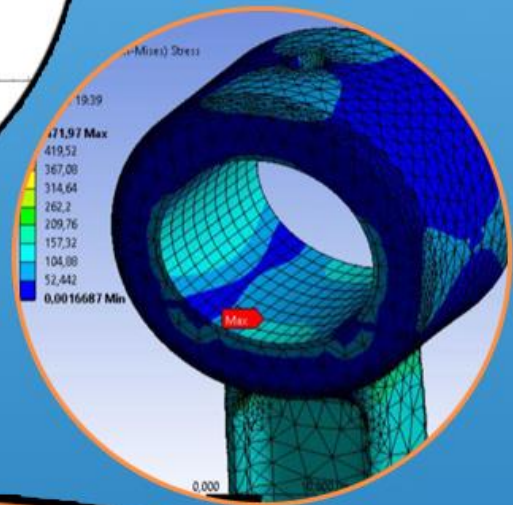
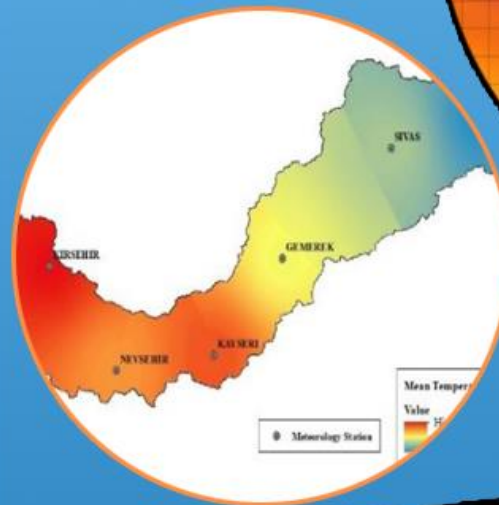
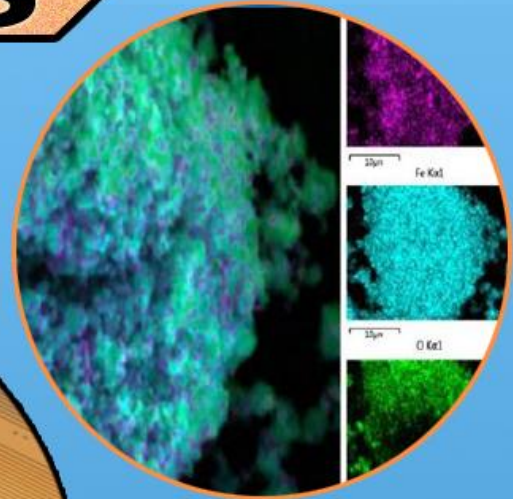
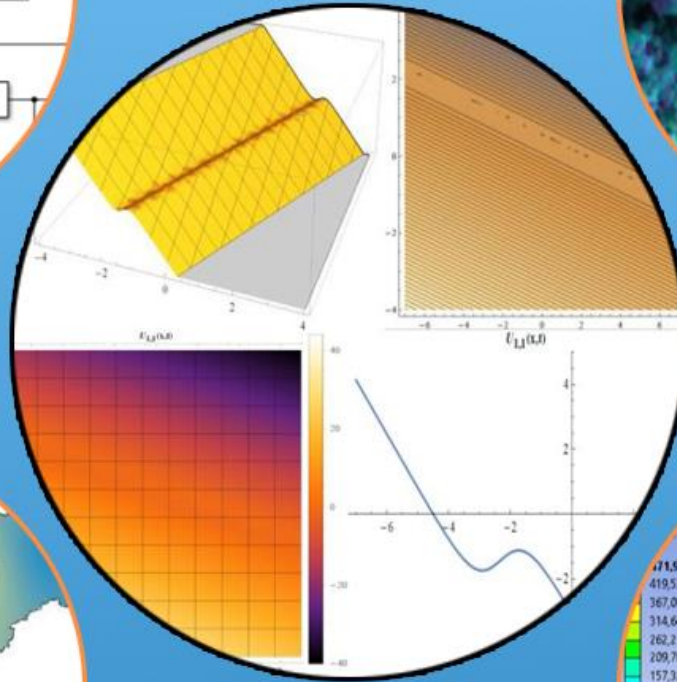
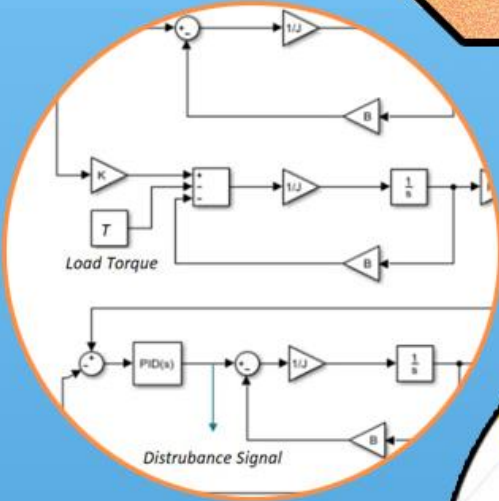


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**TÜRK DOĞA ve FEN DERGİSİ**

TURKISH JOURNAL OF NATURE AND SCIENCE

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# TÜRK DOĞA VE FEN DERGİSİ

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## Türkiye'nin Doğu ve Güneydoğu Bölgelerinde Abort Yapmış Sığır, Koyun ve Keçi Fötüslerinde Pestivirus Enfeksiyonunun Araştırılması

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**Anahtar Kelimeler**  
 Pestivirus,  
 Abort Yapmış Fötüs,  
 Doğu ve Güneydoğu Anadolu, Türkiye.

**Öz:** Bu çalışmada, Doğu ve Güneydoğu Anadolu bölgelerinde bulunan illerde yetiştirilen sığır, koyun ve keçilerin abort olmuş fötüslerinde pestivirus antijen varlığının ticari bir ELISA (AgELISA, IDEXX Laboratories, Inc, Maine, USA) testi ile araştırılması amaçlanmıştır. Elâzığ, Malatya, Tunceli, Bingöl, Bitlis, Diyarbakır, Şırnak ve Hakkâri illerinden 2012 yılı, Ocak-Mayıs ayları arasında 245 sığır, 89 koyun, 36 keçi olmak üzere toplam 370 aborte olmuş fötüs toplandı. Her bir fötüs için, akciğer, karaciğer ve dalak doku numunelerini içeren, bir numune havuzu oluşturuldu. Her bir fötüse ait numune, Ag-ELISA testi ile test edildi. Test sonucunda, 245 sığır numunesinin 81'inde (%33), 89 koyun numunesinin 23'ünde (%25,8) ve 36 keçi numunesinin 8'inde (%22,2) pestivirus antijeni tespit edildi. Bu sonuçlara göre, araştırmanın yapıldığı bölgelerde sığır, koyun ve keçilerde abort yapma nedeni olarak önemli derecede pestivirus enfeksiyonunun sorumlu olduğu ortaya konulmuş oldu.

1

## The Investigation of Pestivirus Infections in Bovine, Ovine and Caprine Foetuses in the East and Southeast Regions of Turkey

**Keywords**  
 Pestivirus,  
 Aborted Foetuses,  
 The East and Southeast Anatolia, Turkey.

**Abstract:** The present study was aimed to investigate the presence of pestiviruses in bovine, ovine and caprine foetuses in the East and Southeast regions of Turkey by antigen enzyme-linked immunosorbent assay (Ag-ELISA) method. For this purpose, 245 bovine foetuses, 89 ovine foetuses and caprine foetuses (in total 370 foetuses) were collected from Elazığ, Malatya, Tunceli, Bingöl, Bitlis, Diyarbakır, Şırnak and Hakkâri provinces during January-May 2012. A pool of foetal tissues (lung, liver and spleen) was created for each foetus. The sample of each foetus was tested with the Ag-ELISA test. Pestivirus antigens were detected in 81 of 245 cattle samples (33%), 23 of 89 sheep samples (25.8%), and 8 of 36 goat samples (22.2%). According to these results, it has been revealed that pestivirus infection is responsible for abortion in cattle, sheep and goats in the investigated regions.

### 1. GİRİŞ

*Flaviviridae* ailesinde bulunan Pestivirus cinsi virüsler tek sarmallı, pozitif polariteli, RNA genomu taşıyan on bir tanınmış tür içerir. Daha önce tanımlanmış olan türlerden bovine viral diarrhoea virus -1 (BVDV-1), Pestivirus A, bovine viral diarrhoea virus-2 (BVDV-2) Pestivirus B, classical swine fever virus (CSFV)

Pestivirus C ve border disease virus (BDV) Pestivirus D olarak yeniden adlandırılmaktadır. Bunlara ek olarak, *Pestivirus* cinsi içinde diğer 7 tür ise Pestivirus E-K olarak yeni sınıflandırmada yer almıştır. Bunlardan Pestivirus E, pronghorn antelope virüs, Pestivirus F,

domuz pestivirusu, Pestivirus G giraffe pestivirusu, Pestivirus H, Hobi benzeri pestivirus aynı zamanda bovine viral diarrhoea virus-3 (BVDV-3) veya atipik ruminant pestivirusu olarak da bilinmektedir. Pestivirus I, Aydın benzeri pestivirus veya koyun pestivirusu, Pestivirus J, rat pestivirusu ve Pestivirus K atipik domuz pestivirusu olarak bilinmektedir [1]. Pestivirusların sınıflandırılması, genetik-antijenik ilişki ve orjin aldığı konakçıya göre yapılmaktadır [2]. Pestiviruslar konakçı tropizmi göstermediği, BVDV 'unun koyunları ve keçileri, BDV'unun ise sığırları enfekte edebildiği gösterilmiştir. Filogenetik analizlerde, 21 Pestivirus A alt tipi (BVDV-1a-u) ve 4 Pestivirus B alt tipi belirlenmiştir (BVDV-2a-d) [3]. Önceden atipik sığır pestivirusları olarak adlandırılan Pestivirus H (BVDV-

3), benzer değişkenliğe sahip olmasına rağmen ancak henüz tanımlanmış alt tipleri olmayan türdür [4]. Sığır pestivirusları koyun, keçi ve domuz gibi diğer evcil hayvan türlerini de enfekte eder [5]. Küçük ruminantların enfeksiyonu olarak bilinen Pestivirus D (sınır hastalığı virusu enfeksiyonunu boğalarda persiste bir enfeksiyon oluşturma yeteneği de dahil olmak üzere sığırlarda normal olarak enfeksiyona da neden olduğu göstermiştir [6]. Sığır pestivirusları, dünya çapında sığır yetiştiriciliğinde ciddi ekonomik kayıplara neden olan önemli enfeksiyöz ajan olarak bilinmektedir [7]. Sığır pestivirus enfeksiyonları genellikle sığırlarda persiste asemptomatik olarak görülür, persiste asemptomatik sığırların etkenin sitopatik efekt oluşturan biyotipi ile süper enfeksiyonu ile mukozal hastalık oluşur. Mukozal hastalığın mortalite oranı daha yüksektir. Sığır pestivirus enfeksiyonları sığırlarda genellikle asemptomatik olarak görülür, ancak tekrar sitopatik efekt oluşturan biyotipi ile enfeksiyon sonucu akut hastalık olarak sığırların mortalite oranı yüksektir. Pestivirus ile enfekte hayvanlarda, transplasental enfeksiyonlar meydana gelebilir. Transplasental enfeksiyonlar embriyonik-fetal ölüm ve abort, embriyonun gelişimsel organ kusurları veya persiste enfeksiyon oluşumu bağışıklık toleransının gelişmesi ve buna bağlı olarak solunum yolu hastalıkları gibi çok faktörlü hastalıklara neden olabilir [8]. Sığırlarda gebeliğin 100-120 gününde meydana gelen fetal enfeksiyon, serebellar hipoplazi, mikroensefalopati, katarakt, mikrooftalmi ve timik aplazi gibi konjenital anormalliklere yol açabilir. Gebeliğin 150 ve 285. günleri arasında meydana gelen enfekte olmuş fetüsler genellikle virustan arınabilir, normal şekilde gelişebilir ve pestivirusa karşı prelokalize nötralize edici antikorlar oluşturabilir. Abortlar, deneysel enfeksiyonlarda gebeliğin 100. gününde virüsle fetüsün enfeksiyonu sonucu gerçekleşir [9].

Dünyada ve Türkiye’de çok değişik bölgelerde pestivirus enfeksiyonlarının sığır, koyun ve keçilerde varlığı ve yaygınlığı serolojik veya virolojik olarak yapılan birçok çalışma ile rapor edilmiştir [14,15,16,17,18,19,20]. Koyunlarda pestivirusa karşı oluşan seropozitifliğin Batı Avusturya’da %29,4 olduğu bildirilmiştir [20]. İran’da abort yapmış fetüsler üzerine yapılan bir çalışmada sığırlarda (111/620) %17,90, koyunlarda (74/525) %14,09, keçilerde (71/442) %16,06, oranında antijen ELİSA testi ile pozitiflik bulmuşlardır [21]. Aynı zamanda Türkiye’de de atık yapmış hayvanlardan sağlanan fetüslerden pestivirus varlığı çalışılmış, abort etiyolojisindeki oranları belirlenmiştir. Albayrak ve ark. [22] Karadeniz Bölgesinde sığır ve koyunlarda abort yapmış fetüslerde pestivirus genom varlığını RT-PCR testi ile araştırmış ve sığır fetüslerinde (6/21) %28,57, koyun fetüslerinde (14/21) %66,66 oranlarında varlığını tespit etmişlerdir. Şevik [23], Türkiye’nin İç, Ege ve Akdeniz Bölgesinden sağladığı abort yapmış sığır, koyun ve keçi fetüslerinde pestivirus varlığını RT-PCR testi ile araştırmış, sığırlarda (61/553) %11, koyunlarda (124/1388) %8,9 ve keçilerde (3/88) %3,4 oranlarında abort etiyolojisinde pestivirus payını tespit etmiştir. Bu araştırma Doğu ve Güneydoğu Anadolu Bölgelerinde yetiştirilen sığır, koyun ve keçi sürülerinde abort yapmış fetüslerde etiyolojideki pestivirus oranını tespit edildiği

ilk çalışma olması nedeni ile önem arz etmektedir. Araştırmadan elde edilen sonuçlara göre, Doğu ve Güneydoğu Anadolu Bölgelerinde keçi fetüslerin %22,2’inde atık nedeni olarak pestivirus tespit edilmiştir. İç, Ege ve Akdeniz Bölgelerinde keçilerde tespit edilen %3,4 oranına göre daha yüksek oranda bulunmuştur. Bunun nedeni Bölgede yetiştirilen keçi ırklarının duyarlılığı ve yetiştirme farklılığı, abort vaka geçmişi, gebeliğin erken veya geç dönemi abort olması gibi nedenlerden kaynaklanmaktadır [13]. Albayrak ve ark. Karadeniz Bölgesinde sığır ve koyun fetüslerinde sırası ile %28,57 ve %66,66 pestivirus oranları tespit etmiş, Şevik [23], İç, Ege ve Akdeniz Bölgelerinden sağladığı sığır ve koyun fetüslerinde sırası ile %11 ve %8,9 oranlarında pestivirus varlığı bulmuştur. Bu çalışmada ise sığır ve koyunlarda sırası ile %33 ve %25,8 oranları ile İç, Ege ve Akdeniz Bölgelerinin oranlarına göre yüksek oranlarda bulunmuştur. Karadeniz bölgesinde tespit edilen oranlara göre sığırlardaki görülme oranı paralel olmasına rağmen koyun pestivirus oranı düşük oranda tespit edilmiştir. Atık etiyolojisindeki sığır ve koyunlardaki pestivirus katkı oranlarının bölgelere göre farklılıklar göstermesinin nedenleri muhtemelen Bölgede yetiştirilen sürülerdeki klinik belirtiler, hayvan ırkları ve yetiştirmedeki bakım ve beslenme şartları ile ilgili yetiştiricilikteki değişikliklerdir [13].

Bu çalışmanın amacı, dünya çapında yaygın olarak enfeksiyonlara sebep olan pestivirusların Sığır, Koyun ve Keçi yetiştiriciliğinin yoğun olarak yapıldığı Elâzığ, Malatya, Tunceli, Bingöl, Bitlis, Diyarbakır, Şırnak ve Hakkâri illerinde yetiştirilen sığır, koyun ve keçi atıklarındaki rolünü ortaya çıkarmaktır.

## 2. MATERYAL VE METOT

### 2.1.Abort Yapmış Fötüs Materyali

Elâzığ, Malatya, Tunceli, Bingöl, Bitlis, Diyarbakır, Şırnak ve Hakkâri illerinden buzağılama ve kuzulama mevsiminde, (Ocak- Mayıs) abort olgularının görüldüğü sığır (n=245), koyun (n=89), ve keçi (n=36), sürülerinden toplam 370 ölü fötüs örneği araştırmanın materyalini oluşturdu (Tablo 1).

Bölgede çoğunlukla Holstein, Friesian ve Simmental ırkı ve/veya melezi sığır ırkları yetiştirilmektedir. Koyunların çoğu Akkaraman ve Morkaraman ırkı olup bölgede genelde yetiştirilen koyun ırklarıdır. Bölgenin keçi ırkı kıl keçisidir. Numune alınan işletmelerde koyun ve keçiler birlikte yetiştirilmekte, bazı işletmelerde sığır yetiştiriciliği de beraberinde yapılmaktadır. Sığır, koyun ve keçi yetiştiricileri tarafından ifade edilen anemnezler alındı Koyun ve keçi sürülerde ölü doğumlar, gelişmemiş zayıf ve zaman zaman anomalili oğlak ve kuzu doğumu şikayetleri çoğunlukla belirtildi.

Sığır, koyun ve keçi abort numunelerinde BVDV antijeninin aranması amacıyla abort yapmış fetüslerin otopsipleri usulüne göre yapıldı. Açılan fetüslerden beyin, akciğer, dalak, böbrek ve karaciğerlerinden alınan doku parçaları 2 mL Phosphate-Buffered Saline ile (1/10) karıştırıldı. MagNa Lyser (Roche, Mannheim,

Germany)'da boncuklu tüplerde 3000 g'de 3 dakika homojenize edildi. Homojenat eppendorf tüplerde 3000 g'de 10 dakika santrifuj edildi. Alttaki katı maddeler ve boncuklar uzaklaştırıldı. Üstte kalan süpernatant inokulum olarak kullanılıncaya kadar -80 °C de saklandı.

**Tablo 1.** Abort olgularının görüldüğü işletmelerinden alınan fötüs örneklerinin sayıları, hayvan türleri ve alındığı iller.

İL	Sığır	Koyun	Keçi	Toplam
Elâzığ	55	2	5	62
Malatya	39	8	4	51
Tunceli	14	5	1	20
Bingöl	36	32	5	73
Bitlis	25	41	13	79
Diyarbakır	48	1	7	56
Şırnak	15	-	-	15
Hakkâri	13	-	1	14
<b>TOPLAM</b>	<b>245</b>	<b>89</b>	<b>36</b>	<b>370</b>

## 2.2. Metod

**Tablo 2.** Yavru atma olgularının görüldüğü işletmelerden alınan abort yapmış fötüs örneklerinin Ag ELISA sonuçları

İL	Sığır (n)	(+)	%	Koyun (n)	(+)	%	Keçi (n)	(+)	%
Elâzığ	55	21	38,18	2	-	-	5	1	20,00
Malatya	39	14	35,89	8	2	25,00	4	-	-
Tunceli	14	3	21,42	5	1	20,00	1	-	-
Bingöl	36	11	30,55	32	8	25,00	5	1	20,00
Bitlis	25	9	36,00	41	12	29,26	13	4	30,76
Diyarbakır	48	16	33,33	1	-	-	7	2	28,57
Şırnak	15	4	26,66	-	-	-	-	-	-
Hakkâri	13	3	23,07	-	-	-	1	-	-
<b>Toplam</b>	<b>245</b>	<b>81</b>	<b>33,00</b>	<b>89</b>	<b>23</b>	<b>25,8</b>	<b>36</b>	<b>8</b>	<b>22,22</b>

## 4. SONUÇ

Çiftlik hayvanı yetiştiriciliğinde, önemli ekonomik kayıplara neden olan abortlar dünya çapında hayvancılığı etkileyen en önemli sorunlardan biridir [10]. Sığır, koyun ve keçilerde abort vakalarının bulaşıcı etiyolojik nedenini oluşturan ajanlar arasında virüsler en önemlisidir. Herpesviruslar ve pestiviruslar abort vakalarına neden olan ana patojenler arasında yer almaktadır [11,12,23]. Bu çalışmada, Elâzığ, Malatya, Tunceli, Bingöl, Bitlis, Diyarbakır, Şırnak ve Hakkâri illerinde yetiştirilen, gebeliğin farklı dönemlerinde abort yapmış toplam 370 adet Sığır, Koyun ve Keçi fötüslerinde pestivirus antijenlerinin varlığı Ag- ELISA testi kullanılarak araştırılmıştır. Amaç atık fötüslerdeki pestivirus etiyolojik rolünü ortaya koymaktır. Teşhis laboratuvarında, abort etiyolojisini adım adım araştırma yaklaşımı tavsiye edilir. Bu maksatla abort etiyolojisi tespitinde, abort vaka geçmişi, gebeliğin erken veya geç dönemi abort olması, mumyalanmış veya taze fetus elde edilmesi, gebe hayvan sayısına göre abort sayısı, ilk vakadan sonra geçen süre, sürülerdeki klinik belirtiler ve yetiştiricilikteki değişiklikler gibi anamnez bilgileri dikkate alınmalıdır [13]. Bu çalışmada kullanılan materyal, hayvan yetiştiriciliğinde bakım ve besleme yönünden önemli değişikliğin olmadığı, yetiştiriciliğin geleneksel yöntemlere göre yapıldığı, hayvanlarda genel klinik belirtilerin zaman zaman görüldüğü, abort vakaları şekillenmiş sürülerden sağlanan fötüslerden oluştu. Örneklerin pestivirus enfeksiyonunun kanıtı için incelenmesinden önce, diğer abort etkenleri yönünden de

İnokulumlarda antijenin varlığı ticari BVDV antijen (Ag) ELISA (BVDV Ag Serum Plus HerdChek IDEXX Laboratories Westbrook, Maine 04092 USA) kiti ile araştırıldı. Test üretici firma protokolüne göre yapıldı.

## 3. BULGULAR

Toplamda 370 atık yapmış fötüs numunesinin doku homojenatlarının Ag ELISA testi sonucunda, sığır sürülerine ait 245 örneğin 81'inde (%33), 89 koyun numunesinin 23'ünde (%25,8) ve 36 keçi numunesinin 8'inde (%22,2), pestivirus antijenlerinin varlığı tespit edildi. Atık Fetuslardan tespit edilen en yüksek pestivirus varlığı oranı sığırlarda Elâzığ'dan sağlanan numunelerden (%38,18), koyun numunelerinde Bitlis'ten sağlanan numunelerde (%29,26), keçilerde ise yine Bitlis yöresinden sağlanan numunelerde (%30,76) tespit edildi (Tablo 2).

incelemeleri yapıldı. Alpay ve ark. sürüde abort geçmişi olan 16 sürüden, abort vakası görülmeyen 6 sürüden sağladığı kan serumlarında koyunlarda %49,7 oranında, keçilerde %3,17 oranında pestivirus seropozitifliği tespit etmiştir [14]. Bu çalışmanın materyalinin tamamını abort geçmişi olan sürülerden sağlanan fötüsler oluşturmaktadır. Pestivirus varlığı antijen-RLISA testi ile araştırıldı. Virüs izolasyonu, pestivirusların kan ve sütte tespiti için altın standart olarak kabul edilmektedir. Ancak günümüzde abort yapmış fötüslerde pestivirus varlığının tanısında, immünohistokimya ve antijen ELISA ile antijen tespiti yanında, polimeraz zincir reaksiyonu (PCR) tabanlı testlerle nükleik asit tespiti artık geniş bir şekilde uygulanmaktadır [1]. Bu çalışmada antijen-ELISA testi ile materyalin tamamının atık tespit edilen sürülerden sağlanan fötüslerde koyunlarda %25,8, keçilerde %22,22 pestivirus varlığı ortaya konulmuştur.

Sonuç olarak abortlar, sığır, koyun ve keçi yetiştiriciliği yapılan işletmelerinde üretim kaybına neden olması ile ciddi ekonomik kayıplara neden olmasından dolayı sürü sağlığı, sürünün devamlılığı açısından ve hayvan yetiştiriciliğinde oldukça önemlidir. Abort nedenleri süt verimini de etkilediğinden özellikle süt üretimi yapılan hayvancılık işletmelerinde abortun etiyolojisi iyi bir şekilde tanımlanmalı ve gerekli koruma-kontrol önlemleri alınmalıdır. Hayvancılık işletmelerinde herhangi bir abort vakası görüldüğünde nedenin bakım ve beslenme şartları ile ilgili olması yanında enfeksiyöz bir etken de olabileceği unutulmamalıdır. Bu yüzden

abortion materyalinin uygun bir şekilde alınıp, laboratuvar teşhisinin yapılması etiyolojinin belirlenmesi korunma ve kontrol açısından önemlidir. Viral hastalıkların hayvancılık işletmelerinde hızlı bir şekilde yayılabileceği ve sürü bazında ciddi kayıplara neden olabileceği düşüncesiyle özellikle primer viral abort etkenlerinin belirlenmesi gerekmektedir. Pestivirusun hayvanlarda persiste enfeksiyon oluşturmaması [24] ve aynı zamanda bölgede atık yapmış ruminant fötüslerinde yaygın atık etkeni tespit edilmiş olması, sürü sağlığı ve devamlılığı yönünden enfeksiyonun hayvan yetiştiriciliğinde göz ardı edilmemesine bu araştırma ile vurgu yapılmaktadır. Bu nedenle bölgede viral etiyoloji yönünden sürüler düzenli kontrol altında tutulmalı, mücadelede sığırlar, koyun ve keçilerin bir arada yetiştirilmesi, enfeksiyonun yaygınlığına katkı sağlayacağından, bu durumun göz önünde bulundurularak, persiste enfekte hayvanların tespit edilmesi, korunma ve kontrol programlarının

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## The Effects of Valproic Acid on NO/cGMP in Pentylene-tetrazole-Induced Acute Epilepsy Model in Rats

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### Keywords

Epilepsy,  
Pentylene-tetrazole,  
Valproic Acid,  
NO/cGMP,

**Abstract:** Epilepsy is a disease which causes neuronal damage and loss of consciousness in consequence of recurrent seizures. Nitric oxide as a neuromodulator in brain is a gas which can penetrate into cells. It has a significant role on physiological cases, pathology of many diseases such as inflammation and degenerative diseases. The purpose of this research is to search the activity of NO/cGMP pathway of valproic acid in an experimental acute epileptic model which is induced with pentylene-tetrazole in rats. 18 adult male Wistar Albino rats were used in the study. The rats were randomly divided into 3 groups (n=6) as control group, pentylene-tetrazole (PTZ+salin) 45 mg kg<sup>-1</sup>, valproic acid (PTZ+VPA) 150 mg kg<sup>-1</sup>. After 24 hours of PTZ application, all rats brain tissues were removed and then cortex and hippocampus were separated. While PTZ increased the hippocampus and cortex NO/cGMP levels compared to control (p <0.01), VPA decreased the hippocampus and cortex NO levels by comparison with PTZ (p <0.001). On the other hand, while VPA decreased cortex cGMP levels (p <0,05) it did not change cGMP levels in hippocampus (p > 0.05). This study has suggested that VPA can show antiepileptic activity via NO/cGMP pathway.

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## Sıçanlarda Pentilentetrazol ile İndüklenen Akut Epilepsi Modelinde Valproik Asidin NO/cGMP Üzerindeki Etkileri

### Anahtar

### Kelimeler

Epilepsi,  
Pentilentetrazol,  
Valproik Asit,  
NO/cGMP

**Öz:** Epilepsi, tekrarlayan nöbetlerle nöronal hasara, bilinç kaybına neden olan bir hastalıktır. Nitrik oksit (NO); beyinde nöromodülatör olarak ve hücreler arasında yayılabilen bir gazdır. Fizyolojik olaylarda, iltihaplanma ve dejeneratif hastalıklar gibi birçok hastalığın patolojisinde önemli rolü vardır. Bu araştırmanın amacı, sıçanlarda pentilentetrazol ile indüklenen deneysel akut epileptik modelde valproik asidin NO/cGMP yolağının aktivitesini araştırmaktır. Çalışmada 18 adet yetişkin erkek Wistar Albino sıçan kullanıldı. Sıçanlar rastgele kontrol grubu, pentilentetrazol (PTZ) 45 mg kg<sup>-1</sup>, valproik asit (VPA) 150 mg kg<sup>-1</sup> olarak 3 gruba (n=6) ayrıldı. PTZ uygulamasından 24 saat sonra tüm sıçanların beyin dokuları çıkarıldı, korteks ve hipokampus ayrıldı. PTZ hipokamp ve korteks NO/cGMP düzeylerini kontrole göre artırırken (p <0,01), VPA hipokamp ve korteks NO düzeylerini PTZ'ye göre düşürdü (p <0,001). Ancak VPA korteks cGMP düzeylerini düşürürken (p <0,05), hipokampus cGMP düzeylerini deęiřtirmedii (p > 0,05). Bu çalışma, VPA'nın antiepileptik aktivitesini NO/cGMP yolu ile gösterebileceğini düşündürmektedir.

### 1. INTRODUCTION

In the central nervous system, NO functions as an intercellular spreadable signalling molecule [1]. NO is

produced from L-arginine in an NADPH-dependent reaction by NO synthase (NOS) in brain. [2]. NO is involved in many physiological and pathological events as a neurotransmitter/neuromodulator in the brain. These

events are involved in the pathology of many diseases such as depression, learning, memory, synaptic plasticity, inflammation, epilepsy, and long-term degenerative diseases [3]. Epilepsy, one of the neurodegenerative disorders, is reported to be caused by NO. Epilepsy is a neurodegenerative disease that affects 3% of the world population [4] and occurs with recurrent seizures [5].

Nitric oxide performs this function by activating the ryanodine receptors. Ryanodine receptors increase the release of intracellular calcium stores and thus neuronal damage occurs [6]. Nitric oxide is a potent stimulator of guanylyl cyclase and causes an increase in intracellular second messenger cyclic guanosine monophosphate (cGMP) levels [7]. Cyclic guanosine monophosphate levels are regulated by cyclic adenosine monophosphate (cAMP) and cyclic nucleotide phosphodiesterases (PDEs) that catalyze the hydrolysis of cGMP [8]. Nitric oxide (NO) is a seizure sensitivity modulator known for its dose-dependent anti-convulsant and pro-convulsant effects in epileptogenesis [9].

Although the mechanism of action of antiepileptic drugs is uncertain, GABA-A receptors are thought to be responsible. One of these drugs, valproic acid (VPA), increases GABA production, reduces GABA transaminase, and inhibits excitatory neurotransmission [10]. They reported that VPA has an anticonvulsive effect on the PTZ-kindled model [11].

It has been suggested that NO may also play a role in the antiepileptic effect mechanism of VPA [12].

In the central nervous system, NO can act as a second messenger, neuromodulator and neurotransmitter, suggesting that NO plays an important role in epilepsy and epileptogenesis. The aim of this study is to investigate the activity of the NO/ cGMP pathway on the antiepileptic efficiency of valproic acid in an experimental acute epileptic model induced by pentylenetetrazole in rats.

## 2. MATERYAL VE METOT

The research was conducted in Sivas Cumhuriyet University Faculty of Medicine Experimental Animals Laboratory. Not having been exposed to stress, 18 Wistar Albino rats, 4-5 months old ( $230 \pm 20$  g), were kept in cages to able to use in the study. All rats in the study were kept at 22-24°C with a 12-hour light/dark cycle, isolated from sound, with  $55 \pm 6$  humidity, and they were fed at an appropriate rate. The experimental application was carried out between 09:00 and 16:00. The light and sound level of the experimental environment were kept under constant control. For experimental procedures, permission was obtained from Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (license number 2020/327).

### 2.1. PTZ-Acute Epilepsy Protocol

A single dose of  $45 \text{ mg kg}^{-1}$  PTZ was applied to rats due to observe acute model epilepsy. After PTZ application, rats were placed in plexiglass cages (40 cmX40 cmX30

cm) and the seizures of each rat were observed within 30 mins. The seizure grade record was used to evaluate the seizures Modifies Racine's Convulsion Scale (RCS) as in the following, at initial phase there was no convulsion; at 1<sup>st</sup> phase there was twitching of vibrissae and pinnae; at 2<sup>nd</sup> phase there was a distinct twitching; at 3<sup>rd</sup> phase there were myoclonic jerks; at 4<sup>th</sup> phase there was tonic-clonic seizure while the animal remained on its feet; at 5<sup>th</sup> phase there was tonic-clonic seizure with loss of the righting reflex; at 6<sup>th</sup> phase there was tonic-clonic seizure with wild climbing and jumping; and 7<sup>th</sup> phase there was a lethal seizure [13].

### 2.2. Drugs

Pentylenetetrazole (PTZ) and valproic acid (VPA) were purchased from Sigma-Aldrich (St Louis, MO, USA). NO and cGMP Elisa kits were provided from Santa Cruz Biotechnology. PTZ and VPA were dissolved in saline and prepared in accordance with the doses stated below. PTZ and VPA were freshly dissolved before administering injections.

### 2.3. Experimental groups and Procedure

18 rats were randomly divided into 3 group (n=6). All injections were performed intraperitoneally.

**Control group:** Rats were administered  $1 \text{ mg kg}^{-1}$  ml single dose of saline and were applied another  $1 \text{ mg kg}^{-1}$  ml single dose of saline dose 30 min after the first injection. 24 hours after, white matter was removed, cortex and hippocampus were separated.

**PTZ group:** Rats in the PTZ group were applied  $1 \text{ ml/kg}$  saline and administered a single dose of  $45 \text{ mg kg}^{-1}$  pentylenetetrazole 30 min after the saline injection. 24 hours after PTZ application, white matter was removed, cortex and hippocampus were separated.

**VPA group:** Rats were applied  $150 \text{ mg kg}^{-1}$  VPA and administered  $45 \text{ mg kg}^{-1}$  pentylenetetrazole 30 min after the VPA injection. 24 hours after PTZ application, white matter was removed, cortex and hippocampus were separated.

### 2.4. Preparation of Brain Tissue Homogenates

24 hours after the experimental protocol was completed, all experimental rats were euthanized. White matter of rats was removed immediately and the NO and cGMP levels in each sample were centrifuged after homogenization and frozen at  $-80^\circ \text{C}$  to measure the NO and cGMP levels with Elisa kit.

### 2.5. Measurement of NO and cGMP

Rat ELISA commercial kits were used to determine the NO and cGMP levels in the supernatants of tissues taken from each group (YL Biont, Shanghai, China). Process protocols were done according to the manufacturer's instructions. Briefly, standard and tissue samples were added to the plate and incubated for 60 minutes at  $37^\circ \text{C}$ .

After the washing step, staining solutions were added and incubated for 15 minutes at 37° C. Stop solution was added and read at 450 nm. Standard curves were drawn to determine the value of the samples. The coefficients of variation within and between plates were found to be less than 10%.

