for both nutrient media. The experiments were performed with five replicates, and the culture medium that provided the best shoot development was determined.

2.5. Rooting of Shoots

The shoots developed in the special nutrient media prepared for regeneration will be transferred to rooting experiments in MS and ½ MS nutrient media containing different concentrations of IBA (0.5, 1, and 2 mg L^{-1}). The rooting experiments will be carried out in sterilized glass jars, with 4-5 shoots placed in each jar.

2.6. Acclimatization

The rooted plants will be washed with clean water to remove any residue from the nutrient media. They will then be transplanted into vials filled with previously sterilized and moistened peat. To prevent sudden moisture loss of the plants, the vials will be placed in a suitable container and covered entirely with cling film before being transferred to the plant growth room. Over the course of three weeks, holes will gradually be made in the cling film to acclimate the plants to external conditions. After three weeks, the plants will be transferred to field conditions.

2.7. Statistical Assessment of Regeneration Studies

The experiments were conducted with five replications. The statistical analysis of the data obtained from the regeneration studies was performed using the SPSS ver. 22 statistical program and One-Way Anova post hoc tests were conducted with the Duncan test (Snedecor and Cochran, 1967).

3. Results and Discussion

Due to the limited vegetative and generative propagation of the immortelle grass, optimization of the micropropagation method is essential. By using different explants, basic nutrient media, plant growth regulators, and culture conditions in micropropagation studies, a large number of plants can be obtained from a few explants (Gupta et al., 2020). Sterilization is difficult in immortelle grass due to the hairy nature of its leaves (Clasquin and Henry, 2002). In addition, blue and red LED lights were used in growth chamber in this study. According to the study of Silva et al., red and blue LED lights provided better growth and development compared to white light (Silva et al., 2014). In another study, it was seen that blue and red light gave better results in shoot development (Ramírez-Mosqueda et al., 2017). In this study, according to the results obtained from the surface sterilization study, 100% contamination was observed in both trials conducted with 15% commercial bleach. In another trial using 25% commercial bleach, 60% contamination occurred in the 10-minute trial and 40% contamination occurred in the 20-minute trial. Although the best result was obtained in the trial with 35% commercial bleach, where 20% contamination was observed in the trials conducted for both 10 and 20 minutes, it was found that the 10-minute trial achieved the best result because the explant browning rate was high in the explants left for 20 minutes (*Table 1*). Morone-Fortunato et al. reported successful results in their micropropagation study of *H. italicum* using explants treated with a 0.1% (w/v) $HgCl_2$ solution for 15 minutes with magnetic stirring (Morone-Fortunato et al, 2010). In another study by Giovanni et al. on the same genus, the best results were obtained by immersing explants in a solution of 1% NaOCl and two drops of Tween 20 for 20 minutes, after being immersed in a 70% ethanol solution for 30 seconds (Giovanni et al., 2003). In the sterilization study of *H. arenarium* by Figas et al., (2016) explants were immersed in a 70% ethanol solution for 1 minute, then immersed in a solution

containing 9% $Ca(OCl_2)$ and Tween 20 for 12 minutes, and finally rinsed with sterilized distilled water to obtain successful results (Figas et al., 2016). In another study on *H. arenarium*, Clasquin and Henry reported a successful sterilization protocol in which explants were stirred on a magnetic stirrer for 30 minutes in a 0.1 M $KMnO_4$ solution, immersed in a 70% ethanol solution for 1 minute, a 15% H_2O_2 solution for 15 minutes, and a 12% NaOCI solution for 10 minutes (Clasquin and Henry, 2002).

Table 1. *Sterilization study results*

Treatment	Contamination**	Browned Explant**
15% bleach – 10 minutes	10.00 ±0.00a	10.00 ±0.00a
25% bleach – 10 minutes	6.00±1.00b	8.00±0.57ab
35% bleach – 10 minutes	2.00±0.57c	2.00±0.57c
15% bleach – 20 minutes	10.00±0.00a	10.00±0.00a
25% bleach – 20 minutes	4.00±0.57bc	6.00±0.57b
35% bleach – 20 minutes	2.00±0.57c	3.00±0.57c

$p \leq 0.01$

**Averages shown with different lowercase letters in each column, according to Duncans multiple test; It is statistically different at the 0.01 significance level.

In vitro micropropagation method, which is one of the vegetative propagation methods, is important for producing seedlings free from diseases and in high quantities. Two different basic nutrient media (MS and Gamborg B5) were used in the micropropagation study conducted in this study. Regeneration and shoot development were provided by adding different doses of BAP, GA and NAA as plant growth regulators to these nutrient media. According to the results of the Duncan test, the number of shoot formations per explant varies between 2 and 12.40 in MS basic nutrient media. The lowest shoot formation per explant was observed in the MS control, while the highest shoot formation per explant was observed in the MS 0.5 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA nutrient medium. No development was observed in the Gamborg B5 nutrient media, and vitrification occurred so that no results could be obtained (*Figure 2*). The percentage of shoot formation varied between 19.99% and 66.66%, with the highest percentage of shoot formation observed in the MS 0.5 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA and MS 1.5 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA nutrient media. The lowest percentage of shoot formation was observed in the MS control nutrient medium (*Table 2*). The average shoot length varied between 0.42 cm and 2.64 cm in MS basic nutrient media. The highest shoot length was observed in the MS 0.5 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA nutrient medium, while the lowest shoot length was observed in the MS control nutrient medium. Since no development was observed in the Gamborg B5 nutrient media, the average shoot length could not be calculated (*Table 2*). As the results we obtained in the study of Clasquin and Henry in *H. arenarium*, no shoot development was observed in the experiments with Gamborg B5, however, callus and vitrification formation were observed (Clasquin and Henry, 2002). Giovanni et al. in their study on *H. italicum* and *H. stoechas*, the best shoot growth was obtained in media containing 2.66 µM BAP in both species (Giovanni et al., 2003). In another micropropagation study of Dimitrova and Nacheva in *H. italicum*, trials were established by adding BAP, KIN and ZT to DKW and MS basic nutrient media. As a result of the study, it was reported that the best shoot growth medium was MS containing 5 µM BAP + 0.005 µM IBA and DKW basic nutrient medium containing 5 µM KIN + 0.005 µM IBA (Dimitrova and Nacheva, 2018). Perrinia et al. (2009) aimed to form callus in the leaves of *H. italicum* and then to achieve shoot development by organogenesis. They performed micropropagation study by combining and modifying MS and NN media as nutrient media. In this study, different concentrations of TDZ and NAA were used as plant growth regulators, and the best shoot growth results were obtained in environments without plant growth regulators. According to the data obtained because of this study, it has been reported that the organogenic capacity of *H. italicum* is high (Perrinia et al., 2009). In another study by Figas et al. in *H. arenarium*, KIN and combinations of KIN and IBA were tried. According to the results they obtained, the best shoot growth was achieved in media containing 5 mg L^{-1} KIN and 0.5 mg L^{-1} IAA (Figas et al., 2016). In Tastekin's master's thesis study, the best shoot growth was obtained in the micropropagation stage of St. John's Wort in a medium containing 0.5 mg L^{-1} BAP and 2.5 mg L^{-1} NAA (Tastekin, 2020). In the micropropagation study performed in mint, Khan et al. showed that the best results were obtained from the combination of BAP and NAA in their trials in which they combined BAP with NAA and IBA (Khan et al., 2021). Hirakawa and Tanno experimented with BA and GA plant growth regulators at different concentrations added to ½MS in their micropropagation study in hops. According to the results they obtained, the best shoot

growth was obtained in the medium containing BA (0.01 mg L^{-1}) at a lower concentration (Hirakawa and Tanno, 2022). In a study conducted by Yesmin (2019) on sugar grass, media were prepared with the combinations of BAP, KIN and NAA added to MS. According to the results obtained in the study, the best shoot growth and shoot length were obtained in media containing 1.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (Yesmin., 2019). In the micropropagation study of Petrova et al. in lemon balm, media were prepared with IBA and NAA combinations of BAP, KIN, ZT and 2-iP added to MS. As the results obtained in sugar grass, the best shoot development and shoot length were obtained in the medium containing 1.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (Petrova et al., 2021). In the micropropagation study of Morone-Fortunato et al. on *H. italicum*, explants were taken from 20 different genotypes and the results obtained from these genotypes were examined. In this study, which used 1 mg L^{-1} BAP and 0.2 mg L^{-1} IBA for shoot development, it was revealed that genotypes gave different responses under the same conditions. According to the results obtained, more than 50% shoot growth was obtained in 16 genotypes, while the result was between 33-47% in the remaining 4 genotypes (Morone-Fortunato et al., 2010).

Table 2. Micropropagation results

Treatment	Average number of shoots per explant**	Percent shoot formation	Average shoot length** (cm)
MS control	2.00±0.44c	19.99 ±2.4b	0.42±0.37c
MS 0.5 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA	12.40±1.80a	66.66±10.2a	2.64±0.19a
MS 1 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA	7.20±1.39b	46.66±12.2ab	1.70±0.25b
MS 1.5 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA	7.00±1.04b	53.33±8.2a	1.88±0.17b
MS 2 mg L^{-1} BAP + 1 mg L^{-1} GA + 0.2 mg L^{-1} NAA	5.40±0.67bc	46.66±12.2ab	2.0±0.17b

$p \leq 0.01$

**According to Duncans multiple tests, the means shown with different lowercase letters in each column; It is statistically different at the 0.01 significance level.

The rooting stage is one of the most important stages for tissue culture studies to achieve results. While the rooting stage can be quite difficult especially in woody plants, it can be achieved relatively easily in herbaceous plants. MS basal nutrient medium was used at ratios of 1/1 and 1/2 for rooting stage of shoots obtained under *in vitro* conditions. Different rates of IBA, which is a plant growth regulator belonging to the auxin group, between 0.5-2 mg L^{-1} were used in these nutrient media. According to the data obtained, all explants were rooted at the end of 30 days. The highest average root length was obtained in the nutrient medium containing 1/1 MS and 1 mg L^{-1} IBA. The lowest root length was obtained in the nutrient medium containing 1/1 MS without plant growth regulator. When the average root number was examined, the highest average root number was observed in the nutrient medium containing 1/1 MS and 1 mg L^{-1} IBA (*Figure 1*). The lowest average root number was observed in the nutrient medium containing 1/2 MS without plant growth regulator (*Figure 1*). Perrinia et al. (2009) reported that they achieved nearly 100% rooting in the MS medium without plant growth regulators in immortelle grass (Perrinia et al., 2009). Figas et al. (2016), on the other hand, set up trials with media containing 0 MS, 0.5 mg L^{-1} IAA and 0.5 mg L^{-1} IAA for rooting in *H. arenarium*. According to the results they obtained, rooting was observed between 85-100% in all media, and when the root length and number of roots were examined, the best result was achieved in the medium containing 0.5 mg L^{-1} IBA (Figas et al., 2016). Again, similar to the results obtained in this study, 100% rooting was obtained in the experiments performed at different concentrations of 0 MS, IAA and NAA in the study of Giovanni et al. on *H. italicum* and *H. stoechas* (Giovanni et al., 2003). In another study, 60-80% successful results were obtained in the trials established with NAA in Dimitrova and Nacheva *H. italicum*, while successful results were between 90-100% in the trials established with IBA (Dimitrova and Nacheva, 2018). In the study of Anrade et al. on *Lavandula vera*, it was tried to achieve rooting by adding different concentrations of IBA and NAA to normal MS and ¼MS nutrient media. According to the results obtained, the best rooting rate was achieved in the medium containing ¼ MS 0.2 mg L^{-1} NAA (Anrade et al., 1999). In the study of El-Banna on thyme, rooting experiments were established by using 0 MS, IBA and NAA at different concentrations. According to the results, while 75% rooting occurred in the control medium, 100% rooting was observed in the medium

containing 1.5 mg L^{-1} IBA, 1 mg L^{-1} NAA and 1.5 mg L^{-1} NAA (El-Banna, 2017). Hirakawa and Tanno reported that they reached the best rooting rate within 2 weeks in ½MS nutrient medium containing 0.05 mg L^{-1} NAA in their study on hops (Hirakawa and Tanno, 2022). In the study of Petrova et al. on lemon balm, 100% rooting was obtained in the control medium without plant growth regulator (Petrova et al., 2021). In a study on sugar grass, Yesmin used 0.2-0.5 mg L^{-1} ratios of IBA, IAA and NAA in MS and ½MS nutrient media for the rooting stage. According to the results obtained, the highest rooting rate was obtained in MS nutrient medium containing 0.2 mg L^{-1} IBA (Yesmin., 2019).

