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Fasulye Tohum Böceği, *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae), Mücadelesinde Bazı Zeolitlerin İnsektisidal Potansiyel Etkisi

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Effectiveness of the Insecticidal Potential of Some Zeolites in the Control of the Bean Seed Beetle, *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae)

ÖZ

Bu çalışmanın amacı depolanmış ürün zararlısı olan *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) ile mücadelede kimyasal kullanımının azaltılmasına alternatif olarak 3A, 4A ve 5A zeolitleri kullanarak zararlı popülasyonunu ekonomik zarar eşiğinin altına düşürmektir. Ülkemizde ve dünyada depo zararlılarıyla mücadelede kalıntı bırakmayan uygulamaların geliştirilmesi ve özellikle baklagillerin muhafaza edilmesinde kullanılan zeolit minerallerini de içeren inert tozların kullanımı önem arz etmektedir. İnert tozlar içinde yer alan zeolit, depolanmış tahıllarda ürüne karıştırılarak zararlılara karşı uzun süreli koruma sağlamaktadır. Sentetik zeolitlerin adsorplama, katalizör olma ve iyon değiştirici özelliklerine göre kullanım alanları oldukça geniştir. Bu amaçla *A. obtectus*'a karşı 3A, 4A ve 5A zeolitler 27 ± 2°C, %65 ± 5 bağıl nem sabit koşulları altında ısıtmalı/soğutmalı inkübatör içerisinde beş farklı dozda (25mg, 50 mg, 75 mg, 100 mg ve 125 mg) test edilmiştir. Uygulama beş tekerrürlü ve her tekerrürde 20 adet ergin olacak şekilde yapılmıştır. Yüzde ölüm oranlarını hesaplamak amacıyla 1, 3, 6, 12, 24, 48, 72, 96, 120 ve 144. saat aralıklarla kontroller yapılmış, canlı ve ölü ergin sayıları kaydedilmiştir. Uygulama öncesi ve sonrası ağırlık kayıpları da değerlendirilmiştir. Çalışma sonucunda, zeolitlerin hepsinin kontrole göre etkili sonuçlar verdiği, ancak ayrı ayrı incelendiğinde 3A ve 5A zeolitlerin 4A zeolite kıyasla *A. obtectus*'a karşı daha etkili oldukları belirlenmiştir. 3A ve 5A zeolitler için istatistiki olarak en yüksek ölüm oranının 125 mg dozda 72. saatte, 4A zeolit için 125 mg dozda 96. saatte olduğu kaydedilmiştir. Tüm dozlar için %100 ölümler 96-144. saatlerde tamamlanmıştır. 3A, 4A ve 5A zeolit dozlarının ve maruz kalma sürelerinin artması *A. obtectus*'un ölüm oranını arttırmış, yumurta sayısını düşürmüş ve dolayısıyla F_1 nesli üretiminde azalmaya sebep olmuştur. Elde edilen sonuçlara göre 3A, 4A ve 5A zeolitlerin *A. obtectus*'un mücadelesi için umut verici olduğu sonucuna varılmıştır.

Anahtar Kelimeler: *Acanthoscelides obtectus*, Chrysomelidae, Zeolit, İnsektisidal etki

ABSTRACT

This study aims to reduce the pest population below the economic damage threshold through the use of 3A, 4A and 5A zeolites, as an alternative to reducing the use of chemicals in the control of the storage pest *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae). In our country and around the world, it is essential to develop residue-free methods in the control of storage pests and to use inert powders, including zeolite minerals, which are used primarily in the preservation of legumes. Zeolite, contained in inert powders, provides long-term protection against pests by mixing with the product in stored grain. Synthetic zeolites have a wide range of applications depending on their adsorption, catalyst and ion exchange properties. For this purpose, 3A, 4A, and 5A zeolites were tested against *A. obtectus* at five different doses (25mg, 50mg, 75mg, 100mg, and 125mg) in a heated/cooled incubator under constant conditions of 27 ± 2°C, 65 ± 5% relative humidity. Five replicates of twenty adults each were used in the experiments. The number of alive and dead adults was recorded at 1, 3, 6, 12, 24, 48, 72, 96, 120, and 144th hours in order to calculate the mortality rates. Their weight losses were also evaluated before and after application. The study's results indicated that all of the zeolites performed better than the control, but 3A and 5A zeolites were more effective against *A. obtectus* than 4A zeolite separately. It was recorded that the highest mortality rate for 3A and 5A zeolites was at the 72nd hour at 125 mg dose, and for 4A zeolite at the 120th hour at 125 mg dose. For all doses, 100% of deaths were completed in 96-144th hours. Increasing 3A, 4A, and 5A zeolite concentrations and exposure times increased the mortality rate of *A. obtectus*, reduced the number of eggs, and, therefore, led to a decrease in F1 generation production. The results obtained show that 3A, 4A, and 5A zeolites can be considered as a promising strategy for the control of *A. obtectus*.

Keywords: *Acanthoscelides obtectus*, Chrysomelidae, Zeolite, Insecticidal effect

Çalışma, birinci yazarın doktora tezinden veriler içermektedir.

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Giriş

Depolanan ürünlerde böceklerin meydana getirdiği zararlar dünya çapında büyük bir soruna neden olmaktadır. Söz konusu zararlar hem ürün kalitesinde kayıplara hem de miktarında önemli düşüşlere yol açmaktadır (de Oliveira Vilela vd., 2021; Mssillou vd., 2022). Fasulye tohum böceği *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae), beyaz fasulye, nohut, soya fasulyesi ve börülce gibi ekonomik açıdan önemli baklagillerin yaygın bir zararlısıdır. Larvalar baklagillerin iç kısmına zarar vererek depolanan ürünün besin ve ticari değerinin yanı sıra çimlenme potansiyelinin de azalmasına neden olmaktadır (Şen vd., 2020; Masoumi vd., 2021). Fosfin ve piretroidler gibi sentetik insektisitler söz konusu zararlının mücadelesinde yaygın olarak kullanılmaktadır (Opit vd., 2016; Abdelgaleil vd., 2021). Kimyasal insektisitlerin artan kullanımı; böceklerde direnç sorunları, gıdalarda ilaç kalıntıları ve insan sağlığı ile ilgili olumsuzluklar, yaban hayatı ve çevre üzerindeki istenmeyen etkileri nedeniyle gün geçtikçe büyüyen bir sorun oluşturmaktadır (Nicolopoulou-Stamati vd., 2016). Bu nedenle son zamanlarda zararlılarla mücadelede çevre dostu tekniklerin ve yeni alternatiflerin araştırılmasına ilgi artmaktadır.

Zeolitler, yapılarında silisyum, alüminyum ve oksijen içeren kristal yapıda, hidrasyona uğramış alüminosilikat içeren mineralleridir (Breck, 1974). Vücutta birikebilen ancak fibrojenik ve toksik etki yapmayan bu tozlar (inert tozların genel tanımı) tarla bitkilerinde, depolanmış ürün zararlılarında ve hayvan beslenmesinde yem katkısı olarak günümüzde kullanılmaktadır (Golob, 1997). Bilinen 50 adet doğal zeolit ve 200 adet sentetik zeolit minerali bulunmaktadır (Gottardi & Galli, 1985). Zeolitler iyon değişimi yeteneği, adsorpsiyon, moleküler elek yapısı, yüksek silis içeriği bulunması, hafif yapıları, küçük kristal boyutlarına sahip gözenekli yapıları gibi pek çok fiziksel ve kimyasal özellikleri bakımından oldukça önemlidir. Bu özellikler zeolitlerin enerji, tarım ve hayvancılık, madencilik, metalürji, inşaat, deterjan ve kâğıt sanayi gibi çeşitli sektörlerde kullanılmasına olanak sağlamıştır (DPT, 2001). Doğal zeolitlerin, depolanmış ürün zararlılarından *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) ve *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) ile mücadelede insektisidal potansiyele sahip oldukları bilinmektedir (Yılmaz Doğu & Emekci, 2023). Zeolitler, böcek kılları arasındaki mesafeden daha küçük çapa sahip parçacıklar içerebilmektedir. Bu parçacıklar vücutta bulunan kılların varlığına rağmen vücut yüzeyine yapışarak böcek derisindeki karbondioksit gazının serbest bırakılmasını engellemektedir. Ayrıca böceklerin stigmalarına yapışarak solunumu engellemekte ve oksijen yetersizliğinden dolayı ölümlerine neden olmaktadır. Böcekler vücutlarına yabancı cisim yapıştığı zaman davranışsal olarak temizlenmeyi denemektedirler. Zeolit bu temizlenme işlemi sırasında vücutta oluşan çiziklere yapışmakta ve nem tutucu özelliğinden dolayı böceğin vücut sıvısını emerek dehidrasyon yoluyla ölümüne yol açmaktadır (Ikeda vd., 1996). Böceklerde bu şekilde ölümün temel sebebinin aşırı su kaybı veya kurumadan kaynaklı olduğu bilinmektedir (Subramanyam & Roesli 2000). Zeolitin moleküler yapısı, silisyum dioksit içeriği, partiküllerin şekli ve boyutu, Alüminyum-Silisyum (Al/Si) oranları, soğurma kabiliyeti ve coğrafi orijinali insektisidal potansiyelini de etkilemektedir (Eroğlu, 2014).

Bu çalışmada, fasulye tohumlarında zarara neden olan *A. obtectus* ile mücadelede kimyasal kullanımını azaltmak amacıyla alternatif olarak 3A, 4A ve 5A zeolitlerin kullanılması ile zararlı popülasyonunun kontrol altına alınması amaçlanmıştır. Böylece insan ve çevre sağlığı için daha etkili bir metot olan çevreyle dost bir mücadele yönteminin kullanılması ve pahalı olan insektisitlerin kullanımının önüne geçilerek ülke ekonomisine katkı sağlanması hedeflenmiştir.

Materyal ve Yöntem

Böceklerin Yetiştirilmesi

Acanthoscelides obtectus erginleri Mayıs 2022'de Selçuk Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümünden temin edilmiş ve o tarihten bu yana popülasyonlar, 27 ± 2°C, %65 ± 5 bağıl nem sabit koşulları altında ısıtmalı/soğutmalı inkübatör içerisinde SDÜ Biyoloji Bölümü Sistematik Entomoloji laboratuvarında üretilmiştir. Böcekler, -18 °C'de dolapta en az 7 gün saklanan fasulye (*Phaseolus vulgaris* L.) taneleri ile beslenmiştir. Fasulye taneleri bahsedilen sabit koşullarda F_1 nesli ortaya çıkana kadar saklanmıştır. Tüm denemeler 1-3 günlük erginler kullanılarak yürütülmüştür. Bu yöntem tüm çalışma süresince takip edilmiştir.

Biyoanalizler

Zeolitlerin ergin *A. obtectus* bireyleri üzerindeki etkisi, doz-ölüm oranı biyoanalizleri ile değerlendirilmiştir. Bu amaçla uygun dozların belirlenmesi için ön denemeler 27 ± 2°C, %65 ± 5 bağıl nem sabit koşulları altında ısıtmalı/soğutmalı inkübatörde yapılmıştır. Elde edilen veriler doğrultusunda beş nihai doz 25 mg, 50 mg, 75 mg, 100 mg ve 125 mg olarak belirlenmiştir. 180 ml'lik steril numune kaplarına üç farklı zeolit (3A, 4A ve 5A) beş farklı dozda (25 mg, 50 mg, 75 mg, 100 mg ve 125 mg) toz halinde ayrı ayrı tartılarak konulmuştur. Her bir deneme kabı içerisine 8 gr fasulye ve 20 adet 1-3 günlük *A. obtectus* bireyleri rastgele eklenmiştir. Deneme kaplarının kapak kısımları uygun havalandırma sağlamak ve böceklerin kaçışını önlemek için tül ile kapatılmıştır. Uygulama yapılmayan fasulye kontrol grubu olarak belirlenmiştir. Her bir doz için bu işlemler beş

tekerrürlü olarak uygulanmıştır. Uygulama öncesi ve uygulama sonrası hassas tartıda böcek ağırlıkları kaydedilmiştir. Tüm kaplar belirlenen dozlarda zeolit uygulamalarının ardından yukarıda bahsedilen koşullar altındaki inkübatöre yerleştirilmiştir (Şekil 1). Canlı ve ölü erginlerin sayısı 1, 3, 6, 9, 12, 24, 36, 48, 72, 96, 120 ve 144. saatlerde kontrol edilerek kayıt altına alınmıştır. Fırçayla rahatsız edildiğinde hareket etmeyen bireyler ölü sayılmış, herhangi bir uzvu hareket eden bireyler ise canlı olarak değerlendirilmiştir. Zeolit uygulanmış ve uygulanmamış örneklerden elektron mikroskop görüntüsü alınmıştır.

Şekil 1.
Acanthoscelides obtectus erginlerinin zeolit denemeleri.

Tablo 1.
Çalışmada kullanılan 3A, 4A ve 5A zeolit içerikleri

Zeolit çeşidi	Gözenek çapı	Dengeleyici katyon
Ürün ismi: **Molecular sieves, 3A** Ürün Numarası: 334286 Marka: SIGALD	3 Å (0,3 nm)	K^+
Ürün ismi: **Molecular sieves, 4A** Ürün Numarası: 688363 Marka: SIGALD	4 Å (0,4 nm)	Na^+
Ürün ismi: **Molecular sieves, 5A** Ürün Numarası: 341029 Marka: SIGALD	5Å (0,5 nm)	Ca^{++}

Zeolitler

Çalışmada kullanılan 3A, 4A ve 5A zeolitler Sigma Aldrich® firmasından temin edilmiştir. Sentetik zeolitler (3A, 4A, 5A) son derece düzgün bir kristal yapıya sahiptir. Yapılarına göre; 5A zeolit kalsiyum alüminosilikat içerir ve 5Å gözenek çapına sahiptir, 4A zeolit sodyum alüminosilikat içerir ve 4Å gözenek çapına sahiptir, 3A zeolit potasyum alüminosilikat içerir ve 3Å gözenek çapına sahiptir (Anonim, 2020) (Tablo 1).

İstatistiksel Analizler

Farklı dozlara ait probit analizleri için JMP Pro 17 paket programı kullanılmıştır. Gözlenen ölüm yüzdesi için Abbott formülü (Abbott, 1925) uygulanmıştır. Abbott formülü;

$$\text{Ölüm oranı (\%)} = \frac{\text{Gözlenen ölüm} - \text{Kontrol ölümü}}{100 - \text{Kontrol ölümü}} \text{x} 100 \quad (1)$$

Farklı doz uygulamalarının ölüm seviyelerini karşılaştırmak ve ayırmak için Tukey testi yapılmıştır.

Bulgular ve Tartışma

Çalışmada farklı zeolitlerin (3A, 4A ve 5A) farklı dozlarının *A. obtectus* erginleri üzerine insektisidal etkilerini belirlemek amacı ile denemeler yapılmış ve ölü-canlı birey sayıları kaydedilmiştir. Böcek ölümlerinin başladığı 6. saatten itibaren veriler oluşturulmuştur ve istatistiksel olarak değerlendirilmiştir (Tablo 2). Doz uygulamalarında ölümler 120-144. saatte tamamlanırken, kontrol grubuna ait ölümler 240-288. saatlerde bitmiştir.

Tablo 2'de uygulanan dozlara bağlı ölüm oranları verilmiş olup, zamana ve doza bağlı olarak ölüm oranlarında artış gözlenmiştir. Sütunlar ve satırlar incelendiğinde, aynı harfi gösteren ortalamalar arasında istatistiksel olarak fark yoktur. 3A zeolit sonuçlarına göre; 6. saatte 75, 100 ve 125 mg dozlarda ölümler başlamıştır. En yüksek ölüm 125 mg dozda 72. saatte gözlenmiştir. 125 mg dozda 96. saatte %100 ölüm gerçekleşmiştir. 120. saatte tüm dozlarda %100 ölüm görülmüştür. 4A zeolit uygulamasında ilk ölümler 6. saatte başlamış, 12. saatte tüm dozlarda ölüm görülmüştür. 125 mg dozda 96. saatte istatistiksel olarak fark görülmüş, ancak 100 ve 125 mg dozlarda 120.saatte %100 ölüm gerçekleşmiştir. 5A zeolitte 24. saatte tüm dozlarda ölüm kaydedilmiştir. 125 mg dozda 72. saatte en fazla ölüm gözlenmiştir. 120. saatte 25 mg hariç diğer dozlarda istatistiksel bir fark gözlenmemiştir.

Tüm sonuçlar bir arada değerlendirildiğinde, 3A ve 5A zeolitlerin 125 mg dozda 72. saatte, 4A zeolitin 125 mg dozda 96. saatte istatistiksel olarak en etkili oldukları belirlenmiştir. 3A, 4A ve 5A zeolitlerin *A. obtectus* erginleri üzerinde insektisidal potansiyeli incelendiğinde en yüksek ölüm oranının 125 mg dozda 72. ve 96. saatlerde olduğu, 120. saatte 3A zeolitin tüm dozlarında, 144. saatte ise 4A ve 5A zeolitlerin tüm dozlarında %100 ölüm gerçekleştiği tespit edilmiştir.

Tablo 2.

3A, 4A ve 5A Zeolitlerin farklı dozlarının ve maruz bırakma sürelerinin (saat) Acanthoscelides obtectus üzerine etkileri

Zaman / Konsantrasyon	3.Saat $X_{3}\pm SE$	6.saat $X_{6}\pm SE$	12.saat $X_{12}\pm SE$	24.saat $X_{24}\pm SE$	48.saat $X_{48}\pm SE$	72.saat $X_{72}\pm SE$	96.saat $X_{96}\pm SE$	120.saat $X_{120}\pm SE$	144.saat $X_{144}\pm SE$
3A ZEOLİT									
25 MG	$0,0 \pm 0,0^{aD}$	$0,0 \pm 0,0^{aD}$	$0,0 \pm 0,0^{eD}$	$0,8 \pm 0,4^{dD}$	$1,8 \pm 0,7^{dC}$	$3,6 \pm 0,8^{cB}$	$19,0 \pm 0,4^{aA}$	$20,0 \pm 0,0^{aA}$	
50 MG	$0,0 \pm 0,0^{aE}$	$0,0 \pm 0,0^{aE}$	$0,4 \pm 0,2^{deE}$	$2,0 \pm 0,3^{cdD}$	$10,6 \pm 1,0^{cC}$	$15,6 \pm 0,8^{aB}$	$18,8 \pm 0,3^{aA}$	$20,0 \pm 0,0^{aA}$	
75 MG	$0,0 \pm 0,0^{aF}$	$0,6 \pm 0,2^{aF}$	$1,4 \pm 0,2^{cdE}$	$3,6 \pm 0,4^{acD}$	$12,8 \pm 0,5^{acC}$	$18,0 \pm 0,5^{aaB}$	$19,6 \pm 0,2^{aA}$	$20,0 \pm 0,0^{aA}$	
100 MG	$0,0 \pm 0,0^{aE}$	$0,4 \pm 0,2^{aE}$	$2,2 \pm 0,2^{acD}$	$5,2 \pm 0,3^{aC}$	$13,2 \pm 0,5^{acB}$	$18,0 \pm 0,5^{aaA}$	$19,8 \pm 0,2^{aA}$	$20,0 \pm 0,0^{aA}$	
125 MG	$0,0 \pm 0,0^{aF}$	$0,6 \pm 0,2^{aE}$	$2,6 \pm 0,2^{aD}$	$5,2 \pm 0,5^{aC}$	$14,6 \pm 0,8^{aB}$	$18,6 \pm 0,5^{aA}$	$20,0 \pm 0,0^{aA}$	$20,0 \pm 0,0^{aA}$	
KONTROL	$0,0 \pm 0,0^{a}$	$0,0 \pm 0,0^{a}$	$0,0 \pm 0,0^{c}$	$0,8 \pm 0,4^{e}$	$1,8 \pm 0,7^{d}$	$3,6 \pm 0,8^{a}$	$5,0 \pm 1,0^{a}$	$6,8 \pm 1,1^{a}$	
4A ZEOLİT									
25 MG		$0,0 \pm 0,0^{aF}$	$0,2 \pm 0,2^{dF}$	$0,8 \pm 0,3^{dF}$	$3,0 \pm 0,4^{cdE}$	$5,8 \pm 0,3^{dD}$	$11,8 \pm 0,3^{aC}$	$17,8 \pm 0,3^{bB}$	$20,0 \pm 0,0^{aA}$
50 MG		$0,0 \pm 0,0^{aG}$	$0,6 \pm 0,2^{cdFG}$	$1,8 \pm 0,3^{cdF}$	$5,2 \pm 0,3^{bcE}$	$8,8 \pm 0,5^{cD}$	$14,2 \pm 0,6^{abC}$	$18,2 \pm 0,3^{abB}$	$20,0 \pm 0,0^{aA}$
75 MG		$0,4 \pm 0,2^{aF}$	$1,6 \pm 0,4^{bcEF}$	$3,4 \pm 0,5^{bcE}$	$6,8 \pm 0,7^{bD}$	$11 \pm 0,8^{bcC}$	$15,6 \pm 0,8^{bcB}$	$18,8 \pm 0,5^{abA}$	$20,0 \pm 0,0^{aA}$
100 MG		$0,4 \pm 0,2^{aG}$	$2,0 \pm 0,3^{abF}$	$4,6 \pm 0,5^{bE}$	$9,4 \pm 0,5^{aD}$	$13,2 \pm 0,3^{abC}$	$17,2 \pm 0,3^{cdB}$	$20,0 \pm 0,0^{aA}$	$20,0 \pm 0,0^{aA}$
125 MG		$0,6 \pm 0,2^{aF}$	$3,0 \pm 0,4^{aE}$	$7,0 \pm 0,7^{aD}$	$11,4 \pm 0,5^{aC}$	$15,2 \pm 0,3^{aB}$	$19,4 \pm 0,4^{dA}$	$20,0 \pm 0,0^{aA}$	$20,0 \pm 0,0^{aA}$
KONTROL		$0,0 \pm 0,0^{a}$	$0,0 \pm 0,0^{d}$	$0,0 \pm 0,0^{d}$	$0,8 \pm 0,3^{d}$	$1,4 \pm 0,5^{e}$	$2,4 \pm 0,6^{e}$	$4,2 \pm 0,7^{c}$	$7,0 \pm 0,9^{b}$
5A ZEOLİT									
25 MG		$0,0 \pm 0,0^{cE}$	$0,0 \pm 0,0^{bE}$	$0,4 \pm 0,2^{bE}$	$1,2 \pm 0,3^{cE}$	$5,2 \pm 0,5^{cD}$	$14,2 \pm 0,3^{bC}$	$18,2 \pm 0,3^{aB}$	$20,0 \pm 0,0^{aA}$
50 MG		$0,0 \pm 0,0^{cE}$	$0,0 \pm 0,0^{bE}$	$1,0 \pm 0,3^{bE}$	$3 \pm 0,3^{bcD}$	$8,8 \pm 0,3^{bC}$	$15,2 \pm 0,3^{abB}$	$19,2 \pm 0,3^{aA}$	$20,0 \pm 0,0^{aA}$
75 MG		$0,2 \pm 0,2^{bcF}$	$0,6 \pm 0,4^{bEF}$	$2,4 \pm 0,6^{bDE}$	$4,2 \pm 0,3^{bD}$	$11,2 \pm 0,6^{bC}$	$15,6 \pm 0,5^{abB}$	$19,4 \pm 0,4^{aA}$	$20,0 \pm 0,0^{aA}$
100 MG		$1,2 \pm 0,4^{abF}$	$3,6 \pm 0,5^{aE}$	$7,2 \pm 0,7^{aD}$	$13,2 \pm 0,6^{aC}$	$15,6 \pm 0,5^{aB}$	$19,2 \pm 0,3^{aA}$	$20,0 \pm 0,0^{aA}$	$20,0 \pm 0,0^{aA}$
125 MG		$2,0 \pm 0,3^{aDE}$	$4,4 \pm 0,2^{aD}$	$8,2 \pm 0,5^{aC}$	$12,6 \pm 0,6^{aB}$	$17,4 \pm 0,5^{aA}$	$17,8 \pm 1,9^{abA}$	$20,0 \pm 0,0^{aA}$	$20,0 \pm 0,0^{aA}$
KONTROL		$0,0 \pm 0,0^{c}$	$0,0 \pm 0,0^{b}$	$0,8 \pm 0,3^{b}$	$1,6 \pm 0,6^{c}$	$3,2 \pm 0^{c}$	$4,4 \pm 1,3^{c}$	$7,0 \pm 1,4^{b}$	$9,8 \pm 1,3^{b}$

Bir sütunda bulunan küçük harfler aynı ise istatistiki olarak bir farklılık yoktur; bir satırda bulunan büyük harfler aynı ise istatistiki olarak bir farklılık yoktur.

Doz-tepki biyoanalizleri

Zamana bağlı olarak farklı zeolit uygulamaları (3A, 4A ve 5A) ve dozlarında (25 mg, 50 mg, 75 mg, 100 mg ve 125 mg) *A. obtectus* popülasyonlarının %50'sinin öldüğü saatleri gösteren LT_{50} eğrileri Şekil 2, 3 ve 4'de gösterilmiştir. Bu eğrilerden yola çıkarak farklı doz ve uygulamaların LT_{50} değerleri ise Tablo 3'de toplu olarak verilmiştir.

Tablo 3.

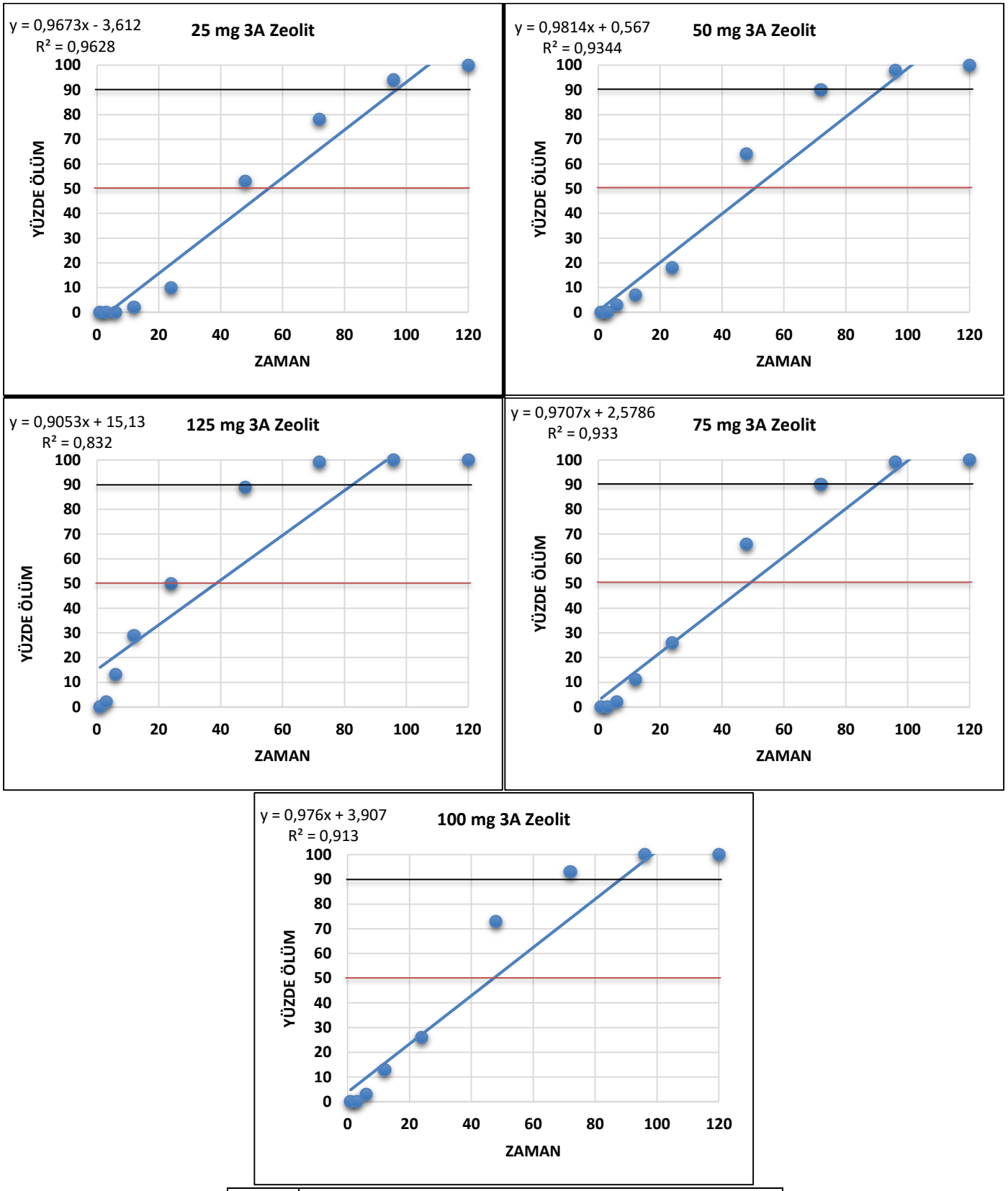
Farklı doz ve zeolit uygulamalarının Acanthoscelides obtectus popülasyonlarının %50'sini öldürdüğü zaman

Doz	Zaman (saat)		
	3A ZEOLİT	4A ZEOLİT	5A ZEOLİT
25 mg	55,4	80,6	79,7
50 mg	50,3	73,3	73,5
75 mg	48,8	67,3	69,4
100 mg	47,2	61,0	52,6
125 mg	38,5	54,7	50,9

Zeolitlerin farklı dozlarının *A. obtectus* erginlerinde meydana getirdiği ölüm (%) oranları incelendiğinde, maruz bırakma süresine bağlı olarak böcek ölüm oranlarında artış gözlenmiştir. Aynı şekilde her maruz bırakma süresinde de doz artışına bağlı olarak ölümlerde artış olmuştur (Şekil 5). Yüksek dozlarda maruz kalma süresi daha kısa, düşük dozlarda maruz kalma süresi daha fazla olduğu için veriler %100 ölüm görülen zamana kadar kaydedilmiştir.

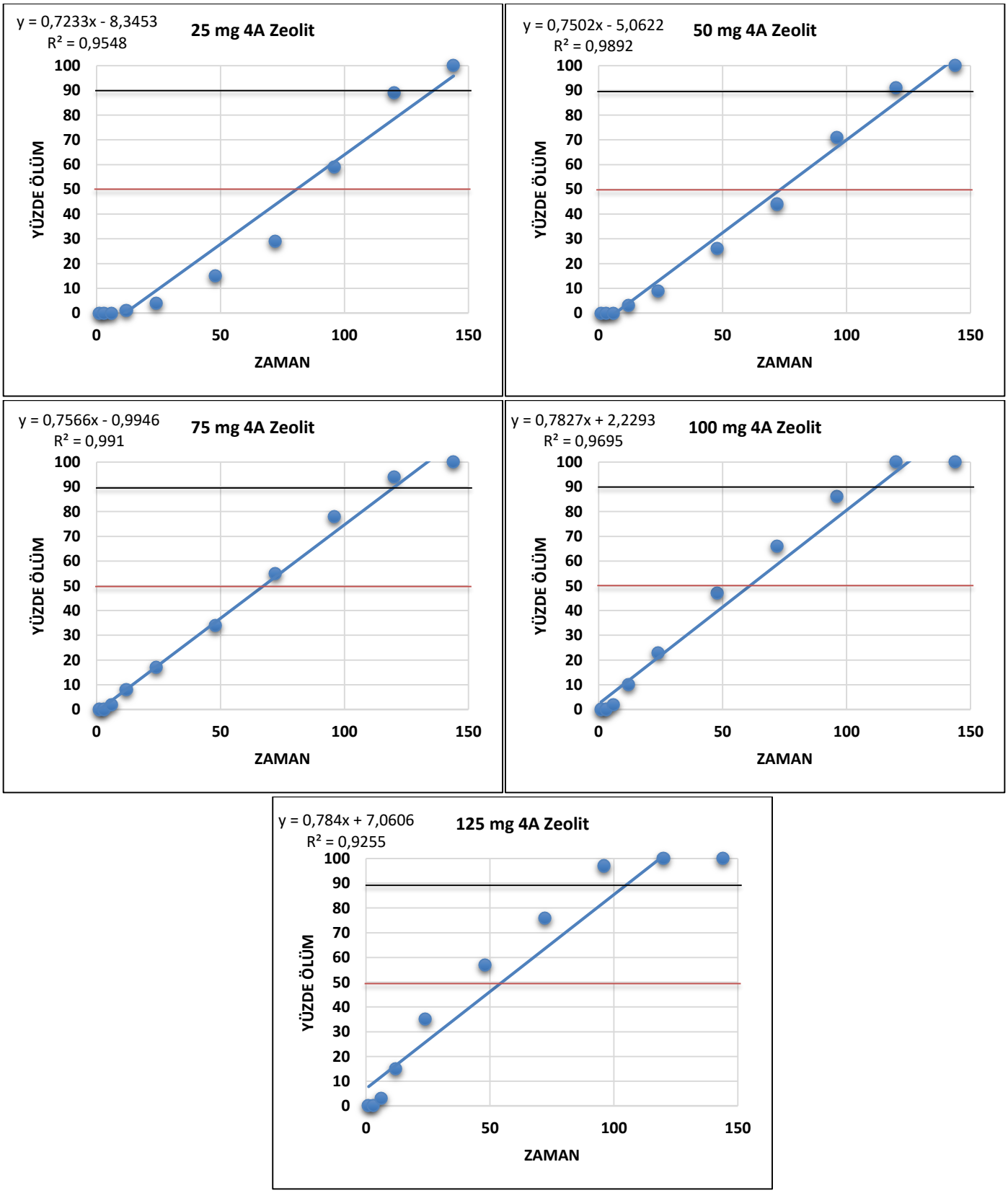
Zeolit uygulamaları sonucunda elde edilen verilere göre ağırlık kaybı incelendiğinde ise, doz miktarı arttıkça böceklerde ağırlık kaybının arttığı gözlenmiştir. Uygulama gruplarında veriler; her bir dozda %100 ölümün görüldüğü zaman kaydedilirken kontrol gruplarında takip süresi 10-12 gün sürmüştür. Zeolitin nem tutucu özelliğinden dolayı farklı dozlarda maruz kalma süresine bağlı olarak daha fazla ağırlık kaybı görülmüştür (Şekil 6).

Elde edilen sonuçlar doğrultusunda üç farklı zeolit arasında değerlendirme yapıldığında en etkili dozun 125 mg olduğu belirlenmiştir. Zeolitler içerik özellikleri bakımından karşılaştırıldığında benzer insektisidal etki göstermiştir.

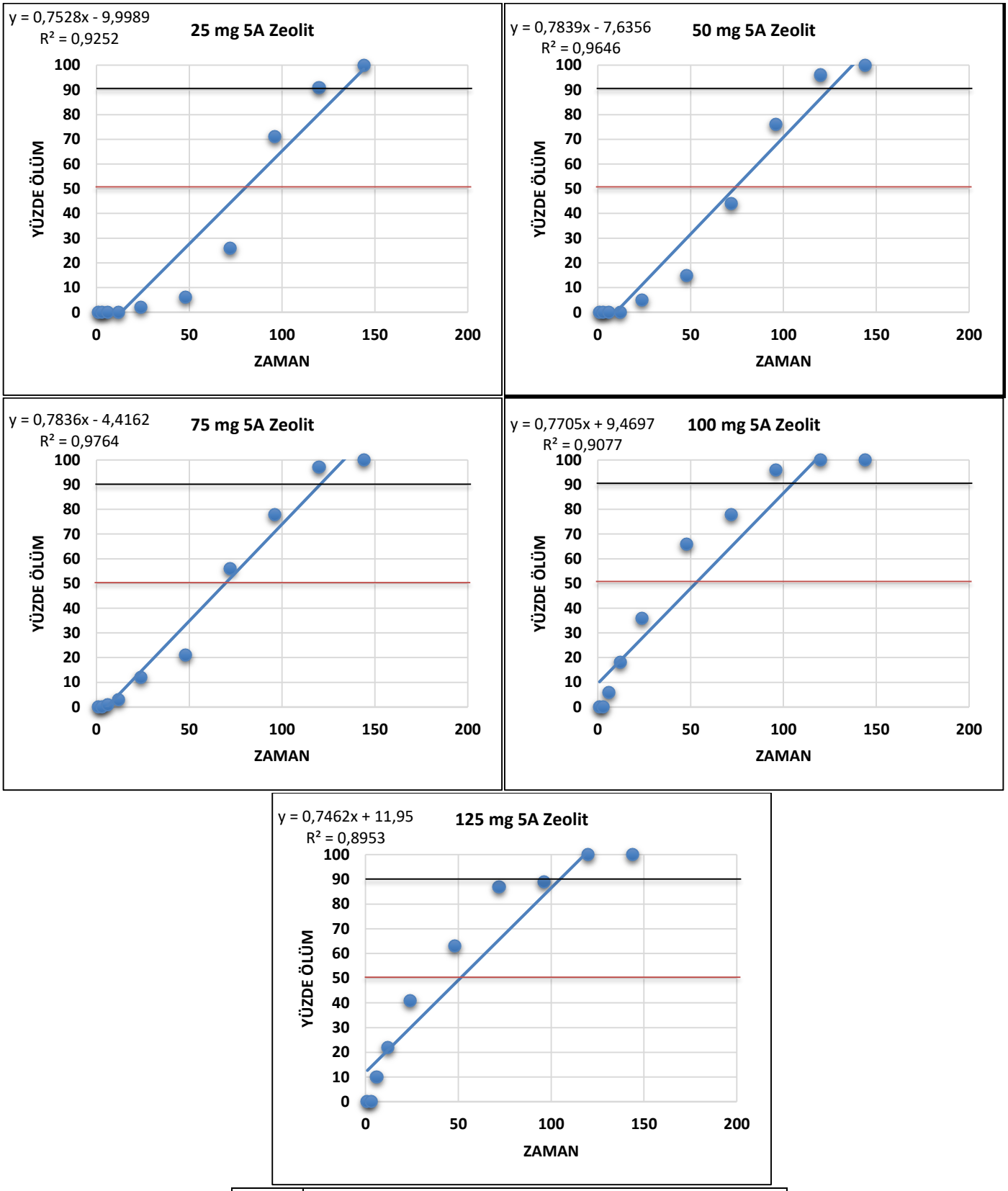


Şekil 2.