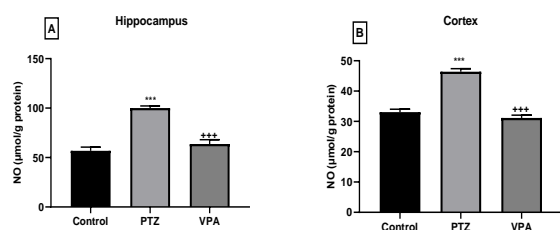
## 2.6. Statistical Evaluation

All experimental results and biochemical analysis were converted into numerical values. Statistical analysis and graphing was used SPSS 25.0 for windows and GraphPad Prism 7. Results were presented as Mean  $\pm$  SEM (standard error of mean). The results were evaluated by One-way ANOVA and followed by Tukey HSD (post-hoc test).  $p < 0.05$  values were defined.

## 3. BULGULAR

### Hippocampus and Cortex NO Levels

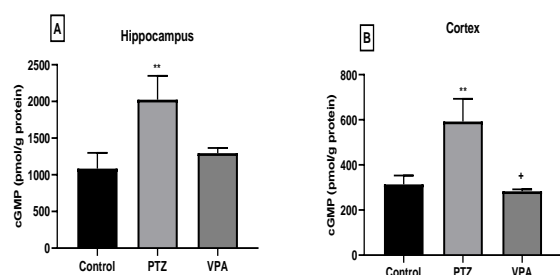
As shown in Figure 1 of our research, a statistically important increase in hippocampus and cortex NO levels were observed in the PTZ administered group compared to the group control ( $p < 0.001$ ). However, a statistical decrease in hippocampus and cortex NO levels was found in the VPA administered group compared to the PTZ administered group ( $p < 0.001$ ).



**Figure 1.** Effect of drugs on NO levels in the Hippocampus and Cortex after PTZ-induced seizure. \* \* \*  $p < 0.001$  different from control; + + +  $p < 0.001$  different from PTZ.

### 3.2. Hippocampus and Cortex cGMP Levels

While a statistically important rise in hippocampus cGMP level was observed in the PTZ group compared to the control group ( $p < 0.001$ ), no significant difference was found between the VPA and PTZ group ( $p > 0.05$ ). However, a statistically significant increase in cortex cGMP level was observed in the VPA group compared to the PTZ group ( $p < 0.05$ ) (Figure 2).



**Figure 2.** Effect of drugs on cGMP levels in the Hippocampus and Cortex after PTZ-induced seizure. \* \*  $p < 0.001$  different from control; +  $p < 0.001$  different from PTZ.

## 4. DISCUSSION

Epilepsy is a most common neurodegenerative disease that trigger progressive neuronal degeneration [14]. Over the past two decades, many studies have been conducted to discern the mechanisms underlying the epileptogenesis process and to treat epilepsy. Many events, such as apoptosis, inflammation, and oxidative stress play a role in the pathogenesis of epilepsy [15]. Nitric oxide, a known neurotransmitter/neuromodulator in the brain, plays a role in many physiological and pathological processes [16]. Nitric oxide is a potent stimulant of guanylyl cyclase, causing increased levels of cyclic GMP (cGMP) [7]. In the pathophysiology of epilepsy, NO shows anticonvulsant or a proconvulsant effects [17,18].

Han et al. [19] found that the 7-nitroindazole (7-NI) which is a NO inhibitor reduced hippocampus NO levels in the PTZ-induced rats, while PTZ caused seizures in rats as a result of an increase in hippocampus NO levels. This has been attributed to the increased density of PTZ's glutamate receptors as the activation of N-methyl-D-Aspartic acid (NMDA) receptors causes an increase in NO levels [20]. The hippocampus is activated after PTZ-induced seizure, as assessed by fos expression. But the mechanism of PTZ to increase NO production in the hippocampus is not clear [21]. PTZ can increase glutamate released by decrease GABA release, and thus stimulate NO production [22]. In our study, there was an important increase in both hippocampus and cortex NO levels in the PTZ group.

Nitric oxide triggers epileptic activity through cGMP formation. Glutamate activation stimulates NMDA receptors, activates nNOS by providing calcium flow to the cytosol, resulting in NO formation. Nitric oxide activates guanylate cyclase to synthesize cGMP, which is thought to initiate seizures [23]. Several studies on the role of NO in epileptic activity have mostly used proconvulsive drugs and NOS inhibitors. Some researchers have suggested that many factors, such as specific proconvulsive drugs, the type and concentrations of NOS inhibitor, method of administration, and employed specific strains or species, influence outcomes [24].

Another study researched the effect of Taurine on cortex NOS level in PTZ-induced rats and found that PTZ increased the cortex NOS level. The reason for this increase was explained by the stimulation of NMDA receptors by glutamate, catalyzing NO production and activating NOS, or by the fact that PTZ increases the NOS level by blocking GABA receptors. Taurine, on the other hand, has been found to significantly reduce the level of NOS [25]. We found that PTZ increased the level of cortex NO of rats, while VPA decreased it. The mechanism underlying this effect was demonstrated by PTZ, a GABA-A receptor antagonist, stimulating the NMDA receptor and activating neuronal nitric oxide synthase (nNOS) [26]. Therefore, in epileptic effect, it is suggested that nNOS increases NO as a result of stimulation with NMDA receptor [27]. Mülsch et al. [28] found that NO levels increased in the amygdala and cortex

during kainate-induced seizures in rats, and NO levels decreased in the group treated with 7-nitroindazole and diazepam. In our study, VPA, which was used as an antiepileptic, reduced NO levels in both the hippocampus and cortex, while decreased the cGMP levels in the cortex, but did not affect the hippocampus cGMP level. In another study, when looking at serum nitrite and nitrate levels in epileptic children using valproic acid or carbamazepine, nitrite and nitrate levels were found to be significantly higher in both valproic acid and carbamazepine groups compared to the control group. Based on these results, it has been suggested that valproic acid and carbamazepine may have an antiepileptic effect through nitric oxide [29]. It has been shown that 7-nitroindazole(7-NI) inhibitor improves the anticonvulsant effect of classic and second-generation antiepileptics, with the exception of tiagabine, felbamate and topiramate. However, the effect of NG-nitro-L-arginine methyl ester has not been clearly found. In the seizure models of pentylentetrazole, picrotoxin and N-methyl-D-aspartate, the inhibitor exhibited both convulsive and anticonvulsive effects depending on the dose. NG-nitro-L-arginine methyl ester enhanced the effectiveness of diazepam and clonazepam, decreased valproate and phenobarbital, but did not affect the anticonvulsant effect of phenytoin and ethosuximide [30].

The data of this research were consistent with previous works view that VPA caused a decrease in NO-cGMP levels in white matter, but an increase in NO-cGMP levels after PTZ-induced seizures.

## 5. SONUÇ

Our results show that NO plays an important role in acute epileptic model. However, VPA can demonstrate its antiepileptic effect by reducing the level of NO/cGMP, thus creating a protective effect against neuronal damage. Further research is needed to answer questions raised about possible involved mechanisms.

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## Conflict of Interest

The authors report no any conflict of interest

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## Investigation of Nonlinear Wave Solutions for Fusion and Fission Phenomenas

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equation,  
Wave  
solutions

**Abstract:** In this study, wave solutions of the (3+1) dimensional Jimbo-Miwa equation and two different phenomena of the solution, fusion and fission, are obtained using the modified exponential function method. In order to get more possible solutions, two different cases are investigated due to the nature of the modified exponential function method. When the resulting solutions are analyzed, trigonometric, hyperbolic and rational functions are obtained. It was checked whether the solution functions found by the Wolfram Mathematica software provided the (3+1) dimensional potential Jimbo-Miwa equation. Two and three dimensional graphs, contour and density graphs of the solution function were get by determining the appropriate parameters.

## Füzyon ve Fizyon Fenomenleri İçin Doğrusal Olmayan Dalga Çözümlerinin İncelenmesi

**Anahtar  
Kelimeler**  
Geliştirilmiş  
Üstel  
Fonksiyon  
Metodu,  
(3+1)-  
boyutlu  
Jimbo-Miwa  
denklemleri,  
Dalga  
çözümleri

**Öz:** Bu çalışmada, (3+1) boyutlu Jimbo-Miwa denkleminin dalga çözümleri ve buna bağlı olarak da çözümün füzyon ve fizyon olmak üzere iki farklı olgusu modifiye üstel fonksiyon yöntemi kullanılarak elde edilmiştir. Daha olası çözümler elde etmek için modifiye edilmiş üstel fonksiyon yönteminin doğası gereği iki farklı durum incelenmiştir. Ortaya çıkan çözümler incelendiğinde trigonometrik, hiperbolik ve rasyonel fonksiyonlar elde edilmiştir. Wolfram Mathematica yazılımı tarafından bulunan çözüm fonksiyonlarının (3+1) boyutlu potansiyel Jimbo-Miwa denklemini sağlayıp sağlamadığı kontrol edildi. Uygun parametreler belirlenerek çözüm fonksiyonunun iki ve üç boyutlu grafikleri, kontur ve hassasiyet grafikleri elde edildi.

### 1. INTRODUCTION

Physics, engineering, health, etc. all events encountered in natural and applied sciences are represented by mathematical models. These types of models are stated in nonlinear partial differential equations. Therefore, it is of great importance to obtain the solutions of such equations. In scientific studies in the literature, there are various methods to investigate the solutions of such equations. Some of these methods in the literature; generalized tanh function method [1], the modified extended tanh-function method [2-3], the generalized Bernoulli sub-equation function method [4-6], The trial

equation method [7-11], the first integral method [12], the modified exponential function method [13-17] and many more methods.

In this study, we consider the (3+1) dimensional Jimbo-Miwa equation given following [18-24],

$$u_{xxx} + 3u_y u_{xx} + 3u_x u_{xy} + 2u_{yt} - 3u_{xz} = 0. \quad (1)$$

Equation (1) given as a mathematical model has been encountered in fusion and fission phenomena which is a subject of nuclear physics. In the literature, there are

results obtained by using various methods regarding the solutions of equation (1) [18-24].

In the second part of this study, the modified exponential function method is introduced. To apply this method PDEs has been reduced to ordinary differential equations (ODEs). In the third chapter, the solutions obtained by applying the determined method (3+1)-dimensional Jimbo-Miwa equation and two- and three-dimensional graphs, contour graphs and density graphs of these results are presented for two cases. In the last part, the conclusion part is given.

## 2. ANALYSIS OF MODIFIED EXPONENTIAL FUNCTION METHOD

Let us consider the general form of the nonlinear partial differential equation for the modified exponential function method as follows;

$$P(U, U_x, U_y, U_z, U_t, U_{xx}, U_{xt}, U_{yy}, U_{xxx}, \dots) = 0, \quad (2)$$

where  $U = U(x, y, z, t)$  is unknown function.

**Step 1.** Taking the independent variables given in equation (1) into consideration, the wave transformation given below is considered,

$$U(x, y, z, t) = U(\xi), \quad \xi = k(x + y + z - ct). \quad (3)$$

The terms  $k$  and  $c$  given here are constants in walking wave transformation. If the expression (3) is substituted by obtaining the derivative expressions in the equation (2) given as the general form of equation (1) by using the wave transformation,

$$N(U, U', (U')^2, U'', U''', \dots) = 0, \quad (4)$$

the general form of the nonlinear ordinary differential equation is gotten.

**Step 2:** According to this method, the solution function of equation (1) is as follows.

$$U(\xi) = \frac{\sum_{i=0}^n A_i [\exp(-\Omega(\xi))]^i}{\sum_{j=0}^m B_j [\exp(-\Omega(\xi))]^j} = \quad (5)$$

$$= \frac{A_0 + A_1 \exp(-\Omega) + \dots + A_n \exp(n(-\Omega))}{B_0 + B_1 \exp(-\Omega) + \dots + B_m \exp(m(-\Omega))},$$

where  $A_i, B_j, (0 \leq i \leq n, 0 \leq j \leq m)$  are constants. The balancing procedure is applied to the nonlinear ordinary differential equation (4) obtained by applying the wave

transformation. In other words, by equating the term with the highest order derivative in the equation (4) and the nonlinear term, the relation between  $m$  and  $n$  is obtained. Equality is found by giving value to the constants in this relation. Thus, the upper limits of the sum symbols in equation (5) are determined.

$$\Omega'(\xi) = \exp(-\Omega(\xi)) + \mu \exp(\Omega(\xi)) + \lambda. \quad (6)$$

The omega function given below is a function used as the power of the exponential function in the solution function. When we solve the Eq. (6), the following families are obtained by He et al [13]:

**Family 1:** When  $\mu \neq 0, \lambda^2 - 4\mu > 0,$

$$\Omega(\xi) = \ln \left[ \frac{-\sqrt{\lambda^2 - 4\mu}}{2\mu} \tanh \left( \frac{\sqrt{\lambda^2 - 4\mu}}{2} (\xi + E) \right) - \frac{\lambda}{2\mu} \right]. \quad (7)$$

**Family 2:** When  $\mu \neq 0, \lambda^2 - 4\mu < 0,$

$$\Omega(\xi) = \ln \left[ \frac{\sqrt{-\lambda^2 + 4\mu}}{2\mu} \tan \left( \frac{\sqrt{-\lambda^2 + 4\mu}}{2} (\xi + E) \right) - \frac{\lambda}{2\mu} \right]. \quad (8)$$

**Family 3:** When  $\mu = 0, \lambda \neq 0$  and  $\lambda^2 - 4\mu > 0,$

$$\Omega(\xi) = -\ln \left[ \frac{\lambda}{\exp(\lambda(\xi + E)) - 1} \right]. \quad (9)$$

**Family 4:** When  $\mu \neq 0, \lambda \neq 0$  and  $\lambda^2 - 4\mu = 0,$

$$\Omega(\xi) = \ln \left[ -\frac{2\lambda(\xi + E) + 4}{\lambda^2(\xi + E)} \right]. \quad (10)$$

**Family 5:** When  $\mu = 0, \lambda = 0$  and  $\lambda^2 - 4\mu = 0,$

$$\Omega(\xi) = \ln(\xi + E). \quad (11)$$

where  $A_0, A_1, \dots, A_n, B_0, B_1, \dots, B_m, E, \lambda, \mu$  are constants.

**Step 3:** After equation (6) is solved, when the equation (5) is written in its place, an algebraic equation system



consisting of coefficients is get. When this system of equations is solved with the help of the Mathematica program, the travelling wave solutions that provide the equation (1) are obtained.

**3. APPLICATION**

Using the traveling wave transformation (3) for equation (1), the following nonlinear ordinary differential equation is obtained,

$$k^2 U''' + 3k(U')^2 - (2c + 3)U' = 0. \tag{12}$$

If  $U' = V$  transform is applied in equation (12), we obtain,

$$k^2 V'' + 3kV^2 - (2c + 3)V = 0. \tag{13}$$

In equation (13), if the equalization term is applied between  $V''$  and  $V^2$  according to the balance procedure,

$$M + 2 = N. \tag{14}$$

If  $M = 1$  so as to satisfy the equation (14),  $N = 3$  is obtained. In this case, the upper limits of the total symbols in the sought solution function in equation (5) are determined. Accordingly, the terms required in the equation (15) are given below.

$$V(\xi) = \frac{\psi}{\phi} = \frac{A_0 + A_1 e^{-\Omega(\xi)} + A_2 e^{-2\Omega(\xi)} + A_3 e^{-3\Omega(\xi)}}{B_0 + B_1 e^{-\Omega(\xi)}}, \tag{15}$$

$$V'(\xi) = \frac{\psi'\phi - \psi\phi'}{\phi^2},$$

$$V''(\xi) = \frac{\psi''\phi^3 - \phi^2\psi'\phi' - (\psi\phi'' + \psi'\phi')\phi^2 + 2(\psi')^2\psi\phi}{\phi^4}.$$

**Case 1:**

$$A_0 = -\frac{1}{3}k(\lambda^2 + 2\mu)B_0,$$

$$A_1 = -\frac{1}{3}k(6\lambda B_0 + (\lambda^2 + 2\mu)B_1),$$

$$A_2 = -2k(B_0 + \lambda B_1), A_3 = -2k B_1,$$

$$c = \frac{1}{2}(k^2(-(\lambda^2 - 4\mu)) - 3).$$

By using the obtained coefficients, the traveling wave solutions of equation (1) have been analyzed considering the following family cases.

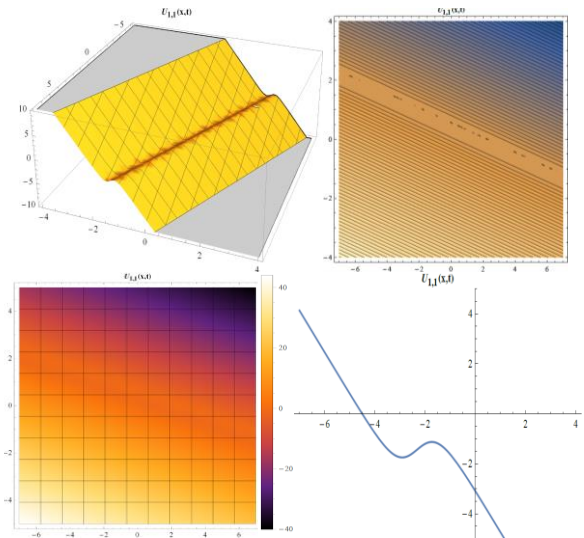
**Family-1:**

$$V_{1,1}(\xi) = -\frac{k(\lambda^2 - 4\mu) \operatorname{sech}^2\left(\frac{1}{2}\phi\right)(\lambda\beta + \alpha - 4\mu)}{3\left(\sqrt{\lambda^2 - 4\mu} \tanh\left(\frac{1}{2}\phi\right) + \lambda\right)^2}. \tag{16}$$

Where  $\alpha = (\lambda^2 - 2\mu) \operatorname{Cosh}[\phi], \beta = \sqrt{\lambda^2 - 4\mu} \operatorname{Sinh}[\phi],$   
 $\phi = \sqrt{\lambda^2 - 4\mu}(EE + \xi).$

Integrate  $\xi$  on both sides of the equation  $U' = V,$

$$U_{1,1}(\xi) = \frac{1}{3}k(\lambda^2 - 4\mu) \left( \frac{3\lambda}{2\mu \operatorname{cosh}(\phi) + \lambda^2 - 2\mu} - EE - \xi \right) + \frac{2k\mu\beta}{2\mu \operatorname{cosh}(\phi) + \lambda^2 - 2\mu}. \tag{17}$$



**Figure 1.** The three dimensional graph, contour graph, density graphs of Eq.(17) for the values  $c = -4, A_3 = 2, B_1 = 1, \lambda = 3, \mu = 1, y = -1, z = 1, k = -1, EE = 0.82$  and two-dimensional graph for  $t = 1.$

**Family 2:**

$$V_{1,2}(\xi) = \frac{k(\lambda^2 - 4\mu) \operatorname{sec}^2\left(\frac{1}{2}\tau\right)(\varsigma - \omega + 4\mu)}{3\left(\lambda - \sqrt{4\mu - \lambda^2} \tan\left(\frac{1}{2}\tau\right)\right)^2}. \tag{18}$$

Where  $\varsigma = \lambda\sqrt{-\lambda^2 + 4\mu} \operatorname{Sin}[\tau], \omega = (\lambda^2 - 2\mu) \operatorname{Cos}[\tau]$   
 $, \tau = \sqrt{-\lambda^2 + 4\mu}(EE + \xi).$

$$U_{1,2}(\xi) = \frac{1}{3}k(\lambda^2 - 4\mu) \left( \frac{3\lambda}{2\mu\cos(\tau) + \lambda^2 - 2\mu} - EE - \xi \right) - \frac{2k\mu\sqrt{4\mu - \lambda^2} \sin(\tau)}{2\mu\cos(\tau) + \lambda^2 - 2\mu}. \tag{19}$$

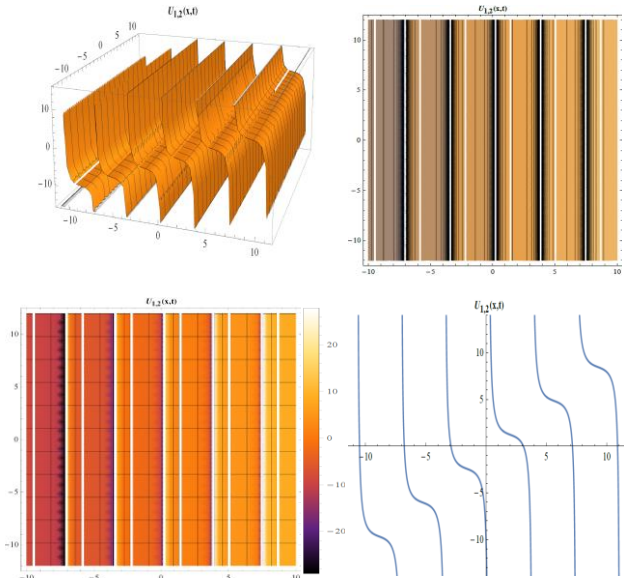


Figure 2. The three dimensional graph, contour graph, density graphs of Eq.(19) for the values  $c = 0, A_3 = 2, B_1 = 1, \lambda = 1, \mu = 1, y = -0.01, z = 0.01, k = -1, EE = 0.82$  and two-dimensional graph for  $t = 1$ .

**Family 3:**

$$V_{1,3}(\xi) = -\frac{1}{6}k\lambda^2(\cosh(\lambda(EE + \xi)) + 2) \operatorname{csch}^2\left(\frac{1}{2}\lambda(EE + \xi)\right). \tag{20}$$

Integrating equation (20) according to  $\xi$ ,

$$U_{1,3}(\xi) = k\lambda \operatorname{coth}\left(\frac{1}{2}\lambda(EE + \xi)\right) - \frac{1}{3}k\lambda^2(EE + \xi). \tag{21}$$

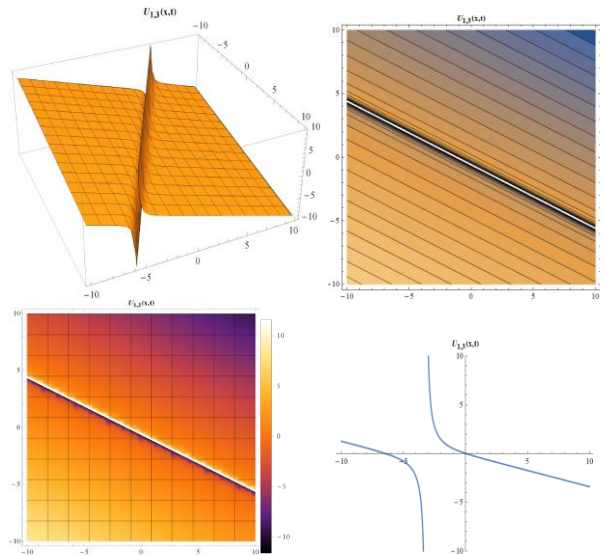


Figure 3. The three dimensional graph, contour graph, density graphs of Eq.(21) for the values  $c = -2, \lambda = 1, \mu = 0, y = 1, z = 1, k = -1, EE = 0.82$  and two-dimensional graph for  $t = 1$ .

**Family 4:**

$$V_{1,4}(\xi) = \frac{1}{6}k \left( \lambda^2 \left( 1 - \frac{12}{(\lambda(EE + \xi) + 2)^2} \right) - 4\mu \right). \tag{22}$$

Integrating equation (22) according to  $\xi$ , we get,

$$U_{1,4}(\xi) = \frac{1}{6}k \left( \frac{\lambda(\lambda(EE + \xi) + 2) + 12\lambda}{\lambda(EE + \xi) + 2} - 4\mu\xi \right). \tag{23}$$

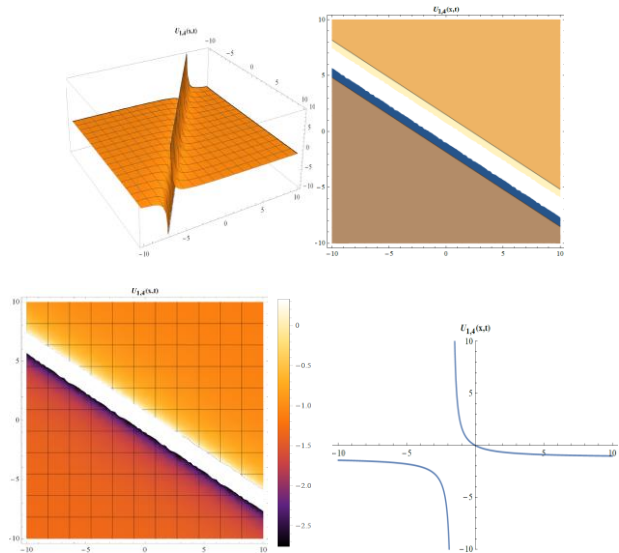


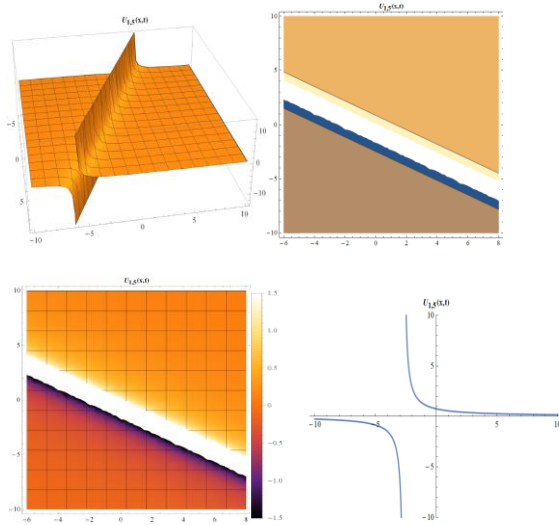
Figure 4. The three dimensional graph, contour graph, density graphs of Eq.(23) for the values  $c = -1.5, \lambda = 2, \mu = 1, y = 1, z = 1, k = -1, EE = 0.82$  and two-dimensional graph for  $t = 1$ .

**Family 5:**

$$V_{1,5}(\xi) = -\frac{2k}{(EE + \xi)^2}. \tag{24}$$

Integrating equation (24) according to  $\xi$ , we obtain,

$$U_{1,5}(\xi) = \frac{2k}{EE + \xi}. \tag{25}$$



**Figure 5.** The three dimensional graph, contour graph, density graphs of Eq.(25) for the values  $c = -1.5, \lambda = 0, \mu = 0, y = 1, z = 1, k = -1, EE = 0.82$  and two-dimensional graph for  $t = 1$ .

**Case 2:**

$$A_0 = -\frac{B_0(2c + 3k^2\lambda^2 + 3)}{6k},$$

$$A_1 = -\frac{B_1(2c + 3k^2\lambda^2 + 3)}{6k} - 2B_0k\lambda,$$

$$A_2 = -2k(B_1\lambda + B_0), \quad A_3 = -2B_1k,$$

$$\mu = \frac{2c + k^2\lambda^2 + 3}{4k^2}.$$

By using the obtained coefficients, the traveling wave solutions of the equation (1) are analyzed by considering the following family cases.

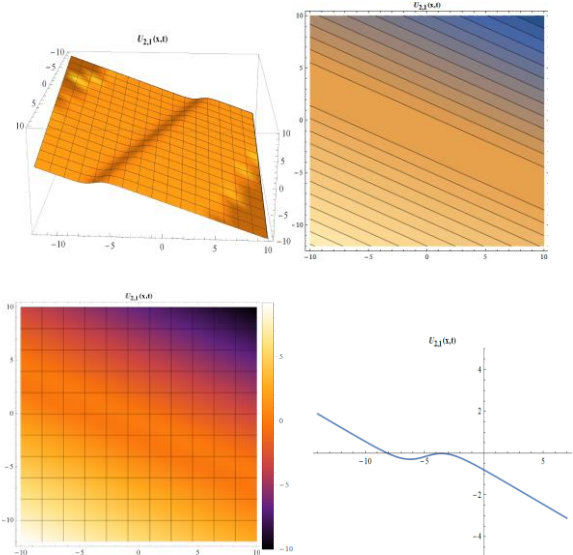
**Family-1:**

$$V_{2,1}(\xi) = -\frac{2c+3}{6k} - \frac{k(\lambda\beta + \lambda^2 - 4\mu)^2}{2(\beta + \lambda)^2}. \tag{26}$$

Where  $\beta = \sqrt{\lambda^2 - 4\mu\alpha}, \alpha = \tanh\left(\frac{1}{2}\phi\right),$   
 $\phi = \sqrt{\lambda^2 - 4\mu}(EE + \xi).$

Integrate  $\xi$  on both sides of the equation  $U' = V,$

$$U_{2,1}(\xi) = -\frac{\beta(2(2c+3)\mu\xi + 3\lambda k^2(\lambda^2 - 8\mu)) + 2(2c+3)\lambda\mu\xi + 12k^2\mu \tanh^{-1}(\alpha) (\sqrt{\lambda^2 - 4\mu(\beta + \lambda)} + 3k^2(\lambda^2 - 4\mu)^2)}{12k\mu(\beta + \lambda)}. \tag{27}$$



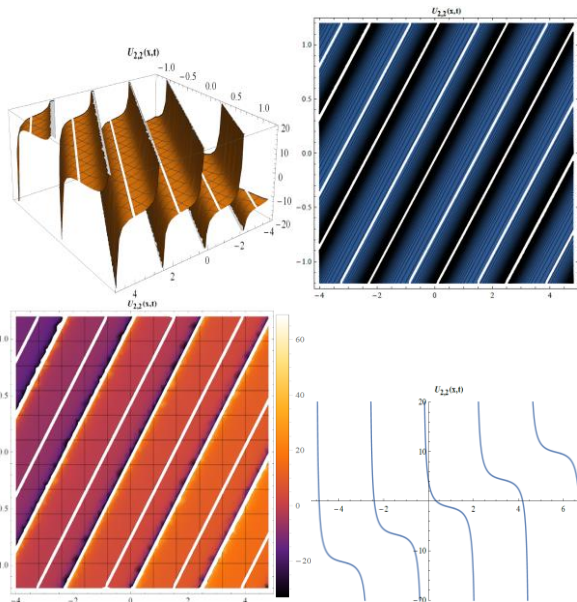
**Figure 6.** The three dimensional graph, contour graph, density graphs of Eq.(27) for the values  $c = -2, \lambda = 2, \mu = \frac{15}{16}, y = 1, z = 1, k = 2, EE = 0.75$  and two-dimensional graph for  $t = 1$ .

**Family 2:**

$$V_{2,2}(\xi) = -\frac{2c+3}{6k} - \frac{k(-\lambda\varsigma + \lambda^2 - 4\mu)^2}{2(\lambda - \varsigma)^2}. \tag{28}$$

Where  $\varsigma = \sqrt{\lambda^2 - 4\mu} \tanh\left(\frac{1}{2}\tau\right), \omega = (2\mu) \text{Cos}[\tau],$   
 $\tau = \sqrt{-\lambda^2 + 4\mu}(EE + \xi).$

$$U_{2,2}(\xi) = -\frac{(2c+3)\xi}{6k} - \frac{1}{2}k(EE + \xi)(\lambda^2 - 4\mu) + \frac{k\lambda(\lambda^2 - 4\mu)}{\omega + \lambda^2 - 2\mu} - \frac{2k\mu\sqrt{4\mu - \lambda^2} \sin(\tau)}{\omega + \lambda^2 - 2\mu}. \tag{29}$$



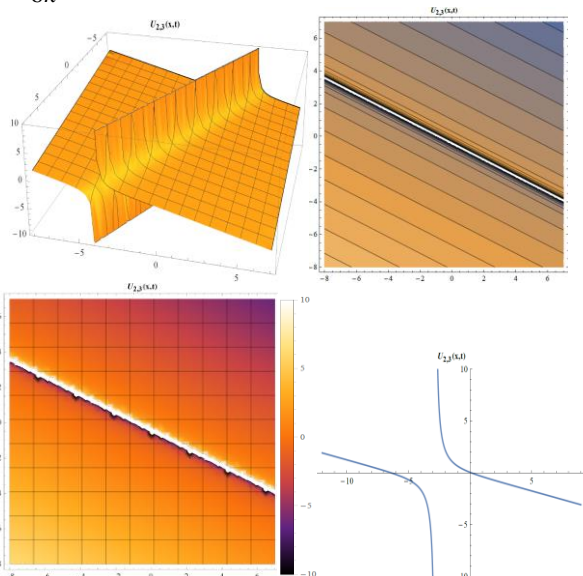
**Figure 7.** The three dimensional graph, contour graph, density graphs of Eq.(29) for the values  $c = 2, \lambda = 1, \mu = \frac{11}{16}, y = -1, z = 1, k = 2, EE = 0.75$  and two-dimensional graph for  $t = 1$ .

**Family 3:**

$$V_{2,3}(\xi) = \frac{2c + 3k^2 \lambda^2 \coth^2\left(\frac{1}{2} \lambda(EE + \xi)\right) + 3}{6k}. \quad (30)$$

Integrating equation (30) according to  $\xi$ ,

$$U_{2,3}(\xi) = k\lambda \left( \coth\left(\frac{1}{2} \lambda(EE + \xi)\right) - \tanh^{-1}\left(\tanh\left(\frac{1}{2} \lambda(EE + \xi)\right)\right) \right) - \frac{(2c + 3)\xi}{6k}. \quad (31)$$



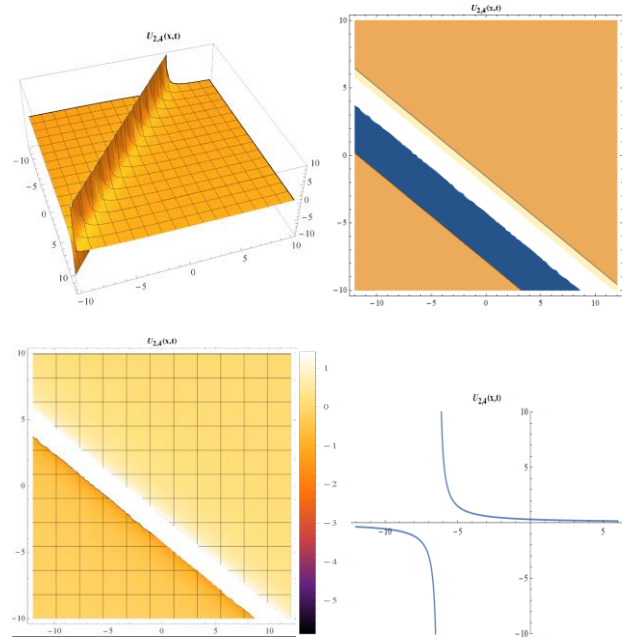
**Figure 8.** The three dimensional graph, contour graph, density graphs of Eq.(31) for the values  $c = -2, \lambda = 1, \mu = 0, y = -1, z = 1, k = 1, EE = 0.85$  and two-dimensional graph for  $t = 1$ .