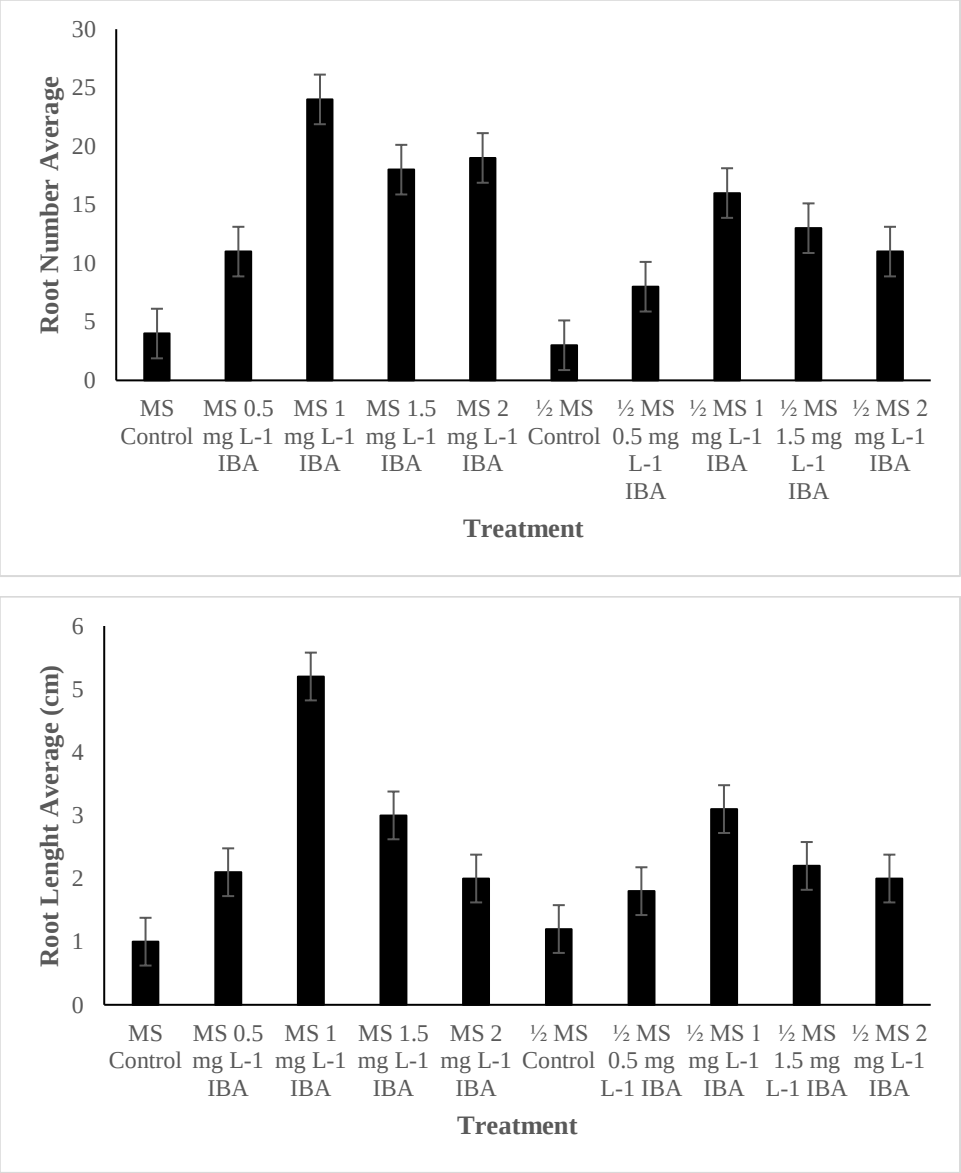

Figure 1. *Rooting study results.*

Rooted plants must go through a gradual acclimation process in order to be transferred to external conditions. This stage, in which plants kept at high humidity and constant temperature *in vitro* conditions are controlled and acclimatized to external conditions, is very important. Especially in herbaceous plants, it is very important to control and monitor the conditions in order to avoid plant losses at this stage. In this study, plants were successfully transferred to external conditions without loss in the acclimatization stage. After the rooted plants were transferred to sterilized peat, after irrigation and leaf moistening, they were covered with airtight cling film and transferred to fully controlled plant growth rooms. After the stretch was kept completely closed during the first week, holes were opened on the stretch and the amount of moisture inside was gradually reduced. The acclimatization process, which continued by increasing the number of holes in the second week, was completed by completely removing the stretch at the end of the third week. Afterwards, the plants were transferred to greenhouse and field conditions and

the acclimatization process was successfully completed and later to field conditions (*Figure 2*). During the acclimatization phase, different soil mixtures and irrigation waters are used according to the plant's demands. In this study, this step was carried out by irrigating sterile peat with distilled water. Hirakawa and Tanno performed the acclimatization process of hops by irrigation with distilled water in a soil mixture containing 3:1:1 peat, vermiculite and akadama (Hirakawa and Tanno, 2022). Petrova et al., on the other hand, carried out the acclimatization stage of lemon balm by irrigating a soil mixture containing soil, peat, perlite, and sand at a ratio of 2:1:1:1 with pure water (Petrova et al., 2021). In Yesmin's study on sugar grass, acclimatization was carried out by irrigating a 1:1 mixture of garden soil and compost with pure water (Yesmin., 2019). Figas et al. watered the *H. arenarium* with water and water containing 25% MS during the acclimatization phase. While 56% acclimatized plants were obtained in irrigation with water, 75% acclimatized plants were obtained in irrigation with 25% MS-containing water (Figas et al., 2016).

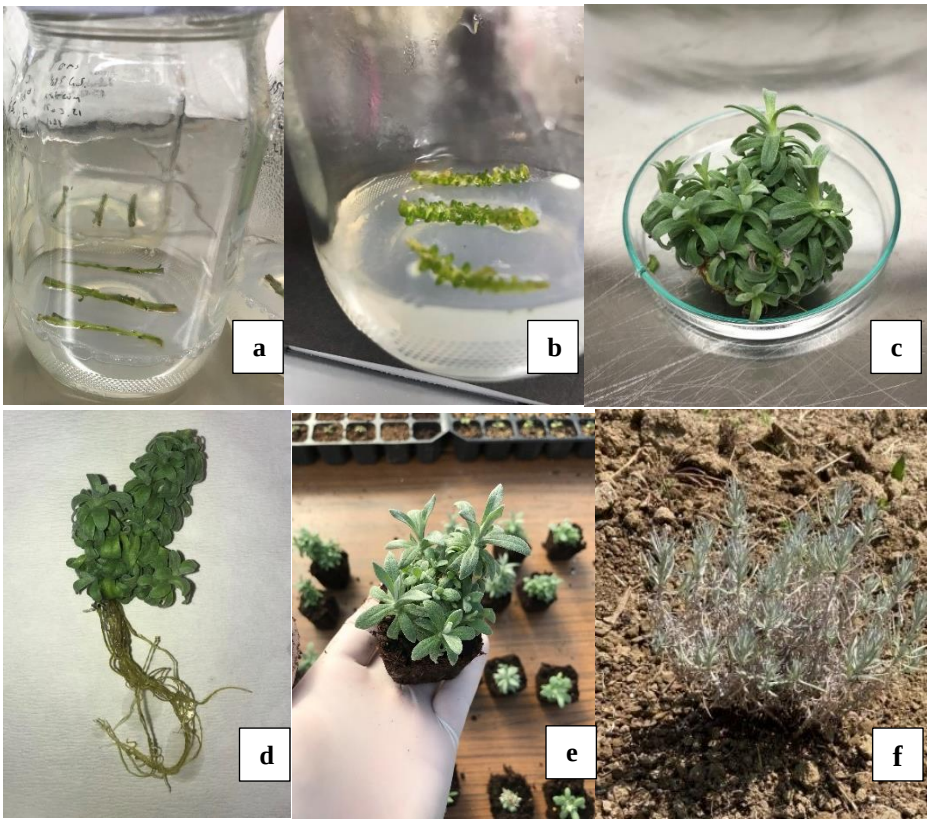

Figure 2. *(a) explants transferred to nutrient media, (b) vitrification in nutrient media containing Gamborg B5, (c) shoot development in 30 days, (d) root development in rooting medium, (e) acclimatized seedlings, (f) 2 months of development in field conditions.*

4. Conclusions

Immortelle grass is a plant that has a very important place among medicinal and aromatic plants that spread naturally in Türkiye. This plant has essential oil and biochemical components used in many sectors, especially in the pharmaceutical, cosmetic and paint sectors. Agriculture is of great importance in our country's economy. For this reason, it is vital to make good use of the agricultural lands owned. Since immortelle grass is not selective in terms of growing conditions, it has the potential to contribute to the country's economy, even in barren lands. For these reasons, it is necessary to encourage the production of healthy and high-quality seedlings of immortelle grass under *in vitro* conditions, their promotion to farmers and cultural agriculture.

According to the information obtained in the literature review for the sterilization of immortelle grass, which is very difficult to sterilize due to its hairy leaves, NaOCl (bleach), which has the lowest cost among the chemicals used and is known to be the least harmful to nature, was preferred. According to the results obtained, it has been observed that the concentration of the commercial bleach solution is as important as the time in order to obtain sterile and healthy plants. Successful results were obtained in the trial, which was kept for a shorter time due to the burning of the plants

kept in high concentration for a long time.

Successful results were obtained by using a lower concentration of plant growth regulator (0.5 mg L^{-1} BAP, 1 mg L^{-1} GA and 0.2 mg L^{-1} NAA) in the micropropagation stage compared to previous studies.

At the rooting stage, as a result of the experiments, 100% rooting was obtained in ½MS basic nutrient medium without plant growth regulator.

The acclimatization stage is crucial for this plant. To prevent plant loss, humidity and temperature should be gradually balanced with external conditions during the controlled acclimation stage. Due to the very high humidity and optimum temperature *in vitro* conditions, sudden moisture loss or heat shock at this stage causes the plant to die.

As a result of the studies carried out, the micropropagation of immortelle grass has been optimized by using lesser amounts of chemicals that are less harmful to nature. In this way, a fast and economical method that can be used to produce of high amounts of seedlings for cultural agriculture has been created. In addition, this study lays the groundwork for future *in vitro*, molecular and secondary metabolite studies in immortelle grass.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Uz, I., Savalan, S.; Design: Uz, I., Savalan, S.; Data Collection or Processing: Uz, I., Savalan, S.; Statistical Analyses: Uz, I., Savalan, S.; Literature Search: Uz, I., Savalan, S.; Writing, Review and Editing: Uz, I., Savalan, S.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Evaluation of Urban Farming System Sustainability in Central Province of Jakarta, Indonesia

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Abstract

The objective of the research was to evaluate the degree of sustainability of urban farming development in Petamburan, Central Jakarta. This research used a descriptive methodology approach using a qualitative approach (1) research preparation stage (2) collection stage (3) data analysis stage to check the degree of sustainability of urban agriculture using the Multi Dimension Scaling (MDS) approach. The study results indicate that urban agriculture in the special capital region of Jakarta is well known and is strongly supported by the residents of Petamburan Village, Tanah Abang District, Central Jakarta. Urban residents generally already have knowledge and insight about urban agriculture. The community support for urban agricultural activities uses their yards to cultivate vegetables, herbs, and other seasonal fruit crops. The development of urban farming in Petamburan, Tanah Abang, Central Jakarta results from the analysis of four dimensioned model MDS is not sustainable. The analysis results of each dimension consist of the ecological dimension 14.55%, the economic dimension 13.85%, the social dimension 13.94%, and the technological dimension 13.43%. In the future, urban farming should pay attention to the supporting factors of the sustainability of agricultural development. The factors include the yard area, types, and variations of cultivated plants, the application of innovation and technology that urban communities can accept and develop, increased counselling and community development, and intensive and tax-free provision for yards with urban farming. The study show that index of urban agriculture sustainability in Petamburan Village, Tanah Abang District, Central Jakarta is very low, including the destructive and unsustainable category depending on the results of multidimensional analysis of both economic, ecological, social, and technological dimensions so that improvements are needed through counselling and motivation for urban agriculture actors.

Keywords: Sustainable, Multidimensional Scaling, Urban Farming, Jakarta

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1. Introduction

The government of the special capital region of Jakarta in Indonesia (here after called as Jakarta) will contribute in spatial planning of the area by allocating a large amount of money in the budget for 2018-30 period for spending in the Research and Development activities related to Urban Farming business. (DKI Jakarta Provincial Government, 2017). These activities will revitalize agricultural sector and support sustainable urban progress with elevated life style, clean lines of environment and promote organic agriculture, beautifying urban landscapes, environmental education facilities for city dwellers, hobbies/pleasures, as well as livelihoods for the urban poor (Abdullah et al., 2017), with varying from crop, livestock and poultry production to aqua cultural practices (Drechsel and Dongus, 2010; De Bon et al., 2010).

Urban farmers streamline resource use by integration of the crop and fish farming sub-sector to increased harvesting of the benefits (Victor et al., 2018). Agricultural conditions in urban areas, especially the special capital region of Jakarta, and their relationship to various environmental problems, need to be designed and formulated comprehensive policies for the development of sustainable farming. Their role in sustainable role in agricultural progress and development in existing conditions have 48.70 % index value or less sustainability (Sampeling et al., 2012). The conversion of agricultural land in urban areas and the occurrence of urbanization to urban areas has resulted in many problems including social, cultural, environmental and economic instability (Peerzado et al., 2019). The ecological function of open space is decreasing due to the increasing expansion and distribution of the built-up area. The concept of urban farming system acts as an alternative to improve the existing conditions in the urban areas and their socio-economics conditions (Abdullah et al., 2017).

Urban farming issues usually revolve around economic food production and foster community welfare, social capital, and involvement of community in food producing systems. (Kullu et al., 2020). Increased infrastructure development in urban areas, growing a large population due to urbanization, have negative impacts on the life styles of the people. It is also observed that agricultural lands around cities are decreasing and with the passage of time less number of people are getting involved in cultural production. Cultivation of beneficial is very necessary for public consumption and improve the supply of oxygen, antidote against air pollution and to stop deterioration of soil (Indrawati, 2017). Biophysical characteristics such as geology and land use have a high sensitivity to the coefficient of erosion intensity produced (Devianti et al., 2023). Biophysical characteristics will influence the response to rainfall that falls in the basin. This response affects the magnitude or small value of characteristic hydrological parameters, such as evapotranspiration, infiltration, surface runoff, and soil water content (Sattari et al., 2020). Understanding the urban life style and agriculture in Jakarta is very important towards the formulation of appropriate government policies for formulation of development plans and integrate urban agricultural systems into an urban lifestyle (Chandra and Dhiehl, 2019).

The existence of urban agricultural land/space has a significantly important role in the agricultural production system and maintains environmental quality and agricultural production maintaining the existence of land/space is not only for the sustainability of the agricultural production system but also for maintaining the quality of the environment. In this case, urban farming provides employment and becomes an additional source of community income and is a buffer for economic stability in critical situations and is directly related to poverty alleviation efforts and a sustainable environment (Sampeling et al., 2012). Urban farming has potential and could be executed yards of residential areas. The function of the garden ecosystem strongly supports the realization of the concept of sustainable urban landscape architecture.

The concept of developing a productive urban landscape can create a sustainable built environment and support food security. Limited land for urban settlements can take advantage of the concept of optimizing very narrow, narrow, medium, wide and very wide land yards (Irwan and Sarwadi, 2015). In socio-economic construction, socio-economic diversity and employment have become sub-constructions for urban agriculture variables. First, socio-economic diversity refers to the variety of business sectors that urban communities can generate through urban farming (Salleh et al., 2021). Therefore, the sustainability in urban farming and its development is significantly influenced by the role and behavior of the components, who support systems. The purpose of this study was to make an analysis of sustainnability level urban farming establishment in Central Jakarta.

2. Material and Methods

2.1. Study area

The research was carried out for 3 (three) months from May to July 2022 in Petamburan Village, Tanah Abang District, Central Jakarta. This area is located at 2.60 meters above sea level (*Figure 1*).

Figure 1. Map of study site

2.2. Research Procedures

Both primary and secondary data were used in the study. The primary data was collected in the form of information on biophysical aspects, economic aspects, and social aspects. Primary data collection was field observations and surveys by distributing questionnaires, and the survey was conducted with 20 respondents involved in urban agriculture, which were taken by simple random sampling. Secondary data was obtained from the previous research based literature and agencies, namely the Petamburan sub-district, Tanah Abang district office, Central Jakarta office, and Central Jakarta City forestry service office.