3A zeolitin farklı dozlarının Acanthoscelides obtectus erginlerinde meydana getirdiği ölüm oranı.

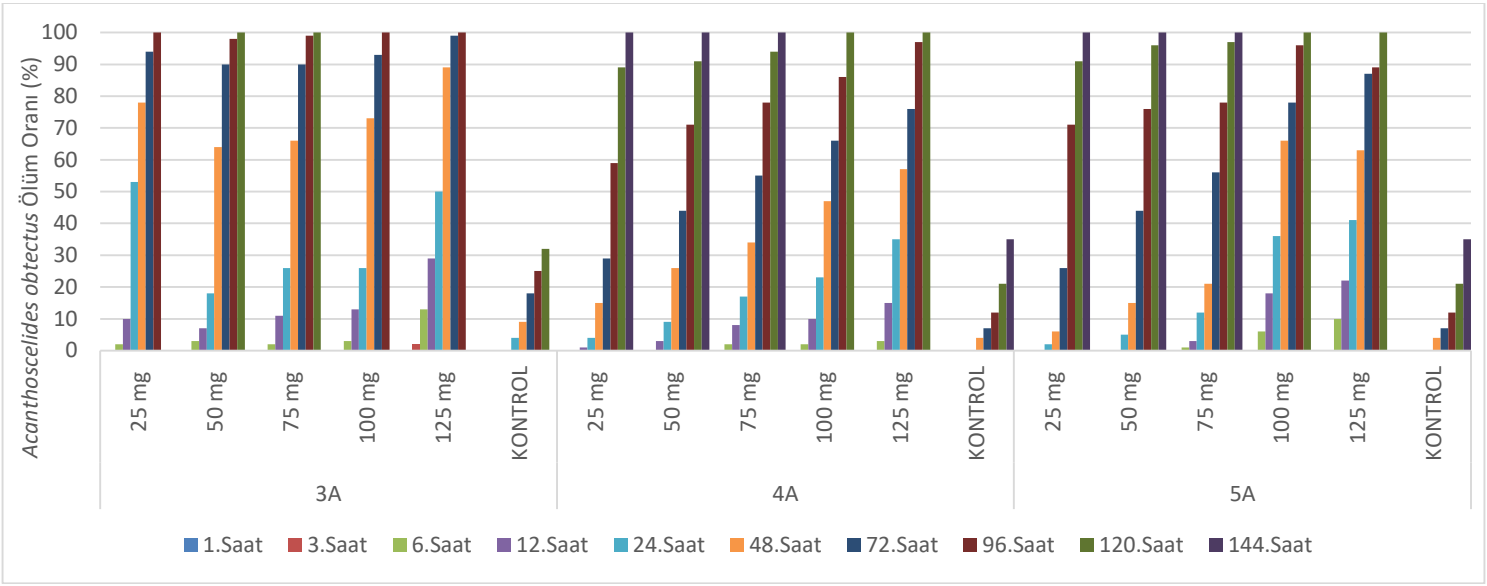


Şekil 3.
4A zeolitin farklı dozlarının Acanthoscelides obtectus erginlerinde meydana getirdiği ölüm oranı.



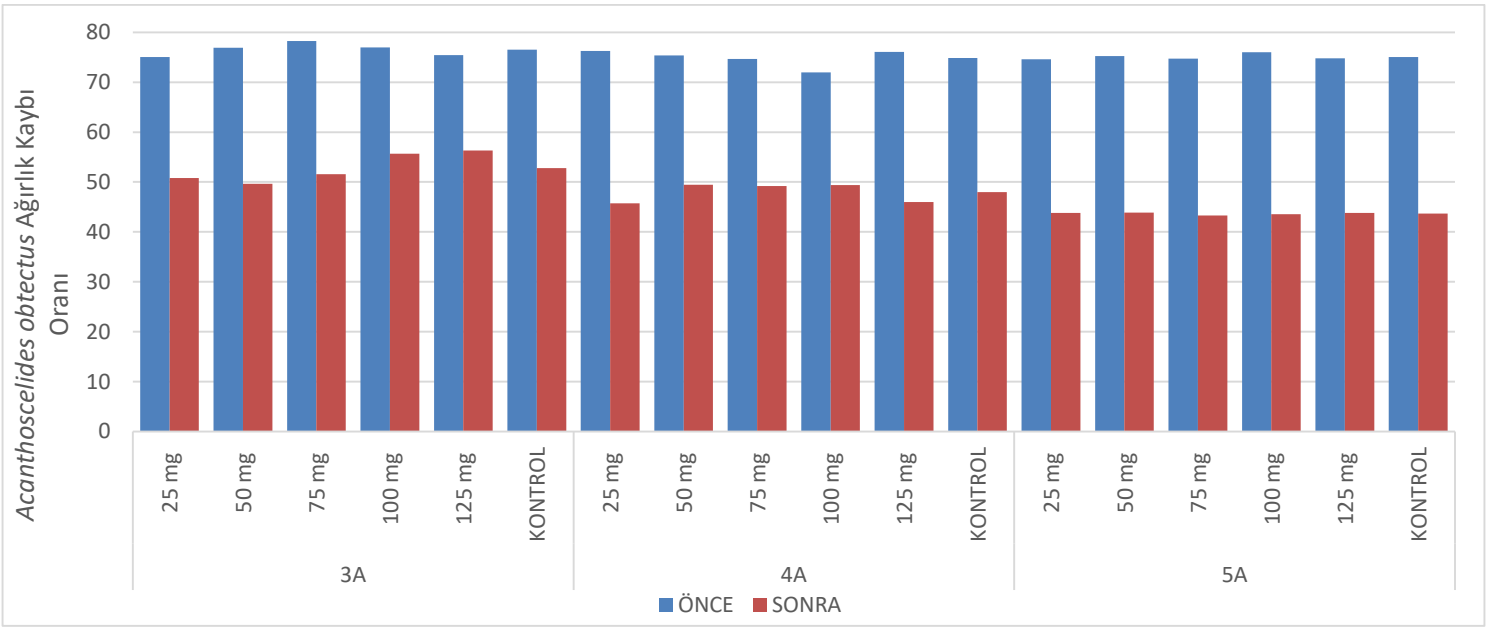
Şekil 4.

5A zeolitin farklı dozlarının Acanthoscelides obtectus erginlerinde meydana getirdiği ölüm oranı.



Şekil 5.

3A, 4A ve 5A zeolit uygulanan Acanthoscelides obtectus erginlerinin doz ve zamana bağlı ölüm oranları.



Şekil 6.

3A, 4A ve 5A zeolit uygulanan Acanthoscelides obtectus erginlerinin uygulama öncesi ve sonrası ağırlık kaybı oranları.

Nem tutucu özellikleri nedeniyle zeolitler böceğin vücut sıvısını emerek ağırlık kaybına sebep olmuştur. Zeolit uygulanmamış ve 125 mg 3A zeolit uygulanmış *A. obtectus*'a ait stereomikroskop ve elektron mikroskop görüntülerinde bu durum açıkça görülmektedir (Şekil 7, 8).

Literatür incelendiğinde, farklı zeolitlerin (doğal veya sentetik) farklı depo zararlıları üzerindeki insektisidal etkileri ile ilgili çalışmaların sonuçlarının bu çalışmayla benzerlik gösterdiği ve yüksek ölüm oranları ile sonuçlandığı görülmektedir (Kljajić vd., 2010a, b; Bodroza-Solarov vd., 2011; Andrić vd., 2012; Perez vd., 2012; Liska vd., 2017; Lü vd., 2017; Eroğlu vd., 2019; Işıklı, 2019).

Floros vd. (2018) tarafından yapılan çalışmada, kuru fasulye üzerine uygulanan çok yüksek kaliteli doğal zeolitlerin (ağırlıkça %92 klinoptilolit içeren zeolitik kaya) farklı dozlarının böcek öldürücü aktivitesi araştırılmış ve düşük dozlardaki doğal zeolitin farklı sıcaklık ve bağıl nem rejimlerinde *A. obtectus*'un kontrolü için umut verici olduğu bildirilmiştir. Kljajić vd. (2010a), doğal zeolitin (Minazel SP) *Sitophilus oryzae, Rhyzopertha dominica* ve *Tribolium castaneum*'a karşı böcek öldürücü etkinliğini araştırmışlardır. Elde edilen verilere göre; yedi günlük uygulamanın ardından 1 g'lık en yüksek uygulama oranında en yüksek etkinlik %62 ile *S. oryzae*'de görülmüştür. 14 ve 21 gün sonra en yüksek etkinliğe aynı uygulama oranıyla *T.*

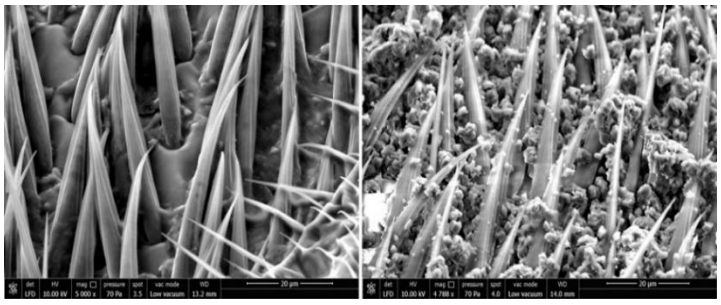
castaneum (%100), *S. oryzae* (%96-98) ve *R. dominica*'da (%70-82) ulaşılmıştır.

Zeolitlerin diatom toprağı ile beraber kullanımları da depo zararlılarına karşı oldukça etkilidir. Kljajić vd. (2010b), buğdaya 0,25, 0,50 ve 0,75 g/kg seçilmiş oranlarda uygulanan iki doğal zeolit formülasyonunun (Minazel plus ve Minazel) ve önerilen oranlarda uygulanan diatomlu toprak formülasyonunun (DE) (Protect-It™) böcek öldürücü etkinliğini test etmişlerdir. En yüksek yetişkin ölüm oranı en uzun maruz kalma süresinden sonra gözlenmiş; bu dönemde, üç zeolit dozaj oranının tümü ve önerilen DE dozajı, *S. oryzae'de %97-100 ve T. castaneum'da* %94-100 ölüme neden olmuştur.

Rumbos vd. (2016), ticari olarak temin edilebilen üç zeolit (Zeoprofeed Land 93, Zeofeed ve yığın zeolit) formülasyonunun buğdaydaki *S. oryzae*, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) ve *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) erginlerine karşı insektisidal potansiyelini araştırmıştır. Zeolitler, 250, 500 ve 1000 ppm olmak üzere üç doz oranında uygulanmış ve böcek ölümü, maruziyetten 2, 7, 14 ve 21 gün sonra değerlendirilmiştir. Zeolit uygulamasına en duyarlı tür *O. surinamensis,* en dirençli olan tür *T. confusum* olarak tespit edilmiştir. Test edilen üç zeolit formülasyonu arasında etkinlik bakımından önemli bir fark görülmemiştir. Zeolit parçacıklarının buğday, mısır, arpa ve pirinç tanelerinde farklı yapışma özelliği gösterdiği vurgulanmıştır.

Şekil 7.
Zeolit uygulanmamış (solda) ve 125 mg 3A zeolit uygulanmış (sağda) Acanthoscelides obtectus'a ait stereomikroskop fotoğrafları (Foto: T. Sarıçam, 2023).

Çalışmamızdaki sonuçlara benzer şekilde, Ibrahim & Salem (2019)'in yaptıkları çalışmada, nano zeolitin *T. confusum* ve *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)'a karşı böcek öldürücü potansiyeli araştırılmıştır. Nano zeolit ile muamele edilen buğday ve börülce tohumlarına maruz kalan *T. confusum* ve *C. maculatus* erginlerinin mortalitesinin yüksek doz ve maruz kalma süresine bağlı olarak arttığı gözlenmiştir. Nano zeolit kutikuladaki çizik ve yarıkların içine girmek suretiyle böceğin tüm vücudunu kaplayarak dehidrasyon yoluyla su kaybına yol açmaktadır. Sürücü (2020), bazı inert tozların (bentonit, halloysit, nobleit, kaolin, sepiolit, zeolit) buğday taneleri üzerinde laboratuvar koşullarında *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)'a karşı toksik ve davranışsal etkilerini araştırmıştır. Elde edilen veriler, nobleit ve kaolin inert tozlarının *S. granarius* üzerinde toksik ve davranışsal etkisi olduğunu göstermiştir.



Şekil 8.
Zeolit uygulanmamış (solda) ve 3A zeolit uygulanmış (sağda) Acanthoscelides obtectus'a ait taramalı elektron mikroskop görüntüleri.

Sonuç ve Öneriler

Sonuç olarak, zeolitlerin yemeklik tane baklagillerde depo zararlılarına karşı kullanımına yönelik araştırmalar yeni başlamış bir konu olmasına rağmen yapılan çalışmalar oldukça başarılı sonuçlar vermiştir. Bu çalışmada kullanılan zeolitlerin nem tutucu özelliği, maruz kalma süresine bağlı olarak böceklerde ağırlık kaybına neden olmuş ve böceğin vücut sıvısını azaltarak dehidrasyon yoluyla ölümüne sebep olmuştur. 3A, 4A ve 5A zeolitler por yapılarından dolayı farklılık gösterdikleri için böcek vücudunda bulunan farklı büyüklükteki moleküllerin geçişini engellemektedir. Çalışmada kullanılan zeolitler arasında en küçük çapa sahip olan ve yoğun yapışma özelliği gösteren 3A zeolit çeşidinin diğer zeolitlere kıyasla oldukça etkili bir öldürme potansiyeline sahip olduğu belirlenmiştir. Zeolitlerin, vücut yüzeyine yapışarak böceklerin hareketini kısıtladığı, normal yaşamsal faaliyetlerini ve uçmalarını zorlaştırdığı gözlenmiş, bu durum dişilerde yumurta bırakma davranışını da olumsuz etkilemiştir. Depo zararlılarının popülasyonlarını kontrol altına almada 3A, 4A ve 5A zeolitlerin etkili bir yöntem olarak kullanılabileceği bu çalışma ile tespit edilmiştir. Türkiye'nin doğal zeolit (Klinoptilolit) rezervlerine sahip olması depo zararlıları ile mücadelede yapılacak olan çalışmalar açısından oldukça önemlidir. Ülkemizde ve dünyada depolanmış ürün zararlılarıyla mücadelede kalıntı bırakmayan, toksisitesi düşük ve hastalık oluşturmayan uygulamaların geliştirilmesinde ve özellikle tahıl ürünlerinin muhafaza edilmesinde kullanılacak etmenler büyük önem taşımaktadır. Baklagil tohum böceklerine karşı doğal inert tozların kullanımı sayesinde, çevreyle dost alternatif bir yol

oluşturularak zararlıların popülasyonlarının kontrol altına alınması sağlanabilecektir. Bu durum gıda güvenliğini de olumlu yönde etkileyen önemli bir faktör olarak değerlendirilmektedir. Zeolitler, çevre dostu olarak gıdalarda kalıntı sorunlarına neden olmamaktadırlar. Kodeks Alimentarius Komisyonu (KAK), gıdalarda zararlı böcekler ile mücadelede zeoliti tavsiye etmiştir. Bitki zararlıları ve hastalık kontrolü için izin verilen maddeler içinde zeolit de yer almaktadır. İleride yapılacak benzer çalışmaların sayısının artması ile depo zararlıları ile mücadelede umut verici sonuçlar elde edilecektir.

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Yazar Katkıları: Konsept - T.S., E.G.A.; Tasarım - T.S., E.G.A.; Denetleme - E.G.A.; Kaynak Sağlama - T.S.; Materyaller - T.S., E.G.A.; Veri Toplama ve/veya İşleme - T.S.; Analiz ve/veya Yorumlama - T.S., E.G.A.; Literatür Taraması - T.S., E.G.A.; Yazım - T.S., E.G.A.; Eleştirel İnceleme - E.G.A.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Teşekkür: Elektron mikroskop görüntüleri için SDÜ Yenilikçi Teknolojiler Araştırma ve Uygulama Merkezine teşekkür ederiz.
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Vegetative Propagation of Wild *Prometheum sempervivoides* (Fischer ex M. Bieb.) H. Ohba by Leaf Cutting: Effects of Auxin and Some Substances

Yabani *Prometheum sempervivoides* (Fischer Ex M. Bieb.) H. Ohba'nın Yaprak Çeliği Yoluyla Vejetatif Çoğaltılması: Oksin ve Bazı Maddelerin Etkileri

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ABSTRACT

The aim of the research is to determine the effects of different rooting contents [control (water), plant growth regulator-rooting hormone (H_1: 0.25 g l^{-1}, H_2: 0.50 g l^{-1} and H_3: 1.0 g l^{-1}), cinnamon powder (T) and *Aloe vera* gel (AV)] and two different planting times on the rooting of leaf cuttings prepared from the stock mother plants of wild *Prometheum sempervivoides* (Fisch. ex M. Bieb.) H. Ohba taxon. Observations and measurements of vitality rate (%), number of rooted cuttings (NR), number of callusing cuttings (NC), maximum root length (MRL), rooting rate (%) and rooting scale (1-5) were made on cuttings whose rooting was completed. The results were evaluated statistically. The most positive rooting effect was determined in the H_3 application, which is the highest dose of the commercial rooting hormone tested in the study. Cinnamon powder application was determined as a more effective natural extract for rooting leaf cuttings of *P. sempervivoides* when compared to *Aloe vera* gel application. At the end of our study, in which the effects of different planting times were also examined, it was determined that planting the leaf cuttings of *P. sempervivoides* as soon as they were taken from the stock mother plant had a positive effect on rooting. The very good quality roots were observed in H_1(0 h), H_2 (0 h), H_3 (0 h) and H_3 (24 h) applications. These applications can be recommended for rooting the leaf cuttings of *P. sempervivoides* succulent plant. It was also concluded that *P. sempervivoides* can be reproduced by vegetative propagation method using leaf cuttings.

Keywords: *P. sempervivoides,* rooting, *Aleo vera,* wild flower, ornamental plants, hormone

ÖZ

Doğal olarak yetişen *Prometheum sempervivoides* (Fisch. ex M. Bieb.) H. Ohba, taksonuna ait anaç bitkilerden hazırlanan yaprak çeliklerinin köklenmesinde, farklı köklendirme içeriklerinin [kontrol (su), bitki gelişim düzenleyici-köklendirme hormonunun üç farklı dozu (H_1: 0.25 g l^{-1}, H_2: 0.50 g l^{-1} ve H_3: 1.0 g l^{-1}), tarçın tozu ve *Aleo vera* jeli] ve iki farklı dikim zamanının uygulanmasının etkilerini saptamak araştırmanın amacını oluşturmaktadır. Canlılık oranı (%), köklü çelik sayısı (NR), kallus oluşturan çelik sayısı (NC), maksimum kök uzunluğu (MRL), köklenme oranı (%) ve köklenme ölçeği (1-5) parametreleri köklenmesi tamamlanan çelikler için yapılmıştır. Sonuçlar istatistiksel olarak değerlendirilmiştir. En olumlu köklenme etkisi, araştırmada kullanılan ticari köklendirme hormonunun en yüksek dozu olan H_3 uygulamasında belirlenmiştir. *P. sempervivoides* yaprak çeliklerinin köklenmesinde toz tarçın uygulamasının *Aloe vera* jel uygulamasına göre daha etkili bir doğal ekstrakt olduğu belirlenmiştir. Farklı dikim zamanlarının etkilerinin de incelendiği çalışmamız sonunda, *P. sempervivoides* yaprak çeliklerinin ana bitkiden alınır alınmaz dikilmesinin köklenme üzerine olumlu etkisi olduğu belirlenmiştir. H_1(0 h), H_2 (0 h), H_3 (0 h) ve H_3 (24 h) uygulamalarında çok kaliteli kökler gözlenmiştir. Bu uygulamalar *P. sempervivoides* sukulent bitkisinin yaprak çeliklerinin köklendirilmesi için önerilebilir. Ayrıca *P. sempervivoides*'in yaprak çelikleri kullanılarak vejetatif çoğaltma yöntemiyle çoğaltılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: *P. sempervivoides,* köklenme, *Aloe vera*, yabani çiçek, süs bitkileri, hormon

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Introduction

Succulent plants, which have an important place among ornamental plants, have been known and used by people since ancient times. In recent years, especially after the changing living conditions with the COVID-19 pandemic (Khayru, 2021), the popularity of succulent plants as ornamental plants has increased. In this increase, succulent plants' unique geometric shapes that form rosettes and their ability to hold high humidity levels play a major role (Cabahug et al., 2018). In addition, when many succulent plants grown in their natural environment are examined, it can be observed that they can withstand extreme conditions and have low irrigation requirements (Sarı, 2021).

Due to the global climate change and the intense drought expected (Bhattacharya, 2019) to be experienced due to this, practices are carried out to reduce water consumption all over the world (Hatfield & Dold, 2019; Zhao et al., 2020; Tahat et al., 2020). Prioritizing the use of natural species in landscape areas due to the decreasing rainfall in recent years is a sustainable method in terms of rational use of water. *Prometheum sempervivoides*, which has eye-catching red flowers, stays in flower for 3 months and during the flowering period, the star-shaped flowers formed by pollen dusts of yellow on red make the flowers of this species even more remarkable. In addition, the color of the flowers of this species turning to burgundy in the seed period after flowering (Fig. 1a, b) and the fact that the plant can attract attention in this color for a long time are among the features that can have a long-lasting effect on designs (Parlakova Karagöz et al., 2020). In this context, it is important to increase in the cultivation studies of *P. sempervivoides*, one of the natural succulent plant species. According to our literature review results, no study was found on the germination or propagation of *P. sempervivoides* species. In addition, it is necessary to increase in the production of these plants by introducing them to the ornamental plants sector, in the shortest possible time and at the highest rate, by revealing the reproduction techniques, transferring them to applications or carrying out breeding studies.

Propagation by cuttings is a method frequently used in vegetative propagation of succulent plants (Anton & Cristescu, 2009; Cabahug et al., 2018). Factors such as rooting medium, plant part to be used as cutting, rooting stimulants, drying time are affected rooting success in the propagation of succulent plants by leaves (Paterson & Rost, 1978; Jeong, 1999; Mihaela et al., 2011; Cabahug et al., 2016a; Khalid & Ahmed, 2022). The use of hormones as rooting stimulants in order to prevent the drying and death of the prepared cuttings, to encourage rapid rooting (Khalid & Ahmed, 2022) and to obtain from rooted-cutting seedlings in series has an important place in horticultural crops. Auxins are the most commonly used of these hormones (Ibrahim et al., 2015; Chaudhari et al., 2018). Synthetic auxin applications are widely used in cutting rooting (Khalid & Ahmed, 2022). It has been reported that synthetic rooting hormones such as indole-3-butyric acid (IBA) and other synthetic growth regulators can affect the environment and people, pollute the environment and cause harmful effects on living things through the food chain (El-Sherif et al., 2017; Sezgina & Kâhya, 2018).

Several studies have reported that biostimulants that can promote physiological processes in plant cells can be a good alternative to the important and widely used synthetic rooting hormones (Hasan et al., 2019; Hamza & AL-Taey, 2020; Al-Khafajy et al., 2020; Khalid & Ahmed, 2022). It has been reported that biostimulants such as coconut water, honey, willow tea (Shield, 2012), *Aloe vera* gel (Hamouda et al., 2012; Uddin et al., 2020), cinnamon powder (Xing et al., 2010), humic acid, seaweed extract (Rajan & Singh, 2021) can be an alternative to widely used synthetic rooting hormones. Comparison of the effects of both synthetic and biological rooting stimulants on the rooting of the leaf cutting of *P. sempervivoides* is important in revealing the propagation techniques, transferring them to applications and culturing this species in the shortest possible time and at the highest rate.

In this context, the aim of the study is to determine the rooting of leaf cuttings of naturally grown *P. sempervivoides* and the effects of different rooting contents on the root formation and rooting performance of the leaf cuttings. With the results of the study, it is aimed to provide information to the literature about the propagation method of *P. sempervivoides*, which has the potential to be an ornamental plant (Dilaver, 2001; Arslan, 2010; Erduran et al., 2010; Gülbağ, 2016; Dilaver et al., 2020; Parlakova Karagöz et al., 2020), to provide data to the cultivation studies and breeding studies and to contribute to the proposal of landscape use opportunities.

Materials and Methods

Description of the Study Area

The research was carried out in a heated glass construction research greenhouse between November 2022 and January 2023. The glass greenhouse where the experiment was conducted has a composite roof, ventilation from the side and the roof, and a steel construction. Temperatures inside the greenhouse are between 17.6 °C and 33.1 °C. Humidity rates in the greenhouse showed values varying between 10% and 46%.

Experimental Materials

In the study, seeds of *Prometheum sempervivoides* (Fischer ex M.Bieb.) H.Ohba, grown naturally in Erzurum city and its

surroundings (Türkiye), were collected (Fig. 1 b), and new plants were grown by germinating in the research greenhouse. These plants grown from seed were used as stock mother plants from which leaf cuttings were taken (Figure 1c). Leaf cuttings taken from stock mother plants in appropriate sizes were used as the main plant material of the study (Fig. 2 a, b).

For the rooting of leaf cuttings, the active ingredient of the commercial plant growth regulator-rooting hormone product is 0.52% 1-Naphtaleneacitic Acid (NAA) + 0.51% 3-Indole Butyric Acid (IBA). This product was obtained from a private company. Three different doses of this commercial product were prepared and applied in the relevant experimental group. Cinnamon powder (Fig. 1d) was obtained from any market, while *Aloe vera* gel was obtained from plants grown in the greenhouse as potted plants at the time of use (Fig. 1e).

The viols in which the leaf cuttings are planted are 24-cell plug trays (viol), 320x500x82 mm in size, the lower diameter of each cell is 55 mm and the upper diameter is 75 mm. Ornamental peat with a pH of 5.06 was used as the rooting medium used in the viols. The cuttings were kept under observation in viols until the experiment was completed.

Figure 1.
Naturally grown wild Prometheum sempervivoides (Fischer ex M.Bieb.) H.Oh. (a); Stage in which the seeds used in the propagation of stock mother plants are collected (b); view from greenhouse grown stock mother plants (c); cinnamon powder application (d), Aloe vera gel application (e)

Table 1.
The details of the applications in the experiment

Application code	Definition	Application code	Definition
Control (0 h)	Control (As soon as the leaf cutting was prepared, it was dipped in tap water and planted.)	Control (24 h)	Control (Leaf cuttings were prepared and kept in room conditions for 24 hours, then dipped in tap water and planted.)
H1 (0.25 g l^{-1}) (0 h)	Leaf cuttings were prepared and planted immediately without waiting and the solution prepared with 0.25 g l^{-1} plant growth regulator-rooting hormone was applied as life water.	H1 (0.25 g l^{-1}) (24 h)	Leaf cuttings were prepared and planted after waiting for 24 hours at room conditions and plant growth regulator-rooting hormone (H1: 0.25 g l^{-1}) solution was applied as life water.
H2 (0.5 g l^{-1}) (0 h)	Leaf cuttings were prepared and planted immediately without waiting and the solution prepared with 0.50 g l^{-1} plant growth regulator-rooting hormone was applied as life water.	H2 (0.5 g l^{-1}) (24 h)	Leaf cuttings were prepared and planted after waiting for 24 hours at room conditions and plant growth regulator-rooting hormone (H2: 0.50 g l^{-1}) solution was applied as life water.
H3 (1.00 g l^{-1}) (0 h)	Leaf cuttings were prepared and planted immediately without waiting and the solution prepared with 1.00 g l^{-1} plant growth regulator-rooting hormone was applied as life water.	H3 (1.00 g l^{-1}) (24 h)	Leaf cuttings were prepared and planted after waiting for 24 hours at room conditions and plant growth regulator-rooting hormone (H3: 1. 005 g l^{-1}) solution was applied as life water.
T (0 h)	Leaf cuttings were prepared and the base of the cuttings was soaked, dipped in cinnamon powder and planted immediately without waiting.	T (24 h)	Leaf cuttings were prepared and kept at room conditions for 24 hours, then the base of the cuttings was soaked, dipped in cinnamon powder and planted.
AV (0 h)	Leaf cuttings were prepared and the base part of the cuttings was dipped in the gel from the fresh *Aloe vera* leaf and planted immediately without waiting.	AV (24 h)	Leaf cuttings were prepared and kept at room conditions for 24 hours, then the base of the cuttings was soaked, dipped the gel from the fresh *Aloe vera* leaf and planted.

Experimental Design

The research was established according to factorial (different rooting content x 2 planting times) arrangement in a randomized plot design. The experiment was established with 4 replications and a total of 288 leaf cuttings were used, 6 for each replication.

The first group of the experiment consisted of control, plant growth regulator-rooting hormone, 3 different doses (0.25 g /L, 0.50 g /L and 1.00 g /L), cinnamon powder and Aloe vera gel applications. The second group of the experiment is the different planting times: The rooting ingredients are applied as soon as the cuttings are taken (0h) and the different rooting contents are applied after the cuttings are taken and kept at room conditions for 24 hours (24 h). The details of the applications were created as in Table 1.

Establishment of the Experiment

Preparation of leaf cuttings: The branches which it was decided to take cuttings from the stock mother plants are determined and the stems are separated from the stock mother plants. The dried, wilted leaves on the stem were cleaned and the leaves were carefully plucked from their bases. Care was taken to leave the bottom parts on the leaf cuttings (Fig. 2 a, b). For the application groups belonging to the 24 h planting time, the leaves were carefully plucked one by one from the stock mother plants and kept on blotting paper at room conditions for 24 hours (Hodzic 2020; Jeong 1999; URL-1). At the other planting time (0 h), the leaf cuttings in the experiment group were planted without waiting.

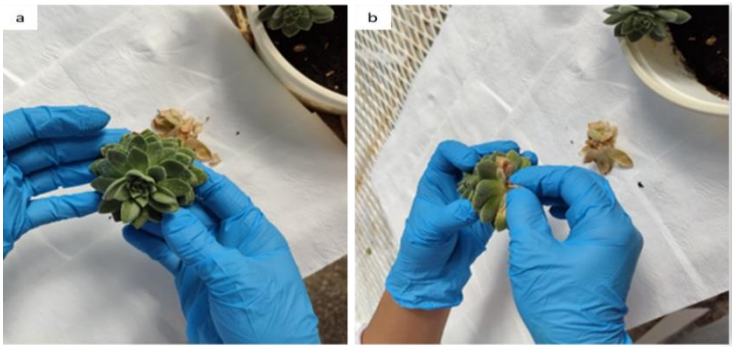

Figure 2.
Preparation of cuttings by carefully separating the leaves from the stock mother plants (a, b)

Preparation of plant growth regulator-rooting hormone doses: 0.25 g, 0.50 g and 1.00 g of the hormone were weighed separately and each was dissolved in 1 liter of water.

Planting of leaf cuttings by making applications: Leaf cuttings belonging to the control application were dipped in tap water (controls at two different planting times) and planted in the medium. Cuttings were planted in viols to be applied at different doses of plant growth regulator-rooting hormone, and certain doses of hormone solutions were given as life water (Fig. 3 a). For T (0h / 24 h) application, the base parts of the leaf cuttings were dipped in cinnamon powder and planted in peat medium (Fig. 1d). For the AV (0h / 24 h) application, the base of the cuttings was dipped into the gel extracted from the leaves of the *Aloe vera* plant, planted in the environment and the experiment setup was carried out (Fig. 1e) (Table 1).

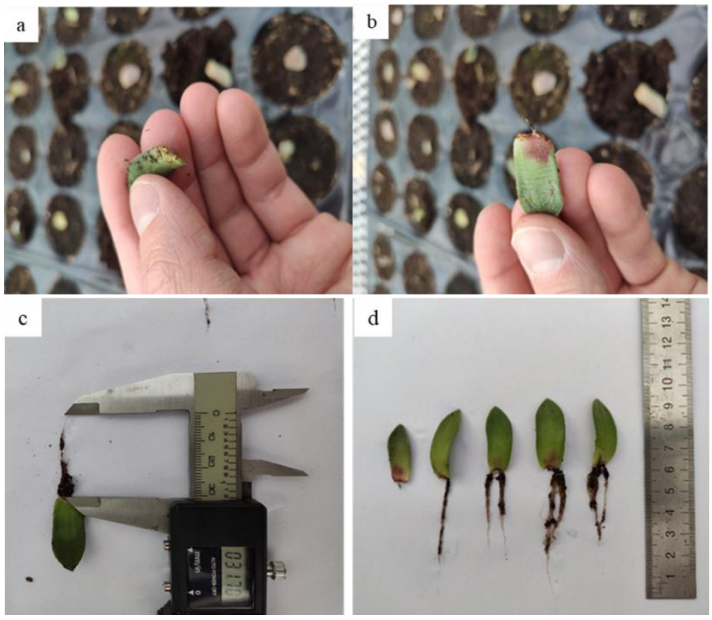

Figure 3.
Callus formation (a), callus and first adventitious root (b) in P. sempervivoides leaf cuttings; measurement of maximum root length (c); the scale prepared with the rooting status of the cuttings in the experiment (d)

Viols were filled with moist peat, and a leaf cutting was planted in each viol cell. Under greenhouse conditions, plant leaf cuttings were rooted in viols at natural day length and light intensity. As the most important maintenance process, water was sprayed on the leaf cuttings twice a day. This application was made in order to prevent moisture loss. Irrigation is done in a way that does not make any difference between applications in case of need.

Evaluated Parameters at the End of the Experiment

Vitality rate (%), number of rooted cuttings (NR), number of callusing cuttings (NC) (Fig. 3a, b), maximum root length (MRL) (Fig. 3c), rooting rate (%) and rooting scale (1-5) (Fig. 3d) such as parameters were measured and evaluated in cuttings rooted in rooting medium (approximately 2 months). Based on the studies of Wang et al. (2005) and Cabahug et al. (2016b), the visual assessment scale according to the 1-5 scale was adapted to take into account only the rooting status of the cuttings. This revised scale is "Very weak root quality = 1; Substandard root quality, not sellable = 2; Roots are of good quality, salable = 3; Very good quality roots = 4; Excellent root quality = 5."

Evaluation of Results

The results were evaluated according to the analysis of variance (ANOVA) in SPSS (Statistical Package for Social Sciences, Version 20.0) statistical program and given in tables after calculating their arithmetic means and standard deviations. Duncan's multiple comparison test (p=0.05 or 0.01) was used to determine the significance of the difference between treatments.