**Family 4:**

$$V_{2,4}(\xi) = -\frac{2c + 3}{6k} - \frac{2k\lambda^2}{(\lambda(EE + \xi) + 2)^2}. \quad (32)$$

Integrating equation (32) according to  $\xi$ , we get,

$$U_{2,4}(\xi) = \frac{2k\lambda}{\lambda(EE + \xi) + 2} - \frac{(2c + 3)\xi}{6k}. \quad (33)$$



**Figure 9.** The three dimensional graph, contour graph, density graphs of Eq.(33) for the values  $c = -1.5, \lambda = 1, \mu = 0.25, y = 1, z = 1, k = 1, EE = 0.82$  and two-dimensional graph for  $t = 1$ .

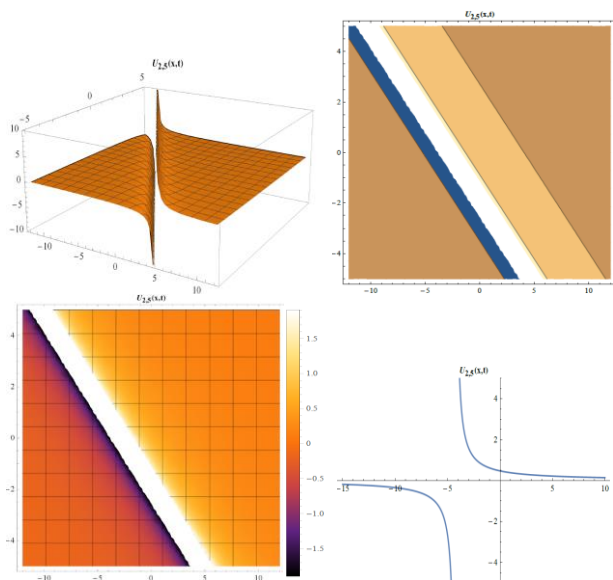
**Family 5:**

$$V_{2,5}(\xi) = -\frac{2c + 3}{6k} - \frac{2k}{(EE + \xi)^2}. \quad (34)$$

Integrating equation (34) according to  $\xi$ , we obtain,

$$U_{2,5}(\xi) = \frac{2k}{EE + \xi} - \frac{(2c + 3)\xi}{6k}. \quad (35)$$





**Figure 10.** The three dimensional graph, contour graph, density graphs of Eq.(35) for the values  $c = -1.5, \lambda = 0, \mu = 0, y = 1, z = 1, k = 1, EE = 0.82$  and two-dimensional graph for  $t = 1$ .

#### 4. CONCLUSION

In this article, MEFM was applied to the (3+1)-dimensional Jimbo-Miva equation considered as a nonlinear mathematical model. In this research, it was seen that the analytical solutions found under the conditions obtained according to the method provided the equation (1). When the analytical solution functions found are analyzed, it is seen that there are hyperbolic and trigonometric functions with periodic functions and rational functions. In particular, obtaining periodic functions is an advantage because such solution functions allow interpretation for an infinite range thanks to the understanding within a specific range of the behaviors represented by the mathematical model. All calculations and graphics were made using Mathematica software program. It has been observed that two and three dimensional graphs obtained by determining the appropriate parameters are suitable for the physical behavior of wave solutions. In addition, contour graphics and density graphics were found with the help of software program for wave solutions. Using this method, if more cases are investigated and different coefficient values are taken, different traveling wave solutions can be get. When the obtained solutions are analyzed, it can be stated that MEFM is effective and reliable in revealing analytical solutions of nonlinear partial differential equations. The obtaining results can help us learn more about the fusion and fission events.

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### 3 Boyutlu Evrişimsel Sinir Ağı Kullanılarak Hiperspektral Görüntülerin Sınıflandırılması

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#### Anahtar Kelimeler

Hiperspektral görüntü sınıflandırma, Derin öğrenme, 2B ESA, 3B ESA

**Öz:** Hiperspektral görüntü sınıflandırma, uzaktan algılanan görüntülerin analizi için yaygın olarak kullanılmaktadır. Bir hiperspektral görüntü, uygulamalarda büyük potansiyele sahip olan yer nesnelerinin zengin spektral bilgilerini ve uzamsal bilgilerini içermektedir. Spektral uzamsal bilgi kullanımı hiperspektral görüntü sınıflandırmasının performansını önemli ölçüde arttırmaktadır. Hiperspektral görüntüler, 3B küpler biçiminde gösterilmektedir. Bu nedenle, 3B uzamsal filtreleme, bu tür görüntülerdeki spektral uzamsal özellikleri eşzamanlı olarak çıkarmak için doğal olarak basit ve etkili bir yöntem sunmaktadır. Bu çalışmada, hiperspektral görüntü sınıflandırması için bir 3B evrişimli sinir ağı (3B ESA) yöntemi önerilmiştir. Önerilen yöntem, derin spektral uzamsal birleştirilmiş özellikleri etkin bir şekilde çıkarmaktadır. Aynı zamanda herhangi bir ön işleme veya son işleme dayanmadan hiperspektral görüntü küpü verileri toplu olarak görüntülemektedir. Hiperspektral görüntü küpü önce küçük üst üste binen 3B parçalara bölünmektedir. Daha sonra bu parçalar, spektral bilgileri de koruyan birden çok bitişik bant üzerinde bir 3B çekirdek işlevi kullanarak 3B özellik haritaları oluşturmak için işlenmektedir. Önerilen yöntem Indian pines, Pavia üniversitesi ve Botswana veri setleri ile test edilmiştir. Deneysel çalışmalar sonucunda, Indian pines için %99,35, Pavia üniversitesi için %99,90 ve Botswana için ise %99,59 genel doğruluk sonuçları elde edilmiştir. Sonuçlar, 4 farklı derin öğrenme tabanlı yöntemle karşılaştırılmıştır. Deneysel sonuçlardan, önerilen 3B ESA yöntemimizin daha iyi performans gösterdiği görülmektedir.

### Classification of Hyperspectral Images Using 3D Convolutional Neural Network

#### Keywords

Hyperspectral image classification, Deep learning, 2D CNN, 3D CNN

**Abstract:** Hyperspectral image classification is commonly used for the analysis of remotely sensed images. A hyperspectral image contains rich spectral and spatial information of ground objects that has great potential in applications. The use of spectral spatial information significantly improves the performance of hyperspectral image classification. Hyperspectral images are shown as 3D cubes. Therefore, 3D spatial filtering offers an inherently simple and effective method to simultaneously extract spectral spatial features in such images. In this study, a 3D convolutional neural network (3D CNN) method is proposed for hyperspectral image classification. The proposed method effectively extracts deep spectral spatially combined features. At the same time, the hyperspectral image cube displays data in aggregate without relying on any pre-processing or post-processing. The hyperspectral image cube is first divided into small overlapping 3D patches. Then these patches are processed to create 3D feature maps using a 3D kernel function on multiple adjacent bands that also preserve spectral information. The proposed method was tested with Indian pines, Pavia university and Botswana datasets. As a result of the experimental studies, the overall accuracy results were obtained 99.35% for Indian pines, 99.90% for the University Pavia, and 99.59% for Botswana. The results were compared with 4 different deep learning-based methods. From the experimental results, it is seen that our proposed 3D CNN method performs better.



## 1. GİRİŞ

Hiperspektral uzak sensörler, elektromanyetik spektrumun yüzlerce sürekli ve dar bant genişliğinde dijital görüntüler yakalayarak hem spektral hem de uzamsal bilgileri içeren 3 boyutlu hiperspektral görüntüler üretmektedir [1]. Hiperspektral görüntülerin zengin spektral bilgileri güçlüdür. Bu nedenle, tarım, astronomi, madencilik, nesne tespiti, askeri gözetim, çevre bilimleri, arazi yangın takibi ve biyolojik tehdit tespiti gibi bir dizi uygulamada yaygın olarak kullanılmaktadır [2, 3]. Hiperspektral görüntülerde, her pikselin sınıflandırılması bu uygulamalarda çok önemli rol oynamaktadır. Bu nedenle, son yıllarda çok sayıda hiperspektral görüntü sınıflandırma yöntemi önerilmiştir. Geleneksel hiperspektral görüntü sınıflandırma yöntemleri genellikle yalnızca spektral bilgilere dayanmaktadır. Bu sınıflandırıcılar arasında k-en yakın komşu [4], lojistik regresyon [5], destek vektör makinesi [6], rastgele orman [7] ve maksimum olabilirlik kriteri [8] temelli sınıflandırıcılar bulunmaktadır. Bu sınıflandırıcılar, spektral fazlalık ve spektral bantlar arasındaki yüksek korelasyon nedeniyle iyi performans gösterememektedir. Ayrıca, bu sınıflandırıcılar hiperspektral görüntülerin önemli uzamsal değişkenliğini korumada başarısız olmaktadır. Bu da düşük performansla sonuçlanmaktadır. Sınıflandırma performansını iyileştirmenin en basit yolu hem spektral hem de uzamsal bilgileri içeren bir sınıflandırıcı tasarlamaktır. Uzamsal bilgi, farklı yapıların şekli ve boyutu ile ilgili ek ayırt edici bilgiler sağlamaktadır. Bu durum uygun şekilde kullanılırsa daha doğru sınıflandırma haritalarına yol açmaktadır. Spektral uzamsal sınıflandırma yöntemleri genel olarak iki kategoriye ayrılabilir. İlki, spektral ve uzamsal özellikleri ayrı ayrı incelemektedir. Başka bir deyişle, uzamsal özellikler, morfolojik işlemler [9], entropi [10], öznelik profilleri [11] ve düşük dereceli temsil [12] gibi çeşitli uzamsal filtreler aracılığıyla önceden çıkarılmaktadır. Daha sonra bu uzamsal özellikler, piksel düzeyinde sınıflandırmayı gerçekleştirmek için spektral özelliklerle birleştirilmektedir. İkinci kategori, ortak özellikler üretmek için genellikle uzamsal özellikleri spektral özelliklerle birleştirmektedir. Örneğin, farklı ölçek ve frekanslarda üretilen bir dizi 3D dalgacık filtresi [13], 3D Gabor filtresi [14] ve 3D saçılım dalgacık filtreleri [15], ortak spektral-uzamsal özellikleri çıkarmak için hiperspektral görüntülere uygulanmaktadır. Hiperspektral görüntüler 3B küpler halinde gösterildiğinden dolayı, ikinci yaklaşım türü daha iyi performans için gerekli olan ortak uzamsal spektral korelasyonu koruyarak daha iyi sonuçlar üretebilmektedir. Bununla birlikte, geleneksel özellik çıkarma yöntemlerinin çoğu, el yapımı özelliklere ve derin olmayan öğrenme modellerine dayanmaktadır. Bu durum sınırlı özellik çıkarma ile sonuçlanmaktadır.

Derin öğrenme tekniklerindeki ilerleme, hiperspektral görüntü sınıflandırmasını yeni bir aşamaya taşımaktadır. Derin öğrenme yönteminin eğitim aşaması, özellikleri otomatik olarak çıkarmakta ve bunları sınıflandırma aşamasında kullanmaktadır. Destek vektör makinesi ve rastgele orman gibi geleneksel hiperspektral görüntü

sınıflandırma yöntemleri, uzamsal özellikleri göz ardı ederken hiperspektral görüntülerin spektral özelliklerini çıkarmaya odaklanmıştır. Tipik bir derin öğrenme modeli olarak, yığılı otokodlayıcı [16] ve derin inaç ağı [17] hem uzamsal hem de spektral bilgiyi çıkarabilmekte ve daha sonra bunları hiperspektral görüntü sınıflandırması için birleştirebilmektedir. Bununla birlikte, yukarıdaki derin öğrenme yöntemlerinin ikisi de girdi olarak tek boyutlu özellik vektörlerini kullanmaktadır. Bu durumda hiperspektral görüntülerdeki uzamsal özellikler tam olarak kullanılmamaktadır. Hiperspektral görüntü sınıflandırma için en sık kullanılan derin öğrenme yöntemlerinden biri de evrişimsel sinir ağlarıdır. Du, Mao ve ark. [18, 19], hiperspektral görüntü sınıflandırması için evrişimsel sinir ağını kullanmıştır. Bu çalışmada, uzamsal özellikler orijinal hiperspektral görüntülerinin ilk birkaç temel bileşen bantlarından yararlanılarak bir 2 boyutlu evrişimsel sinir ağı modeli ile elde edilmiştir. Evrişimsel sinir ağı tabanlı modeller, yığılı otokodlayıcı ve derin inaç ağı modellerine göre gelişmiş sınıflandırma performansı elde edebildiği gösterilen yerel özellikleri tespit etme yeteneğine sahiptir. Ancak, 2 boyutlu evrişimsel sinir ağında (2B ESA), uzamsal ve spektral özellikler genellikle ayrı ayrı çıkarılmaktadır. Bu işlem spektrumdaki bazı ayrıntıları kaybedecek ve bu yöntemlerin farklı nesnelere ayırt etme kapasitesini azaltacaktır. Aynı zamanda, sınıflandırma için önemli olabilecek ortak uzamsal spektral korelasyon bilgilerinden tam olarak yararlanmayı geçersiz kılacaktır. Yani, 2B ESA, spektral boyutlardan iyi ayırt edici özellik haritalarını çıkaramamaktadır.

Bu çalışmada, hiperspektral görüntü sınıflandırması için 3 boyutlu evrişimsel sinir ağı (3B ESA) modeli önerilmektedir. 3B ESA, 3B hiperspektral görüntüye 3B çekirdek uygulayarak, özellik küplerinin hem uzamsal hem de spektral boyutlarındaki yerel sinyal değişikliklerini öğrenebilmektedir. Bu sayede, sınıflandırma için önemli ayırt edici bilgilerden faydalanılmaktadır. Spektral ve uzamsal özellikler eşzamanlı olarak çıkarıldığından, bu çalışma 3B hiperspektral görüntü verilerinin yapısal özelliklerinden tam olarak yararlanmaktadır. Önerilen 3B ESA yöntemi, girdi olarak tüm spektral bantları almaktadır. Herhangi bir ön işlem veya son işlem gerektirmemektedir. Ortaya çıkan derin sınıflandırıcı modeli, uçtan uca bir şekilde eğitilmektedir. Aynı ölçekte, önerilen 3B ESA diğer derin öğrenme tabanlı yöntemlerden daha az parametre içermektedir. Bu, tipik olarak eğitim örneklerine sınırlı erişimi olan hiperspektral görüntü sınıflandırma problemleri için daha uygundur. 3B ESA tabanlı yöntem ile farklı uzak sensörler tarafından yakalanan üç farklı hiperspektral görüntü veri seti üzerinde deneyler gerçekleştirilmiştir. Deneysel sonuçlar literatürdeki farklı derin öğrenme tabanlı tekniklerle karşılaştırılmıştır. Elde edilen deneysel sonuçlar ile önerilen 3B ESA yönteminin karşılaştırılan yöntemlerden daha iyi performans gösterdiği görülmektedir.

Bu çalışmada önerilen yöntemin teorik alt yapısını oluşturan evrişimsel sinir ağı modeli Bölüm 2'de ve

önerilen yöntem Bölüm 3'te anlatılmıştır. Bölüm 4'te kullanılan veri setleri ve deneysel sonuçlar verilmiştir. Bölüm 5'de ise sonuçlar tartışılmıştır.

## 2. EVRİŞİMSEL SİNİR AĞI (ESA)

Görüntü sınıflandırması için derin öğrenme teknikleri son zamanlarda yaygın olarak kullanılmaktadır. ESA, evrişim katmanı, havuzlama katmanı ve tamamen bağlı katmandan oluşan çok katmanlı bir sinir ağıdır. Evrişim katmanı, giriş verileri üzerinde evrişim işlemi gerçekleştiren ESA'nın temel parçasıdır. Evrişim, iki matris, yani alıcı alan (receptive field) ve çekirdek (kernel) arasındaki bir iç çarpım işlemidir. Genel olarak, çekirdek uzamsal olarak giriş verilerinden daha küçüktür. Çekirdek alıcı alan üzerinde kaymakta ve giriş verilerinin bir özellik haritasını oluşturmaktadır. Havuzlama katmanı, özellik haritasının uzamsal boyutunu azaltmaktadır. Bir dizi çıktıyı, yakındaki özellik değerlerinin istatistiklerine göre tek bir değerle değiştirmektedir. En yaygın kullanılan havuzlama tekniği maksimum havuzlamadır ve bir dizi özelliği maksimum değeriyle değiştirmektedir. Tamamen bağlı katman, tüm nöronların birbirini takip eden her katman nöronuna bağlandığı çok katmanlı bir algılayıcıdır. Bu katman, özellikleri çıktıya haritalamak için kullanılmaktadır [20].  $x$  girdileri için tek nöronun çıktısı ( $y$ ) Eşitlik 1.'deki gibi hesaplanmaktadır.

$$y = f(w * x + b) \quad (1)$$

Eşitlik 1.'de,  $w$  filtre ağırlığı ve  $b$  ise bias değeridir.  $f(\cdot)$ , ağırlıklı bir girdi toplamına uygulanan doğrusal olmayan aktivasyonu ifade etmektedir. 2B ESA modeli, giriş verilerini etkinleştirmeden önce iki boyutlu bir çekirdek kullanarak evrişim işlemini gerçekleştirmektedir. Bu, giriş görüntüsünden uzamsal özellikleri çıkarmaya yardımcı olmaktadır. Her nöronun 2B evrişim çıktısı için Eşitlik 1. yeniden Eşitlik 2.'deki gibi formüle edilmektedir [20].

$$y_{mn} = f \left( \sum_r \sum_{i=0}^{h-1} \sum_{j=0}^{w-1} k_{ij} x_{(i+m)(j+n)} + bias_{mn} \right) \quad (2)$$

Eşitlik 2.'de  $y_{mn}$ , ( $m, n$ ) konumunda çıkarılan özelliştir.  $k$ ,  $h \times w$  boyutundaki 2B evrişim çekirdeğidir. 2B görüntü olması durumunda, bu evrişim işlemi, alıcı alandaki tüm özellik haritalarını ( $r$ ) üzerinde gerçekleştirilmekte ve doğrusal olmayan aktivasyon için tüm değerlerin toplamını almaktadır. Bu işlem, çok boyutlu veriler durumunda tüm katmanlar için tekrarlanmaktadır. Veriler üç boyutlu olduğunda (örneğin, video, renkli görüntüler, hiperspektral veya multispektral görüntüler), bunların hem uzamsal hem de spektral veya zamansal boyutları vardır. Bu giriş verileri için 2B evrişim başarısız olmaktadır. Çünkü, geleneksel 2B ESA'da, evrişim işlemleri yalnızca uzamsal boyuttaki özellikleri yakalayan 2B özellik haritalarına uygulanmaktadır. 3B ESA, 2B ESA'da 2B evrişim yerine üç boyutlu evrişim gerçekleştiren 2B ESA modellerinin değiştirilmiş şeklidir. Evrişim işlemleri, 3B verilere uygulandığında hem uzamsal hem de spektral

boyutlardan özelliklerin yakalanması istenilmektedir. Bu amaçla, 3B giriş verilerinden ortak uzamsal spektral özellikleri hesaplamak için 3B evrişim işlemlerinin 3B özellik küplerine uygulandığı 3B ESA önerilmektedir. 3B evrişim, üç boyutlu spektral görüntülerden hem uzamsal hem de spektral özelliklerin çıkarılmasına yardımcı olmaktadır. 3B ESA modelinden çıkarılan özellik Eşitlik 3.'teki gibi formüle edilmektedir [20].

$$y_{mnp} = f \left( \sum_r \sum_{i=0}^{h-1} \sum_{j=0}^{w-1} \sum_{l=0}^{b-1} k_{ijl} x_{(i+m)(j+n)(l+p)} + bias_{mnp} \right) \quad (3)$$

Eşitlik 3.'te  $y_{mnp}$ , ( $m, n, p$ ) konumunda çıkarılan özelliştir.  $b$  spektral boyut boyunca 3B çekirdeğin boyutudur. Çekirdek ( $k$ ), üç boyutludur ve özellikler, 3B giriş verileri üzerinde 3B evrişim gerçekleştirilerek hesaplanmaktadır. Evrişimli sinir ağına en sık kullanılan aktivasyon fonksiyonu  $f(\cdot)$  ReLU'dur. ReLU, eğitim için gradyan iniş tekniklerini kullanırken diğer aktivasyon işlevlerinden daha hızlıdır. ReLU Eşitlik 4.'teki gibi formüle edilmektedir.

$$ReLU(y) = \max(0, y) \quad (4)$$

Softmax, sınıflandırma için bir derin öğrenme modelinin son katmanında genellikle kullanılan başka bir etkinleştirme işlevidir. Softmax, tüm örneklerin olasılık dağılımını üretmekte ve toplamı bire eşittir. Eğitim verilerini kullanarak ağırlıkları güncellemek için ESA'da farklı optimizasyon teknikleri kullanılmaktadır. En yaygın kullanılan optimizasyon gradyan iniş tekniğidir. Adam (uyarlanabilir momentum tahmini), genellikle konveks olmayan problemler için kullanılan başka bir optimize edicidir. Adam, birinci ve ikinci gradyan momentine bağlı olarak ağıdaki her ağırlık değeri için ayrı bir öğrenme oranı sağlar. Adam optimizer'da kullanılan parametreler öğrenme hızı ( $\alpha$ ),  $\beta_1$  ve  $\beta_2$ 'dir.  $\beta_1$  ve  $\beta_2$ , sırasıyla birinci ve ikinci momentlerin üstel azalmasıdır [21]. Özetle, hiperspektral görüntü sınıflandırması için, 2B evrişim işlemi giriş verilerini uzamsal boyutta evrişim işlemine tabi tutarken, 3B evrişim işlemi giriş verilerini hem uzamsal hem de spektral boyutta eş zamanlı olarak evrişim işlemine tabi tutmaktadır. 2B evrişim işlemi ister 2B veriye ister 3B veriye uygulanıp uygulanmadığına bakılmaksızın, çıktısı 2B'dir. Hiperspektral görüntüye 2B evrişim işlemleri uygulandıysa, önemli spektral bilgiler kaybedilirken, 3B evrişim giriş hiperspektral görüntü verilerinin spektral bilgilerini koruyarak bir çıktı hacmi elde edebilmektedir. Bu, zengin spektral bilgi içeren hiperspektral görüntüler için çok önemlidir.

## 3. ÖNERİLEN YÖNTEM

Geleneksel bir 2B ESA, genellikle evrişimli katmanlardan, havuz katmanlarından ve tamamen bağlantılı katmanlardan oluşmaktadır. 2B ESA'dan farklı olarak, bu çalışmada, hiperspektral görüntü sınıflandırması için önerilen 3B ESA, yalnızca evrişim

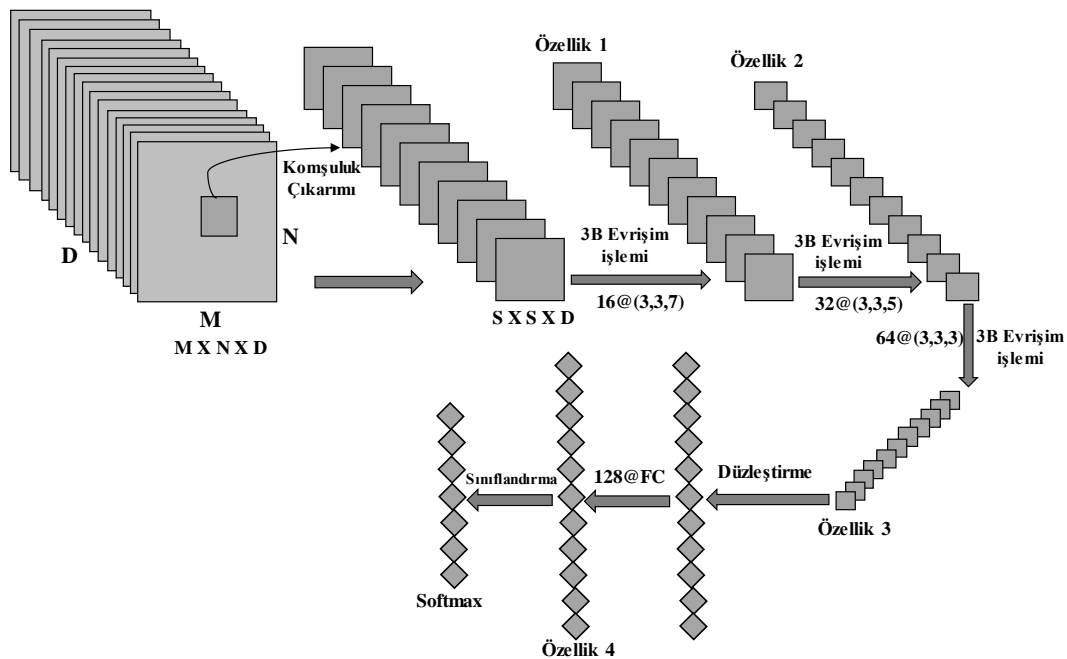
katmanlarından, düzleştirme ve tamamen bağlantılı bir katmandan oluşmaktadır. Hiperspektral görüntülerde uzamsal çözünürlüğü azaltılmasıyla bilinen havuzlama işlemleri önerilen yöntemde uygulanmamaktadır. Önerilen 3B ESA modeli piksel düzeyinde hiperspektral görüntü sınıflandırması için kullanılmaktadır. Spektral uzamsal özellikleri öğrenmek için 3B çekirdeklerle evrişim işlemine tabi tutulmak üzere girdi verileri olarak tüm spektral bantlar boyunca küçük bir uzamsal komşulukta (tüm görüntü değil) piksellerden oluşan görüntü küpleri çıkarılmaktadır. Hiperspektral görüntü  $X$ ,  $M \times N \times D$  boyutunda 3 boyutlu bir küp olarak belirtilmiştir. Burada  $M$  ve  $N$ , görüntünün uzamsal genişliği ve yüksekliğidir.  $D$  ise, spektral bantların sayısını belirtir. Görüntü sınıflandırma tekniklerini kullanmak için, hiperspektral veri küpü, merkezi piksele dayalı gerçek etiketlerin oluşturulduğu küçük üst üste binen 3B uzamsal parçalara bölünmüştür. Uzamsal konumda (a,b) merkezlenmiş ve  $S \times S$  penceresini veya uzamsal boyutu ve tüm  $D$  spektral bantlarını kapsayan hiperspektral veri küpünden 3B komşu parçaları  $S \times S \times D$  oluşturuldu. Hiperspektral veri küpünden üretilen toplam 3B parça sayısı ( $n$ ),  $(M - S + 1) \times (N - S + 1)$  ile bulunur. 3B ESA, 3B evrişim çekirdeğini kullanarak her bir görüntü parçasını 3B evrişim işlemine tabi tutar. Hiperspektral görüntü verileri için önerilen modelde, evrişim katmanının özellik haritaları, giriş katmanındaki çoklu bitişik bantlar üzerinde 3B çekirdek kullanılarak oluşturulur. Böylelikle hem uzamsal hem de spektral özelliklere sahip özellikler çıkarılmış olur. Evrişim işlemi uygulanmış özelliklere doğrusal olmayan bir etkinleştirme işlevi olan ReLU uygulanmaktadır.

Önerilen 3B ESA modelinin akış diyagramı Şekil 1'de gösterilmektedir. Önerilen 3B ESA modelinde üç 3B evrişim katmanı, düzleştirme ve tam bağlantılı bir katman bulunmaktadır. Herhangi bir ön işlem aşaması gerçekleştirilmemektedir. Uygulanan 3B evrişim çekirdeklerinin boyutları ve kullanılan filtreler şu şekildedir. 1. evrişimli katmanda  $3 \times 3 \times 7$  (yani; iki

uzamsal ve bir spektral boyut) boyutunda 16 filtre, 2. evrişim katmanında  $3 \times 3 \times 5$  boyutunda 32 filtre, 3. evrişim katmanında  $3 \times 3 \times 3$  boyutunda 64 filtre kullanılmaktadır. ReLU aktivasyon fonksiyonu tüm evrişim katmanlarında kullanılmaktadır. 3B Evrişim katmanlarından sonra çıkarılan özellikler düzleştirilmektedir. Daha sonra sınıflandırma için tam bağlı katmanlara girdi olarak verilmektedir. Bu modelde, 128 nöron bulunan bir tane tam bağlantılı katman kullanılmaktadır. Tam bağlantılı katmanın (FC) çıktısı, gerekli sınıflandırma sonucunu oluşturmak için basit softmax sınıflandırıcıya verilmektedir. Önerilen model için her katmanda kullanılan çıktı boyutu ve parametre sayısı Tablo 1'de gösterilmektedir. Önerilen modelde, 1.yoğun katmanda en fazla sayıda parametrenin mevcut olduğu görülebilmektedir. Son yoğun katmandaki düğüm sayısı, pavia üniversitesi veri setindeki sınıfların sayısı ile aynı olan 9'dur. Dolayısıyla, önerilen modeldeki toplam parametre sayısı, bir veri setindeki sınıfların sayısına bağlıdır. Pavia üniversitesi veriseti için önerilen yöntemdeki toplam eğitilebilir ağırlık parametresi sayısı 875,369'dur. 0,001 öğrenme oranına sahip kategorik çapraz entropi ve optimizasyon için kullanılan 1e-06 bozunma işlevine sahip Adam optimizör kullanılmıştır. Eğitim süreci, herhangi bir toplu normalleştirme (batch normalization) ve veri arttırma (data augmentation) olmaksızın 256 batch size 300 döngü (epochs) boyunca tekrar etmektedir.

**Tablo 1.** Pavia Üniversitesi veriseti için önerilen 3B ESA modelinin özeti

Katman (Tip)	Çıkış Boyutu	Parametre Sayısı
input_1 (Giriş katmanı)	(5,5,103,1)	0
conv3d_1 (Conv3d)	(5,5,103,16)	1024
conv3d_2 (Conv3d)	(3,3,99,32)	23072
conv3d_3 (Conv3d)	(1,1,97,64)	55360
flatten_1 (Flatten)	6208	0
dense_1 (Dense)	128	794752
dense_2 (Dense)	9	1161
<b>Toplam Eğitilebilir Parametreler : 875,369</b>		



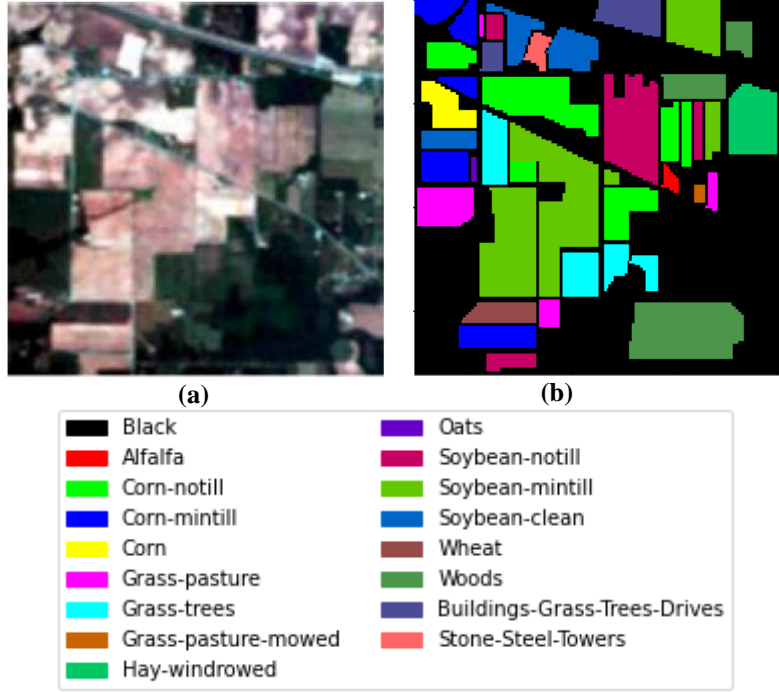
**Şekil 1.** Hiperspektral görüntü sınıflandırması için önerilen 3B ESA modeli

## 4. DENEYSEL VERİSETLERİ VE SONUÇLAR

### 4.1. Verisetleri

Önerilen yöntemin etkinliğini göstermek için Indian Pines (IP), Pavia Üniversitesi (PU) ve Botswana (B) veri setlerini içeren üç gerçek hiperspektral veri seti kullanılmıştır [22]. IP veriseti, Kuzeybatı Indiana'daki Indian Pines test alanında Havadan Görünür / Kızılötesi Görüntüleme Spektrometresi (Airborne Visible/Infrared Imaging Spectrometer-AVIRIS) sensörü tarafından elde edilmiştir. Bu verisetindeki her spektral görüntü  $145 \times$

145 uzamsal boyutundadır. Sensör, 0.4-2.5 mikrometre dalga boyu aralığında toplam 224 spektral bant elde etmiştir. Bu 224 banttan, 24 bant tam su absorpsiyonu (emme) bölgesindedir ve bunlar sınıflandırma işlemi için yararlı değildir. 24 su emme bandı çıkarıldıktan sonra kalan 200 bant deneyler için kullanılmıştır. Şekil 2'de Indian pines verisetinin sahte-renkli görüntüsü ve temel gerçek (ground truth) haritası gösterilmektedir. Tablo 2'de ise IP veri setinin 16 tür arazi örtüsü ve 10249 örnek içerdiği gösterilmektedir.



Şekil 2. Indian Pines veriseti. (a) Indian pines verisetinin sahte-renkli görüntüsü (b) Indian pines verisetinin temel gerçek (ground truth) haritası

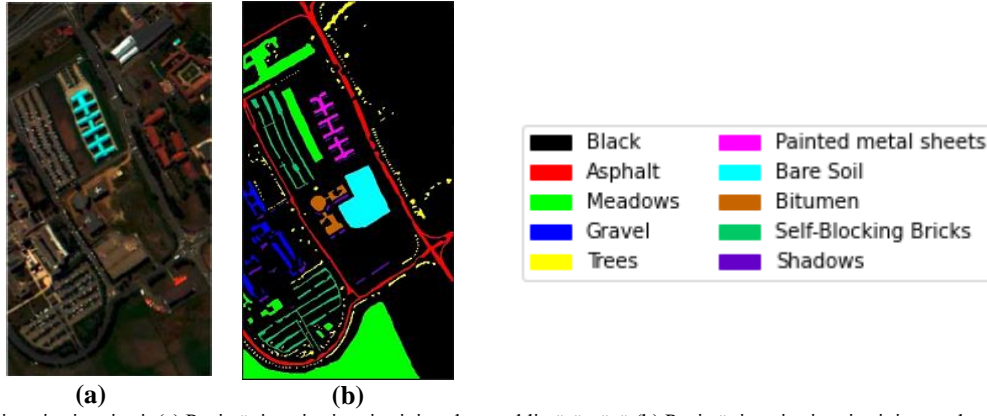
Tablo 2. Indian pines veri seti için sınıflar ve örnek sayıları

Indian Pines (IP) Veriseti					
No.	Sınıf	Örnek Sayısı	No.	Sınıf	Örnek Sayısı
1	Alfalfa	46	9	Oats	20
2	Corn-notill	1428	10	Soybean-notill	972
3	Corn-mintill	830	11	Soybean-mintill	2455
4	Corn	237	12	Soybean-clean	593
5	Grass-pasture	483	13	Wheat	205
6	Grass-trees	730	14	Woods	1265
7	Grass-pasture-mowed	28	15	Buildings-Grass Trees-Drives	386
8	Hay-windrowed	478	16	Stone-Steel-Towers	93

PU veriseti, kuzeydoğu İtalya'daki Pavia Üniversitesi'nden Yansıtıcı Optik Sistem Görüntüleme Spektrometresi (Reflective Optics System Imaging Spectrometer - ROSIS) tarafından toplanan verilerdir. Veriseti, piksel başına 1.3 m uzamsal çözünürlükle 340 piksel genişliğinde ve 610 piksel yüksekliğindedir. 0.43 mikrometre ile 0.86 mikrometre arasında değişen 115 dalga boyuna sahiptir. 12 görüntü bandı çıkarıldıktan sonra, kalan 103 bant deneyde kullanılmıştır. PU veriseti 9 sınıfa ayrılmaktadır. Toplam 42776 örnek içermektedir. Şekil 3'de, PU verisetinin sahte-renkli

görüntüsü ve temel gerçek (ground truth) haritası gösterilmektedir. Ayrıca, PU veriseti için sınıflar ve bu sınıfların içerdiği örnek sayıları Tablo 3'te gösterilmektedir.