The level of sustainability of urban agriculture was determined in multi-dimensional way, namely: ecology, economy, social, and technology with the MDS (Multi-Dimensional Scaling method). The MDS method is a computer-based statistical analysis using RALED-SBH (Rapid Assessment Techniques for Local Economic Development-Sugen Budiharsono) Budiharsono (2007). Software (Local Economic Development Team, BAPPENAS, 2007). The analysis method used is MDS (multi-dimensional scaling) and Rap-Ur-Agri (Rapid Appraisal for Urban Agriculture). The primary factor analysis using leverage factor was followed by the sustainability index (*Table 1*) and agricultural development policy scenario using prospective analysis method. The data analysis stage to see the sustainability of urban farming uses the Multi Dimension Scaling (MDS) approach; the MDS method uses an ordination process which is a modified result of the Rapid Assessment Techniques for Fisheries (RAPFISH) method, then the results were further analysed for several dimensions, namely technological, social, economic and ecological dimensions as presented in the kit diagram.

Table 1. Criteria Index and Sustainable Status

Index Value	Index Category Value
00 – 24.99	Poor : Unsustainable
25 – 49.99	Less : Less sustainable
50 – 79.99	Adequate : Fairly sustainable
80 – 100.00	Good : Very sustainable

Source : Fauzi and Anna (2005)

3. Results and Discussion

3.1. The Population Distribution and Characteristics of Respondents

The population distribution in the Petamburan sub-district, Tanah Abang district of Central Jakarta administration city, is 40 938 people, consisting of 21 024 men and 19 914 women. The average population density in the Petamburan area, Tanah Abang district, Central Jakarta, is 45 487 people/km^2 (BPS, 2020). The increased urban population without supporting balanced provision of food and facilities for housing, employment and infrastructure, to support lives will be catastrophic with decreased food security and increased urban poverty (Lovell, 2010; Listya Cahya, 2014). The number of respondents in this study consisted of 20 respondents, five respondents who were involved in urban farming activities. Population status based on occupation consists of 6 900 people from trade, 18 002 private-sector employees, 136 civil servants, 55 police officers, 230 retirees, 78 carpenters, and 11 211 others. It is well established that the majority of the participants in the integrated farming in urban areas are households with multiple number of house hold members (Gallaher et al., 2013).

3.2. Support for Urban Agriculture

The existence of urban farming in the special capital region of Jakarta is well known and is strongly supported by residents of Petamburan village, Tanah Abang district, Central Jakarta. Urban residents generally already have knowledge and insight about urban agriculture. The form of community support for urban agricultural activities uses their yards to cultivate vegetables, herbs, and other seasonal fruit crops. The utilization of residents yards for urban agriculture will add aesthetic value, improve the micro-climate, and create a sustainable environment. Agricultural system innovations further contribute to the application of urban agriculture in limited areas (vertical cultivation), application with soilless cultivation (hydroponic techniques), and resource management practices (composting techniques) (Sharifi, 2016). The establishment of urban farming in the special province of Jakarta shows that economic factors are the strongest impact to do farming in urban areas, even though the business area is limited. This is influenced by the ease with which crops are marketed at relatively high prices compared to areas outside Jakarta (Mayasari et al., 2015). Based on interviews and observations at the research location, many residents wish to grow vegetables and other seasonal crops. However, limited land, cost, and time are the main obstacles considering the administration of the Government of the Jakarta, the urban village, is to improve urban agricultural programs, which are very useful for enhancing the environment and providing food for families, especially people with low incomes. The government also needs to carry out and improve regular counseling related to urban farming so that urban farming activities can be maximized in Petamburan sub district, Tanah Abang district, Central Jakarta.

3.3. Urban Agriculture Sustainability Status

Observations of the research location on the existing urban agriculture development show that urban farming patterns vary in Petamburan sub-district, Tanah Abang district, Central Jakarta. The community develops urban farming practices by utilizing simple technology and innovations such as planting in pots, polybags, verticulture, simple hydroponic systems, climbing and climbing systems on building walls, home gardens, and direct planting systems in the ground. Plants developed by the local community are ornamental plants, vegetable plants, food crops, annual plants, and herbal plants. The plant cultivation technology used is still very simple and has not utilized an intensive farming system. Urban agricultural development aims only as a hobby, environmental aesthetics, family needs, and a small part of the commercial.

The determination status sustainability of urban farming in Petamburan sub-district, Tanah Abang district, Central Jakarta, based on the attributes assessed on each dimension, namely ecology, economy, social and technology. In general, the condition of urban agriculture in Petamburan sub-district, Tanah Abang sub-district, Central Jakarta, has all dimensions of unsustainable status, namely the technological, social, economic, and ecological, dimensions (*Table 2*). Urban agriculture still requires improvement for sensitive attribute components that affect sustainability, but improvement interventions can be carried out by the government and urban agriculture stakeholders (Abdullah et al., 2017). The indicators for the sustainability of urban farming by selecting 4 dimensions as sustainability indicators have represented the indicators used to evaluate the sustainability of urban farming in Petamburan sub-district, Tanah Abang district, Central Jakarta.

Table 2. Index value and sustainability status of urban farming in Petamburan Village, Tanah Abang District, Central Jakarta Administration City

Dimensions of	Dimensions of	Dimensions of	Dimensions of
Ecology	14.55%	Unsustainable	Petamburan sub district
Social	13.85%	Unsustainable	Tanah Abang district
Economy	13.94 %	Unsustainable	Central Jakarta City
Technology	13.43%	Unsustainable	

Source: MDS Analysis Urban Farming Petamburan Central Jakarta, 2022

Furthermore, analysis of the results of the sustainability of urban farming in every technological, social, economic, and ecological, dimensions in Petamburan village, Tanah Abang sub-district, Central Jakarta using the method is multi dimension scaling presented as follows:

3.4. The Ecological dimensions

Urban farming sustainability in Petamburan sub-district, Tanah Abang district, Central Jakarta, the results mentioned by the analysis of the ecological dimensions show a sustainability index of 14.55%. If viewed from the sustainable category on a scale of 0-100, furthermore, it includes the Bad: Unsustainable criteria. The results of the analysis of the ecological dimensions of the MDS (*Figure 2*).

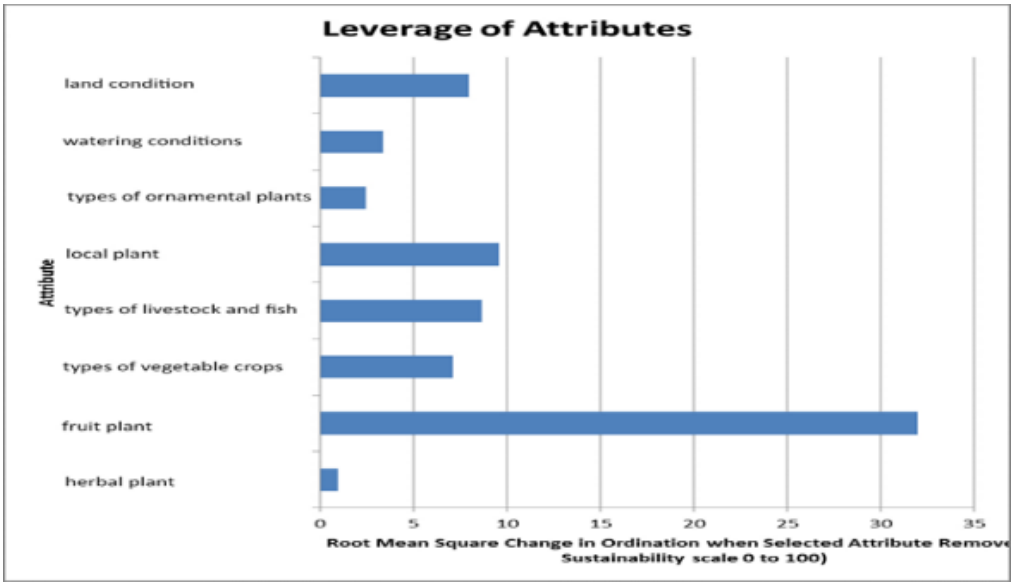

Figure 2. The results of the analysis of leverage the ecological dimension

This environmental condition needs to be maintained or further improved so ecological functions can have a more beneficial impact on a sustainable basis (Abdullah et al., 2017). The attribute values that affect the sustainability index on the ecological dimension with 8 (eight) attributes, including the yard's condition, irrigation conditions, types of ornamental plants, local plants, types of livestock and fish, vegetable crops, and fruit plants and herbal plants. According to Wulandari et al., (2018) the ecological dimension is the most delicate and susceptible attribute, both the area of green open space and the diversity of vegetation; to increase the sustainability of the ecological dimension, it is important to intervene or improve the sensitive attributes.

3.5. The Economic Dimension

The economic dimension of urban farming sustainability in Petamburan sub-district, Tanah Abang district, Central Jakarta administration city, shows a sustainability index value of 13.85%, including inadequate and unsustainable. The value related to sustainability index in economic dimension is smaller than the ecological dimension of 13.85%. In contrast, the other factors, namely the marketing of urban agricultural commodities and the provision of incentives, are presented in (*Figure 3*).

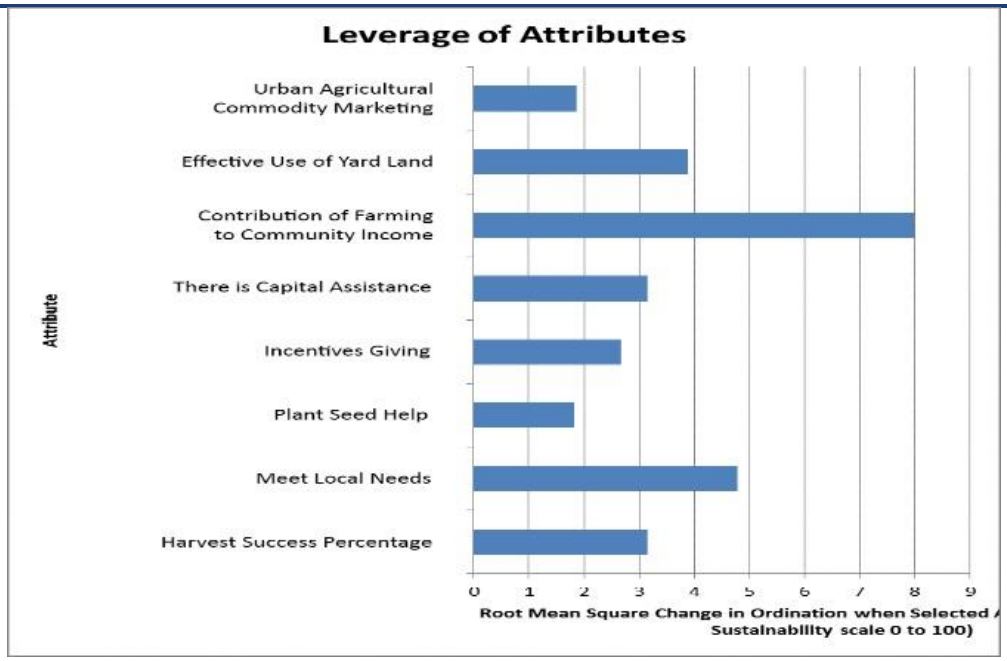

Figure 3. The results of the analysis of leverage the economic dimension

The development of urban farming in Petamburan Village, Tanah Abang sub-district, Central Jakarta implies that it is profitable from the ecological aspect compared to the economic aspect. Urban farming provides employment and becomes an additional source of community income and a buffer for financial stability in critical situations and is directly related to efforts (poverty alleviation) (Sampeling et al., 2012). Therefore, to increase the value related to sustainability index in economic dimension in the future, it is necessary to improve the attributes to increase the value of the index of the economic dimension. One of the minimal dimensions of the financial sustainability factor is the provision of assistance in the form of plant seeds.

3.5. The Social Dimension

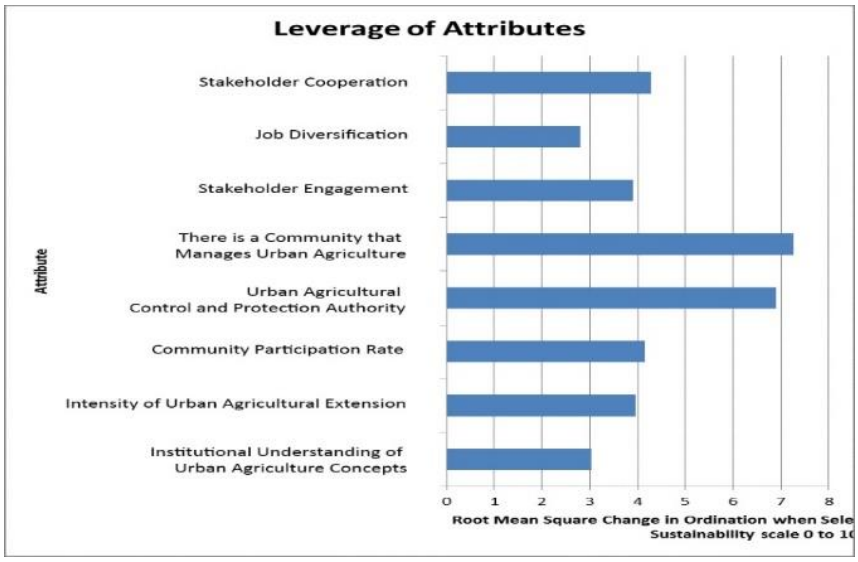

Figure 4. The results of the analysis of the social dimension of leverage

The sustainability index in social dimension was 13.94% less and which, required an increase in level of social dimension, and was necessary to improve attributes affecting its value. The poverty of farmers also causes a lack of sustainability in the social dimension; on average. Children of farmers are not interested and willing to continue farming. Therefore, they inherited their lands or convert their land for non-agricultural activities (Mawarsari and

Noor, 2020). The dominant factors on the sustainability of the social dimension based on the results of the leverage analysis are job diversification, agency understanding of urban agriculture, and the intensity of extension on urban agriculture. The attributes of the social dimension require good management: therefore, the value of this sustainability index could increase in the future (*Figure 4*).

The social service construct consists of three subconstructs: safety and security, community services, and labor or trade. First, security and security represent stakeholders, leaders, and urban communities (Salleh et al., 2021).

