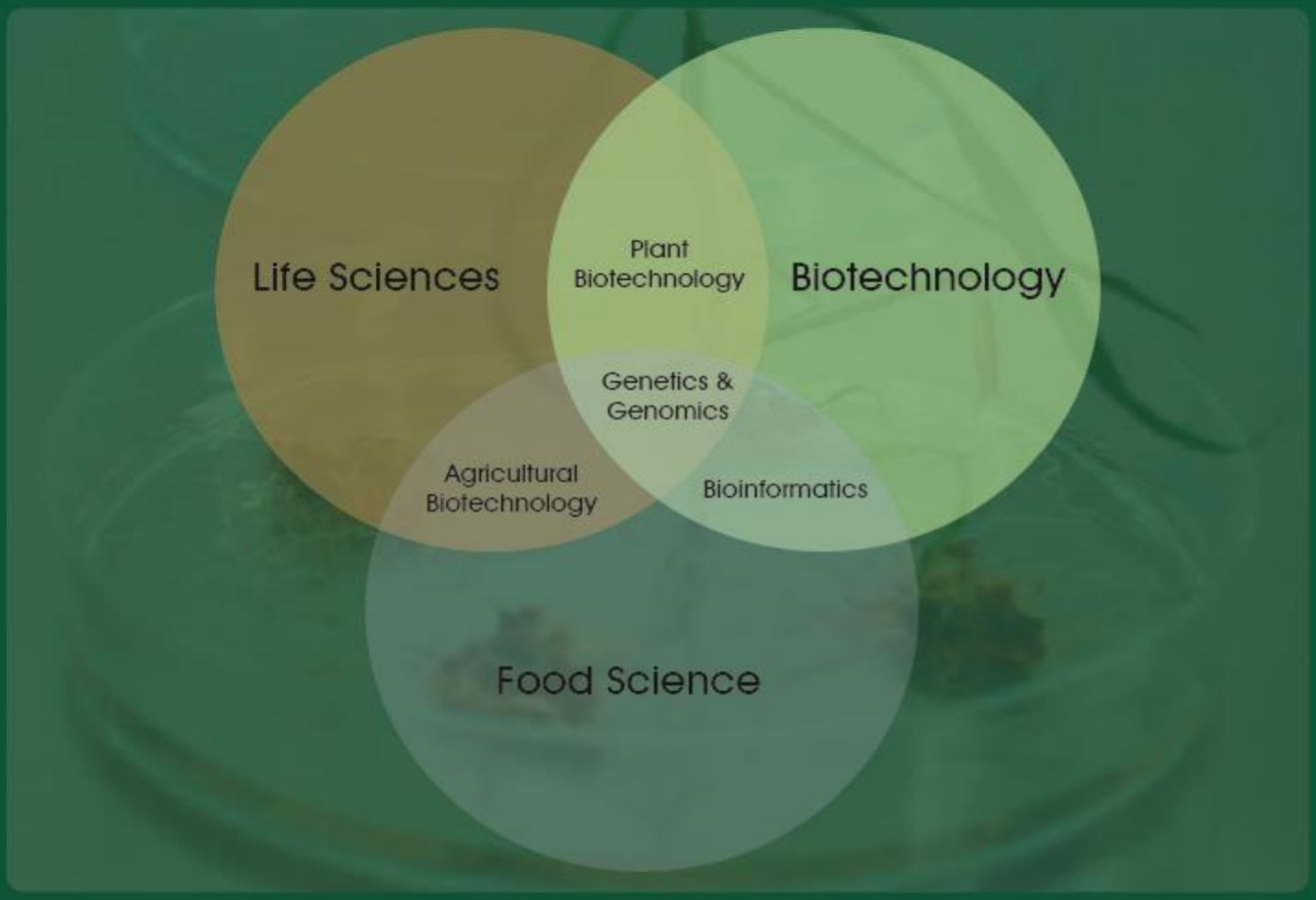
International Journal of Life Sciences and Biotechnology

e-ISSN:2651-4621



Volume/Cilt:6 **2023** **Issue / Sayı:3**

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Bu düşüncelerle 2023 yılı üçüncü sayısını yayınladığımız "International Journal of Life Sciences and Biotechnology" dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Öneri / Katkı ve Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

15.12. 2023
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From The Editor;

Dear Readers and Authors,

As “International Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the 17th issue of the journal. "International Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in the third issue of 2023 "International Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

15. 12. 2023

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İçindekiler/ Contents

Research Articles/ Araştırma Makaleleri

Review Articles / Derleme Makaleler

Düzeltme / Erratum

Araştırma makalesi /Research article

Erdogan H., et al., Sarıtaş Mahallesi Merasının Kentsel Dönüşüm ve Gelişim Amaçlı Tahsis Amacı Değişikliğine Uygunluğunun Araştırılması. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 262-275. DOI: 10.38001/ijlsb.1199393

Sarıtaş Mahallesi Merasının Kentsel Dönüşüm ve Gelişim Amaçlı Tahsis Amacı Değişikliğine Uygunluğunun Araştırılması

Hörünaz Erdoğan[1]*, İbrahim Aydın[1], Fatih Alay[2], Ferat Uzun[1], Ömer Faruk Uzun[3]

ÖZET

Çalışmada, Samsun Büyükşehir Belediyesi tarafından "Kentsel dönüşüm ve gelişim proje alanı" olarak ilan edilen; Atakum ilçesi, Sarıtaş mahallesi 467 ve 567 numaralı mera parsellerinin, tahsis amacı değişikliğine uygunluğu araştırılmıştır. Göz ile tahmin metodu ile mera parsellerinin vejetasyonu incelenerek mera durumu sınıfının belirlendiği çalışma, 2022 yılı Mayıs ayının ikinci yarısında yapılmıştır. Vejetasyon etüdü verilerine göre 467 ve 567 numaralı mera parsellerinin durumları sırasıyla % 59.14 ve % 42.48 ile "İyi" ve "Orta" sınıfta yer almıştır. Bu değerlere göre, Mera Kanunu'nun 14. maddesine göre 467 numaralı parselde tahsis amacı değişikliği mümkün görünmezken, 567 numaralı parselde tahsis amacı değişikliği yapılabileceği anlaşılmıştır. Topografya olarak 467 no'lu parsel % 3 ile % 15, 567 no'lu parsel ise % 5 ile % 35 arasında değişen eğim dereceleriyle engebeli bir yüzeye sahip olduğu görülmüştür. Her iki mera parselinin eğim derecelerinin küçükbaş hayvanların rahatlıkla otlayabileceği değerde olmasına karşın, sığırlar için 467 numaralı parselin uygun fakat 567 numaralı parselin kısıtlayıcı olduğu anlaşılmıştır. Mera parsellerinde tespit edilen azalıcı bitki türleri olan *Lotus corniculatus, Trifolium fragiferum, Trifolium repens, Trifolium hybridum, Medicago sativa, Poa pratensis, Lolium perenne, Dactylis glomerata* ve *Sanguisorba minor*, mahallede hayvancılık yapan işletmeler için suni mera tesisi karışımlarında öncelikle tercih edilebilecek yem bitkisi türleri olarak önerilebilir.

MAKALE GEÇMİŞİ
Geliş
7 Kasım 2022
Kabul
24 Nisan 2023

ANAHTAR KELİMELER
Bitki örtüsü,
kentsel planlama,
kentsel gelişim,
mera durumu,
mera kanunu,
taşınmaz hukuku

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Investigation if Sarıtaş Neighborhood Rangeland is equal to a law for Change Allocation Purpose for Urban Transformation and Development

ABSTRACT
In the study, the equal to a law of rangeland parcels numbered 467 and 567 in Atakum, Sarıtaş neighbourhood, which was declared as "Urban transformation and development project area" by Samsun Metropolitan Municipality, for the change of allocation purpose was investigated. The study, in which the rangeland condition class was determined by examining the vegetation of the rangeland parcels by visual estimation method, was carried out in the second half of May 2022. According to the vegetation survey data, the conditions of the rangeland parcels numbered 467 and 567 were in the "Good" and "Medium" classes with 59.14% and 42.48%, respectively. According to the 14th article of the rangeland law, it is not possible to change the purpose of allocation in the parcel numbered 467, but it is understood that a change in the purpose of allocation can be made in the parcel numbered 567. In terms of topography, it was seen that parcel no. 467 had a rough surface with slopes varying between 3% and 15%, parcel no. 567 between 5% and 35%. Although the slopes of both plots were sufficient for small cattle to graze easily, it was determined that plot number 467 was suitable for cattle, but plot number 567 was restrictive. *Lotus corniculatus, Trifolium fragiferum, Trifolium repens, Trifolium hybridum, Medicago sativa, Poa pratensis, Lolium perenne, Dactylis glomerata* and *Sanguisorba minor,* which are high quality forage plant species identified in rangeland plots, can be recommended as primarily preferred species in pasture plant mixtures for livestock enterprises in the neighbourhood.

ARTICLE HISTORY
Received
7 November 2022
Accepted
24 April 2023

KEY WORDS
Plant composition,
urban planning,
urban development,
rangeland condition,
rangeland law,
real estate law

Giriş

Samsun ilinin nüfusu, 1927 yılında 260 bin civarında iken, günümüzde yaklaşık 1.4 milyon insana ev sahipliği yapmaktadır [1, 2]. Artan şehir nüfusu, yeni yerleşim alanları ihtiyacını ortaya çıkarmıştır. İlin nüfusundaki artış, yerleşim alanının da büyümesine sebep olmuştur. Şehir 1850 yılında 88 hektar, 1950'de 256 hektar, 1985'te ise 1304 hektarlık bir alana yayılmıştır [1]. 2014 yılı itibarıyla Atakum, İlkadım, Canik ve organize sanayi bölgesi de dâhil olmak üzere Tekkeköy ilçeleri sınırları içinde kalan kentsel alan yaklaşık 6418 hektarı bulmuştur [3]. Eski Samsun Merkez olmak üzere doğuda Çınarlık beldesinden batıda Taflan'a kadar yaklaşık 50 km ve deniz çizgisinden Ankara yolu istikametine doğru yaklaşık 20 km derinlik olmak üzere mekân olarak oldukça geniş bir yüzeye yayılmıştır. Samsun'un nüfus artış hızı en yüksek belediyesi olan Atakum ilçesinin nüfusu ise 2008 yılında 107953 iken 2021 yılında 238702 kişiye ulaşmıştır [4, 5]. İlçe, 2015 yılı nüfus artışı oranlarında Karadeniz Bölgesi'nin en çok nüfusu artan, 2016 yılı itibarıyla ise Türkiye'nin nüfusu en çok artan ilçeler sıralamasında 16. sırada yer almıştır. Artvin, Trabzon, Ordu, Giresun, Rize, Amasya ve Sinop illeri başta olmak üzere özellikle çevre il ve ilçelerden sürekli yeni göçler almaktadır [4]. İlçe nüfusunun bu derecede hızlı artışı konut, sanayi, ticaret, sosyo-kültürel tesis, sportif ve rekreasyon alanı ile ulaşım gibi

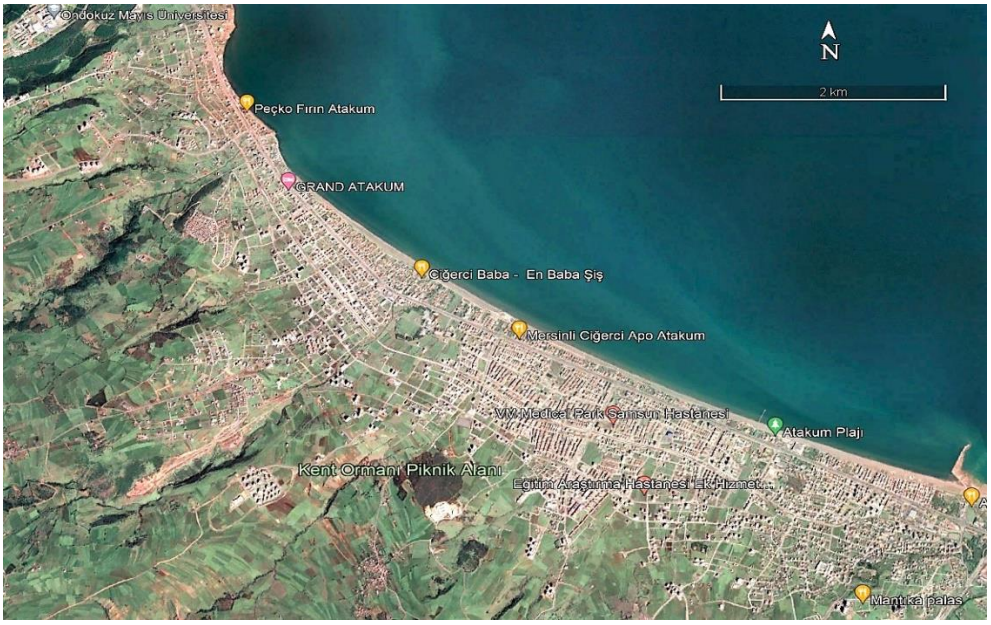
şehirlere ait hizmetlerin üretileceği ilave şehirsel kullanım alanı gereksinimini ortaya çıkarmaktadır.

Şehirlerin hızlı ve plansız gelişmesini önlemek amacı ile 1985 yılından itibaren Türkiye genelinde bir takım tedbirler alınmıştır. Bunlardan birisi de şehirlerde idari düzenlemeler ile şehrin çevresindeki köy ve köy mahallelerin belediye sınırları içine alma, şehirlerin alanını genişletme ve bu alanları imara açarak, hem şehirlerin hizmet alanının ihtiyacını karşılama, hem de muhtemel gelişme bölgesi olacak bu mahallerde planlı yapılaşmayı sağlamaktır [1].

Şehrin yakın çevresine doğru gerçekleşen kentsel gelişim nedeniyle hazırlanan yeni imar planları ile tarım amaçlı kullanılan alanlar, yeni kentsel yaşam alanlarına dönüştürülmektedir. Kentsel gelişim için niteliği değiştirilen tarım alanları içerisinde mera, yaylak ve kışlaklar da yer almaktadır [6, 7, 8, 9]. Ancak en stratejik sektör olan tarımsal üretimde devamlılığı sağlamak, en azından olumsuz etkiyi en aza indirgeyebilmek adına bu tür istek veya talepler için bir takım kanuni düzenlemeler yapılmıştır [1]. Şehirlerin zaman içerisinde ortaya çıkan birtakım ihtiyaçlarını karşılamak amacıyla "kentsel dönüşüm ve gelişim proje alanı" olarak ilan edilen mera, yaylak ve kışlaklardaki tahsis amacı değişikliği işlemleri, Tarım ve Orman Bakanlığı'nın takibinde yürütülmektedir. Tahsis amacı değişikliği sürecinde, oluşabilecek kamu zararının önlenilmesi için, öncelikle kentsel dönüşüm ve gelişim proje alanı olarak ilan edilmesi düşünülen alanın 1/5000 ölçekli haritası ile Mera Komisyonu'na başvurularak uygun görüş alınmaktadır. Çalışma Mera Kanunu'nun 14/ı ve Mera Yönetmeliği'nin 8. maddesi genel hükümleri dikkate alınarak yapılmaktadır [6, 7]. Buna göre Mera durumu ve sınıfı "Çok iyi" ve "İyi" olan mera yaylak ve kışlaklarda tahsis amacı değişikliği yapılmadığından kentsel gelişim için kullanıma açılamamaktadır. Süreç tamamlanıp Mera Komisyonu'nun uygun gördüğü değişiklik istemi daha sonra Tarım ve Orman Bakanlığı'nın onayına sunulmaktadır. Bu çalışma ile, Atakum ilçesi Sarıtaş mahallesi 467 ve 567 numaralı mera parsellerinin, Mera Yönetmeliği'nin 6/c maddesi gereğince mera durumu sınıfının belirlenerek, kentsel dönüşüm ve gelişim sebepli tahsis amacı değişikliği isteminin hukuka uyarlığı araştırılmıştır.

Materyal ve Yöntem

Samsun ili, Atakum ilçesinin 2009 ve 2022 yıllarına ait uydu görüntüleri Şekil 1 ve Şekil 2'de verilmiştir.



Şekil 1 Atakum ilçesinin 2009 yılına ait genel görüntüsü

Figure 1 General view of Atakum district in 2009

Şekil 2 Atakum ilçesinin 2022 yılına ait genel görüntüsü

Figure 2 General view of Atakum district in 2022

Tahsis amacı değişikliği istenilen Atakum ilçesi, Sarıtaş mahallesine ait 467 (Enlem: 41° 18' 59.04" Boylam: 36° 10' 53.40") ve 567 numaralı (Enlem: 41° 18' 55.08" Boylam: 36° 10' 33.60") mera parselleri sırasıyla 327.8 ve 31.6 da'lık alandan oluşmaktadır (Şekil 3). Çalışılan alanın uzun yıllar ortalaması (1929-2021) yıllık yağış miktarı 717.9 mm, ortalama sıcaklık 14.7 ^{o}C ve yağışlı gün sayısı 117.6 gündür [11].

Şekil 3 Tahsis amacı değişikliği istenilen Sarıtaş mahallesi mera parsellerinin görüntüsü

Figure 3 Image of Sarıtaş neighborhood rangeland parcels for which the allocation purpose change is requested

Tarımsal Üretim ve Geliştirme Genel Müdürlüğü ile Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü arasında imzalanan protokol gereği; Samsun İl Tarım Orman Müdürlüğü'nün 24.08.2020 tarih ve 2353594 sayılı yazısı ile Mera Kanunu'nun 14/d maddesi gereği Sarıtaş mahallesinin kullanımına tahsis edilen meranın, mera vasfı kaldırılarak hazine adına tescil edilmesi talebine istinaden bu çalışma yürütülmüştür. Söz konusu mera parsellerinde bulunan bitki türlerinin teşhis edilerek mera durum sınıfının

belirlenmesi için 16.05.2022 tarihinde Samsun ili, Atakum ilçesi, Sarıtaş mahallesinde taşınmaz numarası 3189557 olan 467 ve taşınmaz numarası 3189558 olan 567 numaralı mera parsellerinde vejetasyon etüdü yapılmıştır (Şekil 3).

Mera alanının koordinat ve rakım ölçümleri "South S82 Plus" GPS ile yapılmış ve elde edilen veriler QGis 3.16.8 programı ile değerlendirilerek harita çıktıları oluşturulmuştur (Şekil 3). Alanın eğim, yükseklik ve mesafe ölçümleri ise "Leica DISTO D810" lazer metresi ile yapılmıştır. Arazide yapılan etüt çalışmaları ile toprak yüzeyinin bitki ile kaplılık durumu ve bitki örtüsünü oluşturan türler belirlenmiştir. Mera parsellerinin vejetasyonlarındaki bitki türleri, hâkim bitkilerin çiçeklenme evresine ulaştığı Mayıs ayının ikinci yarısında, "Gözle tahmin metodu" ile "yaprak alanı" esasına göre belirlenmiştir [12, 13].

Mera alanındaki bitkilerin teşhisinde [14, 15]'in vermiş olduğu bilgilerden faydalanılmıştır. Her bir bitki türüne ait veriler, vejetasyonda yer alan toplam bitki sayısına oranlanmak suretiyle türlerin botanik kompozisyondaki oranları hesaplanmıştır. Vejetasyonda teşhis edilen bitki türleri; azalıcı, çoğalıcı ve istilacılar olmak üzere sınıflandırılmışlardır. Alanın "mera durumu" sınıflaması, azalıcıların tamamı, çoğalıcıların ise % 20'si dikkate alınarak [16, 17] ve Mera yönetmeliğinin, Uygulama Esaslarını düzenleyen 2. Bölüm (Uygulama normları)'ün 6/c. maddesindeki kriterlere [7] göre yapılmıştır (Tablo 1).

Tablo 1 Vejetasyonda yer alan azalıcı ve çoğalıcı bitki türlerinin oranlarına göre mera durumu sınıfı

Table 1 Rangeland condition class according to the ratio of decreasing and increasing plant species in the vegetation

Azalıcı + çoğalıcı bitki türlerinin oranı (%)	**Mera durumu**
76-100	Çok İyi
51-75	İyi
26-50	Orta
0-25	Zayıf

Çalışılan meranın eğimi, düz (% 0-2), hafif eğimli (% 3-6), orta eğimli (% 7-12), dik eğimli (% 13- 20), çok dik eğimli (% 21-30), sarp eğimli (%30-45) ve çok sarp eğimli (46+) cetveline göre olarak sınıflandırılmıştır [18].

Bulgular ve Tartışma

Mera lokasyon bulguları

Tahsis amacı değişikliği istenilen mera parselleri 41°18'55.08" - 41°18'59.04" kuzey paralelleri ile 36°10'33.60"-36°10'53.40" doğu boylamları arasında ve yaklaşık 253 metre rakımda yer aldığı belirlenmiştir (Şekil 3).

Çalışılan 467 ve 567 no'lu parseller, Sarıtaş mahallesine 0.55 km, Atakum ilçe merkezine ise 6.6 km uzaklıktadır. 467 no'lu parsel % 3 ile % 15 arasında değişen eğim ve 235 m rakımda, 567 no'lu parsel ise % 5 ile % 35 arasında değişen eğim dereceleriyle dalgalı bir yüzeye sahip olup 229 m rakım değerine sahiptir. Mahallenin küçükbaş hayvan varlığının rahatlıkla otlayabilmeleri için her iki mera parselinin eğim derecelerinin uygun olduğu, sığırlar için ise 467 numaralı parselin uygun fakat 567 numaralı parselin faydalanmayı kısıtlayacak derecede fazla meyilli olduğu söylenilebilir [19, 20]. Bilindiği üzere sığırlar % 10 koyunlar ise % 45 eğim derecesine kadar olan mera alanlarından sorunsuz bir şekilde faydalanabilmektedir [1]

Mera Bitki Türleri ve Mera Durumu Sınıfı Bulguları

Diğer çalılar yanında vejetasyonda keçilerin severek otladığı *Rubus* türlerinin olması merada keçi varlığının yeterli olmadığı ve vejetasyonda bitki süksesyonunun aktif olarak devam ettiğini göstermektedir. Alanın ormana dönüşmesinin engellenmesi ve mera olarak kalabilmesi için keçi varlığının mevcudiyeti zorunlu görünmektedir (Tablo 2). Mera parsellerinin vejetasyonunda yer alan bitki türleri Tablo 2 ve 3'te verilmiştir.

Vejetasyon etüdü verileri ve bu verilerden yapılan hesaplamaya göre; mera durumu sınıfı 467 ve 567 numaralı parsellerde sırası ile % 59.14 ve % 42.48 ile "İyi" ve "Orta" sınıfta yer almıştır [7,16]. Parsellerin vejetasyonlarında yer alan bitki türlerinin aynı sırayla % 40.86 ve % 57.52'sini istilacı türler oluşturmuştur. Meranın 467 numaralı parselinde teşhis edilen azalıcı bitki türleri içerisinde en fazla oranda bulunan yem bitkileri % 7.35 ve % 7.25'lik oranlarla sırasıyla *Poa pratensis* ve *Lotus corniculatus*'tur. Bitki türleri içerisinde ortama en iyi adapte olabilen çoğalıcı tür ise % 8.00'lık oranla *Cynodon dactylon* ve % 5.22'lik oran ile *Plantago lanceolata*'dır. Meranın 567 numaralı parselinde teşhis edilen bitki türleri içerisinde en fazla oranda bulunan azalıcı yem bitkileri % 17.60 ve % 5.63'lük oranlarla sırasıyla *Trifolium fragiferum* ve *Lotus corniculatus*'tur. Bu parseldeki bitki türleri içerisinde yer alan çoğalıcı iki tür olan *Cynodon dactylon* ve *Carex acuta*'nın oranları sırasıyla % 9.00 ve % 2.22'dir.

Tablo 2 467 numaralı mera parselinin vejetasyonunu oluşturan azalıcı, çoğalıcı ve istilacı bitki türlerinin oranları (%)

Table 2 Proportions of decreaser, increaser and invaders plant species forming the vegetation of the rangeland parcel number 467 (%)

Azalıcı Bitki Türleri (%)			
Poa pratensis	7.35	*Trifolium repens*	4.21
Lotus corniculatus	7.25	*Trifolium hybridum*	3.32
Sanguisorba minor	6.27	*Lolium perenne*	3.22
Lotus aegaeus	5.92	*Dactylis glomerata*	2.90
Onobrychis armena	4.27		
Toplam			**44.71**
Çoğalıcı Bitki Türleri (%)			
Cynodon dactylon	8.00	*Carex acuta*	1.21
Plantago lanceolata	5.22		
Toplam			**14.43**
Azalıcı + Çoğalıcı Tür Toplamı			**59.14**
İstilacı Otsu Bitki Türleri (%)			
Bromus tectorum	6.90	*Linum flavum*	0.90
Taraxacum officinale	6.00	*Globularia orientalis*	0.75
Bellis perennis	2.50	*Tanacetum abrotanifolium*	0.70
Medicago lupulina	2.70	*Datura stramonium*	0.70
Trifolium resupinatum	2.00	*Cantaurea iberica*	0.70
Alopecurus myosuroides	2.00	*Plantago lagopus*	0.50
Filipendula vulgaris	2.00	*Dactylorhiza urvilleana*	0.50
Eryngium campestre	1.30	*Lathyrus annuus*	0.20
Verbascum cheiranthifolium	1.20	*Trifolium meneghinianum*	0.19
Muscari armaniacum	1.02	*Anagallis arvensis*	0.10
Toplam			**32.86**
İstilacı Çalı-Ağaç Bitki Türleri (%)			
Paliurus spina-christi	4.70	*Crataegus monogyna*	0.48
Rubus discolor	1.30	*Carpinus betulus*	0.30
Buxus sempervirens	1.02	*Crataegus aronia*	0.20
Toplam			**8.00**
Otsu ve Çalı-Ağaç İstilacıların Toplamı			**40.86**
Genel Toplam			**100.0**

Mera parsellerinde tespit edilen azalıcı bitki türleri olan *Lotus corniculatus, Trifolium fragiferum, Trifolium repens, Trifolium hybridum, Medicago sativa, Poa pratensis, Lolium perenne, Dactylis glomerata ve Sanguisorba minor* bu bölgenin ekolojik şartlarına uyum sağlayarak botanik kompozisyonda varlıklarını devam ettirebilmişlerdir.

Adı geçen türler, Sarıtaş mahallesinde hayvancılık yapan işletmeler için suni mera tesisi karışımlarında öncelikle tercih edilebilecek yem bitkisi türleri olarak önerilebilir.

Tablo 3 567 numaralı mera parselinin vejetasyonunu oluşturan azalıcı, çoğalıcı ve istilacı bitki türlerinin oranları (%)

Table 3 Proportions of decreaser, increaser and invaders plant species forming the vegetation of the rangeland parcel number 467 (%)

Azalıcı Bitki Türleri (%)			
Trifolium fragiferum	17.60	*Trifolium physodes*	1.84
Lotus corniculatus	5.63	*Medicago sativa*	1.46
Lotus tenuis	4.75		
Toplam			**31.28**
Çoğalıcı Bitki Türleri (%)			
Cynodon dactylon	9.00	*Carex acuta*	2.20
Toplam			**11.20**
Azalıcı + Çoğalıcı Tür Toplamı			**42.48**
İstilacı Otsu Bitki Türleri (%)			
Verbascum cheiranthifolium	4.94	*Galium aparine*	1.89
Trifolium resupinatum	4.81	*Eryngium bithynicum*	1.17
Ranunculus arvensis	4.19	*Eryngium billardieri*	1.15
Poa annua	3.87	*Taraxacum aleppicum*	1.15
Bellis perennis	3.82	*Dipsacus laciniatus*	0.97
Bromus distachyon	2.86	*Medicago minima*	0.97
Anagallis arvensis	1.97	*Anthemis cretica*	0.97
Eryngium campestre	1.95	*Brassica nigra*	0.49
Galium aparine	1.95		
Toplam			**31.62**
İstilacı Çalı-Ağaç Bitki Türleri (%)			
Carpinus betulus	4.19	*Crataegus monogyna*	1.25
Quercus cerris	5.71	*Crataegus aronia*	1.15
Salix alba	3.16	*Rubus discolor*	0.97
Rosa canina	1.97		
Toplam			**25.90**
Otsu ve Çalı-Ağaç İstilacıların Toplamı			**57.52**
Genel Toplam			**100.0**

Çalışılan meraların yer aldığı bölgenin iklim değerlerinin özellikle de yağış değerlerinin ülkemiz geneline göre daha yüksek ve yıl içerisindeki dağılımı da daha geniş bir periyoda yayılmıştır [11]. Bu sebeple, vejetasyonda yer alan bazı bitki türleri çeşitli nedenlerle vejetasyondan çekilse bile bunların yerlerini gerek otsu gerekse çalı-ağaç türü bitkilerin

doldurulmuş olduğu görünmektedir. Bu da toprak yüzeyinin tamamının bitki örtüsü tarafından örtüldüğünü ve alanda erozyon riski olmadığını ifade etmektedir.

Sarıtaş mahallesinin mera parsellerinin bitki kaliteleri ve dolayısıyla mera durumu sınıfı Karadeniz Bölgesi'nin geneli dikkate alındığında ortalamanın üzerindedir. Bölge'de daha önceki tarihlerde yürütülen birçok mera çalışmasında; düşük verimliliğe sahip, hayvanlar tarafından lezzetsiz/kaba bulunduğu veya dikenli organlara sahip olduğu için otlanılmayan ya da hayvanlarda çeşitli sağlık problemlerine yol açabilen otsu veya çalımsı yapıya sahip istilacı türlerin bitki örtüsünün çoğunluğunu oluşturdukları bildirilmiştir [21, 22, 23, 24, 25, 26, 27, 28, 29]. Tarım Bakanlığı'nın Bölge için belirlediği ve genel olarak Nisan ayı içerisinde başlayarak müteakip 6 ay süreli otlatma periyodu ve üretilen ot miktarından hareketle meralarda otlatılması gereken hayvan sayısını ifade eden "otlatma kapasitesi" dikkate alınmadan yapılan otlatma, bu durumun en önemli sebepleridir. Ayrıca vejetasyon tipine uygun olmayan hayvan türleri ve manda, sığır, koyun, keçi gibi yaygın olarak yetiştiriciliği yapılan hayvan çeşitliliğinin azaltılarak sadece sığıra dayalı otlatma düzeni bunun en başta gelen sebeplerden olduğu birçok çalışmada ifade edilmiştir [30, 31, 32, 33, 34, 35, 36]. Diğer yandan bitkisel gelişim için meranın aldığı yıllık yağış miktarının nispeten tatmin edici miktarlarda olması, ekosistemdeki süksesyonal dönüşümün daha hızlı gerçekleşmesine bu da ortamda çalı-ağaç türlerinin vejetasyondaki varlığının gittikçe artmasına neden olmuştur. Esasen özellikle Karadeniz Bölgesi'ndeki meraların çalı-ağaç türleri ile istila edilmeleri sürecinin altında yatan en büyük sebep, diğer ekolojik faktörlere ilave olarak otlayan hayvan türlerinin mevcut profilidir. Tabiatın çeşitlilik üzerine kurulan ekolojik yapısı, insan eliyle sadece büyükbaş hayvana, onun da ezici çoğunluğunu sığırların oluşturduğu bir profile evrilmesidir. Sarıtaş mahallesindeki 461 adet sığır, 2 manda ve 247 koyun varlığı mevcuttur [37]. Buna karşılık merada görülmeye başlayan çalı-ağaç türü bitkileri otlayarak onların çoğalmasını sınırlandırarak mera sürdürülebilirliğinin garantisi olan ve bu bitkileri et ve süt gibi hayvansal ürünlere dönüştürmek suretiyle katma değer sağlayabilen keçilerin olmaması, gelecekte bu alanın süksesyon temelli ormanlaşmaya evrilerek otlak alanı niteliğini kaybetme ihtimaline açık olduğunun ve dolayısıyla mera durumu değerinde azalma olabileceğinin bir göstergesidir [27, 32].

Mera Kanunu'nun 14/ı (Ek: 10/09/2014-6552/112 md.) maddesi ve Değişik 4. fıkra: 26/3/2008-5751/3 maddesine göre, "Mera durum sınıfı"nın "İyi" olduğu 467 numaralı

parselin “Kentsel dönüşüm ve gelişim proje alanı olarak kullanılmak üzere tahsis amacının değiştirilmesi” hususuna kapalı olduğu, ancak “Orta” olan 567 numaralı parselin açık olduğu anlamına gelmektedir [6, 7]. Ancak Samsun’un Vezirköprü ilçesi, Doyran ve Kızılcaören mera alanlarında Samsun Büyükşehir Belediyesi’nin istemiş olduğu tahsis amacı değişikliği mera durum sınıfı raporuna binaen Mera Komisyonu tarafından uygun bulunsa ve Valilik Makamı tarafından “Olur” alsa bile, 2018/8 sayılı Cumhurbaşkanlığı Genelgesi [1] kapsamında izin alınmak üzere Tarım ve Orman Bakanlığı’na bildirilen “Karar” uygun görülmemiştir. Eğer ki böyle bir süreç yaşanmayıp 567 numaralı parsel için tahsis amacı değişikliği uygun görülür ise, Mera Kanunu’nun 30/e maddesi gereği, mera alanının Mera Komisyonu’nca belirlenen 20 yıllık ot geliri, Belediye tarafından İl Gıda Tarım ve Hayvancılık Müdürlüğü hesabına yatırılmalıdır. Ot bedelinin yatırılmasından sonraki 2 yıllık süre zarfında alanın kesinleşmiş uygulama imar planı Komisyona sunulması gerekmektedir. Aksi takdirde tahsis amacı değişikliği iptal edilecektir. Hazırlanan imar planlarının tahsis amacı değişikliğine uygun olarak kesinleşmesi durumunda, mera alanının Hazine adına tescilinden sonra Samsun Belediyesi’ne tahsisi sağlanabilir [7]. Diğer yandan 467 numaralı parselde kentsel dönüşüm ve gelişim proje alanı olarak tahsis amacı değişikliği yapılamasa da 4342 sayılı Mera Kanunu’nun 14. maddesi değişik dördüncü fıkrası; (a bendi) Enerji ve Tabi Kaynaklar Bakanlığı’nın talebi üzerine, 3213 sayılı Maden Kanunu ve 6326 sayılı Petrol Kanunu hükümlerine göre, arama faaliyetleri sonunda rezervi belirlenen maden ve petrol çalışmaları için zaruri olan, (f bendi) Ülke güvenliği ve olağanüstü hal durumlarında, (g bendi) Doğal afet bölgesinde yerleşim yeri için ihtiyaç duyulan, (ğ bendi) Enerji piyasası düzenleme kurumunun talebi üzerine, 4628 sayılı Elektrik Piyasası Kanunu, 4646 sayılı Doğalgaz Piyasası Kanunu ve 5015 sayılı petrol piyasası Kanunu hükümlerine göre, petrol iletim faaliyetleri ile elektrik ve doğalgaz piyasası faaliyetleri için gerekli bulunan ve (h bendi) Jeotermal kaynaklı teknolojik seralar için ihtiyaç duyulması halinde bu parsel de ilgili müdürlüğün talebi, komisyon ve defterdarlığın uygun görüşü üzerine, valilikçe tahsis amacı değiştirilebilir ve tescili Hazine adına yapılabilir.

Sonuç

Sarıtaş köyünün “Mera durum sınıfı” “İyi” olan 467 numaralı mera parselinde tahsis amacı değişikliğinin mümkün olmadığı, “Orta” sınıfta yer alan 567 numaralı parselinde ise Mera Komisyonu tarafından alınacak “Karar”, Valilik Makamı’nın “Olur”u ve

Cumhurbaşkanlığı Genelgesi kapsamında oluşturulacak komisyon izni ile "Kentsel Dönüşüm ve Gelişim Proje Alanı" çalışmaları yapılması amacıyla "tahsis amacı değişikliği" yapılabileceği anlaşılmıştır. Diğer yandan tahsis amacı değişiklikleri yapılarak özellikle son on yılda azalan mera alanları sebebiyle, ilave kaba yem ihtiyacının ne kadar olduğu ve telafisi konusunda neler yapılması gerekliliği hakkında bir gelecek vizyonu oluşturabilmek amacıyla ilgili kurumların ciddi bir envanter çalışması yapması faydalı olacaktır.

Acknowledgments / Teşekkürler

Verilerin elde edilmesindeki destekleri için Samsun İl Tarım ve Orman Müdürlüğü Çayır Mera ve Yem Bitkileri Şube Müdürlüğü personelleri; Müdür V. Namık AKGÜL, Ziraat Mühendisleri Hakan UZUN, Nuri DEMİRCİ, Bayram AY ve Mehmet YOLCU'ya teşekkür ederiz.

Samsun Provincial Directorate of Agriculture and Forestry, Meadows, Pasture and Forage Crops Branch Office personnel for their support in obtaining the data; We thank the Director V. Namık AKGÜL, Agricultural Engineers Hakan UZUN, Nuri DEMİRCİ, Bayram AY and Mehmet YOLCU.

Funding / Fon desteği

The author did not receive any support from any organization for the submitted work.

Yazarlar bu çalışma için herhangi bir kuruluştan destek almamıştır.

Data Availability statement / Veri Kullanılabilirliği bildirimi

The author confirms that the data supporting this study are cited in the article.

Yazarlar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.

Compliance with ethical standards / Etik standartlara uyum

Conflict of interest / Çıkar çatışması

The authors declare no conflict of interest.

Yazarlar herhangi bir çıkar çatışması beyan etmemektedir.

Ethical standards / Etik standartlar

The study was conducted based on the ethical standards.

Çalışma etik standartlara uygundur.

Authors' contributions / Yazar katkıları

Makalede adı geçen tüm yazarlar makaleye eşit oranda katkı yapmışlardır. Tüm yazarlar makaleyi incelemiş ve onaylamışlardır.

All authors mentioned in the article contributed equally to the article. All authors have reviewed and approved the article.