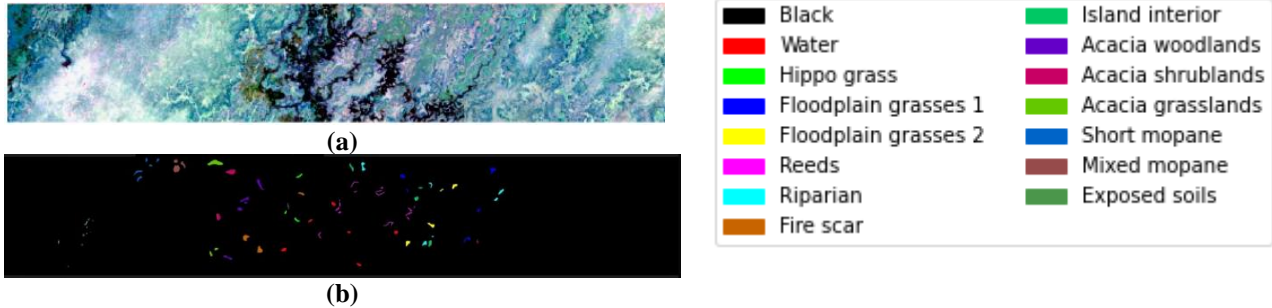
Şekil 3. Pavia üniversitesi veriseti. (a) Pavia üniversitesi verisetinin sahte-renkli görüntüsü (b) Pavia üniversitesi verisetinin temel gerçek (ground truth) haritası

Tablo 3. Pavia üniversitesi veri seti için sınıflar ve örnek sayıları

Pavia Üniversitesi (PU) Veriseti					
No.	Sınıf	Örnek Sayısı	No.	Sınıf	Örnek Sayısı
1	Asphalt	6631	6	Bare Soil	5029
2	Meadows	18649	7	Bitumen	1330
3	Gravel	2099	8	Self-Blocking Bricks	3682
4	Trees	3064	9	Shadows	947
5	Painted Metal Sheets	1345			

B veriseti, 2001 yılında Botswana, Okavango Deltası üzerinde EO-1 üzerinde Hyperion sensörü tarafından toplandı. Elde edilen veriler orijinal olarak 30 m piksel çözünürlüklü 10 nm pencerelerde spektrumun 400–2500 nm bölümünü kapsayan 242 banttan oluşuyordu. Kalibre edilmemiş (Ayarlanmamış) ve su emilimini örten gürültülü bantlar [10-55, 82-97, 102-119, 134-164, 187-220] çıkarıldıktan sonra deneylerde sadece 145 bant

kullanıldı. Kullanılan veriler, arazi örtüsü türlerini temsil eden 14 tanımlanmış sınıftan ve gözlemlerle 1476 x 256 pikselden oluşmaktadır. Toplam 3248 örnek içermektedir. Şekil 4'te, B verisetinin sahte-renkli görüntüsü ve temel gerçek (ground truth) haritası gösterilmektedir. B veriseti için sınıflar ve bu sınıfların içerdiği örnek sayıları ise Tablo 4'te gösterilmektedir.



Şekil 4. Botswana veriseti. (a) Botswana verisetinin sahte-renkli görüntüsü (b) Botswana verisetinin temel gerçek (ground truth) haritası

Tablo 4. Botswana veri seti için sınıflar ve örnek sayıları

Botswana (B) Veriseti					
No.	Sınıf	Örnek Sayısı	No.	Sınıf	Örnek Sayısı
1	Water	270	8	Island interior	203
2	Hippo grass	101	9	Acacia woodlands	314
3	Floodplain grasses 1	251	10	Acacia shrublands	248
4	Floodplain grasses 2	215	11	Acacia grasslands	305
5	Reeds	269	12	Short mopane	181
6	Riparian	269	13	Mixed mopane	268
7	Fire scar	259	14	Exposed soils	95

## 4.2. Deneysel Kurulum

Tüm deneyler Google Colab olarak bilinen çevrimiçi bir platformda gerçekleştirilmiştir. Google Colab, herhangi bir ortamı çalıştırmak için iyi bir internet hızı gerektiren çevrimiçi bir platformdur. Google Colab, kodların Tensör İşlem Birimi (TPU), 35 GB Rasgele Erişim Belleği (RAM) ve veri hesaplaması için 107,77 GB depolama alanı içeren python 3 masaüstü bilgisayarda

çalıştırılması için bir seçenek sunmaktadır. Tüm deneylerde, test-eğitim seti %30-70 oranına bölünmüştür. 0,001 öğrenme oranına sahip kategorik çaprazentropi ve optimizasyon için kullanılan 1e-06 bozunma işlevine sahip Adam optimizör kullanılmıştır. Eğitim süreci, 256 batch size ve 300 döngü (epochs) boyunca tekrar etmektedir. Softmax'ın kullanıldığı son katman hariç tüm katmanlar için bir aktivasyon işlevi olarak ReLU kullanılmaktadır. Üç verisetinin tümü,

evrişim için  $5 \times 5$  komşuluk kullanılmaktadır. Herhangi bir ön işlem ve son işlem gerektirmemektedir. Tüm spektral bant alınarak işlemler gerçekleştirilmektedir. Her modelin sınıflandırma performansını değerlendirmek için genel doğruluk (GD), ortalama doğruluk (OD) ve kappa istatistiği (K) kullanılmıştır. GD, test verilerindeki doğru sınıflandırılmış örnekler ile toplam test örneği sayısı arasındaki oranla hesaplanmıştır. OD her kategorinin doğruluğunun ortalama değeridir. K, temel gerçek (ground truth) haritası ile sınıflandırma haritası arasında güçlü bir anlaşma ile ilgili karşılıklı bilgi sağlayan bir istatistiksel ölçüm metriğidir. GD, OD ve K ölçümlerinin yanı sıra, fl skoru (fl-score), hassasiyet (precision) ve geri çağırma (recall) gibi çeşitli istatistiksel testler de dikkate alınmaktadır.

### 4.3. Deneysel Sonuçlar

Önerilen 3B ESA yönteminin etkinliğini değerlendirmek için, dört derin öğrenme hiperspektral görüntü sınıflandırma yöntemiyle karşılaştırıldı: 3B ESA [1], DBN-LR [17], 2B ESA [19] ve SAE-LR [23]. 3B ESA, 2 tane 3B evrişim katmanı, tam bağlantılı katman ve sınıflandırma katmanından oluşmaktadır. 3B evrişim katmanlarının boyutları pavia üniversitesi için 2 adet  $3 \times 3 \times 7$  ve 4 adet  $3 \times 3 \times 3$  olarak alınmıştır. Pencere boyutu  $S=5$  olarak ayarlanmıştır. Yani, orijinal spektral-uzamsal özellikleri hesaplamak için  $5 \times 5 \times 103$  küplerini çıkarılmıştır. Daha sonra bu küpler 3B ESA için girdi olarak kullanılmıştır. Botswana veri seti için, 2 adet  $3 \times 3 \times 2$  ardından 4 adet  $3 \times 3 \times 2$  evrişim katmanı kullanılmıştır. Indiana pines veri seti için 2 adet  $3 \times 3 \times 7$  ve 4 adet  $3 \times 3 \times 3$  evrişim katmanı kullanılmıştır. SAE-LR, hiperspektral görüntülerin sınıflandırılması için yığılı otokodlayıcı ile lojistik regresyonun birlikte

kullanılmasını öneren yöntemdir. DBN-LR ise, derin inanç ağları ile lojistik regresyonun birlikte kullanılmasını öneren yöntemdir. SAE-LR ve DBN-LR için, orijinal hiperspektral görüntü, sırasıyla temel bileşen analizi yöntemiyle spektral boyut boyunca dört banda ve beş banda indirgenmiştir. Ardından, uzamsal özellikleri oluşturmak için  $5 \times 5 \times 4$  küp ve  $5 \times 5 \times 5$  küp kullanılmıştır. Son olarak, ortaya çıkan uzamsal özellikler spektral özellikler ile birleştirilmiştir. 2B ESA için, hiperspektral görüntü, temel bileşen analizi ile spektral boyut boyunca üç banda indirgenmiştir. Orijinal özellikleri oluşturmak için  $42 \times 42 \times 3$  küp çıkarılmıştır. Ağ, üç evrişim katmanı ve iki havuzlama katmanı içermektedir. Üç evrişim katmanı sırasıyla birinci, ikinci ve üçüncü katmanlar için 36 adet  $5 \times 5$  çekirdek, 72 adet  $6 \times 6$  çekirdek ve 72 adet  $4 \times 4$  çekirdek içermektedir.

Tablo 5, Pavia üniversitesi verisetinden elde edilen farklı yöntemlerin GD, OD ve K olmak üzere üç değerlendirme göstergesine göre sınıflandırma sonuçlarını vermektedir. Önerilen yöntem ile tüm sınıflarda da en iyi sınıflandırma sonucu elde edilmiştir. Önerilen 3B ESA modeli %99,90 ile iki 3B evrişim katmanından oluşan 3B ESA modelinden %0,51, 2B ESA modelinden %0,87, DBN-LR modelinden %0,91 ve SAE-LR modelinden %1,44 daha iyi genel doğruluk sonucu elde etmektedir. Benzer şekilde önerilen model %99,78 ile sırasıyla, 3B ESA modelinden %0,93, 2B ESA modelinden %1,59, DBN-LR modelinden %1,4 ve SAE-LR modelinden %2,11 daha iyi ortalama doğruluk sonucu elde etmiştir. Kappa istatistiği değerlendirme ölçütünde ise yine önerilen yöntem %99,86 ile diğer tüm yöntemlerden en iyi sonucu elde ettiği görülmektedir. Tablo 5 göz önünde bulundurulduğunda, önerilen yöntemin sınıflandırma sonuçları temel gerçeğe (ground truth) çok benzerdir.

**Tablo 5.** PU verisetinde farklı yöntemlerle elde edilen sınıflandırma sonuçları

Sınıf	SAE-LR	DBN-LR	2B ESA	3B ESA	Önerilen 3B ESA
1	98,73	99,05	99,68	99,65	<b>99,90</b>
2	99,55	99,83	99,87	99,83	<b>99,96</b>
3	93,87	95,15	96,31	94,65	<b>99,36</b>
4	98,63	98,83	98,01	99,09	<b>99,89</b>
5	100	99,93	100	100	<b>100</b>
6	97,87	98,71	97,61	99,93	<b>100</b>
7	93,74	96,36	95,63	97,75	<b>99,25</b>
8	96,76	98,20	99,35	99,24	<b>100</b>
9	99,90	99,71	97,25	99,55	<b>99,65</b>
<b>GD</b>	98,46	98,99	99,03	99,39	<b>99,90</b>
<b>OD</b>	97,67	98,38	98,19	98,85	<b>99,78</b>
<b>K</b>	97,98	98,68	98,71	99,20	<b>99,86</b>

Tablo 6, Botswana verisetinden elde edilen çeşitli yöntemlerin GD, OD ve K olmak üzere üç değerlendirme göstergesine göre sınıflandırma sonuçlarını vermektedir. Tablo 6'dan, önerilen yöntemin 6 nolu sınıf için sınıflandırma doğruluğu 3B ESA yönteminden %1,19 daha düşük çıkmıştır. 8 nolu sınıf için sınıflandırma doğruluğu 3B ESA, 2B ESA ve SAE-LR yöntemlerinden %1,64 ve DBN-LR yönteminden ise %1,38 düşük çıkmıştır. 10 nolu sınıf için sınıflandırma doğruluğu DBN-LR ve 2B ESA yöntemlerinden %1,35, 3B ESA yönteminden %1,05 ve SAE-LR yönteminden %1,09 daha düşük çıkmıştır. Geri kalan diğer sınıfların tamamında önerilen yöntem ile başarılı sınıflandırma

sonuçları elde edilmiştir. GD, OD ve K değerlendirme ölçütleri göz önüne alındığında önerilen yöntem ile diğer yöntemlerin tamamından yüksek sonuçlar elde edilmiştir. Önerilen yöntemin %99,59 GD sonucu, SAE-LR, DBN-LR, 2B ESA ve 3B ESA'dan sırasıyla, %1,1, %0,78, %0,71 ve %0,04 daha yüksek olduğu görülmektedir. Önerilen yöntem ayrıca, OD ve Kappa istatistiği açısından sırasıyla, %99,61 ve %99,55 ile diğer yöntemlerin tamamından daha iyi performans gösterdiği görülmektedir. Tablo 6'dan, önerilen yöntemin sınıflandırma sonuçları temel gerçeğe (ground truth) çok benzer olduğu görülmektedir.

**Tablo 6.** B verisetinde farklı yöntemlerle elde edilen sınıflandırma sonuçları

Sınıf	SAE-LR	DBN-LR	2B ESA	3B ESA	Önerilen 3B ESA
1	100	100	99,18	99,64	100
2	100	100	100	100	100
3	100	100	100	100	100
4	99,58	100	99,16	99,45	100
5	94,70	94,84	99,54	98,60	100
6	92,96	95,33	97,36	98,72	97,53
7	99,88	100	100	99,68	100
8	100	99,74	100	100	98,36
9	96,68	96,98	94,99	99,67	100
10	99,74	100	100	99,70	98,65
11	99,47	99,67	100	99,87	100
12	100	100	96,63	99,63	100
13	99,52	99,82	100	99,43	100
14	99,26	100	97,44	100	100
GD	98,49	98,81	98,88	99,55	99,59
OD	98,70	99,03	98,88	99,60	99,61
K	98,36	98,72	98,78	99,51	99,55

Tablo 7, Indian pines verisetinden elde edilen çeşitli yöntemlerin GD, OD ve K olmak üzere üç değerlendirme göstergesine göre sınıflandırma sonuçlarını vermektedir. Tablo 7'den, önerilen yöntemin 12 nolu sınıf için sınıflandırma doğruluğu 3B ESA yönteminden %0,12 ve 14 nolu sınıf için sınıflandırma doğruluğu 3B ESA yönteminden %0,08, DBN-LR yönteminden daha % 0,27 daha düşük çıkmıştır. 12 ve 14 nolu sınıflar için sınıflandırma doğruluğu önerilen yöntemde düşük çıkmasına rağmen, GD, OD ve K değerlendirme ölçütleri göz önüne alındığında diğer yöntemlerin tamamından yüksek sonuçlar elde

edilmiştir. Önerilen yöntemin %99,35 olan GD sonucu SAE-LR, DBN-LR, 2B ESA ve 3B ESA yöntemlerinden sırasıyla, %5,37, %3,44, %3,38 ve %0,28 daha yüksek olduğu görülmektedir. Önerilen yöntem ayrıca, OD ve Kappa istatistiği açısından sırasıyla, %99,61 ve %99,21 ile diğer yöntemlerin tamamından daha iyi performans gösterdiği görülmektedir. Tablo 7 göz önünde bulundurulduğunda, önerilen yöntemin sınıflandırma sonuçları 9 sınıf için temel gerçek (ground truth) ile aynı geri kalan 7 sınıf için ise temel gerçeğe yakın sonuçlar elde edildiği görülmektedir.

**Tablo 7.** IP verisetinde farklı yöntemlerle elde edilen sınıflandırma sonuçları

Sınıf	SAE-LR	DBN-LR	2B ESA	3B ESA	Önerilen 3B ESA
1	85,56	80,90	86,11	95,89	100
2	90,72	93,97	91,37	98,46	98,59
3	91,58	95,13	95,37	98,99	99,19
4	89,81	85,14	98,54	99,14	100
5	96,16	98,05	91,40	99,29	99,31
6	98,98	100	98,05	99,92	100
7	95,29	94,92	97,73	100	100
8	98,75	100	98,44	100	100
9	100	100	50,87	92,31	100
10	94,52	97,37	93,53	98,12	98,63
11	94,79	97,70	97,62	98,96	99,45
12	86,43	84,72	94,89	98,99	98,87
13	99,80	99,35	100	99,82	100
14	97,48	100	99,29	99,81	99,73
15	84,35	84,64	99,59	99,56	100
16	96,76	95,33	98,88	99,38	100
GD	93,98	95,91	95,97	99,07	99,35
OD	93,81	94,20	93,23	98,66	99,61
K	93,13	95,34	95,40	98,93	99,21

Yukarıda sunulan deneysel sonuçlara göre, ilk olarak, ESA tabanlı yöntemler, SAE ve DBN tabanlı yöntemlerden daha iyi sınıflandırma sonuçları elde etmektedir. SAE-LR ve DBN-LR, derin öğrenme mimarisinin kullanımıyla derin özellikler çıkarabilse de, SAE ve DBN'nin girdi gereksinimini karşılamak için görüntü parçalarının tek boyuta düzleştirilmesi gerekmektedir. Düzleştirme işlemi, orijinal hiperspektral görüntüde bulunan uzamsal bilgiden yararlanmada başarısız olmaktadır. Bu durum da biraz daha kötü bir performansa yol açabilmektedir. Daha da önemlisi, SAE ve DBN, özellikleri denetimsiz bir şekilde öğrenmekte ve etiket bilgilerinden tam olarak yararlanamamaktadır. İkinci olarak, 3B ESA, 2B ESA'ya kıyasla spektral-uzamsal özellik öğrenimi için daha iyi çalışmaktadır. 3B

ESA, 3B evrişim işlemleri ile daha ince spektral bilgileri modelleyebilmektedir. Ayrıca 3B hiperspektral görüntü üzerine 3B çekirdek uygulayarak, bir 3B ESA herhangi bir ön işleme dayanmazken, yöntemin uygulanması kolaydır. SAE-LR, DBN-LR ve 2B ESA'da ise spektral boyutluluğu kabul edilebilir bir düzeye indirmek için temel bileşen analizinin kullanılması gereklidir. Son olarak, SAE-LR, DBN-LR, 2B ESA ve 2 evrişim katmanına sahip 3B ESA ile karşılaştırıldığında, önerilen 3B ESA modeli üç veri kümesinin hepsinde daha iyi bir performans elde etti. Özellikle tüm verisetlerinde de, önerilen 3B ESA ile iki evrişim katmanından oluşan 3B ESA, 2B ESA'dan daha iyi genel doğruluk oranlarıyla en iyi sonuçları elde etti. Bu, 3B yaklaşımın geleneksel 2B evrişimlere kıyasla performansta bir artış sağlamaya



yardımcı olduğunu göstermektedir. Hiperspektral görüntü verilerindeki ortak spektral ve uzamsal bilgilerin 3B evrişim işlemleri yoluyla uygun modellenmesi, sınıflandırma işleminde ayırt edici özelliklerin yakalanması için önemlidir.

## 5. SONUÇ

Hiperspektral görüntü sınıflandırması için uygun bir derin öğrenme modelinin tasarımı büyük bir zorluktur. Uzamsal ve spektral özelliklerin birleşik kullanımı, sınıflandırma performansını arttırmaktadır. Bu çalışmada, hiperspektral görüntü sınıflandırmasını iyileştirmek amacıyla, hiperspektral görüntü verilerinde bulunan hem spektral hem de uzamsal bilgilerden tam olarak yararlanan yeni bir 3B ESA hiperspektral görüntü sınıflandırma modeli önerilmiştir. 3B ESA modelinin, sınıflandırma için hiperspektral görüntünün 3B yapısına uyacak şekilde uyarlanabileceği gösterilmiştir. Özellikle, önerilen 3B ESA modeli üç hiperspektral görüntü veri setinde, derin öğrenmeye dayalı dört hiperspektral görüntü sınıflandırma yöntemiyle karşılaştırılmıştır. Deneysel çalışmalar sonucunda sırasıyla genel doğruluk, ortalama doğruluk ve Kappa değerleri, Indian pines için %99,35, %99,61 ve %99,21, Pavia üniversitesi için %99,90, %99,78 ve %99,86 ve Botswana için ise %99,59, %99,61 ve %99,55 olarak elde edilmiştir. Sonuçlar, 4 farklı derin öğrenme tabanlı yöntemle karşılaştırılmıştır. Deneysel çalışmalar, önerilen 3B ESA tabanlı hiperspektral görüntü sınıflandırma yönteminin üç veri setinin tümünde en iyi genel doğruluğu, ortalama doğruluğu ve kappa değerini elde ettiğini göstermektedir.

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## Bazı İlaçların Koyun Dalak Dokusundan Saflaştırılan Glukoz-6-Fosfat Dehidrogenaz Enzimi Üzerine *In Vitro* Etkileri

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**Anahtar Kelimeler**  
 Glukoz 6-fosfat dehidrogenaz, Saflaştırma, İlaç, İnhibisyon, İveral, Gentamisin

**Öz:** Bu çalışmada bazı ilaçların, koyun dalak dokusundan saflaştırılan glukoz-6-fosfat dehidrogenaz enzimi (G6PD; E.C. 1.1.1.49) üzerine *in vitro* etkileri araştırıldı. Çalışmanın ilk aşamasında G6PD enzimi koyun dalak dokusundan *salting out* (amonyum sülfat çöktürmesi) metodu ve 2', 5' ADP-Sepharose 4B afinite kromatografisi ile saflaştırıldı ve enzimin saflık derecesi SDS-PAGE metodu ile kontrol edildi. Çalışmanın ikinci aşamasında iveral, linkomisin, gentamisin, amoksisilin, ampisilin, streptomisin sülfat, novamizol, ketojezik, sefuroksim, sefazolin sodyum ve tylosin ilaçlarının enzim aktivitesi üzerindeki etkileri araştırıldı. Araştırma sonuçları; iveral, gentamisin, streptomisin sülfat ve linkomisin ilaçlarının G6PD enzimini sırasıyla 0,62 mM, 21,6 mM, 173,2 mM ve 231 mM, IC<sub>50</sub> değerleri ile inhibe ettiğini, amoksisilin, ampisilin, novamizol, ketojezik, sefuroksim sodyum, sefazolin sodyum ve tylosin ilaçlarının ise enzim üzerinde önemli bir aktivasyon ya da inhibisyon etkisine sahip olmadıklarını gösterdi.

## *In Vitro* Effects of Some Drugs on Glucose-6-Phosphate Dehydrogenase Enzyme Purified from Sheep Spleen Tissue

**Keywords**  
 Glucose 6-phosphate dehydrogenase, Purification, Drug, Inhibition, Iveral, Gentamicin

**Abstract:** In this study, *in vitro* effects of some drugs on glucose-6-phosphate dehydrogenase enzyme (G6PD; E.C. 1.1.1.49) purified from sheep spleen tissue were investigated. In the first step of the study, G6PD enzyme was purified from sheep spleen tissue by *salting out* (ammonium sulfate precipitation) method and 2', 5' ADP-Sepharose 4B gel affinity chromatography and the purity of the enzyme was checked by SDS-PAGE method. In the second phase of the study, the effects of iveral, lincomycin, gentamicin, amoxicillin, ampicillin, streptomycin sulfate, novamizole, ketogestic, cefuroxime, cefazolin sodium and tylosin drugs on enzyme activity were investigated. Research results showed that iveral, gentamicin, streptomycin sulfate and lincomycin drugs inhibit the G6PD enzyme with IC<sub>50</sub> values of 0.62 mM, 21.6 mM, 173.2 mM and 231 mM, respectively, amoxicillin, ampicillin, novamizole, ketogenic, cefuroxime sodium, cefazolin sodium and tylosin drugs did not have a significant activation or inhibition effect on the enzyme.

## 1. GİRİŞ

Glukoz-6-fosfat dehidrogenaz enzimi (G6PD; E.C. 1.1.1.49) pentoz fosfat yolunun birinci reaksiyonunu katalizleyerek NADP<sup>+</sup> varlığında NADPH üretimi ile birlikte, glukoz 6-fosfatın, 6-fosfoglukono laktone dönüşümünü gerçekleştiren kilit noktada bulunan bir enzimdir [1-3]. Reaksiyon ürünlerinden D-ribuloz 5-fosfat, ATP, CoA, NAD<sup>+</sup>, FAD, RNA ve DNA gibi hayati öneme sahip biyomoleküllerin bileşeni olan riboz 5-fosfata izomerize olur. Reaksiyon sırasında oluşan NADPH ise yağ asitleri, steroidler ve bazı amino asitlerin sentezinde kullanılan önemli bir koenzimdir [4-6]. Glutasyon ve tiyoredoksin sistemleri hücre içi iki ana antioksidan sistemidir. Bir tripeptid olan glutasyonun yapısında bulunan sülfhidril grubu (-SH), hücreleri okside moleküllerin zararlı etkilerine karşı korur. Ayrıca, DNA ve protein sentezine, ksenobiyotiklerin detoksifikasyonuna, amino asit taşınmasına ve enzimatik reaksiyonlara katılır [7-9]. Tiyoredoksin sistemi, DNA sentezinde, hücre sel büyümede, oksidatif stresin neden olduğu hücre sel hasarın önlenmesinde, apoptozda, peroksitlerin olumsuz etkilerinden hücrelerin korunmasında ve transkripsiyon faktör aktivitesinin uyarılmasında önemli bir role sahiptir [10-12]. Hem glutasyonun hem de tiyoredoksinin indirgenmesinde NADPH koenzim olarak görev yapar. Bu nedenle NADPH üretimine sebebiyet veren G6PD enzimi aynı zamanda indirekt antioksidan enzim olarak da bilinir [2, 13].

Günümüzde insan ve hayvan tedavisinde birçok antibiyotik ve ilacın kullanıldığı bilinmektedir [14-16]. Kullanılan ilaçların birçoğunun hedefi metabolizmada önemli görevleri olan düzenleyici enzimlerdir. Metabolizmada kavşak noktalarda yer alan düzenleyici enzimlerin aktivitesinde meydana gelen bir inhibisyon bazı durumlarda bir patojenin ölümünü ya da metabolik bir bozukluğun düzeltilmesini sağlayabilir. İnhibitörler enzimlere dönüşümlü ya da dönüşümsüz olarak bağlanırlar. Bir inhibitörün bağlanması, bir substratın enzimin aktif bölgesine bağlanmasını durdurabilir veya enzimin reaksiyonunu katalize etmesini engelleyebilir. Hastalıkların tedavisinde kullanılan birçok ilaç molekülü enzim inhibitörleridir, bu nedenle keşifleri ve iyileştirmeleri biyokimya ve farmakolojide aktif bir araştırma alanıdır [17,18].

Ancak kullanılan bu kimyasal maddelerin organizmada bulunan enzimler üzerinde olabilecek muhtemel etkilerinin çoğu henüz çalışılmamıştır. Bugüne kadar G6PD enziminin birçok canlı dokusundan saflaştırıldığı, kinetik özelliklerinin belirlendiği ve birçok ilaç ve kimyasal maddenin enzim üzerine etkilerinin tespit edildiği bilinmektedir [2,19-23]. Ancak literatür kaynaklarından araştırdığımız kadarı ile şu ana kadar koyun dalak G6PD enzimi ile ilgili herhangi bir saflaştırma ve kinetik çalışma yapılmamıştır.

Bu çalışmada G6PD enziminin koyun dalak dokusundan saflaştırılması ve bazı ilaçların enzim aktivitesi üzerine etkilerinin araştırılması amaçlanmıştır.

## 2. MATERYAL VE METOT

### 2.1. Materyal

Bu çalışmada kullanılan ilaçlar piyasadan, G6P, NADP<sup>+</sup>, Tris, elektroforez kimyasalları, protein standartları, amonyum sülfat, 2', 5' ADP-Sepharose 4B afinite jeli ve diğer kimyasallar Sigma-Aldrich Co. (St. Louis, MO) ve Merck (Darmstadt, Germany) den temin edildi.

### 2.2. Homojenatın Hazırlanması

Çalışmamızda kullanılan koyun dalak dokusu Bingöl ili Kombine Et ve Süt Kurumundan soğuk zincir kurallarına göre temin edildi. Alınan 15 gram dalak dokusu küçük parçalara ayrılarak 45 mL, 1 M Tris-HCl (pH= 8,0) tamponu içerisinde süspanse edildi. Elde edilen süspanسیون 10.000xg de 1 saat santrifüj edildikten sonra çöktelti atılarak homojenat oluşturuldu [24].

### 2.3. Amonyum Sülfat Çöktürmesi ve Diyaliz

Koyun dalak dokusundan elde edilen homojenat için %0-%90 doygunluk konsantrasyonlarında amonyum sülfat çöktürmesi yapıldı ve G6PD enzimi için en uygun çöktürme aralığı % 40-60 olarak tespit edildi. Enzim bu doygunlukta amonyum sülfat tuzu ile salting out metoduna göre çöktürüldü. Elde edilen süspanسیون 10.000xg'de 15 dakika santrifüj edilerek süpernatant kısmı uzaklaştırıldı. Geriye kalan çökelek 1 M Tris-HCl tamponunda (pH= 8,0) çözüldü ve enzim için aktivite kontrolü yapıldı. Daha sonra enzim çözeltisi diyaliz torbalarına konarak 2 saat 50 mM K-asetat / 50 mM K-fosfat (pH= 7,0) tamponuna karşı diyaliz edildi [25].

### 2.4. Afinite Kolonunun Hazırlanması ve Koyun dalak G6PD Enziminin Saflaştırılması

Amonyum sülfat çöktürme ve diyaliz sonrası elde edilen enzim numunesi 2', 5' ADP Sepharose-4B afinite kromatografisi kullanılarak saflaştırıldı. Öncelikle 2', 5' ADP Sepharose-4B afinite kolonu hazırlandı. 10 mL'lik yatak hacmi için 2 g kuru 2', 5'-ADP sepharose 4B jeli tartılarak, 400 mL destile su ile katı maddelerin uzaklaştırılması için birkaç defa yıkandı. Yıkama esnasında jel şişirilmiş oldu. Şişirilmiş jelin havası su trompu kullanılarak vakum ile alındıktan sonra, dengeleme tamponu (0,1M KH<sub>2</sub>PO<sub>4</sub> + 0,1M K-asetat pH= 6,0) ilave edilerek jel süspanse edildi. Süspanse edilmiş jel, 1x10 cm'lik kapalı sistemden oluşan soğutmalı kolona paketlenildi. Jel çöktükten sonra peristaltik pompa yardımıyla yıkama ve dengeleme tamponu ile yıkandı. Kolonun dengelenmiş olduğu elüat ile tamponun 280 nm'de absorbanlarının ve pH'larının yaklaşık olarak eşitlenmesinden anlaşıldı. Böylece afinite kolonu hazırlanmış oldu. Daha önce hazırlanmış olan enzim numunesi hazırlanan 2', 5'- ADP- sepharose 4B afinite kolonuna tatbik edildi. Afinite kolonu sırası ile 25 mL 0,1M KH<sub>2</sub>PO<sub>4</sub> + 0,1M K-asetat (pH= 6,0), 25 mL, 0,1 M KH<sub>2</sub>PO<sub>4</sub> + 0,1M K-asetat (pH= 7,85) ve 25 mL 0,1M KH<sub>2</sub>PO<sub>4</sub> + 0,1M KCl (pH= 7,85) çözeltileriyle yıkandı. Yıkama işlemi spektrofotometrik olarak takip edildi. Yıkama işlemine kolondan alınan numunenin absorban

değeri ile kör arasındaki fark 0,05 olana kadar devam edildi. Kolon yıkandıktan sonra G6PD enzimi elüsyon tamponu (80 mM  $\text{KH}_2\text{PO}_4$  + 80 mM KCl + 0,5 mM  $\text{NADP}^+$  + 10 mM EDTA pH= 7,85) ile afinite kolonundan elüe edildi [26].

## 2.5. Glukoz 6-Fosfat Dehidrogenaz Enzimi Aktivitesi Ölçümü

G6PD enziminin aktivite ölçümü Beutler metoduna göre spektrofotometrik olarak ölçüldü. Bu metod, G6PD enziminin katalizlediği reaksiyonda  $\text{NADP}^+$ 'nin indirgenmesi sonucu oluşan NADPH'nin 340 nm'de maksimum absorban vermeye esasına dayanmaktadır [27].

## 2.6. Protein Tayini

Kantitatif protein miktarı spektrofotometrik olarak 595 nm'de Bradford metoduna göre yapıldı. Standart grafik sığır serum albümin proteini kullanılarak oluşturuldu [28].

## 2.7. SDS-PAGE ile Enzim Saflığının Kontrolü

Enzimin saflık kontrolü %3-8 kesikli sodyum dodesilsülfat poliakrilamid jel elektroforezi (SDS-PAGE) kullanılarak Laemmli metoduna göre gerçekleştirildi [29].

## 2.8. Kinetik Çalışmalar

Kinetik çalışmalarda iveral, linkomisin, gentamisin, amoksisilin, ampisilin, streptomisin sülfat, novamizol, ketojezik, sefuroksim, sefazolin sodyum ve tylosin ilaçlarının enzim aktivitesi üzerindeki etkileri araştırıldı. Aktivite ölçümleri sonucu inhibisyon etkisi gösteren iveral, lincocin, genta, streptomisin sülfat için farklı inhibitör konsantrasyonlarında % İnhibisyon – [I] grafikleri çizilerek  $\text{IC}_{50}$  değerleri hesaplandı [30].

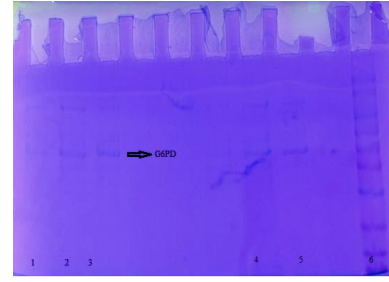
## 3. BULGULAR

Bu çalışmada, koyun dalak dokusundan G6PD enzimi homojenatın hazırlanması, amonyum sülfat çöktürmesi ve 2', 5', ADP-Sepharose-4B afinite kromatografisi kullanılarak 13,68 EU/mg protein spesifik aktivite ile %33 verimle 1302,8 kat saflaştırıldı ve sonuçlar Tablo 1'de gösterildi.