3.5. The Technological Dimension

Analysis of the sustainability dimension of the technology there is a sustainability index value of 13.43%, including the criteria for less and not sustainable. The dominant factor to the sustainability of the technological dimension is the result of the leverage analysis, which is the effort to develop commodities with simple and environmentally friendly technology and knowledge of technology and simple innovation and simple technology of irrigation systems (*Figure 5*).

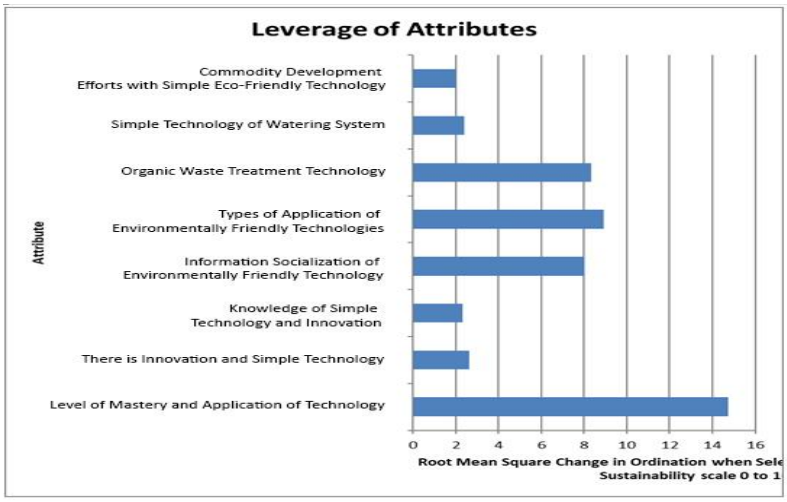

Figure 5. The results of the analysis of the technology dimension leverage

Space outside offices, schools, and residents yards is a potential for the development of urban farming. Changes in land use function in urban areas for the benefit of evolution and development, both industrial and residential, have resulted in shrinking land area. Land and urban space resources are opportunities for ecological, economic, socio-cultural utilization. Urban environmental conditions require capacity analysis and assessing sustainability of urban agriculture as a potential solution to urban problems related to urban life style. Sustainability of urban agricultural development of Petamburan village, Tanah Abang district, Central Jakarta with the MDS model, the results of the analysis of each dimension consist of the ecological dimension 14.55%, the economic dimension 13.85%, the social dimension 13.94%, and the technological dimension 13.43%. The results of the analysis of the level of sustainability on 32 attributes consist of 8 attributes of ecological dimensions, 8 attributes of economic dimensions, 8 attributes of social dimensions, and 8 attributes of the technology dimension. Multi-dimensional analysis results of the sustainability index presented in (*Figure 6*) show that the four dimensions analyzed resulted in all dimensions, including the lousy category of being unsustainable, the sustainability index ranging from 13.43% to 14.55%. The development of urban agriculture in Petamburan village, Tanah Abang sub-district, Central Jakarta requires counseling and motivation for residents to improve; if there is no progress by the local government, the idea of urban farming will continue to shrink. Natural resources and human resources strongly influence the sustainability of urban agriculture. Availability of land, sowing of crops, and available water, along with human resources that influence urban agriculture are the agricultural actors themselves, namely farmers (Mayasari et al., 2015).

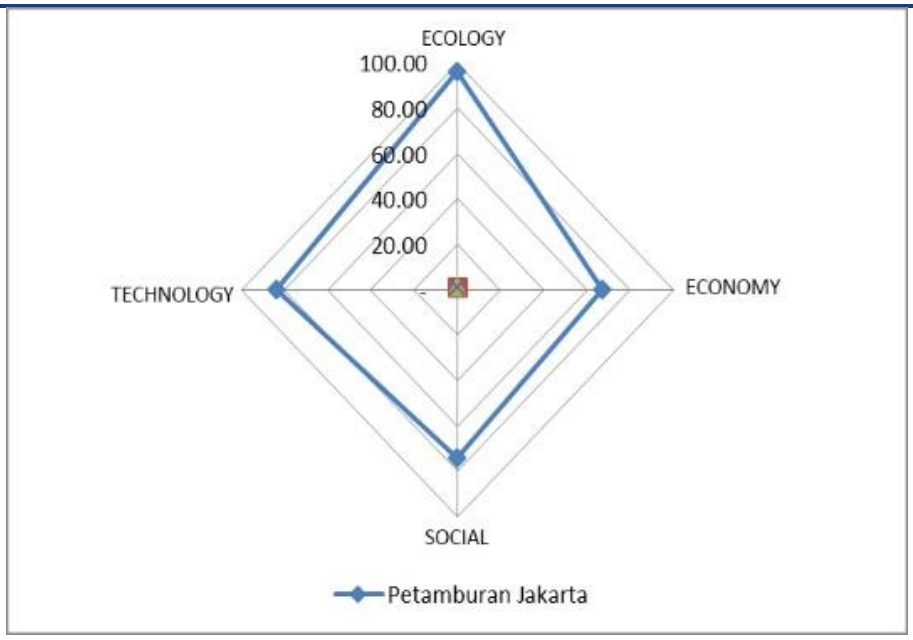

Figure 6. Index four dimensions of sustainability Petamburan urban agriculture in Tanah Abang, Central Jakarta

4. Conclusions

The development of urban farming in Petamburan, Tanah Abang, Central Jakarta results from the analysis of four dimensioned model MDS is not sustainable. The analysis results of each dimension consist of the ecological dimension 14.55%, the economic dimension 13.85%, the social dimension 13.94%, and the technological dimension 13.43%. In the future, urban farming should pay attention to the supporting factors of the sustainability of agricultural development. The factors include the yard area, types, and variations of cultivated plants, the application of innovation and technology that urban communities can accept and develop, increased counselling and community development, and intensive and tax-free provision for yards with urban farming. The results of this research will contribute to literature and provide important data for decision makers on urban farming development not only in Petamburan, Tanah Abang, Central Jakarta but also in other areas as an effort to develop urban farming progress.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper. We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Rini FITRI, Achmad Yozar PERKASA; Design: Hinijati WIDJAJA, Olivia SEANDERS; Data Collection or Processing: Rini FITRI, Reza FAUZI; Statistical Analyses: Olivia SEANDERS, Reza FAUZI; Literature Search: Rini FITRI , Achmad Yozar PERKASA; Writing, Review and Editing: Hinijati WIDJAJA, Achmad Yozar PERKASA.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

A Comparative Study of *Bacillus* Spp. Isolated from Various Sources and Commercial Food Supplements and Evaluation of Some Probiotic Properties

Çeşitli Kaynaklardan ve Ticari Gıda Takviyelerinden İzole Edilen *Bacillus* Türlerinin Bazı Probiyotik Özelliklerinin Karşılaştırmalı Değerlendirilmesi

Burcu KAHRAMAN[1]*, Burcu Mine ŞENOL[2], Enes DERTLİ[3], Muhammet ARICI[4]

Abstract

Bacillus species are gram-positive, aerobic, peritrically flagellated and endospore-forming bacteria. They can be found everywhere in the environment, especially in soil (its common habitat), water, dust or in the air. Probiotics, which have beneficial health effects, constitute an important group of *Bacillus* species. This study aimed to isolate *Bacillus* from various sources, identify it molecularly and determine its probiotic properties.For this purpose, eight *Bacillus subtilis*, *Bacillus coagulans* and *Bacillus clausii* strains among 58 isolates from fish intestine, soil, ripened cheese and commercial probiotic supplements were identified and their probiotic properties were characterized. Firstly, *Bacillus* strains were molecularly identified by 16S rRNA PCR analysis. The growth of *Bacillus* isolates at various temperatures, salt concentrations, and pH levels, as well as tests for esculin hydrolysis, starch hydrolysis, nitrate reduction, and gas generation from glucose, were all investigated to assess the isolates' physiological and biochemical characteristics. In terms of probiotic potential of *Bacillus* isolates; tolerance of bile salt, cell surface hydrophobicity, auto aggregation, antibiotic susceptibility tests were conducted. In all analyses, strains obtained from food supplements showed high levels of hydrophobicity and auto-aggregation properties, and the highest values following these strains were observed in *Bacillus* subtilis strains (F1 and S2) isolated from fish intestines and soil, respectively. All strains showed strong growth features in bile salt conditions. It has been determined that antibiotic sensitivity varies depending on the strain. Overall, high sensitivity to tetracycline has been observed. In summary, this study revealed the potential probiotic properties of *Bacillus* isolates obtained from different sources. The study also compared these probiotic properties with probiotic *Bacillus* strains isolated from food supplements.

Keywords: *Bacillus*, Biochemical characterization, Molecular identification, Probiotics

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Öz

Bacillus türleri gram pozitif, aerobik, peritrik olarak kamçılı ve endospor oluşturan bakterilerdir. Çevrede her yerde; özellikle toprak (yaygın habitatı), su, toz veya havada bulunabilirler. *Bacillus* türleri içerisinde önemli bir grubu ise sağlığa faydalı etki gösteren probiyotikler oluşturmaktadır. Probiyotik *Bacillus*'ları içeren gıdalar ve yemler, genellikle; insanlar için besin takviyesi, hayvanlar için büyümeyi teşvik edici, su ürünleri için ise büyüme düzenleyici veya hastalıklara karşı direnç sağlayıcı olarak kullanılmaktadır. Bu çalışmada, çeşitli kaynaklardan probiyotik *Bacillus* izolasyonu, moleküler tanımlanması ve probiyotik özelliklerinin belirlenmesi amaçlanmıştır. Bu amaçla, balık bağırsağından, topraktan, olgunlaştırılmış peynirden ve ticari probiyotik gıda katkılarından elde edilen 58 *Bacillus* izolatından 8 adet *Bacillus subtilis, Bacillus coagulans* ve *Bacillus clausii* suşu tanımlanmış ve probiyotik özellikleri karakterize edilmiştir. İlk olarak, *Bacillus* suşları 16S rRNA PCR analizi ile moleküler olarak tanımlanmıştır. *Bacillus* cinsine ait izolatların fizyolojik ve biyokimyasal özelliklerini belirlemek için farklı sıcaklıklarda, tuz konsantrasyonlarında ve pH derecelerinde büyümeleri incelenmiş, ardından eskülin hidrolizi, nişasta hidrolizi, nitrat redüksiyonu, glikozdan gaz oluşumu testleri yapılmıştır. *Bacillus* izolatlarının probiyotik potansiyelinin değerlendirilmesi açısından; safra tuzu toleransı, hücre yüzeyi hidrofobikliği, oto-agregasyon, antibiyotik duyarlılık testleri yapılmıştır. Tüm analizlerde, gıda takviyelerinden elde edilen suşlar yüksek düzeyde hidrofobiklik ve oto-agregasyon özellikleri göstermiştir ve bu suşları takip eden en yüksek değerler sırasıyla balık bağırsağından ve topraktan izole edilen *Bacillus subtilis* suşlarında (F1 ve S2) gözlemlenmiştir. Tüm suşlar, safra tuzu koşullarında güçlü gelişme özellikleri göstermiştir. *Bacillus* suşlarının antibiyotik duyarlılığını suşa özgü özellikler belirlemiştir. tetrasikline karşı yüksek düzeyde duyarlılık gözlenmiştir. Özetle, bu çalışma çeşitli kaynaklardan izole edilen *Bacillus* suşlarının potansiyel probiyotik özelliklerini ortaya koymuş ve bu probiyotik özellikler gıda takviyelerinden izole edilen *Bacillus* suşları ile karşılaştırılmıştır.

Anahtar Kelimeler: *Bacillus*, Biyokimyasal karakterizasyon, Moleküler tanımlama, Probiyotikler

1. Introduction

The genus *Bacillus* consists of aerobic, spore-forming, gram positive bacteria that shows heterotrophic or autotrophic growth using a variety of carbon sources. *Bacillus* genus includes around 200 species, some of them have been classified based on new biological data. (Logan and De Vos, 2009). *Bacillus* species are generally considered soil organisms as they have spores that can be isolated from soil. However, it has been shown that *Bacillus* species are not only soil-based and can sustain their viability in many environments through their spores. It has been suggested that *Bacillus* may be an undiscovered gastrointestinal system (GIS) commensal since these bacteria can maintain their viability in the GIS of animals that ingest *Bacillus* spores through digestion (Hong et al., 2009). Because of their spores' outstanding resistance and dormancy, which allows them to survive in any ecosystem longer than vegetative organisms, *Bacillus* species are widely distributed in a variety of habitats. *Bacillus* Probiotics, a significant category of *Bacillus* species that have beneficial effects on health (Sui et al., 2020). Foods and feeds containing probiotic *Bacillus* are generally used as nutritional supplements for humans, growth promoters for animals, and growth regulators or disease resistance providers for aquaculture (Cutting, 2011). Thanks to the antagonistic effects of *Bacillus* species, its use as a biological seed has also been proposed (Güldoğan et al., 2022) It has also been determined that due to the potent antagonistic activities of some *Bacillus* spp. isolates, citrus fruits have the potential to be used as biofungicides in the fight against post-harvest disease agents (Soylu et al., 2022) It has been stated that *Bacillus* probiotics are suitable for human consumption (Urdaci et al., 2004; Nithya and Halami, 2013). Most commercially available probiotic products consist of various microorganisms, especially *Lactobacillus* sp., *Bifidobacterium* sp., *Streptococcus* sp. However, the biggest weakness that makes it difficult to use these species as probiotics in the food industry is their susceptibility to harsh environments. Probiotic bacteria such as *Lactobacillus* and *Bifidobacteria* are highly sensitive to normal physiological conditions such as the highly acidic environment of the stomach and bile salts, and their survival rate under such conditions is 20-40% (Bezkorovainy, 2001). In addition, the viability of these bacteria can be affected by the production method, transportation, and storage conditions (Ljungh and Wadström, 2006; Endres et al., 2009;). In this context, spores formed by *Bacillus* species have an important effect on their evaluation as probiotics, thanks to their durability in difficult conditions. Their survivability in the digestive system and their thermal stability makes *Bacillus* probiotics attractive and their use is increasing (Cutting, 2011).

Although there is a lot of literature on the identification and examination of the properties of commonly used probiotics such as *Lactobacillus* and *Bifidobacteria*, there is a lack of isolation and investigation of the properties of *Bacillus* probiotics. In addition, even though studies on *Bacillus* probiotics have increased in the last 25 years, they have not gained high popularity compared to *Lactobacillus* species.