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Research Article

Karakurt H. U., et al., Evaluation of Differences of Fast and High Accuracy Base Calling Models of Guppy on Variant Calling Using Low Coverage WGS Data. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 276-287. DOI: 10.38001/ijlsb.1308355

Evaluation of Differences of Fast and High Accuracy Base Calling Models of Guppy on Variant Calling Using Low Coverage WGS Data

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ABSTRACT

Long-read sequencing technologies such as Oxford Nanopore Technologies (ONT) enabled researchers to sequence long reads fast and cost-effectively. ONT sequencing uses nanopores integrated into semiconductor surfaces and sequences the genomic materials using changes in current across the surface as each nucleotide passes through the nanopore. The default output of ONT sequencers is in FAST5 format. The first and one of the most important steps of ONT data analysis is the conversion of FAST5 files to FASTQ files using "base caller" tools. Generally, base caller tools pre-trained deep learning models to transform electrical signals into reads. Guppy, the most commonly used base caller, uses 2 main model types, fast and high accuracy. Since the computation duration is significantly different between these two models, the effect of models on the variant calling process has not been fully understood. This study aims to evaluate the effect of different models on performance on variant calling. In this study, 15 low-coverage long-read sequencing results coming from different flow cells of NA12878 (gold standard data) were used to compare the variant calling results of Guppy. Obtained results indicated that the number of output FASTQ files, read counts and average read lengths between fast and high accuracy models are not statistically significant while pass/fail ratios of the base called datasets are significantly higher in high accuracy models. Results also indicated that the difference in pass/fail ratios arises in a significant difference in the number of called Single Nucleotide Polymorphisms (SNPs), insertions and deletions (InDels). Interestingly the true positive rates of SNPs are not significantly different. These results show that using fast models for SNP calling does not affect the true positive rates statistically. The primary observation in this study, using fast models does not decrease the true positive rate but decreases the called variants that arise due to altered pass/fail ratios. Also, it is not advised to use fast models for InDel calling while both the number of InDels and true positive rates are significantly lower in fast models. This study, to the best of our knowledge, is the first study that evaluates the effect of different base calling models of Guppy, one of the most common and ONT-supported base callers, on variant calling.

ARTICLE HISTORY
Received
01 June 2023
Accepted
09 July 2023

KEYWORDS
Oxford nanopore sequencing,
variant calling,
long-read sequencing,
base-calling

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Introduction

Since its development, long-read, single-molecule DNA sequencing Technologies emerged as powerful players in genomics and have proven their ability to resolve some of the most challenging regions of the human genome [1]. Oxford Nanopore Technologies (ONT), especially, provided fast and portable solutions for sequencing. The use of the Oxford Nanopore Sequencing platform has been increasing exponentially for variant calling due to its mobility, easy-to-use structure, accuracy and price. ONT uses electrical signal changes of nucleotides passing through nanopores that are integrated into a semi-conductive surface. Signals are stored as FAST5 files and can be converted to FASTQ files with a procedure called base calling [2]. Guppy, the most common and ONT-approved variant caller which is also used by the MinKNOW operating software of ONT, uses a Hidden Markov Model to generate FASTQ files from FAST5 files [3]. Guppy involves two different built-in models, High Accuracy and Fast models. The fast models are optimized for speed and are designed for applications where quick turn-around times are important, such as in real-time sequencing analysis or rapid diagnostic testing. The high-accuracy models use a more advanced algorithm that provides higher accuracy base-calling but at the expense of longer processing times [4]. These models differ in computation time and computing power requirements. Even though fast models provide significantly faster results, especially when the need for fast result generation, there is not any study that shows the direct effect of different models on variant calling. These kinds of critical cases require clinicians and experimental biologists to know which information on sequencing material they sacrifice to obtain faster results. As a consequence of that, researchers need a guide to have information about the differences between these models. Here, a benchmark study is provided that uses 15 low-coverage human sequencing data sets to provide insight into the model effect on variant calling. In this study, we aimed to investigate the effect of different base-calling models of Guppy on Single Nucleotide Polymorphism and Insertion/Deletion calling by comparing different parameters statistically.

Material and Methods

The study focuses on the “High Accuracy” and “Fast” models of Guppy base caller. Using 15 low-coverage long-read sequencing files (Table 1) from NA12878 Gold Standard Data

[5]; pass/fail ratio, FASTQ quality, true variant discovery and variant quality metrics are compared. FAST5 files were downloaded from Nanopore WGS Consortium [5] using Amazon Web Services (AWS) CLI terminal software [6]. Data sets from different sizes and different laboratories were selected to have a uniform distribution. Data sets are downloaded using AWS S3 Client in Ubuntu 20.04.

Bioinformatic and computational analyses

Base-calling is applied to FAST5 files using Guppy with Fast and High Accuracy built-in models (dna_r9.4.1_450bps_fast and dna_r9.4.1_450bps_hac are used as config files). The base calling process produces 2 different outputs, Pass and Fail. We used FASTQ files in the Pass folder for further steps and then calculated the Pass/Fail Ratios (Supplementary File 2) for each run using R. 16 CPUs are used for base calling processes. In the second step, the number of reads in merged FASTQ files is calculated (Supplementary File 2 / Supplementary Fig 3).

Table 1 Flow Cell Data Used in Guppy Model Analysis

Flowcell ID	Reads	Bases
FAB39075	477495	3014355946
FAB42395	38335	200553219
FAB42260	269507	1583530766
FAB41174	11714	739850920
FAB42476	435934	2655496773
FAB42706	431694	2434471643
FAB43577	427215	2776702333
FAB46664	491945	2335386447
FAB39088	668016	3929822468
FAB39043	442132	2574202451
FAB42316	573736	4047383848
FAB42473	646945	3794243146
FAB42810	322286	2433213020
FAB44989	558539	3962530064
FAB45332	531764	3267600.534

FASTQ files merged using cat command and aligned to the human genome (hg19) using minimap2 [7]. Output SAM files are sorted and indexed using Samtools [8]. Variants are called using Clair3 [9] with default parameters. Called SNPs and InDels are split to separate VCF files using VCFTools [10] (Supplementary File 1). VCF files obtained from Clair3 and filtered using VCFTools are processed using an in-house R function. NA12878 (HG001) truth VCF file [11] is used to compare true and false variants using

Chromosome, Position, Reference Base and Alternative Base (Table 2). For the analysis of InDels, the same procedure as the analysis of SNPs was applied to VCF files (Table 3). For the analysis of false negative rate differences (Supplementary Excel File) between models, the HG001 Truth VCF file is filtered using a BED file, constructed using the regions of VCF files for each dataset. Common variants of each dataset (Table 4 / Supplementary Fig 4-5) are identified and true positive rates for common, "only in fast model output" and "only in high accuracy model output" are calculated.

Results and Discussion

Pass and Fail Ratios

Results indicate that the number of FASTQ files is not significantly changed while Pass/Fail ratios are significantly changed between models (Fig 1 / Supplementary Fig 1). The average of Fold Changes of Pass/Fail Ratios is 0.918 while the P-Value is 0.011 (Effect size is 0.38). The number of generated FASTQ files is not significantly different with a p-value of 0.445 (Effect size is 0.0035). The number of Pass and Fail FASTQ files are also not significantly changed with p-values of 0.078 and 0.077, respectively (With effect sizes of 0.021 and 0.033 respectively).

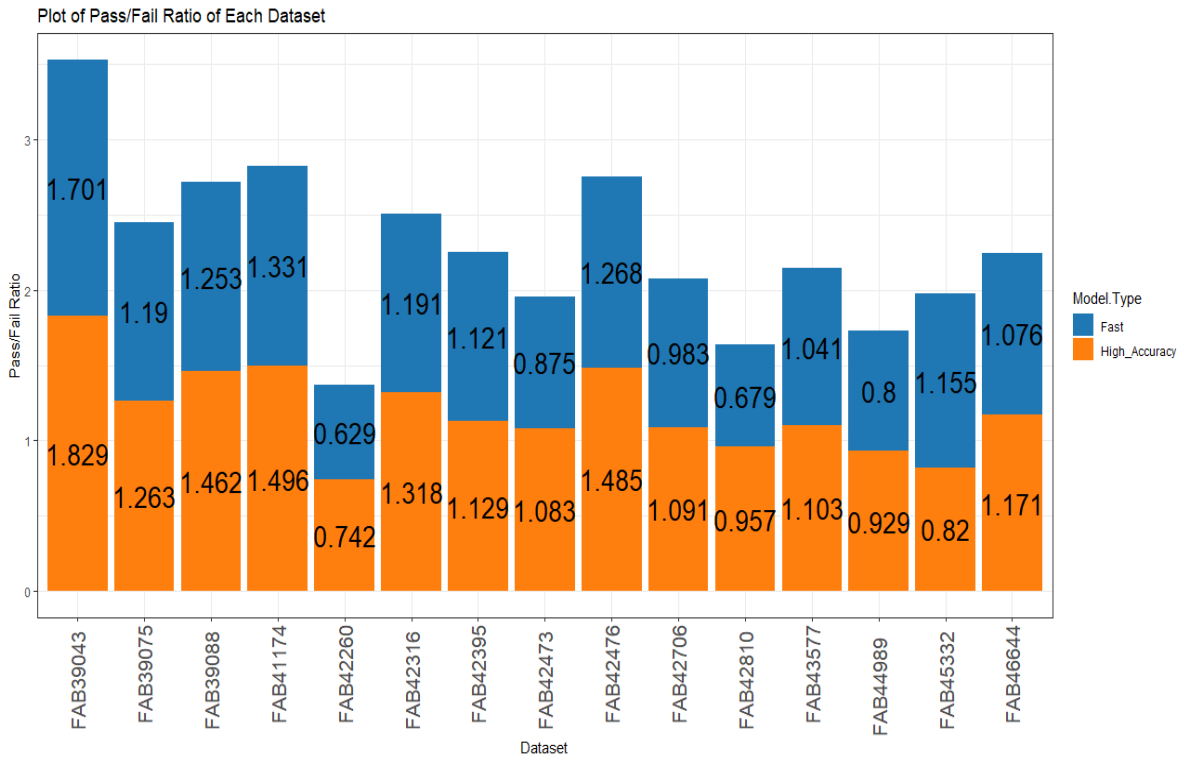

Fig 1 Pass/Fail Ratios of Each Dataset

Comparison of Average Read Lengths of Base Called FASTQ Files

Boxplot of read lengths indicates the average read length between fast and high accuracy models have similar distributions (Supplementary File 2 /Supplementary Fig 2). Paired t-test was applied to the average read lengths and the difference is not significant between models with a p-value of 0.68 (with an effect size of 0.012).

Read Counts in FASTQ Files

The average Fold Change (FC) of read counts is 0.916 while the P-value is 0.24 (effect size is 0.143). Here, it is observed that different models do not have different read counts in FASTQ Files.

Comparison of Single Nucleotide Polymorphisms

The number of called SNPs (Fig 2) is significantly different with 0.475 as Fold Change and 0 as P-value (effect size is 0.44). This result indicated that the number of called SNPs is significantly different between models.

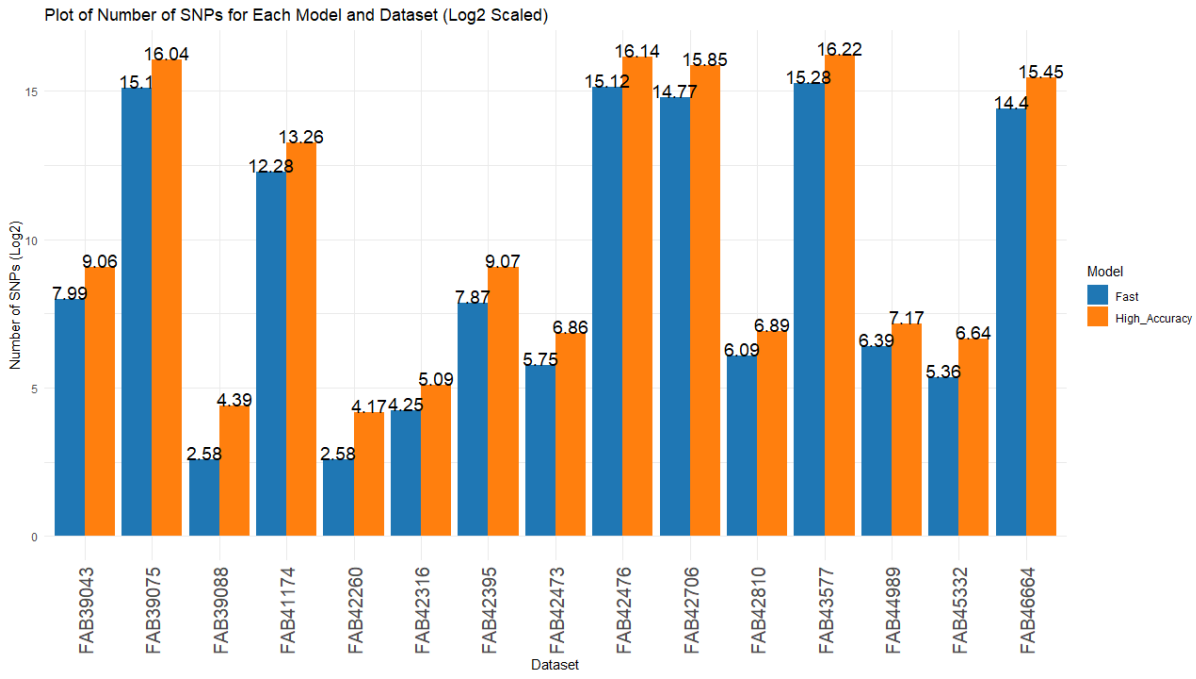

Fig 2 Number of Single Nucleotide Polymorphisms

The same test was applied to true positive rates (Fig 3 / Supplementary Excel File) to test the significance. Even though the number of variants is different, true positive SNP rates are not statistically different between models with 0.97 as Fold Change and 0.22 as P-value (effect size is 0.26).

Due to the high number of variants in the truth VCF file and the dataset's low coverage, the number of false negatives is very high. Even though, different models can be compared since the same methods are applied. Test results indicated that false negative rates are significantly changed with a P-value of 0.013 (effect size = 0.59).

Table 2 Comparison of Variants Obtained with Fast and High Accuracy Models with Truth VCF File (TP: True Positive, FP: False Positive)

Dataset	Model	Number of Variants	Number of TP Variants	Number of FP Variants	TP Ratios
FAB39043	Fast	254	226	28	88.97638
FAB39043	High Accuracy	533	470	63	88.18011
FAB39075	Fast	35192	31144	4048	88.49739
FAB39075	High Accuracy	67430	60538	6892	89.77903
FAB39088	Fast	6	6	0	100
FAB39088	High Accuracy	21	20	1	95.2381
FAB41174	Fast	4989	4210	779	84.38565
FAB41174	High Accuracy	9813	8360	1453	85.19311
FAB42260	Fast	6	3	3	50
FAB42260	High Accuracy	18	12	6	66.66667
FAB42316	Fast	19	17	2	89.47368
FAB42316	High Accuracy	34	34	0	100
FAB42395	Fast	234	205	29	87.60684
FAB42395	High Accuracy	536	466	70	86.9403
FAB42473	Fast	54	43	11	79.62963
FAB42473	High Accuracy	116	103	13	88.7931
FAB42476	Fast	35568	30017	5551	84.39327
FAB42476	High Accuracy	71996	62081	9915	86.2284
FAB42706	Fast	28010	23616	4394	84.31275
FAB42706	High Accuracy	59103	50908	8195	86.13438
FAB42810	Fast	68	62	6	91.17647
FAB42810	High_Accuracy	119	100	19	84.03361
FAB43577	Fast	39673	33783	5890	85.15363
FAB43577	High_Accuracy	76573	65821	10752	85.9585
FAB44989	Fast	84	75	9	89.28571
FAB44989	High_Accuracy	144	122	22	84.72222
FAB45332	Fast	41	33	8	80.4878
FAB45332	High_Accuracy	100	89	11	89
FAB46664	Fast	21621	17779	3842	82.23024
FAB46664	High_Accuracy	44794	37614	7180	83.97107

Comparison of Insertion and Deletions

The number of InDels and true positive rates are significantly different (Figure 4-5) in the context of insertion and deletion calling with p-values as 0, 0.046, respectively (effect sizes are 0.48, 1.16). This result indicates that different models highly affect the results in the context of the number of insertions and deletions more than SNPs and using fast models for InDel calling has more risk to lose variant information.

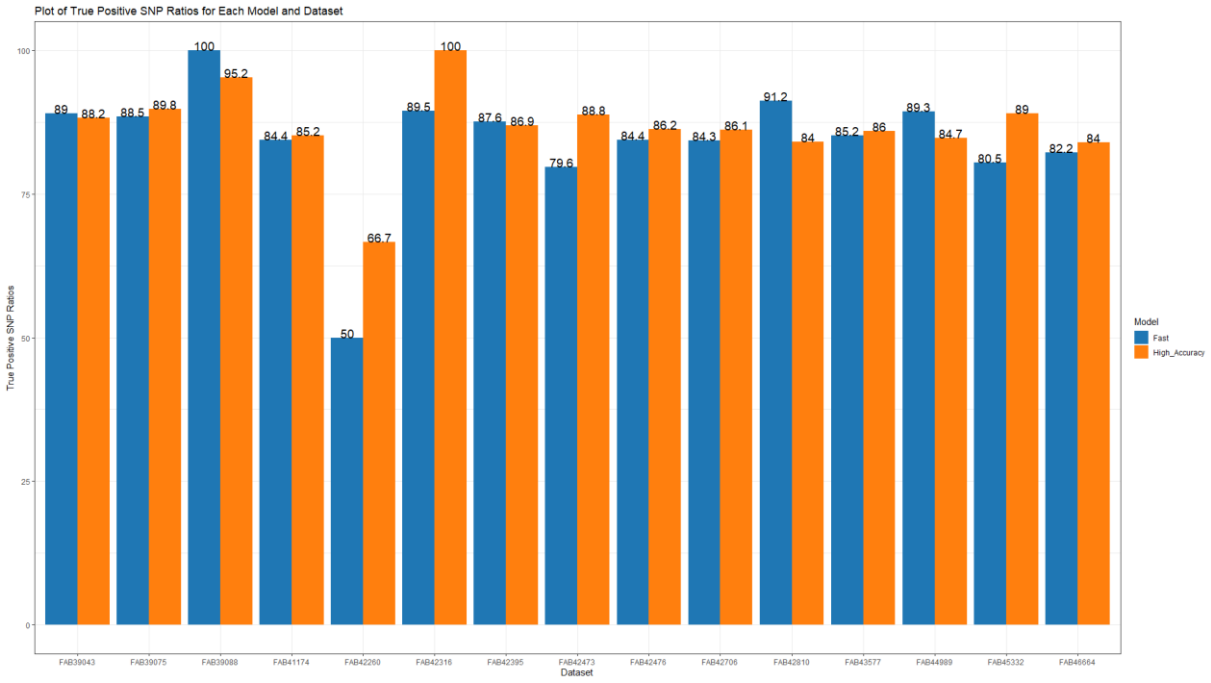

Fig 3 True Positive Ratios of Single Nucleotide Polymorphisms

Table 3 Comparison Results of Called InDels with Truth VCF File (TP: True Positive, FP: False Positive)

Dataset	Model	Number of Variants	Number of TP Variants	Number of FP Variants	TP Ratios
FAB39075	Fast	269	187	82	69.51673
FAB39075	High_Accuracy	493	408	85	82.75862
FAB41174	Fast	17	13	4	76.47059
FAB41174	High_Accuracy	60	46	14	76.66667
FAB42476	Fast	204	154	50	75.4902
FAB42476	High_Accuracy	489	402	87	82.20859
FAB42706	Fast	133	111	22	83.45865
FAB42706	High_Accuracy	361	297	64	82.27147
FAB43577	Fast	248	160	88	64.51613
FAB43577	High_Accuracy	566	406	160	71.73145
FAB46664	Fast	96	69	27	71.875
FAB46664	High_Accuracy	220	180	40	81.81818

Analysis of Common Variants Between Models

Among 21 comparisons (15 SNP and 6 InDel comparisons), it is observed that variants only called in high-accuracy model results have higher true positive rates. True positive rates of variants are only called with fast models and only called with high accuracy models tested using paired t-test and the difference is significant with a p-value of 0.0002 (effect size is 0.98).

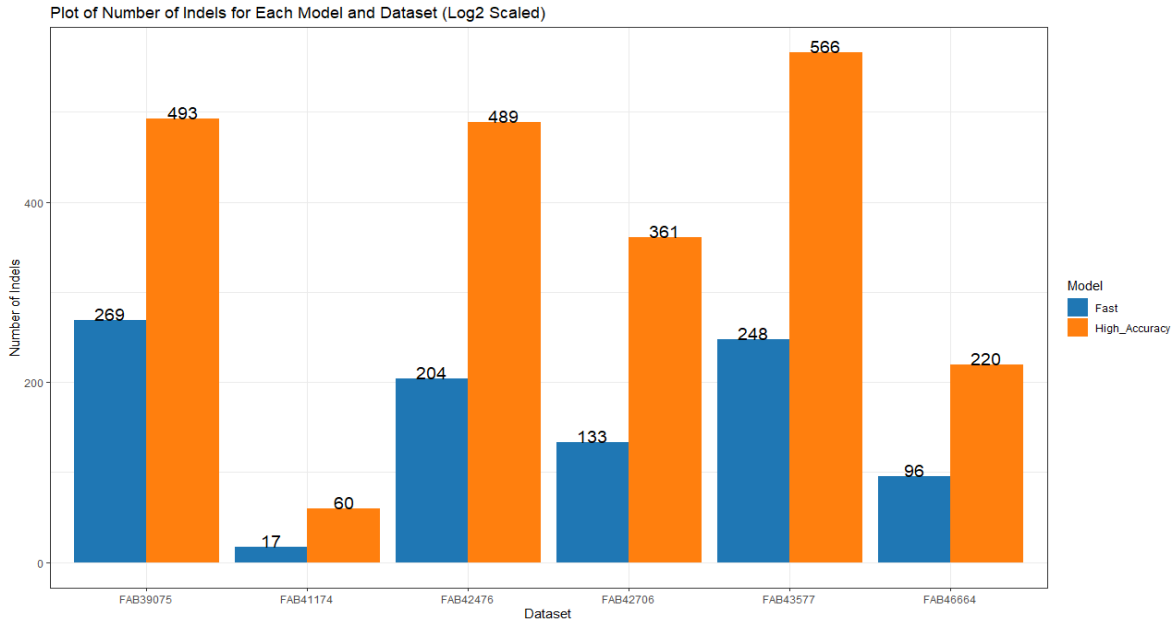

Fig 4 Number of Insertions and Deletions

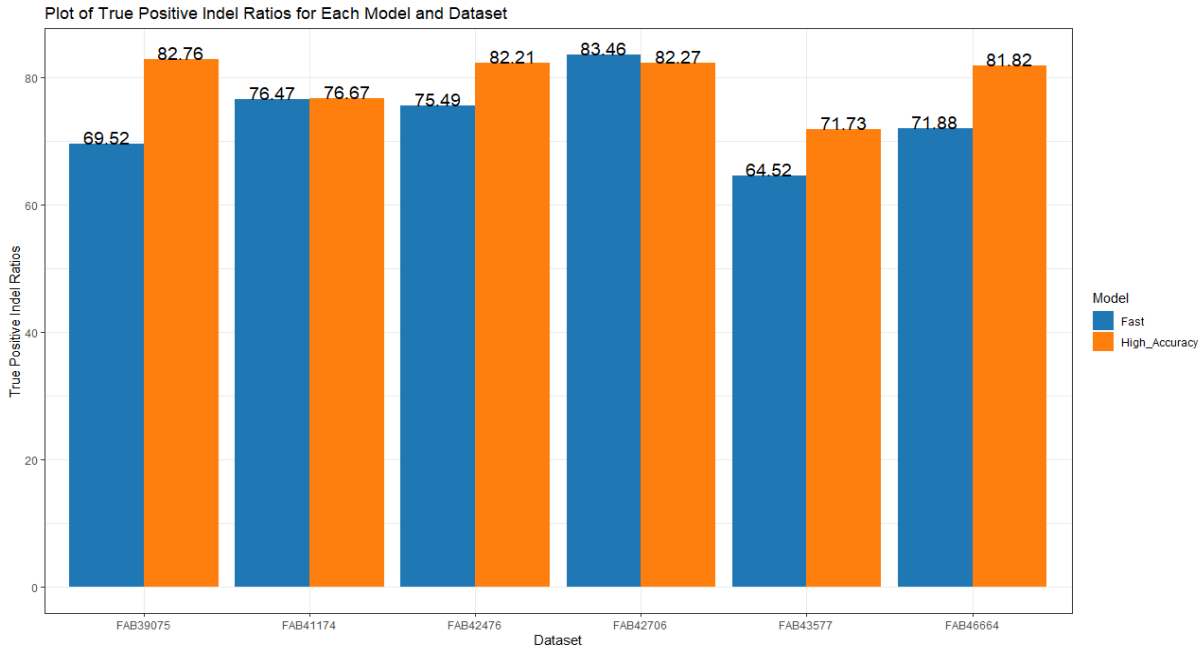

Fig 5 True Positive Ratios of Deletions and Insertions

Table 4 Common Variants Between Models (TP: True Positive, FP: False Positive)

Dataset	Number of Common Variants	TP Rate of Common Variants	Only in Fast Model	Only in High Accuracy Model	TP Rate of Only in Fast Model	TP Rate of Only in High Accuracy Model	Variant Type
FAB39043	150	93.33333	104	383	82.69	86.16	SNP
FAB39075	23725	92.29083	11467	43705	80.65	88.41	SNP
FAB39088	4	100	2	17	100	94.12	SNP
FAB41174	3343	89.41071	1646	6470	74.18	83.01	SNP
FAB42260	3	66.66667	3	15	33.33	66.66	SNP
FAB42316	14	100	5	20	60	100	SNP
FAB42395	155	90.96774	79	381	81.01	85.30	SNP
FAB42473	38	89.47368	16	78	56.25	88.46	SNP
FAB42476	23837	89.7764	11731	48159	73.45	84.47	SNP
FAB42706	18702	90.15079	9308	40401	72.58	84.27	SNP
FAB42810	37	94.59459	31	82	87.1	79.27	SNP
FAB43577	25535	90.75387	14138	51038	75.03	83.56	SNP
FAB44989	41	92.68293	43	103	86.06	81.55	SNP
FAB45332	26	92.30769	15	74	60	87.84	SNP
FAB46664	13816	88.60741	7805	30978	70.94	81.9	SNP
FAB39075	107	89.71963	162	386	56.17	80.83	InDel
FAB41174	14	78.57143	3	46	66.66	76.09	InDel
FAB42476	93	83.87097	111	396	68.47	81.82	InDel
FAB42706	57	96.49123	76	304	73.68	79.60	InDel
FAB43577	98	74.4898	150	468	58	71.15	InDel
FAB46664	40	85	56	180	62.5	81.11	InDel

Analysis of Qualities Common Variants Between Models

The analysis of the qualities of common variants in fast and high-accuracy models indicated that the qualities are not significantly different. (Table 5) and results indicated that the qualities of variants are not significantly different between models.

Conclusion

In this study, 15 different low-coverage data sets from different sequencing experiments (each of them coming from a single flow cell) are used to compare the effects of different built-in base calling models on variant calling. Guppy, the tool that has the best overall performance in benchmark tests [16] and is supported by Oxford Nanopore, is a widely used base caller and to the best of our knowledge, there are not any comparison studies on different base calling models of Guppy. To the best of our knowledge, this study is the first one that analyses the effects of models on variant calling.

This study indicated that the chosen model does not affect true positive and false negative SNP rates significantly while the number of Single Nucleotide Polymorphisms (SNPs), number of Insertions and Deletions (InDels), and true positive and false negative InDel rates are significantly lower in fast models. Also, results indicated that these alterations occur due to significant pass/fail ratio differences, Total read counts and average read lengths of the base called FASTQ files do not significantly change between models.

Table 5 Statistical Analysis Results of Qualities of Common Variants Between Models

Dataset	P-Value	Variant Class
FAB39043	0.845	SNP
FAB39075	0.962	SNP
FAB39088	0.474	SNP
FAB41174	0.95	SNP
FAB42260	0.752	SNP
FAB42316	0.748	SNP
FAB42395	0.72	SNP
FAB42473	0.559	SNP
FAB42476	0.999	SNP
FAB42706	0.169	SNP
FAB42810	0.662	SNP
FAB43577	0.215	SNP
FAB44989	0.818	SNP
FAB45332	0.27	SNP
FAB46664	0.599	SNP
FAB39043	0.389	INDEL
FAB39075	0.555	INDEL
FAB41174	0.065	INDEL
FAB42395	0.232	INDEL
FAB42476	0.288	INDEL
FAB42706	0.832	INDEL
FAB42810	0.935	INDEL
FAB43577	0.3	INDEL
FAB46664	0.512	INDEL

Analyses indicated that High Accuracy and Fast models cause the calling of different numbers of variants but in the context of true positive variants, the difference is not significant for SNPs while it is significant for insertions and deletions. Since there is not a significant difference between read counts and average read lengths, Pass/Fail ratios may be the main reason for this difference. For both models, the differences between false

negative SNPs, true positive SNPs and qualities of common variants between models are not significant. It can be concluded, for SNP calling, the usage of fast models in case of lack of computational power and time limitation, does not create a statistical disadvantage. This study can guide researchers about the applications and differences of built-in models of Guppy. As mentioned, Guppy comes with MinKNOW pre-installed and is the most common choice for clinical and scientific research centres without bioinformatics expertise.

This study has limitations on the number of tested samples. Due to the low number of samples, statistical test results may not be generalized but the properties of tested data are held as uniform. Even though the statistical analyses of the study lack generalizability, the differences are clear for the datasets. For further research, the same analyses can be planned and applied to multiple ONT-based Whole Genome Sequencing and Whole Exome Sequencing experiment results.

It should be also noted that the quality of variant calling is directly associated with experimental procedures and properties of genomic locations (high GC content, CpG Islands etc.). Due to this, it is possible to investigate the effects of models based on these parameters and an application procedure based on experimental steps or genomic locations can be developed.

Abbreviations

ONT: Oxford Nanopore Technologies, NGS: Next Generation Sequencing, SNP: Single Nucleotide Polymorphism, InDel: Insertions and Deletions, TP: True Positive, FP: False Positive, VCF: Variant Call Format, FC: Fold Change

Acknowledgements

The Guppy software downloaded by Bilçağ Akgün from Oxford Nanopore Sequencing Official Website with registered user account. We also thank Dr. Pınar Pir for advises on statistical testing.

Funding

This work is partially funded by TUBITAK as a part of 20AG005 Project.

Data Availability statement

The authors declare that all reported results are obtained computationally. No novel experimental data is reported in this publication. Bash and R commands are provided in https://github.com/ideateknoloji/guppy_models_comparison

Compliance with ethical standards

This study does not involve any experiment, all analyses were applied computationally. According to “TRDizin Etik İlkeleri” guide, ethics committee report is not required" olarak değişecek.

Conflict of interest

The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards. This study does not involve any experiment. Ethical Standards does not apply.

Authors' contributions
In this work, whole study is managed by Hamza Umut Karakurt, analyses coded and applied by Hamza Umut Karakurt, Hasan Ali Pekcan, Ayşe Kahraman and Muntadher Jihad. All analyses applied under the medical supervision of Bilçağ Akgün and technical supervision of Cüneyt Öksür and Bahadır Onay. All authors contributed to the writing section of this article.

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Research Article

Turgut A., et al., Investigation of the Effects of Resveratrol on Paracetamol Toxicity Established in Hep3B Cells. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 288-301.
DOI: 10.38001/ijlsb.1357213

Investigation of the Effects of Resveratrol on Paracetamol Toxicity Established in Hep3B Cells

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ABSTRACT

We therefore wanted to investigate acetaminophen hepatotoxicity by using Hep3B human hepatoma cells exposed to acetaminophen and resveratrol, used as a protective agent. Specifically, we studied the role of some proinflammatory markers and oxidative damage as possible mechanisms of acetaminophen-associated cytotoxicity. The Hep3B human hepatoma cell line was used for this study. In vitro studies (GSH, SOD, CAT, AST, ALT, TNF-alpha, IL-6 and cell viability) were performed by using different methods such as Biochemical analyzer, RT-PCR, ELISA and MTT. Acetaminophen and resveratrol were applied to cells in a different time and doses. Only acetaminophen treatment decreased SOD, CAT and GSH levels in Hep3B cells whereas acetaminophen and resveratrol co-treatment increased these enzymes levels. On the other hand, acetaminophen and resveratrol co-treatment (especially 160 µM dose of resveratrol) lead a severe increase in TNF-alpha and IL-6 levels. In the current study, it was shown that acetaminophen caused liver toxicity and, interestingly, resveratrol applications seriously affected the levels of the above-mentioned parameters. Only, acetaminophen administration may cause abnormal decreases and/or increases in antioxidant enzymes and proinflammatory cytokines levels. Additionally, acetaminophen and high dose resveratrol co-treatment triggered the inflammation and oxidative stress. These results showed that resveratrol have a potential to be an effective agent on the treatment and protection of hepatic damage.

ARTICLE HISTORY
Received
8 September 2023
Accepted
25 October 2023

KEYWORDS
Acetaminophen, hepatotoxicity, Hep3B, oxidative stress, resveratrol, cytokines

Introduction

Paracetamol

Paracetamol (Acetaminophen, N-acetyl-p-aminophenol; APAP) is a drug with analgesic (pain reliever) and antipyretic (fever-reducing) effects. It is metabolized primarily in the liver, kidney and intestine [1]. Exceeding therapeutic doses also increases the amount of released NAPQI, and when glutathione exceeds its NAPQI binding capacity, free NAPQI

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causes liver damage, which can lead to hepatic necrosis by making covalent bonds with molecules in the liver [2]. This damage causes an increase in the number of catabolic enzymes that cause the cell death of Ca^{+2}, which accumulates in the cell with the disruption of the intracellular balance in the liver cells [3]. Apoptosis, nitric oxide, reactive oxygen species and lipid peroxidation are also known to cause toxicity in the liver [4].

Substances that cause liver damage are called hepatotoxins. Hepatotoxins are classified in two ways: "intrinsic" and "idiocentral". Intrinsic hepatotoxins act in a dose-dependent manner, and hepatotoxicity that occurs in this way is called intrinsic hepatotoxicity. Hepatotoxins that act in a non-dose dependent manner are idicentric. Hepatotoxicity, which causes hepatic failure to a large extent, is intrinsic and the best example of this is acetaminophen (APAP or paracetamol). The number of patients exposed to idiocentral hepatotoxins is 1 in 10000. More than 1000 drugs and herbal products are idiocentral hepatotoxic and constitute more than 10% of acute liver failure [5,6].

Resveratrol

Resveratrol is produced from grapes, black mulberry and peanuts in response to pathogenic attack and environmental conditions. Resveratrol, which has a strong antioxidant effect, both scavenges free radicals and prevents the damage caused by these radicals. Resveratrol, which is determined to inhibit lipid peroxidation caused by OH radical, also prevents DNA damage caused by OH and H_2O_2 [7]. Resveratrol is also known to inhibit the oxidation of membrane lipids and reduce oxidative stress. [8,9]. Resveratrol is a substance that shows anti-inflammatory properties by inhibiting the formation of substances that cause inflammation [10]. In addition, resveratrol significantly reduces lipopolysaccharide-induced airway inflammation, chronic edema, and osteoarthritis [11].

Resveratrol also has a cardioprotective effect on the ischemic heart thanks to its anti-inflammatory properties. In studies, when the control group and the group given resveratrol were compared, it was determined that resveratrol reduced myocardial infarction and caused improvements that can be observed in the ventricles after ischemia [12]. In recent years, therapeutic-based scientific studies generally focus on alternative active substances. Resveratrol, a natural polyphenol compound that has many biological activities, is easy and cheap to obtain today, is only one of these active ingredients. Many

scientific studies have shown that resveratrol has many anti-carcinogenic, anti-diabetic, apoptotic, anti-angiogenic, anti-microbial, anti-viral, anti-neurotoxic and similar effects [13,14]. Both *in-vivo* and *in-vitro* studies have been carried out in this area, but there is not enough information in the literature about the changes in various immunological and biochemical parameters and the different dose amounts applied in our *in-vitro* study of paracetamol toxicity and resveratrol efficacy. In the current study, it was aimed to investigate the hepatotoxicity of acetaminophen induced hepatotoxicity in Hep3B human hepatoma cells, where acetamonifen and resveratrol, which we think may have a possible protective effect, were applied. In this study, the role of some proinflammatory parameters and oxidative stress, which is one of the possible causes of acetaminophen-mediated cytotoxicity, were investigated.

Material and Methods

In the present study, the Hep3B (ATCC-HB-8064) Human hepatoma cell line obtained commercially from the American Cell Culture Collection (ATCC) cell bank was used. DMSO, Penicillin, Resveratrol and MTT Cellular viability analysis kit sigma brand, Paracetamol Laboratories UPSA brand, Glutathioneperoxidase (GSH), Superoxide Dismutase (SOD) Assay and Catalase (CAT) Assay ELISA Kit Elabscience brand used in our study were used.

Experimental method

In the present study, it was aimed to investigate the preventive effects of resveratrol, a natural polyphenol compound, on inflammatory and oxidative stress parameters in the paracetamol-induced *in vitro* hepatotoxicity model. For this purpose, liver damage was induced by high-dose paracetamol in Hep3B human hepatoma cells, and then changes in immunological and biochemical parameters such as TNF-alpha, IL-6, SOD, CAT and GSH, which were activated in this damage, were observed with resveratrol application.

Experimental Groups Study;

1. Control group
2. Group treated with 160 µM Resveratrol (R160)
3. 80 µM Resveratrol administered group (R80)
4. Group administered 40 µM Resveratrol (R40)
5. 20 µM Resveratrol administered group (R20)
6. 20 µM APAP +160 µM Resveratrol administered group (APAP+R160)

7. 20 µM APAP + 80 µM Resveratrol administered group (APAP+R80)
8. 20 µM APAP + 40 µM Resveratrol administered group (APAP+R40)
9. 20 µM APAP + 20 µM Resveratrol administered group (APAP+R20)
10. 20 µM of APAP was administered in the administered group.

Application of resveratrol and paracetamol doses

After passage of the Hep3B (ATCC-HB-8064) Human hepatoma cell line obtained commercially from the American Cell Culture Collection (ATCC) cell bank, 1,000,000 Hep3B cells were seeded in 6-well culture dishes (6-well plate). Then, commercially available acetaminophen (APAP-Paracetamol) was dissolved in the medium and added to all experimental groups except the control well as 20 µl. After 1 hour of incubation, resveratrol, a natural polyphenol compound, 20; 40; 80; Fresh solutions dissolved in medium at 160 µM concentrations were given to the cells in equal volumes of 100 µl. For each resveratrol dose, sowing was carried out in 3 different wells. Cells were followed by seeding for 24 and 48 hours for all groups. At the 24th and 48th hours following the cultivation, the cells in the wells were collected by treatment with trypsin again and centrifuged. After discarding the supernatant, it was suspended with 1 ml of medium and counted.