Results and Discussion

In the study, *Prometheum sempervivoides* (Fischer ex M. Bieb.) H. Ohba seeds that grow naturally in Erzurum city (Türkiye) and its surroundings were collected, new plants were grown by germinating and these grown plants were used as stock mother plant. It was observed that the emergence rate of wild seeds of the related species planted in the peat during the germination stage was very low (about 1%). At the end of the experiment, the effects of our applications on the rooting parameters of *P. sempervivoides* leaf cutting were investigated. The results of the effects on the parameters of cutting viability, number of rooted cuttings and number of cuttings forming callus of using different planting times and different rooting contents in rooting of leaf cuttings belonging to the *P. sempervivoides* taxon were given in Table 2.

In general, it is recommended to wait a few days for planting after the cuttings are prepared in succulents for callus formation (Hodzic 2020; Jeong 1999; URL-1). However, this may vary depending on the plant species. The effects of different planting times on the best rooting in the planting technique of the leaf cuttings of the *P. sempervivoides* succulent plant, which is a natural species and has not been studied in its propagation, were also evaluated in this study. In general, it was concluded that planting the leaf cuttings of *P. sempervivoides* succulent immediately after leaving the stem (0 h) has a positive effect on the rooting of the cuttings and this method is also positive for all rooting parameters examined in the experiment. In a study conducted by Ekici (2020), the effects of different planting times on rooting were investigated, including immediate planting and planting after three days of leaf cuttings of the *Sedum album* L. species. It has been reported that the highest rooting rate and number of rooted

shoots were obtained from cuttings planted by waiting for three days. Again, as a result of the same research, the highest plant height, plant diameter, shoot number, shoot length, root length, fresh weight and dry weight were determined in the immediately planted application groups. The findings of our study show parallelism with the findings of the study reported by Ekici (2020).

With the application of different rooting ingredients to leaf cuttings, the highest viability rate was found in the cinnamon powder (T) application with 66.83%, and the lowest viability rate was determined in the *Aloe vera* gel (AV) application (46.33%). The evaluation made according to different planting times, the viability rate of the cuttings in the experimental group, which were planted without waiting after the leaf cuttings were plucked from the stem (0 h), was determined to be higher than those in the experimental group planted by waiting 24 hours (24 h). The highest viability rate was determined in the H_3 application in the cuttings belonging to the 0 h planting time, and it was determined that the viability rate increased in 1.15 times with the H_3 application compared to the control (0 h) application. T, AV and control applications were in the same statistical group. When the effects of different rooting contents on cuttings belonging to 24 h planting time were evaluated, the highest viability rate was determined with cinnamon powder application (T). Control (24h) application and AV application are shown with the same letter, and there is no significant difference between these two applications in terms of viability (Çizelge 2). It is known that synthetic auxin applications are widely used (Khalid & Ahmed, 2022) to promote rapid rooting of cuttings and to obtain from effective roots. We believe that the highest viability rate obtained with the H_3 application can support the knowledge that the effects of synthetic hormones are high and explain the reason for the widespread use of synthetic hormones. Cinnamon application (T) on leaf cuttings of *P. sempervivoides* belonging to the 24 h planting time caused an increase in the viability rate of the cuttings. Surjushe et al. (2008) stated that some plant extracts such as ginger, licorice and cinnamon are used as alternatives to promote rooting (Hameed et al., 2019). Cinnamon contains cinnamic acid, cinnamyl acidate, cinnamon aldehyde, cinnamyl alcohol, tannin, eugeuol and minerals (Gunjan & Anart, 2009). In addition, cinnamon powder contains salicylic acid, which helps the cuttings to root (Shidiki et al., 2019). The explanation for this increase may be due to the rich content of natural plant substances containing natural antioxidants (Mirihagalla & Fernando, 2020)

Table 2

The effects of applications on the viability rate (%), rooted shoot number and callus forming cutting number of P. sempervivoides leaf cuttings

Treatments	Viability rate (%)			Number of rooted cuttings (NR)			Number of callusing cuttings (NC)		
	0 h	24 h	Mean	0 h	24 h	Mean	0 h	24 h	Mean
Control	81.33 ^{bcd}**	18.00 ^{c}***	49.67 ^{D}***	13.33 ^{ns}	1.67 ^{b}*	7.50 ^{BC}**	6.00 ^{b}*	2.33 ^{b}***	4.17 ^{B}***
H1	83.67 ^{bc}	26.00 ^{b}	54.83 ^{C}	14.67	3.00 ^{ab}	8.83 ^{AB}	5.67 ^{b}	3.00 ^{b}	4.33 ^{B}
H2	88.00 ^{ab}	26.00 ^{b}	57.00 ^{BC}	15.33	3.00 ^{ab}	9.17 ^{AB}	7.00 ^{ab}	2.00 ^{b}	4.50 ^{B}
H3	93.67 ^{a}	27.33 ^{b}	60.50 ^{B}	16.67	3.00 ^{ab}	9.83 ^{A}	8.67 ^{a}	1.67 ^{b}	5.17 ^{B}
T	79.67 ^{cd}	54.00 ^{a}	66.83 ^{A}	16.00	4.67 ^{a}	10.33 ^{A}	8.00 ^{a}	9.67 ^{a}	8.83 ^{A}
AV	74.67 ^{d}	18.00 ^{c}	46.33 ^{D}	11.67	2.00 ^{b}	6.83 ^{C}	7.33 ^{ab}	2.33 ^{b}	4.83 ^{B}
Mean	83.50 ^{A}***	28.22 ^{B}		14.61 ^{A}***	2.89 ^{B}		7.11 ^{A}***	3.50 ^{B}	
DRCxPT interaction		$p < .000$			$p < .003$			$p < .001$	

ns: NS: insignificant at p>0.05, statistically significant at the * $p < .05$, ** $p < .01$ and ***$p < .001$ probability level. Note: Values followed by the same small or capital letters are not significantly (5%) different within the columns. DRC: Different Rooting Content; PT: Planting Times

When the general average of the effects of different rooting contents on the number of rooted cuttings is examined, the highest number of rooted cuttings was determined in the applications of cinnamon powder (T) with 10.33 and H_3 with 9.83. However, in terms of the number of rooted cuttings, T and H_3 applications and H_1 and H_2 applications were in the same statistical group. In the evaluation made according to different planting times, the number of rooted cuttings (14.61) in the experimental group planted without waiting after the leaf cuttings were plucked from the stem (0 h) was determined to be higher than the cuttings in the group planted by waiting 24 hours (24 h) (2.89). The effects of different rooting contents on the number of rooted cuttings in the cuttings belonging to the 0 h planting time were found to be statistically insignificant (p>0.05). The highest number of rooted cuttings in cuttings belonging to 24 h planting time was determined as T application. T application and H1, H_2 and H_3 applications are indicated with the same letter, and there is no significant difference between these applications in terms of the number of rooted cuttings (Table 2). In the

study of Ekici (2020), it was determined that the effect of different IBA doses on the propagation of *Sedum album* species by cuttings was not statistically significant ($p > .05$) on all parameters examined. As a result of the same research, higher values were obtained from parameters such as plant height, shoot length, plant diameter, rooted shoot number, root length at the control dose (0 ppm), and it was reported that a higher rooting rate was obtained from cuttings with 50 ppm dose compared to other doses. Khalid and Ahmed (2022) compared the effects of some natural substances (*Aloe vera*, cinnamon and honey) and rooting hormone naphthalene acetic acid (NAA) on the rooting of two fig (*Ficus carica*) cuttings. It was reported that the effect of using natural substances and naphthalene acetic acid (NAA) on rooting properties was similar except for the root number parameter. These findings (Khalid & Ahmed, 2022) showed parallelism with our present study results.

According to the general averages of the effects of different rooting contents on the number of callus-forming cuttings, it was determined that the highest number of callused cuttings was in the T application, with an average of 8.83. While numerically the lowest number of callused steel was obtained from the H_3 application, all applications except the T application were in the same statistical group as the control application. In the evaluation made according to different planting times, the number of callus-forming cuttings (7.11) in the experimental group planted without waiting after the leaf cuttings were plucked from the stem (0 h) was determined to be higher than the cuttings in the group planted by waiting 24 hours (24 h) (3.50). If the effects of different rooting contents on the number of callus-forming cuttings in the 0 h planting time are evaluated, it was determined that the number of callus-forming cuttings in H_3 application was the highest and the number of callus-forming cuttings increased in 1.45 times when compared to the control (0h) application. However, there was no statistically significant difference between the H_3 application and the T application. If the effects of different rooting contents on the number of callus-forming cuttings in the 24 h planting time are evaluated, the highest number of callus-forming cuttings was determined with the T application. Except for the T application, all different rooting content applications and the control application were indicated with the same letter, and no significant difference was found between these applications in terms of the number of callus forming cuttings (Table 2).

When the general average of the effects of different rooting ingredients on the maximum root length is examined, the highest maximum root length was determined in the H_3 application, while the lowest maximum root length was obtained from the AV application. In the evaluation made according to different planting times, the maximum root length (39.34) of the cuttings in the experimental group, which were planted without waiting after the leaf cuttings were plucked from the stem (0 h), was determined to be higher than those in the group planted by waiting 24 hours (24 h) (19.09). When the effects of different rooting contents on the maximum root length of the cuttings at 0 h planting time are evaluated, it was determined that the maximum root length of the H_3 application was higher and the maximum root length increased in 3.06% when compared to the control (0h) application. However, H_3 application and H1 and control (0 h) applications were in the same statistical group. In the evaluation made within 24 h planting time, the highest maximum root length was determined with the H_3 application. With the H_3 application, the maximum root length increased in 146.78% compared to the control (24 h) application. The control (24 h) application and H_1, T and AV were denoted with the same letter, and no significant difference was found between these applications in terms of maximum root length (Table 3). As a result of a previous study, *Aloe vera* gel was shown to be more effective than IBA in rooting *Vitex diversifolia* semiwood cuttings (Shidiki et al., 2019). In the current study, the highest maximum root length was obtained from the application of the highest dose (H_3) of rooting hormone containing 0.52% 1-Naphtaleneacitic Acid (NAA) + 0.51% 3-Indole Butyric Acid (IBA). The effects of auxin on rooting of cuttings are known in previous studies, and the result obtained from our study is an expected result. In addition, this increase may have been achieved by using two different auxin derivatives together. It was determined that there was no statistically significant difference for the highest maximum root length parameter between the Control, H_1, T and AV applications in the group planted by waiting 24 hours (24 h). Uddin et al. (2020) investigated the effect of natural substances on grapevine cuttings by comparing them with synthetic hormones. While the longest root length was observed in *Aloe vera* gel application, it was followed by IBA application and they reported that the lowest root length was measured in the control application. In our study, the maximum root length was determined in the application of the highest dose of auxin hormone. It was determined that there was no significant difference between the other natural substances used in our study and the control application.

When the general average of the effects of different rooting ingredients on the rooting percentage is examined, the highest rooting percentage was determined in T application, while the lowest average rooting percentage was determined in AV and control applications. In the evaluation made according to different planting times, the highest rooting percentage (89.35) was obtained from the cuttings in the experiment group, which were planted without

waiting after being plucked from the stem (0 h). As a result of the evaluation made if it includes 0 h planting time, it was determined that the rooting percentage was higher in H_3 and T applications and the rooting percentage increased in 24.13% when compared to the control (0h) application. As a result of the evaluation made in 24 h planting time, the highest rooting percentage was determined with T application. With the T application, the rooting percentage increased in 3.58 times when compared to the control (24 h) application (Table 3). Hameed et al. (2019), in a study examining the effect of cinnamon extract on root formation and vegetative growth of cuttings taken from *Melaleuca viminalis* L., they found that the application with cinnamon extract had a higher rooting rate than the control. In another previous study (Shidiki et al., 2019), *Aloe vera* leaf extract, IBA, coconut water and *Aloe vera* leaf extract + coconut water were used to propagate semiwood cuttings of *Vitex diversifolia* and *Cordia milleneii*. *Aloe vera* + coconut water has been reported to improve rooting with a higher rooting percentage than IBA (Shidiki et al., 2019). In our study, *Aloe vera* gel was used alone. Combining different natural substances can also be tried in future studies.

Table 3

The effects of applications on the average root length and rooting rate of P. sempervivoides leaf cutting

Treatments	Maximum root length (MRL) (mm)			Rooting rate (%)		
	0 h	24 h	Mean	0 h	24 h	Mean
Control	42.51 ^{ab}***	14.54 ^{c}***	28.53 ^{C}***	80.56 ^{bc}**	16.67 ^{c}***	48.61 ^{C}***
H1	41.25 ^{ab}	15.11 ^{c}	28.18 ^{C}	84.72 ^{bc}	25.00 ^{b}	54.86 ^{BC}
H2	39.96 ^{bc}	21.32 ^{b}	30.64 ^{B}	91.67 ^{ab}	20.83 ^{bc}	56.25 ^{B}
H3	43.81 ^{a}	35.88 ^{a}	39.84 ^{A}	100.00 ^{a}	19.44 ^{bc}	59.72 ^{B}
T	38.04 ^{c}	14.40 ^{c}	26.22 ^{D}	100.00 ^{a}	59.72 ^{a}	79.86 ^{A}
AV	30.45 ^{d}	13.28 ^{c}	21.86 ^{E}	79.17 ^{c}	18.06 ^{bc}	48.61 ^{C}
Mean	39.34 ^{A}***	19.09 ^{B}		89.35 ^{A}***	26.62 ^{B}	
DRCxPT interaction		$p < .000$			$p < .000$	

ns: NS: insignificant at p>0.05, statistically significant at the * $p < .05$, ** $p < .01$ and *** $p < .001$ probability level. Note: Values followed by the same small or capital letters are not significantly (5%) different within the columns. DRC: Different Rooting Content; PT: Planting Times

Table 4.

Rooting quality scores of cuttings according to applications (1-5)

Treatments	0 h	24 h
Control	5	3
H1	4	3
H2	4	3
H3	4	4
T	3	2
AV	2	3

As a result of the applications, the quality scores of the cuttings were given according to their rooting status (Table 4).It was observed that the cuttings belonging to the control (0 h) application had very good rooting and rooting quality, and therefore, a rooting quality score of 5 was given to this experimental group. Good quality leaves and roots were observed in H_1(0 h), H_2 (0 h), H_3 (0 h) and H_3 (24 h) applications. Considering the rooting quality score scale prepared for control (24 h), H_1 (24 h), H_2 (24 h), Av (24 h) and T (0 h) applications, the score of these applications was 3. At both planting times, substandard rooting and rooting quality was determined for the cuttings of the T application and were deemed unsaleable. Therefore, the rooting quality score of T application was determined as 2 (Table 4). In summary, very good quality roots were observed in H_1(0 h), H_2 (0 h), H_3 (0 h) and H_3 (24 h) applications. These applications can be recommended for rooting the leaf cuttings of *P. sempervivoides* succulent plant.

Conclusion

As a result, the effects of the application of the highest dose of IBA+NAA, which is frequently used in cuttings, on the average root length and rooted cutting number parameters were found to be statistically significant. At the same time, it was concluded that the reproduction of the species can be done without using synthetic hormones, and it can be promoted with natural substrates. Cinnamon application caused an increase in the viability of the leaf cuttings of *P. sempervivoides*. It was concluded that cinnamon powder applications can be considered as a more effective natural extract for rooting leaf cuttings of *P. sempervivoides* compared to *Aloe vera* gel application. In plant propagation studies, the rooting percentage may vary depending on the plant type and variety, the rooting environment conditions, the rooting medium used, the type of cutting, the type and dose of plant growth regulator used. In general, it has been

suggested in various studies that in propagation of succulents with cuttings, it is necessary to wait for a while after the cuttings are prepared. At the end of our research, in which we examined the effects of different waiting times (immediate planting and planting with 24 hours waiting), it was determined that planting the leaf cuttings of *P. sempervivoides* as soon as they were taken had a positive effect on rooting. Based on these findings, we suggest that *P. sempervivoides* can be propagated with leaf cuttings by vegetative propagation.

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Effect of Starvation and Refeeding on Gamete Quality and Fertilization in Rainbow Trout (*Oncorhynchus mykiss*) Broodstock

Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Anaçlarında Açlık Döngüsü ve Yeniden Beslemenin Gamet Kalitesi ve Döllenme Üzerine Etkisi

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ABSTRACT

In this study, the impact of starvation and refeeding on broodstocks was observed by examining quality parameters of gametes and fertilization. While the control group fish were fed every day, the male and female members of the group were fed one week apart and placed in starvation after a week of feeding. Weight gain in the control group was the highest. The feed conversion ratio was normal in all groups (0.9-1.3%). Relative fecundity was (935±62 eggs/kg) in control group females; it was found to be (1317±241 eggs/kg) in starving females and statistically different in the groups ($p < .05$). The egg diameter of the group receiving intermittent feeding for a week (3.36±0.2 mm) was found to be the lowest. All spermatological parameters were similar between groups, except for sperm volume. The most sperm count was seen in the group that received one-week intermittent feeding (46.9± 20 ml). The results of fertilization with the control female in the fertilization study based on male individuals showed similarity for the male individuals of the trial group, the highest fertilization rate was seen in fertilization using the control female and control male.

Keywords: Broodfish feeding, Egg quality, Reproductive performance

ÖZ

Bu çalışmada, açlık ve yeniden besleme rejimi uygulanan anaçlarda, bu besleme düzeninin gamet kalitesi ve döllenme üzerindeki etkisi incelenmiştir. Kontrol grubu balıkları her gün beslenirken, deneme grubunun erkek ve dişi bireyleri bir hafta beslenmiş, bir hafta aç bırakılmıştır. Ağırlık artışı en yüksek kontrol grubunda görülmüşken; yemden yararlanma oranı tüm gruplarda normal düzeyde bulunmuştur (%0,9-1,3). Relatif fekondite kontrol grubu dişilerinde 935±62 yumurta/kg; bir hafta açlık rejimi uygulanan balıklarda ise 1317±241 yumurta/kg olarak bulunmuştur ($p < ,05$). En düşük yumurta çapı bir hafta aç bırakılan grupta elde edilmiştir (3,36±0,2 mm). Sperm hacmi dışında tüm spermatolojik parametreler gruplar arasında benzerlik göstermiştir. Sperm miktarı bir hafta açlık uygulanan balıklarda (46,9±20 ml) kontrol grubuna nazaran daha yüksek bulunmuştur. Erkek bireylere dayalı döllenme çalışmasında kontrol dişi ile yapılan döllenme sonuçları deneme grubunun erkek bireyleri için benzerlik göstermiş, en yüksek döllenme oranı kontrol dişisi ve kontrol erkeği kullanılarak yapılan döllenmede görülmüştür.

Anahtar Kelimeler: Damızlık balık besleme, Yumurta kalitesi, Üreme performansı

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Introduction

Rainbow trout's reproductive process is controlled by both photoperiod and temperature. However, the rainbow trout can perceive nutritional status and regulate its reproductive activities accordingly. In individuals whose gonads have reached maturity before the reproductive period, body growth slows down, with most of the energy and nutrients going to the production of vitollogenin, which is necessary for gonad growth (Reading et al., 2018). The broodstock's nutrients during oocyte growth are transferred to the developing eggs. These reserves, taken from the female by mobilization from the yolk sac are used for the development of the embryo until external feeding. Therefore, the feeding regime, ratio and nutritional content of the feed applied before the breeding period have a direct effect on gametes to be obtained from the broodstock fish and on the larval life (Carrillo et al., 2000).

In aquaculture, especially in broodstock fish, the establishment of a species-specific feeding protocol is important both in terms of quality parameters in the gametes to be obtained and in terms of the expenses of the enterprises. Considering the feed expenses included in the production cost with 60-80% of the operating expenses, the establishment of a feeding scheme that will not affect the quality of gametes and larvae in broodstock brings about a cost-effective strategy for fish farmers. Fish are fed daily under culture conditions. To reduce the cost of current feed in broodstock management, it may be beneficial to understand how feed restriction and refeeding affect gonad and gamete development (Izquierdo et al., 2001). The impact of feed restriction on fish reproductive performance is still a matter of contention. Testicular development in zebra cichlid (*Cichlasoma nigrofasciatum*) fish is not affected by feed restriction as stated by Townshend and Wooton (1984). Jobling et al. (1993) report that feed restriction in Arctic charr (*Salvelinus alpinus*) does not influence the number of mature males. The GSI ratio of tilipia fish (*Tilapia zillii*) remained unchanged despite the fed restriction (Coward & Bromage, 1999). It has been shown that feed restriction application in rainbow trout has no effect on GSI and egg quality (Ridelman et al., 1984). However, gonadal maturation in fish species, such as goldfish (*Carassius auratus*, Sohn et al., 1998), European seabass (*Dicentrachus labrax*, Cerda et al., 1994), and Salmonid (Thorpe et al., 1990; Reimers et al., 1993; Hopkins and Unwin 1997) has been observed to be hindered by a decrease in feeding rate. Feed restriction before breeding period has been found to have an impact on gametes, particularly egg productivity, in rainbow trout through previous studies (Imsland & Gunnarsson, 2011; Caldwell et al., 2014; Cleveland et al., 2012).

By sensing the nutritional status of teleost fish, they can regulate their reproductive development and activity accordingly. The quality of gamete may be affected by feed restriction, but it's unclear how. This research aimed in part to address these problems by subjecting rainbow trout to starvation regimes. A feeding trial with two treatments was conducted to assess the impact of starvation periods on growth efficiency and reproductive success before the breeding period. During the control group's daily feeding, the experimental group was given food for seven days and then fasted for the next seven days. The success of fertilization with gametes from both sexes and their quality were investigated using this six-month feeding pattern.

Materials and Methods

Experimental Fish and rearing conditions

This study was carried out between 01.07.2022-15.01.2023 at Istanbul University Faculty of Aquatic Sciences, Sapanca Inland Aquaculture Production Research and Application Unit. A total of 40 females (mean weight 1601±231 g; mean total length 49.7±3.1 cm) and 40 males rainbow trout (*Oncorhynchus mykiss*) (mean weight 670±127 g; mean length 36.7±2.31 cm) aged 2^{+} were used. During the study, the fish were taken care of in four round fiberglass tanks with a diameter of 3 m, a height of 80 cm and a water volume of 3 m^3. The well water with an average temperature of 11.2-13.5 °C was supplied to the system. The experiment was conducted with broodfish raised under natural photoperiod conditions.

Experimental diet

During the study, 8 mm diameter commercial trout broodstock feed supplied from a private company was used to feed the fish (proximate compositions: %48.2 crude protein; %19.3 lipid; %9.6 ash; %1.3 crude cellulose; %92.2 dry matter).

Feeding programme

The male (CM) and female (CF) fish that make up the control group were fed every day during the study (180 days). Male (OWM) and female (OWF) individuals constituting the experimental group were fed every day for a week and starved for a week. All trial groups were fed fish as *ad libitum* by hand twice a daily (09:00; 16:00). The amount of feed delivered to each group was recorded. The tanks were cleaned after evening feeding three days a week. On the days when the gamete maturation would be checked, the fish were not given feed.

Growth Performance

The calculation of feed conversion ratio and growth performance was done using Ricker's formulas (1979).

Weight gain (g) = [final weight (g) – initial weight (g)]
Feed conversion ratio (FCR) = [total feed supplied (g) / weight gain (g)]

Gamete quality analysis

Sperm quality

The spawning activity of male broodfish was monitored every three days during gamete maturation (September to December) by gently pressing the abdomen to check for any sperm. Sperm were obtained by applying abdominal pressure without the use of anaesthetics and stored in clean glass beakers labelled and heated in polystyrene boxes at 4°C until they were analyzed in the lab. Following the measurement of sperm amount (ml), the hemocytometry method was employed to determine sperm density. In brief, a Microcentrifuge tube was used to mix a sperm sample with 0.7% NaCl solution at a ratio of 1:1.000 and analyse it using a Thoma slide (0.00025 mm^3) under a light microscope (Nikon Eclipse E100, <unk> 40). The following equations (ANSCI 2017) were used to determine sperm density (Ekici et al., 2012);
Concentration/ml = (dilution factor) x (count in five squares) x ($0.05x10^6$)

At 12°C, motility parameters were assessed using CEROS II (Hamilton-Thorne) connected to CX41 microscope (Olympus). Images were captured at 60 frames per second using the rainbow trout variables determined in the Hamilton configuration using a digital camera (U-TV1X-2 Tokyo). Sperm motility (Mot,%) and velocity of curvilinear (VCL, <unk> m/sec) were measured in every sperm sample. Sperm with a velocity of less than 20 m/s were classified as immotile. Motility and kinematic parameters were established by using Leja 2 cell chambers with a 20-µl deep chamber (Leja Products). With a dilution rate of 1:500, hatchery water was employed as an activator. All sperm samples were conducted in triplicate by the same operator to minimize errors.

Egg quality

Female individuals were monitored every week for their egg maturation. To observe egg production, stripping was carried out on individuals. Using a towel, the abdomen was dried and gently massaged. Eggs were extracted from clean and labeled stripping jars and than weighed. To prevent eggs from direct sunlight, a sheet was put on the jars. The eggs in each jar were counted by taking a 10-gram sample. Absolute fertility was determined using the following formulas. The determination of relative fecundity was made by dividing absolute fecundity with the total weight (g) of fish (Hunter et al., 1985).
Absolute fecundity (amount⁄Σkg) = [Number of eggs insub−sample×total egg weight (g)] / [Weight of the sub-sample (g)]

The Leica stereo microscope with 20 magnification was used to analyze egg samples (n:10) from each female in the groups. The microscope system software was used to measure egg diameters after photographing all eggs.

Fertilization experiment

Fertilization studies were carried out in a controlled manner for each trial group. To reveal the productivity of female individuals in trial groups; eggs from experimental group was fertilized with the same sperm samples from control group male fish. Again, to reveal the efficiency of male individuals, sperm samples taken from trial group was used for fertilization with eggs belonging to the same female from the control group. The groups in which the fertilization study was performed are given in Table 1.

Table 1.
Fertilization experimental in groups

Groups	Control male	Male (OWM)*
Control female	X	X
Female (OWF)*	X	

*OWF: weekly feeding cycle and a week of fasting female, OWM: weekly feeding cycle and a week of fasting male

Fertilizing the eggs (300 per female) taken from an individual in the trial group was achieved by mixing them with the sperm of the male individual whose spermatologic characteristics were analyzed. Separate incubation trays (30 x 40 cm^2) were used for placing the eggs after fertilization. The incubation trays were filled with water, which was flown continuously (at a rate of 1.5 liters per minute and 10°C). Dead and unfertilized eggs were collected daily from the incubator. The success of fertilization was measured by analyzing the percentage of eyed eggs 16 days after insemination and calculating it based on (number of eyed eggs x initial egg number - 1*100%) (Ekici et al., 2014).

Statistical Analysis

The data obtained at the end of the study are presented with their mean values and standard deviations. The data obtained was analyzed using an ANOVA and then compared by using the Tukey's ($p < .05$) multiple range test in STATISTICA v. 8 program.

Results

The weight gain, calculated according to the date determined as a result of the weighing made at the beginning of the trial and at the end of a total of 6 months, was measured individually, and the values were given with mean and standard deviations. Since the total length of the fish did not differ at the beginning and the end of the experiment, the results were not evaluated.

Growth performance of rainbow trout broodstock

Growth and survival parameters are given in Table 2. Mortality was not recorded in any of the groups. The fasting group had a statistical difference in weight gain ($p < .05$) compared to the control group, which experienced the highest weight gain.

Table 2.
Growth parameters of fish in experimental groups

	CF	CM	OWF	OWM
Initial weight (g)	1588±269	673.5±126	1643.5±257	681±153
Final weight (g)	4352±270[a]	1847±403[A]	3365±540[b]	1145±165[B]
Weight gain	2763	1173	1721	464
FCR	0.9	1.4	1	1.4
Survival (%)	100	100	100	100

CF: control female, CM: control male, OWF: weekly feeding cycle and a week of fasting female, OWM: weekly feeding cycle and a week of fasting male. Each treatment's mean±S.D. value are included in the results. Different superscripts in the same row led to significant differences in values ($p < .05$).

Reproductive performance

Female

In Table 3, the spawning rates of female individuals are shown. Spawning occurred within 177-187 days after the start of experimental feeding.

Table 3.
Spawning parameters of female fish in experimental groups (n:20)

Parameters	CF	OWF
Spawning (days)	180±3	181±3
Spawning females (%)	100	100
Absolute fecundity[2]	4029±432	4389±804
Relative fecundity[3]	935±62[a]	1317±241[b]
Egg diameter (mm)	3.5±0.16	3.36±0.24

CF: control female, OWF: weekly feeding cycle and a week of fasting female, TWF: two week feeding cycle and two weeks of fasting female. 2Mean number of eggs per fish; 3Mean number of eggs per kg body weight. Values in the same row with different superscripts were significantly different ($p < .05$).

In the statistical study conducted on the relative fecundity the control group and the trial group were found to be significant ($p < .05$). However, there was no significant difference between the groups in total fecundity values ($p > .05$). Eggs from broodfish in experimental groups appeared normal, and were similar in shape, and color. However, egg diameters were higher in the control group (3.5±0.16 mm) and lower in the group fed one week apart (3.36±0.24 mm). The egg diameter values of the trial group fed one week apart were compared with the control group and a significant difference was found ($p < .05$).

Male

Table 4 displays the spawning performance of male rainbow trout broodfish. Between 177-187 days after the onset of experimental feeding, spawning occurred.

Table 4.
Spawning parameters of male fish in experimental groups

Parameters	CM	OWM
Spawning (days)	180±3	181±3
Volume (ml/individual)	21.8±7[a]	46.9±20[b]
Spermatozoa density (x10^9/ml)	4.04±0.7[a]	6.5±0.8[b]
Total Motility rate (%)	94.2±2.4	93.42±1.5
Motility duration (s)	24.8±2.9	23.6±1.4
Total Curvilinear velocity, VCL (µm/s)	63.1±9.1	65.7±6.9

CM: control male, OWM: weekly feeding cycle and a week of fasting male. Values in the same row with different superscripts were significantly different ($p < .05$).

In the statistical analysis with the results of sperm density; the group that was fed intermittent for one week was differed from the control group ($p < .05$). The motility rate ranged from 90 to 94 and there was no significant difference between the total motility values of sperm cells between the groups ($p > .05$). In addition, no significant difference was found between the groups in the motility duration of sperm cells ($p > .05$). The total VCL value of sperm cells were 63.1±9.1 µm/sec in the control group, 65.7±6.9 µm/sec in the group fed one week apart. Total VCL values were highest in the one-week intermittent feeding group, but no significant difference was found between the groups in terms of total VCL values ($p > .05$).

Fertilization

In the study, fertilization studies were carried out in a controlled manner for each trial group. To reveal the productivity of female individuals in trial group; eggs from trial group were fertilized with the same sperm samples from control male fish. Again, to reveal the efficiency of male individuals, sperm samples taken from trial group were used for fertilization with eggs belonging to the same female from the control group. In the fertilization study conducted based on female individuals, the highest fertilization rate was observed between control group female and control group male individuals (97.75±1.2%). There was statistically

significant difference between the data obtained in the fertilization study using one week-intermittent female*control male (%. 43±7) ($p > .05$) (Figure 1).

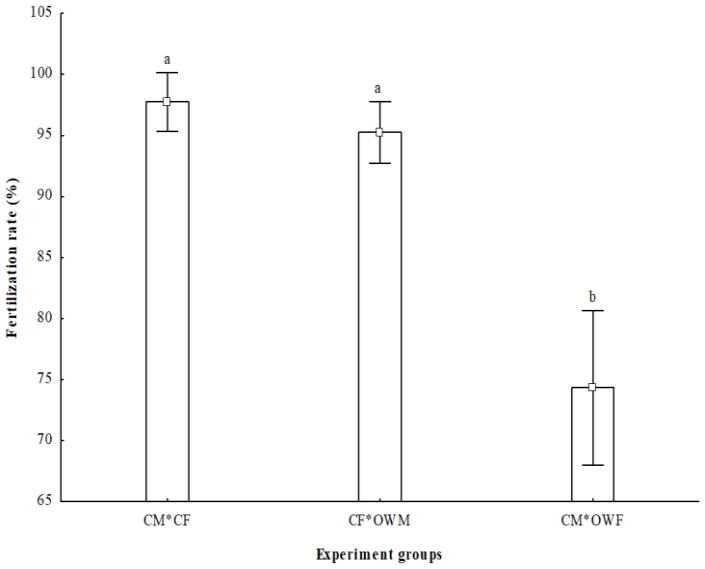

Figure 1.
The percentage (%) of fertilization obtained in the fertilization study based on male and female individuals in the trial groups (Mean±SD). CF: control female, CM: control male, OWM: weekly feeding cycle and a week of fasting male, OWF: weekly feeding cycle and a week of fasting female, the statistical difference between the values indicated by the different letters was found to be significant at the 95% accuracy level according to the Tukey test.

Discussion and Conclusion

By utilizing feed restriction and refeeding cycles in broodstock management of rainbow trout during the 6 months prior to spawning, we were able to achieve a 25% decrease in feed consumption. Live weight gain was observed in both groups, but the control groups of female (2763 kg) and male (1737 kg) fish showed the highest levels. In the study where the growth and reproductive performance of feeding with full ration (100%) and half rations (50%) in Alpine trout (*Salvelinus alpinus*) broodfish was examined; weight gain was achieved in groups fed with a full ration; it was found to be greater than the weight gain in half-ration-fed groups (Imsland and Gunnarsson, 2011). In a study with sea bass (*Dicentrarchus labrax*), broodfish fish were fed with two different feeding rates (0.45% and 1.04 days). Although the starvation cycle is not studied in the study conducted with sea bass, it is thought that in this thesis study, the feeding pattern is subject to a similar restriction as the feed restriction it inevitably brings (Chatzifotis et al., 2011). Before the breeding period in turbot fish (*Scophthalmus maximus*), broodstock fish were fed for 12 months in such a way that the amount of feed increased or decreased according to the months but the total amount of constant feed was determined. At the end of the study, the researchers reported that feeding with low feed rations covering 4 months immediately preceding spawning reduced fish weight by 70% (Bromley et al., 2000). In *Tilapia zillii* fish, more growth was observed in female fish that became broodstocks in high and low feed rationing for about 17 months from the first feeding transition stage compared to fish fed with high feed ration (Coward & Bromage, 1999). In the rainbow trout, the weight gain was found to be less in the feed-restricted groups than in the control group in the results obtained by feeding the broodstock fish regularly as ad-libitum 5 months before the breeding period and feeding by giving 80% of the feed given to the control group by going to feed restriction (Cardona et al., 2019). In contrast to these studies, in the study conducted only with male chinook salmon (*Oncorhynchus tshawytscha*), the fish were fed at intervals of one week for 10-12 months and continued to be fed regularly every day after 10-12 months. Researchers reported that there was no difference between the groups in weight gain in fish (Hopkins & Unwin, 1997). The results obtained in our study were similar to other studies except for the study with chinook salmon. The highest weight gain was seen in the control group of individuals fed regularly daily. Regular feeding has been shown to have a significant effect on weight gain, but this will be re-evaluated by gamete quality.

The feed conversion rate (FCR) may vary between species and within species. FCR, which varies according to the life stage of the fish, the breeding conditions and the feed material, is ideally expressed as 0.5-2 in trout (Davis, 2022). In this study, the FCR value ranged from 0.9 to 1.4. The FCR of this feed determined in each group are similar to the ideal values that should be in fish farming. These results show that the feed given in groups was consumed in a healthy way for use in both weight gain and gamete maturation.

In present the study, although feed restriction did not have a temporal effect on the gonadal maturation of females, it was determined that it caused a change in egg quality. The relative fecundity was found in the control group (935±62 n/kg) and the highest was found in the female group fed one week apart (1317±241 n/kg) and this group statistically different from the control group. When the total amount of eggs taken per individual was evaluated, the highest total fecundity was found in the trial group with one-week interval feeding (4389±804 n/individual). For the control group, 4029±432 n/individuals were found, but there was no significant difference in total fecundity values between the groups. It has been found that changing the normal feeding pattern in rainbow trout and giving 25 and 50% of the daily feed amount decreases in total fecundity (Bromage 1995). In a study where the broodstock fish of the Black Sea trout were fed with commercial feed as ad libitum, the egg

amounts were found as 1476±1043 n/individual (Erbaş & Başçınar, 2013). Erbaş et al. (2013) stated that the fed Black Sea trout once and twice a day without altering the feed ration, and the broodstock group fed once a day produced higher-quality eggs. However, according to studies, decreasing feed intake in rainbow trout can have no negative effect on egg production or egg quality (Cardona et al., 2019). The fecundity values obtained in our study are similar to those of other studies. Even though the relative fecundity results are different, the fact that there was no difference in total fecundity is believed to be linked to the fish's live weight. The lowest relative fecundity in the control group females, which saw the greatest live weight gain, shows that these fish used in the feed consumed more for body weight gain in addition to egg formation. The fact that the total fecundity is similar to the groups in the control group with high kilogram weight gain can be explained by this weight gain. It is thought that intermittent feeding of fish increased relative fecundity, but the reason why fish in this feeding pattern used the feed they received directly to the egg formation did not affect total fecundity because they gained less weight.