**Tablo 1.** Koyun dalak dokusu G6PD enzimi saflaştırma basamakları.

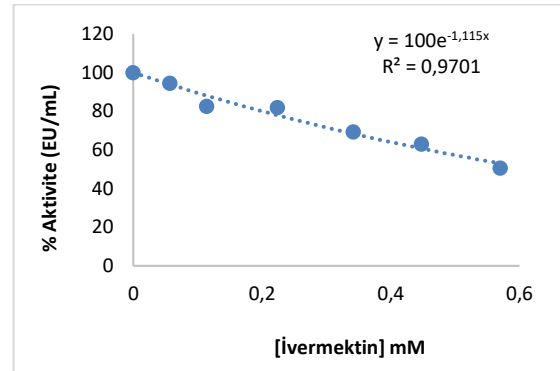
Saflaştırma Basamağı	Toplam Hacim (mL)	Aktivite (EU/mL)	Protein (mg/mL)	Toplam Protein (mg)	Toplam Aktivite	Spesifik Aktivite (EU/mg protein)	%Verim	Saflaştırma Kat sayısı
Homojenat	25	1,128	106,62	2666	28,200	0,0105	100	1
Amonyum sülfat çöktürmesi (%40-70)	11	1,334	108,51	1193	14,674	0,0123	52,36	1,171
Afinite Kromatografisi	7,5	1,259	0,092	0,69	9,44	13,68	33,48	1302,8

Enzim saflık derecesinin tespiti için %3-8 kesikli sodyum SDS-PAGE yapıldı. Jel üzerinde tek bant elde edilmesi enzimin saf olarak elde edildiğini gösterdi (Şekil 1).

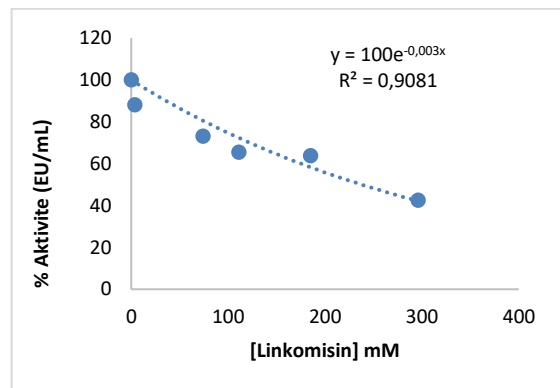


**Şekil 1.** Amonyum sülfat çöktürmesi ve afinite kromatografisi sonrası elde edilen koyun dalak dokusu G6PD enzimi (\*1. 2. 3. 4. ve 5. kuyu: afinite kolonundan elüe edilen saf G6PD enzimi. 6. kuyu: standart proteinler (14 kDa - 175 kDa).

*In vitro* kinetik çalışmalarda farklı konsantrasyonlarda iveral (0,057, 0,114, 0,224, 0,342, 0,448 ve 0,57 mM), linkomisin (3,7, 74, 111, 185, 296 mM), gentamisin (0,66, 1,32, 6,6, 13,2, 33, 39,6 ve 66 mM) streptomisin sülfat (4,29, 8,4, 16,8, 42, 84, 168 ve 210 mM) novamizol (3,2, 6,4, 9,6, 12,8 ve 16 mM), ketojezik (0,78, 1,56, 2,34, 3,1 ve 3,9 mM) amoksisilin (0,114, 0,228, 0,456, 1,14, 2,28, 3,42, 4,56, 5,7 ve 6,84 mM) ampisilin (0,095, 0,19, 0,38, 0,95, 1,9, 2,85, 3,8 ve 7,6 mM), sefuroksim sodyum (0,448, 0,896, 1,344, 1,790 ve 2,24 mM), sefazolin sodyum (0,83, 1,66, 2,49, 3,32 ve 4,15 mM) ve tylosin (0,095, 0,190, 0,285, 0,475 ve 0,95 mM) ilaçlarının enzim aktivitesi üzerindeki etkileri araştırıldı. Enzim üzerinde inhibisyon etkisi gösteren iveral, gentamisin, streptomisin sülfat ve linkomisin ilaçları için % Aktivite-[I] grafikleri çizilerek  $\text{IC}_{50}$  değerleri sırasıyla 0,62 mM, 21,6 mM, 173,2 mM ve 231 mM olarak hesaplandı (Şekil. 2, 3, 4 ve Tablo. 2). Amoksisilin, ampisilin, novamizol, ketojezik, sefuroksim sodyum, sefazolin sodyum ve tylosin ilaçlarının ise enzim üzerinde önemli bir aktivasyon ya da inhibisyon etkisine sahip olmadıkları belirlendi (Tablo. 2).

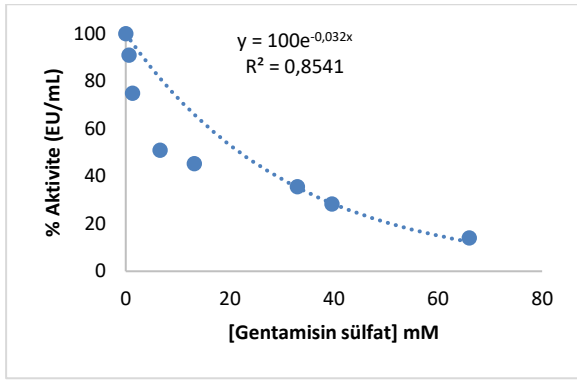


**Şekil 2.** İvermektinin koyun dalak G6PD enzimi üzerine etkisi.

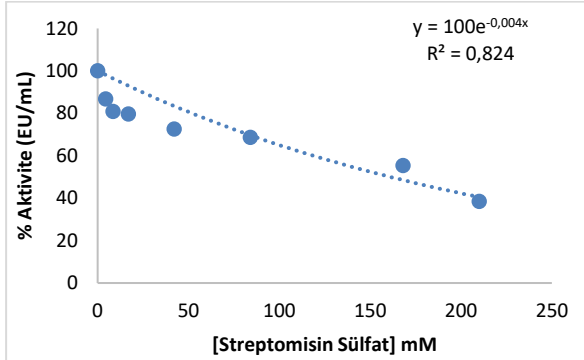


**Şekil 3.** Linkomisin'in koyun dalak G6PD enzimi üzerine etkisi.





Sekil 4. Gentamisin sülfat'ın koyun dalak G6PD enzimi üzerine etkisi.



Sekil 5. Streptomisin sülfat'ın koyun dalak G6PD enzimi üzerine etkisi.

Tablo 2. Bazı ilaçları koyun dalak G6PD enzim aktivitesi üzerine etkileri.

İlaç	IC <sub>50</sub> (mM)	R <sup>2</sup>
iveral	0,62 mM	0,97
gentamisin	21,60 mM	0,85
streptomisin sülfat	173,2 mM	0,82
linkomisin	231 mM	0,91
amoksisilin	-	-
ampisilin	-	-
novamizol	-	-
ketojezik	-	-
sefuroksim sodyum	-	-
sefazolin sodyum	-	-
tylosin	-	-

#### 4. SONUÇ

Pentoz fosfat metabolik yolu hücrelerin büyüme ve gelişiminde önemli rol oynar. Pentoz fosfat yolunun birinci basamak reaksiyonunu katalizleyen allosterik enzimi G6PD aktivitesinin işlevsiz bir durumu, normal hücre çoğalmasının yanı sıra embriyonik ve organizma gelişimini önler. G6PD'nin anormal aktivasyonu tümör oluşumu ile ilişkilidir [31]. Tümör oluşumu, tümör gelişiminin her durumunun yakından ilişkili olduğu dinamik ve karmaşık bir süreçtir. Hızlı büyüyen kanser hücreleri, NADPH üretimi ve nükleik asit sentezi talebini karşılamak için G6PD'yi uyarmak için çok sayıda mekanizma geliştirmiştir [32]. G6PD, lösemi, akciğer kanseri, yumurtalık kanseri, renal hücreli karsinom ve gliomada proliferasyonu, tümör hücrelerinin göçünü, glukoz alımını, NADPH üretimini artırır ve reaktif oksijen türlerini (ROS) azaltır. Birçok çalışmaya göre, G6PD'nin yüksek ekspresyon seviyesi, mesane kanserinin kötü prognozu için bir risk faktörüdür ve G6PD aktivitesindeki artış farklı kanser türleri için kemoterapide ilaç direnci oluşturur. G6PD enzimi birçok hastalığın oluşum, tanı ve tedavisine aracılık ettiği ve etkilediği için G6PD

inhibitörlerinin araştırılması ve geliştirilmesi önem arz etmektedir [33].

İvermektin, lakton grubu bir parazit ilacıdır ve *Streptomyces avermitilis* kültürlerinden elde edilir. İvermektin, parazitlerin sinir sisteminde Gama Amino Butirik Asit (GABA) salgısını artırarak klor kanallarını açık tutar ve impuls geçişini önleyerek parazitin felç olmasına ya da ölmesine neden olur [34]. Gentamisin, çoğunlukla *Pseudomonas*, *Proteus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia* ve Gram-pozitif *Staphylococcus* dahil olmak üzere çok çeşitli bakteriyel enfeksiyonlara karşı etkili bir antibiyotiktir [35]. Streptomisin aminoglikozid sınıfı, hem gram negatif hem de gram pozitif organizmalara ve *Mycobacterium*'a karşı aktif olan bakterisidal bir antibiyotiktir. Mikrobiyal hücrelerin protein sentezini inhibe ederek hücrenin ölümüne yol açar [36]. Linkozamidler gram pozitif koklar (özellikle stafilokoklar, streptokoklar ve enterokoklar), basil ve gram negatif koklara karşı etkilidirler [37]. Amoksisilin ve ampisilin beta laktam grubuna dahil aminopenisilinlerdendir. Bu antibiyotikler penisilin G gibi Gram pozitif bakterilere ve *H. influenza*, *E. coli*, *Proteus mirabilis*, *salmonella*, *shigella* gibi Gram negatif basillere karşı da etkilidirler [38]. Sefuroksim ve sefazolin üst solunum yolu enfeksiyonlarında kullanılan sefalosporin grubu antibiyotiklerdir [39]. Novamizol, metamizol sodyum içeren bir antienflamatuardır [40]. Ketojezik, ketoprofen içeren bir antienflamatuar ve analjeziktir [41]. Tylosin, *Streptomyces fradiae* suşları tarafından üretilen makrolid grubu bir antibiyotiktir [42]. Bu çalışmanın birinci safhasında koyun dalak dokusundan G6PD enzimi amonyum sülfat çöktürmesi ve 2', 5' ADP-Sepharose-4B afinite kromatografisi ile saflaştırıldı. Afinite kromatografisi protein, DNA, RNA ve diğer makromoleküllerin saflaştırılmasında kullanılan ve spesifik etkileşimlere dayanan bir kromatografi yöntemidir. Bu yöntem günümüzde enzim saflaştırmalarında yaygın olarak kullanılmaktadır [12,43-45]. Bu çalışmada afinite kromatografisi kullanılarak kısa zamanda ve yüksek saflıkta enzim elde edildi. Çalışmanın ikinci safhasında insan hekimliğinde ve veteriner hekimlikte yaygın olarak kullanılan, iveral, gentamisin, streptomisin sülfat, linkomisin, amoksisilin, ampisilin, novamizol ketojezik, sefuroksim sodyum, sefazolin sodyum ve tylosin ilaçlarının koyun dalak dokusundan saflaştırılan G6PD enzimi üzerine etkileri araştırıldı. Çalışma sonuçları, iveral, gentamisin, streptomisin sülfat ve linkomisin'in enzim aktivitesini inhibe ettiğini diğer ilaçların ise enzim aktivitesi üzerine herhangi bir etkileri olmadığını göstermektedir. Çalışma sonuçları analiz edildiğinde iveral'ın 0,62 mM IC<sub>50</sub> değeriyle enzim üzerinde en etkili inhibitör olduğu, linkomisin'in ise 231 mM IC<sub>50</sub> değeriyle enzim üzerinde inhibisyon etkisi en düşük ilaç olduğu tespit edildi. Literatürde G6PD enzimi farklı dokulardan çeşitli kromatografik teknikler yardımıyla saflaştırılarak karakterize edilmiş ve bazı ilaç ve organik bileşiklerin enzim aktivitesi üzerine etkileri incelenmiştir [6,46,47]. Çiftçi ve ark. insan eritrositlerinden afinite kromatografisi ile saflaştırdıkları G6PD enzim aktivitesi üzerine melatonin'in *in vitro* ve rat eritrosit G6PD enzimi üzerine *in vivo* etkisini incelemiştir.



Çalışma sonuçları melatonin'in G6PD enzim aktivitesini hem *in vitro* hem de *in vivo* olarak artırdığını göstermiştir [1]. Beydemir ve ark. yapmış oldukları çalışmada G6PD enzimini koyun eritrosit dokusundan afinite kromatografisi ile saflaştırmış ve gentamisin sülfat, penisilin G potasyum ve amikasin antibiyotiklerin enzim aktivitesi üzerine etkilerini incelemişlerdir. Araştırma sonuçları gentamisin sülfat'ın 10,01 mM, penisilin G potasyum'un 12,83 mM ve amikasin'in 41,88 mM, IC<sub>50</sub> değerleriyle G6PD enzimini inhibe ettiğini göstermektedir [48]. Bu araştırmanın sonuçları ile bizim çalışmamızda elde ettiğimiz sonuçlar birbiri ile uyumludur. Temel ve ark. G6PD enzimini rat eritrositlerinden afinite kromatografisi ile saflaştırmış ve gentamisin, klindamisin ve furosemid antibiyotiklerinin enzim aktivitesi üzerine etkilerini araştırmıştır. Araştırma sonuçları gentamisin'in 1,75 mM, klindamisin'in 34,65 mM ve furosemid'in 0,526 mM IC<sub>50</sub> değerleriyle G6PD enzimini inhibe ettiğini göstermiştir [16]. Çağlayan ve Gülçin yaptıkları çalışmada keçi karaciğerinden afinite kromatografisi ile saflaştırdıkları karbonik anhidraz (CA) enzimi üzerine bazı avermektin ilaçlarının inhibisyon etkilerini incelemiştir. Çalışma sonuçları abamectin, doramectin, eprinomectin, and moxidectin ilaçlarının sırasıyla 0.283, 0.153, 0.232, and 0.317 nM, *Ki* değerleriyle enzimi inhibe ettiğini göstermektedir (49).

Sonuç olarak, G6PD enziminin aşırı ekspresyonu bazı hastalıkların patofizyolojisi ile ilişkilidir. Hedefi enzim olan tedavi yaklaşımları için inhibitörler büyük önem arz etmektedir. Bu çalışmada iveral, gentamisin, streptomisin sülfat ve linkomisin'in koyun dalak dokusu G6PD enzimini inhibe ettiği belirlenmiştir. Bu çalışmada elde edilen sonuçlar hedefi G6PD enzimi olan tedavi yaklaşımları için yol gösterici olacaktır.

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## Investigation of Biogas Potential from Animal Waste in Bingöl Province

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**Abstract:** In this study, biogas and energy potential of animal wastes belonging to Bingöl province was investigated. The study in question was based on data from the Bingöl Governorship Provincial Directorate of Agriculture and Forestry for the years 2002-2020. In the light of 18 years of data, it has been determined that annually 741.452 tons of manure can be obtained from an average of 948.144 animals, and 34.225.934 m<sup>3</sup> of biogas can be obtained from this manure, also 161GWh of energy per year can be obtained from this biogas. If these animal wastes were evaluated in the said years, a total of 2898 GWh energy would have been obtained. In addition the evaluating data for 2020 it is understood that the biogas potential installation power of Bingöl province is 25.6 MW. Considering the distribution of biogas energy by districts, it is understood that Karlıova district plays an important role with a share of 31% in total power potential. According to 2020 datas only it is seen that 73.8% of the 303.7 GWh electricity consumed can be met by biogas energy. In addition, according to turkey statistics institute data of 2020 total potential power of Bingöl and its neighboring provinces Diyarbakır, Elazığ, Erzurum, Erzincan, Muş and Tunceli is calculated as 298MW. However, it is understood that only 2.6% of this power (Equal to 7.63MW) is used currently. So it is obvious that the establishment of biogas facilities in related regions will decrease country's dependency on foreign energy.

## Bingöl İli Hayvansal Atık Kaynaklı Biyogaz Potansiyelinin Araştırılması

**Anahtar kelimeler:**  
 Enerji,  
 Biyogaz,  
 Hayvansal atık,  
 Bingöl

**Öz:** Bu çalışmada Bingöl iline ait hayvansal atıkların biyogaz ve enerji potansiyeli araştırılmıştır. Söz konusu çalışma, Bingöl Valiliği İl Tarım ve Orman Müdürlüğü'nün 2002-2020 yıllarına ait verilerine dayanmaktadır. 18 yıllık veriler ışığında, ortalama 948.144 hayvandan yıllık 741.452 ton gübre elde edilebileceği ve bu gübreden 34.225.934 m<sup>3</sup> biyogaz elde edilebileceği, ayrıca yıllık 161 GWh enerji üretilebileceği belirlenmiştir. Söz konusu yıllarda bu hayvan atıkları değerlendirilseydi toplam 2898 GWh enerji elde edilecekti. Ayrıca 2020 yılı verileri değerlendirildiğinde Bingöl ilinin biyogaz potansiyel kurulum gücünün 25.6 MW olduğu tespit edilmiştir. Biyogaz enerjisinin ilçelere göre dağılımına bakıldığında Karlıova ilçesinin %31'lik pay ile önemli bir rol oynadığı anlaşılmıştır. Sadece 2020 verilerine göre tüketilen 303.7 GWh elektriğin %73.8'inin biyogaz enerjisi ile karşılanabileceği görülmektedir. Ayrıca Türkiye İstatistik Enstitüsü verilerine göre 2020 yılı Bingöl ve komşu illeri Diyarbakır, Elazığ, Erzurum, Erzincan, Muş ve Tunceli'nin toplam potansiyel gücü 298 MW olarak hesaplanmıştır. Ancak şu anda bu gücün sadece %2.6'sının (7.63 MW'a eşit) kullanıldığı anlaşılmaktadır. Dolayısıyla ilgili bölgelerde biyogaz tesislerinin kurulmasının ülkenin dışa bağımlılığını azaltacağı aşikardır.

### 1. INTRODUCTION

With the increasing population and the development of technology, the need of energy increases day by day. At the same time with the population increase, environmental

pollution increases, and it is generally known that fossil fuels are exhausted. In this sense, researches on alternative energy sources are increasing day by day. According to 2010 data, fuel and alternative energy sources used in the distribution of electricity generation in



the world are 28% nuclear, 25% solid fuels, 24% natural gas, 17% renewable energy sources excluding biomass, 4% biomass, 3% liquid fuel. By 2020, 23% of electricity production was met from nuclear, 23% if them from solid fuels, 17% of them from natural gas, 30% of them from renewable energy sources excluding biomass, 6% of them from biomass and 1% of them from liquid fuels. In the distribution of electricity generation, it is seen that there is a tendency towards renewable energy by abandoning fossil resources. A study is expected that by 2050 it will have a very high share of 92% of renewable energy including biomass in electrical energy production [1]. In Turkey according to 2010 sources 46.47% of electricity generation was obtained from natural gas, 26.22% of them from renewable energy sources excluding biomass, 18.05% of them from liquid fuels, 9.05% of them from solid fuels[2]. In 2020 26.8% of electricity generation was obtained from natural gas, 50.2% of them from renewable energy excluding biomass, 12.1% of them from liquid fuels, 9.4% of them from solid fuels, 1.5 of them from biomass [3]. Therefore, according to the sources of energy production it is seen that there is a high rate of turn to renewable energy in Turkey. On average 24% of the world's energy needs are met from nuclear energy and therefore, it is seen that investments are made in nuclear energy in our country too. By 2050 it is predicted that nuclear energy will not remain and 92% of energy will be met from renewable energy worldwide. In addition while 36% of energy in the world is met from renewable energy this rate is approximately 51.7% in Turkey in 2020. This ratio shows that Turkey is above the world average in terms of transition to renewable energy. While energy production from biomass increased 1.5 times in the world from 2010 to 2020, it increased approximately 7 times in Turkey. There is a high tendency towards alternative energy sources in our country, especially to reduce foreign dependency. Biogas production from animal wastes should be prioritized as an alternative energy source considering the existence of animals in our country. When evaluated in terms of animal assets, it is known that there are 72 million 271 thousand total animals, including 18 million 158 thousand cattle and 54 million 113 thousand small cattle as of 2020 [4]. If this potential animal waste is evaluated, it is understood that the energy amount of 4.385.371 TEP/year can be met and it will make a great contribution to the country's economy, especially in terms of foreign dependency of energy [5]. Many studies have been carried out in terms of the biogas potential of Turkey. Biogas potential of Elazığ province has been studied and an analysis of return figures of biogas to be produced in the biogas plant has also been conducted [6]. The amount of fertilizer that can be obtained from the wastes of all animal assets of Turkey and the maximum biogas production that can be produced depending on these fertilizer amounts have been evaluated. It has been understood that when this biogas is used for electricity generation, there will be a great decrease in the amount of electrical energy that we depend on. In this respect, it was emphasized that especially agricultural, domestic and animal wastes should be used for energy production [7]. Biomass potential of Eastern Anatolia Region was examined in another study. The amount of dry biomass in cultivated areas in all provinces

in the Eastern Anatolia Region has been calculated. Average thermal values were calculated according to the amount of dry biomass and the amount of energy obtained from it. According to the values obtained, they suggested that necessary researches should be made especially in provinces for the utilization of biomass wastes or forest wastes and that necessary facility should be established and research centers should be established to close our energy deficit on a provincial basis [8]. In the study conducted to evaluate animal wastes in Tokat province, animal assets according to Tokat city center and districts were examined. According to these animal beings, the potential biogas production and the electrical energy to be obtained from it have been revealed. According to the data obtained, the amount of electrical energy produced from animal waste will meet approximately 32% of the electricity consumption of Tokat province in 2007 [9]. In the study carried out for Sivas province, the electrical energy that could potentially be produced according to the general animal number of the province was evaluated. In addition, in the study suggestions were given about the size of the biogas facility that they can establish on a small scale according to the number of animals for cattle, ovine and poultry breeders. In the data presented, the appropriate size of the facility for livestock holdings is suggested as 14, 21, 28 m<sup>3</sup> for 20, 30, 40 animals respectively; for 30 000, 40 000 cattles 356, 474 m<sup>3</sup> respectively. It was emphasized that for small ruminants it should be 16 m<sup>3</sup> for 500 animals and it is more important to establish small-scale biogas plants instead of a single large biogas plant [10]. In the study conducted for the province of Iğdır, the biogas potential from animal waste was evaluated according to the city center and districts. It has been determined that the district with the highest biogas potential is Tuzluca. In addition, they revealed that the biogas potential of Iğdır province constitutes 3.76% of the Eastern Anatolia Region and 0.679% of Turkey in general [11]. In the study conducted for the province of Ardahan, the amount of biogas that can be obtained from animal wastes and the amount of electrical energy that can be produced were determined for the city center and districts. According to the density of animal clusters, locations of the city center and districts to establish a biogas plant have been revealed. In addition, a guide has been prepared for the investments to be made for the biogas plant [12]. In the study conducted for the province of Malatya, the biogas potential that can be obtained from animal wastes was evaluated and the biogas potential of the province was revealed by comparing it with animal assets throughout Turkey [13]. In a statistical study conducted in Turkey, it was calculated that according to the number of chickens, the biogas potential that could be produced in Turkey in 2009 was 390 million m<sup>3</sup>, which is equivalent to an energy of 8.853 million GJ. In addition, the provinces where more than 10 million m<sup>3</sup> of biogas will be obtained annually from chicken waste in Turkey are listed as Bolu, Balıkesir, Sakarya, Manisa, Afyon, Konya, İzmir, Ankara, Çorum and Bursa [14]. In the study conducted for Kahramanmaraş province, biogas production potentials from animal and plant wastes were determined. In this sense, it is predicted that the majority of production will consist of animal wastes. In addition, a mapping was made according to the production potential

of the districts and the places with the highest biogas potential were determined [15]. In the study conducted for Çanakkale province, situations related to electricity generation from biogas in the world and in Turkey have been presented. A comparison of biogas potential with Çanakkale province and other provinces has been made [16]. In the study conducted for Denizli province, biogas production potential from animal wastes and the amount of electrical energy that can be obtained from biogas were revealed according to their districts. In this sense, it is stated that it is important to have a facility made of animal wastes in Çivril district. In addition due to the high number of chickens in Honaz district, it was observed that a biogas production facility was suggested to be established here [17]. In the study conducted for the Thrace region, potential biogas production amounts from animal waste in all provinces and districts were evaluated. In this sense studies were carried out for the whole region with the numerical mapping method. It was stated that Edirne could be the province with the highest potential biogas production from animal wastes in the Thrace region [18]. Potential biogas production that can be obtained from animal wastes in Erzincan province has been determined. The use of potential biogas as an alternative to electricity generation and natural gas has been evaluated. In addition, it has been shown how

Erzincan province will benefit economically in the use of biogas to be produced annually [19]. Again, a study conducted for Erzincan determined where a biogas plant should be established in provincial and district centers [20]. In the study conducted for Kırşehir province, the amount of biogas obtained from animal wastes was determined and where the biogas facility should be established in the province [21]. In the study conducted for Bitlis, biogas production potential from animal wastes in the city center and districts was examined. In this sense, the amount of earning that can be obtained in case of utilizing this potential throughout the province has been presented [22]. In a study, the number of biogas facilities and production amounts in Turkey were examined. In 2017 it was determined that there are 122 licensed Renewable Waste Power Plants with 634.2MW power. It was emphasized that these facilities should increase as the demand for energy increases day by day [23].

Previous studies on biogas energy have been meticulously examined. Among the mentioned studies, there is no study on the biogas energy potential for the province of Bingöl. In this respect, this study will be both an original study and a guide for biogas studies to be carried out in Bingöl Province. And also it is aim to encourage the establishment of biogas facilities in the province of Bingöl and reduce foreign dependency by providing local energy production. Based on literature studies, the biogas potential and related energy data obtained from animal wastes were investigated for the province of Bingöl, which has a high livestock potential. So Animal waste data obtained from Governorship Provincial Directorate of Agriculture and Forestry for the years 2002-2020. Biogas potentials of province and districts were revealed separately. Electrical energy consumed by the region were compared with the electrical energy produced from

biogas. In addition, the data of the provinces neighboring Bingöl for the year of 2020 were evaluated in terms of current installed power.

## 2. MATERIAL AND METHOD

Bingöl province is located in the Upper Euphrates Section of the Eastern Anatolia Region between 41° 20 ' and 39° 56 ' east longitudes and 39° 31 ' and 36° 28 northern latitudes. The land within the provincial borders is quite hilly, and the city center was established on a plain bordered by mountains on all four sides. The majority of rural settlements are located in mountainous areas. In this respect, both bovine and ovine breeding constitute important sources of income in this city. Bingöl is adjacent to Erzincan and Erzurum in the north; Muş in the east; Tunceli and Elazığ in the west and Diyarbakır in the south. There are 7 Districts of Bingöl, Yayladere, Kiğı, Yedisu, Adaklı, Karlıova Solhan and Genç. In the study in question, data belonging to the years 2002-2020 obtained from the Bingöl Governorship Provincial Directorate of Agriculture and Forestry were used. Within the scope of this study, Livestock data for the year 2020 published by Turkish Statistical Institute (TSI) was used [4]. Bovine, ovine and poultry data were used in biogas and energy calculations.

**Table 1.** Animal Presence of Bingöl Province in 2020 [4]

Districts	Bovines	Ovines	Poultry	Total
Adaklı	10.386	48.920	6.800	66.106
Genç	19.357	39.079	6.495	64.931
Karlıova	20.986	348.375	11.760	381.121
Kiğı	3.670	9.976	6.811	20.457
Merkez	60.050	101.398	723.600	885.048
Solhan	19.984	147.291	5.000	172.275
Yayladere	1.783	4.386	382	6.551
Yedisu	8.297	21.427	1.650	31.374
Total	144.513	720.852	762.498	1.627.863

According to 2020 data 60.050 of 144.513 cattle in Bingöl province belong to Bingöl center. The highest number of cattle belongs to Karlıova after the central district, followed by Solhan, Genç, Adaklı, Yedisu and Kiğı. The fertilizer used in the calculations was taken as 3,6 tons year<sup>-1</sup> for cattle, 0.7 tons year<sup>-1</sup> for small cattle and 0.022 tons year<sup>-1</sup> for poultry [24]. The amount of biogas taken from the fertilizer on a ton basis is 33, 58, 78 m<sup>3</sup> ton<sup>-1</sup> for bovine, ovine and poultry respectively [24]. The electrical energy obtained from 1 m<sup>3</sup> of biogas is the same for all three animal species and is 4.7 kWh [25].

## 3. RESULTS

In this study, biogas and energy potential of animal wastes belonging to Bingöl province was investigated. In the study datas belonging to the years between 2002-2020 obtained from the Bingöl Governorship Provincial Directorate of Agriculture and Forestry were used. The land is quite hilly within the province of Bingöl and the city center was established on a plain bordered by mountains on four sides. The majority of rural settlements are located in mountainous areas. In this respect, both bovine and ovine breeding constitute important sources of



income in this city. Especially small cattle breeding is the most common in the region. In Figure 1, the numbers of bovine, ovine and poultry animals belonging to the province of Bingöl and theoretical biogas of them are given for 2002-2020 years. As seen in related figure the annual average bovine number is below 200000, while the average number of sheep and goats is around 500000. It is understood that potential of manure obtained from ovine animals is higher than that of bovine animals. As a matter of fact, while the potential biogas multiplier for

ovines is 58, it is 33 for bovines. So the biogas potential of manure obtained from ovine animals is of higher quality than that of bovine animals. Considering ovines livestock has an important place due to its location, the importance of researching and evaluating potential biogas production can be better understood. In addition as seen from same figure (Figure 1) a significant increase is seen in the number of poultry animals in the last three years.

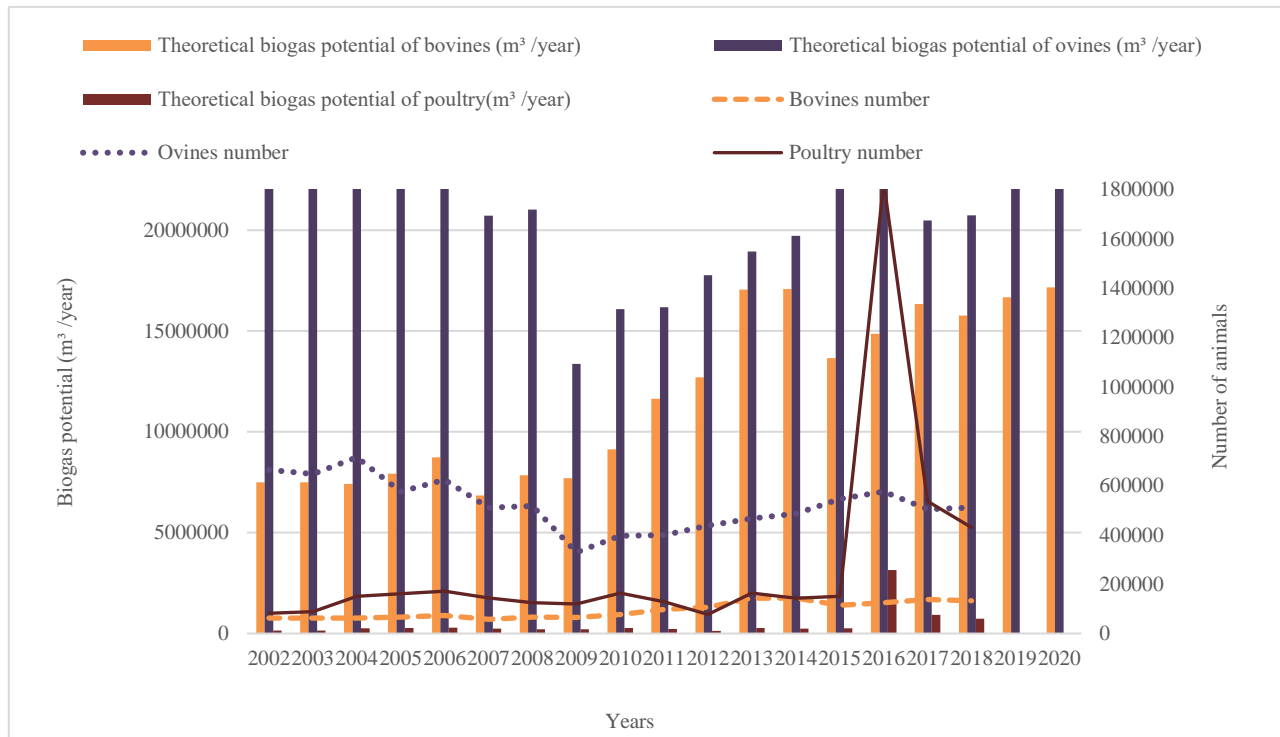


Figure 1. Number of animals and theoretical biogas potential of them for Bingöl province

According to annual average values data from Table 2, there is an annual total of 948.144 animals, including 99.047 bovines, 540.122 ovine, 308.975 poultry. From these animals, in totaly 741.452 tons/year of fertilizer is obtained from all animals, 356.569 tons/year of them obtained from cattles, 378.085 tons/year of them provided from ovine, 6.797 tons/year of them provided from poultry manure. And also 11.766.783 m<sup>3</sup>/year of the total biogas produced annually is obtained by small animals, 21.928.953 m<sup>3</sup>/year of them produced by cattle and 530.197 m<sup>3</sup>/year of them produced by poultry. The relevant energy amounts were determined for bovines, ovines and poultry as 55.35 GWh / year, 103.1 GWh / year, 2.5 GWh / year respectively with an average annual total of 160.9 GWh/year (Table 2). In the light of these values, it was possible to establish a biogas plant with an

installed power of 18.4MW in the 18 year period(from 2002 to 2020). If these animal wastes were evaluated in the said years, a total of 2898 GWh energy would have been obtained. But this energy became idle because the existing potential biogas energy was not utilized.