Biochemical analysis

Following the incubation procedures, the cell suspension was collected in a 15 ml centrifuge tube and centrifuged at 1500 rpm for 3 minutes and the supernatant was discarded. Alanineaminotransferase (ALT) and aspartataminotransferase (AST) liver enzyme levels were measured by an automatic bioanalyzer device from the cell suspension diluted with 5 ml of fresh medium. Then, evaluations were made with Glutathioneperoxidase (GSH), SuperoxideDismutase (SOD) Assay and Catalase (CAT) Assay ELISA Kit according to the manufacturer's recommendation (Elabscience).

The expression levels of the pro-inflammatory cytokines TNF-alpha and IL-6 cytokines were investigated (performed by applying the Real-time PCR method with the primer shown in Table 1).

Table 1 Primer sequences used for the PCR study

Primers	Forward	Reverse
TNF-alfa	5'-CCAGGAGAAAGTCAGCCTCCT-3'	5'-TCATACCAGGGCTTGAGCTCA-3'
IL-6	5'-CGAAAGTCAACTCCATCTGCC-3'	5'-GGCAACTGGCTGGAAGTCTCT-3'
β-aktin	5'- GCAAGCAGGAGTATGACGAG -3'	5'- CAAATAAAGCCATGCCAATC-3'

Statistical method

SPSS 20.0 (IBM, New York, USA) statistical program was used to evaluate the data obtained from the experiments. One-way ANOVA, Tukey and Duncan's Multiplerange analysis tests were used to evaluate the significance of the difference between groups. The limit of significance was accepted as $p< 0.05$.

Results and Discussion

The effect of resveratrol and paracetamol on liver enzymes

When the data obtained was examined, a statistically significant difference emerged between the liver ALT activity of the APAP+R160 group and all other groups ($p<0.05$). However, there was no statistical difference when compared both between the groups and the control group ($p>0.05$). These data gave similar results in AST activities. The letters and numbers in the figure indicate the significance between the groups (Figure 1).

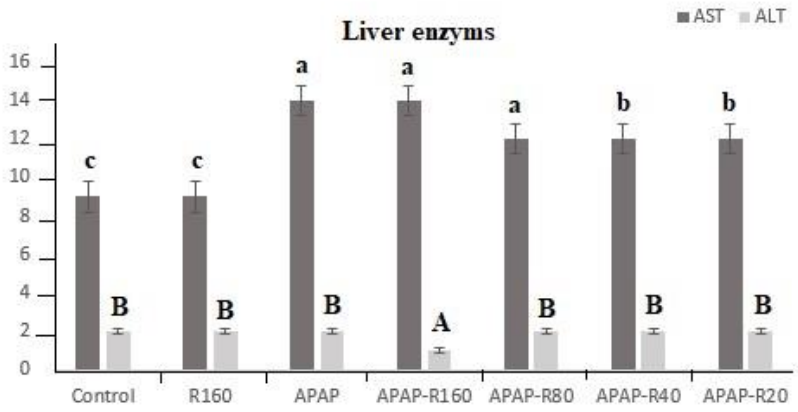

Fig 1 Effects of Resveratrol and Paracetamol on AST and ALT activities (a-c; Shows statistical difference in AST Activity between all experimental groups, A-B; Shows statistical difference in ALT Activity between all experimental groups).

In vitro effect of resveratrol and paracetamol on Hep3B cells

The cellular viability analysis results obtained from the cell culture studies we carried out are shown in Figure 2. When the results obtained were examined, statistically significant

differences were observed between the experimental groups in terms of cell viability. According to the 24th hour MTT results; No significant difference was observed in the control group alone and the groups administered only resveratrol. In other experimental groups, it was determined that paracetamol application significantly reduced the number of viable cells compared to the control and resveratrol-only groups ($p<0.05$). Additionally, the lowest amount of viability was detected in the APAP+R160 experimental group in the groups administered resveratrol together with paracetamol ($p<0.05$). When the 48-hour data obtained from the MTT cell viability analysis test is evaluated; Results parallel to the 24-hour data were determined. In addition to paracetamol having a toxic effect on living cells, high dose resveratrol application also causes serious decreases in the number of living cells.

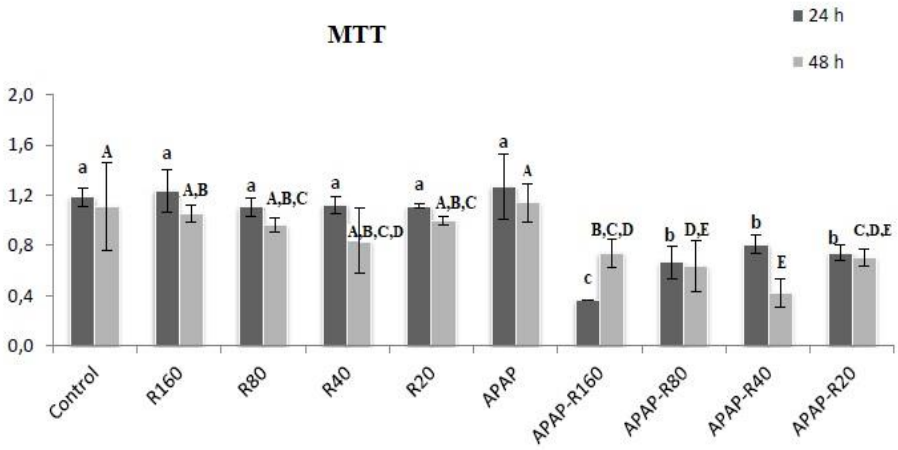

Fig 2 Viability analysis test results of all experimental groups (a-c; shows statistical difference between 24-hour MTT results, A-E; shows statistical difference between 48-hour MTT results).

Effects of resveratrol administration on SOD, CAT and GSH activity

When Figures 3 were examined, it was observed that paracetamol application SOD, CAT and GSH activities were significantly different and at the lowest level in all experimental groups ($p<0.05$). While a significant increase was detected in SOD and CAT activities of high dose resveratrol compared to the control group ($p<0.05$), on the contrary, no increase was detected in GSH activity ($p>0.05$).

In Figure 3B, there was no statistically significant difference in CAT activity between the Control group and APAP+R80 ($p>0.05$). However, all other experimental groups increased or decreased statistically significantly both within themselves and when

compared to the control group. In Figure 3C, GSH levels in the paracetamol applied group were found to be significantly lower compared to the control group ($p<0.05$).

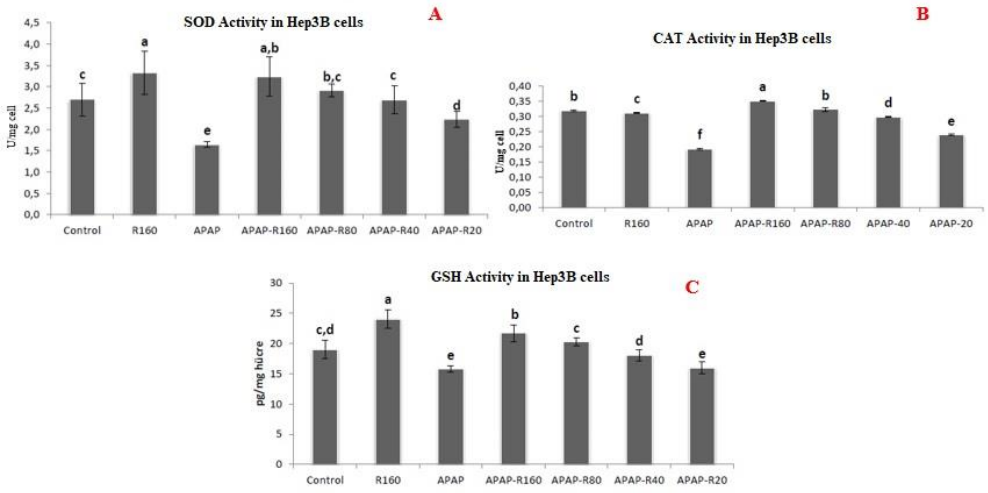

Fig 3 Effects of Resveratrol Administration on SOD, CAT and GSH Activity

Effects of Resveratrol Administration on the Gene Expression levels of Pro-Inflammatory Cytokines

When Figure 4 was examined, a significant increase was observed in the expression levels of pro-inflammatory cytokines IL-6 and TNF-alpha in both paracetamol and high dose resveratrol administered groups ($p<0.05$). This situation seriously triggered inflammation. However, although other doses of resveratrol reduced the amount of IL-6 and TNF-alpha in the APAP applied groups, they could not reduce it to the level of the control group. Partial increases and decreases were observed in other experimental groups. As a result, the data obtained reflects the data we expected in our study.

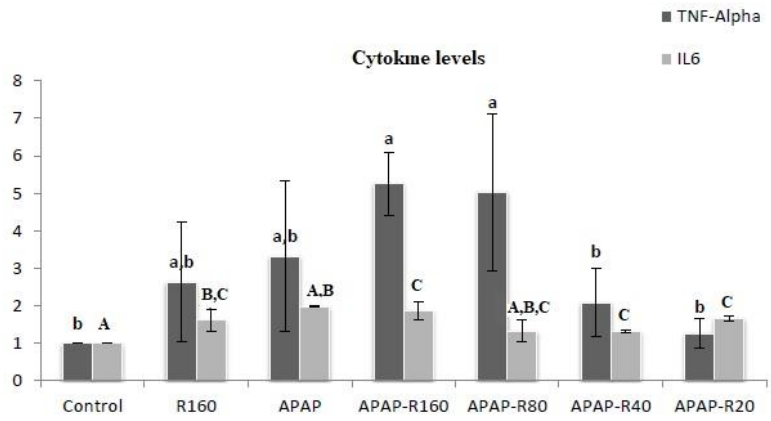

Fig 4 Effects of Paracetamol and Resveratrol applications on expression of the TNF-alpha and IL6 a-b; Shows the statistical difference between the experimental groups in terms of TNF-alpha, 1-3; It shows the statistical difference between the experimental groups in terms of IL-6.

Discussion

Paracetamol is an analgesic and antipyretic drug that is widely used worldwide and has few side effects when taken in appropriate doses [15]. Although it is recommended that the annual paracetamol consumption amount is less than 8 grams per person in some countries (USA, UK, Canada, Australia and New Zealand), this rate can exceed 20 grams per person [16,17]. Due to its high consumption and being an easily accessible drug, the risk of organ toxicity is also quite high. It is an ideal medicine when consumed in sufficient amounts, but when used in high amounts it is very likely to cause fatal liver failure. 2% of the paracetamol taken into the body is excreted unchanged in the urine. However, a small amount of paracetamol is metabolized to N-Acetyl-p-benzoquinone imine (NAPQI) by the cytochrome P-450 enzyme. This metabolite first depletes GSH and then binds to a number of cellular proteins, including mitochondrial proteins, and these bindings initiate liver damage [18]. As a result of these cellular events, consumption of ATP and mitochondrial oxidative stress events are observed. ATP consumption leads to cellularoncotic necrosis in hepatocytes and sinusoidal endothelial cells [19].

In general, fulminant liver toxicity occurs after ingestion of high doses of paracetamol. As an indicator of this damage, elevated liver transaminases (AST and ALT) can be shown. Elevated levels of these enzymes indicate paracetamol-induced liver damage. At the same time, this elevation indicates that the functional integrity of the cell membrane is impaired [20]. Other scientific studies related to the subject have also shown that paracetamol causes elevations in liver AST and ALT enzyme activities [21,22]. It has been found that paracetamol causes significant increases in liver enzyme activities in both experimental animals and *in vitro* studies [23]. In our current study, liver AST and ALT enzyme activities were investigated in order to understand whether paracetamol administration causes toxic effects on liver cells. The data obtained showed that it was statistically higher in the paracetamol administered groups compared to the control group, and this showed us that the hepatotoxicity model we planned in our study was formed. As stated in the findings section, it was determined in the current study that AST and ALT levels in the resveratrol applied groups decreased compared to the paracetamol applied group and approached the control group. In conclusion, this increase in AST and ALT activities is a sign that the toxic dose of paracetamol causes P-450-induced hepatotoxicity [24]. The inhibition of the increase in the activities of these enzymes is the primary

evidence of the hepatoprotective effect of resveratrol at the enzymatic level. In parallel with this, Anundi, et al.. [25] observed periportal and perivenous hepatotoxicity in cell culture with high-dose paracetamol administration.

GSH is one of the very important molecules that play a role in cellular defense against reactive toxic compounds or oxidative stress. Glutathione exists in two forms, oxidized and reduced, and there is a balance between the two forms. In its reduced form, the cysteinethiol group is capable of donating reducing equivalents to unstable molecules such as reactive oxygen products. This mechanism can prevent tissue damage by removing free radical species such as hydrogen peroxide and superoxide radicals. It is known that NAPQI as a mediator of oxidative stress at sufficiently high doses of paracetamol causes a decrease in GSH levels and an increase in lipid peroxidation due to this decrease [26]. In our current study, GSH values were determined in parallel with the known literature. While it was observed to be low in the paracetamol administered groups, significant increases were observed in the resveratrol administered groups. Some scientific studies in which different active ingredients are used as therapeutic agents support our current data. In a study by Manda, et al.; GSH values were found to be decreased in the group with paracetamol-induced liver toxicity compared to the control group, and these values were increased with the applied beta-carotene treatment [27]. Similarly, in another study, GSH level was found to be low in the paracetamol toxicity group, while an increase in GSH level was observed in the L-carnitine administered group [28].

One of the parameters responsible for oxidative stress in paracetamol toxicity is the superoxide radical, and these radicals are a family of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. It protects cells that metabolize oxygen against the harmful effects of superoxide free radicals. Therefore, it is an important antioxidant defense mechanism in almost all cells exposed to oxygen [29]. Some studies have mentioned that the increase of superoxide level in all of the mechanisms that cause the increase of free oxygen radicals has a very important role in the formation of oxidative stress [30]. Antioxidant enzymes such as SOD are easily inactivated by lipid peroxidases or reactive oxygen products, and therefore, decreases in these enzyme activities are detected in paracetamol toxicity. In our current study, it was determined that SOD activity decreased significantly in the groups treated with

paracetamol. In addition, resveratrol administration increased the SOD activities of these groups in a dose-dependent manner. When the literature is scanned, it is determined that there are scientific studies with similar mechanisms. In one of these studies, SOD levels were low in acute liver toxicity induced by paracetamol, but Picroside II given at increasing doses caused an increase in SOD levels in experimental groups [31]. Similarly, in a similar study, it was observed that while the amount of SOD decreased in the toxicity group, the level of SOD increased in the treatment group [32]. Likewise, many scientific studies with similar effects have been conducted and SOD activity has been shown to decrease in acute liver injury [33,34].

Catalase is an antioxidant enzyme commonly found in all prokaryotic and eukaryotic organisms. Catalase catalyzes the conversion of hydrogen peroxide, a strong and stable oxidant, into water and molecular oxygen. Catalase enzyme is also affected by oxidative stress, as are important antioxidant enzymes such as SOD, glutathione peroxidase (GPOX), and glutathione reductase (GR). Antioxidant enzymes such as catalase and SOD are easily inactivated by lipid peroxidases or reactive oxygen products, and therefore, decreases in these enzyme activities are detected in paracetamol toxicity [35]. In our study, parallel to this information, CAT level was found to be decreased in the paracetamol group, and statistically significant increases were observed in CAT levels after resveratrol treatment. It was in agreement with our current findings in other scientific studies, just as in other antioxidant enzymes. In a study performed by Awolede, et al., it was shown that CAT activity decreased in paracetamol-induced acute liver toxicity, but Carica papaya Linn application caused an increase in CAT level [36]. Similarly, in another study conducted in our country, it was stated that CAT levels decreased in the hepatotoxicity model created with paracetamol, but thiamine pyrophosphate application caused improvements in these levels [37].

Considering the data of our own study and the existing literature, we can say that resveratrol reduces oxidative stress in liver cells and increases hepatic antioxidant enzyme activities.

In addition to oxidative stress, the role of proinflammatory cytokines and inflammation precursors TNF-alpha and IL-6 in acute liver injury due to paracetamol toxicity has been demonstrated in scientific studies. TNF-alpha, produced from macrophages in the liver, plays an active role in many liver injuries such as fulminant liver failure [38]. TNF-alpha

can aid tissue repair through its central regulatory role. When scientific studies on this subject were examined, it was observed that the expression level of TNF-alpha increased significantly in acute liver toxicity due to paracetamol [39,40]. Studies have shown that TNF-alpha, which plays a role in the initiation of inflammation, increases significantly in hepatotoxicity, and these levels are significantly reduced with the use of agents with anti-inflammatory properties [41]. In our study, in parallel with the existing literature, the amount of TNF-alpha in the paracetamol-administered group was found to be 3 times higher than the control group, while statistically significant decreases were detected in the resveratrol-administered group.

Similarly, IL-6, which is a pro-inflammatory, has a protective effect on liver damage. It arises through the prevention of mitochondrial dysfunction and suppression of oxidative stress [42]. Similarly, scientific studies carried out in recent years have shown that IL-6 level increases in paracetamol-induced acute liver toxicity, and the therapeutic agents administered following this decrease the IL-6 level [43,44]. In the current study, it was shown that the paracetamol group caused an increase in the IL-6 level, but resveratrol administration showed efficacy even at different doses, as in TNF-alpha, and caused a decrease.

Conclusion

In the current study, it was shown that acetaminophen caused liver toxicity and, interestingly, resveratrol applications seriously affected the levels of the above-mentioned parameters. Only, acetaminophen administration may cause abnormal decreases and/or increases in antioxidant enzymes and proinflammatory cytokines levels. Additionally, acetaminophen and high dose resveratrol co-treatment triggered the inflammation and oxidative stress. These results showed that resveratrol have a potential to be an effective agent on the treatment and protection of hepatic damage.

Abbreviations

ALT: Alanin Aminotrenferaz, APAP: Asetominofen, GSH: Glutatyon, IL-6: İnterlökin-6, INF-α: İnterferon-alfa, KAT: Katalaz, MTT: Hücresel Canlılık Analizi, SOD: Süper Oksit Dismutaz, TNF – α: Tümör Nekroz Faktör – Alfa.

Acknowledgements

The author would like to thank the Department of Biochemistry, Faculty of Veterinary Medicine, for enabling this study to be carried out.

Data Availability statement

The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
In this work, these laboratory studies were carried out by Dr Alpgiray TURGUT, Dr Tubanur Aslan ENGİN and Dr Muhammet TURGUT. While Prof. Dr. Mesut Bünyami HALICI supervised the coordination, the article was organized and finalized by Dr Alpgiray TURGUT.

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Research Article

Demirel, N., Comparison of Food-Based Synthetic Attractants for Capture of Ceratitis capitata (Diptera: Tephritidae) on Persimmon Fruits. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 302-310. DOI: 10.38001/ijlsb.1354718

Comparison of Food-Based Synthetic Attractants for Capture of *Ceratitis capitata* (Diptera: Tephritidae) on Persimmon Fruits

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is an important pest on persimmon fruits in Turkey. The study was conducted in 2016-2018 to determine comparison of food-based synthetic attractants for capture of *Ceratitis capitata* on persimmon fruits in Dörtyol and Antakya districts of Hatay province. The Decis and Maxitrap traps baited with various attractants impregnated in paper handkerchief dispensers were used. As a result of two years of investigations, efficacy of various attractants varied in each of the sampling year. In 2016, the highest mean of C. *capitata* were observed by the combination of ammonium acetate + ammonium carbonate attractant traps, while the lowest mean of *C. capitata* was observed by a single of ammonium carbonate attractant traps. In 2018, the highest mean of *C. capitata* were observed by a single of ammonium carbonate and ammonium bicarbonate attractant traps, while the lowest mean of *C. capitata* was observed by the combination of ammonium acetate + trimethylamine + putrescine and ammonium acetate + trimethylamine + cadaverine attractant traps.

ARTICLE HISTORY
Received
03 September 2023
Accepted
30 October 2023

KEYWORDS
Persimmon,
Ceratitis capitata,
attractant,
Hatay

Introduction

Persimmon, *Diospyros* (Ebaneceae: Ebenales), is an important tropical and subtropical tree [1-3], widespread in Turkey where it comprise approximately 59.491 da with a total produce of 97.560 tons of fruit per annum, and Hatay province's share is 2.330 da and 3.458 tons [4]. The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most important fruit pests throughout the world [5-10]. The Medfly is a polyphagous tropical fruit fly, which attacks more than 350 botanical species [11,12]. The females lay eggs below the skin of the host fruits, which are destroyed by larval feeding [5-7,13]. The Medfly caused significant damages on persimmon fruits in Turkey [13,14,17]. The control program of medfly is based on applications of organophosphate insecticides that are mainly used foliage baiting and cover spraying methods [18]. However, the intensity of insecticide treatments for

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medfly has resulted in the development of resistant populations, harmful effects on human health, beneficial insects and non-target organisms [18-21]. Therefore, development of effective control methods as alternative to chemical control contains traps baited with female and male lures [22-23]. Traps baited with trimedlure and attractants are important tools for detection, monitoring and controlling of the *C. capitata* [13,15,17,24-30]. The trimedlure contained in Jackson traps, and McPhail traps baited with hydrolyzed protein were the primary detection tools used in medfly detection programmes [6,14,17,30-34]. Traps baited with lures are also continuously used to monitor and control population size and spread [15-17,24-26,30,35-36]. The purpose of the current study was to determine comparison of food-based synthetic attractants for capture of *Ceratitis capitata* (Diptera: Tephritidae) on persimmon fruits in Hatay province of Türkiye.

Material and Methods

The studies were conducted in 2016-2018 to determine comparison of food-based synthetic attractants for capture of *Ceratitis capitata* (Diptera: Tephritidae) on persimmon fruits in Hatay province of Türkiye. The Decis type traps and the Maxitrap type traps are usually used for the control of *C. capitata* on various fruits in Türkiye. Therefore, two types traps were used in these studies. In the first year, a study was carried out with Decis type traps which has lateral holes and without bottom entry. In the second year, the study was conducted with Maxitrap type traps which has lateral holes as one-way entrances and invaginated hole in the bottom of the trap. A single and the combinations of two and three attractants, ammonium acetate (AA), ammonium carbonate (AC), ammonium bicarbonate (AB), trimethylamine (TMA), diaminoalkane (cadaverine) (C) and 1,4-diaminobutane (putrescine) (P), were used as synthetic food-based lures in the study (Table 1). An attractant or mixed attractants impregnated into paper handkerchiefs were used. Each of the paper handkerchief package (10x7.5 cm) had a 3-mm diameter hole and contained 25 g attractant or mixed of attractants, 2 ml of 10% propylene glycol to decrease water evaporation and 2 ml of 2% dichlorvos. In the first year, the study was conducted on persimmon orchards in Dörtyol district, which contains more persimmon trees than other persimmon orchard located in Antakya district. Therefore, in the first year, the study was conducted as randomized complete block design with 5 treatments and 10 replications. In the second year, the study was

conducted as randomized complete block design with 5 treatments and 5 replications. In both years, the treatment of ratios were also different to comparison of food-based synthetic attractants for capture of *C. capitata* on persimmon fruits. The distance between two traps was 4 m and the traps were placed at 1.5-2m high on southeastern side of the persimmon trees (1 trap per tree) on 29 June 2016 and 27 August 2018. Attractant and propylene glycol and dichlorvos in the traps were replaced with the new ones in every 45 days. After harvesting of persimmon fruits, traps were removed on 10 November 2016 and 25 November 2018. In addition, they brought to the laboratory and captured adults of *C. capitata* were counted. All data were analyzed by analysis of variance (ANOVA) with using the SAS software and means were separated by using the Least Significant Difference (LSD) Multiple Comparison Tests (P <0.05) [37].

Table 1 Attractants used at persimmon orchards in Dörtyol and Antakya districts of Hatay province.

Treatments (Lures)[a]	2016	2018
	Lures (gr)/trap[z]	Lures (gr)/trap[z]
Ammonium acetate	0.42	7.5
Ammonium carbonate	0.42	7.5
Ammonium bicarbonate	----	7.5
Ammonium acetate+Ammonium carbonate	0.42+0.42	----
Ammonium acetate +Trimethylamine+Putrescine	0.42+0.13+0.01	7.5+3.16+0.05
Ammonium acetate+Trimethylamine+Cadaverine	0.42+0.13+0.01	7.5+3.16+0.05

[a]Lures abbreviations: Ammonium acetate (AA), Ammonium carbonate (AC), Ammonium bicarbonate (AB), Ammonium acetate+Ammonium carbonate (AA+AC), Ammonium acetate + Trimethylamine + Putrescine (AA+TMA+P), Ammonium acetate+ Trimethylamine +Cadaverine (AA+TMA+C). [z]Putrescine and Cadaverine used as (mg) and rest of the treatments (lures) used as (gr).

Results and Discussion

A comparison of food-based synthetic attractants for capture of *C. capitata* on persimmon fruits varied in each of the sampling year. In the first year, a total of 877 *C. capitata* adults were caught by food-based synthetic attractants traps. The highest mean of *C. capitata* were caught by the combination of ammonium acetate + ammonium carbonate attractant traps (F=0,98; P=0,4864) (Figure 1).

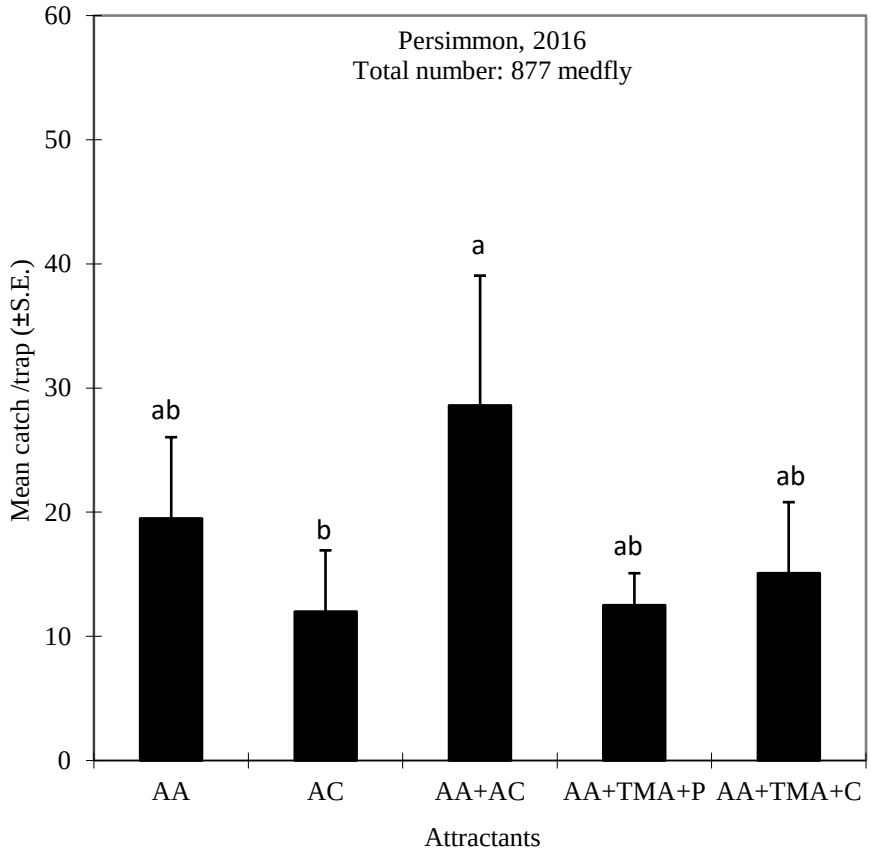

Fig 1 Mean (±SE) catches of medfly adults traps baited with attractants (29 June-10 November, 2016) at persimmon orchard in Dörtyol district. Different letters above bars indicate significant differences according to Least Significant Difference (LSD) ($P < 0.05$).

The lowest mean of *C. capitata* was caught by a single of ammonium carbonate attractant traps. In the second year, a total of 8,804 *C. capitata* adults were caught by attractant traps. The highest mean of *C. capitata* were caught by a single of ammonium carbonate and ammonium bicarbonate attractant traps (F=1.58; P=0,2073) (Figure 2). The lowest mean of *C. capitata* was caught by the combination of ammonium acetate + Trimethylamine + putrescine and ammonium acetate + Trimethylamine + Cadaverine attractant traps.

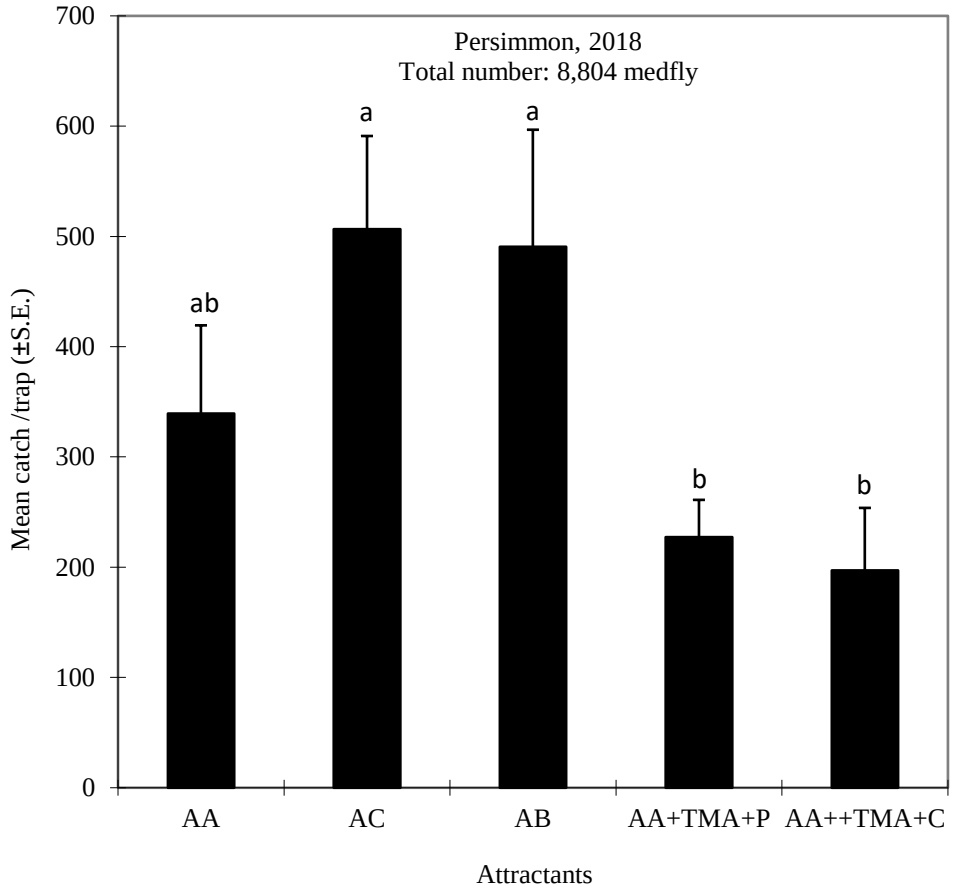

Fig 2 Mean (±SE) catches of medfly adults traps baited with attractants (27 August-25 November, 2018) at persimmon orchard in Antakya district. Different letters above bars indicate significant differences according to Least Significant Difference (LSD) ($P < 0.05$).

Several studies have been conducted to evaluate efficancy of various attractant such as a single of ammonium carbonate for females [15-17,30,38], a different concentrations of ammonium acetate [15,39,40], the combination of ammonium acetate + putrescine [15-17,30,41], ammonium acetate + trimethylamine + putrescine [15,17,30,36,42-45], ammonium acetate + trimethylamine + cadaverine [15-17,24-30] were used for *C. capitata*. Moreover, the food-based baits of diammonium phosphate caught significant number of *C. capitata* [15-17,30,40-46] and the high number of nontarget insects [25]. Previous studies were conducted by Çalıklı (28) to control *C. capitata* with various attractants on pomegranate fruit. In the first year, a total of 2,789 *C. capitata* adults were caught by attractant traps at the 'Katırbaşı' pomegranate orchard. The highest mean of *C. capitata* were caught by the combination of ammonium acetate + ammonium carbonate attractant traps. In the second year, a total of 7,787 *C. capitata*

adults were caught by attractant traps at the ‘Hicaz’ pomegranate orchard. The highest mean of male *C. capitata* was caught by the combination of ammonium acetate + ammonium bicarbonate attractant traps, while the female was caught by the combination of ammonium acetate + ammonium carbonate attractant traps. Moreover, previous studies were conducted by Demirel et al. (15) to evaluated effectiveness of various attractants to *C.capitata* on pomegranate fruits. In the first year, a total of 6,444 medfly adults were caught by attractant traps at the ‘Hicaz’ pomegranate and the highest mean of catches were caught by the combination of ammonium acetate + ammonium bicarbonate attractant traps. The lowest mean of catches were caught by the combination of ammonium bicarbonate + trimethylamine + putrescine, ammonium carbonate + trimethylamine + putrescine and ammonium acetate+ammonium bicarbonate + putrescine, single of ammonium carbonate, ammonium bicarbonate and diammonium phosphate attractant traps. In the second year, a total of 5482 medfly adults were caught by attractant traps at the ‘Katırbaşı’ pomegranate and the highest mean of catches were caught by the combination of ammonium acetate + trimethylamine + diaminoalkane (cadaverine) attractant traps. The lowest mean of catches were caught by the combination of ammonium acetate + trimethylamine + putrescine attractant traps.

Conclusion

The present study was conducted by traps baited with various attractants to determine comparison of food-based synthetic attractants for capture of *C. capitata* on persimmon fruits in Hatay province of Turkey. In the first year, the highest mean of *C. capitata* was observed by the combination of ammonium acetate + ammonium carbonate attractant traps. In the second year, the highest mean of *C. capitata* was observed by a single of ammonium carbonate and ammonium bicarbonate attractant traps.

Abbreviations
Medfly: Mediterranean fruit fly, AA: Ammonium acetate, AC: Ammonium carbonate, AB: Ammonium bicarbonate, AA+AC: Ammonium acetate+Ammonium carbonate, AA+TMA+P: Ammonium acetate + Trimethylamine + Putrescine, AA+TMA+C: Ammonium acetate+ Trimethylamine +Cadaverine

Acknowledgements
The author would like to thank the Faculty of Agriculture for enabling this study to be carried out at persimmon orchard in Dörtyol and Antakya district of Hatay.

Data Availability statement
The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
In these studies were carried out by myself at at persimmon orchards located in Dörtyol and Antakya district of Hatay. In addition, the article was organized and finalized by myself.

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Research Article

Ilhan S. and H. Atmaca, Venom Peptides of Echis carinatus against SARS-CoV-2: Effective Inhibition of Human ACE2 and Mpro. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 311-321. DOI: 10.38001/ijlsb.1338631

Venom Peptides of *Echis carinatus* against SARS-CoV-2: Effective Inhibition of Human ACE2 and Mpro

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ABSTRACT

The search for effective inhibitors against SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has led to re-screening of existing potential molecules. Molecular docking and virtual screening techniques have been employed to identify potential drug candidates. Natural products, known for their wide variety and reduced toxicity, have gained significant attention in these screenings. Snake venom proteins, characterized by their diverse biological activities and unique molecular structures, offer a promising avenue for the discovery of new antiviral molecules. In this study, we focused on the investigation of snake venom proteins isolated from *Echis carinatus*, specifically Schistatin (SCH), Phospholipase A2 (PLA2), Disintegrin (DS), and Echistatin (ECH) for their potential as inhibitors against SARS-CoV-2. Through molecular docking analysis, the binding interactions between these venom proteins and key SARS-CoV-2 targets, the main protease (Mpro), and the ACE2 receptor were examined. Results revealed that PLA2 exhibited the most favorable binding affinity to both Mpro and ACE2, surpassing the reference drug ritonavir (RTV). SCH, DS, and ECH also demonstrated promising binding affinities with both targets. This study sheds light on the unexplored potential of snake venom proteins, specifically PLA2, SCH, DS, and ECH from *E. carinatus* venom, as inhibitors against SARS-CoV-2. The exploration of snake venom proteins presents an intriguing avenue for the discovery of novel drug candidates with broad applications in the treatment of various diseases, including viral infections such as COVID-19.

ARTICLE HISTORY
Received
06 August 2023
Accepted
18 September 2023

KEYWORDS
Venomous snakes, therapeutic toxins, protein interactions, molecular docking

Introduction

Echis carinatus, commonly known as the Indian saw-scaled viper, is a venomous snake species found in various parts of the Indian subcontinent, Middle East, and Central Asia. It is one of several species in the genus , which includes several small to medium-sized venomous snake species from the Viperidae family [1]. Adult *E. carinatus* typically range in length from 40 to 70 centimeters, although some individuals can grow up to 90 centimeters. They have a slender body with a distinctive triangular-shaped head, covered in small scales. The scales on their back possess keels, which give them a rough or "saw-

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like" appearance. They inhabit a variety of ecosystems, including grasslands, scrublands, rocky areas, and semi-desert regions. They also can adapt to different altitudes, ranging from sea level to elevations of around 2,500 [2].

Like other vipers, *E. carinatus* possesses venom that is primarily hemotoxic, meaning it affects the blood and circulatory system [3]. The venom contains a mixture of enzymes, peptides, and other compounds that disrupt blood clotting, cause tissue damage, and can lead to systemic effects in envenomed individuals. It contains several enzymes such as metalloproteinases, phospholipases, and serine proteases, which play a crucial role in the venom's effects on the human body. While many biological activities of snake venom, including anti-viral, have been demonstrated, its potential SARS-CoV-2 inhibitory effects have not been studied [4]–[6].

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has had a significant global impact since its emergence in late 2019. Globally, there have been 767.750.853 confirmed cases of COVID-19, including 6.941.095 deaths, reported to the World Health Organization (WHO) to date [7].

Angiotensin-converting enzyme 2 (ACE2) serves as the entry receptor for the SARS-CoV-2 virus, allowing it to enter and infect human cells. The spike protein on the surface of SARS-CoV-2 binds to ACE2 receptors on host cells, facilitating viral entry, particularly in the respiratory system [8]. ACE2 is widely expressed in various tissues, including the respiratory tract, lungs, heart, kidneys, gastrointestinal tract, and blood vessels. Its presence in the respiratory epithelium makes the lungs a primary target for SARS-CoV-2 infection. Considering the crucial role of ACE2 in the progression of COVID-19, researchers have been actively investigating potential therapeutic strategies that specifically target ACE2 [9]. These strategies encompass various approaches, such as modulating ACE2 expression, enhancing its activity, or developing molecules that can compete with SARS-CoV-2 for binding to ACE2 receptors.