This study aims to identify *Bacillus* species obtained from different sources by molecular identification test (PCR) and to determine their biochemical, probiotic and technological properties. For the probiotic potential of *Bacillus* isolates, it is aimed to conduct in-vitro tests like tolerance of bile salt, cell-surface hydrophobicity test, antibiotic sensitivity test, hemolytic activity and lecitinase activity determination. In addition, the fact that the preparations currently sold in the trade and the *Bacillus* bacteria isolated from probiotic foods were also examined in this study makes the study different and interesting.

2. Materials and Methods

2.1. Isolation of Bacillus strains and molecular identification

2.1.1. Sample collection

Soil samples were collected from Istanbul and Edirne, Turkey in 2020. Following the cleaning of top surface of the soil, eleven soil samples from different locations were taken from approximately 4-5 cm depth with a sterile spatula and placed in sterile plastic bags. For the fish samples, nine fresh fish samples, including sea bream, salmon and sea bass, were purchased from local market, placed in sterile plastic bags and kept at + 4°C until the deriving of the intestinal samples. In addition, two ripened Mihaliç cheese samples were also obtained for *Bacillus* isolation.

Three different probiotic supplements which claiming to contain *B. coagulans*, *B. clausii* and *B. subtilis*, sold commercially in Turkey and the USA were purchased. Two out of the three products were gummy samples, and

one was a suspension. Commercial samples were also subjected to isolation and identification process similar to the soil and food samples.

2.1.2. Isolation of Bacillus strains from distinct samples

Each sample was initially diluted with peptone-water in 1:10 ratio before thoroughly mixing by the stomacher (VWR Star blender LB 400, England). Diluted suspensions in peptone water were heated at 80 °C for 20 minutes to kill vegetative cells. Isolation was carried out by streaking heat treated cultures on TSA (tryptic soy agar) for aerobic spore-formers (Ghosh et al., 2002, Gatson et al., 2006). Then the samples were subjected to serial dilution (up to 10^{-7}), and the 0.1 ml aliquots were aseptically plated on the TSA. The Petrie dishes were incubated at 37°C for 24 and 48 hours. Randomly selected colonies of various morphologies were purified and kept at -80 °C in TSB (tryptic soy broth), which contains 40% glycerol. Pure cultures were subjected to Gram staining to select Gram-positive rod-shaped bacteria. Subsequently, catalase test and spore staining tests were performed in these isolates to represent possible *Bacillus* isolates. Determination of catalase activity was conducted by resuspending the culture in a 3% hydrogen peroxide solution.

2.1.3. Bacterial identification

The isolates were grown in TSB under gentle agitation at 37 °C for 24 hours. The EcoSpin Bacterial Genomic DNA Kit (EcoTech, Turkey) was used to extract DNA. First, with the help of primers specific to *Bacillus* species, Bsub 5F (5'-AAGTCGAGCGGACAGATGG-3') and Bsub3R (5' -CCAGTTTCCAATGACCCTCCCC-3') (Mohd Isa et al., 2020), it was determined whether the isolates were *Bacillus* species or not.

To select distinct strains, randomly amplified polymorphic DNA (RAPD) test was implemented with the GTG 5 primer (5'-GTGGTGGTGGTGGTG-3') (Freitas et al., 2008). The following PCR parameters were used: 30 cycles of 94 °C for 1 minute, 40 °C for 1 minute, and 72 °C for 2 minutes, followed by one cycle of 72 °C for 8 minutes. Fingerprints of the isolates were recorded under UV light after running in agarose gel and similar strains were detected. One representative sample from each similar group formed was used for sequence analysis.

Potential distinct colonies were then subjected to identification process. For this, AMP_F (5'-GAGAGTTTGATYCTGGCTCAG-3') and AMP_R (5'-AAGGAGGTGATCCARCCGCA-3') primers were used the amplify to 16S ribosomal RNA (rRNA) section (Baker et al., 2003). PCR mixtures prepared with 1 µL of DNA template, 5 µL of 5× PCR buffer, 4 µL of dNTPs, 1 µL of 20 mmol/L primers, 0.125 µL of Taq polymerase and up to 50 µL of sterile H_2O. For the amplification of DNA, PCR (Bio-Rad T100 Thermal Cycler, USA) was used with the conditions of: Denaturation at 95 °C for 2 minutes is followed by 20 cycles of 95 °C for 30 seconds, 53 °C for 1 minute, and 72 °C for 30 seconds, with a final extension step at 72 °C for 5 minutes. The PCR products obtained were run on a 1% agarose gel and visualized with a gel imaging system.

Sequention of the samples were conducted at the Medsantek genomics sequencing laboratory. (Medsantek, Turkey). Using the Basic Local Alignment Search Tool (BLAST), the acquired nucleotide sequences were compared to the sequences of *Bacillus* species that were included in the National Center for Biotechnology Information (NCBI) database (Altschul et al., 1990).

2.1.4. Determination of physiological and biochemical properties

The degree of growth of *Bacillus* isolates at various pH (5, 6, 7, 8, and 10) temperature, (5, 30, 40, 65 °C) and salt concentrations (2, 5, 7, 10%) was investigated. In addition, various biochemical tests such as starch hydrolysis, nitrate reduction, esculin hydrolysis, formation of gas from glucose, acid formation from various carbohydrates were performed (Smith, 1981).

2.2. Probiotic properties of Bacillus strains

2.2.1. Growth at different concentrations of bile salt

Growth at changing bile salt concentrations of *Bacillus* isolates analyzed according to the method by Nithya and Halami (2013) with some modifications. The growth of *Bacillus* isolates was tested at different bile salt (0.0, 0.3, 0.5, 1 1.0, and 2.0) concentrations. The samples incubated for 24 hours at 37°C and then the growth of the test cultures was examined by determining the optical density (OD) with using a spectrophotometer (Optizen Pop Bio Uv/Vis Spectrophotometer, Korea).

2.2.2. Cell surface hydrophobicity

The cell surface hydrophobicity of *Bacillus* strains was determined according to the method described previously by İspirli et al. (2015) with some modifications. The cultures grown overnight were obtained by centrifugation (at 3000 and 4 °C for 10 minutes) and suspended again in PBS tampon to observe an OD600 of 1.0. The bacterial cell suspension (3 mL) and 0.6 mL of chloroform were then mixed. This mixture was vortexed for 1 minute and afterwards it is kept undisturbed for 30 minutes to allow separation of phases completely. Then the aqueous phase was separated and OD at 590nm was carefully measured. The percentage of hydrophobicity was calculated according to the following equation:

$$Hydrophobicity\ (\%) = (1 - A_1/A_0) \times 100 \quad \text{(Eq.1).}$$

In this equation A_0 refers to the initial absorbance of the bacterial suspension while A_1 is the absorbance which is measured after 30 minutes of incubation.

2.2.3. Autoaggregation assay

The autoaggregation of *Bacillus* isolates was calculated using to method of Patel et al. (2009). *Bacillus* cultures which were grown at 37 °C for 24 hours in nutrient broth was centrifugated, washed, and resuspended in PBS to get absorbance 0.5 at 595 nm. The 4 ml of cell suspension was mixed by vortex and incubated at 37 °C for 1 h. After the incubation, the upper layer was measured at 595 nm. Then finally autoagregation was calculated as:

$$Autoaggregation\ (\%) = (1 - A_t/A_0) \times 100 \quad \text{(Eq.2).}$$

In this equation A_t represents the absorbance after incubation and A_o the initial absorbance.

2.2.4. Antibiotic susceptibility assay

Antibiotic susceptibility of *Bacillus* isolates determined with disc diffusion method according to the the National Committee for Clinical Laboratory Standards (NCCLS 1997). Resistance of *Bacillus* strains against tetracycline (30 µg), vancomycin (30 µg), rifampicin (30 µg), amoxicillin (10 µg), penicillin G (10 µg), was determined using antibiotic discs. When inhibition zones present around the disks, the length of the disks were measured in centimeters (Chaiyawan et al., 2010).

2.2.5. Lecithinase and hemolytic activity test

For lecithinase test, bacteria are streaked on a medium prepared with egg yolk and incubated at 37 °C for 24-48 hours. The results were evaluated for the formation of a white opaque zone around the colonies (McClung and Toabe, 1947). Hemolysis was determined on Blood agar base (Liofilchem, Italy) supplemented with 5% sheep blood after incubation at 37ºC for 24 hrs α-haemolysis, β-haemolysis or non-haemolytic properties were determined with the examination of the plates (Chaiyawan et al., 2010).

2.2.6. Statistical analysis

Statistical analysis was implemented by one-way analysis of variance (ANOVA). Tukey's multiple comparison test is conducted using JMP 6.0. The values were given with means ± standard deviations. The level of significance was selected to be 0.05.

3. Results and Discussion

3.1. Isolation and identification of distinct strains

3.1.1. Isolation of spore-forming bacteria

In the study, 58 different isolates were selected from colonies belonging to fish, soil and cheese samples. Among these isolates, 35 catalase-positive, gram-positive, rod-shaped and spore-forming strains were evaluated as possible *Bacillus* and selected for further studies.

3.1.2. Identification of Bacillus by 16S rRNA sequence analysis

Following the RAPD PCR analysis, 16S rRNA gene analysis was used to identify different strains. The isolates have 99% sequence similarity with *Bacillus* species according to the 16S rRNA sequencing analysis. Using the

Basic Local Alignment Search Tool (BLAST) Program, the acquired sequences (about 1,500 bp) were deposited in the National Center for Biotechnology Information (NCBI) gene bank and accession numbers are given *Table 1*. The commercial probiotics isolated in this study were *Bacillus subtilis* (PB1), *Bacillus coagulans* (PB2) and *Bacillus clausii* (PB3).

Table 1. Bacillus strains isolated and identified in this study and their sources

***Bacillus* isolates**	**Sources**	**Gene bank accession number**
F1- *Bacillus subtilis*	Fish intestine	OM807211
F2- *Bacillus subtilis*	Fish intestine	OM807212
S1- *Bacillus subtilis*	Soil	OM807213
S2- *Bacillus subtilis*	Soil	OM807214
C1- *Bacillus coagulans*	Kashar cheese	OM867479

3.1.3. Physiological and biochemical properties

Biochemical and physiological properties of isolated *Bacillus* species were given in *Table 2*. Physiological tests were performed to observe temperature, NaCl and pHs effect on *Bacillus* isolates. The findings showed that the *Bacillus* isolates could grow easily in alkaline, salt-containing environments. The best growth was observed between pH 6 and 8. *Bacillus* strains have increasing growth rates from pH 1 to 7 and have promising tolerance with their survival at different acidic-basic degrees (Kavitha et al., 2018). In addition, growth at 10% NaCl concentration exhibited that these species could resist high salt concentrations (Satapute et al., 2012). The best

Table 2. Biochemical and physiological characterization of isolated Bacillus species

Biochemical characteristics	***Bacillus* isolates**							
	P1	**P2**	**P3**	**F1**	**F2**	**S1**	**S2**	**C1**
Gram staining	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+
Ellipsoidal	+	+	+	+	+	+	+	+
Spore formation	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Nitrate reduction	+	-	+	+	+	+	+	+
Egg yolk reaction	-	-	-	-	+	-	+	-
Hydrolysis of starch	+	+	+	+	+	+	+	+
Esculin hydrolysis	+	+	+	+	+	+	+	+
Gas production from glucose	-	-	-	-	-	-	-	-
Growth at pH 5	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+
Growth in NaCl: 2%	+	+	+	+	+	+	+	+
5%	+	+	+	+	+	+	+	+
7%	+	+	+	+	+	+	+	+
10%	+	-	+	+	+	+	+	-
Growth at 5 °C	-	-	-	-	-	-	-	-
30 °C	+	+	+	+	+	+	+	+
40 °C	+	+	+	+	+	+	+	+
65 °C	-	-	-	-	-	-	-	-

temperature values of *Bacillus* isolate to grow have been determined as 30 °C – 40 °C. Studies have shown that the growth ranges of *Bacillus* species vary from mesophilic to moderately thermophilic (54 °C). (Łubkowska et

al., 2023) It was also understood that the isolates did not produce gas from glucose. Total number of bacterial strains could hydrolyze esculin and starch and utilize catalase. Moreover *B. subtilis* and *B.clausii* isolates were able to reduce nitrate, *B. coagulans* isolates were varied according to the subspecies for the reduction of nitrate to nitrite.

3.2. Characterization of probiotic properties of Bacillus strains

3.2.1. Growth at different bile concentrations

The survivability and growth of the strains in the high concentration of bile salts is an important parameter for probiotic selection. Resistance to bile compounds is one of the most widely used assays in survival and growth studies of probiotic organisms reported by FAO/WHO (FAO/WHO, 2002). Bile tolerance tests are usually performed at a concentration of 0.3%, as it is like human bile juice (Conway et al., 1987; Nithya and Halami, 2013). Probiotic strains must be resistant to bile to function in the intestines (Sharma et al., 2023). In this study, growth of *Bacillus* strains was observed in bile salt concentrations of 0.3%, 0.5%, 1% and 2%. According to the results, all the tested *Bacillus* strains were grown with changing concentrations of bile salts (*Figure 1*). It was observed that strains performed well in especially 0.3% and 0.5% bile concentrations. Similar studies also reported that *Bacillus* isolates showed high survival rates even in the presence of 6% and 10% bile (Giri et al., 2012, Thankappan et al., 2015). The PB1 strain showed good resistance to increased bile salt, while the C1 strain was found to be more sensitive. It is noteworthy that *Bacillus subtilis* species were more resistant to bile salt than *Bacillus coagulans* and *Bacillus clausii* species.