In addition to fecundity, one of the quality parameters of female gametes is the egg diameter of the eggs obtained. It is stated that the size of the pre-larva at the stage of hatching is related to the diameter of the egg, and the size of the pre-larvae emerging from large eggs is large (Bromage, 1995). In order for the egg diameter, which is affected by factors such as reproductive period, individual age, genetic structure and nutrition, to be at the desired level, female individuals should be fed with high-quality and adequate nutrition in the period between the two reproductive periods, especially in completing the gametogenesis process (Mananos et al., 2009). For the family Salmonidae, the egg diameter is expressed in the range of 4.9-7.2 mm (Bromage, 1995). Egg diameter in brown trout is 5.2 mm (Gunnes & Gjedrem, 1978); 5-5.3 mm in Black Sea trout (Sonay, 2008); 4.55-5.12 mm (Erbaş & Başçınar, 2013); 4.3±1.8 mm (Gelinçek & Yamaner, 2020); in rainbow trout, 5.4±0.1 mm (Yıldız et al., 2020); has been reported. In this study, egg diameters were found to be 3.5±0.16 mm in the control group; and 3.36±0.24 mm in the one-week intermittent feeding group. The egg diameter data obtained in the study were found to be lower when evaluated intra-species and inter-species compared to other studies. There are studies reporting that feed restriction in rainbow trout leads to larger egg diameters in females and less mortality in eggs (Cardona et al., 2019). Restriction of nutrition in fish during oocyte formation and especially vitellogenesis is known to slow down oocyte growth (Bromley et al., 2000). However, in this study, it is thought that the egg diameters of the eggs in the control group are similar to the results in the fasted fish, and the reason why both results are different from other studies is due to reasons such as different origins of the fish, care conditions, etc. In addition, the highest relative fecundity and the lowest egg diameters obtained in the one-week intermittent feeding group seem to be related to each other. The small egg diameter but the large number of egg production in this group revealed the effect of one-week intermittent feeding.

The amount of sperm differs between individuals of the same species, as well as between each species. In the studies conducted to date, the amount of sperm without any manipulation has been reported as 5-20 ml/individual in the Salmonidae family (Alavi et al., 2008; Dziewulska et al., 2008; Tekin et al., 2007; Yıldız et al., 2021).

In this study, the amount of sperm in the one-week intermittent group differed with control group ($p < .05$). The amount of sperm obtained in the one-week intermittent feeding group was found to be much higher than control group. It was found that the amount of sperm increased significantly in the application of the feeding regime with an interval of one week in rainbow trout broodstock. There are studies showing that the amount of sperm changes with the change in feed amount or feed content (Izquierdo et al., 2001; Davis, 2022). The reason for obtaining more sperm amount in the one-week intermittent feeding group, where the feed content does not change but the feed amount is applied less due to the feeding pattern, is thought to be the fact that the fish spend the feed they consume as soon as they leave the starvation cycle on testicular maturation. The fact that the weight gain was greater in the control group than in the group fed intermittently for one week, but the amount of sperm was less than in this group supports this idea.

The spermatozoa density can vary between 2.42-23.40x10^9 cells/ml in the family Salmonidae (Dziewulska et al., 2008; Lahnsteiner, 2013; Erbaş & Kocabaş, 2013; Gelinçek & Yamaner, 2020; Yıldız et al., 2021). In this study, sperm cell density was highest in the group fed at intervals of one week (6.5±0.8x10^9 cells/ml). The fact that the sperm cell density, which is similar to the sperm amount results, is found to be the highest in the group fed intermittently for one week is thought to be due to the high amount of sperm produced in this group. Although the sperm cell density obtained in the one-week intermittent feeding group was similar to that of Salmonidae family species, the sperm cell densities obtained in all groups were found to be low for rainbow trout. These low results of sperm cell density can be related to the age of the fish and the spawning time. It has been determined that one-week intermittent feeding regime in rainbow trout broodstock also leads to an increase in sperm cell count depending on the amount of sperm.

Sperm motility is one of the most important sperm quality parameters that reveal the fertilization of sperm cells. Sperm motility, which differs within and between species like other gamete quality parameters, is under the influence of many abiotic and biotic factors. The accepted motility in breeding conditions for a successful fertilization study is reported as 70% and above (Mananos et al., 2009). When the motility results were compared, no difference was detected between the groups and it was concluded that the cells in all groups were capable of fertilization with values above 70% and that the intermittent and regular feeding regimen had no effect on the motility values of sperm cells.

When the results obtained from the fertilization studies were evaluated, it was found that there was no difference in the fertilization percentages of the male individuals in the trial group with the control group of female individuals. However, intermittent and regular feeding has been found to affect fertilization in eggs from female fish that have undergone this feeding pattern. The highest percentage of fertilization was seen in gametes obtained from male and female individuals of the control group, and this percentage was statistically different from the other trial group.

Feed used in aquaculture is effective in the performance of vital functions such as survival and growth as well as in gonad development, maturation, reproductive performance and quality parameters of the gametes obtained after maturity (Bromage, 1995). Furthermore, it is also known that the starvation cycle affects gonad and gamete maturation in both sexes (Chatzifotis et al., 2011).

In conclusion, it was determined that feeding the fish regularly every day led to more weight gain in the broodstock fish, but this feeding pattern led to less egg production per kg in the fish, more eggs per kg were produced by feeding the fish one week apart, but the egg diameters produced were smaller and also contributed to the production of more sperm and sperm cells in male individuals. The results obtained with the motility and kinematic parameters of the sperm cells and the results of fertilization were in parallel, and it was concluded that intermittent feeding did not make a difference except for sperm volume and cell density in male individuals, but that the fertilization percentages were low in female individuals, and that although more eggs were produced, the eggs obtained by intermittent feeding did not fully mature. As a result of this study, it is thought that this change in feeding regime, which is made 6 months before the breeding period, should be started earlier than the period applied in this study. Gonadal maturation is influenced by starvation during vitellogenesis, but further research is needed to establish the duration and period of the year during which the starvation re-feeding regime should be implemented.

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Ethical Statement: This study was carried out after the animal experiment was approved by İstanbul University Local Ethics Committee (Decision number: 2022/15).

Peer-review: Externally peer-reviewed.

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Yazar Katkıları: Konsept – GY; Tasarım – NN, GY; Denetleme - GY; Kaynaklar – NN, GY; Malzemeler – NN, GY; Veri Toplama ve/veya İşleme – NN, GY; Analiz ve/veya Yorumlama - GY; Literatür Taraması - NN; El Yazması - GY; Eleştirel İnceleme - GY

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Assessment of Phytochemical Characteristics of Walnut (*Juglans regia*) Leaves: Determination of Nutritional Value and Quantitative Content of Phenolic Compounds

Ceviz (*Juglans regia*) yapraklarının Fitokimyasal Özelliklerinin Değerlendirilmesi: Besin Değeri ve Fenolik Bileşiklerin Niceliksel İçeriğinin Belirlenmesi

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ABSTRACT

This study aimed to assess the nutritional value of walnut leaves (*Juglans regia*), investigate the content of major polyphenolic compounds in walnut leaves, and assess the potential of walnut leaves as a phytogenic supplement to poultry diets. The nutritional composition of dried ground walnut leaves (crude protein, crude fat, crude fiber, calcium, phosphorus, vitamin E, b-carotene) was assessed according to generally accepted methods. The quantitative content of individual groups of polyphenols, such as total phenols, hydroxycinnamic acids, flavonoids, and juglon, was determined spectrophotometrically. The content of the sum of tannins was determined by the titrimetric method. Walnut leaves contain a high content of b-carotene (295.0 µg/g) and natural antioxidant vitamin E (128.8 µg/g). The protein content was noted at the level of 12.5%, the fiber content - 12.86%. In a significant amount, calcium and phosphorus accumulated in walnut leaves - 2.04% and 0.23%, respectively. The content of the total hydroxycoric acids in terms of chlorogenic acid in walnut leaves was 24.3 mg/g, the total content of phenolic compounds in gallic acid equivalent was 14.4 mg/g, flavonoids in rutin equivalent was 20.2 mg/g, and juglone was 2.72 mg/g. The content of total tannins in walnut leaves was 124.5 mg/g. The high nutritional value of walnut leaves compared to alfalfa meal and the presence of a number of biologically active polyphenolic compounds in a significant amount give grounds to consider this phyto-raw material not only as a valuable feed component, but also as a source of natural antioxidants.

Keywords: Walnut, Leaves, Nutritional value, Phenolic compounds, Hydroxycinnamic acids, Flavonoids, Tannins, Juglone

ÖZ

Bu çalışma, ceviz yapraklarının (*Juglans regia*) besin değerini değerlendirmeyi, ceviz yapraklarındaki başlıca polifenolik bileşiklerin içeriğini araştırmayı ve ceviz yapraklarının kanatlı hayvan diyetlerine fitogenik bir katkı maddesi olarak potansiyelini değerlendirmeyi amaçlamıştır. Kurutulmuş ve öğütülmüş ceviz yapraklarının besin bileşimi (ham protein, ham yağ, ham lif, kalsiyum, fosfor, vitamin E, β-karoten) genel olarak kabul edilen yöntemlere göre değerlendirilmiştir. Toplam fenoller, hidroksinamik asitler, flavonoidler ve juglon gibi bireysel polifenol gruplarının niceliksel içeriği spektrofotometrik olarak belirlenmiştir. Toplam tanenlerin içeriği titrimetrik yöntemle tespit edilmiştir. Ceviz yaprakları yüksek β-karoten (295,0 µg/g) ve doğal antioksidan vitamin E (128,8 µg/g) içeriğine sahiptir. Protein içeriği %12,5, lif içeriği ise %12,86 olarak kaydedilmiştir. Ceviz yapraklarında önemli miktarda kalsiyum ve fosfor birikmiştir, sırasıyla %2,04 ve %0,23. Ceviz yapraklarında klorojenik asit cinsinden toplam hidroksikorik asit içeriği 24,3 mg/g, gallik asit eşdeğeri olarak toplam fenolik bileşik içeriği 14,4 mg/g, rutin eşdeğeri olarak flavonoidler 20,2 mg/g ve juglon da 2,72 mg/g olarak belirlenmiştir. Ceviz yapraklarında toplam tanenlerin içeriği 124,5 mg/g olmuştur. Ceviz yapraklarının yüksek besin değeri ve önemli miktarda biyolojik olarak aktif polifenolik bileşiklerin varlığı, bu fito hammaddeyi sadece değerli bir yem bileşeni olarak değil, aynı zamanda doğal antioksidan kaynağı olarak da değerlendirmeyi mümkün kılmaktadır.

Anahtar Kelimeler: Ceviz, Yapraklar, Besin değeri, Fenolik bileşikler, Hidroksinamik asitler, Flavonoidler, Tanenler, Juglon

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Introduction

The introduction of a ban on the use of feed antibiotics in animal husbandry and strict control over their use in the European Union since 2006 prompted a large-scale search for alternatives. The introduction of so-called phytobiotics into diets instead of antibacterial drugs, plant complexes, the use of which makes it possible to obtain environmentally friendly food, has gained immense popularity. Herbal supplements, unlike drugs, can be used for clinically healthy animals almost throughout their lives.

In this context, much attention is paid to the search for local plant materials that have both nutritional value and the presence of biologically active ingredients with antioxidant and antibacterial properties. These criteria are met by the walnut (*Juglans regia*), whose products, such as husks and leaves, are rich in phenolic compounds and are a valuable source of antioxidants (Shah et al. 2018). Walnut is cultivated worldwide not only for its nutritious nuts but also for its valuable by-products, such as husks and leaves, which have numerous applications due to their rich phenolic composition. Walnut cultivation is particularly important in regions like Ukraine, where it contributes significantly to the agricultural economy. According to FAOSTAT, Ukraine occupies the 7th position in the world in the cultivation of walnuts. In the period from 2018 to 2021, 14,218 hectares of new orchards were planted in the country, 41% of their total area was walnut plantations (Mazur & Gontaruk, 2021). These statistics indicate that Ukraine has a sufficient amount of walnut plant material.

Some studies have demonstrated the antioxidant and antimicrobial activity of various walnut parts, including the leaves (Jahanban-Esfahlan et al., 2019). The phenolic composition of walnut leaves, their antibacterial activity and antioxidant potential have been studied, and the dependence of these parameters on the crop variety has been shown (Pereira et al., 2007). It has been reported that walnut leaves have a significant content of phenolic compounds, in particular flavonoids of the flavone and flavonol groups, hydroxycinnamic and hydroxybenzoic acids, and tannins (Nour et al., 2016). Most of the properties of this raw material are determined by the presence of juglone in its composition. It is a natural antibiotic of a number of naphthoquinones, toxic to fungi and plants, inhibits Helicobacter pillory enzymes, exhibits pronounced antioxidant properties, is active against the bacteria *Pseudomonas aeruginosa*, *Candida albicans*, *Helminthosporium sp.*, *S. aureus*, *Bacillus subtilis* and can have a positive effect on the human body, as well as on the body of animals and birds (Chaudhary et al., 2021).

At the same time, the nutritional potential of walnut leaves is insufficiently studied, the details of its chemical components have not yet been fully elucidated. Such information is almost absent in the Ukrainian scientific community. Leaves are readily available in large quantities, and their collection does not endanger the life of plants.

Thus, the literature contains information about the antioxidant and antimicrobial potential of walnut leaves from trees growing in different regions of the world, but there is no characterization of the nutritional value and polyphenolic profile of walnut leaves growing in the forest-steppe zone of Ukraine. The insufficient level of research on the nutrition and polyphenolic complex of walnut (*Juglans regia*) leaves of this region does not allow to fully use it in poultry feed as a phytogenic antioxidant additive.

The aim of the research is to study the nutritional potential and polyphenolic composition of walnut (*Juglans regia*) leaves.

Methods

Experimental studies on the determination of the nutritional composition and content of polyphenolic compounds in phytogenic raw materials were conducted in the conditions of the testing laboratory of the Department of Quality and Safety Assessment of Poultry Feeds and Products of the State Poultry Research Station of the National Academy of Agrarian Sciences of Ukraine (SPRS NAAS) using available laboratory and analytical equipment.

Plant material preparation

The material for research was walnut leaves without petioles, which were collected in early June in dry weather from adult plants growing on the territory of the SPRS NAAS without the use of phytosanitary treatments. The walnut trees were of the 'Bukovynskiy' variety, a commonly cultivated type in Ukraine known for its high yield and resistance to disease. Plant raw materials were dried under natural conditions at ambient temperature (20–24°C) in a dark, well-ventilated room without access to direct sunlight. Samples were ground in an electric mill to a fraction that passed through a 1-mm mesh sieve and then stored at room temperature in paper bags until analysis.

The chemical composition and nutritional value of raw materials from walnut leaves (content of crude protein, crude fat, fiber, calcium, phosphorus, concentration of vitamin E and β-carotene) were studied using generally accepted methods (Ionov & Shapovalov, 2015).

The amount of tannins in terms of tannin was determined by the classical titrometric method, based on the titration of the infusion of raw materials with potassium permanganate in the presence of indigosulfonic acid as an indicator

(Cobzaru et al., 2019).

About 3 g (exact weight) of the tested walnut leaf powder was extracted with distilled deionized water (dd H_2O) in a 250 ml volumetric flask for 4 hours at room temperature. Then the infusion was filtered through cotton wool into a measuring flask with a volume of 250 ml so that particles of raw materials did not enter the flask, the volume of the solution was brought up to the mark with water and mixed.

25 ml of the obtained infusion was placed in a conical flask with a volume of 1 l, 25 ml of indigosulfonic acid solution and 750 ml of distilled deionized water (dd H_2O) were added. For titration, a 0.1 N aqueous solution of potassium permanganate ($KMnO_4$) was used until the blue color of the solution changed to green. Then a few drops were added until the solution turned golden yellow.

In parallel, a control experiment (blank sample) was conducted by titrating a mixture of 25 ml of indigosulfonic acid solution and 750 ml of distilled deionized water (dd H_2O). All samples were analyzed in duplicate. The content of the total amount of tannins in the sample was expressed in milligrams of tannin per gram of dry leaves.

Quantitative assessment of the content of other polyphenolic compounds (hydroxycinnamic acids, phenols, flavonoids, glycosides) in phytogenic raw materials was carried out by the spectrophotometric method at the appropriate wavelength, using the Beer–Lambert law. A SF-26 spectrophotometer and 10-mm quartz cuvettes were used for all optical density measurements.

Determination of the total amount of hydroxycinnamic acids was carried out in terms of chlorogenic acid (Proskurina et al., 2021).

Basic solution. 2.0 g (exact weight) of the test sample was placed in a flask with a capacity of 200 ml, 50 ml of 70% ethanol was added and heated in a reflux water bath for 30 min. After cooling, the extract was filtered through a Buchner filter paper. The filtrate was transferred to a volumetric flask with a capacity of 200 ml and the volume was brought up to the mark with 70% ethanol.

Test solution. 2 ml of the basic solution was added to a 50 ml volumetric flask and the volume of the solution was adjusted to the mark with 70% ethanol. The optical density of the obtained solution was measured at a wavelength of 325±2 nm. As a comparison solution, 70% ethyl alcohol was used.

The total amount of hydroxycinnamic acids was expressed in milligrams of chlorogenic acid per gram of dry leaves, the specific absorption index of chlorogenic acid at 325±2 nm was 531.

The total content of phenolic compounds present in walnut leaf extracts was determined quantitatively in terms of gallic acid (Fedosov et al., 2018).

Basic solution. An exact weight of the raw material (about 1.0 g) was placed in a conical flask with a capacity of 100 ml with a ground stopper, poured 30 ml of 40% ethanol, closed with a stopper and weighed (error ±0.01 g). The flask was connected to a reflux condenser, the contents were heated in a water bath until boiling, and a gentle boiling was maintained for 30 minutes. After that, the flask was cooled, closed with a cork, weighed, adjusted to the initial mass with 40% ethanol, and the contents were filtered through a dry paper filter into a dry flask with a volume of 50 ml.

Test solution. 1.0 ml of the filtrate was collected with a pipette, transferred to a volumetric flask with a capacity of 50 ml and the volume of the solution was brought up to the mark with 40% ethanol. The optical density was measured on a spectrophotometer at a wavelength of 270±2 nm. The comparison solution was 40 % ethanol.

The total content of phenolic compounds was expressed in milligrams of gallic acid per gram of dry leaves, the specific absorption index of gallic acid was 540.

The total flavonoid content was determined spectrophotometrically, in terms of rutin (Vronska et al., 2015).

Basic solution; An exact weight of the sample (approximately 1.0 g) was placed in a 100 ml flask with a ground stopper, and 30 ml of 70% ethanol was added. The flask was connected to a reflux condenser and heated in a boiling water bath for two hours, periodically shaking to wash off particles of raw materials from the walls. After cooling, the contents of the flask were filtered through a paper filter into a 100 ml flask and made up to the mark with 70% ethanol (solution A).

Test solution; 2 ml of basic solution A was poured into a 50 ml volumetric flask, 2 ml of a 3% solution of aluminum chloride in 96% ethanol and 0.1 ml of diluted acetic acid were added, and the volume of the solution was brought up to the mark with 96% ethanol (test solution). After 40 minutes, the optical density of the solution was measured on a spectrophotometer at a wavelength of 415±2 nm. As a comparison solution, a solution containing 2 ml of solution A, 0.1 ml of diluted acetic acid, and made up to the mark with 96% ethanol in a 50 ml volumetric flask was used. In parallel, the optical density of a standard sample of rutin, prepared similarly to the tested solution, was measured. The total flavonoid content was expressed in milligrams of rutin per gram of dry leaves.

Juglone content in walnut leaves was measured by the spectrophotometric method (Kocaçalışkan et al., 2020).

An exact weight of crushed walnut leaves (about 2.0 g) was placed in a 100 ml flask, 50 ml of petroleum ether was added and stirred for 30 minutes on a magnetic stirrer. After filtration, the filtrate was centrifuged at 18,000 rpm in a cooled centrifuge for 15 min. The supernatant was diluted ten times with petroleum ether and its absorbance was recorded by spectrophotometric measurement at a wavelength of 410±2 nm.

The blank sample was petroleum ether. 10 mg of commercially purified juglone was placed in a 50 mL volumetric flask and sufficient petroleum ether was added to make up the volume. A standard curve obtained from a series of pure juglone solutions in the juglone content range of 0.01, 0.02, 0.03, 0.04, and 0.05 mg was used to determine the juglone content in leaves. Juglone content was expressed in milligrams of juglone per gram of dry leaves.

Microsoft Excel software was used for statistical analysis. All determinations were performed in five replicates for each raw material sample and the results were expressed as mean ± error.

Results and Discussion

The growing interest in the powerful biological activity of plant phenolic substances and the potential of using phytobiotics in animal feed has outlined the need to determine the nutritional properties of walnut leaves and evaluate its potential as an alternative to commonly used plant components of poultry feed. Table 1 lists some of the chemical constituents of ground walnut leaves and alfalfa meal that reflect their nutritional value.

Table 1.
Chemical composition of walnut leaves (Bratyshko et al., 2013)

Parameters	Walnut leaves	Alfalfa meal *
β-Carotene, μg/g	295.0	150-250
Vitamin E, μg/g	128.8	170-215
Crude protein, %	12.5	14.2-17.3
Crude fat, %	1.19	2.4-2.7
Crude fiber, %	12.86	22.0-27.1
Ash, %	11.54	9.0
Moisture, %	8.25	10.5
Calcium, %	2.04	0.92-1.22
Phosphorus, %	0.23	0.21-0.26

A high content of β-carotene (295 μg/g) was found in the studied samples of walnut leaves. This is significantly higher than in alfalfa meal, where the concentration of β-carotene can be from 150 to 250 μg/g, depending on the grade. A similar result regarding the content of β-carotene in walnut leaf powder was obtained in the study by Kravchenko & Pop (2014), as well as Panaite et al. (2019).

The studied raw material has a fairly high content of natural antioxidant - vitamin E (128.8 μg/g). A somewhat higher concentration of vitamin E in walnut leaves was noted by Untea et al. (2020) – at the level of 157.54 μg/g. However, according to this indicator, the studied raw material is inferior to alfalfa meal, in which, depending on the class, the content of vitamin E is noted in the range from 170 to 215 μg/g. At the same time, according to other data, the content of this vitamin in alfalfa meal ranges from 27.55 to 83.77 μg/g (Cort et al., 1983).

The content of crude protein in walnut leaves is 12.5%, which is slightly lower than alfalfa meal (14.2-17.3%). Our results are confirmed by the data of Panaite et al. (2019), in whose study this indicator was at the level of 12.83%. In contrast, in another experiment, the mass fraction of protein in walnut leaf powder was 7.7% (Kravchenko & Pop, 2014). This indicates the existence of differences in the composition of nutrients in plant organs of different varieties and grown in different places.

As for fiber, its content in walnut raw materials was significantly lower than in alfalfa flour. 12.86% fiber is noted in the walnut leaf, in alfalfa meal, most of which consists of plant stems, the crude fiber content is almost twice as high and reaches 22-27.1%. At the same time, the study by Panaite et al (2019) noted the content of crude fiber in walnut leaves at the level of 17.41%.

The analysis of phytoraw material showed the content of crude fat at the level of 1.19%. In alfalfa meal, its content is twice as high - 2.4-2.7%. A significantly higher result was also reported by Untea et al (2020), in whose study walnut leaves contained 2.21% crude fat.

Regarding the content of macroelements in the investigated phytogen, calcium and phosphorus accumulated in significant quantities in the walnut raw material. Thus, the calcium content in walnut leaves was more than 2 times higher than in alfalfa meal, and was 2.04%. The phosphorus content in the studied samples of walnut leaves was at a fairly high level (0.23%), which is at the level of the average indicator in alfalfa meal (0.21-0.26%). Our data do not contradict the results obtained by Turfan N. et al. (2020), in whose research, depending on the age of the plants, the content of calcium in walnut leaves was in the range of 1.713-2.206%, phosphorus - 0.236-0.277%. In another experiment, the variation of the content of these macroelements in the leaves of different varieties of walnuts was determined - calcium at the level of 1.23-1.85%,

phosphorus at the level of 0.17-0.37% (Miłek et al., 2022). At the same time, according to Solmaz & Adiloğlu (2017), the content of calcium and, especially, phosphorus in samples of walnut leaves fluctuated within wider limits - calcium from 0.31 to 2.86%, phosphorus from 0.11 to 12.32%.

Walnut (*Juglans regia*) is rich in phenolic compounds, including phenolic acids, tannins, naphthoquinones, and flavonoids. Phenolic compounds, which are the most common secondary metabolites of plants and contain a wide range of molecules with a phenolic structure, exhibit various types of pharmacological activity: antioxidant, antiradical, antibacterial, antiviral, antimicrobial, anti-inflammatory, antitumor, immunostimulating (Nardini, 2022).

The content of the main groups of phenols and juglone in dry ground walnut leaves was determined, the results are shown in Table 2.

Table 2.

Quantitative content of the main groups of phenols in walnut leaves

Group of phenol compounds	Measurement units	Content in walnut leaves
THCAC	mg CGAEs/g DW	24.3±0.34
TPC	mg GAEs/g DW	14.4±0.72
TFC	mg REs/g DW	20.2±0.19
TTC	mg TAEs/g DW	124.5±13.12
TJC	mg J/g DW	2.72±0.571

THCAC: total concentration hydroxycinnamic acid content; TPC: total phenolic content; TFC: total flavonoid content; TTC: total tannin content; TJC: Total juglone content; CGAEs: chlorogenic acid equivalents; GAEs: gallic acid equivalents; REs: rutin equivalents; TAEs: tannic acid equivalents; J: juglone; DM: dry matter; DW: dry weights.

According to the results of research, it was established that the content of hydroxycinnamic acids in walnut leaves in the equivalent of chlorogenic acid is 24.3 mg/g. At the same time, Gutiérrez Ortiz et al (2018) reported that the content of hydroxycinnamic acids, depending on the period of leaf collection, ranged from 23.49 to 68.59 mg/g. Variations in the content of hydroxycinnamic acids from 8.9 to 26.8 mg/g were noted in the leaves of different varieties of walnut (Medic et al., 2022).

The content of total phenols in terms of gallic acid in walnut leaves was observed at the level of 14.4 mg/g in dry weight. According to a general review, dried leaves have a significant amount of total phenolics ranging from 34 to 194 mg/g in gallic acid equivalent (Jahanban-Esfahlan et al., 2019). According to other data, the total content of phenolic compounds in walnut leaves is 25.3 mg/g in dry matter (Santos et al., 2013). In another study, depending on the plant variety and leaf collection period, the total phenolic content of walnut raw materials ranged from 15 mg/g to 88.1 mg/g in gallic acid equivalent (Kocaçalışkan et al., 2020). Miłek M. et al. (2022) noted the variation of the total content of phenols in the leaves of different varieties from 25.6 to 64.78 mg/g. Such a wide range indicates a high dependence of the content of phenols in the studied plant material on a large number of factors, including also geographical location.

The analysis of the total amount of flavonoids showed their content in walnut raw materials in terms of rutin at the level of 20.2 mg/g. A slightly lower result was obtained by Vieira et al. (2019) - 13 mg/g in green leaf and 17.4 mg/g in yellow. However, other authors report a significantly higher content of flavonoids in walnut leaves - 89.62-93.51 mg/g (Turfan et al., 2020), and even 330 mg/g (Chaleshtori et al., 2011).

The content of the amount of tannins in terms of tannin in walnut leaves in our study is 124.5 mg/g in dry matter. According to Cobzaru et al. (2019), the content of this phenolic compound in the walnut leaf ranged from 91.4 mg/g to 124.7 mg/g, depending on the extraction method. At the same time, a significantly lower content of tannins is noted in fresh walnut leaves, which shows seasonal and varietal variations from 1.98 to 9.04 mg/g (Giura et al., 2019).

As can be seen from the data presented in Table 2, the content of juglone in the studied raw material is 2.72 mg/g. Meanwhile, Cosmulescu et al (2011) reported significant differences in juglone content in leaves between cultivars, ranging from 0.054 to 0.228 mg/g. According to other data, the average value of juglone, depending on the variety, was 2.26-3.51 mg/g, and its seasonal variations from 0.21 to 4.46 mg/g of dry leaves were also noted (Kocaçalışkan et al., 2020). In another study, the content of juglone in walnut leaves was 3.57 mg/g, although its individual variations depending on the place of cultivation of the plants ranged from 0.13 to 15.56 mg/g dry weight (Cahalan et al., 2011).

According to the above results of the analysis of the content of nutrients and phenolic compounds in walnut leaves, it can be assumed that the above-mentioned raw material can be considered as a phyto-additive with a positive effect on animal health and productivity. The assessment of the feed properties of the plant showed that walnut leaves can serve as an alternative to alfalfa meal in poultry feeding, and according to a number of researchers, it has high potential as a feed additive to improve the quality of eggs, in particular egg yolk, in quails (Eratalar et al., 2017) and laying hens (Untea et al., 2020; Abbasi Rad et al., 2014).

At the same time, the high content of biologically active polyphenolic compounds found in walnut leaves allows the use of this phytoraw material in feeding animals and poultry in order to improve metabolic processes in their bodies to achieve higher productivity. Some studies have also shown

the high antioxidant capacity of walnut leaves (Jahanban-Esfahlan et al., 2019), as well as its antimicrobial activity against infections caused by *P. aeruginosa* and *E. coli.* (Badiefar et al., 2022), confirming its powerful health benefits the potential of this phytoraw material.

Conclusion

Walnut leaves, in particular the "Bukovynskyi" variety, grown in the forest-steppe zone of Ukraine, are a by-product of the plant and are obtained in large quantities during its growing season. The high content of natural antioxidants, such as β-carotene and vitamin E, as well as significant amounts of essential minerals, such as calcium and phosphorus, highlight the possibility of using walnut leaves as an alternative to conventional feed components. In addition, the rich polyphenolic profile and the presence of bioactive molecules with antioxidant and antimicrobial properties indicate the potential of walnut leaves as an alternative to antibiotics in animal husbandry. Overall, the results of this study provide grounds for considering walnut leaves as a potential phytobiotic feed additive for organic poultry production. Future studies should focus on the effects of walnut leaf supplements on poultry health and performance to validate their use in feed.

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Türkiye'de Pamuk Üretiminin Su Yönetimi Açısından İncelenmesi

Water Management Perspective on Cotton Production in Türkiye

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ÖZ

Pamuk üretimi üzerine yapılan çalışmalar, pamuk yetiştiriciliğinin yüksek su tüketimine sahip olduğunu dolayısıyla, özellikle kurak bölgelerde su kaynaklarının sürdürülebilirliği üzerinde olumsuz bir etkiye sahip olduğunu göstermektedir. Pamuk sulamasında ihtiyaç duyulan suyun az olduğu bölgelerin tespit edilmesi, ülke bazında su kaynaklarının korunması adına önem arz etmektedir. Bu çalışmanın temel amacı, Türkiye'deki pamuk üretiminin yeşil ve mavi su kullanımı üzerinde derinlemesine bir analiz sunarak su ve tarım yönetimi çalışmalarına katkı sağlamaktır. Bu bağlamda ulusal iklim ve bitki verileri sanal su (SS) ve su ayak izi (SA) metodu kapsamında analiz edilmiştir. ArcMap 10.7 yazılımı ve alansal interpolasyon araçları kullanılarak ülke genelinde ekimi yapılan pamuğun yeşil ve mavi su ayak izi yüksek mekânsal ve zamansal çözünürlükle hesaplanmış, sonuçlar 24 il ve 141 ilçe genelinde görselleştirilmiştir. Buna göre, 2019-2023 yılları arasındaki üretim verileri dikkate alındığında Türkiye genelinde yıllık kütlü pamuk üretimine yaklaşık 3.68 milyar m^3 mavi suyun harcandığı görülmektedir. Tarladaki pamuğun birim yeşil ve mavi su ayak izleri ise ortalama 205 ve 1641 m^3/ton olarak bulunmuştur. Hali hazırda Şanlıurfa, Diyarbakır ve Aydın illeri, ülke için en önemli pamuk ekim merkezleri olup, pamuk yetiştiriciliği için tüketilen suyun yarıya yakını Şanlıurfa ilinde kullanılmaktadır. Bu çalışmanın sonuçlarına göre, pamuk üretiminde kullanılan birim mavi su (m^3/ton), bazı bölgelerde iki katına kadar çıkmakta, bu durum özellikle toprak neminin az olduğu kurak bölgelerde aşırı miktarda su tüketilmesine sebep olmaktadır. Buna göre, Türkiye'deki pamuk yetiştiriciliğinin Güneydoğu bölgesinden Çukurova bölgesine kaydırılması, ülkenin su kaynaklarının korunması adına önemli bir strateji olacaktır. Bu çalışmada pamuğun su tüketimini azaltmaya yönelik stratejiler de tartışılmıştır.

Anahtar Kelimeler: Türkiye, Pamuk, Sanal su, Ayak izi, Politika, Yönetim

ABSTRACT

Studies on cotton production have shown that cotton cultivation has high water consumption and thus negatively impacts the sustainability of water resources, especially in arid regions. Identifying areas with lower water requirements for cotton irrigation is important for the conservation of water resources at the national level. The primary aim of this study is to provide an in-depth analysis of green and blue water use in cotton production in Türkiye and contributing to water and agricultural management works. In this context, national climate and crop data were analyzed using the virtual water (VW) and water footprint (WF) methods. Using ArcMap 10.7 software and spatial interpolation tools, the green and blue WF of cotton cultivated across Türkiye were calculated with high spatial and temporal resolution, and the results were visualized for 24 provinces and 141 districts. According to the production data for the years 2019-2023, approximately 3.68 billion m^3 of blue water is used annually for cotton production in Turkey. The unit green and blue WFs of cotton were found to be 205 and 1641 m^3/ton on average, respectively. Currently, Şanlıurfa, Diyarbakır, and Aydın are the most important cotton centers in the country, with nearly half of the water used for cotton cultivation being consumed in Şanlıurfa. According to the results of this study, the unit blue water (m^3/ton) used in cotton production can increase up to two times in some regions, leading to excessive water consumption, particularly in arid areas with low soil moisture. Thus, shifting cotton cultivation from the Southeast region to the Çukurova region would be an important strategy for conserving the country's water resources. Strategies to reduce water consumption in cotton cultivation are also discussed in this study.

Keywords: Türkiye, Cotton, Virtual, Water, Footprint, Policy, Management

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Giriş

Son derece stratejik öneme sahip olan pamuk, sanayi devriminin en temel ürünlerinden birisi olup, tekstil endüstrisi başta olmak üzere çeşitli sektörlerde geniş bir kullanım alanına sahiptir (Telatar vd., 2016). Tarladan kütlü pamuk şeklinde hasat edilen ürünler, çırçırlanma sürecinden sonra lif ve çiğit (pamuk çekirdeği) olarak ikiye ayrılmaktadır. Lif pamuk, tekstil, selülöz kimya sanayi, savaş endüstrisi ve yatak ve dolgu sanayiinde yoğun bir şekilde kullanılmaktadır. Çiğitten elde edilen ham yağ, margarin veya likit yağ şeklinde rafine edilmekte, kalan alt ürünler ise hayvan yemi olarak değerlendirilmektedir (ZMO, 2018).

Küresel pamuk üretimi 2021 yılı itibarıyla, yaklaşık 25 milyon ton/yıl olup, toplam üretim miktarı yılda ortalama %1.5, ekim alanı ise yaklaşık %0.5 artmaktadır (OECD-FAO, 2021). 2021 istatistiklerine göre, Hindistan ve Çin dünyanın en büyük pamuk üreticileri olup, toplam pamuk üretiminin yaklaşık yarısını bu iki ülke karşılamaktadır. Bunların ardından ABD, Brezilya ve Pakistan gibi ülkeler gelmektedir. Küresel pamuk üretiminin %77'si bu ülkeler tarafından karşılanmaktadır. ABD ve Brezilya ise global pamuk ihracatının yarısından fazlasını tek başlarına karşılamaktadırlar (OECD-FAO, 2021).