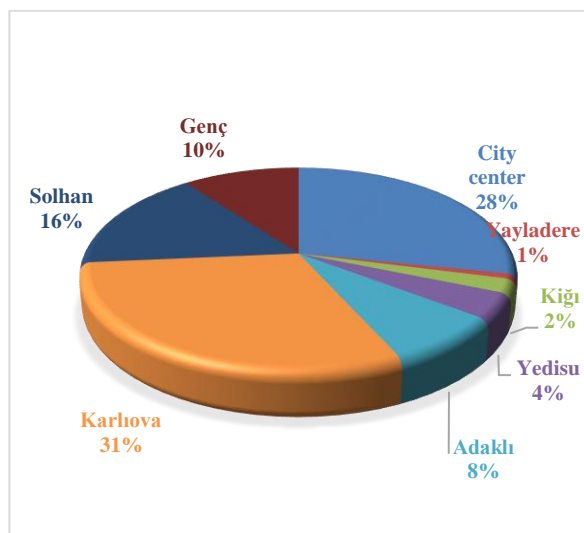
Table 2. Annual average data of 2002-2020

Animal breed	Num. of animals per year	Theoretical Fertilizer Potential (tons / year)	Theoretical Biogas potential (m <sup>3</sup> /year)	Produced Energy (GWh /year)
Bovines	99.047	356.569	11.766.783	55.3
Ovines	540.122	378.085	21.928.953	103.1
Poultry	308.975	6.797	530.197	2.5
Total	948.144	741.452	34.225.934	160.9

**Table 3.** Datas for 2020

Animal breed	Animal number	Theoretical Fertilizer Potential (tons / year)	Theoretical Biogas potential (m <sup>3</sup> /year)	Produced Energy (MWh /year)	Potential power plant capacity(MW)
Bovines	144.513	520.247	17.168.144	80.700	9.2
Ovines	720.852	504.596	29.266.591	137.500	15.7
Poultry	762.498	16.775	1.308.447	6.100	0.7
<b>Total</b>	<b>1.627.863</b>	<b>1.041.618</b>	<b>47.743.182</b>	<b>224.300</b>	<b>25.6</b>

Table 3 shows the animal numbers, potential biogas and energy values for 2020 only. According to the result from Table 2 and Table 3 this is a sign that the number of animals will increase gradually for Bingöl. At the same time the increase in the energy produced by biogas in question will be observed. The evaluating data of 2020 only it is understood that the biogas potential installation power of Bingöl province is 25.6 MW. In this regard, it is observed that there is an increase of approximately 40% in 2020 compared to the power of the facility that can be established for the other 18 years.

**Figure 2.** Distribution of biogas energy by districts

In Figure 2, the distribution of biogas energy by districts is shown in the pie chart. From the related graph, the distribution of total biogas energy in Bingöl province as 31% Karlıova, 28% city center, 16% Solhan, 10% Genç, 8% Adaklı, 4% Yedisu, 2% Kığı, % 1 Yayladere respectively. It is understood that Karlıova has an important place in biogas production in Bingöl province.

**Table 5** Biogas data of neighboring provinces of Bingöl for 2020 (Obtained from turkey statistics institute 02.12.2021)[4]

Data of 2020	Bingöl	Diyarbakır	Elazığ	Muş	Erzurum	Erzincan	Tunceli	Total
Ovine num.	720.852	2.091.344	854.456	1.235.552	904.587	501.027	432.565	6.740.383
Bovine num.	144.550	656.074	209.142	331.881	869.009	124.125	37.086	2.371.867
Biogas potential(MWh/year)	218.264	765.396	279.825	421.077	657.833	164.911	103.249	2.610.555
Potential installed biogas power (MW)	24.9	87.3	31.9	48	75	18.8	11.78	297.68
Installed biogas power(MW)	2.1	2.15	0	0	3.38	0	0	7.63

There are currently two biogas power plants with 3.38MW power in Erzurum named Erzurum and Akşehir biogas power plant. On the other hand, there is currently no biogas facility in Elazığ, Erzincan, Muş and Tunceli that uses animal wastes. According to the relevant table, the total installed power potential of Bingöl and neighboring provinces is 297.68 MW, while all installed facilities are only 7.63MW.

Considering the distribution of biogas energy by districts, it is understood that Karlıova district plays an important role with 31% installed power potential in this matter.

**Table 4.** Electricity Consumption Values in Bingöl Province by Years and Consumer Type (Obtained from board (EPDK)).

Bingöl	2020	2019	2018	2017
Lighting	16.126	14.633	12.469	10.403
Domicile	129.972	122.600	117.778	111.732
Industry	53.042	49.228	63.114	58.029
Agricultural watering	1.308	781	452	426
Business	103.293	118.748	111.723	114.144
Grand Total (MWh)	303.740	305.989	305.535	294.733

According to the electricity consumption values of Bingöl province obtained from EPDK (Table 4), it is seen that the annual consumption potential was in total approximately 294GWh in 2017; 305 GWh in 2018 and 2019 and 303.74 GWh in 2020. It is understood that by using the 224.3 GWh/year biogas potential energy obtained in 2020, 73.8% of the total consumption value of the relevant year,(Which is 303.7 GWh/year according to Table 4), will be met.

In Table 5, the number of animals in Bingöl province and its neighbors, the relevant biogas energy amounts and the current installed power are given for comparison purposes for 2020. The biogas facility established by Sütaş company in Bingöl for cattle manure, chicken manure and vegetable waste has a capacity of 2.1MW, whereas the biogas potential of Bingöl is much higher than this installed power. Similarly It is understood that the biogas potential in Diyarbakır is far above the installed power of 2.15MW.

#### 4. DISCUSSION

In this study, the biogas potential of Bingöl province was evaluated. For this purpose, annual average results were obtained by using the data on animal waste between the years 2002-2020. Also using the data of 2020, the electricity rates that can be met with the biogas potential were examined. In addition, the data of the provinces

neighboring Bingöl for the year 2020 were evaluated. Considering the average values obtained according to 2020, it is understood that the potential biogas power of the province of Bingöl is approximately 25.6MW. It has been understood that 73.8% of the electricity consumed annually can be met if a 25.6MW biogas power plant is established. According to relevant data, the total installed power potential of Bingöl and neighboring provinces is 297.68 MW, while all installed facilities are only 7.63MW. Therefore, it is understood that only 2.5% of the potential of Bingöl and surrounding provinces is used. On the other hand, considering that approximately 27.4% of the electricity produced in Turkey is obtained from natural gas, 17% from coal and 2.2% from biogas [26], it is understood that sufficient attention should be given to biogas studies.

According to the February 2021 biogas production facility pre-feasibility report in Elazığ province, although the biogas installed power of companies that have obtained a biogas license within the scope of Turkey increases every year, it is understood that the total installed power is currently 147MW[27]. Considering that the potential power of Bingöl and neighboring provinces is 297MW according to our own study. Therefore, it is understood that Turkey's total installed power corresponds to only 50% of the potential biogas energy of Bingöl and neighboring provinces. In other words, only the biogas potential of Bingöl and neighboring provinces is twice the current total installed power of Turkey. Therefore, when we consider this in terms of the eastern Anatolian region and Turkey, a large proportion of electrical energy can actually be met from these waste energies. Especially considering that biogas energy is not only caused by animal wastes, but also vegetable wastes and all organic wastes can be evaluated. In this sense, we believe that this issue will be even more important[7]. In this respect, it is clear that the evaluation of biogas energy produced both in the province of Bingöl and the other centers of Turkey will close a large energy deficit of country. With the use of this potential, it will be possible to get rid of the 50% energy dependence on abroad[26]. And also we are of the opinion that biogas energy should be re-evaluated not only throughout Bingöl but also in every region of Turkey.

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## Mısır (*Zea mays* L.): Bir Bitki; İki veya Üç Farklı Ürün

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### Anahtar

### Kelimeler

*Zea mays* L.,  
 Taze mısır,  
 Körpe mısır,  
 Tane özellikleri

**Öz:** Çalışma, mısırdaki bir bitkiden birden fazla farklı ürün elde etme imkânlarını araştırmak amacıyla yürütülmüştür. Bu amaçla, mısır bitkisi üzerindeki koçanlar tüketim amaçlarına göre körpe mısır (koçan püskülü çıkarma dönemi), haşlamalık/közlemelik (süt olum dönemi) ve tane verimi (tam olum dönemi) için farklı dönemlerde hasat edilmiştir. Deneme, “Karadeniz Yıldızı” sert mısır ve “Batem Tatlı” şeker mısır çeşitleri kullanılarak tesadüf blokları deneme desenine göre üç tekerrürlü olarak Isparta koşullarında iki ayrı deneme şeklinde 2019 yılında kurulmuştur. Her iki mısır alt türünde de dekara körpe ve taze koçan sayıları bakımından kontrol ile uygulamalar arasındaki farklılık istatistiksel olarak  $P \leq 0,01$  düzeyinde önemli olurken, koçan özellikleri arasındaki farklar önemsiz çıkmıştır. Sert mısırdaki koçan koparılmayan uygulamada (kontrol) tane verimi bitki üzerinde tek koçan bırakılan uygulamaya göre daha yüksek olurken, koçan ve tane özellikleri bakımından bitki üzerinde tek koçan bırakılan uygulama kontrolden daha yüksek olmuştur. Sonuç olarak; bu uygulamaların başarısı mısır bitkisinin birden fazla koçan bağlama özelliğine bağlı olmakla birlikte, bir bitkiden iki veya üç farklı ürünün elde edilebileceği ve pratikte uygulanabilir bir yöntem olduğu sonucuna varılmıştır.

## Maize (*Zea mays* L.): Two or Three Different Products from One Plant

### Keywords

*Zea mays* L.,  
 Fresh corn,  
 Baby corn,  
 Seed  
 Characteristics

**Abstract:** The study was conducted to investigate the possibilities of obtaining more than one crop from one plant of maize. For this purpose, cobs on the plant were harvested in different stages according to consumption aims for baby corn (ear silk-out time), boiling/roasting (milk maturity stage), and seed yield (full maturity). The experiment was set up as two separate trials according to randomized complete block design with three replications using “Karadeniz Yıldızı” flint corn and “Batem Tatlı” sweet corn in 2019 year in Isparta conditions. In both maize subspecies, the difference between the control and the treatments in terms of the number of baby and fresh ear per decare was statistically significant at the  $P \leq 0.01$  level, while the difference between the ear characteristics was not significant. While the grain yield was higher in unremoved corn treatment (control) than the application with a single cob on the plant, the treatment with single cob on the plant was higher than the control in terms of the ear and grain characteristics. As a result; although the success of the treatments depends on the ear binding properties of more than one of the maize plants, it conclusion has been reached that two or three products can be obtained from one plant and it is a practically applicable method.

## 1. GİRİŞ

Mısır; dünyada ekim alanı, üretimi, kullanımı ve ticareti en fazla olan üç bitkiden biridir. Mısır hızlı gelişmesi, kısa sürede yüksek tane ve sap veriminden dolayı silaj, yeşil ve kesif yem olarak Dünyada ve Türkiye’de hayvansal üretimde yem açığının kapatılmasında önde gelen bitkilerden biridir. Mısırın yüksek melez gücünden dolayı son 20-25 yılda Türkiye’de verimi önemli ölçüde artmış ve silajı son yirmi yılda süt sığırlarının

rasyonlarında temel yem bileşeni haline gelmiştir [1]. Mısırın tanesinden yemeklik yağ, biyoyakıt ve etanol, mısır şurubu, alkol üretimi ile sanayide kullanımı da hızla yükselmektedir. İnsan beslenmesinde haşlamalık/közlemelik, popcorn, körpe mısır, çerez, un, sıvı yağ, nişasta, tatlandırıcı olarak geniş bir kullanım alanına sahiptir [2]. Mısır bu özellikleri ile gelişmiş ülke bitkisi olarak adlandırılabilir. Ülkelerin gelişmişlik düzeyi arttıkça hem üreticiler hem de tüketiciler bilinçlenmektedir. Böylece tarımda hibrit tohum



kullanımı artmakta, mısırın kullanıldığı sanayi kolları gelişmekte ve insan beslenmesinde tüketim tercihleri değişmektedir. Örneğin taze tüketim için atıdışı ve sert mısır yerine şeker mısır tercihi, konserve ve körpe mısır tüketimi, şeker yerine tatlandırıcıların kullanımı ve diğer mısır ürünlerinin tüketimi artmaktadır.

Giderek artan dünya nüfusu ve azalan tarım alanları dikkate alındığında, insanların gıda ve hayvanların yem ihtiyacını karşılamak için tarım alanlarının birim alan verimini artırmak ve bu ürünlerden daha etkin faydalanmak gerekmektedir. Akdeniz iklimi dışında kalan bölgelerde ikinci ürün ve ara ürü tarımının yapılabilme olanaklarının araştırılması ve yaygınlaştırılması ile tarım alanları daha etkin kullanılabilir. Son yıllarda mısır tarımında hibrit tohum kullanımı önemli oranda artmıştır. Mısırdaki iri koçan oluşumu ve yüksek tohum verimi için her bitkinin tek koçan bağlaması istenmektedir. Ancak hibrit tohumlar ekildiklerinde çeşide, iklime ve yetiştirme koşullarına göre 3-4 koçan bağlayabilmektedirler. Bu durumda mısırdan daha etkin faydalanmak için hasat amacına göre (taze tüketim ve tohum) bir koçan ana üretim hedefi için ayrılıp, diğer koçanlar farklı tüketim amacıyla hasat edilebilir. Örneğin şeker mısırdaki bir koçan körpe mısır amacıyla koçan püskülü çıkarma devresinde, sert mısırdaki çok koçan bağlayan bitkilerde bir koçan tane hasadı için bitki üzerinde bırakılıp, bir koçan taze tüketim için süt olum döneminde ve diğer koçanlar körpe mısır olarak hasat edilebilir. Türkiye’de şeker mısırın fazla tanınmaması ve koçanın biraz küçük olması nedeniyle daha iri koçan yapısına sahip olan sert mısır taze tüketim amacıyla kullanılmaktadır. Mısırın yanı sıra fasulye bitkisinin bir kısmı taze tüketim, bir kısmı kuru tane amaçlı, patates bitkisinin bir kısmı erkenden turfanda şekilde, bir kısmı normal hasat zamanda hasat edilerek daha etkin kullanılabilir. Ancak fasulye ve patates gibi bitkilerde bu hasat şekli nihai ürünün verimini azaltabilir. Mısırdaki hibrit bitki ıslahında tek koçanlılık istenen bir özelliktir. Bu nedenle mısır bitkisi üzerinde tek koçan bırakılarak diğer koçanların farklı tüketim amacıyla hasat edilmesi ile hem istenen tek koçanlılığı sağlamış, hem de bitkiden daha etkin faydalanılmış olacaktır. Kara ve ark. [3] şeker mısırdaki ikinci koçanın koparılarak körpe mısır olarak değerlendirmesi ve bunun taze koçan özelliklerine

etkisini araştırdıkları çalışmada, koparılan ikinci koçanların birinci koçan boyutlarına azda olsa pozitif katkı yaptığı, elde edilen koçanların körpe mısır olarak değerlendirilebileceğini bildirmişlerdir. Kara [4] birim alandan daha fazla körpe mısır elde etmek amacıyla ocakta tohum sayısını artırarak yürüttüğü çalışmada en yüksek körpe koçan verimi ve koçan sayısını ocağa 5-6 tohum ekilmesi ile elde etmiştir. Yıldırkan ve Kara [5,6] karasal iklimin hüküm sürdüğü Burdur il merkezinde sulanabilen tarım arazilerde buğday hasadından sonra ikinci ürün koşullarında taze tüketim amaçlı 84-90. günde ve körpe mısır amacıyla 50-76. günde hasadının başarıyla yapılabildiğini ve kullanılan tarım arazisi üzerinde sonraki ürünün ekim zamanını etkilemeden üretim yapılabildiğini bildirmişlerdir.

Bu çalışma; şeker ve sert mısırdaki bitki üzerinde tek koçan bırakılıp, diğerlerinin kullanım amacına göre değişik olum dönemlerinde hasat edilerek bir bitkiden birden fazla ürün elde etme olanaklarını araştırmak amacıyla yürütülmüştür.

## 2. MATERYAL VE YÖNTEM

Araştırma, Isparta merkez ekolojik koşullarında “Batem tatlı” şeker mısır ve “Karadeniz yıldızı” sert mısır çeşitleri kullanılarak 2019 yılında yürütülmüştür. Denemede yer verilen çeşitler, önceki yıllarda farklı amaçlarla yürütülen çalışmalarda bir bitkide birden fazla koçan bağladığı gözlenen çeşitlerden seçilmiştir [20].

### 2.1. İklim Özellikleri

Isparta ili İç Anadolu, Akdeniz ve Ege bölgeleri arasında geçiş iklimi özelliğine sahiptir. Yıllık yağışın büyük bir bölümü kış aylarında yağmur ve kar şeklinde olup, uzun yıllar ortalama yağış miktarı 526,0 mm’dir. Denemenin yürütüldüğü yılın Mayıs-Eylül aylarına ilişkin toplam yağış miktarı 133,8 mm ve 204,7 mm, aynı döneminde aylık ortalama sıcaklık 19,4 °C ve 19,6 °C olmuştur (Tablo 1). Vejetasyon süresindeki gece sıcaklıklarının bir miktar düşmesinden kaynaklanan aylık ortalama sıcaklık değerleri mısır tarımı için düşük görünse de gündüz sıcaklıkları yüksek olması mısırı yetiştiriciliği için uygun koşulları oluşturmaktadır.

**Tablo 1.** Deneme yılına ve uzun yıllara ait iklim verileri\*

İklim verileri	Yıllar	Aylar						Toplam veya Ort.
		Mayıs	Haziran	Temmuz	Ağustos	Eylül	Ekim	
Ortalama sıcaklık (°C)	2019	17,0	20,6	23,4	24,4	18,9	12,6	19,4
	Uzun yıllar	15,6	20,2	23,6	25,8	18,3	13,4	19,5
Toplam yağış (mm)	2019	62,9	29,4	4,1	10,2	1,6	25,6	133,8
	Uzun yıllar	51,4	29,8	14,6	0,3	15,4	38,4	

\*Isparta meteoroloji istasyonu kayıtları

### 2.2. Toprak Özellikleri

Deneme alanı toprağı hafif bazik (pH: 7,6), kireç oranı düşük (%13,6), organik madde içeriği orta (%2,2) ve kumlu-tınlı bir yapıdadır.

### 2.3. Denemenin Kurulması

Şeker ve sert mısır tohumları ayrı iki deneme şeklinde tesadüf blokları deneme desenine göre 3 tekrerrürlü olarak parsel sıra uzunluğu 5 m ve 4 sıra, bloklar arasında 2 m, her parsel arasında bir sıra aralık bırakılarak 26 Nisan 2019’da kurulmuştur. Ekim işlemi; sıra arası 70 cm ve sıra üzeri 18 cm mesafede (70 cm x 18 cm) olacak şekilde elle

her ocağa iki tohum gelecek şekilde 4-5 cm derinliğinde ekilmiştir. Ekimden sonra parsellere damla sulama boruları döşenerek çıkıştan sonra her ocakta bir bitki kalacak şekilde tekleme yapılmıştır.

Uygulamalar: 1. Kontrol, her iki mısır türü için herhangi bir uygulama yapılmayıp geleneksel mısır yetiştiriciliği uygulanmıştır. 2. Uygulama I (U1): Her iki alt türde de bitki üzerinde birden fazla olan koçanlardan en üst koçan süt olum döneminde hasat edilmek üzere bırakılmış ve diğer koçanlar koçan püskülü çıkarma aşamasında körpe mısır (baby corn) olarak hasat edilmiştir. 3. Uygulama II (U2): Sert mısırdan ikiden fazla olan koçanlardan en üst koçan tam olum döneminde tane mısır amaçlı, hemen altındaki koçan süt olum döneminde taze haşlamalık mısır olarak ve diğer koçanlar koçan püskülü çıkarma aşamasında körpe mısır olarak hasat edilmiştir. Bu uygulamada iki koçan bağlayan bitkilerde ikinci koçan süt olum döneminde, tek koçan bağlayan bitkilerin koçanları ise tam olum döneminde hasat edilmiştir.

Toprak analiz sonuçları esas alınarak her iki denemede de saf olarak 20 kg da<sup>-1</sup> azot ve 8 kg da<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> uygulanmıştır [7]. Azotun yarısı (amonyum sülfat formunda %21) ekimle, kalan yarısı boğaz doldurmadan sonra bitki diz boyuna (35-40 cm) ulaştığında, fosforun ise tamamı ekimle birlikte verilmiştir.

Ekimden sonra çıkış yapana kadar ve çıkıştan sonra toprak nem düzeyi kontrol edilerek sulama yapılmıştır. Hasat, her parselde uçlardan 50 cm ve kenarlardan 1'er sıra kenar tesirleri atıldıktan sonra her iki mısır alt türünde de körpe mısır için koçan püskülü çıkışının 2. gününde, haşlamalık veya közlemelik için süt olum döneminin sonuna doğru ve sert mısırdan tane hasadı için tam olum döneminde tane nemi %12-13 iken hasat edilmiştir.

Körpe mısırdan; körpe koçan boyu (cm), körpe koçan çapı (mm), körpe koçan ağırlığı (g) ve körpe koçan sayısı (adet da<sup>-1</sup>) [8], taze mısırlarda; koçan boyu (cm), koçan çapı (mm), koçanda tane sayısı (adet) ve tek koçan ağırlığı (g) [9] ve sert mısırdan; koçan boyu (cm), çapı (mm), ağırlığı (g), koçanda tane sayısı (adet), 1000 tohum ağırlığı (g), hektolitre ağırlıkları (kg<sup>100L</sup>) ve tane verimi (kg da<sup>-1</sup>) [23] değerleri belirlenmiştir.

## 2.4. Verilerin İstatistiki Olarak Değerlendirilmesi

Çalışmadan elde edilen verilerin varyans analizleri SAS istatistik paket programından faydalanılarak ayrı ayrı yapılmış ve istatistik açıdan önemli olduğu ortaya çıkan ortalamalar arasındaki farklılıklar LSD testine (P<0.05, P<0.01) göre karşılaştırılmıştır.

## 3. SONUÇLAR VE TARTIŞMA

### 3.1. Körpe Koçan Özellikleri

Her iki mısır alt türünde de dekara körpe koçan sayıları bakımından kontrol (12390,5 ve 12462,7 adet/da) ile U1 (4420,0 ve 845,3 adet/da) arasındaki farklılık istatistiksel olarak P≤0,01 düzeyinde önemli olurken, koçan özellikleri arasındaki farklar önemsiz bulunmuştur (Tablo 2). TAS 1504-2007 standartlarına göre, her iki mısır alt türüne ait körpe mısır koçanları pazarlanabilir özellikler taşımaktadır. Kontrol uygulamasının körpe koçan sayısının yüksek çıkması; koçanların koparılmasından kaynaklanırken, körpe koçan özellikleri arasında farkın çıkmaması; hem kontrolde hem de U1'de körpe mısır hasadının bitkide koçan püskülünün çıkışının ikinci gününde yapılması ve koçanların gelişimini tamamlamaması ile açıklanabilir. Sert mısırdan körpe koçan özellikleri şeker mısırdan daha yüksek olmuştur. Bu farklılık sert mısırdan genetik olarak hem bitki morfolojisi olarak hem de koçan özellikleri bakımından şeker mısırdan daha büyük yapıda olması ve bu özelliğin körpe koçanda da kendini göstermiş olmasından kaynaklanmaktadır. Kara ve ark. [10] sert mısırdan körpe koçan özelliklerinin şeker ve cin mısırdan daha yüksek olduğunu bildirmişlerdir. Körpe mısırdan koçan özelliklerinin mısır alt türlerine, çeşitlerin erkenci ve geçici olmalarına, koçan bağlama sayısı gibi özelliklerine bağlı olarak önemli ölçüde değişmektedir. Almeida ve ark. [11] ortalama körpe mısır koçan çapının 13,6 mm, boyunun 9,36 cm ve koçan sayısının 6814,6 adet da<sup>-1</sup> olduğunu, Castro ve ark. [12] ortalama körpe mısır koçan çapının 16,5 mm, koçan boyunun 11,4 cm ve Kara ve ark [10] körpe mısır koçan çapının 12,7-13,5 mm arasında, koçan boyunun 9,4-10,4 cm arasında olduğunu tespit etmişlerdir. Çalışmamızda elde edilen körpe koçan özellikleri araştırmalarda belirtilen sınırlar içerisinde yer almıştır. Gözübenli ve Konuşkan [13] ile Lopes ve ark. [14] körpe mısır verim ve koçan özelliklerinin cinslere ve hatta aynı cinsde ait mısır çeşitleri arasında da farklılıklar gösterdiğini bildirmişlerdir.

**Tablo 2.** Şeker ve sert mısırdan alt koçanların körpe mısır özellikleri

Uygulamalar	Şeker mısır			
	Körpe koçan sayısı (da <sup>adet</sup> )	Körpe koçan boyu (cm)	Körpe koçan çapı (mm)	Körpe koçan ağırlığı (g)
Kontrol	12390,5 a	11,3	9,10	12,5
UI	4420,0 b	11,7	9,33	12,3
F değeri	358,85**	1,07	0,49	0,18
LSD (%)	417,5	öd	öd	öd
CV(%)	6,13	2,05	1,44	3,17
Uygulamalar	Sert mısır			
	Körpe koçan sayısı (da <sup>adet</sup> )	Körpe koçan boyu (cm)	Körpe koçan çapı (mm)	Körpe koçan ağırlığı (g)
Kontrol	12462,7 a	15,5	13,9	14,2
UI	845,3 b	15,3	14,3	14,8
F değeri	191,3**	0,17	0,63	0,99
LSD (%)	263,9	öd	öd	öd
CV(%)	4,88	4,40	2,17	3,06

\*\* : P≤0.01 düzeyinde önemli, öd: Önemli değil

UI: Bitki üzerinde birden fazla olan koçanlardan en üst koçan taze mısır olarak bırakılmış ve diğer koçanlar körpe mısır olarak hasat edilmiştir.

### 3.2. Taze Koçan Özellikleri

Her iki mısır alt türünde de (şeker ve sert mısır) en üst koçanlar taze mısır olarak hasat edilmiştir. Kontrol uygulamasının dekara koçan sayıları (12021,3 ve 12229,0 adet/da) U1 den (6015,7 ve 4890,3 adet/da) ve sert mısırdaki U2 den (5524,7 adet/da) daha yüksek olurken, taze koçan özellikleri bakımından istatistiksel olarak fark çıkmamıştır (Tablo 3). Araştırmada sert mısırdaki U1 ile U2 uygulamasının dekara koçan sayıları arasında istatistiksel olarak fark çıkmamıştır. Kontrol uygulamasında taze koçan sayısının yüksek çıkması; koçanların tamamının sayılmasından kaynaklanırken, taze koçan özellikleri arasında farkın çıkmaması; süt olum dönemi sonunda hasat edilen taze mısırlarda nişasta dolun döneminin başlamamış olmasından kaynaklandığı düşünülmektedir. Çünkü nişasta dolun dönemi (sarı olum) verim ve verimle ilişkili komponentleri doğrudan etkilemektedir [15]. Taze tüketim amaçlı mısırın pazarlanmasında etkili olan en önemli iki koçan özelliği boyu ve çapıdır. Amerikan (US) tüketici standartları sınıflandırmasına (US Fancy, US No. 1 ve US No. 2) göre şeker mısırdan elde edilen taze koçanların %65'i, sert mısırdan elde edilen koçanların ise %80'i pazarlanabilir koçan sınıfına girmiştir. Şeker mısır

kardeşlenme özelliğine sahiptir, bu nedenle kardeşlerden ve aynı bitkiden çıkan ikinci koçanlar pazarlanabilir koçan sınıfı dışında kalmıştır. Sert mısırın taze koçan özellikleri şeker mısırdan daha yüksek olmuştur. Bu farklılığın iki alt türün genetik özelliklerinden kaynaklandığı düşünülmektedir. Kara ve ark. [3] şeker mısırdaki koparılan ikinci koçanların taze mısır boyu, çapı ve ağırlıklarını pozitif etkilediğini bildirmiştir.

Kara ve Akman [16] Isparta koşullarında şeker mısırın koçan boyunun 19,2-20,9 cm, koçan çapının 45,6-47,5 mm, koçandaki tane sayısının 713-720 adet, tek koçan ağırlığının 320,3-329,7 g, Sakin ve Azapoğlu [17] Tokat koşullarında şeker mısırın koçan boyunun 19,6-20,8 cm ve tek koçan ağırlığının 181,0-260,5 g ve Yıldırkan ve Kara [5] Burdur koşullarında şeker mısırın koçan boyunun 17,5- 22,0 mm, çapının 43,6-50,3 mm, koçanda tane sayısının 515,6-750,4 adet, ağırlığının 201,1-315,4 g ve taze koçan sayısının 10384,5-10732,0 da<sup>adet</sup> arasında değiştiğini bildirmişlerdir. Mısırdaki koçan özelliklerinin ekolojik koşullara, çeşitlere, kardeşlenme özelliklerine, bakım işlemlerine ve koçan bağlama sayısı gibi özelliklere bağlı olarak değişebileceği pek çok araştırmacı tarafından bildirilmiştir [18, 19].

**Tablo 3.** Şeker ve sert mısırdaki taze koçan özellikleri

Şeker mısır					
Uygulamalar	Koçan sayısı (da <sup>adet</sup> )	Koçan boyu (cm)	Koçan çapı (mm)	Koçan ağırlığı (g)	Koçanda tane sayısı (adet)
Kontrol	12021,3 a	15,3	38,6	184,7	499,3
UI	6015,7 b	15,9	39,3	187,6	527,6
F değeri	156,06**	1,00	0,57	0,22	3,75
LSD (%)	477,1	öd	öd	öd	öd
CV(%)	6,52	4,44	2,63	4,35	6,07
Sert mısır					
Uygulamalar	Koçan sayısı (da <sup>adet</sup> )	Koçan boyu (cm)	Koçan çapı (mm)	Koçan ağırlığı (g)	Koçanda tane sayısı (adet)
Kontrol	12229,0 a	18,1	39,0 b	198,6	579,5
UI	4890,3 b	18,6	41,6 a	203,3	576,3
UII	5524,7 b	18,3	40,3 ab	200,1	569,8
F değeri	153,5**	1,29	1,21	2,49	1,54
LSD (%)	675,0	öd	1,53	öd	öd
CV(%)	2,38	3,34	2,05	3,48	4,06

\*\* $P \leq 0,01$  düzeyinde önemli, öd: Önemli değil  
 UI: Bitki üzerinde birden fazla olan koçanlardan en üst koçan taze mısır olarak bırakılmış ve diğer koçanlar körpe mısır olarak hasat edilmiştir.  
 UII: Sert mısırdaki bitki üzerinde ikiden fazla olan koçanlardan en üst koçan tane mısır, hemen altındaki koçan taze mısır ve diğer koçanlar körpe mısır olarak hasat edilmiştir.

### 3.3. Sert Mısırdaki Verim, Koçan ve Tane Özellikleri

Sert mısırdaki birden fazla koçan bağlayan bitkilerde koçan koparılmasının koçan boyu, çapı, ağırlığı, koçanda tane sayısı, tane verimi, bin tane ağırlığı, hektolitre ağırlığı, tane genişliği ve tane boyuna etkisi istatistiksel olarak önemli olmuştur (Tablo 4). Araştırmada koçan koparılmayan kontrol uygulamasında tane verimi bitki üzerinde tek koçan bırakılan uygulamalara göre daha yüksek olurken, incelenen koçan ve tane özellikleri bakımından bitki üzerinde tek koçan bırakılan uygulamalarda kontrole göre daha yüksek değerler belirlenmiştir. Kontrol uygulamasında tane veriminin yüksek olması birden fazla koçan bağlayan bitkilerdeki koçanların koparılmamasına ve dolayısıyla daha fazla koçan sayısına bağlanabilir. U2 uygulamasında koçan ve tane iriliği özelliklerinin yüksek olması, bitki üzerinde en üst koçanın bırakılması ve bir C<sub>4</sub> bitkisi olan mısırın üst

yapraklarındaki fotosentez ürünlerinin daha kısa yoldan tek bir koçana taşınması ile ilişkili olduğu ve bunun koçan ve tane iriliğine yansımaları ile açıklanabilir. Akdoğan ve Kara [20] sert mısırdaki ortalama tane genişliğini 4,86-5,19 mm, tane boyunu 9,24-9,64 mm, koçanda tane sayısını 622,2-631,5 adet, bin tane ağırlığını 384,3-397,1 g, hektolitre ağırlığını 72,8-73,2 kg ve tane verimini 1202,1-1263,2 kg da<sup>-1</sup> arasında, Öztürk ve Büyükgöz [21] Trabzon ili yerel mısır popülasyonlarında koçan uzunluğunu 10,85-21,95 cm, koçan çapını 3,34-4,71 cm, koçanda tane sayısını 193,1-534,5, bin tane ağırlığını 270,6-397,0 g ve tane verimini 319,3-1167,1 kg da<sup>-1</sup> arasında belirlemişlerdir. Bu çalışmada belirlenen sonuçlar araştırmacıların bildirdiği sınırlar içerisinde yer almaktadır. Mısırdaki tane verimi, koçan ve tane özellikleri gibi parametreler çeşitlere, iklim faktörlerine, agronomik işlemlere ve bitki üzerinde yürütülen farklı uygulamalara göre değişebilir [21, 22, 23].

**Tablo 4.** Sert mısırdaki tane özellikleri ve verim

Uygulamalar	Koçan boyu (cm)	Koçan çapı (mm)	Koçan ağırlığı (g)	Koçanda tane sayısı (adet/koçan)	Tane verimi (kg da <sup>-1</sup> )
Kontrol	20,6 b	46,3 b	236,3 b	547,3 b	1160,3 a
UII	22,3 a	48,0 a	248,1 a	558,4 a	895,0 b
F değeri	4,10*	4,57*	5,90*	7,99*	19,62**
LSD (%)	1,21	0,96	6,24	8,06	226,83
CV(%)	2,70	6,02	6,50	2,58	6,11

Uygulamalar	1000 tane ağırlığı (g)	Hektolitreye ağırlığı (kg <sup>100L</sup> )	Tane genişliği (mm)	Tane boyu (mm)
Kontrol	320,4 b	63,9 b	8,75 b	8,85 b
UII	338,0 a	65,6 a	9,36 a	9,01 a
F değeri	7,62*	6,20*	5,19*	4,43*
LSD (%)	12,30	1,23	0,51	0,12
CV(%)	7,29	3,71	4,57	6,90

\*, \*\*: Sırsıyla P≤0.05 ve P≤0.01 düzeyinde önemli, öd: Önemli değil  
 UII: Sert mısırdaki bitki üzerinde ikiden fazla olan koçanlardan en üst koçan tane mısır, hemen altındaki koçan taze mısır ve diğer koçanlar körpe mısır olarak hasat edilmiştir.

#### 4. SONUÇ

Mısırın birden fazla koçan bağlayan bitkilerinde farklı tüketim amaçlarına göre değişim olum dönemlerinde hasat edilmiş ve dekara koçan sayısı dışında hem körpe mısır hem de taze mısır koçan özelliklerini etkilememiştir. Çeşitlere göre ekimden itibaren 55-70. günlerde körpe mısır, 81-102. günlerde taze mısır hasadı yapılmıştır. TAS 1504-2007 standartlarına göre, her iki mısır alt türüne ait körpe mısır koçanları pazarlanabilir özelliklere sahip olmuş ve US Fancy, US No. 1 ve US No. 2'ye göre taze koçanların %65 (şeker mısır) ile %80'i (sert mısır) pazarlanabilir koçan sınıfına girmiştir. Tane amaçlı mısır üretiminde bitki üzerinde tek koçan bırakılması koçan ve tane özelliklerini pozitif etkilemiştir.