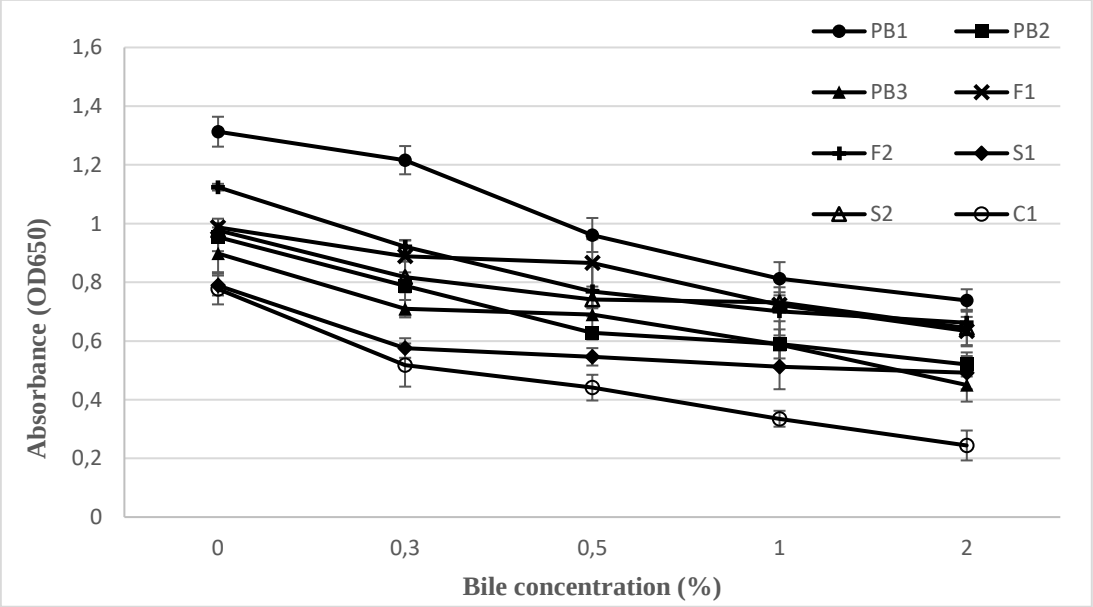

Figure 1. Growth of Bacillus isolates at different concentration of bile salt

3.2.2. Cell surface

It is very important that probiotic strains adhere to epithelial cells of the intestine to colonize in the gastrointestinal system, which prevent elimination by peristalsis, and provide an advantage for competition in microflora (Kos et al., 2003). The chloroform adhesion capacity was used to determine the hydrophobicity of *Bacillus* strains. (*Figure 2a).* The adhesion percentage of *Bacillus* strains showed differences between 51.18% and 25.7% among the strains tested. The strains PB1 and PB3 from commercial probiotic products showed the highest hydrophobicity capacity. *Bacillus subtilis* F1 strain, which is the fish isolate, gave the closest value to these with 44.6%. The lowest hydrophobicity capacity was observed for fish isolate F2 which was nearly half that of the highest degrees of hydrophobicity tested. In general, significant statistical differences were observed between the degree of hydrophobicity of the isolates. Previous studies evaluating the hydrophobicity of *B. coagulans*, *B. licheniformis*, *B. flexus*, *B. subtilis* isolates used xylene as the hydrocarbon and reported adhesion values ranging from 30% to 90%. (Nithya and Halami, 2013). In another analysis performed using toluene, the cell surface

hydrophobicity value varied between 73.62 and 95.3% among the *Bacillus* strains tested (Dabire et al., 2022). Solvents such as xylene and ethyl acetate have also been used in other studies, resulting in different degrees of hydrophobicity of *Bacillus* strains (Patel et al., 2009; Nithya and Halami, 2013). In this study all isolates exhibited remarkable affinity to chloroform, a monopolar acidic solvent and electron acceptor. Since fish and soil and cheese isolates showed different adhesion degrees among themselves, no generalized results were obtained that would allow a comprehensive comparison in terms of isolates source.

3.2.3. Auto-aggregation assay

Auto-aggregation is a crucial functional trait of probiotic strains, along with hydrophobicity. It was stated that the isolates' surface characteristics, such as auto-aggregation and hydrophobicity, contributed to the adhesion property. The surface properties such as auto-aggregation and hydrophobicity exhibited by the isolates contribute to the adhesion property (Kos et al., 2003). In general auto-aggregation ability is related to cell adhesion properties and also provide their ability to survive and endurance in the digestive system. (Vlková et al., 2008). The auto-aggregation activity of the isolates varied from 28.4 to 35.4% (*Figure 2b*). *Bacillus subtilis* PB2 which is the commercial probiotic isolate showed the highest auto-aggregation activity. Similar to cell surface hydrophobicity,

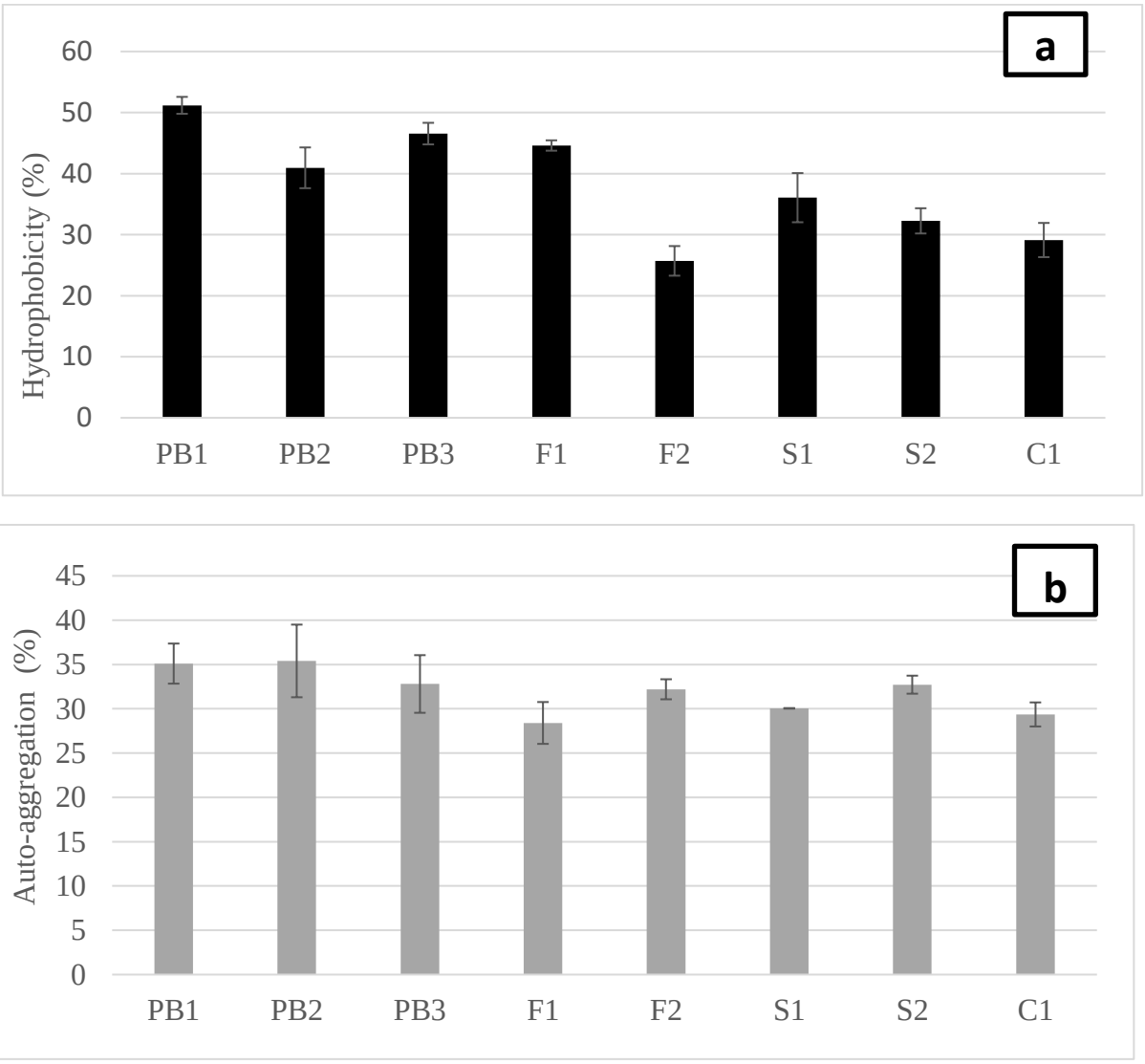

Figure 2. Cell surface hydrophobicity (a) and auto-aggregation (b) properties of the Bacillus isolates

commercial probiotic strains showed higher auto-aggregation activity. However, no statistically significant difference was observed between the auto-aggregation values of other isolated *Bacillus* strains. In this study, we report that *Bacillus* strains isolated from fish (F2) intestine and soil (S2) showed good auto-aggregation percentage

with 32.2% and 32.7% levels, respectively. Previous studies have reported a wide range of auto-aggregation values for *Bacillus* species ranged between 20% to 98%. (Nithya and Halami, 2013; Nwagu, et al., 2020; Dabire et al., 2022). As stated in other studies, these properties can provide colonization of *Bacillus* in the gastrointestinal tract and competition against pathogens (Thankappan et al., 2015).

3.2.4. Antibiotic susceptibility assay

The safety of probiotics is of primary importance, as their resistance to antibiotics can be one of the possible threats. The presence of transferable antibiotic resistance genes generates a safety hazard (Sharma et al., 2014). *Table 3* displays the outcomes of the bacterial strains' tests for antibiotic sensitivity. All the eight isolates were susceptible (>1cm of zone of inhibition) to all tested antibiotics which are tetracycline, vancomycin, rifampicin, amoxicillin, penicillin G in various degrees. Patel et al. (2009) stated in their study that vulnerability against antibiotics is an important probiotic feature. The *Bacillus* isolates examined in the study do not show antibiotic resistance, which is an important finding in terms of inability to transfer the plasmid gene that triggers pathogenicity and enterotoxin formation.

Almost all isolates were more sensitive (S+++) to tetracycline and sensitive (S++) to Vancomycin. In addition, the isolates exhibited different susceptibility degree to rifampicin, penicillin G and amoxicillin antibiotics according to the bacterial type. *Bacillus* strains were sensitive to antibiotics which indicates that these isolates evaluated as probiotics are safe. These findings are consistent with previous research (Zeng et al., 2022; Lei et al., 2023). Previous studies examined different probiotic products that were commercially available and found that some of them contained a different strain of *Bacillus* than indicated, and that the bacteria showed high levels of resistance to antibiotics such as penicillin G, tetracycline, rifampin and ampicillin (Green et al., 1999; Hoa et al., 2000; Senesi et al., 2001).

Table 3. Diameters of inhibition zone (cm) exhibited against test bacteria of standard antibiotics

Isolates	Vancomycin (30 µg)	Rifampicin (30µg)	Penicillin G (10 µg)	Amoxicillin (10 µg)	Tetracycline (30 µg)
PB1	1.85±0.07^{bc}	1.40±0.00^{f}	1.50±0.00^{e}	1.55±0.07^{c}	2.55±0.07^{bc}
PB2	1.85±0.21^{bc}	1.55±0.07^{e}	1.55±0.07^{de}	1.55±0.07^{c}	2.65±0.07^{b}
PB3	1.75±0.07^{c}	2.50±0.00^{cd}	1.75±0.35^{cde}	2.25±0.35^{b}	2.90±0.14^{a}
F1	2.10±0.00^{a}	2.60±0.14^{c}	2.25±0.07^{ab}	2.75±0.07^{a}	2.45±0.07^{c}
F2	2.05+0.07^{ab}	2.85±0.07^{b}	2.45±0.21^{a}	2.35±0.21^{b}	2.60±0.14^{bc}
S1	2.00±0.14^{ab}	2.45±0.07^{d}	2.05±0.07^{bc}	2.30±0.00^{b}	2.50±0.00^{bc}
S2	2.05±0.07^{ab}	3.05±0.07^{a}	1.85±0.07^{cd}	2.85±0.07^{a}	3.05±0.07^{a}
C1	1.90±0.14^{abc}	1.60±0.00^{e}	1.65±0.07^{de}	1.65±0.07^{c}	2.55±0.07^{bc}

*Where, inhibition zone diameter <0.5 cm, resistant (R); inhibition zone diameter between 0.6-1.5 cm Susceptibility (S+), inhibition zone diameter between 1.6-2.5 cm Susceptibility (S++), and inhibition zone diameter > 2.6 cm Susceptibility (S+++). **Different letters show significant ($p < 0.05$) differences between samples.

3.2.5. Lecithinase and hemolytic test

Strong hemolytic and/or lecithinase activity could be a sign that cytotoxic phopholipases are present, which affect the virality of bacteria. (Sorokulova et al., 2008). Therefore, the absence of hemolytic and lecithinase activities in these isolated bacteria is important in terms of being evaluated as probiotics. Our findings show that all *Bacillus* isolates from commercial supplements together with *Bacillus subtilis* F1 and *Bacillus coagulans* C1 were lecithinase negative which is important parameter for consideration as probiotic but *Bacillus subtilis* S1, S2, and F2 showed lecithinase activity. However, it has been stated that not all lecithinase positive strains are necessarily toxigenic (Obi, 1980). Hemolytic activities of 8 tested isolated were assessed on blood agar plates. Analysis of hemolytic capacity of tested samples demonstrated that no strains showed α hemolytic or ß hemolytic activity on sheep blood agar. Hemolytic activity is critical parameter for evaluating the biosafety of probiotics. Hemolysis and erythrocyte abnormalities can be caused by certain pathogenic bacteria that lyse red blood cells (Mondal et al., 2023). In the present study, *Bacillus* isolates not show any lysis of the blood cells. The strains showed γ hemolytic, or negative depending on strain specific conditions. Likewise, several investigators have

shown that distinct *Bacillus* strains exhibit no hemolytic activity (Banerjee et al., 2017, Pahumunto et al., 2021, Zeng et al., 2021).