The 3-chymotrypsin-like protease (Mpro), also known as the main protease, is a cysteine protease that plays a vital role in the replication of coronaviruses, including SARS-CoV-2. It plays a crucial role in processing viral replicase polyproteins into functional non-structural proteins (NSPs), which are essential for viral replication and transcription [10]. 3CLpro has been identified as a promising target for the development of antiviral drugs against coronaviruses. Inhibiting 3CLpro activity can disrupt viral replication and

potentially halt the spread of the virus. Researchers have been actively studying the structure and function of 3CLpro to identify potential inhibitors. Structure-based drug design approaches, such as virtual screening and molecular docking, have been utilized to identify small molecules that can bind to and inhibit 3CLpro activity [11].

Molecular docking is considered a valuable tool in the early stages of drug discovery, as it provides information about the potential binding modes of small molecules with the target protein. It assists researchers in the selection and optimization of drug candidates, guiding further development and experimental testing to identify potential therapeutics for a variety of diseases [9].

In the current study, the research focused on examining the interactions between venom peptides derived from *E. carinatus* (ECVPs), namely Schistatin (SCH), Phospholipase A2 (PLA2), Disintegrin (DS), and Echistatin (ECH), with ACE2 and Mpro receptors. The objective was to investigate the potential of these peptides as anti-viral molecules targeting SARS-CoV-2.

Material and Methods

Receptor preparation

The receptor proteins chosen for this study were the SARS-CoV-2 main protease 3CLpro with the PDB ID 6LU7 and ACE2 with the PDB ID 1R42. The three-dimensional (3D) structures of Mpro and ACE2 were downloaded from the Protein Data Bank (PDB) using the PDB format (https://www.rcsb.org/). Preprocessing steps, including load distribution, hydrogenation, and removal of water molecules, were performed using the PyMOL software. Hydrogen atoms were added to the receptor molecule using the MG Tools plugin in the AutoDock Vina program. The final structures were saved in PDB format for further analysis.

Ligand preparation

The three-dimensional structures of the identified snake venom proteins, including SCH (PDB ID: 1RMR), PLA2 (PDB ID: 1OZ6), DS (PDB ID: 1Z1X), and ECH (PDB ID: 2ECH)

were retrieved in PDB format from PubChem. The ligand structures underwent water removal, hydrogenation, and load distribution adjustments using AutoDock Vina 4.2.5.1 software.

Molecular docking

AutoDock Vina was employed for high-throughput molecular docking. The grid center for Mpro was set at X=21.41, Y=3.62, and Z=21.94, with a grid box size of 60 Å×60 Å×60 Å. For ACE2, the grid center was set at X=19.81, Y=-5.57, and Z=14.73, with the same grid box size. These parameters were used for all 4 proteins. Multiple docked conformations were generated, and the setup was optimized and calibrated accordingly. The secondary structures of the docked molecules were visualized using PyMOL for further analysis.

Results

Docking of Mpro protease with ECVPs

Molecular docking scores of SCH, PLA2, ECH, and DS with Mpro were- 92.998, -116.513, -27.197 and -81.601 Kcal/mol, respectively (Table 1). Among ECVPs, PLA2 showed the best binding affinity (-116.513 Kcal/mol) with Mpro as compared to RTV with the docking score of-73.550 Kcal/mol. SCH and DS showed similar binding affinities with Mpro with docking scores of-92.998 and-81.601 Kcal/mol, respectively. The lowest docking score was ECH with-27.197 Kcal/mol (Table 1).

PLA2 also formed hydrogen bonds with His48, and Asp49, and steric interactions with Leu106, Tyr52, and Asp49 (Figures 1A and B). SCH formed hydrogen bonds with Gly43, Asn46, and steric interactions with Ala41, Asp44, Asn46, Asp48 residues while interacting with MPro (Figures 2A and B). DS formed steric interactions with Arg59, Asp48, Lys39, Ala41, Leu46 residues (Figures 3A and B). ECH formed only hydrogen bonds with Arg24, Ala23, Arg22, Asp29, and steric interactions with Arg22 and Arg24 with MPro (Figures 4A and B).

Docking of ACE2 receptor with ECVPs

Molecular docking scores of SCH, PLA2, ECH, and DS with ACE2 were -58.260, -78.067, -39.800 and -61.540 Kcal/mol, respectively (Table 1). PLA2 showed a higher docking score (-78.067 Kcal/mol) than RTV (-108.731 Kcal/mol), indicating a better binding than the reference drug. DS also showed a similar binding energy with RTV with a docking score of -61.540 Kcal/mol. Although the binding energies of SCH and ECH were lower than the reference drug, they were favorable and were -58.260 and -39.800 Kcal/mol, respectively.

Table 1 Molecular docking analysis of the interaction of ECVPs with ACE2 and MPro.

	ACE2			Mpro		
	Docking Score (Kcal/mol)	**Honda**	**Steric interactions**	**Docking Score (Kcal/mol)**	**Hbonds**	**Steric interactions**
Schistatin (SCH)	-92.998	Gly43, Asn46	Ala41, Asp44, Asn46, Asp48	-58.260	Lys39, Asp48, Asn46, Asn63	Gln47, Ala41, Arg59
Phospholipase A2 (PLA2)	-116.513	His48, Asp49	Leu106, Tyr52, Asp49	-78.067	Gly30, Cys29	His48, Cys45, Cys44, Cys29
Echistatin (ECH)	-27.197	Arg24, Ala23, Arg22, Asp29	Arg22, Arg24	-39.800	Arg22, Arg24, Asp29	Arg24, Ala23
Disintegrin (DS)	-81.601	-	Arg59, Asp48, Lys39, Ala41, Leu46	-61.540	Lys39, Leu46, Asp48, Asn63	Ala41, His47
Ritonavir (RTV)	-73.550	Asn98, Ile3, Thr96, Pro1	-	-108.731	Gln2	Asn98, Ile3, Thr96,

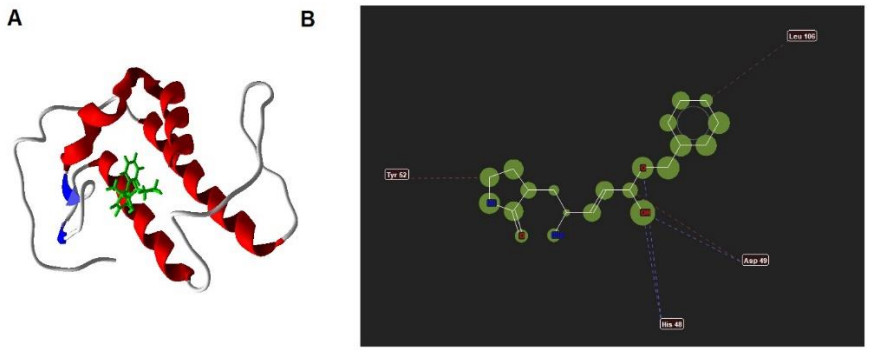

Fig 1 (A) 3D-Molecular docking images of PLA2 and MPro. (B) Amino acid residues with which PLA2 interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

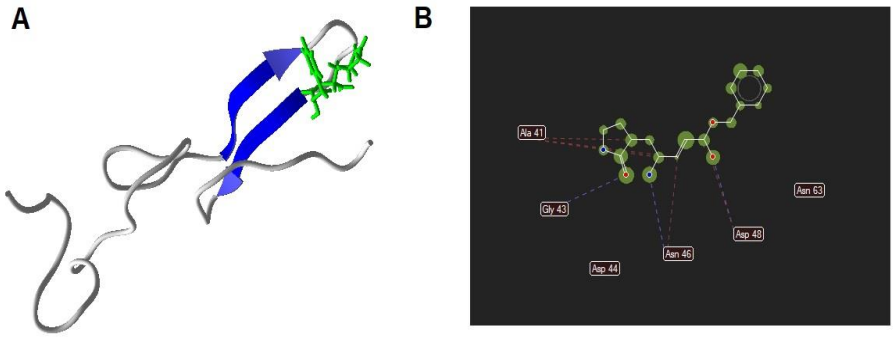

Fig 2 (A) 3D-Molecular docking images of SCH and MPro. (B) Amino acid residues with which SC interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

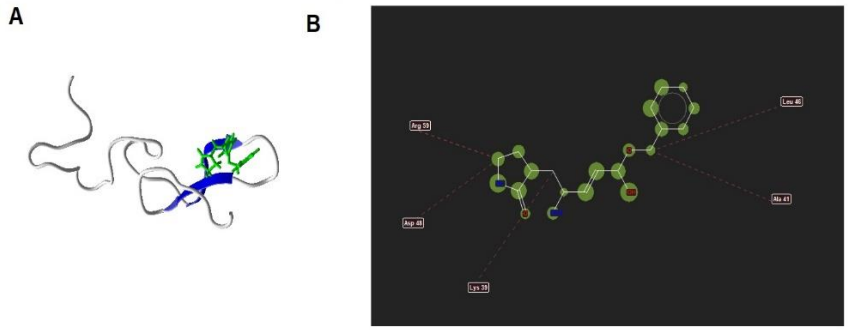

Fig 3 (A) 3D-Molecular docking images of DS and MPro. (B) Amino acid residues with which DS interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

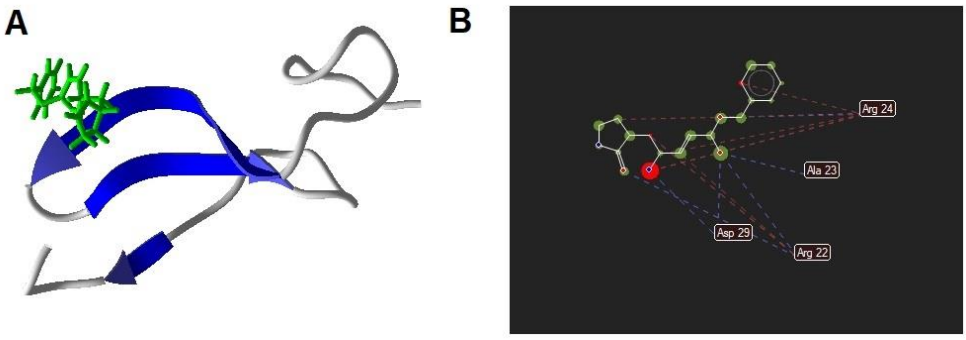

Fig 4 (A) 3D-Molecular docking images of ECH and MPro. (B) Amino acid residues with which ECH interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

PLA2 formed hydrogen bonds with Gly30, and Cys29 residues and steric interactions with His48, Cys45, Cys44, Cys29 residues while interacting with ACE2 (Figures 5A and B). It was the molecule with the lowest binding energy due to its high steric interactions. DS formed hydrogen bonds with Lys39, Leu46, Asp48, and Asn63 residues and steric

interactions with Ala41 and His47residues (Figures 6A and B). The number of hydrogen bonds formed by SCH was high and these bonds were formed with Lys39, Asp48, Asn46, and Asn63 residues. SCH also formed steric interactions with Gln47, Ala41, and Arg59 residues (Figures 7A and B). ECH formed hydrogen bonds with Arg22, Arg24, Asp29 and steric interactions with Arg22, Arg24 and Asp29 residues while interacting with ACE2 (Figures 8A and B).

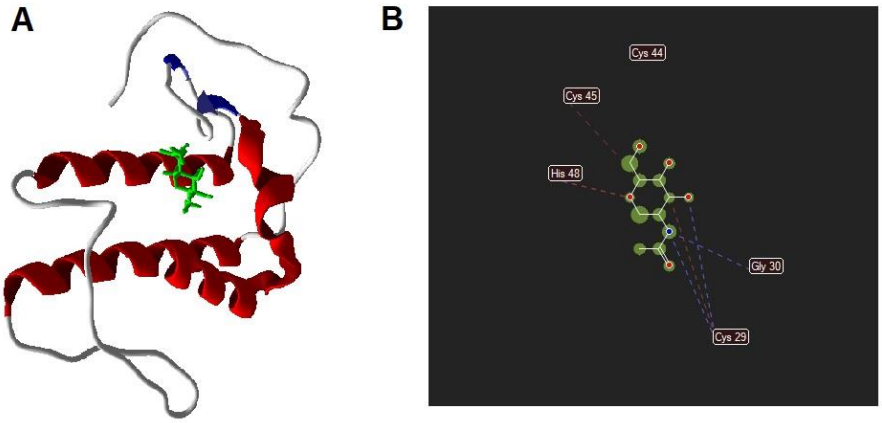

Fig 5 (A) 3D-Molecular docking images of PLA2 and ACE2. (B) Amino acid residues with which PLA2 interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

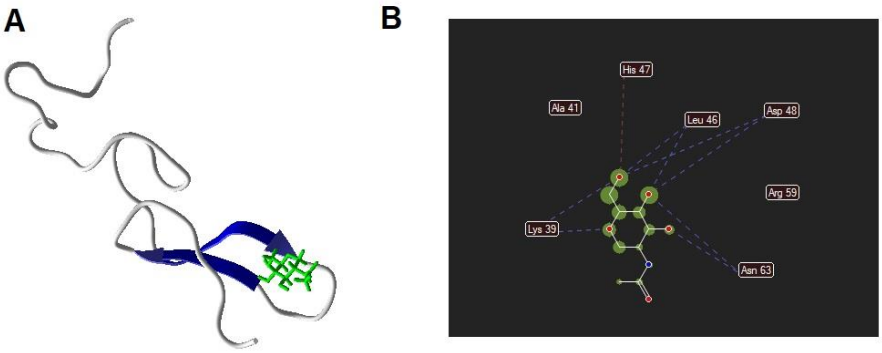

Fig 6 (A) 3D-Molecular docking images of DS and ACE2. (B) Amino acid residues with which DS interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

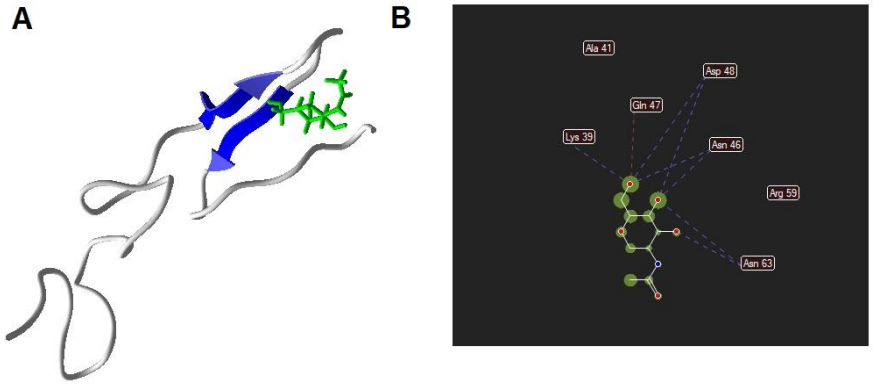

Fig 7 (A) 3D-Molecular docking images of SCH and ACE2. (B) Amino acid residues with which SCH interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

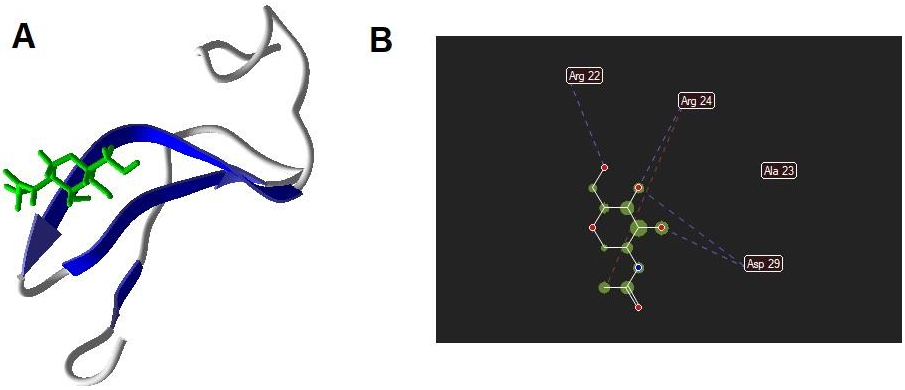

Fig 8 (A) 3D-Molecular docking images of ECH and ACE2. (B) Amino acid residues with which ECH interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

Discussion

Many studies use molecular docking to screen large compound libraries and identify potential SARS-CoV-2 inhibitors [9]–[11]. These studies usually include the virtual screening of databases of small molecules, in which compounds with the best insertion scores and binding interactions are selected as potential inhibitors, and these detected compounds are then subjected to experimental assays to confirm their inhibitory activities.

The majority of these screenings are for natural products. Natural products are considered the best drug candidates because of their wide variety, abundance in nature, and less toxicity [12]. Snake venom proteins have gained attention as potential drug candidates due to their diverse biological activities and unique molecular structures. Several

components of snake venom have been investigated for their therapeutic potential in various areas of medicine, including pain management, cardiovascular disorders, cancer, and neurological conditions [13]–[15]. Tirofiban is an antiplatelet drug that received approval from the US Food and Drug Administration (FDA) in 1998 and from the European Medicines Agency (EMA) in 1999. Tirofiban is derived from echistatin, a disintegrin toxin found in *E. carinatus* venom, and is used in the treatment of acute coronary syndrome [16]. It blocks platelet aggregation via bonding with the fibrinogen receptor. Overall, snake venom proteins represent a fascinating source of bioactive molecules that offer potential therapeutic applications. Continued research and exploration in this field may uncover novel drug candidates and contribute to the development of new treatments for various diseases such as COVID-19.

The antiviral proteolytic fraction was isolated from the venom of *E. carinatus sochureki* and investigated for its activity against the Sendai virus [4]. However, the potential effects of ECVPs on SARS-Cov-2 have not been studied. In the current study, we used the molecular docking technique to determine the possible interactions between ECVPs and Mpro and also the ACE2 receptor. Among the tested ECVPs, PLA2 had the most negative value, indicating the best binding affinity with ACE2 than the reference drug RTV. PLA2 can be isolated from *E. carinatus* venom by chromatography and electrophoresis combination [17]. In the literature, PLA2s obtained from different snakes have been characterized and their antiviral activities have been demonstrated [4], [18]. However, the antiviral activity of PLA2 from *E. carinatus* has not been studied. In this study, it was determined that the PLA2 contained in *E. carinatus* may show better activity than the reference drug. SCH, ECH, and DS showed similar binding affinities with RTV. SCH is a disintegrin homodimer with 64 amino acids. Observations indicate that disintegrins, acting as antagonists, disrupt the functioning of integrins, thereby serving as agents with anticancer and antithrombotic properties. Some of the disintegrins obtained from snake venoms have been shown to have antiviral activity, but there is no study yet on SCH [19]. SCH showed -92.998 and -58.260 Kcal/mol binding scores with ACE2 and MPro, respectively, indicating a favorable interaction with both receptors. ECH is a 49 amino acid protein, a potent inhibitor of platelet aggregation [20]. There is no study in the literature investigating the antiviral activity of ECH. Thus, the present study was the first to investigate the potential anti-Sars-Cov2 activity of ECH.

Conclusion

The findings of this study highlight the potential of snake venom proteins, specifically PLA2 SCH, DS and ECH from *E. carinatus* venom, as promising candidates for the development of antiviral agents against SARS-CoV-2. Through molecular docking analysis, these venom proteins demonstrated favorable binding affinities with key targets involved in SARS-CoV-2 infection. Further experimental investigations are needed to validate their antiviral activities and evaluate their efficacy in combating COVID-19. The exploration of snake venom proteins as a source of bioactive molecules offers a fascinating avenue for the discovery of novel therapeutic options against viral infections and may contribute to the development of effective treatments for diseases such as COVID-19.

Abbreviations
SARS CoV 2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; SCH: Schistatin; PLA2: Phospholipase A2; DS: Disintegrin; ECH: Echistatin; Mpro: Main protease; RTV: Ritonavir; WHO: World Health Organization; ACE2: Angiotensin-converting enzyme 2; NSPs: Non-structural proteins; ECVPs: *Echis carinatus* venom peptides; 3D: three-dimensional; PDB: Protein Data Bank; FDA: US Food and Drug Administration; EMA: European Medicines Agency

Acknowledgements
Funding
The authors did not receive support from any organization for the submitted work.

Data Availability statement
The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
All authors contributed equally to the study.

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Research Article

Aksu D.A., Memon A., Identification of Drought-Responsive Genes in Turkish Bread Wheat (*T. aestivum* L.) Cultivar Gerek 79 by mRNA Differential Display Analysis. International Journal of Life Sciences and Biotechnology, 2023. 6(3): p. 322-334. DOI: 10.38001/ijlsb.1302905

Identification of Drought-Responsive Genes in Turkish Bread Wheat (*T. aestivum* L.) Cultivar Gerek 79 by mRNA Differential Display Analysis

Diğdem Aktopraklıgil Aksu[1*], Abdulrezzak Memon[2]

ABSTRACT

Wheat is one of the most important cereal crops in the world. Many parts of the world depend on wheat as a source of food and animal feed. Drought stress negatively affects its development and greatly reduces its production. Drought response is a complex genetic mechanism involving multiple genes, transcription factors, miRNAs, hormones, proteins, co-factors, ions, and metabolites. Understanding the genetic basis of the drought tolerance mechanisms is very important for genetic improvement of this trait in wheat. Wheat is also an important cereal crop in Turkey. In this study, it is aimed to identify drought stress-regulated genes in bread wheat (Triticum aestivum cv. Gerek 79) and gene expression profiling using mRNA differential display (mRNA DD) was performed for seedling leaves of control and drought-stressed plants (62.4% of RWC). The comparative profiling study showed a total of 20 differentially- expressed cDNA bands and 10 of them were cloned and sequenced. The inserts with poor quality reads were eliminated. The nucleotide sequences of the remaining two cDNAs named G1 and G2 were subjected to similarity analysis. G1 and G2 showed a high degree of homology the mRNA of purple acid phosphatase and glycosyltransferase family 92 protein-like sequences of Triticum aestivum and some other plants, respectively. Purple acid phosphatases have been shown to be involved in plant responses to abiotic and biotic stresses. Similarly, the role of glycosyltransferases in thermotolerance has been reported in rice besides their functions in cellular homeostasis and detoxification pathways in plants. These reports and our findings have laid a foundation for further investigation of G1 and G2 cDNA clones. The investigation of differential expression of these gene fragments corresponding to purple acid phosphatase and glycosyltransferase family 92 protein-like sequences under drought conditions at the RNA level is ongoing. Further characterization of these genes could be important in understanding the functions of these gene/s in drought response.

ARTICLE HISTORY
Received
26 May 2023
Accepted
11 September 2023

KEYWORDS
Wheat,
drought,
mRNA differential display,
gene expression

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. Many parts of the world depend on wheat as a source of food and animal feed. Drought is a major environmental stress that negatively affects its development and greatly reduces its production [1]. The development of wheat varieties with improved drought resistance can help ensure food security and improve the sustainability of agricultural systems. Plants respond to drought stress at the morphological, physiological and molecular levels. The developmental stage, age, plant genotype, severity and duration of

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drought influence their responses [2]. Molecular response to drought stress is very complex [3]. Numerous genes, transcription factors, microRNAs, metabolites, stress hormones, co-factors and drought-related proteins [4] are involved in this response. A large number of genes induced during water deficit function in the stress signaling pathways and the protection of cells from stress by production of functional proteins such as aquaporins and osmoprotectants. Although many genes and signaling pathways involved in drought response have been identified, the complex gene network that mediates plant responses to drought stress remains largely unclear. Therefore, understanding the molecular mechanisms of drought stress tolerance is very important for genetic improvement of this trait. The identification of drought-responsive genes and understanding of their functions in stress adaptation provide insight into the molecular mechanisms underlying the plant's response to drought stress. So far, hundreds of genes involved in the key processes of the plant response to drought have been identified using different genetic and genomic techniques [5]. These genes play crucial roles by strictly controlling the physiological and biochemical responses to stress during a water deficit. Different gene expression technologies such as differential display reverse transcription-polymerase chain reaction (DDRT-PCR), SAGE, SSH, cDNA-AFLP, microarray and RNAseq have been employed to study stress-related genes in many plant species [6-10].

mRNA Differential Display (DD) technique is a powerful technique for the identification and isolation of differentially expressed eukaryotic genes [11]. Several abiotic and biotic stress-related genes have been identified in plants using this genome-wide transcript profiling technique. For example, the stress responsive *psb A* gene was isolated from rice (*Oryza sativa* L.) using DD [12]. In a study performed by [13], a desiccation-responsive small GTP-binding protein (Rab2) was characterized from the desiccation-tolerant grass *Sporobolus stapfianus* using RT-PCR product identified with DD. A cDNA clone, *Bnuc1*, encoding a nuclease gene was isolated from salt-stressed barley leaves by using the DD technique [14]. The different members of a heat shock protein gene family, HSPI 6.9 were identified in wheat using a modified version of DD [15]. The DD technique was successfully used for detecting transcriptome changes in durum wheat upon exposure to Cd and identified *NADH dehydrogenase subunit 1* and *PsaC* genes [16]. Several transcripts differentially induced by exogenous ABA

treatment were identified in bread wheat using DD profiling [17].

The present study aimed to investigate the genetic response to drought tolerance in bread wheat. The differentially expressed genes upon drought stress were profiled on a whole-genome scale using mRNA differential display technique in bread wheat (*Triticum aestivum* cv. Gerek 79). Here we report some expressed sequence tags (ESTs) that were found to be activated in response to drought stress in the bread wheat cultivar Gerek 79.

Materials and Methods

Plant materials and stress treatment

Gerek 79 (moderately drought resistant) wheat variety was obtained from the Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, Eskişehir and used as plant material. Seeds were washed under running tap water and initially surface sterilized with 70% (v/v) ethanol for 1-2 min followed by treatment with 10% commercial bleach (sodium hypochlorate) containing a few drops of Tween-20 for 15 min. After that, the seeds were thoroughly washed with sterilized water at least six times. The sterilized seeds were then placed between wet sterile filter papers at room temperature for 3 days under dark conditions for germination. Seedlings were transferred to plastic pots with a top diameter of 18 cm and height of 20 cm containing soil/sand mixture (flower soil:garden soil:sand; 1.5:1.5:1). They were grown under white fluorescent light, 108- 135 μmol m^{-2} s^{-1} at 25ºC with 65% relative humidity by watering daily with tap water for 30 days until the pre-flowering stage. When they reached the pre-flowering stage, they were divided into two groups; one treated with drought-stress and one without drought stress as a control. Drought stress was given by completely withholding irrigation until they reached the desired relative water content (RWC) value. The RWC was measured in the daytime using the flag leaf and calculated as follows;

RWC (%) = [{(FW – DW)/ (TW – DW)}] ×100, where FW is the fresh weight of the leaves, TW is the weight at full turgor and DW is the dry weight of the leaves [18].

Plant leaf samples (control and drought-stressed) were harvested and immediately plunged in liquid nitrogen and stored at -80˚C for RNA isolation. Three replicates were performed for control and drought-stressed plants.

Total RNA isolation and DNase I digestion

Total RNA extraction from the collected leaf samples was performed using the hot phenol method [19]. Genomic DNA contamination was removed by digesting total RNA samples with 10 units of DNase I enzyme using the MessageClean Kit (GenHunter Corporation, Nashville, TN, USA) following the manufacturer's instructions. Digested samples were extracted with phenol:chloroform (3:1) and ethanol precipitated. RNA pellets were dissolved in DEPC- treated ddH_2O. The quantity of RNA samples was measured using a NanoDrop1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Extracted RNAs were stored at -80°C until use.

mRNA differential display analysis

Single-stranded cDNA synthesis was performed using RNAImage Kit (GenHunter Corporation, Nashville, TN, USA) according to the manufacturer's instructions. For each RNA sample, three reverse transcription reactions were performed with three different H-T11M primers (M= G, C or A). The template RNA (0.2 µg/reaction) was added to tubes containing 4 µl of 5X reverse transcription buffer, 1.6 µl of 25 µM dNTPs, 2 µl of2 µM H-T11M primer, and 9.4 µl of ddH_2O. Reactions were incubated at 65^{o}C for 5 min and 37^{o}C for 10 min. After that, 1 µl of MMLV reverse transcriptase (100 u/µl) was added to each tube and incubated at 37^{o}C for 20 min and 75^{o}C for 5 min. The reactions were stored on ice or -20^{o}C until use.

Differential display (DD) PCR amplification was performed on single-stranded cDNA samples using H-T11M primer (M= G, C or A) in combination with the arbitrary H-AP1-8 and H-AP17-24 primers from RNAimage Kits (GenHunter Corporation, Nashville, TN, USA) following the manufacturer's instructions. Briefly, one-tenth of single-stranded cDNAs were subjected to PCR in a 20 µl reaction mixture composed of 10 µl ddH_2O, 2 µl of 10X PCR Buffer (100 mM Tris.HCl, pH 8.4; 500 mM KCl; 15 mM $MgCl_2$ and 0.01% (w/v) gelatine), 1.6 µl of dNTPs (25 µM), 2 µl of H-AP primer (2 µM), 2 µl of H-T11M primer (2 µM, same one used in the RT-PCR), 0.2 µl of α-(^{33}P)dATP (2000 Ci/mmole) and 0.2 µl of Taq DNA polymerase (5 u/µl) under the following cycle conditions: 40 cycles of 94°C for 30 sec, 40°C for 2 min, 72°C for 30 sec for amplification, followed by 72°C for 5 min for final extension. The reactions were stopped by adding 3.5 µl of stop solution (95% formamide; 10 mM EDTA, pH 8.0; 0.09% (w/v) xylene cyanol FF; 0.09% (w/v) bromophenol blue). Prior to loading,

PCR products were denatured at 80°C for 2 min and separated on 6% denaturing DNA sequencing gels. After electrophoresis, DNA gels were dried and exposed to X-ray film at -80°C for 2-3 days. The radioactively-labeled cDNA bands were visualized on X-ray films after exposure. Changes in cDNA bands (differential expression) between control and drought-stressed samples were detected for each set of primers. The autoradiograph was properly aligned with the gel and the differentially expressed bands were excised from the gel. Elution of cDNA fragments from gel slices was carried out with 100 µl ddH_2O by incubating at 100°C for 15 min. The gel debris was removed by centrifugation. The cDNA fragments were ethanol precipitated and then resuspended in 10 µl ddH_2O. 4 µl of cDNA fragments were reamplified by PCR with the same primer combinations, in the absence of radiolabeled nucleotides, under the same conditions used for the initial reaction. The reamplified products were electrophoresed on a 1.5% (w/v) agarose gel and visualized under a UV transilluminator.

Cloning, sequencing and homology analysis of differentially expressed cDNAs

The reamplified bands were directly subcloned into the PCR-TRAP vector using PCR-TRAP Cloning System (GenHunter Corporation, Nashville, TN, USA) as instructed. Colony-PCR was performed with Lgh and Rgh primers to verify the presence of inserts. The PCR products were electrophoresed on a 1.5% (w/v) agarose gel. Plasmid DNAs were isolated from overnight bacterial cultures of positive clones using alkaline lysis method [20]. Sequencing of cloned inserts was performed on a CEQ8800 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA, USA) using vector-specific primers. Alignment of insert sequences was performed by BioEdit software [21]. The homology search was performed using the Basic Local Alignment Search Tool (BLAST) [22] provided by the National Centre of Biotechnology Information (NCBI) at National Institutes of Health (NIH) (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

In this study, it is aimed at identifying drought stress-related genes in wheat by using mRNA DD. The seedlings were grown as mentioned above until the pre-flowering stage. When they reached the pre-flowering stage they were divided into two groups; one group served as a control and the other group was subjected to drought-stress by withholding irrigation until plants reached 62.4% of RWC (Figure 1).

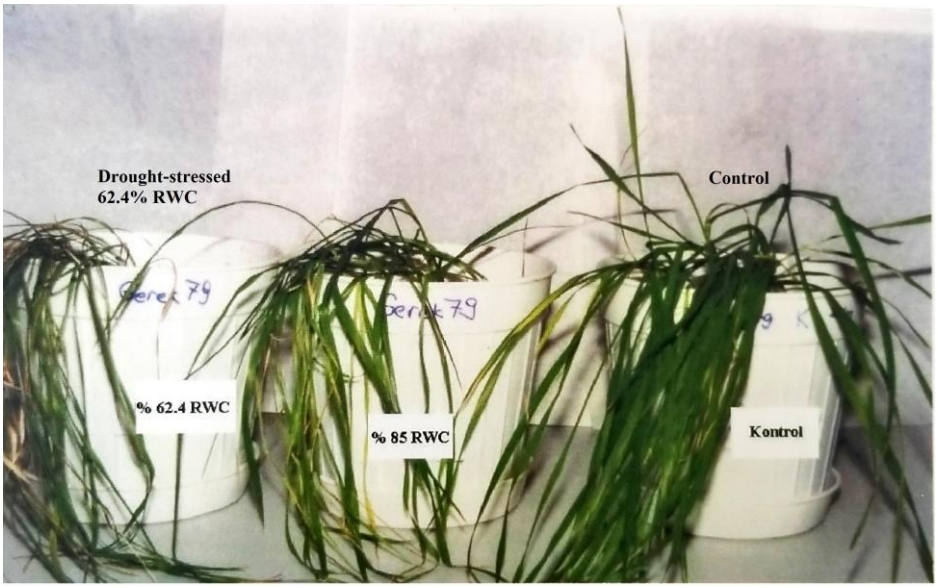

Fig 1 Control and drought-stressed (62.4% RWC) Gerek 79 plants

mRNA DD analysis was performed to analyze changes in gene expression between control and drought-stressed leaf samples and identify differentially expressed transcripts under drought conditions. In total, 48 different combinations of anchored and arbitrary primers were used in the mRNA DD analysis (Table 1).

A total of 20 bands were identified to be differentially expressed (unique and/or upregulated in stress condition) between control and stressed samples and excised from the gels. All of them were reamplified. The randomly selected 10 bands were cloned into the PCR-TRAP vector and sequenced. Eight of the cloned inserts were not sequenced well and subjected to similarity analysis. The nucleotide sequences of the remaining two differentially expressed cDNAs named G1 and G2 were subjected to similarity analysis. The reamplified G1 and G2 fragments with sizes of 324 and 268 bp, respectively separated on 1.5% agarose gel are shown in Figure 2. The DD-RT- PCR gel and DDRT-PCR autoradiographies showing G1 and G2 fragments are given in Figure 3 and Figure 4, respectively. In BLAST analysis, G1 and G2 showed a high degree of homology to the mRNA sequence of purple acid phosphatase and glycosyltransferase family 92 protein-like sequence of *Triticum aestivum* and some other plants, respectively.

Purple acid phosphatases (PAPs) belong to the metallophosphatase superfamily of proteins. They hydrolyze phosphate esters and anhydrides under optimal acidic

conditions [24]. Besides plants, PAPs have also been found in fungi, bacteria, unicellular eukaryotes and mammals [25].

Table 1 Primers used in mRNA differential display

Anchored Primers	
Primer	**Sequence (5′–3′)**
H-T11A	AAGCTTTTTTTTTTTA
H-T11G	AAGCTTTTTTTTTTG
H-T11C	AAGCTTTTTTTTTTC
Arbitrary Primers	
Primer	**Sequence (5′–3′)**
H-AP1	AAGCTTGATTGCC
H-AP2	AAGCTTCGACTGT
H-AP3	AAGCTTTGGTCAG
H-AP4	AAGCTTCTCAACG
H-AP5	AAGCTTAGTAGGC
H-AP6	AAGCTTGCACCAT
H-AP7	AAGCTTAACGAGG
H-AP8	AAGCTTTTACCGC
H-AP17	AAGCTTACCAGGT
H-AP18	AAGCTTAGAGGCA
H-AP19	AAGCTTATCGCTC
H-AP20	AAGCTTGTTGTGC
H-AP21	AAGCTTTCTCTGG
H-AP22	AAGCTTTTGATCC
H-AP23	AAGCTTGGCTATG
H-AP24	AAGCTTCACTAGC

PAPs exist as a multigene family in many plants such as Arabidopsis (29 *AtPAPs*), rice (26 *OsPAPs*) and tomato (25 *SlPAP*s) [26-28]. PAPs are mainly involved in Pi homeostasis but they also have roles in root growth modulation, carbon metabolism, symbiotic association, reproductive development and response to environmental stresses [29-35].