Türkiye İstatistik Kurumu (TÜİK) verilerine göre, 2022 yılında Türkiye'de yaklaşık 573 bin hektarlık alanda 2.25 milyon ton kütlü pamuk üretimi yapılmıştır. Bu, yaklaşık 1.02 milyon ton lif pamuğa denk gelmektedir. Son yıllardaki rakamlara bakıldığında, Türkiye'nin lif pamuk üretiminin 0.8-1 milyon ton/yıl arasında değiştiği görülmektedir. Türkiye'de pamuk üretimi, 23 ilde yapılmakta olup, bu üretimin yaklaşık %87'sini Şanlıurfa, Diyarbakır, Aydın, Hatay, Adana ve İzmir illeri karşılamaktadır (Özüdoğru, 2023). Türkiye'de küt ve lif pamuğun verim ortalaması son altı sezon (2018-2024) için sırasıyla 4.75 ve 1.81 ton/ha olarak hesaplanmıştır (Özüdoğru, 2023).

Dış ticaret istatistikleri Türkiye'nin pamukta net bir ithalat ülkesi olduğunu göstermektedir. İhracat miktarları da düşüldüğünde 2023/24 sezonunda, yurt içi kullanımın yaklaşık %52'sine denk gelen net 0.837 milyon ton lif pamuğun yurt dışından ithal edildiği görülmektedir. Buna karşılık Türkiye'nin lif pamuk ihracatı 0.1-0.2 milyon ton arasında değişmektedir. Ülkemizde, pamuk üretiminin tüketimi karşılama oranı ortalama %45'tir. Türkiye'de lif pamuk ihracatının az olması, iç piyasadaki hazır giyim sektörünün yüksek ihtiyacına bağlanmaktadır (Özüdoğru, 2023). Uluslararası Pamuk İstişare Komitesi'nin (ICAC) güncel (2023/2024) verilerine göre ise, Türkiye, dünyanın en büyük yedinci (lif) pamuk üreticisi (0.75 milyon ton), beşinci en yüksek verimli ülkesi (1.67 ton/ha) ve dördüncü en çok tüketen (1.78 milyon ton) ülkesidir (ICAC, 2024).

Pamuğun uluslararası piyasası genellikle lif pamuk üzerinden yürümekte olup, ekonomik değeri dış etkilere bağlı olarak dalgalanmasına karşın genellikle 1500-2000 USD/ton arasında değişmektedir. 2010/2011 yıllarındaki petrol ve ilişkili polyester fiyatlarındaki aşırı artıştan dolayı pamuk ürününe talep artmış ve uluslararası piyasadaki pamuk fiyatları yaklaşık iki katına kadar çıkmıştır. Ancak, özellikle polyester ve diğer sentetik liflerin piyasada alıcı bulması sonucu, ABD doları bazında her ne kadar artsa da, son yıllarda pamuğun reel fiyatının düştüğü bildirilmiştir (OECD-FAO, 2021).

Pamuk, su tüketimi yüksek olan bir tarımsal üründür. Pamuğun bitkisel gelişimin sağlanabilmesi için gerekli su; iklim, toprak ve sulama yöntemine göre değişmekte olup bu miktarın 700-1300 mm arasında olduğu bilinmektedir (Dağdelen vd., 2005). Dolayısıyla pamuk bitkisinin su tüketimi bölgeler arasında ciddi farklılıklar sergilemektedir. Bölgenin toprak nemi veya yağış durumu da dikkate alındığında pamuk yetiştiriciliğinde kullanılan sulama suyu miktarında Türkiye genelinde kayda değer farklılıklar mevcuttur. GAP sulamalarının yaygınlaşması ile birlikte, Güneydoğu Anadolu bölgesi, son yıllarda önemli bir pamuk merkezi olmuştur.

Ülkemizde ekimi yapılan pamuk bitkisinin farklı özelliklerinin çalışıldığı birçok araştırma mevcuttur. Pamuğun bitkisel özellikleri Bozbek ve Ünay (2005), Bayhan vd. (2015) ve Haliloğlu (2016) tarafından çalışılmış; Memiş ve Özpınar (2021) pamukta bitki koruma uygulamalarını analiz etmiştir. Sulama yönetiminin bitki kalitesine etkisi; Ektiren ve Değirmenci (2018), Tunalı vd. (2019), Üzen vd. (2019), Yazdıç ve Değirmenci (2018) gibi araştırmacılar tarafından detaylandırılmıştır. Pamukta su stresi ve sulama ile ilgili özellikler; Ertek ve Kanber (2002), Ödemiş vd. (2018), Tanrıverdi vd. (2018), Erten ve Dağdelen (2020) tarafından araştırılmıştır. Çopur (2018), Eski ve Kayalak (2018), Caner ve Engindeniz (2020), üretim ile ilgili yaklaşım ve tahminleri çalışmış; Candemir vd. (2017) ve Özüdoğru (2021) ise pamukta üretim ekonomisini analiz etmişlerdir. Dağdelen vd. (2005), Aydın Ovası koşullarında, yüzey sulama yöntemi ile pamuğun su tüketimini analiz etmişlerdir. Sarı ve Dağdelen (2010), damla sulama yönteminde farklı arazi uygulamalarının su ve verim arasındaki ilişkilerini çalışmışlardır. Yeşil vd. (2023); kumaş üretimi, çırçırlama, nakliye ve tüketici kullanımları gibi endüstriyel süreçlerindeki su ayak izini raporlamışlardır. Son olarak, pamuk bitkisinin iklim değişikliğinden etkilenmesi (Ünay & Başal, 2005), gübre kullanımı (Cevheri vd., 2021) ve uzaktan algılama (Kılıçaslan vd., 2023) ile ilgili çalışmalar da mevcuttur. Ancak, ülkemizdeki pamuk üretiminin su kaynakları üzerindeki etkisinin su yönetimi açısından değerlendirilmesi ile ilgili kayda değer çalışmalar oldukça kısıtlıdır.

Türkiye'de pamuk ve tekstil sektörünün su ayak izinin (SA) değerlendirildiği birkaç tez çalışması mevcuttur. Alper (2015), Adana, Aydın, Diyarbakır, İzmir, Şanlıurfa ve Antalya illerindeki pamuğun sanal su içeriğini hesaplamış, ancak bu çalışma, pamuğun su ayak izi konusundaki ilk çalışmalardan biri olmasından dolayı su ayak izindeki değişimler ve alt SA parametreleri yeterince analiz edilmemiştir. Başkılıç (2023), tekstil sektöründeki su ayak izi üzerinde örnek bir uygulama yürütmüştür. Ancak, söz konusu çalışma endüstriyel açıdan yapılan bir çalışma olduğu için, mevcut çalışmanın kapsamı dışındadır. Engin (2019); 2016 ve 2018 yılları için Şanlıurfa, Aydın, Hatay, Diyarbakır, Adana ve İzmir illerinde pamuğun mavi ve yeşil su ayak izlerini net sulama suyu ihtiyacı ve sulama randımanını gözeterek analiz etmiştir. Ancak söz konusu çalışma, il bazında bir yaklaşım sergilemiş ve çalışmada nispeten sayılı iklim ve yağış istasyonları kullanılmış, dolayısıyla alansal değişiklikler haritalandırılmamış, aylık değişimler yansıtılmamış, ayrıca konu, su yönetimi açısından yeterince tartışılmamıştır. Avanoz (2020), Türkiye'de ekimi yapılan tarımsal ürünlerin su ayak izini 2008-2018 yılları için il bazında raporlamıştır. İraz (2021) tarafından Fırat havzası; Erdem (2021) tarafından ise Seyhan, Ceyhan ve Asi havzasındaki tarımsal üretimin su ayak izi analiz edilmiştir. Ancak bu çalışmalarda geniş bir ürün yelpazesine yer verildiği için pamuktaki su yönetimi yeterince tartışılmamıştır.

Su ayak izinin hesaplanması üzerine ilk uluslararası çalışmalar Hoekstra, Mekonnen, Chapagain ve ark. (2006; 2011; 2012; 2017) tarafından yürütülmüştür. Muratoğlu ve ark. (2019, 2020b, 2020a; 2022, 2023; 2019) tarafından yapılan çalışmalar ise Türkiye'deki tarımsal faaliyetlerin detaylı su ayak izlerinin hesaplandığı ve görselleştirildiği ilk uluslararası çalışmalardandır. SA konusunda artan uluslararası farkındalık ve GAP bölgesinde artan pamuk tarımı, pamuk üretiminin ülkemizin su güvenliği açısından detaylı bir şekilde gözden geçirilmesi gerekliliği ortaya çıkarmıştır.

Bu çalışmanın temel amacı, Türkiye'nin ulusal pamuk üretiminin, su kaynaklarının sürdürülebilirliği yönünden analiz edilmesi, ilgili sorunların tespit edilmesi ve gerekli çözüm önerilerinin getirilmesidir. Türkiye'de pamuk yetiştiriciliğinde, optimum su yönetiminin sağlanması adına ilgili araştırmacı ve kurumlara, güncel veri ve metodolojiler kapsamında bilgi sağlanması, bu çalışmanın temel hedeflerindendir. Bu bağlamda, pamuk yetiştiriciliğinin su tüketimi son yıllarda uluslararası literatürde sıklıkla kullanılmaya başlanan Su Ayak İzi (SA) metodolojisi kapsamında analiz edilmiştir. Türkiye'de ekimi yapılan pamuğun mavi ve yeşil SA değerleri belirlenerek, son dört yıllık (2019-2022) ekili alan ve üretim miktarı verileri kullanılarak il ve ilçe bazında analizler yapılmıştır. Elde edilen sonuçlar alansal ve zamansal değerlendirmelere tabi tutularak özellikle mavi (yüzey ve yeraltı) su kaynaklarının sürdürülebilirliği yönünden gerekli yaklaşımlar geliştirilmiştir. Mevcut çalışmada, Türkiye'de yetiştirilen pamuğun mavi ve yeşil su tüketimi hesapları, bugüne kadarki en yüksek alansal çözünürlük ve en güncel veriler ile gerçekleştirilmiş ve su ayak izi miktarları uzun dönem iklim verileri ile ortaya koyulmuştur. Analizlerin doğruluğunun artırılması amacı ile çok sayıda iklim ve yağış istasyonu kullanılmıştır. Elde edilen bulguların, ülkedeki su yönetimi, tarımsal planlama ve sürdürülebilirlik çalışmalarına katkı sağlaması hedeflenmektedir.

Materyal ve Yöntem

Su ayak izi (SA) metodu

Su ayak izi (SA) metodu, ürün ve süreçlerin arka planında bulunan suyun nicelleştirilmesi amacı ile son yıllarda ortaya koyulan modern araçlardan biridir. SA metodunda suyun türünün dikkate alınarak direkt ve dolaylı su tahsisinin modele katılması klasik su kullanım hesaplarına karşı avantaj sağlamaktadır. Bundan dolayı, SA metodolojisi son yıllarda geniş bir araştırma alanı bulmuş, bölgesel ve küresel su yönetimi açısından kayda değer sonuçlar ortaya koymuştur. Bu açıdan, özellikle tarım sektörünün ve ilgili süreçlerin SA analizlerinin yapılması son yıllarda su güvenliği açısından önem kazanmıştır.

SA metodolojisi, temelde *sanal su* (SS) yaklaşımına dayanmaktadır. SS ise, *nihai bir ürünün elde edilebilmesi için işlemden geçen toplam su* olarak tanımlanmaktadır. Sanal suyun, son ürün içerisinde hapsolması gerekmez, ürün üretilirken herhangi bir süreçten geçtiyse sanal su hesaplarına dahil edilir. Bu yaklaşım, 90'lı yıllarda Allan (1998) tarafından stratejik bir kaynak olarak ortaya koyulmuştur. Akabinde, ulusların sanal su ticareti konusu önem kazanmaya başlamıştır. Nihayetinde, 2000'li yıllarda Chapagain ve Hoekstra (2004) tarafından SA kavramı geliştirilmiş ve ileriki yıllarda bu yaklaşımın teorisi detaylandırılmaya başlanmıştır. Pamuk da, yüksek su ayak izi ve uluslararası değeri ile SA çalışmaları kapsamında önemli olduğu değerlendirilen ve ilk çalışılan (Chapagain vd., 2006) ürünlerdendir. Günümüzde, en güncel SA metodu, "Su Ayak İzi Değerlendirme Kılavuzu" (İng: Water Footprint Assessment Manual) adlı esere dayanmaktadır. SA metodu ve SS yaklaşımı ile ilgili diğer hususlar bu çalışmanın kapsamı dahilinde olmayıp, detaylı bilgi Hoekstra vd. (2011) ve Muratoglu (2022a, 2022b) çalışmalarından elde edilebilir.

SA metodu, işlemden geçen suyu üç farklı renk altında incelemektedir. Bunlar; yeşil, mavi ve gri sulardır. *Yeşil su, hidrolojik olarak toprak nemi depolama sisteminde biriktirilen yağış miktarını ifade eder*. Her ne kadar taşınabilme özelliği olmasa da diğer metotların aksine, toprak suyu bir su kaynağı olarak kabul edilir. *Mavi su ise*

akışkan özelliği gösteren yüzey ve yeraltı sularını simgeler. SA metodunda mavi suyun yeri ve konumundan ziyade ulaşılabilme özelliği önemlidir. Bu bağlamda, yeşil ve mavi su kaynakları her ne kadar farklı su türleri olsa da nihai ürünün su ihtiyacını karşılamaları yönünden birbirlerini tamamlayıcı özelliğe sahiptirler. Mavi su, farklı sektörlerde saklanabilme, iletilebilme ve dağıtılabilme özelliğine sahip olduğu için son derece değerli bir su kaynağı iken, yeşil su toprağın üst kısımlarında depolanması ve bitki kökleri tarafından kullanılabilmesinden dolayı bölgesel bir özelliğe sahiptir. *Mavi ve yeşil SA ise, bir ürünün yaşam döngüsünde bu kaynaklardan çekilen toplam su miktarını ifade etmektedir*. Son olarak *gri su*, bu çalışmanın kapsamı dışında olup *belli kontaminantlar ile kirletilen suyun doğal konsantrasyonlara geri çekilebilmesi için eklenmesi gereken temiz su* miktarını ifade etmektedir (Muratoglu, 2019).

Bu çalışmadaki yeşil ve mavi SA hesapları Hoekstra vd. (2011) tarafından önerilen metodolojiye dayanılarak yapılmıştır. Buna göre tarımsal ürünlerin toplam su ayak izinin (gri SA hariç), tarla koşullarında bitki su tüketimine (BST) eşit olduğu kabul edilmiştir. BST ise, bitkisel evapotranspirasyon (ET_c) hesapları ile modellenmektedir. BST, ilk olarak bitki kökleri ile ulaşılabilecek bölgedeki yeşil su kaynaklarından karşılanmakta, kalan miktar ise teorik olarak sulama ile bitkiye mavi su cinsinden verilmektedir. Buna göre, ET_c, iki alt bileşene ayrılabilir:

$$ET_c = ET_{mavi} + ET_{yeşil} \quad (1)$$

Burada; ET_c, evapotranspirasyon (mm); ET_{mavi}, sulama suyundan gerçekleşen evapotranspirasyon (mm); $ET_{yeşil}$ ise toprak neminden gerçekleşen evapotranspirasyon (mm) olarak verilebilir.

Yeşil sudan gerçekleşen evapotranspirasyonun modellenmesi için önerilen en ideal yöntem "*toprak su dengesi*" (TSD) metodudur. Ancak, geniş uzamsal sınırlarda yapılan çalışmalarda, topraktaki su dengesinin deneysel olarak yeterli hassasiyet ile takip edilmesi neredeyse imkansızdır. Buna göre, birçok çalışmada, daha pratik bir yöntem olan "*etkili yağış*" (P_{eff}) metodu kullanılmaktadır. *Etkili yağış, bitkilerin gelişim periyodu içerisinde su tüketimlerini karşılamaya yardımcı olan toplam yağış fraksiyonu* (Pathwardhan vd., 1990) olarak tanımlanmaktadır. Literatürde birçok etkili yağış metodu tavsiye edilmiş olup, bunlardan SA hesaplarında en güvenilir olanının USDA-SCS (1993) metodu olduğu belirtilmiştir (Muratoglu vd., 2023). Bu çalışma kapsamında etkili yağış, CROPWAT programı yardımı ile söz konusu metot kullanılarak hesaplanmıştır. Buna göre etkili yağış aylık olarak Denklem 2'deki gibi modellenmiştir. BST, ilk olarak toprak neminden karşılandığına göre, eğer bitkinin toplam ihtiyacı kadar su, toprak nemi formunda bitki kökleri tarafından ulaşılabilir bölgede mevcut ise etkili yağış miktarı evapotranspirasyondan büyük olacağı için sulama suyu ihtiyacı (SSİ) olmayacaktır. Bu durumda, su tüketiminin tümü yeşil sudur. Eğer yeterli miktarda toprak nemi yok ise, ET_c-P_{eff} kadar mavi suya gerek duyulacaktır (Denklem 4). Nihai olarak, yeşil ve mavi sudan gerçekleşen evapotranspirasyon miktarları sırasıyla Denklem 5 ve 6'da verilmektedir. Söz konusu bağıntılar, bitki su tüketiminin ideal koşullarda gerçekleştiği ve herhangi bir su stresi olmayan durumlar için geçerlidir. Dolayısıyla, bu koşul mevcut çalışmanın varsayımlarından bir tanesidir. Kısıntılı sulamanın verim ve su tüketimine etkisi bu çalışmanın kapsamı dışındadır.

$$P_{eff} = \begin{cases} P(125 - 0.2P)/125; & P \leq 250mm \\ 125 + 0.1P; & P > 250mm \end{cases} \quad (2)$$

$$BST = ET_C \quad (3)$$

$$SS\dot{I} = \begin{cases} 0;\ P_{eff} > ET_C \\ ET_C - P_{eff};\ ET_C > P_{eff} \end{cases} \quad (4)$$

$$ET_{yeşil} = min(ET_c, P_{eff}) \quad (5)$$

$$ET_{mavi} = max(0, ET_c - P_{eff}) \quad (6)$$

Burada; P, yağış yüksekliği (mm); P_{eff}, etkili yağış (mm), BST; bitki su tüketimi ve $SS\dot{I}$, sulama suyu ihtiyacı (mm) olarak verilebilir.

Yeşil ve mavi su yükseklikleri belirlendikten sonra toplam ve birim SA hesaplarına geçilir. SA hesabı yapılırken genellikle su hacminden bahsedilir. Dolayısıyla birim su yüksekliğinin, belirli bir tarım alanı (ha) kapsamında kullanılan su hacmine (m^3) Denklem 7 ve 8 kullanılarak dönüştürülmesi gerekmektedir.

$$SA_{yeşil} = 10 \sum ET_{yeşil}\, xA \quad (7)$$

$$SA_{mavi} = 10 \sum ET_{mavi}\, xA \quad (8)$$

$$SA_{toplam} = SA_{yeşil} + SA_{mavi} \quad (9)$$

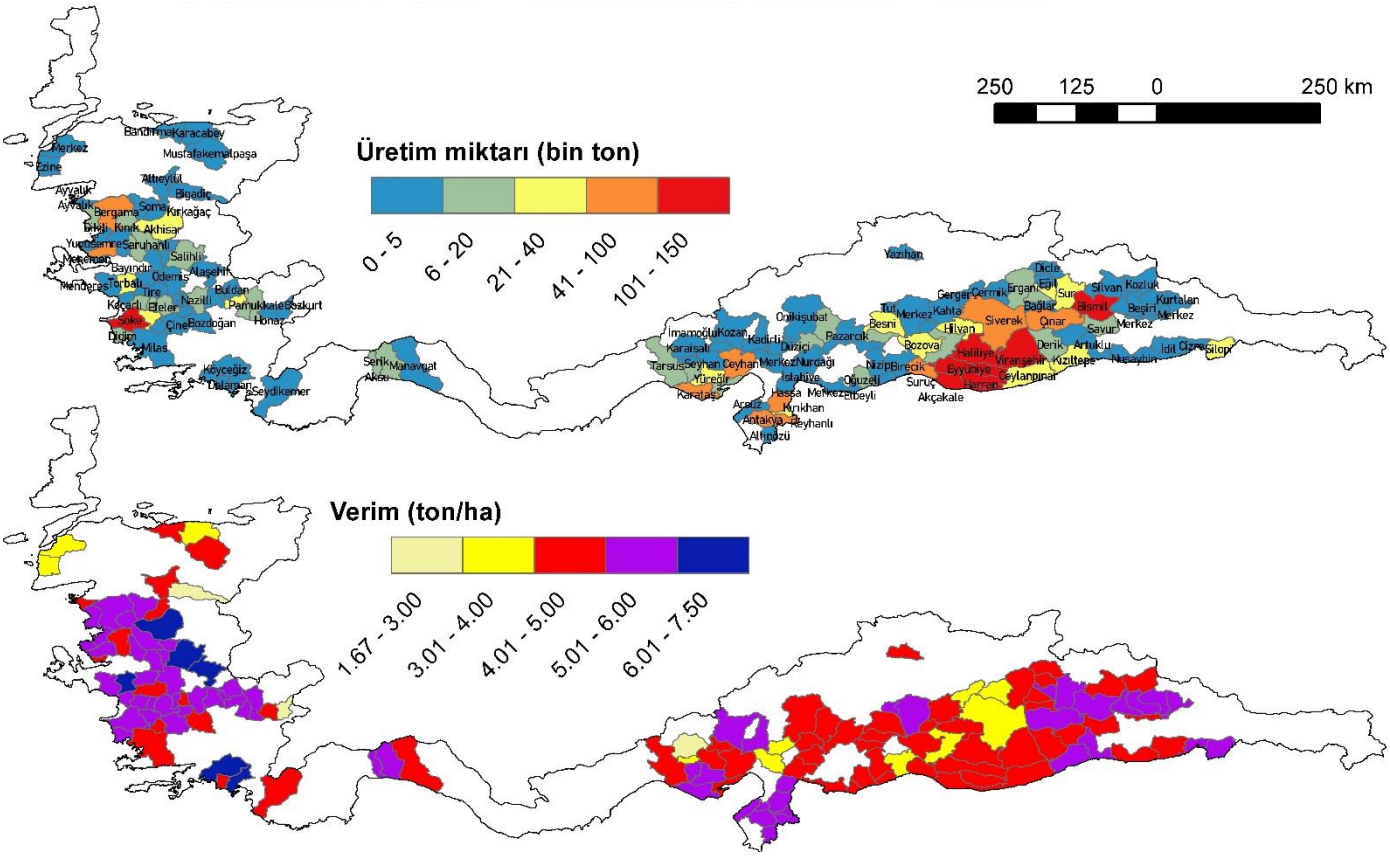
Burada; SA, su ayak izi (m^3), A ise ekilen alan (ha) olarak alınmalıdır.

Toplam su ayak izi miktarı, *belirli bir bölgede tüketilen toplam su* hacmini verdiği için su tüketimine yönelik genel bir çerçeve sunmaktadır. Bu yolla su mevcudiyeti ile tüketimi arasında kolaylıkla ilişki kurulabilir. Ancak, farklı ürünlerin su ayak izlerinin karşılaştırılması veya aynı ürünün farklı bölgelerdeki SA değişimlerinin araştırılması için bu yeterli değildir. Bunun için birim su ayak izi parametreleri geliştirilmiştir. Bunlardan en sık kullanılanı *sanal su muhtevası*dır (SSM). Bu parametre, tarımsal ürünlerin birim kütledeki su içeriğini göstermekte ve sıklıkla m^3/ton cinsinden ifade edilmektedir. Buna göre, SSM, *bir ton tarımsal ürünün yetiştirilebilmesi için harcanması gereken suyun metreküp cinsinden karşılığıdır*. Ancak, SA hesabından SSM hesabına geçilebilmesi için bitkisel üretim miktarı veya verimi kullanmak gerekmektedir (Denklem 10).

$$SSM = \frac{SA}{T} \quad (10)$$

Burada; SSM, sanal su muhtevası (m^3/ton); SA, su ayak izi (m^3), T ise bitkisel üretim miktarıdır (ton).

Şekil 1.
Çalışma alanı



Şekil 2.
Kütlü pamuk üretim miktarı ve veriminin ilçe bazlı dağılımı

Çalışma alanı, veri ve veri analizi

Bu çalışma, Türkiye'nin ülke sınırları dahilinde gerçekleştirilmiştir. Çalışmada kullanılan pamuk üretim miktarı, ekili alan ve verim istatistikleri Türkiye İstatistik Kurumunun 2019-2023 arasındaki dört yıllık verilerine dayanmaktadır (TÜİK, 2024). Bu bağlamda pamuk ekimi yapılan 24 il ve 141 ilçe bu çalışmanın kapsamına alınmıştır. Iğdır'ın Aralık, Karakoyunlu ve Merkez ilçelerinde yapılan pamuk ekimleri oldukça düşük olduğu için, sonuçların daha iyi yansıtılabilmesi amacıyla işleme alınmamıştır (Şekil 1).

Bu çalışma kapsamında uzun dönem aylık yağış gözlemleri kullanılmıştır. Bu veriler CLIMWAT 2.0 (FAO, 2024) yazılımının veri tabanından çekilmiştir. Pamuk yetiştiriciliğin yapıldığı bölge ve yakın çevresinde toplam 59 yağış istasyonunun verisi kullanılmıştır. Çalışmada kullanılan 91 adet istasyonun bitkisel su tüketimleri (ET_c) ise Türkiye'de Sulanan Bitkilerin Su Tüketim Rehberinden (TAGEM, 2017) alınmıştır. Bu veriler, ideal koşullarda uzun dönem bitki su

tüketimi değerlerini (mm) yansıtmaktadır.

Bu çalışmada, sonuçların görselleştirilebilmesi ve veri analizinin sağlanması amacıyla ArcMap 10.7 yazılımı kullanılmıştır. Noktasal yağış ve bitkisel evapotranspirasyon değerlerinin sürekli dağılımının ortaya koyulması ve bölgesel (il, ilçe) ortalamaların türetilebilmesi için alansal interpolasyon araçları ve (ordinary) Kriging yaklaşımı kullanılmıştır.

Küt ve lif pamuk istatistikleri genellikle birbirleri ile karıştırılabilmektedir. Bu çalışmada, tarımsal üretim ile ilgili değerlerde pamuk bitkisi veya kütlü pamuk kastedilmektedir. Uluslararası piyasa veya fiyatlandırmada ise lif pamuk ürünü kastedilmektedir. Bu çalışmada esas alınan TÜİK bitkisel üretim verilerine bakıldığında kütlü pamuktan lif pamuk üretim verimi %37, çiğit üretiminin ise %60 olarak gerçekleştiği görülmektedir. Bu bağlamda kütlü pamuktan lif ve çiğit verimi aşağıdaki gibi ifade edilebilir:

$$L = 0.37K \quad (11)$$

$$Ç = 0.60K \quad (12)$$

Burada; K, kütlü pamuk üretim miktarı (kg), L, pamuk lifi üretim miktarı (kg), Ç ise çiğit üretim miktarıdır (kg).

Bulgular ve Tartışma

Bitkisel üretim ve verim değerleri

Çalışma periyodu (2019-2023) içerisinde, Türkiye genelinde yıllık ortalama 460.7 bin hektar (ha) alanda 4.87 ton/ha verim ile 2.24 milyon ton (Mton) kütlü pamuk üretimi yapılmıştır. Bu, 0.83 Mton lif pamuğa denk gelmektedir. En düşük kütlü pamuk üretimi 2020 yılında 1.77 Mton; en yüksek üretim ise 2022 yılında 2.75 Mton olarak gerçekleşmiştir. Pamuk üretiminin en yoğun olduğu iller sırasıyla Şanlıurfa, Diyarbakır, Aydın, Hatay, İzmir, Adana, Manisa, Denizli, Mardin ve Adıyaman olarak verilebilir. Şanlıurfa, Türkiye'deki pamuk üretiminin %37.6'sını tek başına karşılamaktadır. Ulusal pamuk üretiminin yarısı ise Şanlıurfa ve Diyarbakır illerinden tedarik edilmektedir. Şanlıurfa'daki pamuk verimi 4.42 ton/ha ile Türkiye ortalamasının altında, Diyarbakır'daki verim ise 5.17 ton/ha ile ortalamanın üstündedir. Aydın'ın Söke; Diyarbakır'ın Bismil ve Şanlıurfa'nın Akçakale ilçeleri sırasıyla Türkiye'de en çok pamuk üretimi yapılan alt bölgeleri olup her birindeki üretim, Türkiye'nin toplam üretiminin yaklaşık %6'sının biraz üstündedir. Şanlıurfa'nın Viranşehir, Eyyübiye, Harran ve Haliliye ilçelerinden her biri ise ulusal pamuk üretiminin yaklaşık %5'ine denk gelmektedir. Çınar, Kırıkhan, Suruç, Siverek, Antakya, Karataş, Bergama, Reyhanlı ve Ceyhan ilçeleri de pamuk üretiminin kayda değer (~%2-3) olduğu diğer ilçelerdendir. Türkiye'de kütlü pamuk üretim miktarı ve veriminin ilçe bazlı dağılımı Şekil 2'de verilmiştir. En yüksek pamuk verimi, Köyceğiz, Dalaman, Alaşehir, Salihli, Akhisar, Torbalı, Kınık ve Seyhan (6-7 ton/ha) gibi ilçelerde elde edilmiştir. Şekil 2'de (alt) sarı ve kırmızı ile gösterilen ilçeler, nispeten düşük pamuk verimi; mor ve mavi ile gösterilen ilçeler ise yüksek pamuk verimi sergilemektedirler.

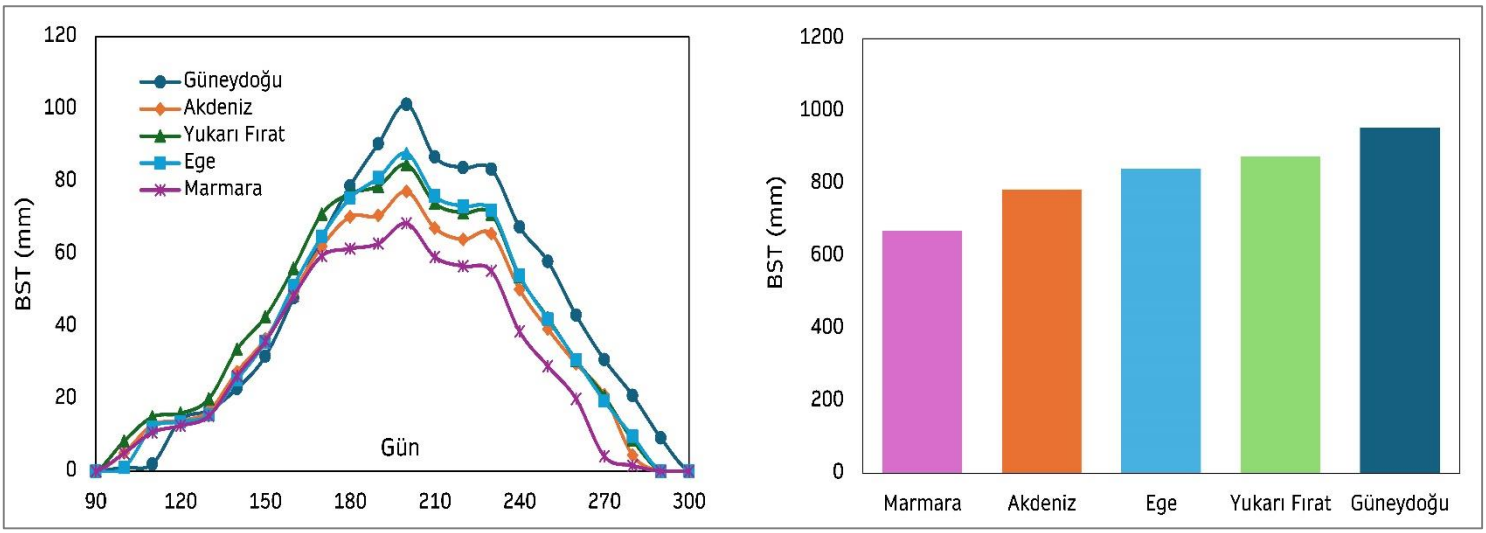
Bitki su tüketimleri

Ülkemizde pamuğun bitki su tüketimi 541-1153 mm arasında değişmekte olup, en düşük su tüketimi Yalova ili Çınarcık ilçesinde, en yüksek ise Adıyaman ili Kahta ilçesinde gerçekleşmektedir. Şekil 3'te Türkiye'nin 5 farklı bölgesindeki pamuk yetiştiriciliğinin 10'ar günlük ve sezonluk su tüketim değerleri karşılaştırılmış; Şekil 4'te ise aylık ve toplam değerler sürekli yüzey haritası şeklinde gösterilmiştir. Buna göre, Marmara bölgesi (669 mm) en düşük BST yüksekliğini sergilemekte, akabinde Akdeniz (883 mm) ve Ege (840 mm) bölgeleri gelmektedir. Güneydoğu Anadolu (953 mm) ve Yukarı Fırat (874 mm) bölgeleri en yüksek BST değerlerini yansıtmaktadır. Genellikle Nisan ayının ikinci on gününde başlayan pamuk ekimi, Güneydoğu bölgesinde Mayıs ayını bulmaktadır. Marmara bölgesinde vejetasyon süresi erken tamamlanan pamuk, Güneydoğu Anadolu bölgesinde daha geç toplanmaktadır. Nisan-Haziran periyodunda, BST değerleri arasında çok yüksek fark bulunmamakta; ancak Haziran-Ekim ayları arasında BST miktarında bölgeler arasında kayda değer varyasyonlar bulunmaktadır. Güneydoğu ve Yukarı Fırat bölgeleri, iklim koşulları ve pamuğun bitkisel özelliklerinden dolayı nispeten yüksek evapotranspirasyon değerleri sergilemektedir.

SA miktarları ve SSM değerleri

Türkiye'de pamuk üretiminin yeşil, mavi ve toplam SA miktarları sırasıyla 0.46, 3.68 ve 4.14 milyar m^3 (Gm^3) olarak hesaplanmıştır. İl ve ilçelerdeki mavi ve yeşil su akışları Şekil 5'te, il bazında mavi ve yeşil SA ve SSM miktarları, mavi su kullanımları ve yeşil su oranları Şekil 6'da, ilçe bazında farklı SA parametrelerinin harita üzerindeki dağılımı ise Şekil 7'de gösterilmiştir. Bu çalışmada elde edilen sonuçlara göre, pamuk tarımındaki mavi suyun %46'sı 1.7 Gm^3 ile Şanlıurfa'da kullanılmaktadır. En yüksek mavi su tüketimleri sırasıyla Şanlıurfa'nın Akçakale, Viranşehir, Eyyübiye, Harran ve Haliliye; Diyarbakır'ın Bismil; Aydın'ın Söke ilçelerinde gerçekleşmektedir (Şekil 6). En yüksek yeşil su tüketimleri ise Söke, Bismil, Kırıkhan (Hatay), Viranşehir, Akçakale ve Eyyübiye ilçelerinde mevcuttur.

Etkili bir su yönetimi açısından, toplam su ayak izi skorlarının bölgesel veya havza bazında su mevcudiyeti ile birlikte değerlendirilmesi tavsiye edilmektedir. Bu çalışmada, pamuk özelinde suyun iki temel rengi, su yönetimi açısından farklı incelemelere tabi tutulmaktadır. Yeşil su, sadece doğal yollardan depolanabilirliği, taşınamaz ve iletilemez olma özelliklerinden dolayı son derece lokal bir kaynaktır. Bölgeler arası yeşil su mevcudiyeti ise toprak, bitki, iklim ve coğrafya karakteristiklerine bağlı olarak değişmektedir.



Şekil 3.