4. Conclusions

In this investigation, five potential probiotics from fish intestine, soil, and cheese and three probiotics from commercial food supplements were successfully isolated. Commercial food supplement isolates generally exhibited the best probiotic properties among all the isolates, as they are expected to deliver on their promises. All isolates displayed antibiotic susceptibility and exhibited good survival at 0.3% bile salt concentration. Analysis of hemolytic activity of tested strains demonstrated that isolates were non-hemolytic. Due to its strong bile salt resistance, highest hydrophobicity, and good auto-aggregation activity among the five isolates, *Bacillus* subtilis F1 demonstrated the most promise for practical application. This strain will be further tested in different food formulation especially for the development of probiotic confectionery products.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Arıcı M.; Design: Kahraman B., Dertli E.; Data Collection or Processing: Kahraman B., Şenol B. M.; Statistical Analyses: Kahraman B.; Literature Search: Kahraman B., Şenol B. M.; Writing, Review and Editing: Kahraman B., Arıcı M., Dertli E.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Entegre Zararlı Yönetimi Programlarında Kullanılacak Bitki Koruma Ürünlerinin Risk Değerlendirmesi ve Seçimi

Risk Assessment and Selection of Plant Protection Products to be Used in Integrated Pest Management Programmes

Cem ERDOĞAN[*1]

Öz

Türkiye'de tarımsal üretimin biyolojik çeşitliliği koruyarak sürdürülebilir bir şekilde yürütülmesi önem arz etmektedir. Ülkemiz tarım sektörünün üretimde yaşadığı, bazılarının ise kronikleştiği çeşitli sorunları bulunmaktadır. Verimli tarım alanlarının azalması, artan gıda talebi, genç neslin tarımdan uzaklaşması, girdi maliyetleri, kırsal kalkınma, küçük aile işletmeciliğinin ve buna bağlı olarak parçalı arazi yapısının yaygın olması ve iklim değişikliği gibi tarımsal üretimi ve verimliliği etkileyen pek çok sorun bulunmaktadır. Üretimde verimliliği etkileyen sebeplerden birisi de tarımsal üretimde görülen hastalık, zararlı ve yabancı otlardır. Biyolojik çeşitliliği koruyarak sürdürülebilir bir şekilde tarımsal üretim yapılmasının yolu Entegre Zararlı Yönetiminin (IPM) uygulanmasından geçmektedir. Entegre Zararlı Yönetiminde tarımsal zararlılara karşı bütün mücadele yöntemlerinin uyumlu bir şekilde kullanılması esas olup, biyolojik, biyoteknik ve kültürel önlemlere öncelik verilmektedir. En son çare olarak, Bitki Koruma Ürünlerinin (BKÜ) uygulanması istenmektedir. Bu nedenle, kullanılacak BKÜ'lerin insan sağlığına zararlı olmaması, çevre kirliliğine yol açmaması, parazitoid, predatör, pollinatörleri olumsuz etkilememesi ve ürünlerde kalıntıya sebep olmaması gerekmektedir. Yani BKÜ uygulamalarının gelişi güzel, bilinçsizce değil, akla, bilime, bilgiye dayalı ve biyolojik çeşitliliğin korunmasına yardımcı olacak şekilde sürdürülebilir olarak yürütülmesi esastır. Entegre Zararlı Yönetiminde kullanılacak BKÜ'lerin seçiminde, insan ve çevre sağlığı açısından oluşabilecek riskleri ortaya koyabilmek amacıyla aktif maddelerin memeli hayvanlar, balıklar, hedef dışı organizmalar (parazitoitler, predatörler) ve balarılarına etkileri ile topraktaki kalıcılıkları göz önüne alınarak risk değerlendirmeleri yapılmakta olup, biyolojikler, böcek gelişme düzenleyicileri gibi BKÜ'lere öncelik verilmektedir. Yürütülen risk değerlendirmesi çalışmaları sonucunda 495 aktif madde, 204 adet karışım, 13 adet biyolojik preparat, 21 adet biyolojik mücadele etmeni ve 1 adet safener olmak üzere toplam 734 adet BKÜ incelenmiş ve uygun olanlar entegre mücadele teknik talimatlarında üreticilerimizin ve ülke tarımımızın hizmetine sunulmuştur.

Anahtar Kelimeler: Aktif madde, Bitki koruma ürünleri, Biyolojik çeşitlilik, Entegre zararlı yönetimi, Sürdürülebilirlik

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Abstract

In Türkiye, it is important to carry out agricultural production in a sustainable manner by protecting biodiversity. Our country's agricultural sector has various problems in production, some of which have become chronic. There are many problems affecting agricultural production and productivity such as decreasing arable agricultural areas, increasing food demand, young generation moving away from agriculture, input costs, rural development, prevalence of small family farming and consequently fragmented land structure and climate change. One of the reasons affecting productivity in production is diseases, pests and weeds in agricultural production. The way of sustainable agricultural production by protecting biological diversity is through the implementation of Integrated Pest Management (IPM). In Integrated Pest Management, it is essential to use all control methods against agricultural pests in harmony, and biological, biotechnical and cultural measures are prioritised. As a last resort, the application of Plant Protection Products (PPPs) is desired. For this reason, the PPS to be used should not be harmful to human health, should not cause environmental pollution, should not adversely affect parasitoids, predators, pollinators and should not cause residues in the products. In other words, it is essential that the application of PPPs should not be carried out randomly and unconsciously, but in a sustainable manner based on reason, science, knowledge and in a way that will help the conservation of biological diversity. In the selection of PPPs to be used in Integrated Pest Management, risk assessments are made by considering the effects of active substances on mammals, fish, non-target organisms (parasitoids, predators) and honey bees and their persistence in soil in order to reveal the risks that may occur in terms of human and environmental health, and priority is given to PPPs such as biologics and insect growth regulators. As a result of the risk assessment studies carried out, a total of 734 PPPs, including 495 active substances, 204 mixtures, 13 biological preparations, 21 biological control agents and 1 safener, were examined and the appropriate ones were presented to the service of our producers and our country's agriculture in the integrated control technical instructions.

Keywords: Active ingredients, Plant protection products, Biodiversity, Integrated pest management, Sustainability

1. Giriş

Türkiye'de tarımsal üretimin biyolojik çeşitliliği koruyarak sürdürülebilir bir şekilde yürütülmesi ülkemiz tarımı açısından oldukça önemlidir. Ülkemizde tarımsal üretimde 657 zararlı organizma mevcut olup (Anonim, 2023a), bu zararlı organizmalardan resmi olarak 335'ten fazlası ile mücadele yapılmaktadır. Türkiye'de 24 milyon hektar ekilebilir alan, 30 farklı agroekolojik bölge, 165 ticari bitki türü bulunmaktadır (Kaymak ve ark., 2015). Türkiye Cumhuriyeti Devleti 2.2 milyon kayıtlı tarım işletmesi, 6 milyon civarında tarım çalışanı ile yaklaşık olarak 140 milyon ton tarımsal ürün üretmektedir (Birişik ve ark., 2015). Ülkemizde ekonomik gelişmenin sürdürülebilir olması, ve çevre kirliliğini azaltılabilmesi için tarım sektörü, tarım arazileri korunmalı ve katma değerli üretim teşvik edilmelidir (Çetin ve ark., 2020).

Gıda güvenliği ve güvenilirliği birbirinden ayrılmayan stratejik alanlardır. Uzmanlar artan dünya nüfusu ve azalan verimli tarım alanları nedeniyle bitkisel üretimde verimliliğin arttırılması gerekliliğine dikkat çekmektedirler. Günümüzde insan ve çevre sağlığına uygun olan teknikler kullanılarak üretilen gıdalara olan talep artmakta olup, tüketici tercihlerine uygun üretim yapmakta üreticiler tarafından dikkate alınmak zorundadır (Sayın ve ark., 2021).Tarımsal üretimin başlangıcından bu yana, yani 10.000 yıl dan fazla bir süredir tarımsal alanlarda görülen hastalık, zararlı ve yabancı otlar önemli bir sorun olmuştur ve günümüzde de sorun olmaya devam etmektedir. Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) ile Ekonomik İşbirliği ve Kalkınma Örgütü (OECD) tarafından hazırlanan bir raporda "her yıl dünya genelinde potansiyel olarak tarımsal üretimin yaklaşık % 26 ila %40'ının hastalık, zararlı ve yabancı otlar nedeniyle kaybedilmekte olduğunun altı çizilerek, bu zararlılarla mücadele edilmediği takdirde bu kayıpların iki katına çıkabileceği belirtilmektedir " (OECD/FAO, 2012). Hasattan sonrada kayıplar devam etmekte ve depolama esnasında da ortalama %14'lük bir ek kayıp daha oluşabilmektedir (Özdem ve Karahan, 2018). İyi senaryoda bile bitkisel üretimimizin yaklaşık %40'nı kaybetme riski ile karşı karşıyayız. Bu duruma ilave olarak tarım sektörü, artan gıda talebi, dünya nüfusundaki hızlı artış, erozyon, tuzlanma ve yoğun kullanımın yanı sıra şehirleşme nedeniyle verimli tarım arazilerinin giderek azalması, ülkemizde küçük aile işletmeciliğinin ve buna bağlı olarak da parçalı arazi yapısının yaygın olması, biyolojik çeşitlilik kaybı, toprak ve su kaynaklarının sürdürülebilirliği, çevre kirliliği, iklim değişikliği, bitkisel üretimi sınırlayan zararlı organizmaların sayısının artması, bu zararlılarla mücadelede kullanılan bitki koruma ürünlerine (BKÜ) karşı görülen direnç gelişimi, BKÜ'lerin faydalı böcekler üzerine olan olumsuz etkileri, kalıntı, verim düşüklüğü, tarımsal girdilerin fiyatlarındaki aşırı artışlar, genç nüfusun tarımdan uzaklaşması vb. Gibi çok sayıda küresel risklerle karşı karşıya bulunmaktadır. (Erdoğan, 2021). Ukrayna Rusya savaşı gibi jeopolitik riskler ve küresel ticaretin hızlanmasıyla birlikte belirsizliklerde artmaktadır.

Tüm bu riskler göz önüne alındığında tarımsal üretimde verimliliği etkileyen ana unsurlardan olan hastalık, zararlı ve yabancı otlarla mücadelenin gerekliliği daha açık ortaya çıkmaktadır. Dünyada ve ülkemizde tarımsal zararlılarla mücadelede en çok tarımsal ilaçların yani Bitki Koruma Ürünlerinin kullanıldığı görülmektedir. BKÜ'ler uygulaması kolay, hızlı, kısa sürede etkili ve hedef zararlıları kontrol altına alabilmeleri nedeniyle yaygın olarak kullanılmaktadır. BKÜ kullanılmasının çeşitli avantaj ve dezavantajları bulunmakta olup, tarımsal üretimde verimliliği etkileyen zararlılarla mücadelede kullanılacak BKÜ'lerin sürdürülebilir bir şekilde, biyoçeşitliliği ön planda tutarak, insan ve çevre sağlığı açısından zarar oluşturmayacak bir şekilde kullanılması gerekmektedir.

BKÜ'ler bilinçsiz ve yanlış kullanıldığında insan ve çevre sağlığını olumsuz yönde etkilemektedirler. Doğal denge üzerine olumsuz etkileri, ilaçlama sırasında gerekli korunma tedbirleri alınmadığı takdirde üreticiler ve sıcakkanlılarda akut veya kronik zehirlenmeler yapabilmekte, doğal düşmanlar, balarıları ve polinatör böcekler ve toprakta yaşayan mikro ve makroorganizmalar üzerine olumsuz etkiler meydana getirebilmektedirler. Ayrıca yeraltı sularına bulaşma, uygulanma sırasında sürüklenme yoluyla, yağmur ve sulama suyu ile toprak yüzeyinden akarak ve drenaj ile akarsu, göl ve diğer su kaynaklarını kirleterek, balıklar ve suda yaşayan (algler, su pireleri, sucul bitkiler gibi) canlıların olumsuz etkilenmesine, hatta bu canlıların kitle halinde ölümlerine yol açabilmektedirler. Aynı şekilde kuşlar ve yaban hayvanlarına da olumsuz etkileri olmaktadır. Tarım ilaçlarının aşırı kullanımı hastalık, zararlı ve yabancı otlarda direnç gelişimlerine neden olabilmekte, bitkilerde ise kalıntı ve fitotoksisiteye sebep olabilmektedir. Rachel Carson tarafından 1962 yılında yazılan 'Sessiz Bahar' adlı kitap, bilim insanlarının ve kamuoyunun çevre kirliliğine ilişkin tutumunu değiştirmede bir dönüm noktası olarak kabul edilmektedir (Carson, 1962). Carson'un bu yayınından sonra tarımsal üretimde zararlı olan hastalık, zararlı böcek ve yabancı otlarla mücadelede Entegre Zararlı Yönetimi stratejisi doğmuştur diyebiliriz. Bu nedenle, tarımsal

üretimin vazgeçilmez parçalarından biri olan bitki koruma uygulamalarının gelişi güzel, bilinçsizce değil, akla, bilime, bilgiye dayalı ve biyolojik çeşitliliğin korunmasına yardımcı olacak şekilde sürdürülebilir olarak yürütülmesi esastır (Erdoğan, 2021). Yaklaşık altmış yıldır, Entegre Zararlı Yönetimi (IPM) tarımsal üretimdeki zararlıları çevreye zarar vermeden yönetmek için kabul edilen bir stratejidir (Abrol ve Shankar, 2012). İlk kez Stern ve ark. (1959) tarafından IPM yani Entegre Zararlı Yönetimi kavramı kullanılmıştır. İlk uygulamalarda biyolojik ve kimyasal mücadelenin beraber kullanımı şeklinde değerlendirilmiş olup (Ünal ve Gürkan, 2001), geniş spektrumlu insektisitlerin kullanılması sonucu doğal düşmanların olumsuz etkilenmesi, zararlı böcek popülasyonlarında görülen hızlı artışlar üzerine, Smith ve Reynolds (1966) tarafından Entegre Zararlı Yönetimi, zararlı popülasyonlarını azaltmak ve bunları ekonomik zarar seviyesinin altında tutmak için bütün mücadele yöntemlerinin uyumlu bir şekilde kullanılması olarak tarif edilmiştir. Avrupa Birliği Pestisitlerin Sürdürülebilir Kullanımı Çerçeve Direktifi tarafından 'Entegre Zararlı Yönetimi', mevcut tüm bitki koruma yöntemlerinin dikkatli bir şekilde değerlendirilmesi ve ardından zararlı organizma popülasyonlarının gelişimini engelleyen bitki koruma ürünleri ile diğer mücadele yöntemlerinin kullanımını ekonomik ve ekolojik olarak gerekçelendirilen seviyelerde tutan, insan ve çevre sağlığı için riskleri azaltan ya da minimuma indiren uygun önlemlerin entegre edilmesi anlamına gelir" şeklinde tarif edilmektedir (Direktif 2009/128 / EC) (Anonymous, 2009). Entegre zararlı yönetimi, zararlılarla mücadele için dünya çapında kabul edilmiş bir politika ve hükümetler tarafından büyük ölçekli IPM programlarının 60'tan fazla gelişmiş ve gelişmekte olan ülkede uygulandığını görüyoruz (FAO, 2011). Entegre mücadelenin yürütülmesi ile ulaşılması planlanan temel hedefleri, sürdürülebilir bitkisel üretimin devamlılığının sağlanması, bitkisel üretimde verim artışının sağlanması, kaliteli ve pestisit kalıntısı bulunmayan ürün elde edilmesi, doğal düşmanların korunması ve desteklenmesi, biyolojik çeşitliliğin korunması ve desteklenmesi ve çiftçilerin kendi tarlası, bahçesi ve bağının doktoru/uzmanı olmasının sağlanması olarak sıralayabiliriz.