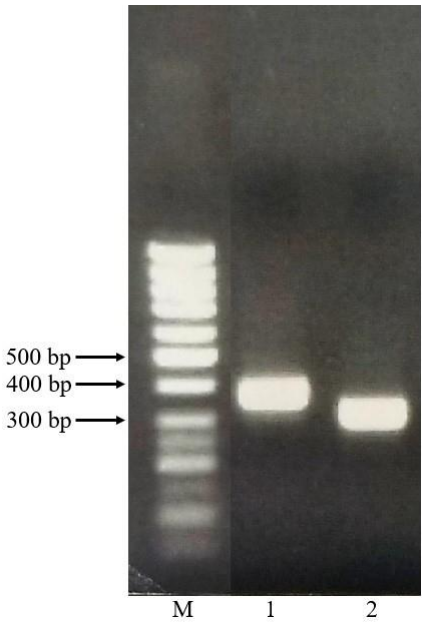

Fig 2 Agarose gel electrophoresis of reamplified G1 and G2 fragments M: GeneRuler 50 bp DNA Ladder (Thermo Fisher Scientific). Lane 1 and Lane 2 represent reamplified G1 and G2 fragments, respectively

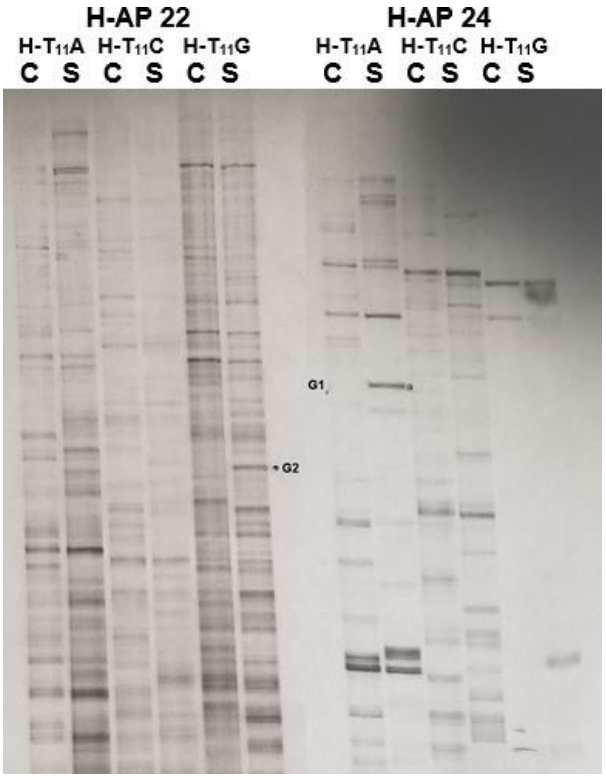

Fig 3 DD-RT-PCR gel showing G1 and G2 fragments C and S indicate cDNA band patterns from untreated and drought-stressed (62.4% RWC) plantsamples, respectively

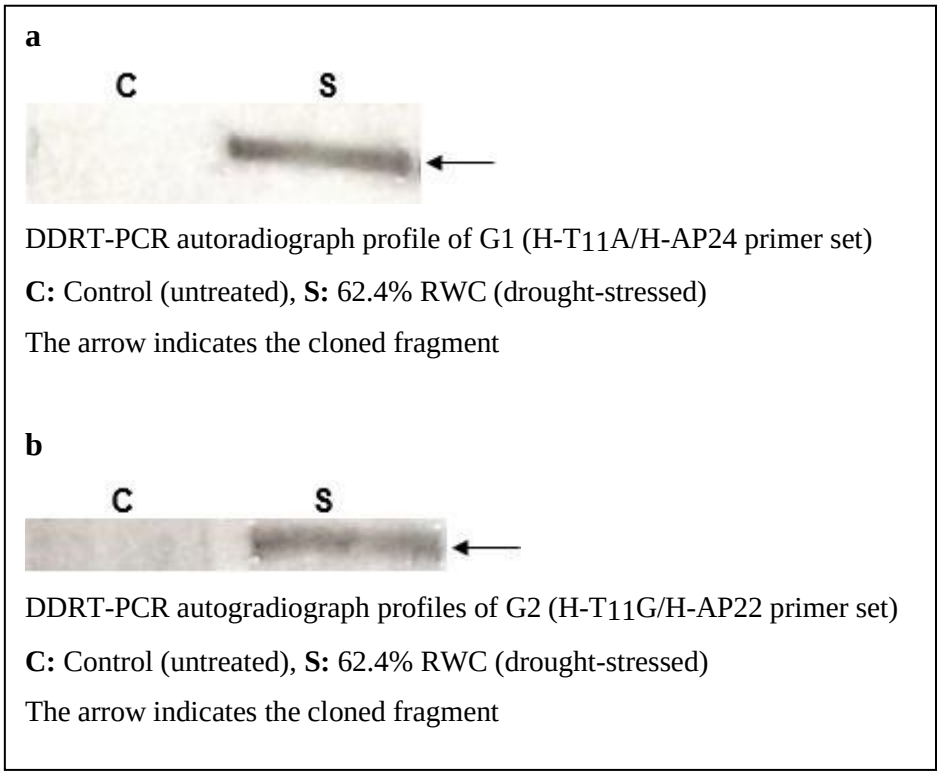

Fig 4 DDRT-PCR autogradiograph profiles of **(a)** G1 (H-T11A/H-AP24 primer set) and **(b)** G2 (H- T11G/H-AP22 primer set)

Although they are ubiquitously present in plants, a comprehensive dissection of their roles is still elusive. It was reported that the overexpressing AtPAP15 enhances salt and osmotic stress tolerance and reduces the phytate content in the leaves of Arabidopsis [34]. Recently, functional characterization of the AtPAP17 and AtPAP26 genes has shown their roles in salt tolerance [36]. It was shown that PgPAP18 gene, a heat-inducible novel purple acid phosphatase 18-like gene isolated from pearl millet, plays a defensive role against abiotic stresses such as heat and salt [37]. In another study, GmPAP3 gene from soybean has been shown to be induced by NaCl stress and oxidative stress [38]. As far as we know, the induction of the PAP gene upon dehydration in bread wheat has not been reported before. The reports showing plants PAPs' roles under environmental stress conditions and our findings suggest that the gene fragment corresponding to PAP could play an important role in wheat subjected to drought stress.

The glycosyltransferases (GTs; EC 2.4.x.y) catalyze the transfer of sugar moieties to acceptor molecules such as sugars, nucleic acids and lipids. Plants have an important

and functionally diverse family of GTs. Their major roles are the biosynthesis of cell wall polysaccharides and glycoproteins. They are also important for cellular homeostasis and detoxification of mycotoxins and xenobiotics [40].

The role of glycosyltransferase in thermotolerance in some cereals has been shown before. 1,704 candidate genes (CGs) identified by meta-QTL analysis in wheat were subjected to in silico expression analysis. The analysis results identified 182 differentially expressed genes, which included 36 CGs with known functions previously reported to be important for thermotolerance such as UDP-glucosyltransferase, zinc finger transcriptional factor and K homology domain etc. [41]. Dong et al. (2020) characterized GSA1, which encodes a UDP-glucosyltransferase and redirects the metabolic flux between branches of the phenylpropanoid pathway under abiotic stress in rice. This results in the accumulation of flavonoid glycosides, which protect rice against abiotic stress [42]. To our knowledge, there is no study showing the direct involvement of *Triticum aestivum* glycosyltransferase family 92 protein in drought stress response. Our findings suggest that the gene fragments corresponding to purple acid phosphatase and glycosyltransferase family 92 protein-like sequences could play an important role in plants subjected to drought stress. These results have laid the foundation for further investigation of these clones. The investigation of the differential expression of these putative genes under drought conditions at the RNA level is ongoing. Further characterization of these genes could be important in understanding the functions of these gene/s in drought response. They may be used as candidate genes in molecular breeding approaches to improve drought tolerance of wheat.

Conclusion

Understanding the genetic basis of drought tolerance in wheat is very important for genetic improvement through breeding. mRNA differential display was used to identify candidate drought responsive genes in bread wheat (*Triticum aestivum* cv. Gerek 79). It was found that two clones named G1 and G2 showed a high level of sequence homology to the purple acid phosphatase and glycosyltransferase family 92 protein-like sequences of *Triticum aestivum* and some other plants, respectively. These clones are likely to be related to drought stress responses. Further characterization of these genes could be important in understanding their function in drought response.

Abbreviations
BLAST: Basic local alignment search tool; DDRT-PCR: Differential display reverse transcriptase polymerase chain reaction; DW: Dry weight; EST: Expressed sequence tag; FW: Fresh weight; NIH: National Institutes of Health; RWC: Relative water content; TW: Turgor weight.

Acknowledgements
The authors would like to thank the Scientific and Technological Research Council of Turkey (TUBITAK) and the Istanbul University Scientific Research Project Foundation for funding. The authors would also like to thank Ömer Kaçar for his technical assistance in this study.

Funding
This work is fully funded by the Scientific and Technological Research Council of Turkey (TUBITAK) (Project no. TUBITAK TOGTAG TARP/2463) and partially funded by the Istanbul University Scientific Research Project Foundation (Project No. T-84/11112002).

Data availability statement
The author confirms that the data supporting the findings of this study are included within the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
In this study, the laboratory experiments were conducted by Diğdem Aktopraklıgil Aksu. Prof. Dr. Abdulrezzak Memon received funding from TUBITAK and supervised her work.

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Derleme makale / Review makale

Can, B. and A. Gurel, Nanopartiküllerin Bitki Sistemlerinde ve Bitki Doku Kültürlerinde Uygulamalarına Yönelik Genel Bir Bakış. International Journal of Life Sciences and Biotechnology, 2023 6(3): p. 335-370.
DOI: 10.38001/ijlsb.1293031

Nanopartiküllerin Bitki Sistemlerinde ve Bitki Doku Kültürlerinde Uygulamalarına Yönelik Genel Bir Bakış

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ÖZET

Dünya nüfusunun hızla artmasıyla birlikte, bitkiye ve bitkisel materyallere duyulan ihtiyaç da artma göstermiştir. Bitki biyoteknolojisi, bu ihtiyaçların karşılanması için iyi bir alternatiftir. Bitki biyoteknolojisinin en önemli kısmını oluşturan bitki doku kültürleri, farklı amaçlara yönelik birçok tekniği içermektedir. Bitki doku kültürü teknikleri; mikroçoğaltım, genetik manipülasyon, biyoaktif bileşik üretimi ve bitki gelişimi vb. alanlarda bitki biyolojisinin temel yapıtaşlarından biri olarak kabul edilmektedir. Nanoteknoloji, nano boyutlu yeni malzemelerin (nanomalzeme) üretimi, bunların tasarımını ve uygulamasını ele alan multidisipliner bir bilim dalıdır ve temelini nanopartiküller oluşturmaktadır. Nanopartiküllerin, bitki sistemlerinde ve bitki doku kültürlerindeki uygulamalarının bitki büyüme ve gelişme fizyolojisi üzerinde çeşitli etkileri mevcuttur. Bu alanlarda en çok çalışılan nanopartiküller; sırasıyla metal/metal oksit bazlılar, karbon bazlılar, kuantum noktaları, silikon ve polimerik nanopartiküllerdir. Bitki sistemlerinde nanopartiküllerin kullanıldığı çalışmalar incelendiğinde; tohum çimlenmesi, bitki büyümesi ve verim, sürgün rejenerasyonu, kök/sürgün uzunluğu ve biyokütle artışı gibi parametrelerde olumlu sonuçlar alındığı, fizyolojik/biyokimyasal aktiviteler açısından da indükleyici etkilerin belirlendiği raporlanmıştır. Ayrıca genetik modifikasyonun sağlanması, biyoaktif bileşiklerin üretiminin iyileştirilmesi, bitki korumanın sağlanmasının yanı sıra biyotik ve abiyotik strese karşı dayanıklılığı artırma gibi etkileri de belirlenmiştir. Son yıllarda, nanopartiküllerin bitki doku kültürlerinde gerçekleştirilen uygulamaları ile de eksplantlardan kontaminantların yok edilmesi, kallus indüksiyonu, sürgün rejenerasyonu, organogenez, somatik embriyogenez, somaklonal varyasyon, *in vitro* çiçeklenme, genetik transformasyon ve sekonder metabolit üretimine yönelik başarılı sonuçlar alınmıştır. Nanopartiküllerin bitki sistemlerinde ve bitki doku kültürlerindeki uygulanma başarısı, kullanılan nanopartikül çeşidine, dozuna ve üzerinde çalışılan bitki türüne bağlı olduğu ortaya konulmuştur. Bu derleme, nanoteknolojinin bitki sistemlerine ve bitki doku kültürlerine entegre edilmesine yönelik mevcut çalışmaların incelenerek, nanoteknoloji kullanımının olumlu yönlerinin ortaya konulmasını amaçlamıştır.

MAKALE GEÇMİŞİ
Geliş
5 Mayıs 2023
Kabul
14 Temmuz 2023

ANAHTAR KELİMELER
Bitki,
doku kültürleri,
biyoteknoloji,
nanoteknoloji,
nanopartikül

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An Overview of Applications of Nanoparticles in Plant Systems and Plant Tissue Cultures

ABSTRACT
With the rapid increase in the world population, the need for plants and plant materials has also increased. Plant biotechnology is a good alternative to meet these needs. Plant tissue cultures, which constitute the most important part of plant biotechnology, include many techniques for different purposes. These techniques; micropropagation, genetic manipulation, bioactive compound production, and plant growth, etc. it is accepted as one of the basic building blocks of plant biology in fields. Nanotechnology is a multidisciplinary science that deals with the production, design, and application of nano-sized new materials (nanomaterials), and its basis is nanoparticles. Applications of nanoparticles in plant systems and plant tissue cultures have various effects on plant growth and development physiology. The most studied nanoparticles in these areas are; metal/metal oxide-based, carbon-based, quantum dots, silicon, and polymeric nanoparticles. When the studies using nanoparticles in plant systems are examined; It has been reported that positive results were obtained in parameters such as seed germination, plant growth and yield, shoot regeneration, root/shoot length, and biomass increase, and inducing effects were determined by physiological/biochemical activities. Also, effects such as providing genetic modification, improving the production of bioactive compounds, providing plant protection as well as increasing resistance to biotic and abiotic stress have been determined. In recent years, successful results have been obtained for the elimination of contaminants from explants, callus induction, shoot regeneration, organogenesis, somatic embryogenesis, somaclonal variation, *in vitro* flowering, genetic transformation, and secondary metabolite production with the applications of nanoparticles in plant tissue cultures. It has been revealed that the success of the application of nanoparticles in plant systems and plant tissue cultures depends on the type of nanoparticle used, its dose, and the plant species studied. This review aims to reveal the positive aspects of the use of nanotechnology by examining the existing studies on the integration of nanotechnology into plant systems and plant tissue cultures.

ARTICLE HISTORY
Received
5 May 2023
Accepted
14 July 2023

KEYWORDS
Plant,
tissue cultures,
biotechnology,
nanotechnology,
nanoparticles,

Giriş

Bitki biyoteknolojisi; bitkilerin verim ve kalitesini artırmak, bitki verimliliğini sınırlayan hastalık, zararlı ve stres faktörlerini engellemek, azaltmak veya ortadan kaldırmak amacıyla genetik mühendisliği ile birlikte hücre ve doku kültürü teknolojilerinin kullanıldığı bir süreçtir. Bu kapsamda bitki doku kültürleri; farklı özelliklere sahip birçok tarımsal türde çeşitli yöntem ve prosedürler yoluyla uygulanarak kaliteli üretimin hızlandırılmasına, bitkilerin daha verimli değerlendirilmesine ve ayrıca *ex situ* korumanın sağlanmasına önemli katkıda bulunmaktadır. Tarımsal öneme sahip ürünlerde, iklim değişikliğinin etkileri ve buna bağlı olarak gelişen olan farklı biyotik ve abiyotik streslerin yol açtığı biyolojik çeşitlilik ve ürün kayıpları göz önüne alındığında, bitki biyoteknolojisinin bu sorunlara sunduğu avantajlar son derece ilgi çekicidir [1].

In vitro ya da steril kültürler olarak da adlandırılan bitki doku kültürleri hem temel hem de uygulamalı çalışmaların yanı sıra ticari uygulamalar için de önemli bir araç olarak

benimsenmiştir. Bitki doku kültürleri; hücre, doku ya da organların ve bunların bileşenlerinin, kontrollü, uygun kültür koşulları altında, genellikle mikroçoğaltım, klonal üretim ve *in vitro* rejenerasyonları gerçekleştirmek üzere steril, simüle edilmiş besin ortamlarında kültüre alınarak hücrelerin veya bitki kısımlarının büyüme ve gelişmelerinin araştırıldığı tekniklerdir [2]. Bitki doku kültürlerinin çalışma konuları içerisinde sitoloji, embriyogenez, morfogenez, patoloji ve germplazm koruma, genetik manipülasyon, büyük ölçekli mikroçoğaltım ve klonal üretim, patojen içermeyen bitkilerin ve yararlı metabolitlerin üretimi gibi bitki biyolojisinin hem temel hem de uygulamalı alanları yer almaktadır [3]. Nanopartiküller, benzersiz fizikokimyasal özelliklere sahiptir ve biyomoleküllerle işlevselleştirme için çok yönlü yapı iskeleleri sağlamaktadır [4]. Son yıllarda nanopartiküller bitkilerde, tohum çimlenmesini iyileştirmek, büyümesini ve verimini artırmak, genetik modifikasyonunu sağlamak, biyoaktif bileşik üretimini iyileştirmek ve bitki korumasını sağlamak gibi birçok araştırmaya konu olmaktadır [1].
Bu derlemede, nanoteknolojide önemli bir yere sahip olan olan nanopartiküller incelenmiş, bunların bitki sistemleri ve bitki doku kültürleri ile olan ilişkileri yapılan çeşitli araştırmalar kapsamında ele alınarak, nanopartiküllerin uygulama alanlarının aydınlatılmasına çalışılmıştır.

Nanopartiküllerin Özellikleri ve Bitki Sistemlerinde Genel Kullanılma Potansiyelleri

Teknik bir terim olarak ‘nano’, 10^{-9} ya da milyarda bir anlamındadır ve nanoteknoloji, 1-100 nm boyutları arasında çalışmaktadır [1]. Nanoteknoloji, nano boyutlu malzemeler üretmek için atomik, moleküler ve makromoleküler ölçekte malzemelerin tasarımını, üretimini ve uygulamasını ifade eden multidisipliner bir bilim dalıdır. Nanomalzemeler olarak tanımlanan bu yapılar, yapımında kullanılan hammaddenin özelliklerine, kimyasal bileşimlerine, boyut, şekil ve uygulama alanlarına göre farklı kategorilerde sınıflara ayrılabilmekte ve bunlar; nanokristaller, nanopartiküller, nanotüpler, nanoteller, nanoçubuklar ve nanofilmler olarak adlandırılmaktadır. Nanomalzemeler arasında nanopartiküller, birim başına çok büyük yüzey alanına sahip olmaları nedeniyle uygulamalar arasında özellikle dikkat çekmektedir [5]. Boyutları 10-100 nm arasında olan nanopartiküller ise nano boyutlu malzemelerin, dolayısıyla nanoteknolojinin temelini oluşturmaktadır [6]. Nanopartiküller; spesifik şekilleri, ayarlanabilir gözenek

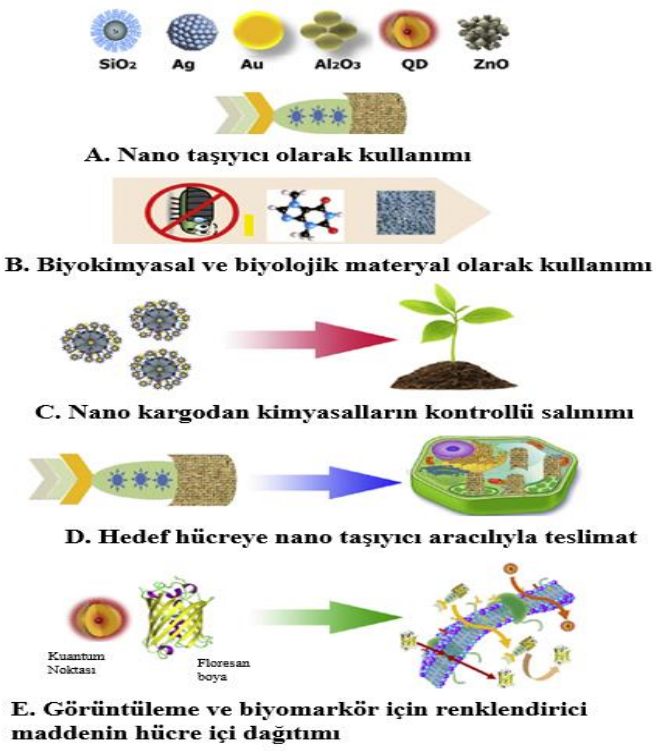
boyutları, geliştirilmiş yüzey alanı ve yüksek reaktiviteleri ile bilinirler [7]. Uygulama ve kullanımlarına bağlı olarak nanopartiküller; çubuk, tüp, küre, misel ve kabuk şekillerinde bulunabilirler. Nanopartiküllerin, kimyasal kompozisyonlarına göre sınıflandırılması ise; karbon bazlı (karbon nanotüpler, fulleren vb.), metal (Au, Ag, Cu vb.), metal oksit (ZnO, CuO, TiO_2 vb.), kuantum noktaları, silikon bazlı, polimerik (dendrimer, lipozom vb.) ve nanojeller (sentetik, doğal ve hibrit) şeklindedir [8] (Şekil 1).

Şekil 1 Nanopartiküllerin çeşitli kategorilerde gruplandırılması [8]
Fig 1 Grouping of nanoparticles into various categories [8]

Nanopartiküller, belirli metal tuzlarının indirgenmesi yoluyla oluşturulan ve bu indirgenme sürecinde bağımsız hareket edebilen nano ölçekli sistemlerdir. Küçük sistemlere nüfuz etme kapasitesine sahip olan bu partiküller, farklı biyokimyasal ve fizyolojik yolları tetiklemek üzere bitki içerisindeki uzak bölgelere ulaşabilirler. Bu nanopartiküllerin boyutu ve konsantrasyonu, kullanılan bitki türüne özgüdür. Nanopartiküller; kalite iyileştirme, büyüme ve besin değerini geliştirme, gen koruma vb. dahil olmak üzere bitki sisteminin çeşitli alanlarında yaygın olarak kullanılmaktadır [9]. Bitki sistemlerine nanopartiküllerin entegre edilmesi ile tarım kimyasallarının (gübre, pestisit, herbisit) kontrollü bir şekilde salınımı, biyomolekülerin (protein, aktivatör) hedefe yönelik iletimi gibi programlanabilir özelliklerin kazandırılması mümkündür [5]. Nanopartiküllerin bitki sistemlerinde translokasyon kabiliyetinin iki ana özelliğe bağlı olduğu bildirilmiştir: (1) nanopartiküllerin boyutu ve (2) nanopartiküllerin yüzey yükü.

Boyutlarına bağlı olarak, çoğu nanopartikül, bitkiler içinde yer değiştirebilmektedir. Bununla birlikte, negatif yüzey yüküne sahip nanopartiküller, bitki içine entegrasyonları daha kolay gerçekleşmekte ve bitki içinde yer değiştirme potansiyeline sahip olmaktadırlar, böylece bitkinin büyümesini ve korunmasını desteklerler [10].

Nanopartiküller, bitkilerde gösterdikleri önemli etkilerinden dolayı, son yıllarda tarım sektöründe de kendilerine yer bulmuşlardır. Bitki sistemlerinde yapılan çeşitli uygulamalar; I) nanomalzemelerin nanotaşıyıcı olarak kullanılması, II) böcek kovucular, böcek ilaçları ve gübrelerin nanomalzemeler ile birleştirilmesi ya da kapsülize edilmesi, III) nano taşıyıcılardan kimyasalların (böcek kovucular, böcek ilaçları ve gübreler vb.) hedefe kontrollü salınımı, IV) biyoaktif moleküllerin bitki hücrelerine nano taşıyıcı aracılı teslimi, V) görüntüleme ve biyomarkör amaçlı kullanılan renklendirici maddelerin (kuantum noktaları, floresan boyalar vb.) nano taşıyıcılar aracılığı ile hücre içi teslimi ve, VI) hastalıklara karşı direncin artırılması şeklinde sayılabilir (Şekil 2) [9].



Şekil 2 Nanopartiküllerin bitkilerdeki çeşitli uygulama alanları [9]

Fig 2 Various applications of nanoparticles in plants [9]

Nanopartiküllerin bitkisel sistemlerde abiyotik stres faktörlerine karşı uygulanmaları

Nanopartiküller; genellikle tohum çimlenmesini iyileştirmek, bitki büyümesini ve verimini artırmak, bitki genetik modifikasyonunu sağlamak, biyoaktif bileşiklerin üretimini iyileştirmek ve bitki korumasını sağlamak için yaygın olarak kullanılmaktadır [11 12]. Bitkilerde nanopartikül uygulamaları, çoğunlukla biyotik ve abiyotik strese karşı bitkilerdeki dayanıklılığı artırmaya ve dayanıklılık ile ilişkili yolakları harekete geçirmeye yöneliktir [13, 14, 15]. Bitkiler durağan oldukları için tuzluluk, kuraklık, yüksek ve düşük sıcaklıklar, ağır metaller, taşkın, yüksek ve düşük ışık yoğunlukları, ultraviyole (UV) vb. çevresel stres koşullarıyla yüzleşmek zorundadırlar. Bu tip stres koşulları, makromoleküllerin parçalanmasına ve membran yapısının bozulmasına yol açan hücre toksisitesini tetikleyerek bitki büyümesini inhibe edici reaktif oksijen türlerinin çoğalmasına neden olmakta ve bunun sonucunda sitotoksisite ve genotoksisite meydana gelerek, bitkilerin büyümesi inhibe edilmektedir [16, 17].

Bitki büyümesi; su basıncı, tuzluluk, kuraklık, yüksek sıcaklık ve ultraviyole (UV) radyasyon gibi çeşitli çevresel koşullardan (abiyotik stres koşulları) etkilenmektedir. Bu nanopartiküllerin boyutu, şekli, yüzey yükü ve geniş yüzey alanı, bitkilerin büyümesini artırmaktadır. Nanopartiküller, ayrıca bitkilerin çevresel streslere karşı korunmalarında da önemli bir role sahiptirler, çünkü bu partiküllerin ROS'u (Reaktif Oksijen Türeleri) temizleyen antioksidan enzimleri taklit edebilme özelliği bulunmaktadır. Reaktif oksijen türleri, oldukça yüksek reaktiviteye sahip moleküller olup başta mitokondri olmak üzere hücre organellerinde gerçekleşen, normal metabolizmanın sonucu olarak veya yaşlanma, radyasyon, inflamasyon ve kimyasal ajanlara maruz kalma gibi nedenlere bağlı olarak üretilirler [19, 20]. Nanopartiküller, bitki verimini düzenlemek ve abiyotik stres koşulları altında tolerans mekanizmalarını artırarak tarımsal üretimin mevcut ve gelecekteki sınırlamalarının üstesinden gelmek için etkili ve umut verici bir araç olarak kabul edilirler [18]. Çeşitli çalışma bulgularına dayanılarak nanopartiküllerin bitki büyümesini kolaylaştırmanın yanı sıra çevresel streslere karşı da toleransı oluşturma potansiyeline sahip oldukları ifade edilmektedir [10, 21].

Bitki sistemlerinde nanopartiküllerin çevresel stres faktörlerine karşı kullanımları kuraklık, ısı, tuzluluk ve UV stresi alt başlıkları halinde aşağıdaki gibi açıklanmaya çalışılmıştır:

a) Kuraklık stresi: Bitkilerdeki kuraklık stresi üzerine yapılan çalışmalarda, farklı nanopartikül çeşitlerinin kuraklık stres toleransı üzerinden olumlu sonuçları belirlenmiş

ve bu çalışmalar sonucunda kuraklık stresi altındaki fidelerde, büyümeyi ve fizyolojik parametreleri iyileştirdikleri bildirilmiştir. TiO_2 nanopartikülünün, tohum çimlenmesi ve fide büyümesi üzerindeki etkileri araştırılmış ve kuraklık stresi altındaki bitkilerde biyokütleyi artırma, bağıl su içeriğini (RWC) koruma ve antioksidan enzimleri artırmaya yardımcı olduğu gösterilmiştir. Fe_2O_3 nanopartiküllerinin karbonhidrat metabolizmasını ve stoma hareketlerini değiştirerek kuraklık stresine karşı toleransı artırdığı, ZnO nanopartiküllerinin ise fotosentetik pigment bozunmasını azalttığı, böylece fotosentez hızını ve stoma hareketlerini artırdığı kanıtlanmıştır. Nişasta ve sükroz sentezi de UDP glikoz pirofosforilaz, fosfoglukoizomeraz ve sitoplazmik invertaz gibi anahtar enzimlerin yönlendirmesiyle artmakta, kuraklık stresi altındaki bitki sistemlerinin daha iyi performans göstermesi sağlanmaktadır. Bu durum, ZnO'yu kuraklık stresinin olumsuz etkilerini azaltmak için potansiyel bir nano ajan yapmaktadır [22].

b) Isı stresi: Nanopartiküller, ısı stresini azaltmak amacıyla bitki sistemlerinde kullanıldıklarında olumlu sonuçlar alınmıştır. Isı stresinin etkilerini azaltmak üzere farklı konsantrasyonlarda nanopartiküller uygulandığında, bitki büyümesinde ve hidrasyonda artışlar meydana gelmiştir [23]. Wang ve ark. (2022) tarafından gerçekleştirilen bir çalışmada, mısır bitkisinde CeO_2 nanopartiküllerinin uygulanması sonrasında H_2O_2'nin aşırı üretilmesine ve HSP70 geninin düzenlenmesine yol açılmış, ayrıca nanopartiküllerin hücrede stoma açıklığını düzenleyerek ısı stresinin etkilerini azalttığı belirtilmiştir [24]. Kareem ve ark., 2022 yılında maş fasulyesinde yaptıkları araştırmalarında nano ZnO partiküllerinin yüksek sıcaklık koşullarında bulunan bitkilere uygulanmasının klorofil aktivitesini, gaz değişim parametrelerini ve enzimatik dengeyi artırdığı ve bunun da bakla sayısında, boyutunda ve toplam tane veriminde artışa neden olduğu bildirilmiştir [25]. Isı stresi altındaki buğday fidelerinde ZnO ve TiO_2 uygulaması hem kök hem de sürgün parametrelerinde membran stabilitesini ve antioksidan savunma mekanizmasını iyileştirmiştir [26].

c) Tuzluluk stresi: Tuzluluk stresi altındaki bitkilerin büyümelerini ve hayatta kalmalarını iyileştirmek için etkili bir araç olarak nanopartiküller, son yıllarda çok dikkat çekmektedir. Nanopartiküllerin tuzluluk stresi altındaki bitkilerde yararlı etkileri olduğu farklı çalışmalarda gösterilmiştir. Nanopartiküllerin bitki sistemlerinde hormonal konsantrasyonları, antioksidan enzim aktivitesini, iyon homeostazını ve gen ekspresyonunu etkilediği için tuzluluk stresine karşı bitkinin tepkilerini manipüle

edebildiği bildirilmiştir [27, 28]. Uygulanan nanopartiküllerin boyutuna, şekline ve konsantrasyonlarına ek olarak, bu etkiler farklı çevre koşulları ve farklı bitki türleri arasında da değişiklik gösterebilmektedir. Tuzluluk stresine maruz kalan bitkilerde gözlenen potasyum\sodyum oranının yüksek bulunması, tuzluluk stresine karşı bitki dayanıklılığı için en kritik faktörlerden biri olarak kabul edilmektedir. Bu stres altındaki bitki, nanopartikül uygulamasına tabi tutulması sonucunda, bitki hücrelerindeki ozmotik potansiyelin artması ile iyileştirilebilmektedir [29]. Farhangi-Abriz ve Torabian'ın 2018 yılında yapmış oldukları araştırmaya göre, SiO_2 nanopartiküllerinin yapraktaki K^+ iyon konsantrasyonunu artırarak tuz stresi altındaki soya fasulyesinin fide büyümesini artırdığı gösterilmiştir [30]. Ayrıca tuzluluk stresi de dahil olmak üzere abiyotik streslere yanıt olarak bitkiler tarafından reaktif oksijen türlerinin (ROS) üretildiği bilinmek ile birlikte stres altındaki bitkiler, hücrelerindeki aşırı ROS ile başa çıkmak için antioksidan enzimler geliştirmektedirler. Birçok çalışmada, nanopartiküllerin antioksidan enzim düzeylerini artırdığı gösterilmiştir. Bu da partiküllerin antioksidan özelliklere sahip oldukları için bitkilerdeki oksidatif stresin yaratmış olduğu koşulların üstesinden gelmelerine yardımcı oldukları bildirilmiştir [31, 32, 33].

d)Ultraviyole stresi: Dünya yüzeyinde, alınan ultraviyole-B (UV-B) radyasyonunun artışı ile ürün bitkilerinin veriminde dünya çapında düşüşler meydana gelmektedir. Yüksek UV-B seviyesinde (ROS) konsantrasyonu artmakta, antioksidan enzim aktivitesi değişmekte, fotosentetik hız düşmekte, DNA ve hücre zarları zarar görmekte ve mikrotübül yapısı bozulmaktadır. Bütün bunların sonucunda ise bitkilerin büyümesi olumsuz etkilenmektedir. Bu nedenle, UV-B'nin bitkiler üzerindeki olumsuz etkilerini azaltmak için yeni stratejilere ihtiyaç duyulmuştur. Nanopartiküller; fotosentezi ve flavonoid birikimini artırarak, antioksidanları taklit ederek veya antioksidan enzim aktivitelerini iyileştirerek, bitki hücrelerinde mikrotübül depolimerizasyonunu önleyerek ve oksidatif stresin azalmasını sağlayarak UV-B stresini önemli ölçüde azaltmaktadırlar [34].

Nanopartiküllerin bitki sistemlerindeki olumsuz etkileri; çeşitli biyokimyasal, fizyolojik ve moleküler engellerle ilişkilidir. Bitkilerde yüksek konsantrasyonlarda nanopartikül bulunması; klorofil içeriğini inhibe edebilmekte, terlemeyi ve fotosentetik oranları azaltabilmekte ve elektron taşıma aktivitesini düşürebilmektedir [10, 17, 35].

Bitki büyümesine ve gelişmesine olumsuz yönde etki etmeyen güvenli nanopartikülleri tasarlama ve sentezleme gereksiniminin karşılanması durumunda, bitkilerde nanopartiküller ile ilişkili alım ve mobilizasyon mekanizmaları hakkında bilgi eksikliğinin giderilmesinin yanı sıra, multidisipliner yaklaşımların katkılarıyla da nanopartiküllerin kullanım alanlarının daha çok çeşitleneceği ve kullanımlarının giderek artacağı öngörülmektedir.

Farklı Nanopartikül Çeşitlerinin Bitki Sistemlerinde ve Bitki Doku Kültürlerindeki Uygulama Alanları

Nanopartiküllerin özelliklerine bağlı olan bitki-nanopartikül etkileşimi, bitkilerde farklı morfolojik ve fizyolojik değişikliklere neden olmaktadır. Yapılan araştırmalar sonucunda, nanopartiküllerin bitki sistemlerinde olumlu ve olumsuz etkilerinin var olduğu, bunun da kullanılan nanopartikül çeşidine, konsantrasyonuna ve nanopartikül uygulanan bitkinin türüne özgü olduğu belirlenmiştir. Bu bilgiler doğrultusunda; bitki sistemlerinde uygulanan nanopartiküllerin tohum çimlenmesi, kök ve sürgün uzunluğu, bitki büyümesi ve fotosentezdeki rolü üzerinde bazı çalışmalar yapılmıştır. Nanopartiküllerin bitki doku kültürlerinde kullanımlarıyla ilgili olarak da tohum çimlenmesi, büyümenin iyileştirilmesi, genetik transformasyon, genetik modifikasyon, kontaminasyonun eliminasyonu, bitki koruma, *in vitro* rejenerasyon, (kallus, organ ve embriyo), somaklonal varyasyon ve sekonder metabolit üretimi vb. konularında çeşitli araştırmalar gerçekleştirilmiştir [11, 12]. Bitki doku kültürlerinde ve bitki sistemlerinde nanoteknolojinin kullanımından olumlu sonuçlar alındığını gösteren çok sayıda çalışma mevcuttur. Bu nedenle makalenin bu kısmında, bitki doku kültürlerinde ve bitki sistemlerinde kullanılan nanopartikül çeşitlerinin tanıtılması esas alınarak, uygulama alanlarının belirlenmesine çalışılmıştır.

Metalik/metal oksit bazlı nanopartiküller

Bitkide büyüme ve gelişme çalışmalarında kullanılan metalik nanopartiküller, metal oksitler ve saf metaller olmak üzere iki geniş kategoride ele alınmaktadır [36].

Bitki sistemlerinde uygulamalar

Bitki büyümesi üzerindeki etkileri araştırılan nanopartiküller arasında gümüş nanopartikülleri (Ag NP'ler), çinko oksit nanopartikülleri (ZnO NP'ler), altın nanopartikülleri (Au NP'ler), silika nanopartikülleri (SiO_2 NP'ler), nanoselenyum nanopartikülleri (Se NP'ler), seryum oksit nanopartikülleri (CeO_2 NP'ler), bakır ve bakır

oksit nanopartikülleri (Cu ve CuO NP'ler), demir nanopartikülleri (Fe NP'ler), nikel oksit ve nikel hidroksit nanopartikülleri (NiO ve $Ni(OH)_2$ NP'ler), alüminyum oksit nanopartikülleri (Al_2O_3 NP'ler), indiyum oksit (In_2O_3 NP'ler), kobalt oksit (CoO, Co_2O_3, Co_3O_4 NP'ler) ve neodimyum oksit nanopartikülleri (Nd_2O_3 NP'ler) yer almaktadır [37]. Tüm metal oksit nanopartikülleri, bitkilerin büyüme ve gelişimi üzerinde çeşitli etkilere sahiptirler. Bu etkiler; tohum çimlenmesi, sürgün/kök gelişimi, biyokütle üretimi ve fizyolojik/biyokimyasal aktiviteler açısından indükleyici niteliktedir [36]. Metalik nanopartiküllerin bitkilerde inhibe edici özelliği; morfo-anatomik, fizyolojik ve genetik bileşenleri etkilemesi ve daha yüksek konsantrasyonlarda ise toksisiteye neden olmasıyla ilişkilendirilmiştir [38, 39]. Metal ve metal oksit bazlı nanopartiküller ile yapılan çalışmalar incelendiğinde; farklı bitki türlerinin farklı konsantrasyonlarda, farklı nanopartikül çeşitlerine farklı tepki verdikleri gözlenmiştir [40].

Dekontaminasyon ajanı olarak kullanım: Abdulrazaq'ın 2018 yılında yapmış olduğu bir çalışmada, domates bitkisinde kurşuni küf hastalığı (*Botrytis cinerea*) üzerinde ZnO ve CuO nanopartiküllerinin ve nanopartikül-kekik (*Origanum vulgare*) uçucu yağ karışımlarının antifungal aktivitesi araştırılmıştır. ZnO ve CuO nanopartikülleri 5, 50, 100, ve 200 mg L^{-1} konsantrasyonlarda uygulanmıştır. Buna ek olarak, 50 ve 200 mg L^{-1} konsantrasyonda ZnO ve CuO; 0, 10, 20, 40, 80, 160, 320 ve 640 µl L^{-1} kekik uçucu yağı ile karıştırılarak yapraklara uygulanmış ve kurşuni küf oluşumu incelenmiştir. Denemeler sonunda iki nanopartikülün de kurşuni küf kolonisi oluşumu üzerinde baskılayıcı etkiye sahip olduğu gösterilmiştir [41]. Mahna ve ark.'nın (2008) yapmış oldukları çalışmada ise, Arabidopsis tohumları, patates yaprakları ve domates kotiledonlarının yüzey dezenfeksiyonu üzerine gümüş nanopartikülleri ile muamelenin etkisi araştırılmıştır. Eksplantların 1 ve 5 dakika süreyle 100 mg L^{-1} Ag nanopartikülleri ile muamele edilmesinin tohum, yaprak veya kotiledonların dekontaminasyonu (%100) için ideal olduğu ve bu işlemin eksplant canlılığı üzerinde herhangi bir olumsuz etkisinin bulunmadığı raporlanmıştır. Bununla birlikte, daha yüksek konsantrasyonlarda Ag nanopartikülleri ile muamele yapıldığında ise tohum çimlenmesi, yaprak ve kotiledon sağ kalımının azaldığı bildirilmiştir [42].

Shokri ve ark.'nın 2013 yılında gül nodal segmentlerini kullanarak yapmış oldukları çalışmada, eksplantların 0, 100, 200 ve 400 ppm konsantrasyonlarında gümüş nanopartiküllerine daldırıldıkları denemeler sonucunda, 200 ppm'lik konsantrasyon

kullanımının dekontamine kültürler elde etmek için en iyi daldırma işlemi olduğu belirlenmiştir [43].