Farklı bölgelerdeki pamuk yetiştiriciliğinin su tüketim değerleri

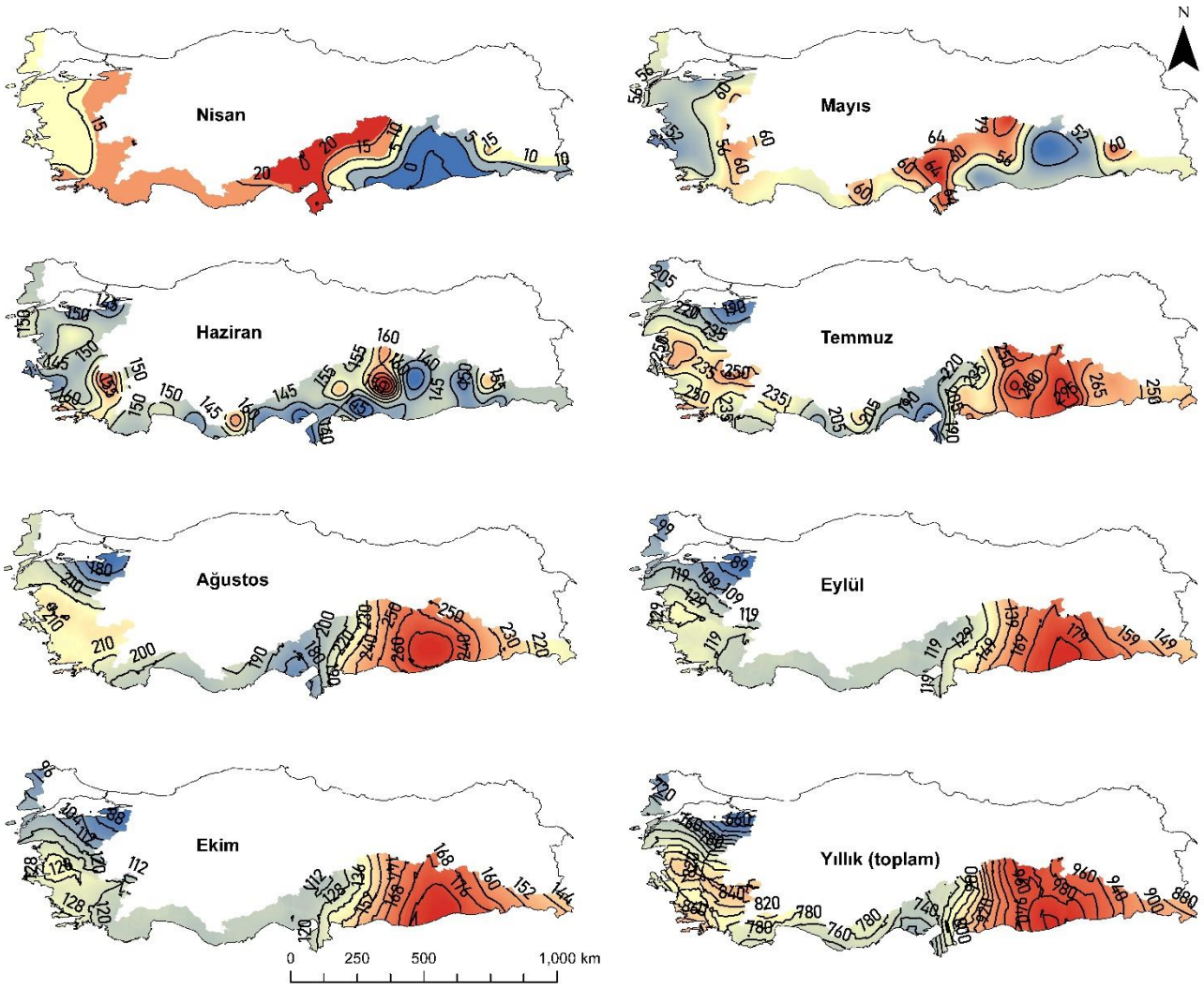
Su yönetiminin en temel amaçlarından biri, sulama suyunu ve ilgili alt yapı, bakım ve onarım maliyetlerini minimuma indirmek sureti ile toprak neminden maksimum fayda sağlamak olmalıdır. SA analizi sonuçları bu açıdan değerlendirilirse mavi su kullanımında kayda değer düşüşler elde etmek mümkündür.

SA (m^3) sonuçları, her ne kadar ürünlerin mavi su içeriklerinden etkilense de aynı zamanda üretim miktarının da bir fonksiyonudur. Toplam su ayak izinin düşürülmesi, üretim miktarlarında da bir düşüş anlamına geleceğinden, etkili su yönetimi açısından tek başına bir amaç olmamalıdır. Dolayısıyla tarımsal ürünlerin daha az sulama suyu tükettiği bölgelerin belirlenebilmesi için birim SA parametrelerine ihtiyaç vardır. Bu açıdan Şekil 6 ve 7'de m^3/ton ve m^3/ha cinsinden su ayak izi parametreleri de yansıtılmıştır. Burada, SSM veya kütle başı su ayak izi (m^3/ton), bir ton pamuk üretilmesi için gerekli (mavi veya yeşil) su hacmini ifade etmektedir. Benzer bir şekilde m^3/ha cinsinden su ayak izi parametresi ise alan başı harcanması gereken su hacmini göstermektedir. Ton başı su ayak izinin, hektar başı su ayak izine karşı avantajı, bitkisel üretim veriminin de bu parametre içinde kodlanmış olmasıdır. Bu bağlamda bölgeler arası karşılaştırma yapılırken SSM (m^3/ton) farklılıkları son derece önemlidir.

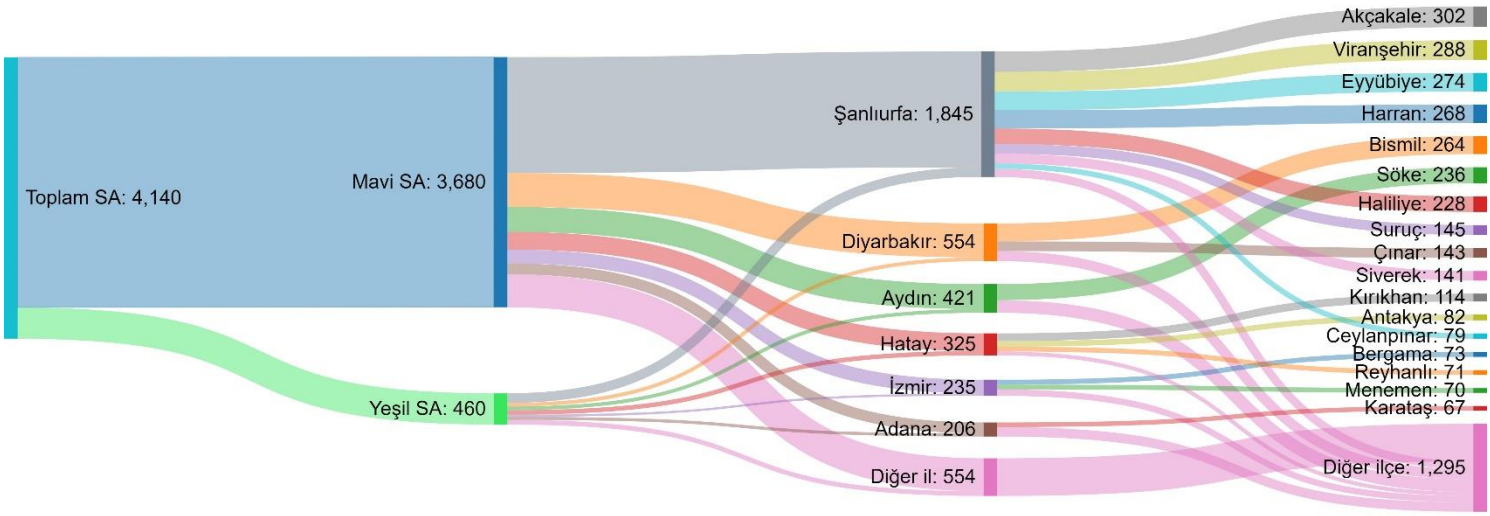
Mevcut çalışmanın sonuçlarına göre, Bozkurt (Denizli) ve Karaisalı (Adana) ilçeleri, sırasıyla 4288 ve 2845 m^3/ton ile mavi SSM parametresinin en yüksek olduğu ilçelerdir. Nitekim, bu ilçeler, sırasıyla 1.67 ve 2.11 ton/ha değeri ile Türkiye'de pamuk tarımının en düşük verim ile gerçekleştirildiği ilçeleri belirtmektedir. Bu ve benzer bölgelerde, pamuk tarımının bu verimler ile yapılması, su kaynaklarının sürdürülebilirliği adına elverişsiz bir durum olarak karşımıza çıkmaktadır. Bununla birlikte, bu ilçelerdeki istatistiklere bakıldığında, pamuk üretiminin 10 ton'un altında kaldığı ve toplamda kayda değer bir su kullanılmadığı görülmektedir. Bu bağlamda, etkili bir su yönetimi çerçevesinin çizilmesi için hem mavi SSM değerinin düşük olduğu, hem de yoğun tarım yapılan ilçelerin belirlenmesi gerekmektedir. Buna göre, Şanlıurfa'nın Akçakale, Suruç, Eyyübiye, Bozova ve Haliliye ilçeleri, sırasıyla Türkiye'de pamuk tarımının en çok mavi su tükettiği ilçelerden olup, mavi SSM değerleri de 2000 m^3/ton değerine yakın veya üzerindedir. Buralardaki yeşil SSM değerleri de oldukça düşük olup 200 m^3/ton'un altındadır. Şanlıurfa ilinin geneline bakıldığı zaman ise mavi ve yeşil SSM değerleri sırasıyla 2018 ve 167 m^3/ton olarak bulunmuştur. Su kaynakları açısından bakıldığında, Şanlıurfa'daki pamuk, aşırı yüksek mavi; aynı zamanda bölgesel iklim karakteristikleri gereği oldukça düşük yeşil su tüketim değerlerine sahiptir. Şanlıurfa, burada tipik bir örnek olarak sunulmuştur. Diyarbakır ve Mardin bölgeleri de bu kadar uç olmasa da benzer karakteristik sergilemektedirler.

Pamuk tarımında su tüketiminin düşürülmesi için önerilen stratejiler

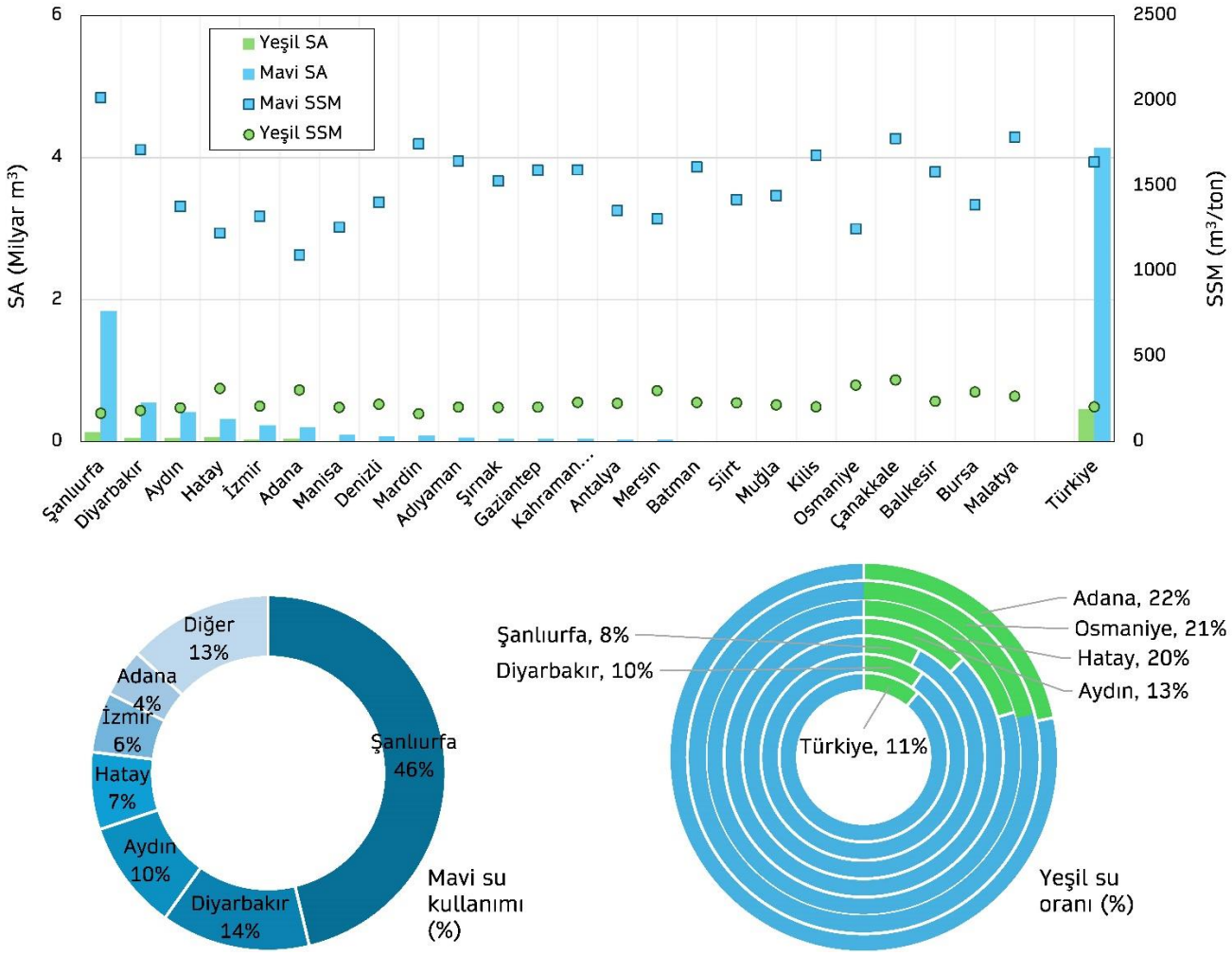
Bir önceki bölümde, bazı bölgelerde yakın üretim verimlerine sahip olunmasına rağmen, bitki su tüketimlerinde, iklim, topoğrafya, bitkisel karakteristikler vb. nedenlerden dolayı kayda değer farklılıkların olduğunu ortaya konmuştur. Bu bölümde ise, ülkedeki su kaynaklarının korunması adına pamuk özelinde geliştirilmesi gereken stratejiler incelenecektir. Bu kapsamda ilk dikkat edilmesi gereken husus, yoğun pamuk tarımında seçilmesi gereken bölgelerin gözden geçirilmesidir. Buna göre Şekil 8'de Türkiye genelinde aylık ve yıllık teorik (uzun dönem) yeşil ve mavi SA yükseklikleri paylaşılmıştır. Söz konusu haritaların evapotranspirasyon ve etkili yağışın bir fonksiyonu (Denklem 5 ve 6) olduğu unutulmamalı, geleceğe yönelik planlamalarda bu haritalar bitkisel verim (Şekil 2) ile değerlendirilmelidir.



Şekil 4.
Türkiye'de pamuk yetiştiriciliğinin su tüketim haritası (mm)



Şekil 5.
Türkiye'de pamuk tarımının mavi ve yeşil su akışları



Şekil 6.

Pamuk üretiminin il bazında mavi ve yeşil su ayak izi göstergeleri

Bitki gelişim periyodunun geç başlamasından dolayı Nisan ve Mayıs aylarında Güneydoğu bölgesinde yoğun bir yeşil ve mavi su kullanımı söz konusu değildir. Ayrıca, bu bölgede nispeten düşük yağış ve bununla ilişkili toprak neminin az olmasından dolayı, yılın geri kalan ayları boyunca (Ekim hariç) yeşil su ayak izi oldukça düşük seyretmektedir. Türkiye genelinde pamuğun yeşil su ayak izinin en yoğun olduğu merkezler iki farklı bölgede yoğunlaşmaktadır. Bunlar sırasıyla Adana ve Edirne illeri olup, buralara yakın bölgeler de benzer karakteristik sergilemektedir. Mavi su ayak izi yüksekliği dağılımına bakıldığında ise yine benzer coğrafi sebeplerden dolayı Güneydoğu bölgesindeki değerlerin oldukça yüksek olduğunu görmekteyiz. Daha kapsamlı bir değerlendirme yapılabilmesi için il ve ilçe bazındaki ortalamaların Türkiye geneli sonuçlar ile kıyaslanması önemli görülmektedir.

Yeşil ve mavi su yükseklikleri, sanal su muhtevası değerleri, verim ve toplam ulusal üretimi karşılama miktarları Şekil 9'da Türkiye ortalamaları ile normalize edilerek boyutsuzlaştırılmıştır. Buna göre şekildeki her bir parametre, ilgili parametrenin o il ve ilçedeki miktarının Türkiye ortalamasından farkının yüzdesini göstermektedir. Karşılama oranı ise, o bölgedeki üretimin Türkiye'deki üretimin (ton) % kaçını karşıladığını ifade etmektedir. Negatif deviasyonlar, ilgili parametrenin Türkiye ortalamasının ne kadar altında (%); pozitif deviasyonlar ise ne kadar üstünde olduğu göstermektedir. İl ve ilçelerin sıralanışı ise mavi SSM parametresinin düşüklüğüne göre yapılmıştır. Sonuç olarak Şekil 9'daki grafiklerin sağ tarafı pamuk ekiminin su kaynakları yönünden en elverişsiz; sol tarafı ise en elverişli olduğu bölgeleri göstermektedir. Buna göre, Siverek, Karaköprü, Mazıdağı, Hilvan ve Derik ilçeleri su kaynaklarının korunması yönünden Türkiye'nin en elverişsiz bölgeleridir. Benzer bir şekilde Şanlıurfa, Malatya, Çanakkale, Mardin ve Diyarbakır illeri, mavi su tüketimleri en yüksek olan şehirleri temsil etmektedir. Kilis ve Adıyaman'da yapılacak yoğun pamuk tarımı, Türkiye'nin su tüketimini kayda değer bir şekilde değiştirmeyecek; Batman'dan itibaren grafikte sola doğru gösterilen şehirlerdeki tarım ise mavi su tüketimini önemli ölçüde düşürecektir. Çalışmamız kapsamında Türkiye'de pamuk ekimine en elverişli ilçeler ise Seyhan, Yüreğir, Karataş, Arsuz, Belen, Yumurtalık, Ceyhan gibi ilçeler; Adana, Hatay, Osmaniye ve Manisa gibi şehirlerdir.

Mevcut çalışma kapsamında yapılan analizler, SA

çalışmalarının önemli sonuçlarından birini daha ortaya çıkarmaktadır. Buna göre, Çanakkale ili, Türkiye'de SA parametreleri yönünden sıra dışı bir yere sahiptir. Şekil 4'teki evapotranspirasyon miktarları ve Şekil 8'deki yeşil ve mavi SA yükseklikleri haritaları, Çanakkale bölgesindeki pamuk ekiminin alan başına oldukça az su tükettiği bir merkez olarak öne çıkarmaktadır. Buna göre, Çanakkale ilinde mavi su yüksekliği, kayda değer bir şekilde düşüktür (640 mm). Ancak, üretim verimindeki azlıktan dolayı (3.60 ton/ha) mavi SSM oldukça yüksek çıkmakta, bu durumu Çanakkale ilini en dezavantajlı illerden biri olma konumuna (Şekil 9) getirmektedir. Buna göre, Çanakkale ve yakın bölgelerde eğer pamuk verimi modern zirai metotlarla iyileştirilip, Türkiye ortalamalarına yaklaştırılabilirse, gelecekte buranın önemli bir pamuk üretim merkezi olma potansiyeli vardır. Ancak bu çalışma kapsamında mevcut verimler ile Çanakkale bölgesinde pamuk tarımının önemli ölçüde su kaybı yaşanmasına sebep olduğu sonucuna varılmıştır.

1980'li yıllara kadar Türkiye'deki pamuk üretiminin büyük çoğunluğu Akdeniz, ardından Ege bölgelerinde gerçekleştirilmekte idi. Ancak GAP sulamalarının başlaması ile birlikte 1995 yılından itibaren Güneydoğu'daki pamuk üretiminde kayda değer artışlar ortaya çıkmış, 2000 yılından bugüne, GAP sulamalarının artması ile Ege ve Akdeniz bölgesindeki pamuk ekim oranları önemli ölçüde azalmış, pamuğun yarısından çoğu artık GAP bölgesinde üretilmeye başlanmıştır. Harran ovasındaki pamuk ekiminin, toplam ürün deseni içerisindeki yerinin 2000'li yıllardan bugüne kadar alan bazında % 75-90 arasında değiştiği raporlanmıştır (Çopur, 2018).

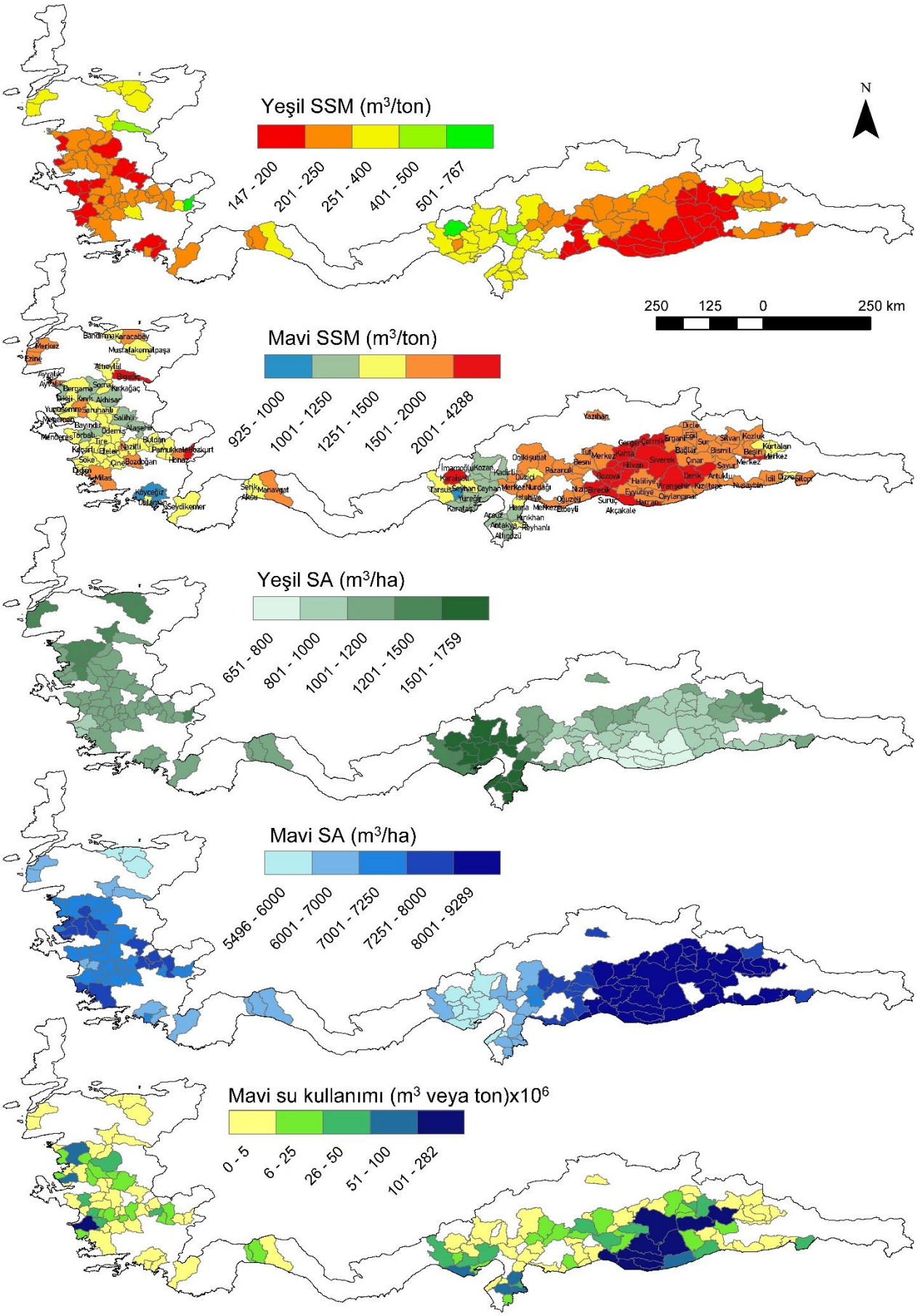
Ancak bu çalışma kapsamında yapılan analizler, ülkedeki geniş alan ve iklim çeşitliliği düşünüldüğünde Güneydoğu bölgesinde oldukça yoğun bir şekilde yapılan pamuk tarımının su kaynakları yönünden önemli miktarda kayıplara sebep olduğunu ortaya koymaktadır. Güneydoğu'da pamuk tarımına en elverişli bölge ise, nispeten yüksek yeşil su muhtevası ile Siirt-Batman hattıdır. Gelecekte su yapılarının planlanmasında pamuk yönünden bu bölgeye ağırlık verilebilir. Ancak, temel sonuçlarımız ulusal üretimin genelinin Adana-Hatay-Osmaniye bölgesine kaydırılmasının gerekli olduğunu ortaya çıkarmıştır. Adana ve Şanlıurfa, ulusal pamuk tarımının su tüketimi açısından iki uç örnek olarak ortaya çıkmaktadır. Ortalama bir ton pamuk yetiştiriciliğinde Şanlıurfa ilinde kullanılan su, Adana ilinden yaklaşık 923 m^3 daha fazladır. Ülkedeki pamuk tarımının ağırlıklı olarak bu bölgeye taşınması durumunda, toplam mavi su tüketiminin yaklaşık %30'una denk gelen 1.1 Gm^3 değerinden fazla suyun tasarruf edilmesi mümkündür.

Su tüketimini düşürmeye yönelik diğer uygulamalar

Bitkisel üretim veriminin iyileştirilmesi, su kaynaklarının korunmasına en az diğer parametreler kadar etki etmektedir. Ekim zamanı, bitki sıklığı (Bozbek & Ünay, 2005) gibi tarla ve arazi yönetimi uygulamaları, ürün kalitesinin yanında verimi de direk olarak etkilemekte, bu da kullanılan suyun minimize edilmesini sağlamaktadır. Farklı pamuk türlerinin tarlalarda denemesinin yapılmasının (Ekinci & Gençer, 2015; E. Karademir vd., 2015; Mustafayev vd., 2005) ve farklı azot ve fosfor uygulamalarının (Ç. Karademir vd., 2005) verimi iyileştirildiği görülmüştür. Benzer bir şekilde sulama aralığı ve sulama düzeylerinin (Dağdelen vd., 2009) farklı bölgelerdeki pamuk için optimize edilmesi suretiyle kayda değer miktarlarda su tasarrufu sağlanabilir. Bu kapsamda toprağın su tutma kapasitesini artıracak organik ve inorganik maddelerin uygulanması ve su hasadı gibi tekniklerinin geliştirilmesi yerinde olacaktır. Bu sayede yeşil su miktarı artırılacak olup, yeşil suyun kullanımı maksimize edilebilecektir.

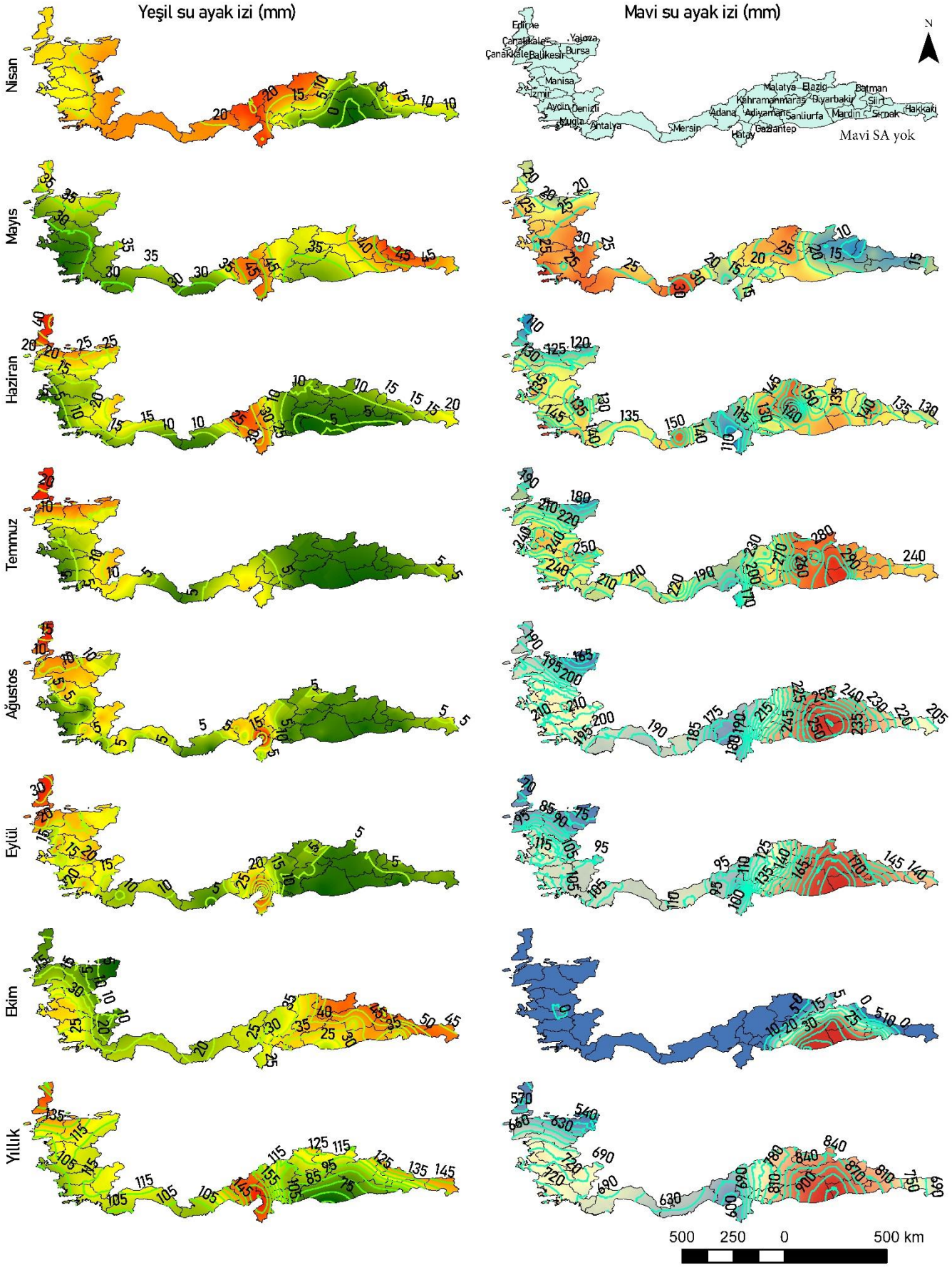
Kısıntılı sulama uygulamaları da su ayak izinin düşürülmesini sağlayan önemli tedbirlerdendir. Sulama suyundaki kontrollü eksiltmeler ile (Tunalı vd., 2019) her ne kadar bitki veriminde kısmi düşüşler yaşansa bile, bitki su tüketimi kayda değer miktarlarda azaltılabilir. Cheng vd. (2021) yaptıkları global analizlerde, kısıntılı sulama metotları ile WP (birim su başına tarımsal ürün üretim miktarı) değerlerinde ortalama %5.3'lük artış olmasına karşın, verimde %20.2'lik bir kayıp yaşanacağını ifade etmişlerdir. Bununla birlikte fide ve çiçeklenme aşamasında kısıtlı sulama yapılması durumunda ise WP'de %16.2'lik artış sağlanabileceği gösterilmiştir. Zoidou vd. (2017) kısıtlı sulamanın pamuğun sanal su muhtevasını (m^3/ton) %35'e kadar azaltabileceği; buna karşın mahsul veriminde % 30'a varan verim kayıpları yaşanabileceği göstermişlerdir. Ahmad vd. (2021) ise kısıntılı sulama ile pamuk veriminde %8-9 arasında verim kaybı görülmesine karşın su kullanım veriminde (kg/m^3ha) %75'e varan artış gözlemlemişlerdir. Li vd. (2019) ise diğer çalışmalara paralel olarak kısıtlı sulama ile su verimliliğinin artacağını, fakat pamuk veriminin azalacağını ifade etmişler ve kısıtlı sulama ile sulama suyundan %37 oranında tasarruf sağlanabilmesine karşın bitki üretim veriminde %17.5 düşüş yaşanacağı göstermişlerdir. Söz konusu konu oldukça önem taşımakta olup, farklı gelişim aşamalarında uygulanacak olan kısıtların daha detaylı incelenmesi gerekmektedir. Bu sayede daha yüksek verimlerin elde edilmesi olasıdır.

Mevcut çalışmada uzun dönem iklim verileri ve 2019-2023 bitkisel üretim verileri baz alınmıştır. Ancak, iklim değişikliğinin Türkiye geneli pamuk verimini % 14'e kadar düşüreceği (Gürkan vd., 2017) tahmin edilmektedir. Bu durum, gelecek yıllarda pamuğun su ayak izinde önemli değişiklikler olacağını ortaya koymakta ve ekim için uygun bölgelerin değişebileceği sonucunu doğurmaktadır. Bu açıdan, farklı bölgelere adapte olmuş pamuk türleri üzerinde yapılacak zirai çalışmalar (Köken & İlker, 2020) önem arz etmektedir.



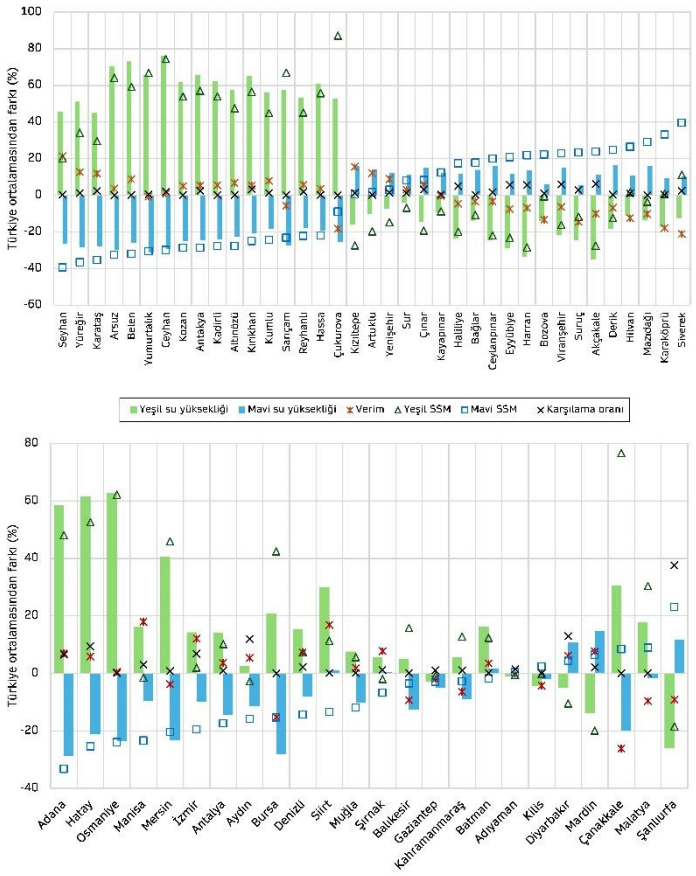
Şekil 7.

Pamuk üretiminin ilçe bazlı alansal su ayak izi göstergeleri



Şekil 8.

Türkiye genelinde pamuk üretiminin teorik ve zamansal su ayak izi yükseklikleri



Şekil 9.
Pamuk üretiminin yeşil ve mavi su yükseklikleri, sanal su muhtevası değerleri, verim ve toplam ulusal üretimi karşılama oranları

Sonuçların önceki çalışmalar ile kıyaslanması

Engin (2019) tarafından yapılan çalışmada, pamuktaki yeşil su ayak izi oldukça düşük hesaplanmış, mavi su ayak izi değerleri ise bu çalışma ile tutarlı görünmemektedir. Alper (2015) ile kıyaslandığında, bu çalışmada ulaşılan mavi SSM değerleri daha düşük çıkmıştır. Ancak, bu çalışmada elde edilen yeşil ve mavi SSM dağılımları, Avanoz (2020) tarafından CROPWAT programı kullanılarak hesaplanan bitkisel su tüketim değerleri ile oldukça tutarlı görünmektedir. Bu çalışmada elde edilen ülke ortalamaları, Chapagain vd. (2006) tarafından hesaplanan, pamuğun mavi su tüketimleri (874 mm) ve etkili yağış (90 mm) değerleri (Şekil 8) ile oldukça tutarlıdır. Esetlili vd. (2022) tarafından Küçük Menderes havzası özelinde elde edilen sonuçlara göre, yeşil SSM (292 m^3/ton) her ne kadar bu çalışma ile uyumlu olsa da, mavi SSM (2331 m^3/ton) değerleri, pamuğun o bölgedeki bitkisel evapotranspirasyon değerleri göz önüne alındığında yazarlar tarafından oldukça yüksek hesaplanmıştır. Bu çalışmada elde edilen ulusal ortalama değerler Muratoglu ve Avanoz (2021) tarafından raporlanan 2008-2019 yılları arasındaki yeşil (244 1728 m^3/ton) ve mavi SSM (1728 m^3/ton) değerleri ile de paralellik arz etmektedir. Pamuk üretiminin Dicle-Fırat bölgesinde sürdürülebilirliği ile ilgili kaygılar Pilevneli vd. (2023) tarafından da not edilmiştir. Bu çalışmanın Türkiye özelinde yapılan diğer çalışmalardan farkı, çok sayıda yağış ve bitki istasyonlardaki alansal varyasyonların yansıtılması ve en güncel bitkisel üretim verilerinin kullanılması suretiyle bugüne kadarki en geniş çözünürlükteki sonuçların yansıtılması ve bu sonuçların bölgesel ve ulusal su güvenliği açısından irdelenmesidir.