Bu yöntem biyolojik mücadele, habitatın zararlılara uygun olmayacak şekilde değiştirilmesi, kültürel uygulamaların değiştirilmesi ve dirençli çeşitlerin kullanımı gibi tekniklerin kombinasyonu yoluyla zararlıların veya zararlarının uzun vadeli önlenmesine odaklanan ekosistem tabanlı bir stratejidir. Zararlıları uygun bütün yöntemlerin kullanılmasıyla kontrol etme yaklaşımıdır. Bu yöntemde, pestisitler yalnızca hedef organizmaları uzaklaştırmak amacıyla, standart olarak belirlenmiş kurallara göre kullanılır. Kimyasal mücadele ekonomik ve ekolojik olarak bir zorunluluk bulunması koşuluyla, en son başvurulması gereken mücadele yöntemidir. Bunun dışında kimyasal mücadelenin gelişi güzel uygulanmaması gerekir.

BKÜ'leri kullanma zorunluluğu ortaya çıktığında; uygun BKÜ'yü, doğru zamanda, doğru teşhis edilmiş zararlıya karşı, doğru dozda ve doğru uygulama yöntemiyle, çevreyi ve insan sağlığını koruyacak şekilde kullanmak gerekmektedir. Ruhsatlı olan her BKÜ entegre zararlı yönetiminde kullanılamamaktadır. Bunun içinde mevcut ruhsatlı olan BKÜ'ler değerlendirmeye alınarak, risk durumları hesaplanmalı ve entegre zararlı yönetiminde kullanılabilecek olan BKÜ'ler belirlenmelidir. 2008-2010 ve 2015 yıllarında yapılan risk değerlendirmesi sonucunda 495 aktif madde, 204 adet karışım, 13 adet biyolojik preparat, 21 adet biyolojik mücadele etmeni ve 1 adet safener olmak üzere toplam 734 adet BKÜ incelenmiş ve uygun olanlar entegre mücadele teknik talimatlarında üreticilerimizin ve ülke tarımımızın hizmetine sunulmuştur.

2. Materyal ve Metot

Entegre zararlı yönetiminde kullanılacak ilaçlar Matthews (1984)'e göre belirlenmiştir. Entegre Zararlı Yönetimi sisteminde yalnızca insan sağlığı ve çevre üzerinde daha düşük etkiye sahip BKÜ'lerin kullanımına izin verilmektedir. Tavsiye edilecek BKÜ'lerin değerlendirilmesinde, insan ve çevre sağlığı açısından oluşabilecek riskleri ortaya koyabilmek amacıyla aktif maddelerin memeli hayvanlar, balıklar, hedef dışı organizmalar (parazitoitler, predatörler) ve balarılarına etkileri ile topraktaki kalıcılıkları göz önüne alınarak değerlendirmeler yapılmaktadır. Elde edilen bu veriler sınıflandırma kriterlerine göre az ya da yüksek riskli olarak sınıflandırılmaktadır. Sınıflandırma kriterleri ve formül yardımıyla her bir aktif madde için ayrı hesaplama yapılmıştır. Toplam risk değerinin hesaplanması Eşitlik (1)'e göre yapılmıştır.

$$\text{Toplam Risk} = \frac{\text{Balık} + \text{Hedef dışı org} + \text{Bal arısı}}{3} + \text{Memeli hayvanlar} + \text{Topraktaki kalıcılık} \qquad \text{(Eş. 1)}$$

Sınıflandırmayı düzenlemek için, balık, hedef dışı organizmalar ve balarılarına olan etkileri toplanarak üçe bölünmüş ve tek bir değere indirilmiştir. Daha sonra elde edilen bu veri ile memeli hayvanlar ve topraktaki

kalıcılıklarına ait sınıf değerleri toplanarak "Toplam Risk Değeri" elde edilmiştir (*Tablo* 1). Karışım ilaçların değerlendirilmesinde risk puanı yüksek olan sınıf değerleri dikkate alınmıştır. Bazı aktif maddelerin faydalı organizmalara yan etkileri ile ilgili yeterli araştırma sonucu bulunmadığından, bunların değerlendirilmesinde en yüksek risk puanı esas alınmıştır. Yine yapılan değerlendirmelerde herhangi bir verisi bulunmayan kriterler içinde en yüksek risk puanının kullanılmasına karar verilmiştir. Direnç gelişimini azaltmak için BKÜ seçimi ve tavsiyesinde dikkate alınmak üzere aktif maddelerin "etki mekanizmaları" da çizelgelere ilave edilerek, entegre mücadele teknik talimatlarında yayınlanmışlardır (Anonim, 2010). Tarımsal zararlılarla mücadelede, Entegre Zararlı Yönetiminde kullanılabilecek BKÜ bulunmadığı durumlarda, geçici olarak tavsiye edilen BKÜ'ler önerilmiştir. Entegre Zararlı Yönetiminde kullanılabilecek BKÜ'ler ruhsatlandırıldığında, geçici olarak tavsiye edilen BKÜ'ler Entegre Mücadele Teknik Talimatlarından çıkartılacaktır. BKÜ'ler Elde edilen toplam risk değerine göre aşağıdaki şekilde sınıflandırılmıştır. Ürün ve zararlı bazında değerlendirilen BKÜ'ler içinde 1, 2 ve 3 sınıf değeri alan tarım ilacı bulunmadığı takdirde konu başlığının altın "Entegre Mücadele Programına uygun tarım ilacı bulunmamaktadır" ifadesi eklenmiştir (Anonim, 2010).

Tablo 1. BKÜ'lerin toplam risk değerlerine göre sınıflandırılması (Anonim 2010).

Table 1. Classification of the PPPs according to their total risk value (Anonymous 2010).

Risk Değeri	Sınıf Değeri	Açıklama
3,0-5,9	1	Güvenli olarak tavsiye edilen tarım ilaçları
6,0-7,0	2	Kontrollü olarak tavsiye edilen tarım ilaçları
7,1-10,0	3	Geçici olarak tavsiye edilen tarım ilaçları
>10,0	4	Entegre mücadele programı için uygun değildir

Risk değerlendirmelerinde ayrıca pesticide manualden (Tomlin, 1997) yararlanılmıştır. BKÜ'lerin bal arılarına etkilerinin değerlendirilmesinde Anonim (2008a) yararlanılmıştır. BKÜ'lerin memelilere etkilerinin değerlendirilmesinde Anonim (2008b,c,d), balıklara etkilerinin değerlendirilmesinde Anonim (2008e), topraktaki kalıcılığının değerlendirilmesinde Anonim (2008f), hedef dışı organizmalara yan etkilerinin değerlendirilmesinde Boller et al. (2006)'dan yararlanılmıştır. Bunlara ilave olarak IRAC: (Insecticide Resistance Action Committe), HRAC: (Herbicide Resistance Action Committe), FRAC: (Fungicide Resistance Action Committe) ve Pestisitlerin kanserojen risklerinin değerlendirilmesinde IARC (Uluslararası Kanser Araştırma Ajansı), Pesticide Manual (2003) ve verilerinden faydalanılmıştır. Yine Pesticide Manual (Thirteenth Edition) version 3.0, IOBC, EPA, EFSA ve Pesticide Properties DataBase'den yararlanılmıştır (Anonim, 2008g).

3. Araştırma Sonuçları ve Tartışma

Entegre zararlı yönetimi çalışmaları konusunda ülkemizin 50 yıldan fazla tecrübesi bulunmaktadır. Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı bünyesinde entegre mücadele programlarında kullanılmak üzere önemli ürün gruplarına yönelik birer Entegre Mücadele Teknik Talimatı yayınlanmış bulunmaktadır. Mevcut olan Elma Entegre Mücadele Teknik Talimatı'na Armut ve Ayva ürünleri eklenmiş ve yine mevcut olan, Kiraz Entegre Mücadele Teknik Talimatı'na da Vişne'nin, Şeftali ürünlerinin ve Çeltik, Nar ve Ceviz Entegre Mücadele Teknik Talimatlarının da eklenmesiyle 2017 yılında toplam 19 adet talimat yayınlaşmıştır. 2022 yılında da Badem, Çilek, İncir, Yaprağı Yenen Sebzeler, Açık Alan Domates ve Ayçiçeği Entegre Mücadele Teknik Talimatları eklenerek toplam 29 üründe 25 adet teknik talimat üreticilerin ve ülkemiz tarımının hizmetine sunulmuştur (Anonim, 2023b).

Ülkemizde entegre mücadele programlarında ilaç seçimi Matthews (1984)'e göre yapılmaktadır**.** Entegre Mücadele Teknik Talimatlarında kullanılacak tarım ilaçlarının seçimi çalışmalarına ilk başlandığında oluşturulan komisyon değerlendirmelerinin Matthews (1984)'e göre yapıldığını belirterek, herbisitlerle ilgili olarak yapılan değerlendirmelerde faydalı böceklerle ilgili yeterli bilgi olmaması nedeni ile değerlendirmeler diğer kriterler göz önünde bulundurularak yapılmıştır. Yine karışım içeren pestisitlerle ilgili yeterli bilgiler bulunamadığı için bu tip ilaçlar değerlendirmeye alınmamıştır. Yapılmış olan ilk komisyon değerlendirmesinde karışım ilaçların değerlendirilmediği anlaşılmakta olup, veri eksikliğinden dolayı mevcut ruhsatlı pestisitlerin hepsi değil sınırlı sayda pestisitlerin değerlendirildiği görülmektedir (Anonim, 1997). Bu durum son derece normal olup çok önemli bir kilometre taşı olarak yerini almıştır. Ülkemizde bu çalışmaların başlamasına vesile olmuştur. Bu ilk

değerlendirmelerden sonra ülkemizde ruhsatlı pestisitlerin sayısı artmış buna ilaveten başka ürün gruplarında da entegre mücadele teknik talimatları oluşturulması ihtiyacı doğmuştur. Bu noktada ikinci komisyon ilk toplantısı olan 2008 yılında toplanarak yine ilk komisyondaki gibi değerlendirmelerin Matthews (1984)'e göre yapılması konusunda karar almıştır. Komisyon toplantısında 1997 yılından sonrada yeni tavsiyeler ruhsatlandırıldığı ve revizyon çalışmasında bir bütünlük sağlayabilmek için, 1997 yılında değerlendirilen aktif maddelerde dahil olmak üzere var olan bütün aktif madde ve karışımların yeniden değerlendirilmesi benimsenmiştir. Bu ikinci komisyon çalışmaları sırasında 460 aktif madde ve 155 karışım halindeki tarım ilaçları değerlendirmeye tabi tutulmuştur. Güncel gelişmeler dikkate alınarak incelenen tüm aktif madde ve karışımların etki mekanizması ve kanser oluşturma riskleri de değerlendirilmiştir. Riskli bulunan aktif maddelere entegre mücadele teknik talimatlarında yer verilmemiştir. Entegre mücadelenin bir bileşimi olan entegre direnç yönetimi kapsamında direnç gelişimini önlemek için bitki koruma ürünleri seçimi ve tavsiyesinde dikkate alınmak üzere aktif maddelerin "etki mekanizmasıları da çizelgelere eklenmiştir. Bu konuda "Insecticide Resistance Action Committee, Fungicide Resistance Action Committee ve Herbicide Resistance Action Committee" tarafından yayınlanmış olan listelerden yararlanılmıştır. Çizelgelerde yer alan etki mekanizması sütununa ait "Pestisitlere karşı direnç gelişimini azaltmak için birbiri ardına yapılacak uygulamalarda etki mekanizması sütununda yer alan farklı harf ya da rakama sahip aktif maddelerin seçilmesine özen gösterilmelidir" şeklindeki açıklama, sayfanın altında dip not olarak verilmiştir.

2010 yılından sonra ruhsatlanan BKÜ'ler içinde aynı değerlendirmeler yapılmış ve 2010 yılından sonra ruhsat almış olan 35 aktif madde, 54 adet karışım, 13 adet biyolojik preparat, 21 adet biyolojik mücadele etmeni ve 1 adet safener olmak üzere toplam 124 adet daha BKÜ incelenmiştir.

4. Sonuç

Türkiye'nin entegre zararlı yönetimi konusunda yapmış olduğu araştırmaları, ülke tarımının hizmetine sunduğu entegre zararlı yönetimi programları, Entegre Zararlı Yönetimi konusunda elli yıldan fazla deneyimi, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı bünyesinde ürünlere özel hazırlanmış 25 adet Entegre Mücadele Teknik Talimatı, Gıda ve Kontrol Genel Müdürlüğü, Bitki Sağlığı ve Karantina Daire Başkanlığı ile Bitki Koruma Ürünleri Daire Başkanlığı bünyelerinde hazırlanan güncel "Entegre Mücadele" ve "Bitki Sağlığı Uygulama Program"ları, BKÜ ile ilgili mevzuatlar ve BKÜ takip sistemi ile BKÜ veri tabanı gibi uygulamalar ile Tarım ve Orman Bakanlığı'nın hazırlamış olduğu yasal mevzuatlar bakımından oldukça önemli gelişmiş bir kapasiteye sahip olduğu görülmektedir. Biyolojik çeşitliliğin korunması ve sürdürülebilir tarımsal üretimin yapılabilmesi için entegre zararlı yönetim programlarının titizlikle uygulanması gerekmektedir. Arzu edilen hedef, insan ve çevre sağlığının korunması ile biyolojik çeşitliliğin korunduğu, gıda güvenliği ve güvenli gıdaya arzın sürdürülebilir bir tarımsal üretimle garanti altına alındığı, sağlıklı, kaliteli ve güvenilir ürünlerin elde edildiği bir üretim sürecinin gerçekleşmesidir. Çevre ve insan sağlığının korunması için, mevcut olan eğitim yayım faaliyetlerine ağırlık verilerek ya da güncel gelişmelere göre yeni konularda eklenerek, üreticilerimizin kendi tarla ve bahçelerinin uzmanı olması sağlanmalıdır.

Teşekkür

Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı ve Gıda Kontrol Genel Müdürlüğü, Bitki Koruma Ürünleri Daire Başkanlığı ile Bitki Sağlığı ve Karantina Daire Başkanlığı ve Zirai Mücadele Merkez Araştırma Enstitüsü Müdürlüğü'ne ve teknik talimatların hazırlanmasında emeği geçen bütün araştırmacılara teşekkürlerimi sunarım.

Etik Kurul Onayı

Bu çalışma için etik kuruldan izin alınmasına gerek yoktur.

Çıkar Çatışması Beyanı

Yazar herhangi bir çıkar çatışması olmadığını beyan etmektedir.

Yazarlık Katkı Beyanı

Makale tek yazarlıdır.

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