Bitki doku kültürlerinde uygulamalar

Antioksidan savunma sistemi: Bitkiler, oksidatif hasarı önlemek ve aynı zamanda metal oksit toksisitelerine karşı direnci artırmak için enzimatik ve enzimatik olmayan bileşenleri içeren antioksidan savunma zırhlarını geliştirmişlerdir [44]. Poborilova ve ark. (2013)'na ait bir çalışmada, tütün hücre süspansiyon kültürlerine Al_2O_3 nanopartiküllerinin (10–100 μg mL^{-1}) eklenmesinin, reaktif oksijen (ROS) ve reaktif azot (RNS) türlerinin üretimi yoluyla hücre canlılığını önemli ölçüde azalttığı bildirilmiştir [45]. Iannone ve ark. (2016)'nın yapmış oldukları çalışmalarda; Fe_3O_4 nanopartikülleri ile muamele edilen buğday bitkilerinde süperoksit dismutaz (SOD), katalaz (CAT) ve peroksidaz (POX) enzimlerinin aktivitelerinde artışlar kaydedilmiştir [46]. Benzer bir durum, Okupnik ve Pflugmacher (2016) tarafından *Hydrilla verticillata* bitkisi ile gerçekleştirdikleri çalışmada TiO_2 nanopartikül kaynaklı katalaz ve glutatyon redüktaz aktivitelerinde artışların meydana geldiği ifade edilmiştir. Nanopartikül kaynaklı toksisiteye karşı bitki savunmasının altında yatan kesin mekanizma tam olarak bilinmemekle birlikte, bitkinin farklı bölümlerinde metal oksit nanopartiküllerinin absorpsiyonu ve translokasyonunun; konsantrasyonlarına, çözünürlüklerine ve maruz kalma sürelerine bağlı olduğu ifade edilmiştir [47].

Tabay 2021 yılında, CuO ve ZnO nanopartiküllerinin mısır bitkisinde büyüme, gelişme ve bazı genlerin ekspresyonu üzerine etkilerini incelediği bir araştırma gerçekleştirmiştir. Çalışmada, mısır fidelerine 3 farklı konsantrasyonda CuO (250, 500, 1000 mg L^{-1}) ve ZnO (100, 200, 400 mg L^{-1}) nanopartikülleri uygulanmış ve kök-gövde uzunluğu, malondialdehit (MDA) miktarı, hidrojen peroksit (H_2O_2) ve süperoksit anyon ($O_2^{\cdot -}$) seviyesi, süperoksit dismutaz (SOD), katalaz (CAT), peroksidaz (POD), glutatyon redüktaz (GR) aktivitesi, glutatyon (GSH) miktarı ve ayrıca *sod, cat, pod, gr, gst, gpx* gibi antioksidan sistemle ilişkili genlerin ekspresyonlarında meydana gelen değişimler incelenmiştir. Sonuç olarak, uygulanan CuO ve ZnO nanopartiküllerinin kök-gövde uzunluğunu, klorofil a ve b miktarını azalttığı, MDA miktarını, H_2O_2 ve $O_2^{\cdot -}$ seviyesini ise artırdığı belirlenmiştir. Ayrıca nanopartikül uygulamalarının (tüm konsantrasyonlarda) fide kök ve yapraklarındaki SOD enzim aktivitesini düşürdüğü tespit edilmiştir. Ek olarak, mısır fidelerinin kök ve gövdelerindeki CAT enzim aktivitesinin

CuO nanopartikül uygulaması ile azaldığı, ZnO nanopartikül uygulaması ile arttığı gözlenmiştir. POD enzim aktivitesinin ise fidelere uygulanan CuO nanopartikülü ile arttığı, ZnO nanopartikül uygulaması ile azaldığı kaydedilmiştir. CuO ve ZnO nanopartiküllerin hem kök hem de yapraklarda GR aktivitesi ve GSH miktarını artırdığı raporlanmıştır. Son olarak, uygulanan nanopartiküllerin mısır kök ve yapraklarında SOD, CAT, POD, GR, GST, GPX gibi antioksidan sistemle ilişkili genlerin ekspresyonunu artırıp ya da azalttığı gözlenmiş ve bu nanopartiküllerin uygulanmasının fidelerde toksik etkiye yol açtığı, fidelerin antioksidan sistemini harekete geçirdiği tespit edilmiştir [48].

Dekontaminasyon ajanı olarak kullanım: Bitki doku kültürlerinde eksplantların sterilizasyonu ve aseptik koşulların korunması, *in vitro* kültürün önemli bir bölümünü kapsamaktadır. Farklı mikroorganizmaların, *in vitro* besin ortamlarında bitki hücrelerine kıyasla daha hızlı büyümeleri sonucunda, bitki hücrelerinin büyümesini inhibe ettikleri bilinmektedir. Geleneksel yöntemde, bitki organ ve dokuları, kontaminasyonu en aza indirmek için farklı antibiyotikler ve antifungal solüsyonlarla sterilize edilmektedir [6].

Nanopartiküller, özellikle *in vitro* kültürlerde sıkça karşılaşılan ve en önemli problemlerden biri olan kontaminasyon kaynağı olarak görev yapan mikroorganizmaları yok etme kapasitesine sahiptirler. Nanopartiküllerin antibakteriyel etki gösterebilmeleri için, bakteriyel hücrelerle elektrostatik çekim, Van der Waals kuvvetleri, reseptör ligand ve hidrofobik etkileşimleri sağlaması gerekmektedir. Bu etkileşimlerin sağlanabilmesi için bakteri hücreleri-nanopartikül teması şarttır. Bu temastan sonra; nanopartiküller bakteri zarını geçerek bakteri hücresinin zarının şeklini ve işlevini bozarlar. Nanopartiküller hücre zarını geçtikten sonra bakteride; oksidatif strese, heterojen değişikliklere, bozulmuş hücre zarı geçirgenliğinin yol açtığı elektrolit denge bozukluklarına ve ayrıca DNA, lizozomlar, ribozomlar ve enzimler gibi bakterilerin hücresel temel bileşenleri ile etkileşime girerek de enzim inhibisyonuna ve protein deaktivasyonuna sebep olurlar [6, 49]. Ek olarak, nanopartiküller doku kültürü ortamında antimikrobiyal olarak kullanıldıklarında; bitkilerin sağlıklı büyüme şansını ve verimini artırdığının gözlenmesi, dikkatleri bu konuda yapılan çalışmaların üzerine çekmiştir [9]. Nanopartiküllerin antibakteriyel özelliği yeni bir konu olmamakla birlikte; en bilinenleri Ag nanopartikülleri, TiO2 nanopartikülleri ve ZnO nanopartikülleridir ve ayrıca üzerinde en derin ve iyi çalışılmış nanopartikül türleridirler. Özellikle gümüş nanopartikülleri, gümüşün çok geniş spektrumlu bir antibiyotik olması ve bakteri direncinin neredeyse hiç

bulunmaması gibi özelliklerinden dolayı en çok ilgi göreni ve üzerinde çalışılanıdır [49]. Gümüş iyonlarının güçlü antibakteriyel, antifungal ve antiviral aktiviteye sahip oldukları ve mikroorganizmaların hücre yapısını tahrip ettikleri ifade edilmiştir [50]. Gümüş nitrat gibi gümüş bazlı bileşiklerin de mikroorganizmalar açısından oldukça toksik olduğu kabul edilmektedir [6].

Odunsu bitkiler söz konusu olduğunda, *in vitro* kültürlerde kontaminant kaynağının yok edilmesi büyük bir zorluktur. Rostami ve Shahsavar (2009) tarafından bu alanda yapılan bir çalışmada, gümüş nanopartiküllerinin kültür ortamına 4mg L^{-1} konsantrasyonda eklenmesinin, zeytinin *in vitro* kontaminasyonlarını ortadan kaldırmada etkili olduğu bulunmuştur. Bu çalışmada, 9 yıllık zeytin bitkisinin nod ve sürgün uçları eksplant kaynağı olarak kullanılmış ve 1 dakika boyunca %70 EtOH, ardından 10 dakika boyunca %10 Clorox ile muamele edilmiştir. Bu sterilizasyon yöntemi ile %51,4 dekontamine kültürler elde edilmiş, fakat eksplantlar EtOH ve Clorox'a maruz bırakıldıktan sonra 60 dakika boyunca 100 mg L^{-1} gümüş nanopartikülleri ile muamele edilmeleri durumunda ise kontaminasyon tamamen ortadan kaldırılmakla birlikte, eksplantlardan çok düşük bir sağ kalım yüzdesi (%18) sağlanmıştır. Öte yandan, kültür ortamına gümüş nanopartiküllerinin (4 mg L^{-1}) eklenmesi, zeytin eksplantlarındaki içsel kontaminantları baskılamış ve eksplantların büyümeleri üzerinde herhangi bir olumsuz etki oluşturmamıştır [51]. Gümüş nanopartikülleri, Sarmast ark. (2012) tarafından doku kültürlerinde *Araucaria excelsa* eksplantlarının dekontaminasyonu için başarıyla kullanılmıştır [52]. Bunu destekleyici bir çalışma olarak geçici daldırma sistemlerinde (TIS) kontaminasyonun ciddi bir sorun olduğu gerçeğinden yola çıkarak, Spinoso-Castillo ve ark.'nın (2017) yapmış oldukları bir çalışmada, vanilya bitkisinde sürgün rejenerasyonu için MS sıvı ortamında farklı konsantrasyonlarda (0, 25, 50, 100 ve 200 mg L^{-1}) gümüş nanopartikülleri kullanılmıştır. 50, 100 ve 200 mg L^{-1} gümüş nanopartiküllerini içeren besin ortamlarında bakteriyel kontaminasyonun azaldığı, 25 ve 50 mg L^{1} gümüş nanopartikülleri bulunduran ortamlarda büyüme uyarımının gözlendiği, 100 ve 200 mg L^{-1} gümüş nanopartikülleri içeren ortamlarda ise inhibisyon tespit edildiği raporlanmıştır [53]. Başka bir çalışmada ise; Abdi ve ark. (2008), *Valeriana officinalis*'te bakteriyel kontaminasyonu kontrol etmek için ilk kez Ag nanopartiküllerini kullanmışlardır. Serada yetiştirilen bitkilerden sağlanan tek nodlu eksplantların, 1 dakika %70 EtOH, 1 dakika %10 Clorox ve ardından 180 dakika boyunca 100 mg L^{-1} Ag

nanopartikülleri ile yüzey dezenfeksiyonu yapılmıştır. Bu işlem sonunda, %89 dekontamine kültürler elde edilmiştir. *Valeriana officinalis* bitkisinde Ag nanopartikülleri ile muamele edilen eksplantlarda sürgün çoğaltım katsayısında ve köklenmede bir artış gözlenmemiştir [54].

Helaly ve ark. (2014) yaptıkları bir çalışmada, 3 yaşındaki muz bitkilerinden elde edilen sürgün ucu eksplantları, %80 NaOCl, %95 EtOH ve son olarak %0,1 $HgCl_2$ şeklinde ilerleyen ve her bir ajan ile 15 dakika muamelenin söz konusu olduğu sterilizasyon aşamalarına dahil edilmişlerdir. Bu işlemler sonunda mikrobiyal kontaminasyonu önlemede başarısız olunmuştur. Araştırmacılar bunlara ek olarak, kullandıkları MS besin ortamına 100 mg L^{-1} Zn nanopartiküllerinin dahil edilmesine karar vermişler ve kontaminasyon içermeyen kültürler elde etmişlerdir [55].

Shokri ve ark. tarafından 2013 yılında yapılan çalışmada, *in vitro* kültürlerde gül bitkisinde ana sınırlayıcı faktör olan bakteri kontaminasyonlarının önlenmesi amacıyla gümüş nanopartiküllerin etkisi araştırılmıştır. Çalışmada, nodüler gövde segmentleri eksplant olarak kullanılmış ve iki farklı yöntem uygulanarak dekontamine hale getirilmeye çalışılmıştır. Bunlardan ilki; gümüş nanopartiküllerinin 0, 50, 100 ve 150 ppm konsantrasyonlarda kültür ortamına ilave edilmesidir. Bu yöntem sonunda, direkt olarak ortama eklenen 100 ppm konsantrasyonunun bakteriyel kontaminasyonu ve fenolik eksüdasyon oranını azalttığı belirlenmiştir [43].

Bao ve ark.'nın (2022) begonya (*Begonia* × *tuberhybrida Voss*) bitkisinde yapmış oldukları çalışmada, farklı eksplant tipleri (petiol, nod ve çiçek salkımı) üzerinde cıva klorür ve kalsiyum hipoklorit gibi yaygın sterilizasyon ajanları yerine bakır nanopartiküllerini kullanmışlardır. Bu nanopartiküllerin, sterilizasyon ajanı olarak, somatik embriyo oluşumu, somatik embriyo morfolojisi, antioksidan aktivite ve karbonhidrat içeriği üzerindeki etkilerini belirlemek için yaprak sapı, çiçek salkımı ve nod eksplantları yaklaşık 1 mm uzunluğunda ince hücre tabakaları halinde enine kesilmiş ve somatik embriyo indüksiyon ortamında kültüre alınmışlardır. Çalışma sonunda araştırmacılar, bakır nanopartiküllerin cıva klorür ve kalsiyum hipokloritin yerini alabildiğini ve ayrıca petiol, çiçek salkımı ve nod eksplantlarının yüzey dezenfeksiyonunda etkili olduğubelirtilmiştir. Bakır nanopartiküllerinin, cıva klorür veya kalsiyum hipoklorite kıyasla, eksplantların embriyogenik kallus ve somatik embriyogenezini artırdığı bildirilmiştir. Çiçek salkımı ve gövde nodu eksplantlarında, en

yüksek somatik embriyo sayısı (36,67 – 38 embriyo) ve kotiledon şeklindeki somatik embriyo (%47,37–49,09) elde edilmiştir. Bakır nanopartikül ile muamele edilmiş çiçek salkımlarının ve gövde nodlarının eksplantlarından türetilen somatik embriyo kümelerinin antioksidan enzim aktiviteleri ve nişasta içerikleri, cıva klorür ve kalsiyum hipoklorit ile steril edilenlere kıyasla enzim aktivitesi açısından yüksek ve şeker içeriğinin ise daha düşük olduğu bulunmuştur. Farklı işlemlerden elde edilen bitkicikler, seraya transfer edildikten sonra morfoloji, *in vitro* köklenme veya aklimatizasyon aşamalarında hiçbir farklılık gözlenmemiştir [56].

In vitro rejenerasyon (kallus indüksiyonu, organogenez ve somatik embriyogenez): Nanopartiküllerin çeşitli çalışmalarda; kallus oluşumu, sürgün rejenerasyonu ve büyümesinin yanı sıra somatik embriyogenez üzerine de olumlu etkileri gösterilmiştir. *Tecomella undulata* bitkisinde yapılan çalışmada, gövde eksplantları 10 mg L^{-1} Ag nanopartikülleri içeren ve 2,5 mg L^{-1} BAP ve 0,1 mg L^{-1} IAA ile güçlendirilmiş MS ortamında kültüre alındıklarında, sürgün indüksiyon yüzdesi, sürgün sayısı ve kallus oluşumunun arttığı ortaya konmuştur [52]. Bununla birlikte, gümüş nanopartiküllerinin, *Vanilla planifolia*'da sürgün rejenerasyonu [53], *Solanum nigrum*'da yüksek frekanslı kallus oluşumu (%89) [57], *Prunella vulgaris*'te kallus çoğaltımı [58] ve *Linum usitatissimum*'da artan embriyogenez oranı (%50) [59] gibi büyüme parametreleri üzerinde çeşitli etkilerinin bulunması, *in vitro* kültürlerde etkili olduklarını kanıtlamaktadır. Ayrıca gümüş nanopartiküllerinin organogenez üzerindeki olumlu etkileri, etilen üretiminin inhibisyonu ile ilişkilendirilmektedir [37]. Yapılan çalışmalar sonunda, ZnO nanopartiküllerinin de benzer etki gösterdikleri kaydedilmiştir. Javed ve ark.'nın (2016) domates bitkisinde gerçekleştirdikleri ZnO nanopartikül çalışmasında maksimum bitki rejenerasyonu ve kallus büyümesi gözlemlenirken [60], ZnO nanopartiküllerinin *Stevia rebaudiana*'da en yüksek sürgün oluşumunu (%89,6) teşvik ettiğini bildirmiştir [61]. Muz bitkisi ile yapılan başka çalışmada ise ZnO nanopartiküllerinin maksimum somatik embriyo oluşumunu tetiklediği ve kontrole kıyasla bu embriyolardan sürgün ve kök uzunluğunun arttığı bildirilmiştir [55]. *Verbena bipinnatifida* fidelerinin *in vitro* kültürleri sırasında kullanılan bakır sülfat nanopartiküllerinin de kök ve sürgün uzunluğunu artırdığı bildirilmiştir [62]. Ayrıca CuO nanopartiküllerinin ve Co nanopartiküllerin de *Mentha longifolia*'daki sürgünlerin sayısını ve uzunluğunu artırdıkları belirlenmiştir [63].

Phong ve ark. (2022) tarafından gerçekleştirilen çalışmada, *Passiflora edulis* Sims f. *edulis* bitkisinde gümüş nanopartiküllerinin sürgün oluşumu, *in vitro* çiçeklenme ve meyve oluşumu üzerindeki etkileri araştırmışlardır Bitkinin internodları, ince hücre tabakası tekniği kullanılarak kültüre alınmış ve besin ortamına gümüş nanopartikülleri eklenmiştir. Eklenen gümüş nanopartiküllerinin sürgün sayısında önemli role sahip olduğu belirlenmiş ve elde edilen sürgünlerin, sürgün çoğaltımı için kullanılması planlanmıştır. Bu aşamada sürgünler 1 mg L^{-1} konsantrasyonda metatopolin ve 5 mg L^{-1} gümüş nanopartikülleri ilave edilmiş MS besin ortamında kültüre alınmıştır. Kültür sonunda eksplantlarda, maksimum sürgün sayısı (13.67 sürgün/eksplant) kaydedilmiştir. Bu sürgünlerin sürgün uçları bir sonraki adımda çiçek indüksiyonunun sağlanması için kullanılmıştır. Çiçek indüksiyonu için, 7 mg L^{-1} bir konsantrasyonda gümüş nanopartiküller ile takviye edilmiş MS ortamında kültüre alınan sürgün uçlarının; maksimum çiçeklenme oranı (%51,67), sürgün başına maksimum çiçek sayısı (2,33 adet), maksimum çiçek açma oranına (%100) sahip oldukları belirtilmiştir. Bu çiçekler, kendi kendine döllenerek meyve oluşturmuşlardır. 90 günlük kültür sonunda da en yüksek meyve verme oranına (%56,67), meyve sayısına (1,67 meyve) ve meyve çapına (1,13 cm) ulaşıldığı belirlenmiştir [64].

In vitro kültürlerde elisitör amacıyla kullanılan nanopartiküllerin bitki fizyolojisi üzerine olan etkileri değişkendir. Arslan'ın (2022) yapmış olduğu bir çalışmada, yeşil sentez yöntemi kullanılarak üretilen Ag nanopartiküllerinin *Salvia Sclarea* bitkisinde sürgün rejenerasyonu ve sekonder metabolit içeriklerine olan etkileri araştırılmıştır. Eksplant olarak bitkinin nodal segmentleri farklı konsantrasyonlarda (0, 25, 50, 100 mg L^{-1}) AgNP veya AgNO3 ilave edilmiş, 1 mg L^{-1} MT (meta-topolin) ve 0,2 mg L^{-1} IAA içeren MS besin ortamlarında kültüre alınmıştır. Kültür sonrası elde edilen verilere göre; AgNP ve AgNO3 uygulamalarının eksplant başına düşen sürgün sayısında istatistiksel olarak farklılık olmadığı, fakat sürgün uzunluğu, sürgün yaş ve kuru ağırlığı bakımından farklılık gösterdiği belirlenmiştir. Sürgün uzunluğu bakımından ise en iyi sonuç kontrol grubundan elde edilmiş olup (18.29 mm), 25 mg L^{-1} AgNP içeren ortam (14. 08 mm) ile kontrol uygulaması aynı grupta yer almıştır. Sürgün yaş ağırlığı bakımından incelendiğinde ise kontrol uygulaması ve 25 mg L^{-1} AgNP konsantrasyonundan elde edilen değerlerin (5.33-7.53 mg) en iyi sonuç verdiği gözlemlenmiştir. Sürgün kuru ağırlığı bakımından ise en yüksek sonuç 50 mg L^{-1} AgNP (0,74 mg) içeren ortamdan elde

edilmiştir. Denemede ayrıca, rejenere sürgün yapraklarının sekonder metabolit içeriğine bakılmış ve besin ortamına AgNP ve AgNO3 ilavesinin yapraklardaki sekonder metabolit içeriklerini ve miktarlarını artırdığı tespit edilmiştir [65].

T. undulata'da yapılan diğer bir çalışmada ise, bitkinin nodal eksplantları 60 µg L^{-1} Ag nanopartikülleri, 2,5 mg L^{-1} BAP ve 0,1 mg L^{-1} IAA ile güçlendirilmiş MS ortamında kültüre alındıklarında; sürgün sayısı, uyarılmış sürgünlerin uzunluğu ve üretilen sürgünlerin yüzdesi önemli artış göstermiştir [66]. Buna ek olarak, Ag nanopartiküller ile yapılan muamelede, eksplant yaşlanmasını geciktirdiği ve yaşama oranını artırdığı belirlenirken, daha yüksek konsantrasyonlarda (60 µg L^{-1}'in üzerinde) ise sürgün rejenerasyonu üzerinde negatif etkiye sahip olduğu belirlenmiştir [3]. *Brassica nigra* bitkisinde yapılan bir çalışmada, MS ortamına ZnO nanopartiküllerinin (500–1500 mg L^{-1}) eklenmesi tohum çimlenmesini önemli ölçüde inhibe ettiği ve ZnO nanopartiküllerinin varlığında bitkinin sürgün ve kök uzunluklarını olumsuz etkilediği belirtilmiştir. Öte yandan, 1–20 mg L^{-1} ZnO nanopartiküllerini içeren MS ortamında *B. nigra*'nın gövde eksplantlarının büyümesi ve kök oluşumu gözlenmiştir [67]. Anwaar ve ark.'nın gerçekleştirdikleri bir çalışmada ise CuO nanopartikülleri (15-20 mg L^{-1}) ile muamelenin çeltik çeşitlerinde organogenezi artırdığı tespit edilmiştir [68]. *Daucus carota*'da yapılan bir çalışmada, Fe_3O_4 içeren MS ortamında somatik embriyo sayısında azalma gözlemlenmiştir [69]. TiO_2 nanopartiküllerinin de sitokinin ve giberellik asit gibi bitki büyüme düzenleyicileri ile benzer bir rol oynayabileceği bildirilmiştir [70]. Domokos-Szaabolcsy ve ark. (2012), *Nicotiana tabacum* bitkisinde yapmış oldukları çalışmada, selenyum nanopartiküllerini ve selenatı karşılaştırmışlardır. Selenyum nanopartiküllerinin organogenezi uyardığı ve kök sisteminin gelişimini %40'a kadar artırdığı, selenatta ise böyle bir etkinin gözlenmediği raporlanmıştır [71].

Sekonder metabolit üretimi: Sekonder metabolitler, insanlar için tıbbi öneme sahip bileşiklerdir. Bu durum, bitkilerde sekonder metabolit üretiminin artırılması ihtiyacını doğurmuştur. Bitki hücre, doku ve organ kültürleri yoluyla sekonder bileşiklerin içeriğini iyileştirmek için yapılan uygulamalardan bazıları; besin ortamı bileşiminin değiştirilmesi, prekürsörlerin ve elisitörlerin sağlanması ve çevresel koşulların değiştirilmesini içermektedir [37, 73]. Diğer uygulamalara ek olarak nanopartiküller de bu bileşiklerin artan üretimi için kullanılabilmektedir. Nanopartiküllerin bitkilere ve *in vitro* kültürlere uygulanmasının, önemli sekonder metabolitlerin üretimini sağlayabileceği çeşitli

araştırmalarla öne sürülmüştür. Örneğin, nanopartiküllerin optimize edilmiş bir şekilde bitki doku kültüründe uygulanması sonucunda; gümüş nanopartiküllerin, *Echinacea purpurea* hücre süspansiyon kültürlerinde kikorik asit içeriğini [74] ve çemen otu bitkisinde diosgenin konsantrasyonunu [75] artırdığı, ayrıca fenoliklerin ve fitoaleksinlerin biyosentezini de etkilediği raporlanmıştır. Bunlara ek olarak alüminyum oksit (Al_2O_3) nanoparçacıklarının eklenmesi, tütünün hücre süspansiyon kültürlerindeki fenolik içeriği önemli ölçüde artırmıştır [53].

Amir ve ark.'nın 2019 yılında yapmış oldukları çalışmada, sekonder metabolitleri nedeniyle tıbbi açıdan önemli ve tehdit altında bir tür olan *Caralluma tuberculata* kullanılmış ve kallus kültürleri yoluyla sürdürülebilir biyokütle ve sekonder metabolit üretimi için *in vitro* kültürlere farklı oranlarda gümüş nanopartiküller ve bitki büyüme düzenleyicileri eklenmiştir. Sonuçlar, çeşitli gümüş nanopartikül konsantrasyonlarının, MS ortamında bitki büyüme düzenleyicileri ile birlikte kallus proliferasyonunu önemli ölçüde etkilediğini ve kallus yaş (kontrol grubuna göre 0.637mg L^{-1} artış) ağırlığını arttırdığı göstermiştir. En yüksek yaş (0.78 g L^{-1}) ve kuru (0.051 g L^{-1}) kallus biyokütle birikimi, 0,5 mg L^{-1} 2,4-D, 3,0 mg L^{-1} BA ve 60 µg L^{-1} Ag nanopartikülleri içeren MS besi ortamından elde edilmiştir. Bunun yanı sıra, 90 µg L^{-1} Ag nanopartikülleri içeren MS besin ortamı ile oluşturulan kallus kültürlerinin fitokimyasal analizine bakıldığında ise; kalluslarda sırasıyla daha yüksek fenolik (TPC:3,0 mg), flavonoidler (TFC:1,8 mg), fenilalanin amonyak (PAL: 5,8 U mg^{-1}) üretimi ve antioksidan aktivitesi (%90) gözlenmiştir. Ayrıca, süperoksit dismutaz (SOD: 4,8 U mg^{-1}), peroksidaz (POD: 3,3 U mg^{-1}), katalaz (CAT: 2,5 U mg^{-1}) ve askorbat peroksidaz (APX: 1,9 U mg^{-1}) gibi antioksidan enzimlerin aktivitelerini de artırdığı tespit edilmiştir. Çalışma sonunda, gümüş nanopartiküllerin, tıbbi açıdan önemli olan *C. tuberculata*'nın kallus kültürlerinde biyoaktif antioksidanların artırılması için etkin bir şekilde kullanılabileceği sonucuna varılmıştır [76].

Al-Oubaidi ve Mohammed-Ameen (2014) tarafından yapılan bir çalışmada, gümüş nanopartikülleri ile muamele edilen *Calendula officinalis*'in kallus kültürlerinde uçucu yağların miktarlarının arttığı bildirilmiştir [77]. Benzer şekilde, titanyum oksit nanoparçacıklarının eklenmesi, *Cicer arietinum*'un kallus kültürlerinde klorojenik asit, sinamik asit, gallik asit, o-kumarik asit ve tanik asit miktarını artırmıştır [3]. Ayrıca, farklı nanopartiküllerin kombinasyonlar şeklinde uygulanmasının bitkilerde metabolit içeriğini

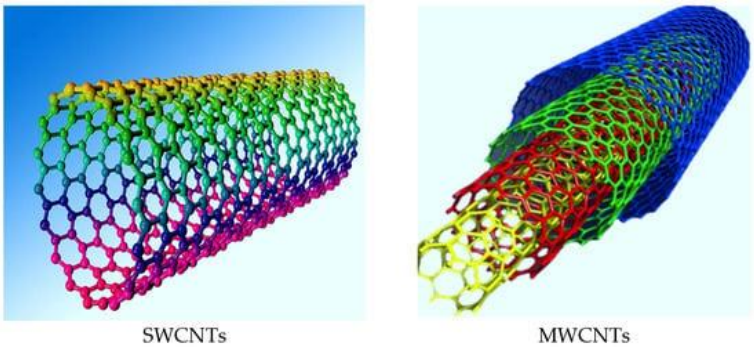
artırmada etkili olduğu da kanıtlanmıştır. Örneğin, Au-Ag nanopartiküllerin 1:3 oranlarında eklenmesi ile *Prunella vulgaris* kallus kültürlerinde toplam fenolik bileşiklerin birikiminin arttığı belirlenmiştir [58]. Bitkilerde sekonder metabolit üretiminin artırılması için kullanılan nanopartikül çeşitleri arasında Ag nanopartikülleri, ZnO nanopartikülleri, $CuSO_4$ nanopartikülleri, Fe nanopartikülleri ve TiO_2 nanopartikülleri yer almaktadır [62]. Nanopartiküllerin uygulanması ile *Arabidopsis thaliana* fidelerindeki antosiyaninler [78], *Stevia rebaudiana*'nın sürgün kültürlerindeki steviol glikozitler [60], *Satureja khuzestanica* sürgün kültürlerindeki rosmarinik asit ve kafeik asit [79], Aloe vera süspansiyon hücrelerinde aloin [80], *Hyoscyamus reticulatus*'un saçaklı kök kültürlerinde hiyosiyamin ve skopolamin [81] ve *Capsicum frutescens* hücre süspansiyon kültürlerinde kapsaisin [82] gibi sekonder metabolitlerin üretimini artırdığı raporlanmıştır.

Ancak nanopartiküllerin bitkilerde sekonder metabolitlerin üretimini artırdığına dair çalışmalar bulunmasına karşın, bitkilerdeki sekonder metabolizmanın hızlandırılmasının altında yatan mekanizma henüz tam olarak anlaşılamamıştır. Bu konu üzerine birkaç hipotez sunulmuştur. Bu hipotezler arasında, ROS'un neden olduğu stresle başa çıkmak için sekonder metabolizmayı tetikleyen nanopartiküller tarafından sekonder metabolitlerin üretilmesi ve sekonder metabolitlerin biyosentezinde yer alan çeşitli genlerin daha yüksek eksprese edilmesi bulunmaktadır. Kaveh ve ark. (2013), Ag nanopartikülleri ile muamele edilmiş bitkilerde talinol biyosentetik yolunda yer alan genlerin daha fazla aktive olduklarını bildirmişlerdir [83]. Sonuç olarak, nanopartiküllerin farklı bitkilerde ve bitki hücre kültürlerinde biyoaktif bileşiklerin üretimini önemli ölçüde etkiledikleri ve bu nedenle değerli metabolitler üretmek üzere bitki hücre, doku ve organ kültürlerinde standart elisitörler olarak kullanılabilecekleri de ileri sürülmüştür [37].

Karbon bazlı nanopartiküller (çok duvarlı ve tek duvarlı nanotüpler)

Karbon bazlı nanaopartiküller, yüksek elektriksel ve termal iletkenlik, mekanik dayanım gibi benzersiz fizikokimyasal ve yapısal özellikleri taşıyan bir nanomalzeme sınıfıdır [84]. Bu nanomalzeme; metalli veya metalsiz, küçük boyutlu ve optik, elektriksel ve manyetik özelliklere sahiptir [85]. Karbon bazlı nanaopartiküller iki gruba ayrılmaktadır: çok duvarlı karbon nanotüpler (MWNT’s-Multi-walled carbon nanotubes) ve tek duvarlı karbon nanotüpler (SWNT- Single-walled carbon nanotubes) [86] (Şekil 3). Yapısal

olarak, çok duvarlı karbon nanotüpler (MWNT'ler), eş eksenli olarak yerleştirilmiş birçok silindirden oluşmaktadır (Şekil 3). Her silindir, içi boş bir çekirdeği çevreleyen tek bir grafen tabakasına sahip olup MWNT'lerin 2-100 nm aralığında dış çapı ve 1-3 nm'lik iç çapı bulunmaktadır [87]. Bunun yanı sıra, tek duvarlı karbon nanotüpler (SWNT'ler) ise tek bir grafen silindirden oluşmakta ve çapları 0,4 ile 2 nm arasında değişmektedir [96]. Sarmallıklarına ve çaplarına göre SWNT'ler ya metalik ya da yarı iletken olabilmektedirler [88, 89].



Şekil 3 Karbon nanotüpleri (tek duvarlı-SWCNT ve çok duvarlı-MWCNT karbon nanotüpler) [90]

Fig 3 Carbon nanotubes (single-walled-SWCNT and multi-walled-MWCNT carbon nanotubes) [90]

Bitki sistemlerinde uygulamalar

Nanoteknolojinin hızlı bir büyüme ivmesine sahip olması, nanomalzemelerin özellikle nanopartiküllerin canlı organizmalarla etkileşiminin incelenmesi gerekliliğini de beraberinde getirmiştir. Bu iki nanotüp tipi (çok duvarlı ve tek duvarlı karbon nanotüpler), bitkilerde kullanılan ve incelenen çeşitli karbon nanomalzemelerdendir ve bitki gelişimini hem olumlu hem de olumsuz yönde etkileyebilmektedir [91]. Bu konuda yapılan birçok çalışma, MWCN nanotüplerin önemli DNA hasarına, mikronükleus oluşumuna ve kromozom sapmalarına neden olduğunu bildirmiştir [92, 93]. Karbon bazlı nanotüplerin, agregasyon nedeniyle bitki hücrelerinde fitotoksik etkilere sahip olduğu, hücre ölümüne ve doza bağlı bir şekilde ROS birikmesine neden olduğu da bilinmektedir [94, 95]. Ayrıca karbon nanopartikülleri, transgenik bitkiler elde etmek için kullanılabilen bir nanomalzemedir [85]. Bu tip tepkiler; farklı bitki türleri [94], kullanılan farklı nanopartikül çeşitleri [96] ve bitki gelişiminin değişik aşamaları arasında da farklılık göstermektedir.

Bitki doku kültürlerinde uygulamalar

Bitki doku kültürlerinde MWCNT'ler ile yapılan çalışmalara bakıldığında, *A. thaliana*'nın süspansiyon hücre kültüründe, çok duvarlı karbon nanotüp aglomeralarının (yığınlarının) olası toksisiteleri analiz edildiğinde, hücre kuru ağırlıkları, hücre canlılıkları, hücre klorofil içerikleri ve süperoksit dismutaz (SOD) aktiviteleri değerlerinde düşüş meydana geldiği belirlenmiştir. Bu da MWCNT aglomeralarının *A. thaliana* hücre süspansiyonu için toksik olduğunu göstermiştir [96].

Xiao-ming Tan ve ark.'nın (2009) yapmış oldukları bir başka çalışmada da çok duvarlı karbon nanotüplerin bitki hücreleri üzerindeki olası toksik etkileri araştırılmıştır. Süspanse haldeki çeltik hücreleri (*Oryza sativa* L.) MWCNT'lerle kültüre alındıklarında reaktif oksijen türlerinin (ROS) arttığı ve hücre canlılığının ise azaldığı gözlemlenmiştir. Primer bir antioksidan olan askorbik asit, kültür süspansiyonuna dahil edildiğinde ROS içeriğinin azaldığı ve hücre canlılığının arttığı bildirilmiştir. Geçirimli Elektron Mikroskobu (TEM) altında yapılan incelemelerde hücre duvarlarıyla temas halinde olan tüpler gözlemlenmiş ve hücre duvarlarında MWCNT'lere sahip süspanse haldeki çeltik hücrelerinin, mikrobiyal patojenlerin yaşam döngülerini tamamlamalarını önlemek üzere ROS savunma yanıtı kaskadı olan aşırı duyarlı bir tepkiye maruz kaldıkları rapor edilmiştir [97]. Mohamed Lahiani ve ark.'nın 2013 yılında üç önemli bitki türü olan soya fasulyesi, arpa ve mısırın *in vitro* çimlenmesi üzerine yapmış oldukları çalışmada, MWCNT'lerin etkileri araştırılmıştır. Çalışma sonucunda, çok duvarlı karbon nanotüplerin tohum çimlenmesini, büyümesini ve üç önemli ürün bitkisinin gelişimini etkilediği ve MWCNT'lere maruz kalan tohumların erken çimlendikleri gözlenmiştir. Benzer şekilde, MWCNT'lerin tohum yüzeylerinde birikmesi, tüm ürün bitkilerinde tohum çimlenmesini aktive etmiştir. MWCNT'lerin mısır, arpa ve soya fasulyesinin tohum katlarına nüfuz etme yeteneği, Raman spektroskopisi ve TEM kullanılarak MWCNT'ye maruz kalan tohumların içindeki nanotüp aglomeratlarının saptanmasıyla kanıtlanmıştır. Ters transkripsiyon polimeraz zincir reaksiyonu (RT-PCR) analizi, MWCNT'lerle kaplanmış soya fasulyesi, mısır ve arpa tohumlarında çeşitli su kanalı proteinlerini kodlayan genlerin ekspresyonunun, kaplanmamış kontrol tohumlarına kıyasla arttığını ortaya koymuştur [98]. Begum ve ark. (2011)'nın yapmış oldukları çalışmada ise grafenin *in vitro'*da kök ve sürgün büyümesi, biyokütle, hücre ölümü ve

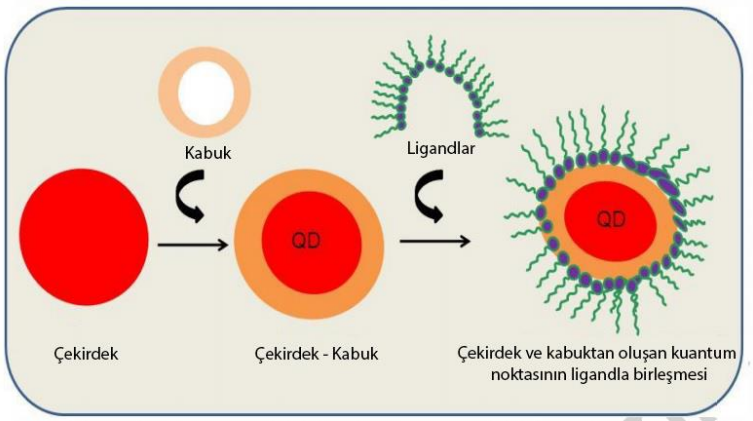
reaktif oksijen türleri (ROS) gibi parametreler üzerindeki etkileri, 500 ila 2000 mg L^{-1} konsantrasyon aralığında araştırılmıştır. 20 günlük maruziyet sonrası, morfolojik ve fizyolojik analizlerin sonucunda, grafenin, kontrole kıyasla bitki büyümesini ve biyokütleyi önemli ölçüde inhibe ettiği gösterilmiş ve grafen ile muamele edilmiş bitkilerin yaprak sayısı ve boyunun, doza bağlı bir şekilde azaldığı bildirilmiştir. ROS ve hücre ölümünde konsantrasyona bağlı bir artışın yanı sıra nekrotik lezyonların semptomlarını gösteren önemli etkiler de tespit edilmiştir. Bu durum, oksidatif stres nekrozunun aracılık ettiği lahana, domates ve kırmızı ıspanak üzerindeki grafen kaynaklı olumsuz etkileri işaret etmiştir. Aynı koşullar altındaki marul fidelerinde ise önemli toksik etki gözlenmemiştir [95]. Ghorbanpour ve Hadian yapmış oldukları çalışmada, çok duvarlı karbon nanotüplerin (25–500 μg mL^{-1}) *Satureja khuzestanica*'nın yaprak eksplantlarında kallus oluşumu üzerindeki etkisini araştırmışlardır. Burada kallus büyümesinin, 25-50 μg mL^{-1} MWCNT ile desteklenmiş B5 ortamında önemli ölçüde teşvik edildiği (%64,5-%77,2) belirlenmiştir. 100-500 μg mL^{-1}'de çok duvarlı karbon nanotüplerin varlığında ise kallus biyokütlesinin azaldığı gözlemlenmiştir [79]. Khodakovskaya ve ark.'nın arkadaşlarının çalışmasında ise, 100 μg mL^{-1} çok duvarlı karbon nanotüplerin 1 mg L^{-1} 2,4-D içeren MS besin ortamına dahil edilmesinin tütün eksplantlarının kallus büyümesini artırdığı bildirilmiştir (kontrol grubu üzerinde %64 artış). Karbon nanotüplerinin kullanımı sonucu, hücre bölünmesi (CycB), hücre duvarı uzantısı (NtLRX1) ve su taşınımı (NtPIP1) ile ilgili genlerin yüksek aktivite göstermeleri nedeniyle kallus büyümesinin arttığı yapılan çalışmada rapor edilmiş, ancak karbon nanotüp muamelesinin (10–600 mg L^{-1}) *Arabidopsis*'te hücre canlılığını ve kuru ağırlığı azalttığı bildirilmiştir [99].