Kısıtlamalar

Bu çalışma kapsamında yapılan varsayımlardan kaynaklanan birtakım kısıtlamalar mevcuttur. Öncelikle, bitki su tüketiminin (BST), ideal koşullardaki evapotranspirasyona (ET_c) eşit olduğu varsayılmış, su stresi ve kısıntılı sulama durumları göz önüne alınmamıştır. Ayrıca, bu çalışmada elde edilen sonuçlar, net bitki su tüketimini yansıtmaktadır. Bu bağlamda sulama randımanının %100 olduğu varsayılmış, farklı sulama yöntemlerinin su ayak izine etkisi dikkate alınmamıştır. Tarlada pamuğun sulanması sırasında kullanılan enerjinin su ayak izi, hesaplara dahil edilmemiştir. Su kirliğinin bir göstergesi olan gri su ayak izi analizleri bu çalışma kapsamı dışında tutulmuştur. İklim değişikliğinin olası etkileri göz önünde bulundurulmamıştır. Fabrikada kütlü pamuğun lif ve çiğit olarak ayrıştırılmasında kullanılan enerji ve tüketilen suyun ayak izi hesaplarda dikkate alınmamıştır. Bu çalışmada pamuk su tüketimi açısından incelenmiş, nihai ürün kalitesi ve ticaret ile ilgili hususlar göz önünde bulundurulmamıştır.

Bu çalışmada kütlü pamuğun su ayak izi analizleri yapılmıştır. Lif, çiğit, yağ, kumaş vb. alt ürünlerin sanal su muhtevalarının belirlenmesi için kütlü pamuğun SSM değerlerinin, ilgili alt ürün fraksiyonuna bölünmesi ve bu süreçlerde de işlemden geçen su miktarının da eklenmesi tavsiye edilmektedir. Tekstil endüstrisindeki alt proseslerdeki su ayak izi değerleri için Başkılıç (2023) çalışması referans alınabilir.

Sonuç

Bu çalışmada, Türkiye'de ekimi yapılan kütlü pamuğun su ayak izi analizleri yapılmış, işlemden geçen yeşil (toprak nemi) ve mavi su (yüzey ve yeraltı suyu) kaynakları yüksek çözünürlüklü alansal ve zamansal değerlendirmelere tabi tutulmuştur. Bu çalışmanın temel vizyonu, ulusal pamuk tarımında tüketilen su kaynaklarının miktarındaki bölgesel farklılıkların görselleştirilmesi ve analiz edilmesi sureti ile su yönetimi çalışmalarına katkıda bulunmaktır. Bu bağlamda, GAP bölgesi, Dicle-Fırat havzası veya Şanlıurfa-Diyarbakır bölgelerindeki yoğun pamuk tarımının düşük yeşil su ve oldukça yüksek mavi su tükettiği tespit edilmiştir. Temel bulgularımıza göre, ülkedeki pamuk ekiminin Adana, Hatay, Osmaniye illerinde veya Seyhan, Yüreğir, Karataş, Arsuz, Belen, Yumurtalık, Ceyhan, Kozan, Antakya vs. gibi yeşil su muhtevası yüksek olan ilçelerde (Şekil 9)

yoğunlaştırılmasının, sulama suyu tüketiminde % 30'lara varan düşmeler ve bu suretle GAP bölgesindeki su kaynaklarının sürdürülebilirliğine önemli katkılar sağlayacağı tespit edilmiştir. Uzun yıllar büyük alt yapı maliyetleri ile inşa edilen GAP sulamalarında, bölgesel su tüketimi daha düşük ürünler yetiştirecek şekilde ağırlıklandırılması ve tarımda fırsat maliyetine dikkat edilmesi elzemdir. Ülkemizdeki su kaynaklarının tasarruf edilebilmesi ve sürdürülebilirliğin artırılması için, bu çalışmada sunulan şekil, grafik ve temel çıktıların su yönetim otoriteleri tarafından gelecek planlamalarına dahil edilmesi tavsiye edilmektedir. İleriki çalışmalarda, pamuk tarımının yeraltı suyu kaynakları üzerinde oluşturduğu baskılar ve iklim değişikliğinin pamuk tarımının su tüketim paternleri üzerindeki potansiyel etkisinin analiz edilmesi tavsiye edilmektedir.

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Enhancing Growth of Upland Rice in Low-Phosphorus Soil by Leveraging Root Morphological Traits

Düşük Fosforlu Topraklarda Kök Morfolojik Özelliklerinden Yararlanarak Yayla Pirincinin Büyümesinin Artırılması

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ABSTRACT

Low phosphorus (P) in the upland ecosystems negatively influence rice growth and cause significant yield losses. Upland rice to effectively adapt to low P in upland agroecosystems requires a suite of novel root traits. However, studies to identify these traits in upland rice grown in low P agroecosystems have received limited attention in Kenya. In the present study, nine (9) upland rice cultivars were screened to identify root traits that support low-P soil growth in a cement tank. Upland rice cultivars showed significant ($p \leq 0.05$) variation in number of root tips (NRT), number of root branching points (NBP), total root length (TRL), whole root network area (NA), average root diameter, root volume (RV), root surface area (RSA), first-order root length (FORL), and second order root length (SORL). BW01 and ITA01 recorded the highest NRT, NBP, TRL, RV, NA, RSA, FORL, and SORL, while NERICA04 had the lowest, representing 5.8, 8.0, 7.6, 6.8, 9.0, 5.8, and 9.3 differences in these traits under low P soil. NRT was significantly and positively correlated with NBP, TRL, NA, RV, RSA, and FORL, indicating the role of these traits in foraging for soil nutrients. Principal component analysis (PCA) showed that NRT, NBP, TRL, RSA, and SORL are important and effective root traits for selection in rice breeding under low P soil. BW01 and ITA01 recorded well-developed root systems, indicating that they are more P-efficient than the P-inefficient NERICA04 under low P soil conditions. Therefore, BW01 and ITA01 can be targeted for cultivation in P-deficient soils and used as trait donors to improve P-inefficient rice cultivars.

Keywords: Low phosphorus, Phosphorus efficiency, Root trait variation, Rice breeding, Upland rice

ÖZ

Yayla ekosistemlerdeki düşük fosfor (P) seviyeleri, pirincin büyümesini olumsuz etkileyerek önemli verim kayıplarına neden olur. Yayla pirincinin yayla tarımsal ekosistemlerindeki düşük P'ye etkili bir şekilde uyum sağlaması için bir dizi yeni kök özellikleri gerekmektedir. Ancak, Kenya'da düşük P içeren agroekosistemlerde yetiştirilen yayla pirinçlerinde bu özellikleri belirlemeye yönelik çalışmalar sınırlı ilgi görmüştür. Bu çalışmada, bir çimento tankında düşük P'li toprakta büyümeyi destekleyen kök özelliklerini belirlemek amacıyla dokuz (9) yayla pirinç çeşidi taranmıştır. Yayla pirinç çeşitleri, kök uç sayısı (NRT), kök dallanma noktası sayısı (NBP), toplam kök uzunluğu (TRL), tüm kök ağ alanı (NA), ortalama kök çapı, kök hacmi (RV), kök yüzey alanı (RSA), birinci derecede kök uzunluğu (FORL) ve ikinci derecede kök uzunluğu (SORL) açısından anlamlı ($p \leq 0,05$) varyasyon göstermiştir. BW01 ve ITA01, NRT, NBP, TRL, RV, NA, RSA, FORL ve SORL için en yüksek değerleri kaydederken, NERICA04 en düşük değerlere sahip olup, düşük P toprakta bu özelliklerde sırasıyla %5,8, %8,0, %7,6, %6,8, %9,0, %5,8 ve %9,3 oranlarında farklılık göstermiştir. NRT, NBP, TRL, NA, RV, RSA ve FORL ile anlamlı ve pozitif bir şekilde ilişkilidir, bu da bu özelliklerin toprak besin maddeleri için forajlamadaki rolünü göstermektedir. Temel bileşen analizi (PCA), NRT, NBP, TRL, RSA ve SORL'nin düşük P toprakta pirinç ıslahı için önemli ve etkili kök özellikleri olduğunu göstermiştir. BW01 ve ITA01, iyi gelişmiş kök sistemleri kaydederek, düşük P toprak koşullarında P-verimsiz NERICA04'ten daha P-verimli olduklarını göstermiştir. Bu nedenle, BW01 ve ITA01, P bakımından fakir topraklarda yetiştirilmek ve P-verimsiz pirinç çeşitlerini iyileştirmek için özellik donörleri olarak hedeflenebilir.

Anahtar Kelimeler: Düşük fosfor, Fosfor verimliliği, Kök özellik varyasyonu, Pirinç ıslahı, Yayla pirinci

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Introduction

Rice is a principal cereal crop that provides the global population with calories. Increased preference of rice in sub-Saharan Africa (SSA) due to a shift in consumer preference away from traditional crops toward rice consumption has triggered rice production to lag behind local demand for rice in Kenya. Therefore, there is a need to maximize production in traditional irrigated ecosystems and expand cultivation into upland ecosystems to meet Kenya's demand for rice. In upland acidic soils, free iron and aluminum oxides bind to native and applied phosphorus (P), whereas in calcareous soils, abundant calcium and magnesium compounds bind tightly to inorganic phosphates, making P inaccessible to plant roots (Alewell et al., 2020). This has contributed to over 80% of the soils in western Kenya becoming P deficient (Jama & Straatan, 2006). Therefore, P deficiency is one of the factors that limit rice production in upland rice ecosystems. The drought occasioned by climate change is likely to contribute to enhanced P deficiency because P mobility decreases with the decline in soil moisture in upland ecology (Marin et al., 2021). Low P levels in the soil are likely to affect rice seedling establishment and development because soil foraging by roots is insufficient to acquire immobile P. One strategy that can reduce P limitations is to search for varieties or cultivars with novel root traits that yield well under low P soil conditions (Rakotoson et al., 2020; Anandan et al., 2022).

Plants can withstand low P in soil by developing root morphological changes that enhance P uptake and increase internal P use efficiency (Kale et al., 2021a; Dinh et al., 2023). Previous studies have shown that rice root morphological and biomasses vary under P-deficient conditions (Wissuwa et al., 2020; Kale et al., 2021b; Anandan et al., 2022; Ranaivo et al., 2022). The genotypic differences in P uptake observed in rice are attributed to variations in the root growth and, to a lesser extent, differences in the quantity of P acquired per root size or root efficiency (Mori et al., 2016; Wissuwa et al., 2020). Rapid seedling root development drives differences in P acquisition ability during early rice seedling stages (Pariasca-Tanaka et al., 2015). The variation observed in these studies indicates that there is an opportunity to exploit root traits to improve the performance of rice cultivars under low P conditions. Although significant root morphological traits that enhance rice growth have been identified in low P soil, the characteristics of many rice varieties and cultivars grown by farmers in upland ecology in Kenya is unknown. Therefore, the yield of upland rice in P-deficient upland ecology is extremely low. Consequently, there is a need to search for root functional phenotypes that confer better establishment of upland rice in low P soil. This is likely to support better crop establishment, sustain rice production, and reduce the economic burden of the farmers that rely on fertilizer imports. The present study aimed to screen nine (9) upland rice varieties/cultivars in a cement tank to identify novel root traits that support growth under low P conditions.

Materials and Methods

Plant materials

Nine rice cultivars sourced from the Kenya Agricultural Research Organization were used in this study. These cultivars were named as BW01, IR01, IR02, IR64, ITA01, Komboka, Mnuri, NERICA01, and NERICA04, respectively.

Phenotyping for phosphorus stress in a cement tank

Low-P soil was collected from the top 15 cm of an unfertilized farm, native grassland vegetation at the University of Eldoret (0.584° N 35.309° E. 2100 masl). The soil was air-dried, sieved through a 2 mm mesh to remove coarse fragments, and thoroughly mixed. Soil pH was measured in a 1:2 (w/v) soil to distilled water suspensions, following the method described by Anderson and Ingram (1993). Total organic carbon was determined using the Walkley-Black method (Nelson and Sommers 1982), whereas Kjeldahl nitrogen was measured according to Jackson (1962). Total P was quantified using the ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extraction method as per Soltanpour and Workman (1979). Approximately 10 g of soil was placed in a beaker, mixed with 20 ml of extraction solution containing 1 M AB and 5 mM DTPA, shaken for 15 min, and then filtered. The filtrate was analyzed for P content using a colourimeter. For calcium measurement, about 0.5 g of sieved soil was placed in a beaker with 40 ml of 0.5 N HCl, left at room temperature for 5 min, and the filtrate was analyzed (Sahrawat 1987). The physical characteristics of the sieved soil were determined using the hydrometer method as described by Bouyoucos (1962). The soil exhibited the following properties: pH-H_2O 5.73, organic carbon (%) 1.89, total nitrogen (%) 0.15, available P (mg/kg) 5.38, Na (m/kg) 427, Ca (mg/kg) 1770, sand (%) 61, clay (%) 25, and silt (%) 14.

The phenotyping of root morphological traits of nine rice cultivars was carried out in a cement tank following the procedure described by Anandan et al. (2022). The cement tank was filled with air-dried, and sieved soil. Fifteen rice seeds per cultivar were sterilized by adding 10 ml of 10% sodium hypochlorite to a beaker for 10 min. Seeds were rinsed five times with distilled water to remove any sodium hypochlorite. The seeds were imbibed in water for 24 h to accelerate germination. Soaked rice seeds for each cultivar were directly seeded in a cement tank on 1st February 2023 following a completely randomized design with three

replicates. Seeds were sown in a single-row plot measuring 80 cm long and spaced 20 cm between plants within a row and 20 cm between the rows. NERICA04 was used as a P-sensitive control to identify P-efficient cultivar in this study. Weeds were manually uprooted during the experiment. No fertilizers or chemicals were applied to the experimental setup.

The soil was irrigated daily, and 15 days after sowing, the seedlings were thinned, leaving only two seedlings per hill. On the 45th day, five plants per cultivar were uprooted and placed in individual plastic bags. The roots were washed with distilled water and stored at 4°C for further analysis. Five intact root samples, including first- and second-order roots, were randomly selected for morphological measurements. Root classification followed the standard method, where first-order roots are the most distal and unbranched, and second-order roots originate at the junction of two first-order roots (Pregitzer, 2002; Freschet & Roumet, 2017). The samples were arranged in water on a transparent tray measuring 30 × 20 × 3 cm, and scanned at 300 dpi using an HP scanner (hp300 version). The following root traits were recorded: number of root tips (NRT), number of root branching points (NBP), total root length (TRL, mm), whole root network area (NA, cm²), average root diameter (ARD, mm), root volume (RV, cm³), root surface area (RSA, cm²), first-order root length (FORL, mm), and second-order root length (SORL, mm). These traits were recorded for each replication and analyzed using RhizoVision version 2 (Seethepalli et al., 2021).

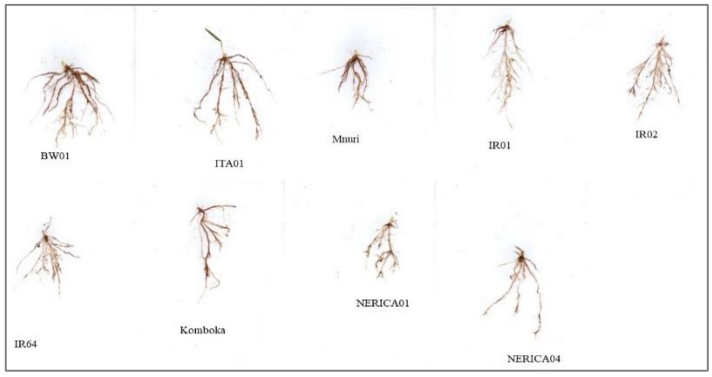

Figure 1.
Root morphological traits of nine rice cultivars grown under low phosphorus soil conditions.

Statistical Analysis

Phenotypic data: NRT, NBP, TRL, NA, ARD, RV, RSA, FORL, and SORL were subjected to one-way analysis of variance (ANOVA) to determine the response of genotype variation in low-P soil. ANOVA was performed using IBM SPSS Statistics for Windows, version 23.0 (Armonk, NY: IBM Corp), based on a general linear model at $p \leq .05$. Cultivar means in low-P soil were compared using Tukey's significance difference test. Pearson's correlation coefficient was used to determine the relationship among the root traits using IBM SPSS version 23. Principal component analysis (PCA) was performed on root morphological traits to identify novel root traits that can be used to select genotypes for cultivation and breeding to improve P use efficiency under low-P conditions. PCA analysis was executed using the GenStat (GenStat, 2003)

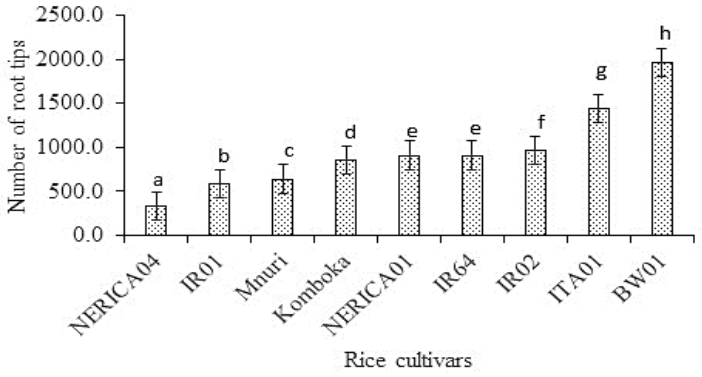

Figure 2.
Number of root tips of rice cultivars grown under low soil phosphorus soil conditions. Bar graph columns with different letter(s) sindicate that the means differ significantly at p ≤ .05.

Results

Root morphological trait variation among rice cultivars

One-way ANOVA revealed highly significant ($p \leq .05$) variation in NRT, NBP, TRL, NA, ARD, RV, RSA, FORL, and SORL among the upland rice cultivars, as presented in Table 1 and Figure 1. There was a 5.8-fold difference in NRT, ranging from 336 for NERICA04 to 1865 for BW01 (Figure 2). In low-P soil, NBP varied significantly, with an 8-fold difference varying from 216.7 in NERICA04 to 1775.3 in BW01 (Figure 3). BW01 recorded the highest TRL at 505.4 mm, whereas NERICA04 had the lowest at 66.6 mm, showing a 7.6-fold difference among cultivars under low-P soil (Table 2). The rice cultivars showed a 6.8-fold variation in NA, with BW01 having the highest of 63.0 cm², while NERICA04 had the lowest of 9.3 cm² (Table 2). The rice cultivars exhibited a 1.9-fold in average root diameter, with Mnuri recording the highest value of 2.76 mm, while IR02 had the lowest value of 1.46 mm (Table 2). The root volume (RV) varied significantly, with BW01 recording the highest value of 65.48 cm³, whereas IR01 had the lowest value of 4.54 cm³, indicating a 14.4-fold difference among rice cultivars (Table 2). IR02, NERICA04, IR64, and NERICA01 recorded RV values less than 10.0 cm³, with no significant differences among them. The rice cultivars exhibited a 9-fold difference in RSA, with BW01 recording the highest value of 449.4 cm², while IR01 and NERICA04 had the lowest values of 47.97 cm² and 54.12 cm², respectively, in low-P soil (Figure 4). BW01 recorded the highest FORL at 120.81 mm, whereas NERICA04 and IR01 had the lowest value at 20.78

mm and 22.30 mm, respectively, showing a 5.8-fold difference among rice cultivars (Table 2). The SORL of the rice cultivars showed a 9.3-fold difference in response to low-P soil, with BW01 recording the highest value of 304.86 mm, while NERICA04 and IR01 had the lowest values of 32.72 mm, and 33.70 cm, respectively (Table 2).

Table 1.

Analysis of variance for root morphological traits among nine rice cultivars in low phosphorus soil

Parameters	Genotype			
	Df	Mean square	F value	P value
NRT	8	709134.0	4310.4	< .001
NBP	8	725571.8	1697.6	< .001
TRL	8	57052.7	1587.2	< .001
NA	8	948.7	272.9	< .001
ARD	8	0.5	8.7	< .001
RV	8	1287.8	853.6	< .001
RSA	8	48826.4	859.8	< .001
FORL	8	3932.9	250.4	< .001
SORL	8	23884.6	870.7	< .001

NRT = number of root tips, NBP = number of root branching points, TRL = total root length, NA = whole root network area, ARD = average root diameter, RV = root volume, RSA = root surface area, FORL = First order root length, SORL = second order root length. $p \leq .05$

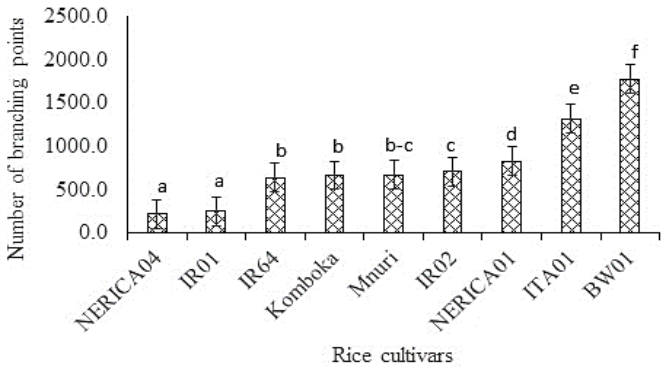

Figure 3.

Number of root branching points of rice cultivars grown under low phosphorus conditions. Bar graph columns with different letter(s) sindicate that the means differ significantly at $p \leq .05$.

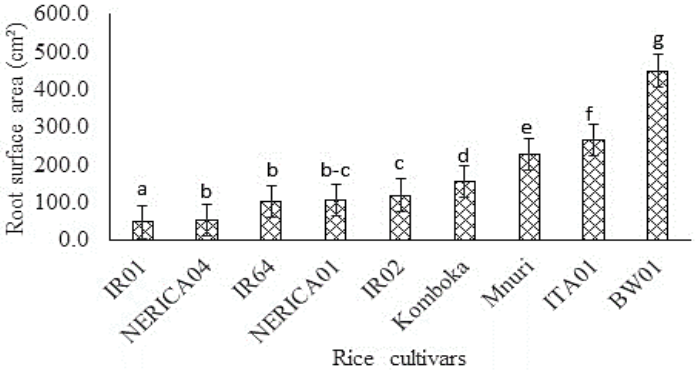

Figure 4.

Root surface area of rice cultivarss grown low soil phosphorus soil conditions. Bar graph columns with different letter(s) sindicate that the means differ significantly at $p \leq .05$.

Table 2

Mean values of root morphological traits of nine rice cultivars s grown in low-phosphorus soil

Genotype	Mean					
	TRL (mm)	NA (cm^2)	ARD (mm)	RV (cm^3)	FORL (mm)	SORL (mm)
BW01	505.5^{g}±1.0	63.0^{f}±1.0	2.4^{de}±0.3	65.5^{f}±0.8	120.8^{f}±0.9	304.9^{g}±0.4
IR01	82.0^{b}±0.5	11.1^{a}±3.6	1.7^{ab}±0.1	4.5^{a}±0.1	22.3^{a}±1.6	33.7^{a}±0.4
IR02	272.4^{e}±10.3	22.7^{c}±1.3	1.5^{a}±0.2	7.1^{b}±1.4	105.7^{e}±2.4	107.2^{c}±8.9
IR64	201.8^{c}±14.3	17.6^{b}±2.5	1.6^{ab}±0.1	8.8^{b}±0.1	61.0^{cd}±10.5	79.8^{b}±10.6
ITA01	387.2^{f}±1.9	48.0^{e}±1.0	1.8^{ac}±0.4	24.3^{d}±0.3	103.0^{e}±0.6	226.5^{f}±1.1
Komboka	207.8^{c}±1.2	25.5^{c}±2.1	2.1^{bd}±0.3	14.9^{c}±0.7	45.5^{b}±3.5	126.2^{d}±1.2
Mnuri	218.9^{d}±1.0	32.1^{d}±0.7	2.8^{e}±0.3	43.2^{e}±3.2	54.5^{c}±0.3	135.4^{e}±2.2
NERICA01	208.2^{c}±1.8	17.2^{b}±1.4	1.7^{ab}±0.1	9.3^{b}±0.7	63.6^{d}±3.0	83.5^{b}±6.9
NERICA04	66.6^{a}±1.2	9.3^{a}±1.3	2.2^{cd}±0.2	8.3^{b}±0.3	20.8^{a}±0.4	32.7^{a}±0.7

NRT = number of root tips, NBP = number of root branching points, TRL = total root length, NA = whole root network area, ARD = average root diameter, RV = root volume, RSA = root surface area, FORL = First order root length, SORL = second order root length. Means in the same column followed by a different letter(s) differ significantly at $p \leq .05$.

Table 3

Person's correlation coefficients for pairwise root morphological traits for nine rice cultivars in response to low soil phosphorus

	NRT	NBP	TRL	NA	ARD	RV	RSA	FORL	SORL
NRT	1								
NBP	0.974**	1							
TRL	0.967**	0.982**	1						
NA	0.908**	0.948**	0.953**	1					
ARD	0.045	0.195	0.161	0.398	1				
RV	0.684*	0.767*	0.753*	0.872**	0.737*	1			
RSA	0.876**	0.927**	0.922**	0.983**	0.511	0.940**	1		
FORL	0.880**	0.874**	0.931**	0.799**	-0.091	0.544	0.745*	1	
SORL	0.929**	0.965**	0.968**	0.996**	0.353	0.847**	0.976**	0.822**	1

NRT = number of root tips, NBP = number of root branching points, TRL = total root length, NA = whole root network area, ARD = average root diameter, RV = root volume, RSA = root surface area, FORL = First order root length, SORL = second order root length. ** significant at $p \leq .01$, * significant at $p \leq .05$.

Table 4

Principal component analysis showing eigenvectors, percent and cumulative variation of root morphological traits of nine rice cultivars at low phosphorus soil.

	Components	
Root traits	PC 1	PC2
ARD	0.00008	0.00394
FORL	0.04466	-0.02850
NA	0.02337	0.04746
NBP	0.68318	0.50959
NRT	0.67340	-0.6916
RSA	0.16311	0.44071
RV	0.02148	0.11457
SORL	0.11954	0.20349
TRL	0.18950	0.1007
Eigen values	7.311	1.468
% of Variance	81.238	16.307
Cumulative %	81.238	97.545

NRT = number of root tips, NBP = number of root branching points, TRL = total root length, NA = whole root network area, ARD = average root diameter, RV = root volume, RSA = root surface area, FORL = First order root length, SORL = second order root length.

Correlation coefficients of root morphological traits

Table 3 shows the Pearson's correlation coefficients of the root morphological traits for rice cultivars in low P soil conditions. In the low P soil, NRT significantly and positively correlated with NBP (r = 0.95, $p < .0001$), TRL (r = 0.94; $p < .0001$), NA (r = 0.84; $p = .001$), RV (r = 0.47; $p = .042$), RSA (r =0.77; $p = .002$), FORL (r = 0.77; $p = .002$), and SORL (r = 0.86; $p < .0001$), except for the average root diameter. NBP significantly and positively correlated with all root morphological traits under low P soil conditions, except for ARD. ARD showed a positive significant correlation only with RV (r = 0.54; $p = .024$). RV showed a positive significant correlation with RSA (r = 0.88; $p < .0001$) and SORL (r = 0.72; $p = .004$), while FORL positively and significantly correlated with SORL (r = 0.68, $p = .007$). The two principal component analyses (PCA) revealed a total variability of 97.55%, as shown in Table 4. PC1 and PC2 accounted for 81.24% and 16.31% of variability at low-P soil conditions, respectively. NBP, NRT, and TRL contributed to variation in PC1, whereas RSA and SORL were associated with PC2 (Table 3; Figure 5).

Discussion

Plants alter root morphological features as an adaptation to improve P acquisition under low P soil conditions (Gutierrez-Alanis et al., 2018). In this study, there were significant differences in root morphological traits: NRT, NBP, TRL, NA, ARD, RV, RSA, FORL, and SORL among the upland rice germplasm grown under low P soil. These variations may have arisen from evolutionary adaptations of upland rice to diverse habitats or indirect selection by farmers over the years. Previous findings indicate that rice landraces collected from different agroecological zones exhibit variations in root morphology, and are well adapted to survive low levels of P in the soil (Panda et al., 2021; Anandan et al., 2022). The observed variation indicates the presence of novel traits that can be harnessed for improved growth of P-sensitive rice cultivars such as NERICA04 under low P soil. These findings are in agreement with previous studies, which reported high variation in root number, total root length, total root surface area, root average diameter, root volume and root tips, and root and shoot biomasses in diverse rice lines under low P soil conditions (Anis et al., 2018; Solangi et al., 2020; Kale et al., 2021; Anandan et al., 2022; Ranaivo et al., 2022).

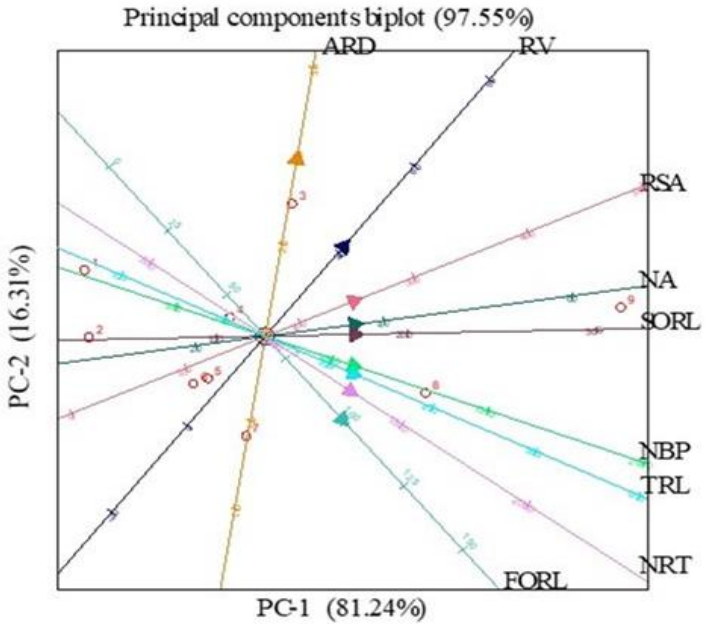

Figure 5.
Principal component analysis of nine root morphological traits of in nine rice cultivars grown at low phosphorus conditions. ARD = average root diameter, RV = root volume, RSA = root surface area, NA = whole root network area, SORL = second order root length, NBP = Number of root branching points, TRL = Total root length, NRT = Number of root tips, FORL = First order root length. Numbers in figure represent genotypes as follows: 1 = NERICA04, 2 = IR01, 3 = Mnuri, 4 = Komboka, 5 = NERICA01, 6 = IR64, 7 = IR02, 8 = ITA01, 9 = BW01.

BW01 and ITA01 recorded higher NRT, NBP, TRL, RV, RSA, FORL, and SORL than NERICA04 and IR01, indicating that they are well adapted to low P soil conditions. Therefore, BW01 and ITA01 are P-efficient rice cultivars, whereas NERICA04 and IR01 are P-inefficient. NERICA04 exhibits slow seedling growth and root development under P-limiting conditions, as reported by Wissuwa et al. (2020) and Ranaivo et al. (2022), which aligns with the findings of this study. Previous findings in Arabidopsis, rice, and cotton have revealed that increasing the root tip density, lateral root density and length, total root length, and number of root tips under low P stress increases the capacity of P to be absorbed (Fitter et al., 2002; Gutierrez-Alanis et al., 2018; Kayoumu et al., 2022; Dinh et al., 2023). De Bauw et al. (2020) similarly reported that root tips and lateral root types are key drivers of P uptake in upland rice. Rice cultivars with high root phenes, such as NRT, NBP, TRL, RV, RSA, FORL, and SORL are suitable for low P conditions.

In this study, NRT showed a significant positive correlation with NBP, TRL, NA, RV, and RSA, demonstrating that root morphological traits work synergistically to enhance P foraging in low P soil. Previous studies identified multiple traits, including root volume, total surface area, number of root tips, and total root length, that contribute to phosphorus deficiency tolerance in rice (Panda et al., 2021; Anandan et al., 2022). Kaysar et al. (2022) reported a close relationship among root length, root number, root volume and root porosity when sourcing water and nutrients from the soil in different rice cultivars under subtropical conditions. These findings align well with those of Anis et al. (2018), Kale et al. (2021b), and Anandan et al. (2022), who reported significant positive correlations among root traits in rice under low P. The strong positive correlation among the root traits demonstrates the potential to improve P-inefficient cultivars. PCA identified NBP, NRT, TRL, RSA, and SORL as important root traits that enhance P stress tolerance in upland rice. These results are consistent with previous studies by Fitter et al. (2002) and Kayoumu et al. (2022), which identified the number of root tips and total root length as key traits for improving phosphorus uptake in Arabidopsis and cotton genotypes. Therefore, NRT, NBP, RSA, and SORL are valuable root traits that can be leveraged in breeding programs to improve the growth of P-sensitive upland rice germplasm in low-P soils.

In conclusion, nine rice cultivars revealed wide variation in root morphological traits, with most traits showing significant positive correlations, indicating that they are synergistically linked in enhancing growth under low P soil. PCA showed that NRT, NBP, TRL, and RSA are key traits to target for selection in rice breeding under low soil P supply. BW01 and ITA01 recorded higher NRT, NBP, TRL, and RSA than NERICA01, NERICA04, IR01 and IRO2, among others, under low soil P conditions. Therefore, BW01 and ITA01 are suitable candidates for cultivation in P-limited upland ecosystems, and as potential donors of novel root traits to improve P-inefficient upland rice cultivars.

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Is Tea Waste A Promising Co-substrate for Optimizing The Cultivation, Growth, and Yield of Charleston Pepper (*Capsicum annuum* L.)?

Çay Atığı, Çarliston Biberi (*Capsicum annuum* L.) Yetiştiriciliğinde, Büyüme ve Verimi Optimize Etmek İçin Umut Verici Yardımcı Bir Substrat mıdır?

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ABSTRACT

To address growing concerns about sustainable agriculture and waste management, this study aimed to explore the viability of tea waste as an eco-friendly alternative substrate for cultivating Charleston peppers (*Capsicum annuum*), with the goal of optimizing plant growth and yield while reducing soil dependence, lowering cultivation costs, and repurposing agro-industrial waste. Six different substrate combinations were evaluated: 1) Tea waste, 2) Tea waste + Manure, 3) Tea waste + Soil, 4) Manure + Soil, 5) Tea waste + Manure + Soil, and 6) Tea waste + Manure + Soil + Perlite. Data were analyzed using both multivariate and univariate analyses to assess significant differences among treatments. Notably, significant differences in stem diameter were observed among plants grown on different substrates (one-way MANOVA, p <.05). However, plant height and chlorophyll content remained unaffected by substrate type. Although leaf structure exhibited considerable variation across treatments, no significant difference in dry matter content was observed. These results demonstrate that tea waste, especially when combined with other materials, is a promising sustainable substrate for Charleston pepper cultivation, potentially reducing soil dependence and agro-industrial waste.

Keywords: Pepper, Organic substrate, Manure, Tea waste substrate, Perlite

ÖZ

Sürdürülebilir tarım ve atık yönetimi konusundaki artan endişelere yanıt olarak, bu çalışma, Çarliston biberi (*Capsicum annuum*) yetiştiriciliğinde çay atığının çevre dostu alternatif bir substrat olarak kullanılabilirliğini araştırmayı ve bu sayede bitki büyümesini ve verimini optimize ederek toprağa bağımlılığı azaltmayı, yetiştirme maliyetlerini düşürmeyi ve tarımsal sanayi atıklarını yeniden değerlendirmeyi amaçlamıştır. Bu amaçla, altı farklı substrat kombinasyonu değerlendirilmiştir: 1) Çay atığı, 2) Çay atığı + Gübre, 3) Çay atığı + Toprak, 4) Gübre + Toprak, 5) Çay atığı + Gübre + Toprak ve 6) Çay atığı + Gübre + Toprak + Perlit. Elde edilen veriler, uygulamalar arasında anlamlı farklılıkları değerlendirmek amacıyla hem çok değişkenli hem de tek değişkenli analizler kullanılarak incelenmiştir. Farklı substratlarda yetiştirilen bitkiler arasında gövde çapında anlamlı farklılıklar gözlenmiştir (tek yönlü MANOVA, p <.05). Ancak, bitki boyu ve klorofil içeriği substrat tipinden etkilenmemiştir. Yaprak yapısı uygulamalar arasında önemli farklılıklar göstermesine rağmen, kuru madde içeriğinde anlamlı bir fark gözlenmemiştir. Bu sonuçlar, özellikle diğer materyallerle kombine edildiğinde çay atığının, Çarliston biberi yetiştiriciliği için toprak bağımlılığını ve tarımsal sanayi atıklarını potansiyel olarak azaltabilecek sürdürülebilir bir substrat olduğunu göstermektedir.