Sonuç olarak; i. Her iki mısır alt türünde de birden fazla koçan bağlayan bitkilerde tek koçan bırakılarak diğer koçanların farklı tüketim amacıyla erken dönemlerde koparılmasının, körpe ve taze koçan özellikleri üzerine olumsuz bir etkisinin olmadığı, bununla birlikte sert mısırdaki tam olum döneminde tane ve koçan özelliklerini olumlu etkilediği belirlenmiştir. ii. Tek koçan bırakılarak diğer koçanların koparılmasının sert mısırın tane veriminde bir düşüşe neden olmasına rağmen tane boyutlarına pozitif yansımaları nedeniyle tohumluk mısır üretiminde bu yöntem uygulanabilir. iii. Mısırdaki bu uygulamaların başarısı bitkilerin birden fazla koçan bağlama özelliklerine bağlı olmakla birlikte, bir bitkiden iki veya üç farklı ürünün elde edilebileceği ve pratikte uygulanabilirliğinin olduğu sonucuna varılmıştır.

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# Türk Doğa ve Fen Dergisi

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### Homogeneity and Trend Analysis of Temperature Series in Hirfanlı Dam Basin

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Test,  
Hirfanlı Dam  
basin

**Abstract:** Climates are constantly changing on a temporal and spatial scale, so they are not static. In recent years, global warming and changes in climate have shown more and more effects on the hydrological cycle and water resources, and their effects have become so noticeable that they hinder sustainable life. For this reason, the studies on the investigation of the main causes of the observed changes in the climate, the evaluation of climate change as a process and the determination of the effects that will emerge, have increased over time. In the present study, the homogeneity of annual and seasonal temperature series in Hirfanlı Dam basin were examined by using the Pettitt Test (PT), and the trends were examined with the Spearman's Rho (SR) test and Mann Kendall (MK) test. Hirfanlı Dam basin, which is located in the semi-arid climate region where climate change can be seen due to its location, was chosen as the study area. The temperature data of the Gemerek, Kayseri, Kirsehir, Nevşehir, Sivas and Zara meteorological stations in the basin between 1965 and 2017 were analyzed. It was noted that summer temperatures increased throughout the basin. Significant trends to increase were also detected in spring and autumn. The trend to increase was statistically significant at a 95% confidence level in all stations except for the Zara in terms of annual temperatures. Trend maps were prepared for the basin by using the results obtained here and the Geographical Information Systems. It was reported that the tendency to increase in annual temperature series was because of the increase in summer temperatures at intense levels throughout the basin.

### Hirfanlı Baraj Havzasında Sıcaklık Serilerinin Homojenlik ve Eğilim Analizleri

#### Anahtar

#### Kelimeler

Trend analizi,  
Homojenlik  
analizi,  
Spearman's Rho  
Test,  
Mann Kendall  
Test,  
Hirfanlı Baraj  
havzası

**Öz:** İklimler durağan olmamakla beraber zamansal ve mekânsal ölçekte sürekli değişim halindedir. Son yıllarda küresel ısınma ve iklimde gözlenen değişimler, hidrolojik çevrim ve su kaynakları üzerinde gün geçtikçe daha fazla etkisini göstererek, günümüzde sürdürülebilir yaşamı engelleyecek boyutlarda hissedilebilir hale gelmiştir. Bu sebeple, iklimde gözlenen değişimlerin temel sebeplerinin araştırılması, iklim değişikliğinin süreç olarak değerlendirilmesi ve ortaya çıkacak etkilerin belirlenmesine yönelik yapılan çalışmaların sayısı zamanla artmaktadır. Çalışmada, Hirfanlı baraj havzasındaki yıllık ve mevsimlik sıcaklık serilerinin homojenlikleri Pettitt Testi (PT), eğilimleri ise Spearman's Rho (SR) ve Mann Kendall (MK) testleri kullanılarak araştırılmıştır. Yarı kurak iklim bölgesinde yer alan ve konumu gereği iklim değişiminin görülebileceği Hirfanlı Baraj havzası çalışma alanı olarak seçilmiştir. Havzada içerisinde bulunan Gemerek, Kayseri, Kırşehir, Nevşehir, Sivas ve Zara meteoroloji gözlem istasyonlarına ait 1965-2017 yılları arasındaki sıcaklık verileri analiz edilmiştir. Havza genelindeki yaz sıcaklıklarının arttığı dikkat çekmektedir. İlkbahar ve sonbahar mevsimlerinde de anlamlı artma eğilimleri belirlenmiştir. Yıllık ortalama sıcaklıklarda ise Zara dışındaki bütün istasyonlardaki artma eğilimleri istatistiki olarak %95 güven düzeyinde anlamlıdır. Elde edilen sonuçlar ve coğrafi bilgi sistemleri kullanılarak havzaya ait trend haritaları hazırlanmıştır. Yıllık sıcaklık serilerindeki artma eğiliminin havza genelinde yoğun bir şekilde yaz sıcaklıklarından artmasında kaynaklandığı ifade edilmiştir.

## 1. INTRODUCTION

Different climatic conditions occurred in Turkey due to global warming, as it is the case in other countries in the world. Due to being surrounded by seas on three sides, has a fragmented topography and orographic features, the effects of global climate change occur differently in various regions of our country [1]. Climate parameters need to be analyzed in order to determine the effects of climate change and to take necessary measures [2, 3]. Hydro-meteorological parameters were investigated during the observation periods in order to investigate the effects of global climate change on our country [4-20]. Partal and Kahya [21] examined the trends of precipitation from 1929 to 1993 of 96 stations. Uçgun [22] conducted trend analysis of precipitation, temperature, evaporation and flow data obtained from stations in the Kızılırmak basin. Yerdelen [23] investigated the trends of Susurluk basin flows using the Sequential MK. It is stated that there is a downward trend in river flows in the basin. Simsek et al. [24] investigated the seasonal and annual trends of temperature, humidity, wind speed and precipitation data of Hatay. In Antakya, they determined an increase in temperature, a decrease in wind speed, an increase in temperature and precipitation and a decrease in humidity values for the Iskenderun. Zeybekoglu and Karahan [25] investigated the trends of annual maximum rainfall intensity series for 206 stations using the MK, SR and Innovative Trend Analysis.

Southeast, Central Anatolia, Aegean and Mediterranean Regions, which have the characteristics of arid and semi-arid climates under the threat of desertification and which are expressed as semi-humid regions due to their lack of sufficient water resources, are expected to be affected more by the increase in temperature [26, 27]. From the past to the present, research has been carried out for seven geographical regions using temperature data, which is the subject of this study also. Toros [28] evaluated low and high temperature data and precipitation data of 18 stations. When daytime and nighttime temperatures are compared, they determined that there are significant increases in night temperature. Kadioglu [29] used temperature data measured at 18 stations between 1929 and 1990 and investigated local and regional trend analysis with the MK. Altin et al. [30] conducted trend analysis using rainfall and temperature series of 33 stations in the Central Anatolia region between 1975 and 2007 using the MK test in the

analysis, and observed that the precipitation decreases in the winter and spring months and tends to increase in the summer and autumn months. Kizilelma et al. [31] determined that there were significant increases in the maximum and minimum temperature trends for the central Anatolia region, and that there were increases in the values of the mean temperatures at all stations, except the Ürgüp. Dogan et al. [32] reported in their study that the trends achieved a milestone in the 1950-2006 period based on the analysis of trends in temperature series in Turkey. Ulke and Ozkoca [33] investigated the changes in temperature series of Sinop Ordu and Samsun provinces located in the central Black Sea Region over time using the MK Test and Sen's Trend Slope Test. As a result of their findings, they stated that the temperatures in the region are in an increasing trend. Gumus [34] investigated precipitation and temperatures in the Seyhan-Ceyhan river basin. As a result of annual and seasonal scale evaluations, it has been determined that temperatures tend to increase throughout the basin.

In this study, Hirfanli Dam basin, which is located in the semi-arid climate region where climate change can be seen due to its location, was chosen as the study area. Seasonal and annual temperature series analyzes were made by using PT, SR and MK Tests.

## 2. MATERIALS AND METHHOD

### 2.1. Study Area

The Hirfanli Dam basin, which is a sub-basin of the Kizilirmak River basin, is about 27,092 km<sup>2</sup> in areal size (Fig.1.) and located between 33.3-38.7°E longitudes and 38.3-40.1°N latitudes. The Hirfanli Dam, which was built on the Kizilirmak River in 1959 for flood control and hydropower purposes, has a surface area of 263 km<sup>2</sup> and reservoir volume of 5,980 hm<sup>3</sup> at normal water surface level. The Hirfanli Dam basin has a high and mountainous plateau with the altitude varying between 799-3880 m. The east part of the basin is the hilliest region of the basin, which consists of high peaks and is bordered by mountainous areas. Plateaus, wide plains, and meadows are more common in the west part of the basin. Agriculture is a major economic sector in the study area where wheat, barley, potato and sugar beet are the main agricultural products [35, 36].

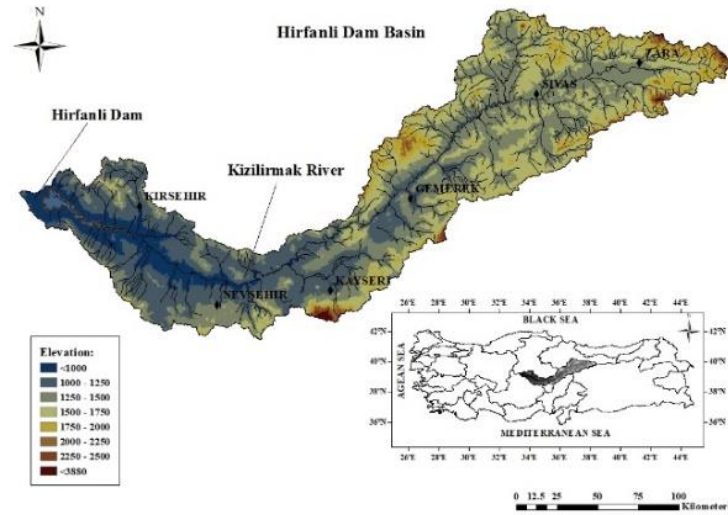


Figure 1. Hirfanli Dam basin

Annual and seasonal mean temperature data between 1965 and 2017 were obtained from the Turkish State Meteorological Service. Table 1 gives the geographical details of the six meteorological stations used in the

study. Statistical characteristics of annual and seasonal mean temperature (minimum, maximum and mean) are shown in Table 2.

Table 1. Geographical information of the meteorological stations in the Hirfanli Dam basin

Station Name	Station ID	Latitude (N)	Longitude (E)	Elevation (m)
Gemerek	17162	39.11	36.04	1173
Kayseri	17196	38.44	35.29	1093
Kırşehir	17160	39.09	34.10	1007
Nevşehir	17193	38.35	34.40	1260
Sivas	17090	39.45	37.01	1285
Zara	17716	39.54	37.45	1348

Table 2. Statistical information of temperature series in the Hirfanli Dam basin (°C)

		Gemerek	Kayseri	Kırşehir	Nevşehir	Sivas	Zara	Basin
Annual	Min.	6.94	8.41	9.43	8.49	6.64	5.94	7.64
	Max.	11.77	13.23	13.78	13.64	11.99	11.27	12.61
	Mean	9.63	10.52	11.46	10.69	9.17	8.63	10.02
Winter	Min.	-7.63	-6.87	-3.80	-4.73	-7.93	-9.03	-6.39
	Max.	2.47	4.13	4.70	5.40	2.87	2.60	3.67
	Mean	-1.66	-0.46	0.93	0.71	-1.89	-2.30	-0.78
Spring	Min.	6.80	8.17	7.70	7.13	6.37	5.23	7.38
	Max.	11.63	13.07	12.73	12.40	11.10	10.83	13.36
	Mean	9.20	10.23	10.52	9.83	8.67	7.96	9.40
Summer	Min.	17.90	18.73	19.83	17.73	16.90	16.03	17.86
	Max.	22.20	23.70	24.30	23.03	21.93	20.90	22.59
	Mean	20.15	21.07	21.95	20.50	19.29	18.61	20.26
Autumn	Min.	8.30	8.87	10.37	9.37	8.33	7.73	8.94
	Max.	13.63	13.67	15.00	14.63	12.90	12.30	13.36
	Mean	10.81	11.24	12.44	11.70	10.60	10.26	11.18

The basin is dominated by convective and frontal precipitation in general. The mean temperature is around 10.02°C and also the annual temperature decreases from the upstream to the downstream due to the increase in altitude. In the basin the hottest months are July and August, and the coldest months are January and February, and the difference between the highest temperature value and the lowest temperature value is over 10°C. While the temperature is around 21~22°C in July and August, the temperature drops to around -2~-

1°C in January and February. As can be seen, the mean temperature drops below 0°C in winter months.

According to Table 2, the maximum temperature is during the summer at 20.26°C; the minimum temperature is seen in the winter at -0.78°C. In addition, the mean temperature values of basin in autumn and spring were 11.18°C and 9.40°C, respectively. The spatial distribution of seasonal and annual temperature in the basin was given in Figure 2.

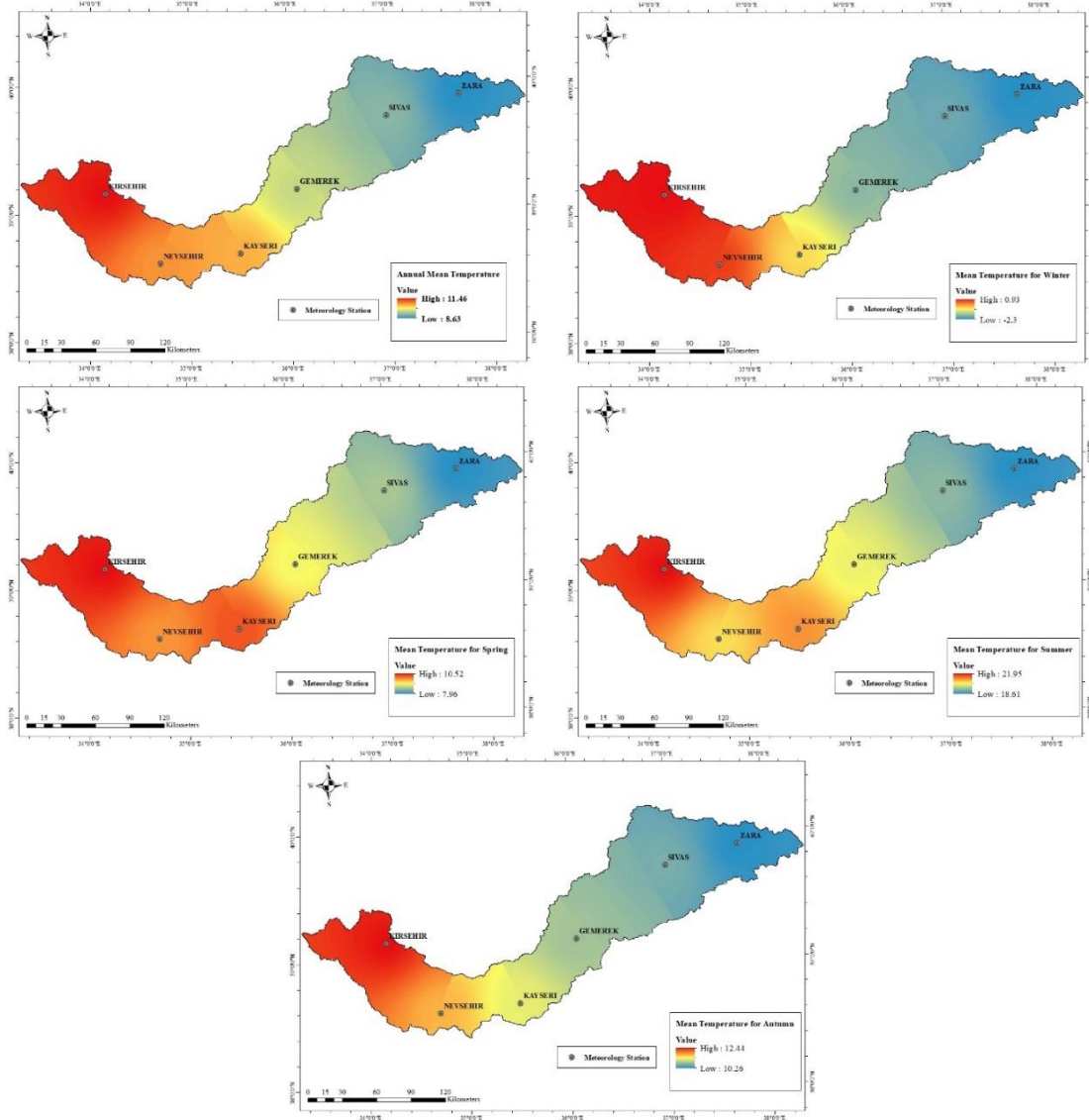


Figure 2. Spatial distribution of annual and seasonal temperature series

## 2.2. Pettitt Test (PT)

This non-parametric method developed by Pettitt [37] to determine the change point in a time series can find the change point on a monthly or annual scale [37]. The null hypothesis ( $H_0$ ) states that the series has an independent and random distribution, the alternative hypothesis states that there is a sudden change. The test statistic is associated with the Mann-Whitney statistic [38]. The critical values of this test are given in Table 3 [37].

Table 3. Critical values of  $X_E$

N	20	30	40	50	70	100
%95	57	107	167	235	393	677
%99	71	133	208	293	488	841

Observation values  $Y_1, \dots, Y_n$  values are listed as  $r_1, \dots, r_n$ .

$$X_k = 2 \sum_{i=1}^k r_i - k(n+1); k = 1, \dots, n \quad (1)$$

$X_k$  values are plotted graphically by means of Equation 1. In Equation 2, the absolute maximum value of  $X_k$  determines the change point.

$$X_E = \max_{1 \leq k \leq n} |X_k| \quad (2)$$

If the result of the homogeneity test is smaller than the value determined as the critical value, that data set is called homogeneous. The confidence level was chosen as 95% in the study. The critical value at this level of confidence was calculated as 256.

## 2.3. Spearman's Rho (SR) Trend Test

SR method is a simple and fast method used to investigate whether a linear trend exists. The purpose of the SR test is to investigate the existence of a linear relationship between the two observation series [39, 40]. Using Equation 3, the  $r_s$  value for the SR test statistic is calculated [41, 42].

$$r_s = 1 - \frac{6[\sum_{i=1}^n (R(x_i) - i)^2]}{(n^3 - n)} \quad (3)$$

If the observation period ( $n$ ) exceeds 30 years, the  $Z$  value is calculated using Equation 4.

$$Z = r_s \sqrt{n-1} \quad (4)$$

If the  $Z$  value at a selected  $\alpha$  significance level is greater than the  $Z_\alpha$  value determined from the standard normal distribution table, the  $H_0$  (No trend) hypothesis based on the fact that the observation values do not change over time is rejected and it is concluded that there is a certain trend.

#### 2.4. Mann Kendall (MK) Trend Test

The MK test is independent of the distribution of variables [43, 44]. Whether there is a tendency in the time series is tested by the null hypothesis ( $H_0$ : no trend) [21, 45, 46]. The pairs  $x_i, x_j$  in the series  $x_1, x_2, \dots, x_n$  are divided into two groups. The test statistic ( $S$ ) is expressed by Equation 5, where for  $i < j$  the number of pairs with  $x_i < x_j$  is  $P$  and the number of pairs with  $x_i > x_j$  is  $M$ . Kendall correlation coefficient with Equation 5; variance is calculated by Equation 7. If there are equal values in observations in the series, the variance value is calculated using Equation 8.

$$S = P - M \quad (5)$$

$$\tau = \frac{S}{\left[ \frac{n(n-1)}{2} \right]} \quad (6)$$

$$\sigma_s = \sqrt{\frac{n(n-1)(2n+5)}{18}} \quad (7)$$

$$\sigma_s = \sqrt{\frac{n(n-1)(2n+5) - \sum t_i(t_i-1)(2t_i+5)}{18}} \quad (8)$$

Standardized MK test statistics are calculated by Equation 9.

$$\begin{aligned} \frac{(S-1)}{\sigma_s} & ; S > 0 \\ 0 & ; S = 0 \\ \frac{(S+1)}{\sigma_s} & ; S < 0 \end{aligned} \quad (9)$$

If the absolute  $Z$  obtained by Equation 5 is less than the critical  $Z$  of the normal distribution corresponding to the selected  $\alpha$  significance level, the  $H_0$  is accepted; otherwise, the existence of the trend is determined. Positive values indicate the presence of an increasing trend, while negative values indicate a decreasing tendency [47].

### 3. RESULTS AND DISCUSSION

The homogeneity of annual and seasonal temperature series was tested by the PT at the significance level of 0.05 across the Hirfanli Dam basin. Six meteorological stations for the period 1965-2017 were analyzed, with the test results and break years given in Table 4.

**Table 4.** Results of PT

	Annual	Winter	Spring	Summer	Autumn
Gemerek	358 (1994)	124	310 (1994)	394 (1985)	172
Kayseri	466 (1998)	222	384 (1999)	596 (1995)	462 (1988)
Kirsehir	424 (1992)	104	252	494 (1995)	360 (1991)
Nevsehir	456 (1996)	146	294 (1993)	598 (1989)	336 (2000)
Sivas	408 (1993)	166	286 (1998)	448 (1991)	328 (1986)
Zara	276 (1994)	164	172	358 (1994)	150

According to Table 4, it was determined that the annual, spring and summer temperatures of Gemerek were not homogeneous. The years when the break occurred were determined as 1994, 1994 and 1985, respectively. For Kayseri, annual, spring, summer and autumn temperature values are not homogeneous and the breaking years are 1998, 1999, 1995 and 1988, respectively. In the Kirsehir temperature data, non-homogeneous values belong to the annual, summer and autumn temperature results. While the deterioration in the annual temperature values started in 1992, the deteriorations in the mean temperature values of summer and autumn began in 1995 and 1991. It was seen that the annual, spring, summer and autumn temperature values for Nevsehir were not homogeneous and the breaking years were 1996, 1993, 1989 and 2000, respectively. In

the Sivas mean temperature data, non-homogeneous values belong to the annual, spring, summer and autumn mean temperature results. While the deterioration in the annual temperature values started in 1993, the deteriorations in the mean temperature values of spring, summer and autumn began in 1998, 1991 and 1986. It is concluded that the annual and summer temperatures of Zara are not homogeneous. Breakings in the annual and summer temperature values occurred in 1994.

Annual and seasonal temperature results of SR and MK of the Hirfanli Dam basin are shown in Table 5. In this study, the critical  $Z$  value was chosen as 1.96, which corresponds to 0.05 significance level.

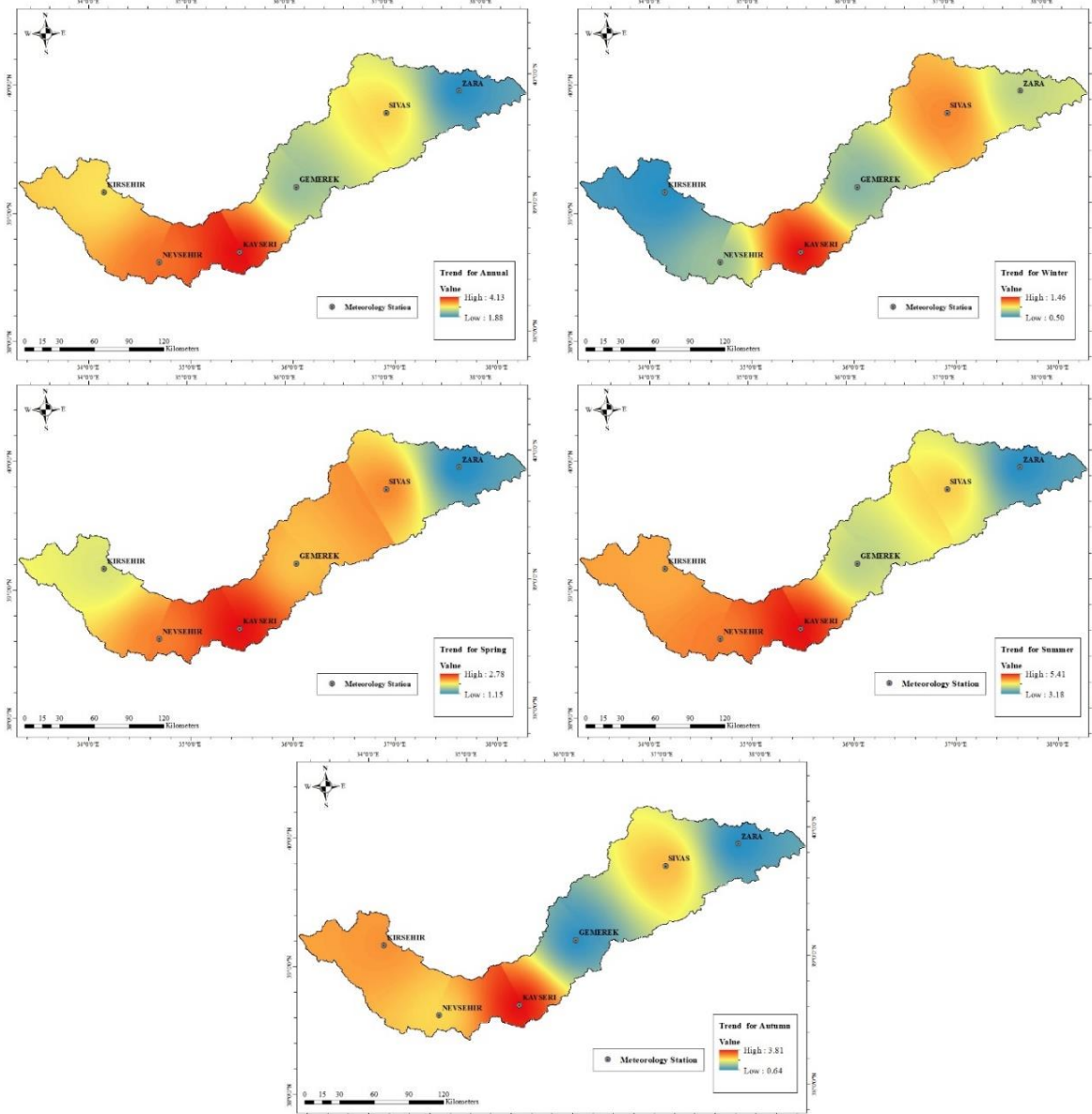


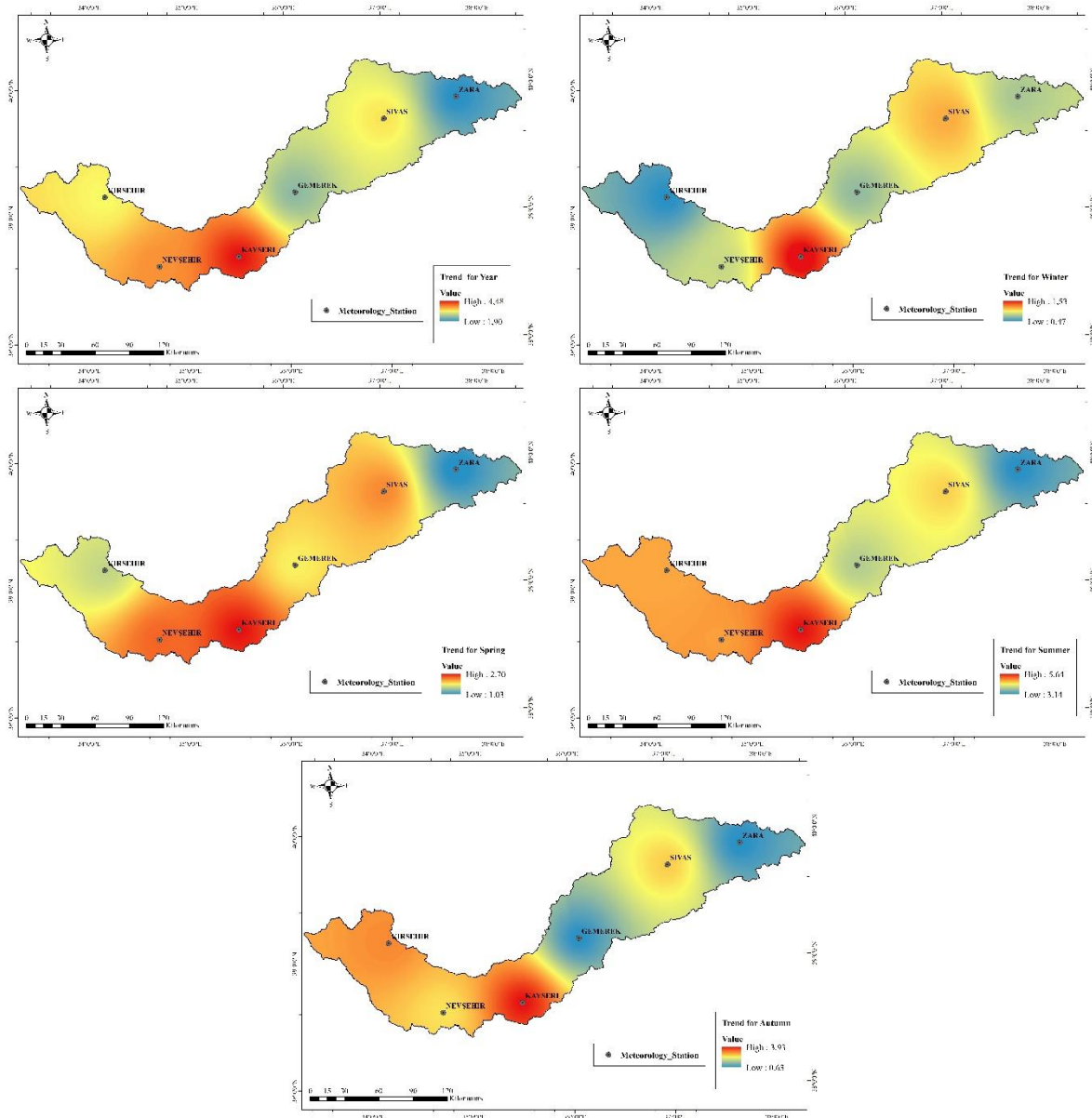
**Table 5.** Results of SR and MK Trend Tests

		Annual		Winter		Spring		Summer		Autumn	
		Z	Trend	Z	Trend	Z	Trend	Z	Trend	Z	Trend
Gemerek	SR	2.42	Δ	0.68	-	2.18	Δ	3.91	Δ	0.68	-
	MK	2.43	Δ	0.69	-	1.91	-	3.90	Δ	0.63	-
Kayseri	SR	4.13	Δ	1.46	-	2.78	Δ	5.41	Δ	3.81	Δ
	MK	4.48	Δ	1.53	-	2.70	Δ	5.64	Δ	3.93	Δ
Kirsehir	SR	3.12	Δ	0.50	-	1.83	-	4.71	Δ	2.96	Δ
	MK	3.18	Δ	0.47	-	1.62	-	4.86	Δ	3.14	Δ
Nevsehir	SR	3.72	Δ	0.74	-	2.43	Δ	4.94	Δ	2.47	Δ
	MK	3.82	Δ	0.83	-	2.45	Δ	4.92	Δ	2.45	Δ
Sivas	SR	3.21	Δ	1.23	-	2.39	Δ	4.52	Δ	2.68	Δ
	MK	3.32	Δ	1.16	-	2.31	Δ	4.62	Δ	2.62	Δ
Zara	SR	1.88	-	0.82	-	1.15	-	3.18	Δ	0.64	-
	MK	1.90	-	0.75	-	1.03	-	3.14	Δ	0.63	-

According to Table 5, significant increasing trends in annual, spring, summer and autumn temperatures were determined at Kayseri, Nevsehir and Sivas. Statistically significant trends of increase in annual, spring and summer temperatures in the Gemerek temperature series. On the other hand the MK result of the Spring for Gemerek, a significant trend could not be determined.

Significant positive trends were found in the annual, summer and autumn temperature series in the results of Kirsehir. In Zara, a significant increasing trend was identified for just summer temperatures.

**Figure 3.** Annual and seasonal trend maps for SR Results.



**Figure 4.** Annual and seasonal trend maps for MK Results.

The increasing trend in spring and summer temperatures determined in Gemerek will cause an increase in the annual temperatures. The increasing trend in annual temperatures in Kirsehir is due to the increasing trend in summer and autumn. In addition, the reason for the increase in annual temperatures of Kayseri, Nevsehir and Sivas is due to the significant increases in spring, summer and autumn temperatures.

In the studies carried out for Turkey, an increasing trend was reported in the temperature series [48-51]. The existence of an increasing trend in annual and seasonal temperature series has been revealed by various trend analysis methods in the Central Anatolia region and the Kizilirmak River basin [31, 52-55]. The findings of this study, obtained by SR and MK methods for the Hirfanli dam basin, show parallelism with the studies carried out for the Kizilirmak River basin, of which it is a sub-basin, and the Central Anatolian region. In addition, annual and seasonal trend maps of the basin were prepared using geographic information systems and Z scores of SR and MK (Figs. 3-4).

#### 4. CONCLUSION

In this study, the annual and seasonal temperature series of the Hirfanli Dam basin in the period 1965-2017 were investigated using the PT, SR and MK. According to the homogeneity test results, deterioration was detected in the annual and summer mean temperatures of all stations in the basin. All stations in the basin showed homogeneous characteristics in the winter temperature series. In the trend analysis results, the significant increase trend in the annual temperature series, except for Zara, is remarkable in the basin. Another remarkable point is that significant increase trends were detected in all stations in summer temperatures throughout the basin. Also, significant increases were determined in the spring and autumn temperature series at the several stations in the basin. There is no significant trend in winter temperature series for all stations. In addition, the results of trend analysis methods (SR and MK) are generally compatible with each other.

Homogeneity analysis and trend analysis results support each other at all stations, other than the Zara. In other words, the reason for the deterioration of homogeneity may be the increasing trend. However, it is thought that this situation determined at the Zara is due to its highest altitude in the basin and being under the influence of the climate of the Eastern Anatolian region.

In the Hirfanli Dam basin, which has a semi-arid feature, it is possible that severe drought events will be seen as a result of the temperature increase caused by global climate change. For this reason, it is of great importance to evaluate the basin in terms of climatic parameters, drought and water resources.

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## Traditional Uses of Some Food Plants in Suruç (Şanlıurfa, Turkey)

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Şanlıurfa,  
Turkey

**Abstract:** This research was carried out throughout a period of approximately two years between 2019-2020 and aimed to identify wild food plants frequently used by people living in Suruç district (Şanlıurfa) and some of its surrounding villages. 64 taxa belonging to 27 families were identified through these interviews that we conducted with 44 people. The demographic information of the interviewees, both the local and scientific names of the plants they use for food purposes, the parts of the plants used and the methods of preparing the plants were recorded. The plants used by the habitants of the region were scientifically diagnosed at Harran University herbarium and were recorded as herbarium material. The families of the plant taxa, their scientific and local names, their patterns of use and use value (UV) of the plants were calculated and presented in a table. The families with the widest taxa discovered in our research throughout the region are Asteraceae with 10 taxa (16%), Lamiaceae with 10 taxa (16%), Malvaceae with 5 taxa (8%), Brassicaceae with 4 taxa (6%), Fabaceae with 4 taxa (6%), Apiaceae with 3 taxa (5%) and Rosaceae with 3 taxa (5%); while genus discovered to have the widest taxa are *Mentha* L. with 3 taxa, *Crocus* L., *Euphorbia* L., *Malva* L., *Papaver* L., *Salvia* L. with 2 taxa. Food plants are usually consumed as food after their stems are peeled, their leaves are used to make salads; they are cooked by adding tomato paste, rice or egg, used as a spice and brewed like tea. Most common plant parts used for food purposes are above ground, leaf, stem, root and fruit. Some cultivated and natural plants in Suruç district are used for food purposes. Our literature review has revealed that the food plants used in Suruç district are also used both for food and medicinal purposes in different regions of our country.