Tek duvarlı karbon nanotüpler (SWCNT), bitki biyoteknolojisinde üzerine az çalışılmış nanopartiküllerdir. Bu nedenle az sayıda literatür bilgisi mevcuttur. Nanotüplerin bitkiler üzerindeki etkilerini belirlemeye yönelik protokollerin geliştirilmesine yardımcı olmak için, tek duvarlı karbon nanotüplerin altı bitki türünde (lahana, havuç, salatalık, marul, soğan ve domates) kök uzaması üzerindeki etkileri araştırılmış ve bu bitki türlerinde kök uzamasıyla ilişkili olarak farklı sonuçlar alınmıştır [100]. Bitki doku kültürlerinde SWCNT uygulamaları incelendiğinde, incir [101] ve mısır [102] gibi çeşitli bitki türlerinde fide büyümesini arttığı bildirilmiştir [103]. Ayrıca, Mohammed ve ark. (2011)

tarafından domates fidelerinde yapılan bir çalışmada da SWCNT nanosistemlerine maruz kalma durumunda bazı olumlu etkilerin elde edildiği ifade edilmiştir [104].

Kuantum nokta nanopartikülleri

Kuantum noktaları, endüstriyel ve biyolojik uygulamalarda kullanılan yarı iletken nanopartiküllerdir. Ekosisteme salınan önemli miktardaki kuantum noktaları, canlı organizmalarda, özellikle kültür bitkilerinde olası toksik etkileri nedeniyle endişe yaratmaktadır [78]. Buğday fidelerinde yapılan bir çalışmada, bitki kök hücrelerinde CdTe (Kadmiyum Tellürid) kuantum noktaları birikimi programlanmış hücre ölümüyle sonuçlanmıştır. Ayrıca kuantum noktalarının floresan özelliğinin, partikül yapısal bütünlüğünü ve su ya da besin ortamında toplanma eğilimlerini temsil ettiği bildirilmiştir [105].



Şekil 4 Kuantum noktasının şematik görünümü [106]

Fig 4 Schematic view of the quantum dot [106]

Bitki sistemlerinde uygulamalar

Djikanovic ve ark. (2012) yapmış oldukları çalışmada, CdSe (Kadmiyum Selenür) kuantum noktalarının *Picea omorika*'nın hücre duvarında bulunan selüloza bağlanabildikleri ve dolayısıyla hücre duvarı için bir biyomarkör olarak kullanılabilecekleri belirtilmiştir [107]. Santos ve ark.'nın (2013), DNA onarımı ile ilgili bir dizi test ve gen ekspresyonunu ele aldıkları öncü bir çalışmada, 10 nM^3 merkaptopropanoik kaplı-CdSe/ZnS kuantum noktalarının yonca hücreleri için sitotoksik ve genotoksik olduğu gösterilmiş, ayrıca CdTe kuantum nokta nanopartiküllerinin ve UV-B radyasyonunun, buğday fidelerinin köklerinde ve sürgünlerinde antioksidan savunma sistemini etkilediği raporlanmıştır [108].

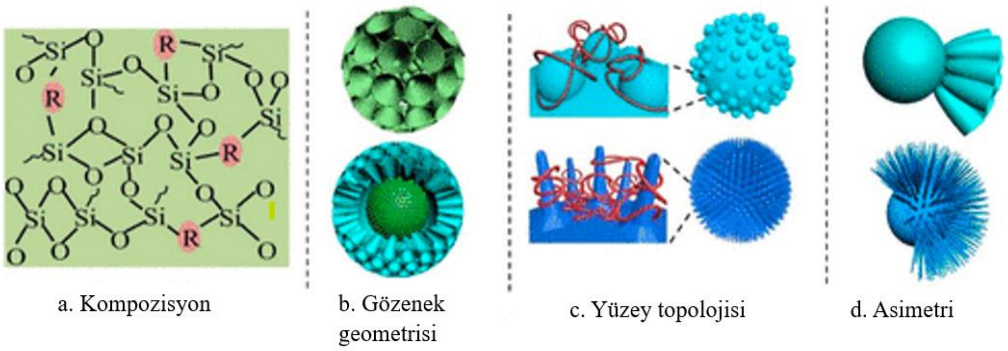
Bitki doku kültürlerinde uygulamalar

Borovaya ve ark.'nın (2016) yapmış oldukları çalışmada, sentezlenen CdS kuantum noktaları (0,097-0,049-0,025-0,012-0,006-,003 ve 0,002 mg mL^{-1} stok çözeltiler halinde) *Nicotiana tabacum* bitkisinden elde edilen protoplast kültürlerinde kullanılmıştır. Bu çalışmada, biyolojik olarak sentezlenen CdS (Kadmiyum Sülfit) kuantum noktalarının çok hassas bir model tür olan *Nicotiana tabacum* protoplastları üzerindeki toksik etkisi araştırılmış ve düşük konsantrasyonlarda biyolojik olarak sentezlenen CdS nanopartiküllerinin herhangi bir toksik etki oluşturmadığı ortaya konmuştur [109]. Santos ve ark.'nın (2013) yapmış oldukları çalışmada ise, merkaptopropanoik asit kaplı CdSe/ZnS kuantum noktalarının eklenmesi ile ortaya çıkan etkilerin değerlendirilmesi için *Medicago sativa* bitkisinin hücre süspansiyon kültürleri kullanılmıştır. Yapılan bu çalışma sonucunda, üstel büyüme fazı aşamasında 100 nM merkaptopropanoik asit kaplı kuantum noktaları eklendiğinde, hücre büyümesinin önemli ölçüde azaldığı ve hücrelerin %50'sinden daha azının merkaptopropanoik asit kaplı kuantum noktalarının eklenmesinden 72 saat sonra canlı kaldıkları bildirilmiştir. *Medicago sativa* hücrelerinde yapılan optik ve konfokal görüntülemede, merkaptopropanoik asit kuantum noktalarının hücrelerin sitoplazmasında ve çekirdeğinde biriktiği ortaya konulmuş ve *Medicago sativa* hücrelerinin, reaktif oksijen türlerinin (ROS) üretimini doza ve zamana bağlı şekilde artırdığı bildirilmiştir. Ayrıca çalışma sonunda, bitki hücrelerinde merkaptopropanoik asit kaplı CdSe/ZnS kuantum noktalarının sitotoksisitesinin, kuantum noktalarının özelliklerine, dozuna ve çevresel uygulama koşulları gibi bir dizi faktöre bağlı olduğu ve *Medicago sativa* hücreleri için güvenli doz aralığının 10-50 nM arasında olduğu tespit edilmiştir [108].

In vitro koşullar altında gelişmiş organlarda ve bitkiciklerde gözlenen değişiklikler olarak da ifade edilen somaklonal varyasyonlar; genellikle kromozom sayısında, kromozom yapısında, DNA dizisinde, DNA metilasyonunda, mitoz bölünmede ve transposable elementlerin aktivasyonundaki değişikliklerle ilişkilendirilirler. Somaklonal varyasyonlar, bitki doku kültürlerinde hem avantajlara hem de dezavantajlara sahiptirler [110, 111]. Nanopartiküllerin mitotik indeks ve DNA bütünlüğü üzerindeki etkileri, bitkilerde protein ve DNA ekspresyonunu değiştirmektedir [112, 113]. Nanoteknoloji, hastalıkların hızlı tespiti ve moleküler tedavisinde, ayrıca bitkilerin besinleri emme kabiliyetinin artırılmasında kullanılabilecek bir inovasyon potansiyeline sahiptir [114].

Silikon bazlı nanopartiküller

Küçük boyutlu moleküller, daha geniş ve gelişmiş uygulamalar için birçok endüstride kullanılmaktadır. Bu uygulamalar arasında, biyomedikal ve tarım endüstrilerinde nanopartiküllerin kullanımına daha fazla önem verilmektedir. Tarımda, nanogübreler şeklinde temel makro ve mikro besinler kullanılarak mahsulün verimi önemli ölçüde artırılmaya çalışılmaktadır. Silika, toprakta bol miktarda bulunan [115], bitkilerin kök ucu yoluyla topraktan emilen ve gövdelerinde biriken bir bileşiktir. Bitkilerde hastalık ve zararlılara karşı dayanıklılık, dona karşı dayanıklılık, bitkinin su kullanımında iyileşme ve toksisitenin azaltılması gibi konularda önemlidir [116, 117]. Bunun yanı sıra, bitkinin hastalıklı kısımlarının çevresinde birikerek iyileştirici bir etki göstermektedir. Silikon, temel bir mikro besindir ve yüzyıllar boyunca bitki büyümesini artırdığı bilinmektedir [118, 119]. Bitki türlerinin çoğunluğunda silisyum birikimi, ksilemden sürgünlere kadar pasif difüzyon ve su akışı yoluyla gerçekleşmektedir [120, 121].



Şekil 5 Silika bazlı nanopartiküllerin bileşimi ve mimarisinin şematik gösterimi [122]

Fig 5 Schematic representation of the composition and architecture of silica-based nanoparticles [122]

Bitki sistemlerinde uygulamalar

Silisyum, köklerdeki etkisine benzer şekilde, yaprakların terleme oranını (%20–35) ve ksilemdeki su akış hızlarını azaltmaktadır, bundan dolayı mısır bitkisinin üzerinde yapılan çalışmalarda, su kullanım verimliliğinin artmasına yol açtığı gösterilmiştir [123, 124, 125]. Bunu destekleyici bir çalışma ise Rathinam ve ark. (2011) tarafından gerçekleştirilmiştir. Çalışmada, silika, toprakla karıştırılarak mısır bitkilerine verilmiştir. *In vivo* olarak, nanosilikanın gövde yüksekliği, gövde genişliği, yaprak sayısı ve silika

içeriği gibi temel parametreler üzerindeki etkisi analiz edilmiştir. Nanosilikanın mısır mahsulü üzerindeki etkisinin her açıdan arttığı bulunmuştur [126].

Toprak, aynı zamanda çeltik ve şeker kamışının üretimi için sınırlayıcı bir faktördür. Bu sınırlamanın üstesinden gelmek üzere çeltik ve şeker kamışı dahil olmak üzere çeşitli ürün bitkileri için silikon bazlı gübreler rutin olarak tavsiye edilmektedir. Çevre dostu bir tarımsal element olan silisyumun değerli bir gübre olarak uygulanması Japonya'da ortaya çıkmıştır. Araştırmacılar tarafından silikanın bitki büyümesi ve verimi üzerindeki etkisini incelemek üzere bir silikon kaynağı olarak, kalsiyum silikatlar [121] ve silisik asit [123] kullandıkları belirtilmiştir [9].

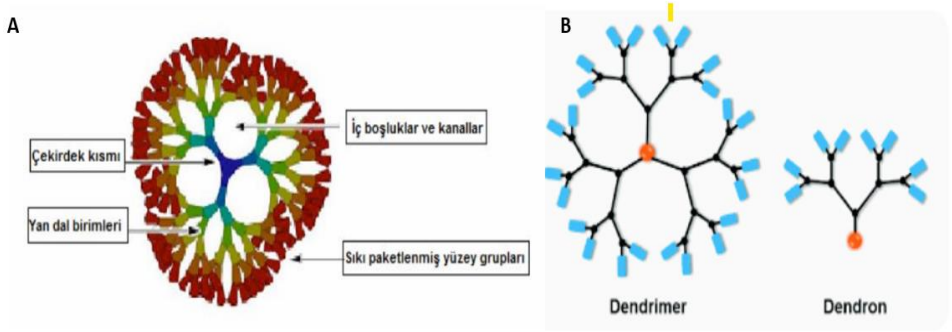
Bitki doku kültürlerinde uygulamalar

Rathinam ve ark. (2011) tarafından mısır bitkisi üzerinde nanosilikanın mahsül iyileştirici özelliği üzerinde durulmuştur. *In vitro'*da nanosilika tuzlarının uygulanmasının, mısır mahsulünün tohum çimlenmesini (%2-11), su kullanım verimliliğini (%53'e kadar) ve toplam klorofil içeriğini (%13-17) artırdığı rapor edilmiştir [126].

Polimerik nanopartiküller

Polimerik nanaopartiküller, farklı polimer tipleri (doğal ya da sentetik) ile hazırlanan, hazırlanma yöntemine göre nanokapsül veya nanoküre olarak adlandırılan, etkin maddenin partikül içerisinde çözündürüldüğü, hapsedildiği veya absorbe edildiği ya da bağlandığı matriks sistemlerdir. Polilaktik asit (PLA), Poliglikolik asit (PGA) ya da bunların kopolimeri olan Poli(d,l-laktik-ko-glikolik asit) (PLGA) gibi biyobozunur ve biyouyumlu polimerlerden üretilen nanopartiküller yaygın olarak araştırılan sistemlerdir. Nanopartiküller, nanoboyutlu yapılarından dolayı mikropartiküler sistemlere kıyasla hücre içine daha fazla alınırlar [127].

Nanoteknolojide polimerlerin bir sınıfı olan dendrimerler [128], nano boyutlu küresel makromoleküllerdir. Bu polimerlerin küresel olmasının nedeni ise yüzeyindeki dendronların (büyüyen dallanmış polimerik kollar) bir çözücü varlığında çözünmeleri ve küre şekline sahip olmalarıdır. Dendrimer örnekleri arasında PAMAM [Poli (amido amin)], PPI [Poli (propilen imin)], POPAM [Poli (propilen amin)] vb. bulunmaktadır [129].



Şekil 6 A Dendrimer kısımları [130] **B** Dendrimer ve dendron terimleri arasındaki farkların şematik gösterimi [131]

Fig 6 A Dendrimer parts [130] **B** Schematic representation of the differences between the terms dendrimer and dendron [131]

Bitki sistemlerinde uygulamalar

Santiago-Morales ve ark.'nın (2014) yapmış olduğu çalışma, amin sonlu G3 PAMAM dendrimerinin çavdar, domates ve marul gibi tek çenekli ve çift çenekli türlerin normal tohum çimlenmesini etkilediğini göstermiştir. Bulgular sonucunda, test edilen dendrimerlerin (veya nano boyutlu küresel makromoleküllerin) tohum çimlenmesi üzerinde olumsuz etkileri olduğu ve yüksek bitki sistemleri üzerinde sitotoksik etkiler ürettiği raporlanmıştır [131].

Bitki doku kültürlerinde uygulamalar

Pasupathy ve ark. (2008), poli(amidoamin) dendrimerini kullanarak bitkilerde yeni bir gen aktarım yönteminin geliştirilmesini hedeflemişlerdir. Araştırmacılar tarafından yeşil floresan proteini (GFP) kodlayan plazmit DNA'nın çim hücrelerine başarıyla dahil edildiği raporlanmış ve transfeksiyon verimliliğinin, hücre kültürü ortam pH'ını ve dendrimerin plazmit DNA'ya olan molar oranını optimize ederek daha da arttığı bildirilmiştir. Çalışmada mevcut teslimat sisteminin kullanımının, başarılı rejenerasyon sistemlerine sahip hemen hemen tüm bitki türlerini kapsayacak şekilde genişletilebileceği öne sürülmüştür [132].

Sonuç

Bu derlemede kapsamında, bitki sistemlerinde ve bitki doku kültürlerinde nanopartiküllerin avantajları hakkında bilgilendirme yapmak, bu alana olan ilgiyi arttırmak ve yeni uygulamaların keşfedilmesine yardımcı olmak hedeflenmiştir.

Nanoteknoloji, nanopartiküllerin benzersiz fizikokimyasal özelliklere sahip olması nedeniyle bitki sistemleri ve bitki doku kültürlerinde yeni ve geniş bir uygulama alanı sunmaktadır. Nanopartüküller, bitkilerin büyümesini arttırmanın yanı sıra, aynı zamanda kuraklık, tuzluluk ve sıcaklık değişiklikleri gibi çevresel streslere karşı da koruma sağlamaktadır. Bitkilerde çeşitli olumlu sonuçlara yol açan nanopartiküllerin, bitki büyümesi ve gelişmesine yönelik etkilerini gösteren mekanizmalar hakkında çok az çalışma yapılmıştır. Ancak nanobilim, bitkilerdeki nanopartiküllerin hareket tarzını anlaşılmasına yol açan yeni fikirlere katkı sağlamaya devam etmektedir. Bitkilerdeki nanopartiküllerin fizyolojik, biyokimyasal ve moleküler mekanizmalarının uygun şekilde aydınlatılması, büyüme ve gelişmeye yönelik çalışmaların iyileştirilmesine öncü olacağı bir gerçektir. Ayrıca bu nanopartiküllerin etki şekillerini, biyomoleküllerle olan etkileşimlerini ve gen ifadelerini araştırmak için fazla çalışmaya ihtiyaç duyulmaktadır. Nanomalzemelerden özellikle de nanopartiküllerin bir taraftan bitkilerde ve bitki biyoteknolojisi üzerindeki etkileri detaylı şekilde ele alınmaya devam edilmeli, diğer yandan da nanoteknolojinin multidisipliner doğası gereği pek çok avantajının olabileceği göz önünde bulundurulmalıdır. Bu kapsamda, bitkilerle ilişkili farklı uygulama alanlarının kısa sürede keşfedilme potansiyeli yüksek olduğu ifade edilebilir.

Abbreviations/Kısaltmalar

NP: Nanopartikül/Nanoparticle, ark.: Arkadaşları/ Et All, UV: Ultraviyole/Ultraviolet, DNA: Deoksiribo Nükleik Asit/ Deoxyribo Nucleic Acid, ROS: Reaktif Oksijen Türleri/Reactive Oxygen Species, RSN: Reaktif Azot Türleri/Reactive Nitrogen Species, CdTe: Kadmiyum Tellürid/ Cadmium Telluride, CdSe: Kadmiyum Selenür/ Cadmium Selenide, CdS: Kadmiyum Sülfit/Cadmium Sulfide, SOD: Süperoksit Dismutaz/ Superoxide Dismutase, CAT: Katalaz/Catalase, POD: Peroksidaz/ peroxidase, APX: Askorbat Peroksidaz/ Ascorbate Peroxidase, CuO: Bakır Oksit / Copper Oxide, ZnO: Çinko Oksit/ Zinc Oxide, Fe: Demir/Iron, Au: Altın/Gold, Ag: Gümüş /Silver, Cu: Bakır/Copper, SiO_2: Silika/Silica, K: Potasyum/ Potassium, Se: Selenyum/Selenium, CeO_2: Seryum Oksit/Cerium Oxide, NiO: Nikel Oksit/Nickel Oxide, $Ni(OH)_2$: Nikel Hidroksit/Nickel Hydroxide, Al_2O_3: Alüminyum Oksit/Aluminum Oxide, In_2O_3: İndiyum Oksit/ Indium Oxide, Co: Kobalt/ Cobalt, CoO: Kobalt Oksit/ Cobalt Oxide,Nd_2O_3: Neodimyum Oksit/ Neodymium Oxide, $CuSO_4$: Bakır (II) sülfat/ Copper(II) sulfate ,MDA: Malondialdehit/Malondialdehyde, H_2O_2: Hidrojen Peroksit/ Hydrogen Peroxide, $O_2^{\cdot-}$: Süperoksit Anyonu/ Superoxide Anion**,** GR: Glutatyon Redüktaz/Glutathione Reductase, GSH: Glutatyon/ Glutathione, TiO_2: Titanyum dioksit/Titanium Dioxide, EtOH: Etanol/Ethanol, µg: Mikrogram/Microgram, mg: Miligram/Milligram, L: Litre/Liter, mL: Mililitre/Milliliter, µL: Mikrolitre/Microliter, NAOCl: Sodyum Hipoklorit/Sodium Hypochlorite, $HgCl_2$: Civa Klorür/Mercury Chloride, ppm: Milyonda Bir/Parts Per Million, MWNT: Çok

Duvarlı Karbon Nanotüp/Multi-walled carbon nanotube, SWNT: Tek DuvarlıKarbon Nanotüp/ Single-walled carbon nanotubes, BA: Benzil Adenin/Benzyl Adenine, BAP: 6-Benzilaminopurin/ 6-Benzylaminopurine, IAA: Indol-3-Asetik Asit/ Indole-3-Acetic Acid, 2,4-D: 2-4 Diklorofenoksi Asetik Asit/ 2-4 Dichlorophenoxy Acetic Acid, MS: Murashıge Skoog, MT: Meta-Topolin/ Meta-Topolin, TEM: Geçirimli Elektron Mikroskobu/ Transmission Electron Microscope, RT-PCR: Ters Transkripsiyon Polimeraz Zincir Reaksiyonu/ Reverse Transcription Polymerase Chain Reaction, PLA: Poliaktik Asit/ Polylactic Acid, PGA: Poliglikolit Asit/ Polyglycolic Acid, PLGA: Poli-d,l-laktik-ko-glikolik Asit/ Poly-d,l-lactic-co-glycolic Acid, PAMAM: Poli-amido amin/Poly-amido amine, PPI: Poli-propilen imin/ Poly-propylene Imine, POPAM: Poli-propilen Amin/ Poly-propylene Amine, GFP: Yeşil Floresan Proteini/ Green Fluorescent Protein

Acknowledgments / Teşekkürler

We would like to thank Ege University and the Department of Bioengineering for making it easy to access resources while writing this review.

Bu derleme yazarken, kaynaklara ulaşımda kolaylık sağlayan Ege Üniversitesine ve Biyomühendislik Bölümüne teşekkür ederiz.

Funding / Fon desteği

The author did not receive support from any organization for the submitted work.

Yazar, gönderilen çalışma için herhangi bir kuruluştan destek almamıştır.

Data Availability statement / Veri Kullanılabilirliği bildirimi

The author confirms that the data supporting this study are cited in the article.

Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.

Compliance with ethical standards / Etik standartlara uyum

Conflict of interest / Çıkar çatışması

The author declare no conflict of interest.

Yazar herhangi bir çıkar çatışması beyan etmemektedir

Ethical standards / Etik standartlar

The study is proper with ethical standards.

Çalışma etik standartlara uygundur.

Authors' contributions / Yazar katkıları

During the study, Buse CAN conducted a literature search, Buse CAN and Aynur GÜREL wrote the review.

Çalışma sırasında Buse CAN literatür araştırması yapmış ve derlemeyi Buse CAN ve Aynur GÜREL kaleme almıştır.

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DÜZELTME

2021 yılı 4. Cilt 3. Sayıda yayınlanan; “Yaban Eriği (*Prunus spinosa* L.) Özütlerinin Antioksidan, Enzim İnhibisyonu ve Antiproliferatif Etkinliklerinin İncelenmesi”, **“Investigation of Antioxidant, Enzyme Inhibition and Antiproliferative Activities of Blackthorn (*Prunus spinosa* L.) Extracts”** başlıklı makalede yer alan; “This work was supported by Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, Project number: FYL 2014/222 as MSc Thesis of MS.” ifadesinde proje numarası (project number) sehven yanlış yazılmıştır ve makalede sorumlu yazar tarafından hatalı olarak yazıldığı beyan edilmiştir. Yapılan bu hatadan dolayı yazarlar, okuyuculardan özür dilemektedir. Makalede yer alan hatanın giderilmesi amacıyla bu düzeltme metni sunulmuştur.

Bildirilen Proje numarası düzeltilmiş hali:

“This work was supported by Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, Project number: FYL 2014/212 as MSc Thesis of MS.”

ERRATUM

The text you provided seems to be a correction statement related to an article titled **"Investigation of Antioxidant, Enzyme Inhibition and Antiproliferative Activities of Blackthorn (*Prunus spinosa* L.) Extracts,"** published in Volume 4, Issue 3 of the year 2021. The correction addresses an error in the project number mentioned in the article. The corrected project number is "FYL 2014/212" instead of the previously reported "FYL 2014/222." This correction is being made by the responsible author(s) to rectify the mistake and apologize to the readers for any confusion caused by the error.

The corrected reported project number is:

"This work was supported by Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, Project number: FYL 2014/212 as MSc Thesis of MS."

Research article

Sönmez, M., et al., Investigation of Antioxidant, Enzyme Inhibition and Antiproliferative Activities of Blackthorn (Prunus spinosa L.) Extracts. International Journal of Life Sciences and Biotechnology, 2021. 4(3): p. 360-380. DOI: 10.38001/ijlsb.851220

Investigation of Antioxidant, Enzyme Inhibition and Antiproliferative Activities of Blackthorn (*Prunus spinosa* L.) Extracts

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ABSTRACT

Natural products have a key role for drug discovery in pharmacology and medicine. *Prunus spinosa* L. (blackthorn) grown in Çanakkale province in western Turkey, is known as a medicinal plant, a rich source of biologically active compounds such as phenolics, flavonoids and anthocyanidins. The flower and fruit extracts of the plant are subjects of many studies, but they usually lack details of its potential for bio-inhibition studies. Thus, this study aimed to investigate the antioxidant, enzyme inhibition and antiproliferative activity studies of the methanol, ethyl acetate, dichloromethane, and *n*-hexane extracts of the plant. The ethyl acetate and methanol extracts showed better antioxidant activity with DPPH, FRAP, CUPRAC, and TEAC assays. Enzyme inhibition studies of the extracts were performed using *β*-lactamase, proteases and tyrosinase. The methanol (FL) and ethyl acetate (FL and L) extracts at the concentration of 10 mg/mL, displayed better inhibition against *α*-chymotrypsin, trypsin, and papain with values of 22.6%, 34.7% and 92.1%, respectively. Furthermore, the methanol and ethyl acetate extracts have displayed higher cytotoxic effect against cancer cells such as Hep3B and HT29 compared to healthy cells (HUVEC) using MTT assay. The findings suggest that *P. spinosa* L. extracts and their components may be potential for further investigations of novel drug candidates from natural sources.

ARTICLE HISTORY
Received
05 January 2021
Accepted
18 March 2021

KEY WORDS
Prunus spinosa L., enzyme inhibition, proteases, cytotoxicity, antioxidant

Introduction

Medicinal plants that are generally used in the health area, are the most important primary sources of naturally occurring bioactive compounds in the pharmaceutical industry [1]. There is an increasing interest to use these plants to prevent and treat chronic diseases like cardiovascular (CVD) [2,3]. Therefore, searches must continue to find valuable lead compounds from medicinal and aromatic plants [4]. Up to now, it was calculated that

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about 40% drugs in the pharmaceutical markets are derived from plant bioactive components [5]. There are numerous medicinal plants that have not been investigated by the researchers. Among those plants, *Prunus spinosa* L. possesses effective and valuable secondary metabolites [5,6] for which limited information is available. *P. spinosa* L. (Blackthorn or sloe, and also its name is "çakal eriği" in Turkish) which belongs to the Rosaceae family, is growing worldwide [6,7]. The taxonomy of the genus is complicated due to polymorphism and wide ecological tolerance of the species. There has been no taxonomic study of genus *Prunus* in Turkey since Browicz's work, so it is indicated that the studies are not enough to understand its genus and species [8,9]. It is widely used as a source of natural bioactives which have an important role to prevent diseases such as cardiac, hypertensive etc. [3]. It has reported that its leaves and flowers are used for constipation and diuretic and also flowers are used as laxatives in Turkey [7]. According to ethnopharmacological sources, the researchers have been interested in the flowers of this plant due to its medicinal usages such as vasoprotective, diuretic, anti-inflammatory and, spasmolytic activities [5,10,11]. The fruits of the plant have also been used for mild inflammation of oral and pharyngeal mucosa [3,5,6]. Based on the literature reports, highly rich biologically active compounds such as flavonoids, a large group of secondary metabolites, have been identified in *P. spinosa* L. extracts. Especially anthocyanins are present in the fruits and flowers of the plant. The active extracts were demonstrated for its importance on human health [10,12,13]. The proanthocyanidins that are known with their antioxidant activity, anti-inflammatory, and enzyme inhibition, have been investigated in blackthorns [14]. Furthermore, total phenolics and antioxidant activities of the fruit and flower extracts from some *Prunus* species including *P. spinosa* L. have been investigated in several studies by Folin-Ciocalteu (FC) method and DPPH, ABTS and, FRAP assays.

The enzyme inhibitory potentials of the compounds in the extracts can be determined with the therapeutically important enzymes to find the new inhibitors for drug discovery program. For instance, proteases can be given as an example due to their over activity in diseases such as cancer and relationship with neurodegenerative disorders, inflammatory, cardiovascular, and viral diseases [15-17]. The cellular roles of proteases make them prime targets in drug developments. Such important roles have been highlighted in previous works [15-17]. Inhibitors of serine proteases derived from various plants have

been reported for their roles as repressors in tumor cell growth [18-20]. Another example is tyrosinase that possesses a role in melanin synthesis. In hyperpigmentation, hydroxylation from *L*-tyrosine to 3,4-dihydroxyphenylalanine (*L*-DOPA) is catalyzed by tyrosinase and tyrosinase like enzymes [21]. The overproduction of melanin results in discoloration. Therefore, finding effective tyrosinase inhibitors is of prime importance for developing skin whitening agents [22,23]. Recently, the researchers have focused on the determination of new tyrosinase inhibitors from natural sources like plants for medicinal, cosmetic and food industries due to their bioavailability and non-toxic properties on human health [24-27]. Different extracts obtained from leaves of *Prunus* species were investigated for their tyrosinase enzyme inhibition activities and compared to other plant extracts [28]. Anti-tyrosinase activities at low doses of valuable plant bioactive components including coumarins, anthocyanidins, chalcones, flavanols etc. were reviewed in the latest study [29]. Lastly, β-lactam antibiotics including penicillin derivatives are well known antibiotic drugs. β-Lactamases that hydrolyzes β-lactam rings are important enzymes whose inhibitors like clavulanic acid, tazobactam and sulbactam are preferred for the studies of drug discovery and development [30,31]. Therefore, identification of natural inhibitors or synthetic derivatives and their combination with β-lactamase inhibitors have gained an interest to prevent infectious diseases. To determine new bioactive inhibitors from medicinal plants such as leaves of *P. africana*, anti β-lactamase activity of the extracts was investigated in the literature [32]. As stated, plant components could be proposed as an important source for enzyme inhibition studies [21]. Consequently, there are much needed novel enzyme inhibitors from medicinal plants for prevention of those diseases and to be used in the food and cosmetic industry and biotechnological applications. Antitumor potential of *P. spinosa* L. was also investigated and associated with the enriched phenolic compounds in the fruit extracts [33,34]. In addition, water extract obtained from the fruit of *P. spinosa* was investigated for its apoptotic effect against human colon carcinoma cell line (HT29) with dose- and time-response studies [33]. It is considered that *P. spinosa* L. extracts especially isolated from flower gains importance for the treatment of cancer due to these limited, but promising *in vitro* results.

In the light of these explanations, this study aimed to investigate the biologically active extracts such as flowers, fruits and leaves of *P. spinosa* L growing in Turkey (Fig. 1). The

content of total phenolics and antioxidant activities of the extracts were evaluated by using Folin Ciocalteu method and DPPH (1,1-diphenyl-2-picrylhydrazyl), CUPRAC (Cupric Reducing Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity with ABTS radical cation) assays. Enzyme inhibitory potentials of the extracts were performed against selected enzymes including proteases (trypsin, α-chymotrypsin, and papain), tyrosinase and β −lactamase. Antiproliferative activities of the extracts on Hep3B (human hepatoma cell line), HT29 (human colon carcinoma cell line) and HUVEC (primary human umbilical vein endothelial cells) were succesfully carried out. To our knowledge, this is the first study that is based on the combination of antioxidant, enzyme inhibition and antiproliferative activities of valuable *P. spinosa* L extracts.

Fig 1 Flowers, fruits and leaves of *Prunus spinosa* L.

Materials and methods

Chemicals and reagents

HPLC grade solvents including *n*-hexane, dichloromethane, ethyl acetate, methanol, DMSO were purchased from Sigma-Aldrich and Merck. Biological activity reagents were indicated in our previously reported study; the reference was given in the experimental section. Additionally, human hepatoma carcinoma (Hep3B) and human colon carcinoma (HT-29) cell lines were provided from Cardiff University and Animal Cell Culture Collection (HUKUK, Ankara, Turkey), respectively. HUVEC (Human Umbilical Vein Endothelial Cells) was provided from Bilkent University, Ankara, Turkey). Dulbecco's modified Eagle's medium (DMEM), L-Glutamine, trypsin/ethylenediaminetetraacetic acid (EDTA) solution and, Fetal Calf Serum (FCS) were from Gibco (USA). Silica gel plates (0.25 mm, 60GF 254) were used to perform qualitative analysis with DPPH spraying.

Plant material and preparation of the extracts

Prunus spinosa L. was collected from Panayır place-Ayvacık which is a town and district of Çanakkale in Turkey. The geographic coordinates of Ayvacık are 39° 36' 4" North, 26° 24' 17" East. All parts were identified in the Herbarium Application Research Center and Botanic Garden of Çanakkale Onsekiz Mart University. Taxon's name was recorded as *P. spinosa* L. subsp. *dasyphylla* (Schur) Domain and the altitude was defined as 270 m. It spreads in the bushes and under the forest. It scatters up to 1700 m above sea level. To prepare the plant extract, all parts that were dried without sunlight in the air, were grinded by a blinder and stored at +4 ºC in the refrigerator. The extraction was performed with different solvents including *n*-hexane, dichloromethane, ethyl acetate and methanol, respectively, by Soxhlet extraction. The solvents were removed by using a rotary evaporator under vacuum and stored at +4 ºC for further analysis.

Phenolic content determination

To determine the total phenolics, the most known method was applied to plant samples by using Folin-Ciocalteu (F-C) reagent. The details were reported in our previous studies [35,36]. The concentration of the extracts was prepared as 1 mg/mL. Ferulic acid was used for preparing a standard calibration curve. Total phenolic contents of the extracts were measured at 725 nm by spectrophotometrically using the Perkin Elmer lambda 25 UV spectrophotometer. The data was expressed as mg ferulic acid/100 g extract.

Determination of antioxidant activity

Qualitative analysis

Firstly, plant extracts were analyzed qualitatively using thin layer chromatography (TLC). To detect the active extracts, DPPH solution (4 mg/50 mL in methanol) was prepared. Then, each extract was run on silica gel plates in the mix of proper solvents using *n*-hexane/ethyl acetate and dichloromethane/methanol and sprayed by DPPH solution. The changings of color from purple to yellow were observed at 254 nm following 30 min (Camag UV Cabinet 4). Hereby, active extracts were successfully determined with this assay [36,37].

Quantitative analysis

For antioxidant activity studies, we used DPPH, CUPRAC, FRAP and TEAC assays. DPPH radical scavenging activity was carried out with small modifications by the method of Shoeb *et al.* 2007 as described in our previous work [36,38]. For this purpose, the serial

dilutions (1-0.001 mg/mL) were prepared to be used in the assays. Then, sample solution (500 µL) was treated with DPPH solution (500 µL; 8 mg/100 mL in methanol) for half an hour and the absorbance values were determined at 517 nm spectrophotometrically. IC_{50} values for each solution were determined and then, scanning using a DPPH solution was performed in the range of proper concentrations to identify the most active value. Quercetin was used as a positive standard. CUPRAC assay was implemented according to the literature [39]. Briefly, freshly prepared samples at 0.1 mg/mL concentrations were reacted with copper (II) chloride, alcoholic solution of neocuproine and buffer solution with ammonium acetate for half an hour at the room temperature. The measurement of the absorbances was performed at 450 nm. Quercetin was used as a standard. The data was determined as quercetin equivalent flavonoid concentration. FRAP assay was performed with small modifications [40]. In brief, freshly prepared working solution using TPTZ, ferric chloride, acetate buffer was reacted with 500 µL sample (0.1 mg/mL) and stood at 37 ^{o}C for 10 min and then, the absorbance values were determined at 593 nm. To prepare the standard curve, $FeSO_4.7H_2O$ was used. FRAP values were identified as mmol Fe^{+2}/g of sample. Finally, TEAC assay was applied to the prepared solutions of the extracts according to the literature [41]. In this assay, the experiment was performed using ABTS radical cation. The absorbances for different volumes (50, 75 and 100 µL) of the solutions (1 mg/mL) were determined at 734 nm to calculate the percentage of inhibitions.