Anahtar Kelimeler: Biber, Organik substrat, Gübre, Çay atığı substratı, Perlit

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Introduction

Tea (*Camellia sinensis*), a globally cultivated perennial crop with a worldwide production of 27.2 million tons in 2020 and 28.2 million tons in 2021, is projected to experience significant market growth of 54.4%, increasing from USD ~96 billion in 2020 to USD ~148 billion by 2027 (Debnath et al., 2021; Industry Research, 2022; FAOSTAT, 2023). According to FAOSTAT (2023) China was the largest tea producer with 13.7 million tonnes in 2021, followed by India (5.4 million tonnes), Kenya (2.3 million tonnes), and Türkiye (1.4 million tonnes). Tea leaves are processed into different types (i.e., green, white, yellow, oolong, black, and dark tea) based on the degree of fermentation, ranging from non-fermented to post-fermented (Ho et al., 2008; Wong et al., 2022; Shi et al., 2023). Black and green teas are the most popular, accounting for approximately 75% and 15% of global tea consumption, respectively (Debnath et al., 2021; Shi et al., 2023).

As global tea consumption rises, so does the amount of biomass waste generated during harvesting and processing. Tea consumption was estimated at 6.3 million tons in 2020 and is expected to reach 7.4 million tons by 2025 (Duarah et al., 2024).The increase in tea consumption has resulted in a corresponding growth in tea waste, including discarded leaves, buds and stems. This waste disposal poses environmental risks if not properly managed (Debnath et al., 2021; Duarah et al., 2024). India, the second-largest tea producer, reported producing approximately 0.2 million tons of tea waste, representing 22.2% of its total production of 0.9 million tons (Wasewar et al., 2009; Debnath et al., 2021). Improper disposal of tea waste can contribute to environmental pollution, affecting water, soil, and air quality (Debnath et al., 2021). In response to these environmental concerns, various studies have explored the potential and sustainable utilization of tea waste in diverse fields, such as environmental remediation (e.g., bioremediation and soil amendment) (Kaliaperumal et al., 2023; Zou et al., 2023), energy generation (e.g., biogas production) (Seth et al., 2023), fabrication of polymer composites (Prabhu et al., 2021), development of electrical devices for energy storage (e.g., supercapacitors) (Ratnaji & Kennedy, 2020), and its application as a bio-manure (Karataş, 2022; Seth et al., 2023). Tea waste contains similar components and quantities to regular tea, with notable levels of nitrogen (4.5%), potassium (4.6%), and phosphorus (0.6%), and it has been proven pathogen-free and non-phytotoxic (Manyuchi et al., 2018; Sui et al., 2019; Debnath et al., 2021; Seth et al., 2023). Several studies have shown that the use of tea waste as a co-substrate can significantly improve the growth and yields of both tomato and oyster mushrooms compared with control treatments (Pane et al., 2016; Karataş, 2022).

Peppers (*Capsicum annum* L.), an exotic vegetable with a unique flavor, are a valuable source of vitamins and bioactive compounds (Anaya-Esparza et al., 2021). These compounds, including provitamins A, E, and C, phenolic compounds, and carotenoids, offer several health benefits, including anti-inflammatory, antidiabetic, antimicrobial, and immunomodulatory properties (Sagar et al., 2018; Coman et al., 2020; Samtiya et al., 2021). Peppers, which range in color from red to yellow depending on ripeness and pigments (chlorophylls or carotenoids), are categorized as either hot or sweet and are cultivated in subtropical climates worldwide (Anaya-Esparza et al., 2021).

This study conducted the first comprehensive evaluation of tea waste as a substrate for Charleston pepper cultivation, investigating its effects on growth, yield, and optimization potential in various combinations: 1) Tea waste alone, 2) Tea waste + manure, 3) Tea waste + soil, 4) Tea waste + manure + soil, and 5) Tea waste + manure + soil + perlite. Additionally, a control substrate of 6) Manure + soil, commonly used for Charleston pepper cultivation, was included for comparison. This study aims to provide complementary data on the potential of tea waste as a sustainable alternative substrate, with the goal of reducing reliance on soil, lowering cultivation costs, and promoting the valorization of agro-industrial waste.

Material and Methods

The study was conducted at the Recep Tayyip Erdoğan University Faculty of Agriculture research greenhouse in 2016. The experimental setup used organic tea waste sourced from a tea factory in Rize as the primary growing medium. Composted barn manure was obtained from a local producer in Rize. The manure used was derived from year-old, composted cattle manure. The perlite used in the mixture was coarse agricultural perlite with a particle size of 3-6 mm. The peat used was a fine-textured sphagnum moss peat with a pH of 6 and was sterilized to be free of pathogens such as nematodes and fungi (Klasmann TS1). Both perlite and peat were acquired from the “Tartes” company.

The soil characteristics analyzed in this research were as follows: pH = 4.7 (indicating acidity), EC = 0.73 dS/m (non-saline), organic matter content = 1.93% (low), lime content = 0.21% (low), and phosphorus content = 2.19 mg/kg (very low). The soil was classified as clayey.

The plant material used in this study consisted of the “Charliston 341” “Yalova Charleston” variety obtained from the Torun Seed Company. This variety is characterized by its yellow-green color, thick fruit wall, and sweet taste, and it is classified as a Charleston-type pepper.

Table 1.
Compositions of different substrates applied to Charleston pepper (Capsicum annuum L.) cultivation.

SUBSTRATE	Ratios (v/v)
Manure + Soil (control)	1:1
Tea waste	1
Tea waste + Manure	2:1
Tea waste + Soil	2:1
Tea waste + Manure + Soil	2:1:1
Tea waste + Manure + Soil + Perlite	2:1:1:1

The experiment involved the preparation of five different media using tea waste (T), perlite (P), soil (S), and composted barn manure (M) at varying volume ratios (v/v) (Table 1). A mixture of manure and soil (M + S) was used as the control. The experiment was designed as a randomized block trial with three replications, each containing five plants.

- Seed sowing occurred on March 24, 2016, in black plastic pots (18x16 cm), with 3-4 seeds planted per pot.
- After germination, two seedlings were left in each pot, and one was used for seedling measurements. The time at which 50% of the seeds germinated was recorded as the germination time.
- Measurements were taken when the seedlings had 3-4 true leaves and included cotyledon width, cotyledon length, leaf width, leaf length, leaf area, leaf chlorophyll content, hypocotyl length, seedling height, seedling stem diameter, and dry matter content. These measurements were conducted on five samples.
- Flowering dates were recorded when 50% of the plants had flowered.
- Plant and leaf measurements were taken on August 9, 2016, and fruit harvesting was conducted on August 10, 2016. For green peppers that had reached harvest maturity, measurements included fruit width, fruit length, peduncle length, average fruit weight, and dry matter content. These measurements were taken from five fruits. Additional mature Charleston peppers were harvested once and categorized into three groups: red, orange, and green. For each group, measurements of fruit width, length, fruit count, total fruit weight, and average fruit weight were taken.
- Leaf measurements were performed on 10 fully grown leaves to assess leaf blade width, length, area, leaf chlorophyll content, and dry matter content. At the end of the experiment, plant height and stem diameter were recorded for each of the five plants.

Irrigation was carried out using a filtered bucket, based on the drying of the soil in the pots. To more precisely determine the impact of environmental conditions on plant development, no commercial manure was used.

- The dry matter content was determined by drying the leaves in an oven at 70°C until the weight of the fresh leaves stabilized.
- Leaf area measurements were taken on fully developed leaves randomly selected from each plant. The leaf area was measured using the WinDIAS image analysis system (Delta-T Devices, UK) and an HP Scanjet G2410 scanner (Hewlett-Packard, Palo Alto, California, USA).
- Chlorophyll levels were measured using a Konica Minolta SPAD-502 Plus chlorophyll meter (Konica Minolta, Tokyo, Japan). Width and length measurements were performed using a digital caliper and millimeter ruler.

Statistical analysis

The data from three replications are presented as Mean ± SD, are displayed in descending order in the figures to clearly illustrate the decrease in observed values across different substrate treatments. To evaluate significant variations in the cultivation, growth, and yield characteristics of Charleston pepper across various substrates, one-way ANOVA was used if the data followed a normal distribution, as verified using the Shapiro-Wilk test. For data that did not meet the normality assumption, the Kruskal-Wallis test was applied. Prior to conducting principal component analysis and dendrogram analysis, the data were standardized using the log + 0.1 transformation (Alkan et al., 2019). All statistical analyses were performed using R software.

Results

Duration to T_{50}

Seed germination

The mean number of days required for T_{50} germination of control seeds was 20.3 ± 0.6 days, which was significantly higher than the Tea Waste + Manure + Soil treatment and Tea Waste + Manure + Soil + Perlite treatment (one-way ANOVA, $F_{(5,12)} = 17.6$, $p < .05$). The overall maximum number of days was observed for tea waste treatment, with 8.4% more time required for T_{50} germination (Figure 1).

Flowering

The control treatment, Manure + Soil, produced T_{50} flowering in 55.0 ± 1.7 days, which was significantly shorter than that of all other treatments (one-way ANOVA, $F_{(5,12)} = 32.1$, $p < .05$). The control treatment was followed by Tea Waste + Soil treatment, which took 58.0 ± 1.0 days to T_{50} flowering (Figure 1).

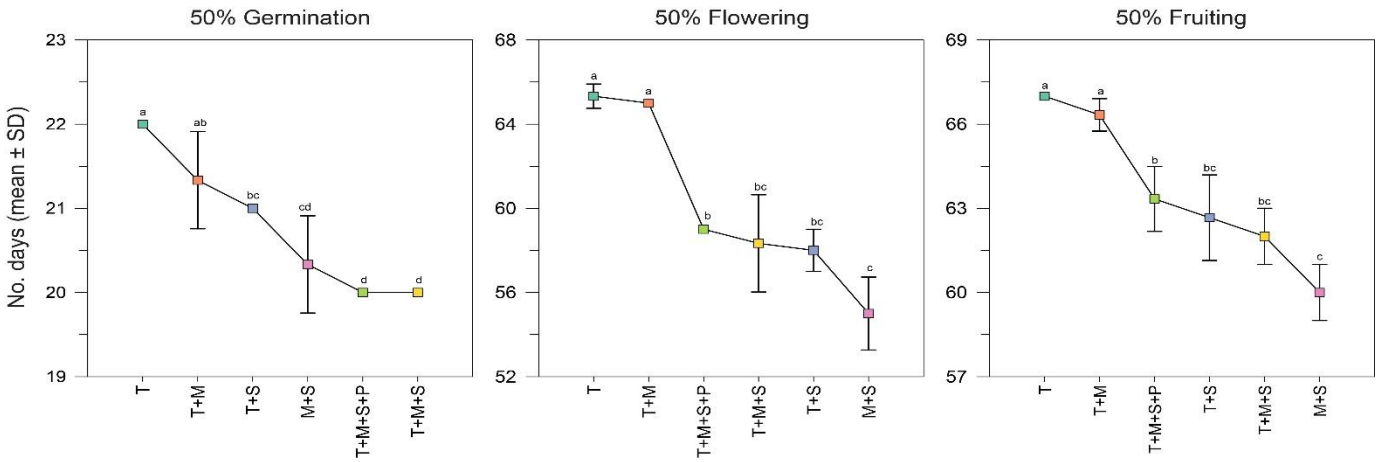

Figure 1.

Mean (± s.d.) time taken for 50% (T_{50}) of seeds/seedlings to achieve germination, flowering, and fruiting of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

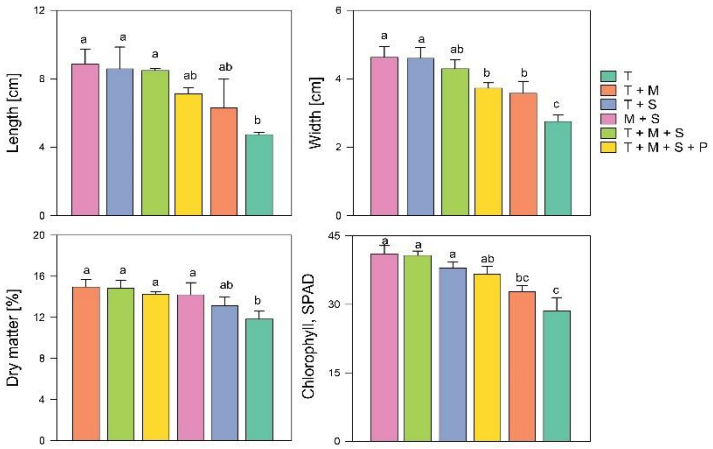

Figure 2.

Physical properties Seedling leaf of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

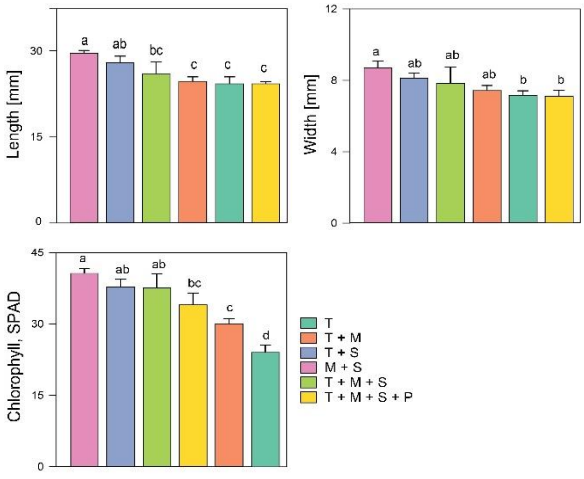

Figure 3.

Physical properties of Cotyledons of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Fruiting

Similarly, flowering, the control treatment of Manure + Soil took a shorter time to provide T_{50} fruiting, which was 60.0 ± 1.0 days, followed by Tea Waste + Manure + Soil treatment with 62.0 ± 1.0 days) and Tea Waste + Soil treatment 62.7 ± 1.5 days (Figure 1). The T_{50} fruiting was significantly different between treatments (one-way ANOVA, $F_{(5,12)} = 21.3$, $p < .05$).

Physical properties Seedling leaf and Cotyledon

The mean (± s. d.) of the seedling leaf and cotyledon leaves under different treatments are provided in Table 1. The control treatments, Manure + Soil and Tea Waste + Soil, provided the seedling leaves with the highest length and width sizes, which differed significantly from the other treatments (Figure 2). On the other hand, on the other hand, the smallest length and width of the seedling leaves were treated with Tea Waste, followed by Tea Waste + Manure. The highest chlorophyll contents were observed in the control treatment (Manure + Soil) followed by Tea Waste + Manure + Soil treatment, while the Tea Waste treatment had the lowest chlorophyll contents (Figure 2). The highest dry matter content of seedling leaves was provided by the Tea Waste + Manure treatment, followed by the Tea Waste + Manure + Soil and Tea Waste + Manure + Soil + Perlite treatments, which were significantly similar (one-way ANOVA, P > 0.05). Likewise, the Tea Waste treatment provided the poorest results and the lowest dry matter content among the treatments (Figure 2).

The Cotyledon response to different treatments was consistent with seedling leaf, providing better results in the control, Manure + Soil, and Tea Waste + Soil treatments. Furthermore, the lowest chlorophyll was observed in Charleston pepper cultivated under Tea Waste treatment. However, the smallest Cotyledon length and width were provided by Tea Waste + Manure + Soil + Perlite followed by Tea Waste (Figure 3).

Physical properties of plants and leaves

The plant size of the Charleston pepper also showed significant variations between different treatments, with the highest height of the plant being achieved by the Tea Waste + Manure treatment, followed by the Tea Waste + Soil. The smallest plant size was observed for Tea Waste + Manure + Soil + Perlite followed by the control treatment, Manure + Soil treatment (Figure 4). The highest chlorophyll contents were recorded in leaves treated with Tea Waste + Manure and Tea Waste + soil. On the other hand, the control treatment (Manure + Soil treatment) provided the leaves with the lowest chlorophyll content (Figure 4).

The length, width, and dry matte of leaves did not significantly differ between treatments (P > 0.05). The highest length and width of leaves were recorded for Tea Waste + Soil followed by Tea Waste + Manure + Soil and then the control treatment, Manure + Soil (Figure 5). However, the highest dry matter content of leaves was recorded in the Tea Waste + Manure + Soil + Perlite treatment, followed by the Tea Waste + Manure + Soil treatment and then the Control treatment, Manure + Soil.

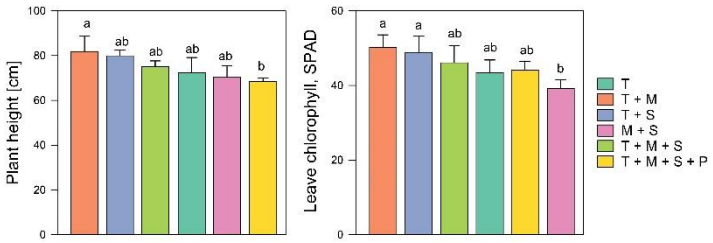

Figure 4.

Physical properties, plant height, and leaf chlorophyll content of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

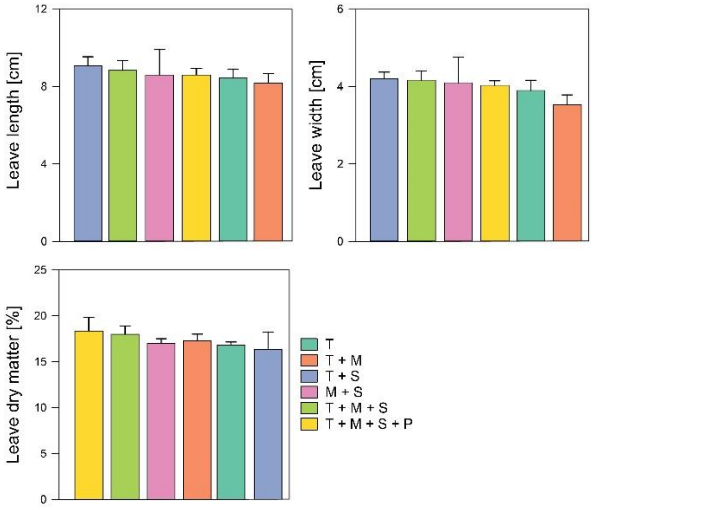

Figure 5.

Physical properties of leaves of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Fruit number per plant

At 139 days, the fruits were harvested and categorized (as green, yellow, or red), and counted for each substrate. The highest mean number of total fruits (green, yellow, and red) per plant was observed in the Tea Waste + Soil substrate, with an average of 18 fruits per plant. This was followed by Tea Waste + Manure + Soil + Perlite substrate, with an average of 15 fruits per plant. The lowest mean count of total fruits per plant was recorded for the Tea Waste + Manure substrate, with an average of 11 fruits per plant.

The mean counts of fruits (green, yellow, or red) among different substrates showed significant differences. However, in the subsequent analysis using the Tukey test, the differences among the different substrates were not significant. Nonetheless, significant differences were observed for the green fruits, with the Tea Waste substrate exhibiting a significantly higher number of green fruits ($F_{(5,12)}$ = 12.59, p <.001; Figure 6). The mean weight of a single Charleston pepper did not differ significantly among the different substrates (Green: one-way ANOVA, $F_{(3,6)}$ = 1.78, p =.251, Yellow Charleston pepper: Kruskal-Wallis One-Way ANOVA, H = 4.02, p =.547; and Red: one-way ANOVA, $F_{(5,12)}$ = 0.578, p =.717).

Physical characteristics of the Charleston pepper

The physical properties of the Charleston pepper differed greatly between treatments. The highest-width Charleston pepper was produced using the Manure + Soil treatment, followed by Tea Waste + Manure + Soil and Tea Waste + Soil. The Charleston pepper was the smallest width in the Tea Waste treatment. The dry matter of the Charleston pepper also exhibited significant variations. The highest dry matter content was provided by Tea Waste + Manure + Soil + Perlite, followed by Tea Waste and Tea Waste + Soil. The dry matter of Charleston peppers obtained using the Manure + Soil treatment was significantly smaller than that of other types (Table 2).

PCA and dendrogram analysis

The first and second principal components explained up to 60% of the variation in the data (Figure7). The number of significant correlations was 17, and six were negatively correlated. Negative correlations were found between fruit weight (green) and Fruit width with a Pearson correlation of -0.65. A significant positive correlation between Fruit width and red Charleston pepper count was recorded (a Pearson correlation of 0.49. However, the green fruit and red Charleston pepper counts were negatively correlated, with a Pearson correlation of -0.82 (Figure 7).

The cluster analysis dendrogram identified three clusters. The first cluster was created by Tea Waste treatment and Tea Waste + Manure treatment, which contributed up to

97.1%. This cluster exhibited the highest dissimilarity with the others. The second cluster was created by the control group, Manure + Soil (Figure 8). This second cluster exhibited the highest similarities with the third cluster compared to the first cluster. In the third cluster, the highest similarity was recorded between the Tea Waste treatment and the Tea Waste + Manure + Soil, at 98.2%.

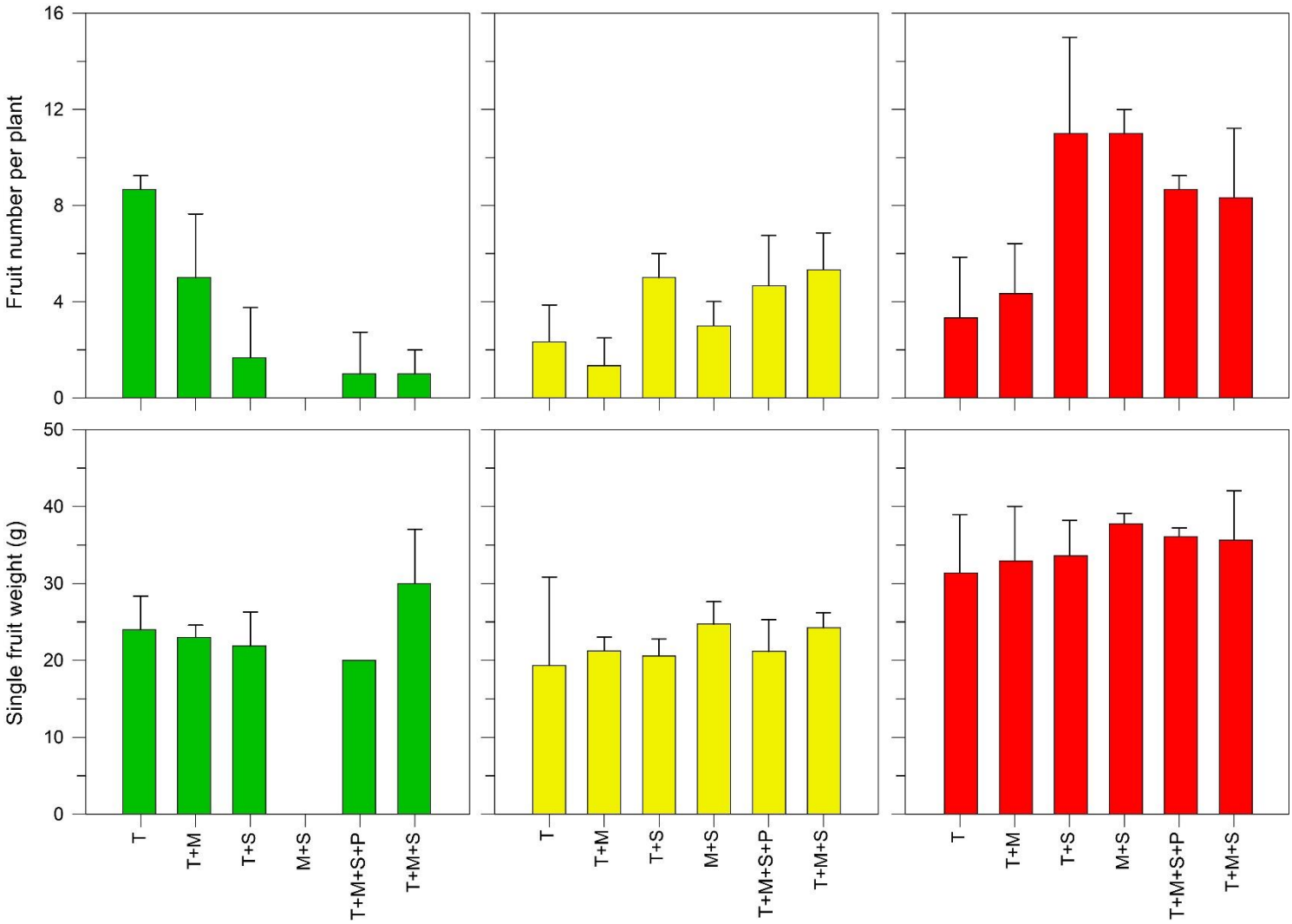

Figure 6.

Fruit count per plant and mean (± s. d.) weight of individual Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite. The green, yellow, and red colors indicate the fruit colour stage.

Table 2.

Effects of different substrates on the physical properties (Mean ± s. d.) of Charleston pepper (Capsicum annuum L.) harvested at T_{50} fruiting stage.

SUBSTRATE	Fruit width (mm)	Fruit length (cm)	Fruit stem length (mm)	Fruit dry matter (%)	TSS (%)
Tea waste	30.16 ± 2.97^{a}	13.95 ± 0.61	40.60 ± 0.25	9.90 ± 0.29	6.87 ± 0.59
Tea waste + Manure	31.16 ± 2.07^{a}	14.73 ± 0.91	38.78 ± 2.52	6.06 ± 5.25	4.17 ± 3.61
Tea waste + Soil	34.01 ± 1.72^{ab}	15.13 ± 0.49	41.91 ± 3.04	9.29 ± 0.36	7.47 ± 0.50
Manure + Soil	36.58 ± 1.33^{b}	14.75 ± 0.59	45.18 ± 2.73	8.27 ± 0.42	6.57 ± 0.38
Tea waste + Manure + Soil	34.07 ± 0.45^{ab}	14.41 ± 0.84	42.63 ± 2.92	9.20 ± 0.72	7.00 ± 0.00
Tea waste + Manure + Soil + Perlite	32.87 ± 1.25^{ab}	15.32 ± 1.22	39.08 ± 4.47	10.06 ± 0.49	6.87 ± 0.42

TSS: total soluble solid content

Substrates with the same letter are not significantly *different from each other.*

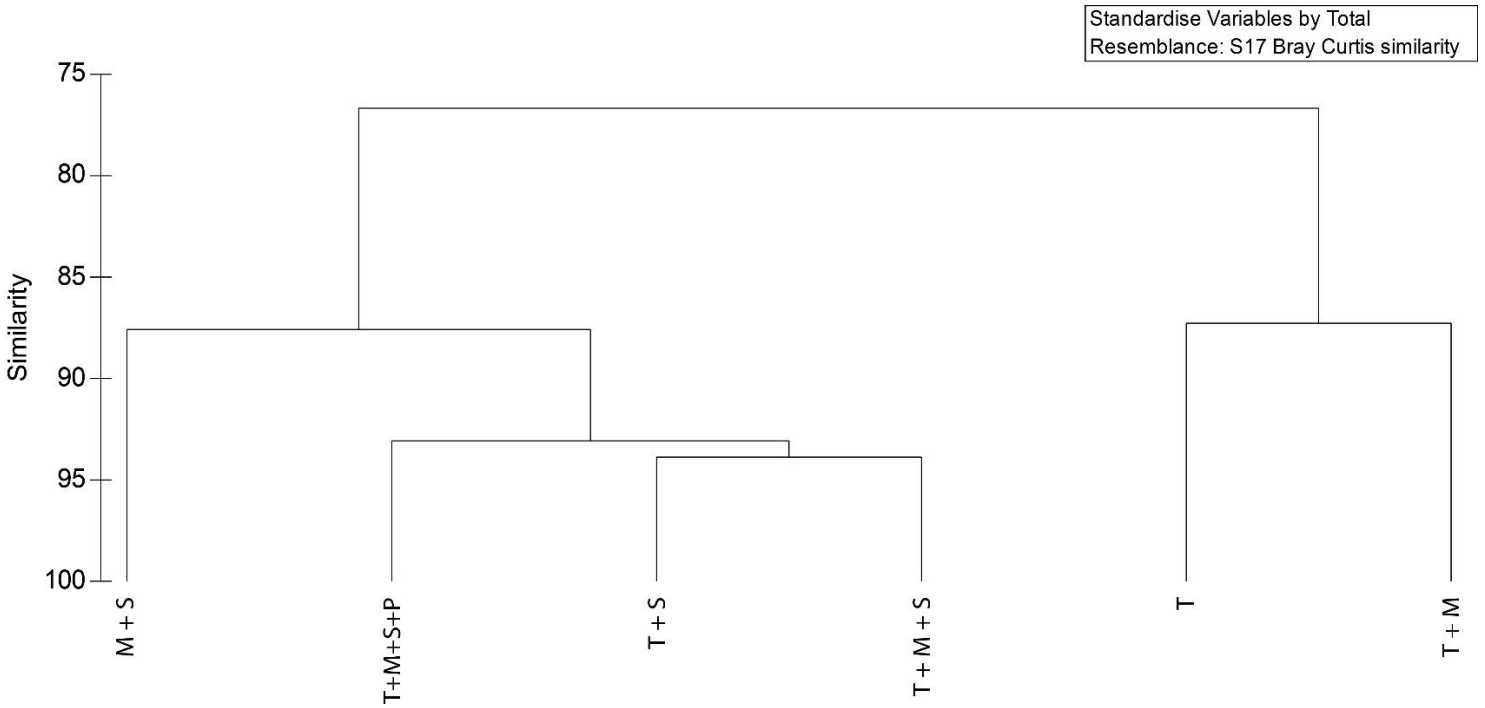

Figure 8.

A dendrogram based on data provided in Figure 7 revealing the similarities and dissimilarities among Charleston pepper (Capsicum annuum L.) plants cultivated on various substrates. In the dendrogram, T, tea waste; M, manure; S, soil; P, perlite.

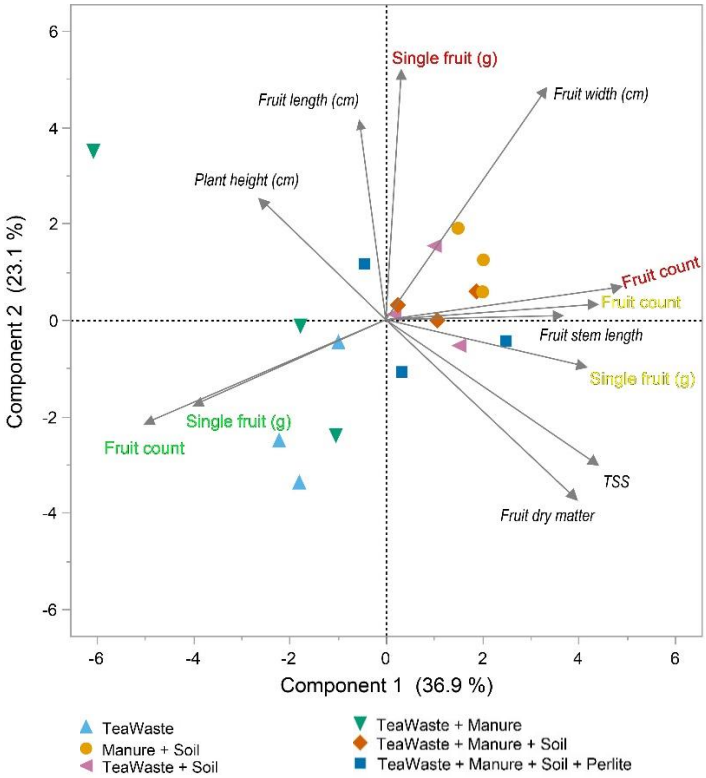

Figure 7.

Principal component analysis revealed a correlation between the different types of Charleston pepper cultivated on different substrates. The green, yellow, and red colors indicate the fruit colour stage and values in italic belong to the fruiting stage of T_{50}. TSS stands for total soluble solid content (%).

Discussion

The findings of this study highlight the potential of tea waste as a sustainable co-substrate for Charleston pepper cultivation, especially when combined with other materials such as soil and manure. This is consistent with the results reported by Duarah et al. (2024), who also demonstrated the effectiveness of tea waste in enhancing soil fertility and plant growth when used as part of an integrated substrate mix. In this study, it was observed that high concentrations of tea waste (e.g., tea waste treatment) initially hindered seed germination and early plant growth. This can be attributed to the high polyphenol and tannin content in tea waste (Sökmen et al., 2018; Duarah et al., 2024; Wang et al., 2024), which might have allelopathic effects and potential phytotoxicity in young seedlings. These compounds are known to delay germination and inhibit root elongation, a phenomenon previously documented in studies on organic waste materials in agriculture (De Almeida et al., 2014; Nahed et al., 2015; Wang et al., 2024). However, when used as part of a balanced co-substrate, tea waste has significant benefits for early-stage pepper growth, likely due to the synergistic interactions between tea waste and other organic materials (Debnath et al., 2021; Kumar et al., 2023). The combination of soil, manure, and perlite in the growing medium not only dilutes the phytotoxic effects of polyphenols but also enhances the physical properties of the substrate (Kumar et al., 2023). This balanced mixture may improve aeration, water-holding capacity, and nutrient availability, promoting more vigorous early growth, as

evidenced by increased seedling height and leaf number. In this study, the inclusion of perlite, for example, could contribute to better root oxygenation and drainage, mitigating potential waterlogging issues often associated with the use of organic waste as a substrate (Pane et al., 2016; Karataş, 2022; Kumar et al., 2023).

Tea leaves, which are rich in nitrogenous compounds (Wang et al., 2020; Debnath et al., 2021), release nitrogen into the soil as they decompose, which can improve leaf color and plant health over time, even without the use of commercial manure (Peksen & Yakupoglu, 2009). Nitrogen is a vital macronutrient for pepper plants, particularly during the vegetative stage, as it is essential for chlorophyll synthesis, leaf development, and biomass accumulation (Hunde, 2020; da Silva Magalhães et al., 2023).

Tea waste enhances soil structure by improving porosity, water retention, and cation exchange capacity (CEC), which are crucial for plant growth, while also helping to retain moisture, reduce irrigation needs, and form stable soil aggregates that facilitate root penetration and nutrient access (Debnath et al., 2021; Kumar et al., 2023). The presence of lignocellulosic compounds in tea waste further supports its use as a co-substrate (Barathi et al., 2017). These compounds enhance the structural integrity of substrates, allowing for better root anchorage and water infiltration (Sial et al., 2019). Thus, combining tea waste with faster-decomposing materials like manure might offer a balanced nutrient supply throughout the growth cycle, making it a valuable resource for sustainable agriculture, particularly where high-quality organic amendments are limited.

Conclusion and Recommendations

This study highlights the potential of tea waste as a sustainable alternative substrate for Charleston pepper cultivation, particularly when combined with components like soil, organic manure, and perlite. While high tea waste concentrations initially inhibited seed germination and early vegetative growth—probably due to its limited immediate nutrient availability and the presence of inhibitory compounds—its use as part of a balanced mixture showed significant benefits. The combination of tea waste with soil and manure promoted early seedling development, enhanced leaf coloration, and improved overall plant vigor, suggesting a gradual release of nutrients, particularly nitrogen, as tea waste decomposes over time.

Based on these findings, the use of tea waste as a co-substrate is recommended, rather than as the sole medium for Charleston pepper cultivation. Further research is needed to optimize tea waste as a sustainable substrate by exploring pre-treatment methods like composting or microbial inoculation to enhance nutrient release. Additionally, testing different substrate compositions with materials such as biochar or compost could help identify optimal mixtures for various crops. Investigating the long-term effects of tea waste on soil health, including nutrient cycling and microbial activity, is also crucial, as is studying crop-specific responses to ensure broader agricultural applicability. Addressing these areas will further validate tea waste as an eco-friendly alternative to synthetic fertilizers and promote more sustainable agricultural practices.

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