Determination of enzyme inhibitory potentials of the extracts

To evaluate the enzyme inhibitory potentials with various enzymes containing proteases (α-chymotrypsin, trypsin and, papain), tyrosinase and *β*-lactamase (penicillinase), the experiment was performed by the method of Rahman *et al.* 2001 [42]. Detailed procedure was also given in our reported study [43]. Stock (10 mg/mL) and diluted (1 mg/mL) solutions for all the parts of the plant were used in the assays. The experiment was conducted in a 96-well plate and total volume was determined as 200 µL. The absorbances were measured by using Molecular Devices Spectramax Plus 384 ELISA microplate reader. Control reactions using PMSF as a positive control were run without inhibitors.

Protease inhibition assay was applied to the extracts using *α*-chymotrypsin, trypsin and, papain with their specific chromogenic substrates [43]. The optimized volumes for *α*-

chymotrypsin, trypsin and papain were determined as enzyme (50 µL), Tris-HCl buffer (pH 7.5) (40 µL), sample (10 µL and 30 µL for papain) and substrate (100 µL). The absorbances of the mixtures incubated at 37 ºC were measured at 410 nm. Tyrosinase inhibition assay was applied like protease inhibition assay. Briefly, the experiment was carried out using a dopachrome method in which L-DOPA was used as a substrate. The reaction was held at 25 ºC for 10 min and activity of tyrosinase was measured spectrophotometrically at 475 nm. *β*-Lactamase inhibition assay that was similar to protease assay, was performed by using penicillinase and nitrocefin. The conditions for 1 mg/mL solutions of the plant extracts were optimized as enzyme (10 µL), Tris-HCl buffer (pH 7.5) (160 µL), sample (10 µL) and substrate (20 µL) for this assay. The volume of the sample (10 mg/mL) was increased from 10 µL to 50 µL. After 10 min incubation at 30 ºC, the absorbance was determined at 495 nm.

Cell viability

To investigate the antiproliferative activities of the extracts, the most-known method (MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)) was applied. Firstly, Hep3B, HT29 and, HUVEC cells were used (50.000 cells in each 96-well) and, cultured overnight at 37 ºC in a humidified incubator (5% (v/v) CO_2 in air). Then, the cells were treated with different concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL in DMSO) of the extracts for 24 h and 48 h. According to our study, the method was carried out [43].

Results

Phenolic contents in the extracts

The total phenolics of *P. spinosa* L. for all extracts was calculated for the first time according to Velioğlu's method as equivalent to ferulic acid [35]. The higher amounts of phenolics were found in the extracts of methanol (FR) (19.00 ± 0.01 mg FAE/100 g extract), ethyl acetate (FL) (15.00 ± 0.01 mg FAE/100 g extract), and methanol (FL) (12.70 ± 0.12 mg FAE/100 g extract). On the other hand, dichloromethane extracts obtained from flowers and leaves of the plant possessed the lower phenolics (Table 2). In this study, total phenolic content possessed the highest value following Soxhlet extraction with methanol due to the efficiency of the solvent polarity. Table 1 shows that the total phenolics in *n*-hexane extracts (FL, FR and L) were significantly higher than

dichloromethane extracts that were found between 0.40 ± 0.03 mg FAE/g and 2.50 ± 0.08 mg FAE/g.

Antioxidant activity

Qualitative analysis

Before investigation of the extracts in biological studies, the method is based on the reduction of 2,2-diphenyl-1-picrylhydrazyl free radical, the active extracts including strong antioxidants give the yellow spots on the TLC plates, was successfully applied to the extract solutions obtained from *P. spinosa* L. and changings of the colors were observed following the spray with DPPH solution. According to our observations, the spots on TLC plates for active extracts such as ethyl acetate and methanol extracts of flower, leaves and fruit and *n*-hexane extract (L) of the plant changed from purple to white and yellow.

Quantitative analysis

Determining the radical scavenging activities of the *P. spinosa* L. extracts, well-known SET based methods such as DPPH, ABTS, FRAP and CUPRAC were used. The alterations of the solvent polarity, extraction method and the type of assay can affect the results of antioxidant activities (Table 1). Following the qualitative analysis of plant extracts using DPPH, inhibitory concentrations (IC_{50}) of the different concentrations prepared from extracts were determined as mg/mL by the measurement of absorbances at 517 nm. According to the results of DPPH assay, the highest antioxidant capacity was provided by ethyl acetate extracts (FR = 327 ± 0.13 µg/mL, FL = 387 ± 0.08 µg/mL, L = 463 ± 0.08 µg/mL) and methanol extracts (FL = 476 ± 0.06 µg/mL, FR = 480 ± 0.05 µg/mL), except methanol extract (L = 830 ± 0.02 µg/mL) that was determined with its highly phenolic content. Then, it was followed by dichloromethane and *n*-hexane extracts. In CUPRAC assay, the highest activity as quercetin equivalent flavonoid concentration was observed in ethyl acetate (FR) (0.36 ± 0.01) and *n*-hexane (FL) (0.34 ± 0.05) extracts, respectively. However, increasing activity between CUPRAC values of methanol flower and fruit extracts have not been determined. As given in the results of FRAP assay, methanol (L) (666.30 ± 0.01 mM Fe^{+2}) and dichloromethane (FR) (651.00 ± 0.06 mM Fe^{+2}) extracts displayed better scavenging activity. According to ABTS assay, the similar results have been determined in methanol (FR) and ethyl acetate extracts (FR). The values were between 151.03 ± 7.90 and 136.87 ± 9.71 mM Tr/g. The highest percentage of

inhibition values for sample volume (100 µL) were obtained with methanol and ethyl acetate extracts, 48.94% and 54.16%, respectively. In conclusion, methanol and ethyl acetate extracts showed higher antioxidant activity as a dose-dependent manner, compared to other extracts. The ability of scavenging free radicals and reduction of metal ions of active extracts has been explained clearly by the synergic effects of the components. These results were shown in Table 1. It should be noted that antioxidant assay may not generally correlate with each other.

Table 1 Total phenolic content and antioxidant activities of *P. spinosa* L. extracts

Extracts	TPC (mg FAE/100 g extract)	DPPH (IC_{50} = µg/mL)	CUPRAC (QERFC)	FRAP (mM Fe^{+2}/g extract)	ABTS/TEAC (mM TR/g extract)
M-FL	12.70 ± 0.12	476 ± 0.06	0.16 ± 0.01	518.20 ± 0.01	142.19 ± 2.39
M-FR	19.00 ± 0.01	480 ± 0.05	0.18 ± 0.02	273.80 ± 0.01	149.46 ± 15.11
M-L	9.00 ± 0.01	830 ± 0.02	0.30 ± 0.01	666.30 ± 0.01	136.87 ± 9.71
EAA-FL	15.00 ± 0.01	387 ± 0.08	0.25 ± 0.02	423.30 ± 0.01	144.57 ± 9.66
EAA-FR	1.25 ± 0.04	327 ± 0.13	0.36 ± 0.01	570.90 ± 0.03	151.03 ± 7.90
EAA-L	12.50 ± 0.07	463 ± 0.08	0.27 ± 0.02	378.50 ± 0.02	144.62 ± 5.16
DCM-FL	0.40 ± 0.02	580 ± 0.01	---	---	142.17 ± 16.81
DCM-FR	2.50 ± 0.08	670 ± 0.04	0.09 ± 0.01	651.00 ± 0.06	138.59 ± 11.45
DCM-L	0.40 ± 0.03	800 ± 0.02	0.17 ± 0.02	320.00 ± 0.01	45.64 ± 10.30
H-FL	0.80 ± 0.00	850 ± 0.01	0.34 ± 0.05	235.50 ± 0.01	73.33 ± 4.89
H-FR	0.80 ± 0.01	1000 ± 0.02	---	---	15.52 ± 3.64
H-L	3.80 ± 0.01	970 ± 0.03	---	---	22.36 ± 3.99

M-FL: Methanol Flower; M-FR: Methanol Fruit; M-L: Methanol Leave; EAA-FL: Ethyl Acetate Flower; EAA-FR: Ethyl Acetate Fruit; EAA-L: Ethyl Acetate Leave; DCM-FL: Dichloromethane Flower; DCM-FR: Dichloromethane Fruit; DCM-L: Dichloromethane Leave; H-FL: n-Hexane Flower; H-FR: n-Hexane Fruit; H-L: n-Hexane Leave. Total Phenolic Content (TPC). Data is expressed as mg of ferulic acid equivalent (FAE)/100 g of extract. [b]Data is expressed as the value of percentage of DPPH inhibition (%). [c]Data is expressed as the IC50 value of DPPH (µg/mL) [d]CUPRAC (QERFC) (Quercetin equivalent of flavonoid concentration). [e]Data is expressed as mM Fe^{+2}/g extract. [f]Data is expressed as mM of Trolox equivalent per gram of sample. The results are expressed as mean ± standard error

Enzyme inhibition studies

In this study, proteases, tyrosinase and *β*-lactamase enzymes inhibitory potentials of the extracts of *P. spinosa* L. were determined via using different reaction temperatures (25 ºC and 37 ºC) and different wavelengths in a microplate reader. Among the reactions, maximal velocity (Vmax) values as milliunits per min for each of extracts were

determined and inhibition values (%) were calculated by using these values. According to protease enzyme inhibition results as given in Table 3, methanol extract (L) demonstrated that the highest inhibition (20.77%) against *α*-chymotrypsin when compared to methanol extract (FL) (0.39%) at 1 mg/mL solutions on the contrary of 10 mg/mL dose of the same extracts. In the reaction rate graph, Vmax values obtained with trypsin enzyme were determined as 74.22 (inhibition of 9.50%) and 183.30 (inhibition of 34.70%) for 1 and 10 mg/mL doses of flower methanol and ethyl acetate extracts, respectively. This correlation was similar to the inhibition of papain enzyme with methanol (FR) and ethyl acetate (FL) extracts in papain enzyme inhibition study.

Table 2 Proteases enzyme inhibition of *P. spinosa* L. extracts

Proteases												
Extracts	**α-Chymotrypsin**				**Trypsin**				**Papain**			
	1 mg/mL		10 mg/mL		1 mg/mL		10 mg/mL		1 mg/mL		10 mg/mL	
	Vmax	% Inh	Vmax	% Inh	Vmax	% Inh	Vmax	% Inh	Vmax	% Inh	Vmax	% Inh
M-FL	38.06	0.39	43.61	22.67	74.22	9.50	359.00	-	32.92	5.15	13.08	77.34
M-FR	35.32	5.34	69.54	14.25	80.64	1.68	285.00	-	21.07	39.29	34.95	39.45
M-L	30.40	20.77	71.55	11.78	76.62	6.58	289.00	-	27.01	22.18	19.29	66.58
EAA-FL	39.32	-	21.66	20.36	77.45	5.57	183.30	34.70	28.29	18.49	-	-
EAA-FR	36.60	4.61	21.15	22.24	94.08	-	220.70	21.34	32.52	6.30	20.67	64.19
EAA-L	38.88	-	21.60	20.58	89.00	-	221.20	21.20	27.55	20.62	4.56	92.10
CT		-		-		-		-	34.82	-	57.74	-

M-FL: Methanol Flower; M-FR: Methanol Fruit; M-L: Methanol Leave; EAA-FL: Ethyl Acetate Flower; EAA-FR: Ethyl Acetate Fruit; EAA-L: Ethyl Acetate Leave; CT-Control; Inh: Inhibition; - (dashed line) indicates no inhibition. Asterisk (*) indicates the volume of sample as 10 µL in the reaction. Control includes (enzyme + substrate + DMSO + buffer solution). Extract includes (enzyme + sample solution + substrate + buffer solution)

In tyrosinase enzyme assay, the major inhibition was determined with methanol (FR and L) extracts for 1 and 10 mg/mL doses, respectively. Although some of the extracts obtained higher Vmax values at 1 mg/mL, there was no inhibition against penicillinase enzyme (*β*-lactamase), the inhibition by all the extracts was provided with changing values from 7.79% to 12.85%. When *n*-hexane extracts (FL, FR and L) were applied to the reaction media containing enzyme and its chromogenic substrate, *n*-hexane extract (FL) showed inhibitory activity against proteases, tyrosinase and penicillinase enzymes at 1 and 10 mg/mL doses (Table 2,3). According to the literature, enzyme inhibitory abilities of blackthorns can be associated with proanthocyanidins [31].

Table 3 Tyrosinase and penicillinase enzyme inhibition of *P. spinosa* L. extracts

Extracts	Tyrosinase				*β*-Lactamase (Penicillinase)			
	1 mg/mL		10 mg/mL		1 mg/mL		10 mg/mL	
	Vmax	% Inh.	Vmax	% Inh.	Vmax	% Inh.	Vmax	% Inh.
M-FL	73.01	-	44.15	16.14	68.63	3.66	68.59	8.35
M-FR	57.87	13.53	43.36	17.64	80.99	-	67.68	9.56
M-L	61.33	8.36	33.02	37.28	76.19	-	67.86	9.32
EAA-FL	61.42	8.23	36.31	31.03	69.15	2.93	65.22	12.85
EAA-FR	61.55	8.03	39.38	25.20	73.68	-	66.82	10.71
EAA-L	64.67	3.38	36.82	30.06	72.11	-	69.01	7.79
H-FL	62.21	7.05	56.86	-	72.60	-	44.38*	15.20*
CT	66.94	-	65.00	-	71.25	-	71.25	-

M-FL: Methanol Flower; M-FR: Methanol Fruit; M-L: Methanol Leave; EAA-FL: Ethyl Acetate Flower; EAA-FR: Ethyl Acetate Fruit; EAA-L: Ethyl Acetate Leave; Hexane Flower; CT-Control; Inh: Inhibition; - (dashed line) indicates no inhibition. Asterisk (*) indicates the volume of sample as 50 µL in the reaction. Control includes (enzyme + substrate + DMSO + buffer solution). Extract includes (enzyme + sample solution + substrate + buffer solution)

Antiproliferative activity of *P. spinosa* L. extracts

To investigate the antiproliferative activities of *P. spinosa* L. extracts on Hep3B and HT29 cancer cells and HUVEC cells at various concentrations for the treatment of 24 h and 48 h. For this reason, we evaluated *in vitro* cell growth of the cancer cells at increasing doses of each extracts. When the extracts were applied to the cells at five different concentrations that were changing from 62.5 to 1000 µg/mL, the cell growth was inhibited at proper dose of the extracts. For instance, although the cytotoxicity of all methanol extracts on HT29 cancer cells was observed for all doses at two different time points, the components of methanol (FL and L) extracts inhibited the cell growth as a dose-dependent (between 250 and 1000 µg/mL) for 24 h compared to DMSO as a positive control. However, the effect of methanol (FR) extract on HT29 cancer cells significantly reduced the cell viability at 1000 µg/mL for 24 h and 48 h treatments. Also, the percentage of cell viability of methanol extracts (FR) was found as approximately 60% and 50% on HT29 cancer cells at 62.5 and 1000 µg/mL doses in time-response manner study, respectively. Whereas methanol extracts displayed different effects on Hep3B cells. The

antiproliferative effect was observed at an active time points, especially for 48 h. The methanol extract of fruit decreased the cell viability on Hep3B cells nearly 65% at 1000 µg/mL dose for 48 h. Most strikingly, methanol (L) extract showed dramatically inhibition on cell viability of Hep3B cells for all doses for 48 h and the percentage of cell viability was determined as 55% at 1000 µg/mL and 500 µg/mL doses. The methanol extracts did not show any proliferative effect on healthy HUVEC cells. The inhibition profiles of methanol and ethyl acetate extracts on HT29, Hep3B and HUVEC cells were given in Fig 2-4.

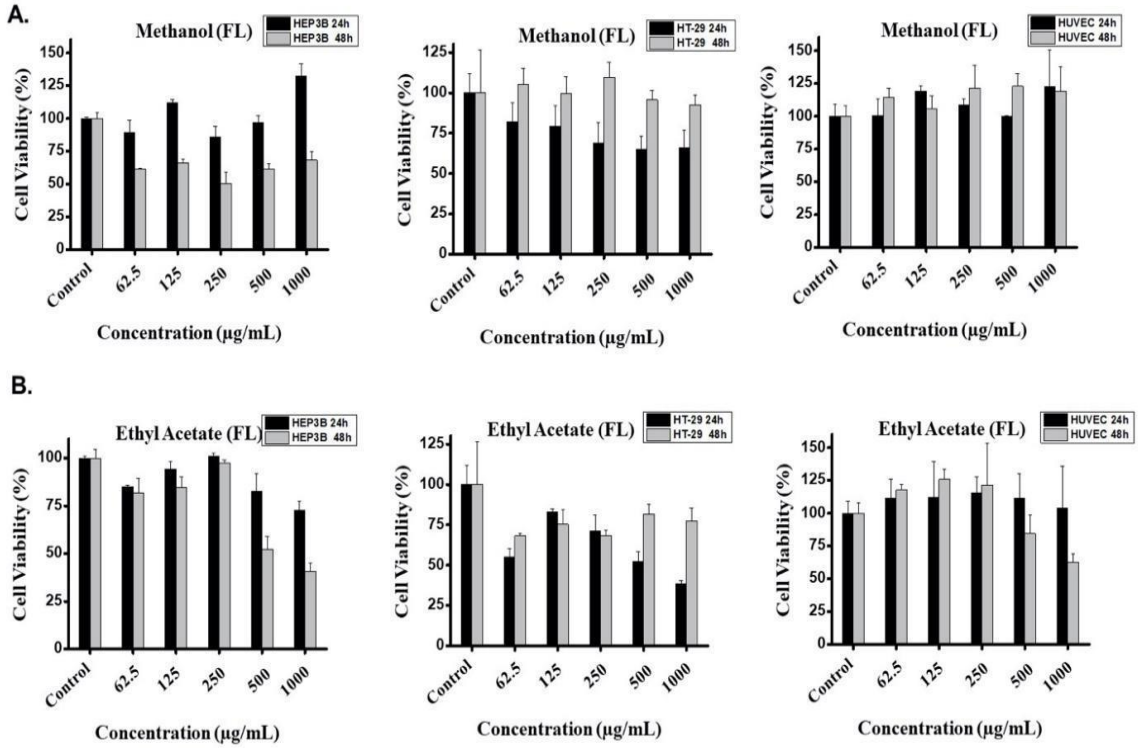

Fig 2 A. Antiproliferative effect of methanol extract (FL) on Hep3B and HT29 cancer cells and HUVEC healthy cells. **B.** Antiproliferative effect of ethyl acetate extract (FL) on Hep3B and HT29 cancer cells and HUVEC healthy cells

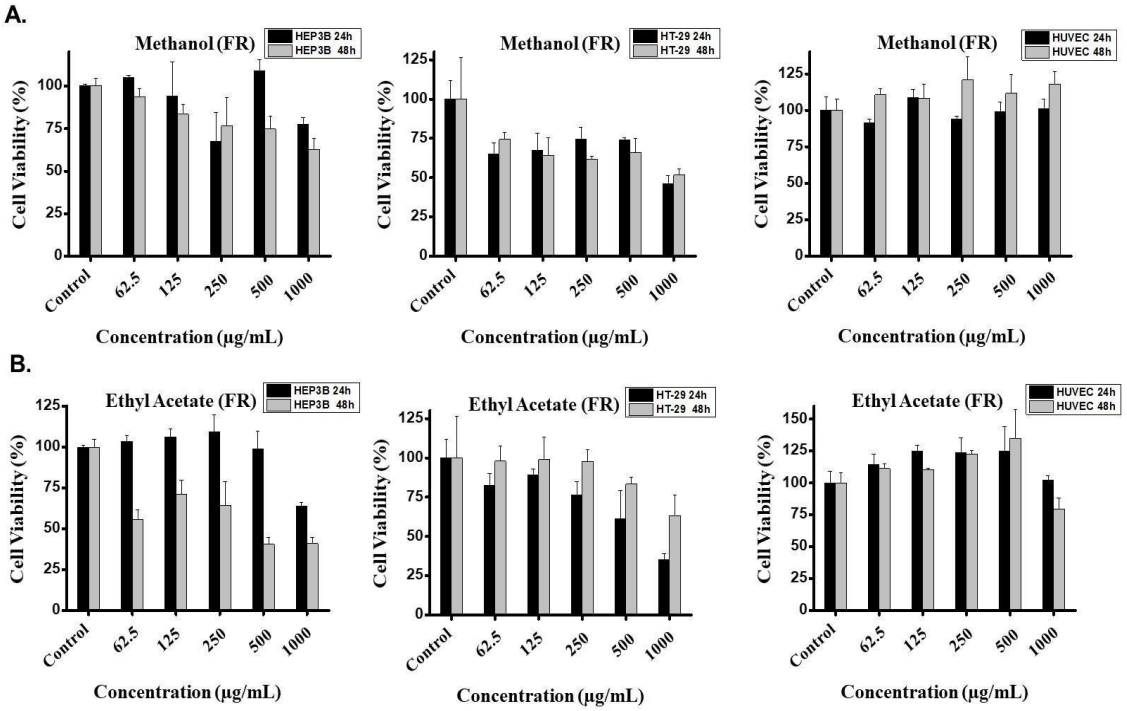

Fig 3 A. Antiproliferative effect of methanol extract (FR) on Hep3B and HT29 cancer cells and HUVEC healthy cells. **B.** Antiproliferative effect of ethyl acetate extract (FR) on Hep3B and HT29 cancer cells and HUVEC healthy cells

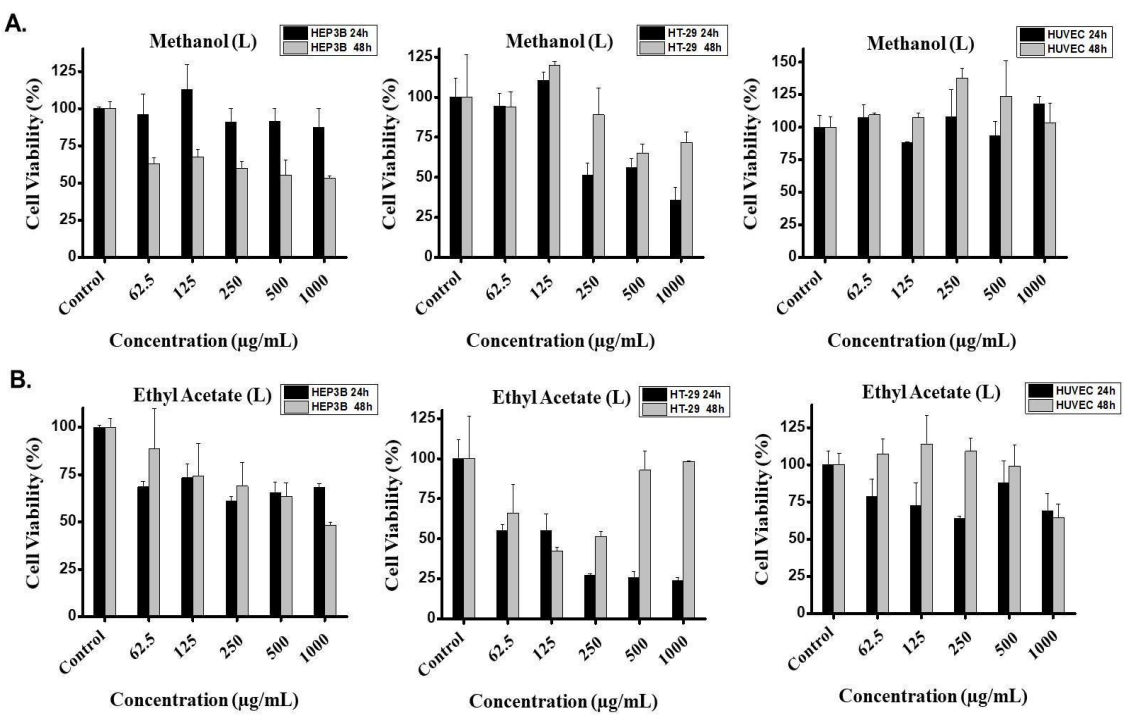

Fig 4 A. Antiproliferative effect of methanol extract (L) on Hep3B and HT29 cancer cells and HUVEC healthy cells. **B.** Antiproliferative effect of ethyl acetate extract (L) on Hep3B and HT29 cancer cells and HUVEC healthy cells

In the cytotoxicity results of ethyl acetate extracts for all parts of *P. spinosa* L., there was a major inhibition on cell viability of cancer cells. Although the ethyl acetate extract (L) displayed linearly inhibition on HT29 cells from changing doses 62.5 to 1000 µg/mL for 24 h, the inhibition correlation was observed at 250, 500 and 1000 µg/mL doses on Hep3B cells for 48 h. The ethyl acetate extract (FR) at 1000 µg/mL verified the cytotoxicity on HT29 cells for 24 h and 48 h. Furthermore, the ethyl acetate extract (FL) showed the antiproliferative effect on HT29 and Hep3B cells at 500 and 1000 µg/mL doses in time response manner study with MTT assay (Fig. 2). When HT29 and Hep3B cells were exposed to dichloromethane extract (FR) of *P. spinosa* L., significant inhibition was determined at 250 µg/mL (approximately 50%) for 24 h (Fig. 5).

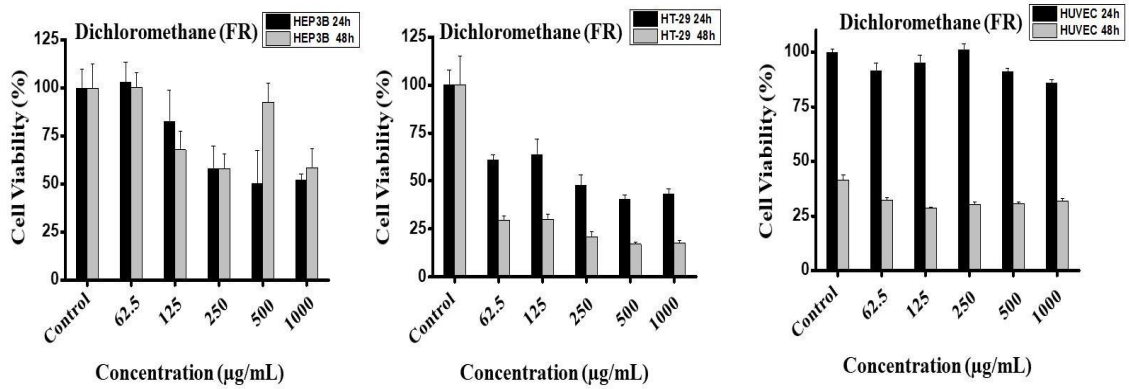

Fig 5. Antiproliferative effect of dichloromethane extract (FR) on Hep3B and HT29 cancer cells and HUVEC healthy cells. The decrease of cell viability on HT29 cancer cells was observed at both 24 h and 48 h treatments between 62.5 and 1000 µg/mL doses. Whereas dichloromethane extract displayed toxicity on healthy cells for 48 h. The cell viability on HUVEC cells at related doses was remarkably decreased. These results may contribute to short time treatment like 24 h

Additionally, although *n*-hexane extracts (FL and L) reduced the cell viability of both cancer cells at different doses, we observed the toxic effect on healthy cells. The methanol and ethyl acetate extracts have a greater cytotoxic potential on studied cancer cells than dichloromethane extracts (FL and L) and *n*-hexane extracts. Consequently, the decreasing of cell viability on cancer cells were observed with dose- and time-dependent studies.

Discussion

Plant phenolics are known with their benefits to human health. They play an important role for antioxidant activity. F-C reagent is used for determining the amount of phenolics [44]. According to the literature findings, total phenol contents of *P. spinosa* L. extracts

(FR and FL) were investigated by several researchers and the results were generally expressed as equivalent to gallic acid [34,45-47]. In the literature, the total amounts of phenolics in the extracts of *P. spinosa* L. have been indicated in alternative equivalents. For instance, in one of the studies, the researchers reported the content of phenolics of methanol extract prepared from fruits of the plant as equivalent to mg gallic acid (2548 ± 18 mg GA/100 g extract) [47]. In the another study, the extracts including dichloromethane, ethyl acetate, ethanol and water obtained from branches, leaves and fruits of *P. spinosa* L. have been studied for determining antioxidant capacities and the amount of total phenolics. The findings showed that the ethanol and ethyl acetate extracts of branches (732.24 ± 6.41 mg GA/g and 499.23 ± 1.99 mg GA/g) and ethanol extract of fruit (359.11 ± 2.54 mg GA/g) had rich phenolic components. Some of the researchers reported that the total phenolic amount of *P. spinosa* L. fruit samples obtained from different solvents such as acetone, ethanol and water determined as changing values from 26.78 ± 4.44 to 19.98 ± 1.28 GAE/g dry weight [34]. Although the results were calculated at different equivalents in this study, the phenolic content of methanol extract (FR) was determined as 19.00 ± 0.01 FAE/100 g that was nearly similar to literature found expressed as GAE [34]. However, synergism in the components of the extract may affect results in the increasing values. Based on the obtained data, active extracts were investigated with *in vitro* enzyme inhibition and cytotoxicity studies on cancer cells to continue analyzing phytochemical behavior and anticancer potentials. To identify qualitative analysis of antioxidant activity in the extracts, TLC that is a simple, easy, and known method was carried out [36,37]. Indeed, the activity depends on the solvent extraction changing polarity of the solvents and it is related with the studied parts of the plant. When the different types of solvents were used, increasing activity was observed on the results in *in vitro* applications. In conclusion, as it is understood from the qualitative analysis, the methanol and ethyl acetate extracts of *P. spinosa* L. might contribute to the major radical scavenging capacities with DPPH. In the previously reported study, it was indicated that the extracts obtained from *P. spinosa* L. showed changing antioxidant activity from low to moderate in the qualitative analysis with DPPH [48]. Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) have a role in deactivation of radicals by antioxidants. To obtain more comparison results, the antioxidant capacity was measured by using SET based methods in this study [49,50]. In

one of the studies, the ethyl acetate extract (FL) of *P. spinosa* L. was identified with its highly antioxidant activity in DPPH and FRAP assays compared to methanol extract [3]. Whereas the ethanol extract (FR) of *P. spinosa* L. possessed the good antioxidant activity in DPPH (257.84 ± 6.57 μg/mL), FRAP (0.10 ± 0.01 Fe^{+2}) and ABTS (184.43 ± 3.88 μg/mL) assays than water and acetone extracts in a reported study [34]. The phenolic compounds and DPPH scavenging activity correlation have been confirmed in one of the reported studies [45]. Additionally, although blackthorn fruits have not been preferred to be used as food products due to their properties such as acidity and bitter taste, rich components having antioxidant properties have been determined in the studies [51]. These results may be led for further studies of *P. spinosa* L. extracts and/or its constituents.

Several studies have been performed to identify the naturally occurring active compounds from the plants and to be used for biological applications. For instance, coumarin based two compounds isolated from the plants have been reported for its tyrosinase inhibitory activity [52,53]. Therefore, showing of *in vitro* enzyme inhibition studies performed with plant extracts may lead to the new concepts for the discovery of plant-based therapeutics. In the literature, there are several studies performed with the medicinal plants to inhibit these selected enzymes [29]. One of the studies suggested that the highest tyrosinase inhibitory activity had been provided by white tea extract compared to green and black tea extracts [21]. Most of the plant protease inhibitors have been determined in preclinical studies. For instance, *Solanum tuberosum* L. inhibited proteases such as trypsin, α-chymotrypsin, and papain at 0-20 mg/mL dose. In addition, *Vigna unguicalata* L. had been determined as a potent inhibitor for trypsin at 2 μg/mL [17]. Valuable proteases inhibitors isolated from plants like *Bauhinia rufa* trypsin inhibitor that inhibited trypsin at 2.9 nM, tested for various diseases [17]. The antioxidant activity of the plant samples can specify the tyrosinase inhibition potential [25,54]. However, in our study, methanol extracts (FR and L) of *P. spinosa* L. exhibited remarkable inhibition against tyrosinase enzyme (13.53% and 37.48%) compared to other studied extracts. These *in vitro* results of methanol fruit extract could be related with its higher total phenolic content (19.00 ± 0.01 mg FAE).

Our preliminary data from *P. spinosa* L. extracts could be shown potential anticancer effects for further studies. Thus, in addition to antioxidant activity and enzyme inhibition

studies, cell viability results were evaluated with time- and dose-response manner studies. We were interested in studying liver and colon cancers, because the liver is a detoxifying organ, colon cancer is also chosen because it is a part of the digestive system when taken as a supplement. Herein, these obtained preliminary results may contribute to determine the anticancer potential of *P. spinosa* L. active components. The extracts including phenolics have gained an interest because of some activities including antioxidant, anticancer etc. According to previously reported study, *P. spinosa* Trigno ecotype extract investigated on human colon cancer cells such as HCT116 and SW480 cells for its cytotoxic and apoptotic activities, the promising results were obtained [55]. According to our study, the results demonstrate a higher cytotoxicity against HT29 cancer cells with methanol extracts (FL and FR) than ethanol extract (L) at the decreasing doses. It was reported that chlorogenic and caffeic acids decreased the cell proliferation on colon cancer cell lines [56,57]. In the literature, the results generally were associated with the bioactive components of the plant. Therefore, it is pointed out the contents of the extracts and synergic effects of the compounds. *P. spinosa* L. active extracts may be potential candidates for further pharmaceutical research.

Conclusion

The findings reveal that methanol and ethyl acetate extracts of *P. spinosa* L. growing in Çanakkale/Turkey seem to have antioxidant activity, enzyme inhibition and antiproliferative properties. The workflow was given in Fig. 6. Solvent extraction affects the isolation of the compounds from the plant. Therefore, the active extracts may have potential for use by related industries for the preparation of natural-based products. We hope that this study may lead to new investigations of *P. spinosa* L. to be used in pharmaceutical applications.

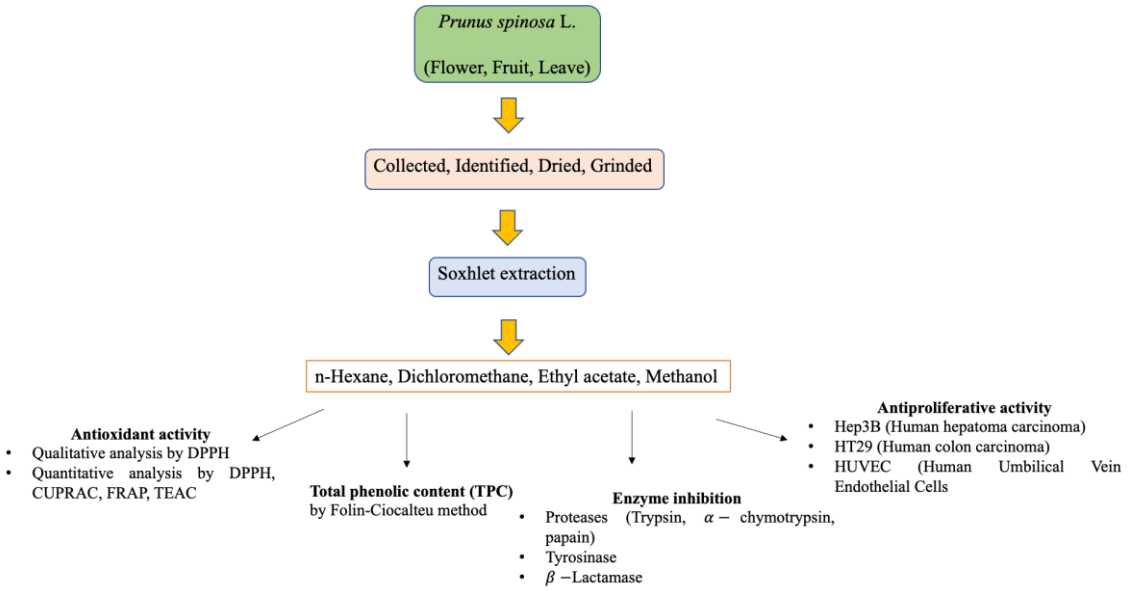

Fig 6 The workflow applied to our study

As stated above, the studies on this medicinal plant have been increasing day by day. Thus, a future prospect of *P. spinosa* plant is the isolation of active components that can be used in the treatment of various cancers and other diseases. Thus, the studies will show a new avenue as well as synergic effect of the components in the extracts. We also thought that the further investigations of the plant against different types of therapeutic enzymes and on human cancer cells may keep light to determination of effect mechanism.

Conflict of interest
The authors declare no conflicts of interest.

Acknowledgments
This work was supported by Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, Project number: FYL 2014/212 as MSc Thesis of MS. We thank Çanakkale Onsekiz Mart University for financial support. The authors also thank Ersin Karabacak for the identification of the plant.

Abbreviations
1,1-diphenyl-2-picrylhydrazyl (DPPH), Quercetin equivalent of flavonoid concentration (QERFC), FAE (ferulic acid equivalent), Total Phenolic Content (TPC), Cupric Reducing Antioxidant Capacity (CUPRAC), Ferric Reducing Antioxidant Power (FRAP), Trolox Equivalent Antioxidant Capacity with ABTS radical cation (TEAC), Hydrogen Atom Transfer (HAT), Single Electron Transfer (SET)

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Table 1 Possible effects of genetically modified organisms

Table 2 Methods of tissue culture in tomato plants

Fig. 1 Amount of GMO products in the world

Fig. 2 Countries with the highest number of GM cultivation in the world

Citation Inside the Text

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Example: Potatoes produced on a global scale are used in many basic areas [1, 2, 3]. Fifty percent of primarily produced potatoes are used for fresh consumption, such as baking, frying, boiling [4, 5, 6, 7].

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Kocacaliskan, I. and I. Tailor, Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. The Journal of Horticultural Science and Biotechnology, 2001. 76 (4): p. 436-440.

Example of an article with 3 authors;

Segura-Aguilar, J., I. Hakman, and J. Rydström, The effect of 5OH-1,4-naphthoquinone on Norway spruce seeds during germination. Plant Physiology, 1992. 100 (4): p. 1955-1961.

Example of articles with 4 or more authors

Arasoglu, T., et al., Synthesis, characterization, and antibacterial activity of juglone encapsulated PLGA nanoparticles. Journal of applied microbiology, 2017. 123 (6): p. 1407-1419.

Example for the book;

Kocalishkan, I., Allelopathy. 2006, Ankara, Turkey: Our Office Press-In Turkish.

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Kaya, Y., F.Z. Huyop, and M.F. Edbeib, Genetic Diversity in Plants, in Advances in Biosciences, F.Z. Huyop and S. Mohammed, Editors. 2019, Penerbit UTM Press. Malaysia: Malaysia. p. 04-24.

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DECLARATION: This work is part of Gülfidan KUYUMCU's MA thesis. Articles on 27-29 September 2017 held in Bayburt in Turkey was presented as a Oral Presentation I. International Organic Agriculture and Biodiversity Symposium

Genetic Analysis Related To Organized Genetic Changes in Potato And Processed Potatoes

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