## Suruç'ta (Şanlıurfa-Türkiye) Bazı Gıda Bitkilerin Geleneksel Kullanımları

### Anahtar

### Kelimeler

Suruç, Gıda  
bitkileri,  
Geleneksel  
kullanım,  
Etnobotanik,  
Şanlıurfa,  
Türkiye

**Öz:** Bu araştırma 2019-2020 yılları arasında yaklaşık iki yıllık bir sürede, Suruç ilçesi (Şanlıurfa) ve çevresindeki bazı köylerinde yaşayan insanların kullandığı yabani gıda bitkilerin tespit edilmesi için yapılmıştır. 44 kaynak kişi ile yapılan görüşmelerde 27 familyaya ait 64 takson tespit edilmiştir. Görüşme yapılan kişilerin demografik bilgileri, gıda amaçlı kullandıkları bitkilerin yerel adları ve bilimsel adları, kullanılan bitki kısımları ve bitkileri hazırlama yöntemleri kayıt edilmiştir. Bölge halkının kullandığı bitkilerin Harran Üniversitesi herbaryumunda teşhisleri yapılmış ve herbaryum materyali haline getirilmiştir. Bitki taksonların familya, bilimsel ve yöresel adları, bitkilerin kullanım şekilleri ve kullanım değerleri (UV) hesaplanarak tablo halinde sunulmuştur. Bölgede yaptığımız araştırmada en çok takson içeren familyalar şöyledir; Asteraceae 10 takson (% 16), Lamiaceae 10 takson (% 16), Malvaceae 5 takson (% 8), Brassicaceae 4 takson (% 6), Fabaceae 4 takson (% 6), Apiaceae 3 takson (% 5), Rosaceae 3 takson (% 5), en çok takson içeren cinsler ise; *Mentha* L. 3 takson, *Crocus* L., *Euphorbia* L., *Malva* L., *Papaver* L., *Salvia* L. ise 2 takson içermektedir. Gıda bitkileri en çok gövdesi soyulduktan sonra yenilir, yaprakları salata yapımında kullanılır, salçalı, pirinçli, yumurtalı yemeği yapılır, baharat olarak kullanılır ve çay olarak demlenip içilir. Gıda amaçlı en çok kullanılan bitki parçaları; toprak üstü, yaprak, gövde, kök ve meyve gibi organlardır. Suruç ilçesinde bazı kültür ve doğal yetişen bitkiler gıda amaçlı kullanılmaktadır. Literatür araştırmamızda, Suruç ilçesinde kullanılan gıda bitkileri ülkemizin farklı bölgelerinde gıda ve tıbbi amaçlı kullanıldığı görülmüştür.

## 1. INTRODUCTION

Our plants, which naturally grow in spring, are widely used in many regions of our country, particularly in Eastern Anatolia and the Aegean region for food purposes besides being a source of medication. Wild plants used for food purposes may either be consumed raw, or by adding eggs or yogurt, may be boiled or cooked by adding rice; they are also widely used in salads [1-4]. Wild food plants were also essential in ancient times for human survival. For example, people in besieged settlements during the war in Bosnia and Herzegovina benefited from plants to survive. The World Health Organization (WHO) has specified that wild plants consumed for food purposes will meet the daily vitamins (particularly A and C) and mineral needs of individuals [5].

Ethnobotany refers to the relationships of people in human societies with plants. In this context, "ethno" is used for human studies whereas "botany" refers to plant research or plant science. Ethnobotanical information has been compiled by trial and error methods over a long period of time and has reached the present day. It is inevitable that the culture of benefiting from plants will eventually disappear before it can be transferred to the younger generations due to the increase in migration to cities and the development of technology. Therefore, it is an extremely important requirement to record this knowledge before it is completely lost [6-7].

When our country is examined in terms of plant existence, it is possible to mention that it is among the richest countries in the world. According to the information included in the book titled 'Türkiye Bitkileri Listesi' there are 167 plant families, 1320 genera and 11707 taxa of plants in Turkey. Among the 11707 taxa in our country, 3649 are endemic [8-11]. This study aimed to record the use of wild plants for food purposes in Suruç district and its surrounding villages and to shed light on future research on wild food plants.

## 2. MATERIALS AND METHODS

Our research was carried out by interviews held with local people living in Suruç district of Şanlıurfa province (Figure 1) and some of its surrounding villages (Aybastı, Bahçe, Yalınca, Zeyrek and Ziyaret) over a period of about two years (2019-2020). The villages we researched were further transformed into neighborhoods by law [12].

### 2.1. Study of Area

Suruç, a district of Şanlıurfa province, is located in the southwest of the province and is 45 km from the city center. Surrounded by the Güvercik, Devres and Cudi mountains, the altitude of the district is 537 m. Syria is located to the south of the district. The population of the district has approached 103.000 and its surface area is 706 km<sup>2</sup>. The region is located in a semi-arid climate zone. The annual average temperature is 18 °C and the average precipitation is 457 mm. The summer months

are hot and dry while winter months are warm. The hottest period of the region is experienced in July and August [13-15].

90% of the total surface area of the district comprises of agricultural lands whereas remaining 10% is meadow, pasture, forest and non-agricultural areas. Cotton, wheat, barley, legumes and corn are grown in the district. In addition, although not much, olives, pistachios, sesame, chickpeas, vegetables, fruits, especially pomegranates are grown in the district [16].

### 2.2. Plants Materials

For the purpose of this study we have interviewed 44 people using food plants in the region. Personal information such as age, educational background, profession of these people were recorded. In addition to Kurdish, limited Arabic and Zazaki are spoken around the Suruç district. During our trips in the region, we asked local people to show us the plants they used for food purposes; we recorded the local names of the plants they used, the parts of the plant they used and how they prepared it for food. We collected the plants used in the region and took photographs of some of them. The plants we diagnosed were scientifically named and were recorded as herbarium material at Harran University. The plants are currently preserved in the Herbarium of Harran University (HUH). The plants we recorded at the end of the research are exhibited in the table on the basis of family names (Table 1). Family names, scientific and local names of plants, plant parts used for food, patterns of use and use value (UV) each plant are exhibited respectively in the table 1. The book titled "Flora of Turkey and the East Aegean Islands" were used for scientific naming of the plants we recorded [17-20].

### 2.3. Calculations

Use value indicating the degree of relative importance of native species is a quantitative method [21]. and is calculated according to the formula below and exhibited in the table accordingly (Table 1).

$$\text{Use Value (UV)} = T / K$$

T: The number of citations to each taxon

K: Indicates the number of people providing information

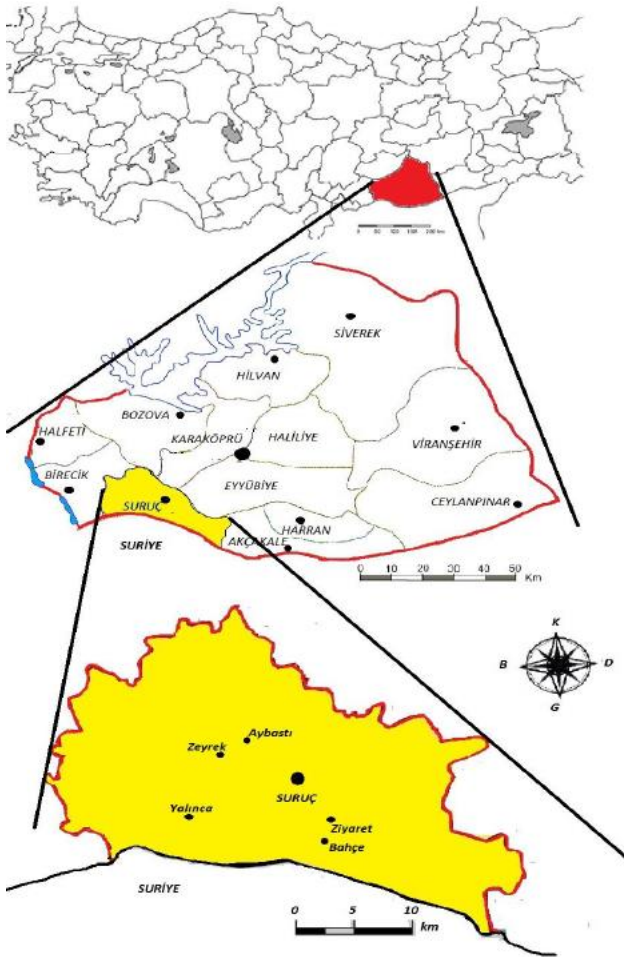


Figure 1. Maps of the study area

### 3. RESULTS AND DISCUSSION

Our interviews, held within the scope of our research in Suruç district, were conducted face-to-face with the people providing information (Figure 2).

#### 3.1. Demographic Characteristics of Study Participants

Mean age of the people who provided us information was 49. Examining the educational background of 44 people who provided us information, 10 were found out (23%) to have received no education at all, 19 (43%) were found out to be primary school graduates, 2 (4%) were secondary school graduates, 3 (7%) were high school graduates and 10 (23%) were university graduates. Elder people have been observed to be more experienced and acquainted about plants.



Figure 2. Interviews with local people (Suruç)

#### 3.2. Interviews with Locals and Literature Review

Examining the profession of 44 people who provided us information, 30% were found out to be farmers, 19% were tradesmen, 17% were workers, 9% were housewives and 25% were from other professions.

For the purpose of the ethno-botanical study we conducted around Suruç district, it was recorded that local people of the region used 64 taxa belonging to 27 families of plants for food purposes (Table 1). No study have been conducted specific to wild food plants within the scope of our research area, however there are a few studies conducted in the immediate vicinity [22-23]. We have concluded in our study that the plant families with the widest taxa were Asteraceae (16%) and Lamiaceae (16%) with 10 taxa each. Among other plant species available in the research area, Malvaceae has 5 taxa (8%), Brassicaceae has 4 taxa (6%), Fabaceae has 4 taxa (6%), Apiaceae has 3 taxa (5%) and Rosaceae has 3 taxa (5%) (Figure 3). The genera of plants with the widest taxa discovered in our research area are *Mentha* L. with 3 taxa, *Crocus* L., *Euphorbia* L., *Malva* L., *Papaver* L., *Salvia* L. with 2 taxa each (Figure 3).

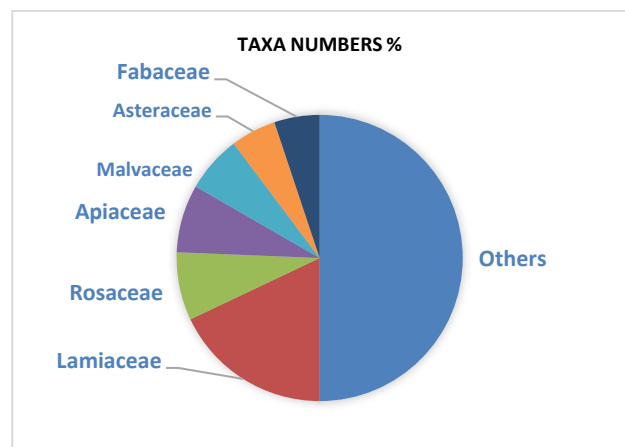


Figure 3. Plant families with the widest taxa

Most common food plants that we have identified throughout our research are; *Allium sativum* L., *Mentha longifolia* (L.) L. subsp. *typhoides* (Briq.) Harley, *Mentha x piperita* L. *Glycyrrhiza glabra* L., *Gundelia tournefortii* L., *Juglans regia* L., *Nigella sativa* L., *Thymus fallax* Fisch. & C.A. Mey. *Portulaca oleracea*



L., *Thymbra spicata* L., *Urtica dioica* L. and *Vitis vinifera* L.

Local people have been determined to either peel the stems of the food plants and consume them raw (7 use-report), use the leaves to make salads (6 use-report), cook them by adding tomato paste (7 use-report), rice or eggs (7 use-report), use them as a spice to flavor their meals (3 use-report) or brew them to serve as tea (18 use-report). The above-ground, leaves, stems, roots and fruits of some cultivated and naturally grown plants in Suruç district are used for food purposes. Plants are generally consumed fresh, however there are also examples where they are either dried and/or frozen for further consumption in the winter season.

Some plants that are traditionally used for food purposes in Suruç district have been reported to show different activities in studies carried out in the laboratory environment. For instance; *Rosa canina* is recorded to have antidiabetic, antioxidant, anti-inflammatory and antinociceptive activity; *Rhus coriaria* and *Salvia* species are recorded to have antioxidant activity and gastrointestinal diseases, *Malva* species are recorded to have antioxidant and antiulcerogenic activity; *Urtica dioica* are recorded to have anti fungal, anti analgesic, immunomodulatory, antimicrobial, antidiabetic, anti ulcer activity, depurative, diuretic and *Helichrysum* is reported to have antinociceptive and anti-inflammatory activity [24-34]. Local names of plants available in Turkey vary according to dialect and local languages [35]. As the local people around Suruç speak Kurdish, the plants are also known by their Kurdish names.

Literature review revealed that food plants used throughout the district are used for therapeutic purposes in many different parts of our country [36-39]. "Use value" (UV) has been calculated in many ethnobotanical studies [40-42]. The plants with the highest use value (UV) identified in the research area are as follows (Table 1); *Mentha x piperita* (0.44), *Allium sativum* (0.43), *Thymus fallax* (0.43), *Glycyrrhiza glabra* (0.39), *Urtica dioica* (0.39), *Nigella sativa* L. (0.38), *Mentha longifolia* subsp. *typhoides* (0.37), *Gundelia tournefortii* (0.36), *Juglans regia* (0.36), and *Polygonum cognatum* (0.36).

It has been determined in studies that Euphorbia and Equisetum taxa, which are used by the people of the region, show toxic effects [43-44]. People do not know about the toxic effects of these plants.

#### 4. CONCLUSION

64 wild and cultivated plant taxa belonging to 27 families determined as a result of face-to-face interviews held with local people in and around Suruç district are used for food purposes.

Most common plant parts used in the region for food purposes are above ground, leaves, stem, root and fruits. Food plants are usually consumed as food after their stems are peeled, their leaves are used to make salads,

their above-ground parts are cooked by adding tomato paste, rice or egg or used as a spice and brewed like tea.

Some cultivated and natural plants in Suruç district are used for food purposes. Our literature review has revealed that the food plants used in Suruç district are also used both for food and medicinal purposes in different regions of our country.

The studies carried out in the Eastern and Southeastern Anatolia Regions revealed that the local names of the plants used in this region for food purposes are similar to the local names of the plants used in Suruç.

It was recorded that the food plant with the highest calculated use value was *Mentha x piperita* with 0.44. Other plants with the highest use value are It was recorded that the food plant with the highest calculated use value was *Mentha x piperita* with 0.44. Other plants with the highest use value are *Allium sativum*, *Thymus fallax*, *Glycyrrhiza glabra*, *Urtica dioica*, *Nigella sativa*, *Mentha longifolia*, *Gundelia tournefortii* (0.36), *Juglans regia* (0.36), and *Polygonum cognatum* respectively.

The traditional use of plants in the region is decreasing day by day due to migration. In addition, the vegetation of the region is being destroyed due to agricultural activities and herding. Plant protection measures should be implemented urgently in the region.

Consumption of food plants by human raises the question of whether these plants are safe. We think that the wild plants that we have reported will be a resource for those working on food safety. We believe that ethnobotanical studies carried out in provinces and districts of the region where this kind of research has not yet been carried out will be rather beneficial.

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**Table 1.** Food plants used in the region

Familya	Scientific name / voucher specimen	Vernacular name(s)	Use parts	Place and form of use	UV
Amaryllidaceae	* <i>Allium sativum</i> L. SY-	Sarımsak	Fruit	Used to flavor food	0.43
Anacardiaceae	<i>Rhus coriaria</i> L. SY -	Sumak	Fruit	Used as a spice	0.22
Apiaceae	<i>Eryngium creticum</i> Lam. SY 1099	Çistok	Leaves, Stem	The stem is consumed after peeling; the leaves are used to make salads	0.04
Apiaceae	<i>Ferula orientalis</i> L. SY 1051	Kingor	Root	It is cooked by adding tomato paste; it is brewed and served like tea	0.23
Apiaceae	<i>Malabaila secacul</i> (Mill.) Boiss. SY 1100	Harık	Aerial parts	The plant is boiled and cooked by adding rice and consumed with yogurt; the leaves are used in salads	0.02
Asparagaceae	<i>Ornithogalum narbonense</i> L. SY 1096	Akbandır	Leaves	Cooked with eggs	0.02
Asteraceae	<i>Achillea oligocephala</i> DC. SY 1088	Civanperçemi	Aerial parts	Brewed and served like tea	0.02
Asteraceae	<i>Carduus pycnocephalus</i> L. SY 1101	Kerbeş	Stem	The stem is consumed raw after peeling	0.02
Asteraceae	<i>Carthamus tinctorius</i> L. SY 1020	Aspir, Diken, Zafur	Flower	Added to soups and local rice pudding called "zerde"	0.04
Asteraceae	<i>Centaurea iberica</i> Spreng. SY 1118	Trevir, ex Hesteriyezer, Çakırdiken	Aerial parts	It is cooked by adding tomato paste	0.03
Asteraceae	<i>Echinops orientalis</i> Trautv SY 1010	Şekerok	Stem	The stem is consumed raw after peeling	0.04
Asteraceae	<i>Gundelia tournefortii</i> L. var. <i>armata</i> Freyn & Sint. SY 1119	Kereng, kenger	Stem	The stem is consumed raw after peeling; cooked with eggs	0.36
Asteraceae	<i>Helichrysum orientale</i> (L.) Vaill. SY 1041	Ölmez çiçek	Aerial parts	Brewed and served like tea	0.05
Asteraceae	<i>Matricaria chamomilla</i> L. SY 1009	Papatya	Aerial parts	Brewed and served like tea	0.04
Asteraceae	<i>Notobasis syriaca</i> (L.) Cass. SY 1120	Kerbeş, Kırbeş	Stem	The stem is consumed raw after peeling	0.02
Asteraceae	<i>Onopordum carduchorum</i> Bornm. & Beauverd SY 1125	Kulındor, Qulındor	Stem	The stem is consumed raw after peeling	0.07
Boraginaceae	<i>Alkanna orientalis</i> (L.) Boiss. SY 1140	Mıtmıtok, Fısfıso	Flower	Its flowers are absorbed by children	0.05
Boraginaceae	<i>Anchusa azurea</i> Mill. SY 1139	Guriz	Leaves, Stem	Cooked with eggs; consumed by adding it to rice	0.28
Brassicaceae	<i>Alyssum strictum</i> Willd. SY 1137	Nanieçuçuka	Aerial parts	Consumed by adding it to rice	0.09
Brassicaceae	<i>Capsella bursa-pastoris</i> (L.) Medik. SY 1049	Çobançantası, otu, Kuşkuş otu	Kuş Aerial parts	Brewed and served like tea	0.06
Brassicaceae	<i>Lepidium draba</i> L. SY 1126	Kıniberk	Aerial parts	It is cooked by adding tomato paste	0.02
Brassicaceae	<i>Thlaspi perfoliatum</i> L. SY 1121	Nanieçuçuka	Aerial parts	Used in salads	0.02
Cannabaceae	<i>Celtis australis</i> L. SY 1124	Dağdağan	Fruit	Consumed as a fruit	0.14
Equisetaceae	<i>Equisetum arvense</i> L. SY 1061	Atkuyruğu, Kırkkilitotu	Aerial parts	Brewed and served like tea	0.02
Ericaceae	<i>Erica vulgaris</i> L. SY 1076	Taşakçilotu	Leaves, Flower	Brewed and served like tea	0.02
Euphorbiaceae	<i>Euphorbia cheiradenia</i> Boiss. & Hohen. SY 1098	Heşül	Branch, Flower	Used to add a pleasant smell and taste to molasses	0.05
Euphorbiaceae	<i>Euphorbia falcata</i> L. SY 1097	Heşül	Branch, Flower	Used to add a pleasant smell and taste to molasses	0.02
Fabaceae	<i>Astragalus allepicus</i> Boiss. SY 1130	Gunpisik	Fruit	Consumed as a fruit	0.08
Fabaceae	<i>Glycyrrhiza glabra</i> L. SY 1019	Meyan, Biyan, Biyam balı	Roots	The roots are boiled and drunk as juice	0.39

Fabaceae	<i>Prosopis farcta</i> (Banks & Sol.) J.F.Macbr. SY 1134	Hurnif, Çeti	Fruit	Consumed as a fruit	0.02
Fabaceae	<i>Pisum sativum</i> L. SY 1111	Bezelye	Seed	Fresh seeds are consumed raw	0.04
Iridaceae	<i>Crocus cancellatus</i> Herb. subsp. <i>damascenus</i> (Herb.) B.Mathew SY 1103	Pivok	Bulb	Eaten raw	0.03
Iridaceae	* <i>Crocus sativus</i> L. SY 1032	Zahferan, Safran	Stylus	Sprinkled on top of the local pudding named "zerde" to add a pleasant smell and color	0.06
Juglandaceae	* <i>Juglans regia</i> L. SY -	Ceviz, Koz, Goz	Seed	Consumed as an appetizer; used in making cakes and desserts	0.36
Lamiaceae	<i>Clinopodium congestum</i> (Boiss. & Hausskn.) Kuntz SY 1107	Pungietehta	Aerial parts	Used in salads	0.05
Lamiaceae	<i>Melissa officinalis</i> L. SY 1085	Melisa, oğulotu, Acem otu	Leaves	Brewed and served like tea	0.04
Lamiaceae	<i>Mentha longifolia</i> (L.) L. subsp. <i>typhoides</i> (Briq.) Harley SY 1117	Nane, Pung	Aerial parts	Brewed and served like tea	0.37
Lamiaceae	* <i>Mentha x piperita</i> L. SY 1028	Nane, Pung	Aerial parts	Brewed and served like tea	0.44
Lamiaceae	<i>Mentha pulegium</i> L. SY 1064	Kaya yarpuzu, Punge tahte, Tüylü nane	Aerial parts	Brewed and served like tea	0.21
Lamiaceae	<i>Rosmarinus officinalis</i> L. SY 1006	Kuşdili, Biberiye	Leaves	Brewed and served like tea	0.11
Lamiaceae	<i>Salvia fruticosa</i> Mill. SY 1026	Adaçayı	Leaves	Brewed and served like tea	0.19
Lamiaceae	<i>Salvia multicaulis</i> Vahl SY 1104	Rehan	Aerial parts	Brewed and served like tea	0.20
Lamiaceae	<i>Thymbra spicata</i> L. SY 1033	Zahter, Yalancı kekik	Aerial parts	Used as a spice; brewed and served like tea	0.35
Lamiaceae	<i>Thymus fallax</i> Fisch. & C.A. Mey. SY 1046	Kekik	Aerial parts	Used as a spice	0.41
Malvaceae	* <i>Abelmoschus esculentus</i> (L.) Moench SY -	Bami	Fruit	The fruits are consumed by boiling	0.02
Malvaceae	<i>Althaea officinalis</i> L. SY 1069	Hatmi, Hiro	Flower, Leaves	Brewed and served like tea	0.10
Malvaceae	* <i>Hibiscus sabdariffa</i> L. SY -	Narçeçeği	Flower	Brewed and served like tea	0.12
Malvaceae	<i>Malva parviflora</i> L. SY 1116	Tollik	Aerial parts	Cooked with adding tomato paste and eggs	0.14
Malvaceae	<i>Malva sylvestris</i> L. SY 1067	Kömeç, Ebegümeçi	Tollik, Aerial parts	Cooked with adding tomato paste and eggs	0.19
Moraceae	<i>Morus nigra</i> L. SY 1082	Karadut	Fruit	Consumed as a fruit; used in making fruit juice and molasses	0.27
Oleaceae	* <i>Olea europaea</i> L. SY-	Zeytin	Fruit	Fruit is eaten	0.24
Papaveraceae	<i>Papaver dubium</i> L. SY 1141	Zingilzava	Whole plant	Cooked with adding tomato paste and eggs	0.16
Papaveraceae	<i>Papaver somniferum</i> L. SY 1081	Haşhaş	Leaves, Seed	Sprinkled on the cake	0.12
Pedaliaceae	* <i>Sesamum indicum</i> L. SY -	Susam, Küncü	Seed	Sprinkled on cakes, breads and pastries	0.29
Poaceae	<i>Triticum aestivum</i> L. SY 1144	Gennim	Seed	Used as flour	0.25
Poaceae	* <i>Zea mays</i> L. SY -	Mısır	Seed	Consumed as an appetizer; used in making cakes and desserts	0.32
Polygonaceae	<i>Polygonum cognatum</i> Meisn. SY 1142	Tırşok	Aerial parts	Used in salads	0.36
Portulacaceae	<i>Portulaca oleracea</i> L. SY 1024	Semizotu, Parpar	Leaves	Used in salads	0.28
Ranunculaceae	<i>Nigella sativa</i> L. SY 1030	Çörekotu, Kara çörek	Seed	Sprinkled on cakes, breads and pastries	0.38
Rosaceae	<i>Amygdalus communis</i> L. SY 1147	Beivf	Fruit, Seed	Consumed as a fruit; consumed as an appetizer	0.32
Rosaceae	<i>Crataegus monogyna</i> Jacq. SY 1047	Alıç, Guviç	Flower, Fruit	Consumed as a fruit; brewed and served like tea; used for producing vinegar	0.37
Rosaceae	<i>Rosa canina</i> L. SY 1008	Kuşburnu	Fruit	Consumed as a fruit; jam is made	0.29
Urticaceae	<i>Urtica dioica</i> L. SY 1021	Isırgan	Leaves	Cooked with adding tomato paste and eggs	0.39
Vitaceae	* <i>Vitis vinifera</i> L. SY -	Üzüm, Tiri	Fruit	Consumed as a fruit; used for producing vinegar	0.35

\*Cultivated plants



## Vegetative Propagation of Cotton (*Gossypium* spp.) By Rooting

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### Keywords

Clone  
 production,  
 Cotton,  
 Hybrid vigor,  
 Rooting

**Abstract:** Cotton plant is produced with seeds. However, its genetic structure is suitable for vegetative production. In this study, rooting method used to propagate cotton plant as vegetative. Experiment was conducted in climate chamber at East Mediterranean Transitional Zone Agricultural Research Institute in 2020. Stems of Askabat 100 (*G. barbadense* L.), Stoneville 468 (*G. hirsutum* L.) cultivars and their F<sub>1</sub> hybrid were used as plant materials while 2000 ppm IBA (Indole butyric acid) concentration used as chemical materials. As a result of the study, it was determined that cotton genotypes rooted and formed shoots in different rates. Moreover, F<sub>1</sub> hybrid produced more clones than their parents, followed by Askabat 100 and Stoneville 468. Also, F<sub>1</sub> hybrid were showed that superior heterotic effects as heterosis and heterobeltiosis in term of investigated properties. This result showed that cotton cultivars and hybrids can be propagation as clone using by rooting, and these clones could be used as rootstocks again in clone production.

## Pamuk (*Gossypium* spp.) Bitkisinin Köklendirme Yöntemiyle Vejetatif Çoğaltılması

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### Anahtar

Kelimele  
 Klon üretimi,  
 Pamuk,  
 Hibrit gücü,  
 Köklendirme

**Öz:** Pamuk bitkisi tohumlarla üretilir. Ancak genetik yapısı vejetatif üretime uygundur. Bu çalışmada, pamuk bitkisinin vejetatif olarak çoğaltılması amacıyla köklendirme yöntemi kullanılmıştır. Deneme, 2020 yılında Doğu Akdeniz Geçit Kuşağı Tarımsal Araştırma Enstitüsü Müdürlüğü'nün iklim odasında yürütülmüştür. Bitki materyali olarak Askabat 100 (*G. barbadense* L.), Stoneville 468 (*G. hirsutum* L.) çeşitleri ile bunların F<sub>1</sub> hibritlerine ait gövde parçaları, kimyasal material olarak ise 2000 ppm IBA (İndol butirik asit) konsantrasyonu kullanılmıştır. Çalışma sonucunda pamuk genotiplerinin sürgün oluşturduğu ancak farklı oranlarda köklendiği belirlenmiştir. Ayrıca F<sub>1</sub> melezi, ebeveynlerinden daha fazla kök üretmiş, bunu Askabat 100 ve Stoneville 468 çeşitleri izlemiştir. Dahası F<sub>1</sub> melezleri incelenen özellikler yönünden önemli heterotik etki (heterosis ve heterobeltiosis) göstermiştir. Bu sonuç, pamuk çeşitlerinin ve hibritlerinin köklendirme yoluyla klon olarak çoğaltılabileceğini ve bu klonların klon üretiminde tekrar anaç olarak kullanılabileceğini göstermiştir.

### 1. INTRODUCTION

The *Gossypium* L. genus contains more than 50 species in the world. One of them is *G. hirsutum* L. (Upland cotton) which different varieties of it were cultivated in Turkey. Turkey is major *G. hirsutum* L. producers with Greece and Spain in Europe. Moreover, Bulgaria has been producing some amount of cotton. Farming of cotton starts with its seed planting, and quality seeds are bases of modern farming of cotton. But, preparing of quality seeds starts with breeder seeds, and takes minimum five years to serve for farmers with systematic and its expensive methods after breeding studies that takes 8-12 years.

Cotton is having sexual reproduction system, but it can rapidly propagation by vegetative methods too such as T or Shield budding method [1]. However, because of easy sowing of cotton seeds with machine, in traditional, grafting, transplantation and pruning practices are not widely used in cotton farming. Whereas, vegetative propagation of cotton is get some advantages. For example, because of every new individual (clone) has the same genetic constitute with its parent, breeders will can save their important hybrids or genotypes along with years in the greenhouse. Moreover, cotton farming will can be done with clones some areas having problems such as salty, droughty and contaminated with *Verticillium dahliae* Kleb. In this areas, if varieties have strong root development, tolerant to disease with high

yield and fiber quality, can be propagated with rooting, furthermore because have strong root system these cultivars can be used as rootstock plant in grafting methods.

On the other hand, for production of cotton seed, rooting, grafting or combination of rooting with grafting can be used in greenhouse. This production is very important. When breeders use this method, will can offer seeds having same genetic potential which produced from lots of clone plants to farmers. So, farmers will know about yield and quality potential of their field before harvest. There are a lot of studies on propagation of cotton by vegetative. Numerous cotton clones have been produced via micro-propagation from shoot [2], cotyledon explants [3] and hypocotyl of cotton seedlings [4]. Moreover, cotton was propagation as vegetative by grafting method in Turkey [1, 5]. The aim of this work is to search possibility of vegetative propagation on cotton (*Gossypium* spp) by rooting.

## 2. MATERIALS AND METHODS

### 2.1. Plant Materials

Stoneville 468 (*G. hirsutum* L.), Askabat 100 (*G. barbadense* L.) and their F1 hybrid were used as plant material in this experiment. Askabat 100 needs longer vegetation period for productions cotton, but produced lower seed cotton yield, lint yield and ginning out-turn in Turkey. On the other hand, Stoneville 468 is an upland cultivar having extra seed cotton yield; lint yield and ginning out-turn than Askabat 100, but lower fiber qualities.

### 2.2. Field Experiment and Climate Chamber Applications

This experiment was conducted at East Mediterranean Transitional Zone Agricultural Research Institute in 2020 (37° 38' N; 36° 37' E, altitude: 568 m), in climate chamber between 15 October 2020 and 30 November 2020, according to Randomized Complete Block Design (RCBD) with three replications. Plant stems of 22 weeks were used in study. The stems are divided into pieces with at least 4 nodes. Genotypes were represented by 100 plant stem parts in replications. Plant pieces were kept in indole butyric acid (2000 ppm) for 5 minutes and then transferred to perlite medium prepared before in the climate chamber. Then the climatic room conditions were fixed at 28±2 °C temperature and 70-80% humidity. With the counts made on 30 November 2020, the number of rooting plants, root number, root length, shoot number and shoot length were determined. Data were analyzed for number of rooting plant, root number per plant, root length per plant (cm), shoot number per plant, shoot length per plant (cm). Then, means of investigated properties were compared by Least Significant Degree at 0.05 (LSD0.05). In addition, hybrid vigor (heterosis and heterobeltiosis) values were determined by the following Eq.1 and Eq.2 formulas at below.

$$\text{Heterosis (\%)} \quad Ht = ((F_1 - MP) / MP) * 100, \quad (1)$$

$$MP = (\text{Line parent} + \text{Tester parent}) / 2$$

$$\text{Heterobeltiosis (\%)} \quad Htb = (F_1 - \text{Better Parent}) / \text{Better Parent} * 100 \quad (2)$$

## 3. RESULTS

### 3.1. Rooting Stages

In the study, the visuals about rooting can be seen in Figures 1, 2, 3, 4, 5 and 6. Plant parts with or without wood (bark) texture developed callus and rooted (Figure 1 and Figure 2).



Figure 1. Callus formation



Figure 2. Rooting from callus

Thick plant parts formed mostly roots and long shoot (Figures 3 and 4). IBA 2000 dose and duration were sufficient for callus formation (Figures 1 and Figure 2). Furthermore, plant parts forming roots and shoots adapted to pots filled with 1/2 peat + 1/2 soil (Figure 6).





**Figure 3.** Rooting from green and non-green cuttings



**Figure 4.** Advanced roots



**Figure 5.** Clone plant with developed roots and shoots



**Figure 6.** Clone plant in pots

### 3.2. Number of Rooting Plant

The variance analysis regarding the number of rooting plants is shown from According to Table 1, the number of rooting plants differed according to their genotypes. The F1 hybrid was the most rooted genotype with 54.88%, and it was followed by Askabat 100 with 49.22% and Stoneville 468 with 26.88% (Table 2). According to Ouma et al. [4], cotyledon node explants of Stoneville 474 have rooted on sucrose based medium + IBA and +/- activated charcoal after a 25, 39 and 65 cumulative days in culture. It is a plant that can reproduce by cotton grafting and micro propagation methods. This study showed that the stem of cotton plant can be rooted and reproduced vegetative. This finding is very important. Wood texture or green plant body parts did not differ and were rooted.

The method will help breeders easily reproduce the plants they want to use for many years in seed production. In addition, they will be able to reproduce and protect important breeding materials in a short time. Moreover, seeds of commercial varieties will be propagated from clones of a plant and given to farmers. Thus, efficiency and quality losses will be reduced. On the other hand, triploid genotypes developed by interspecies hybridization will be easily reproduced by the method and can be used for many years in polyploidy studies.

### 3.3. Root Number Per Plant

Differences of cultivars were found important for average root number per plant (Table 1). The root number per plant has changed from 19.22 (Stoneville 468) to 37.56 (F1 hybrid).

**Table 1.** Variance analysis for the rooting application

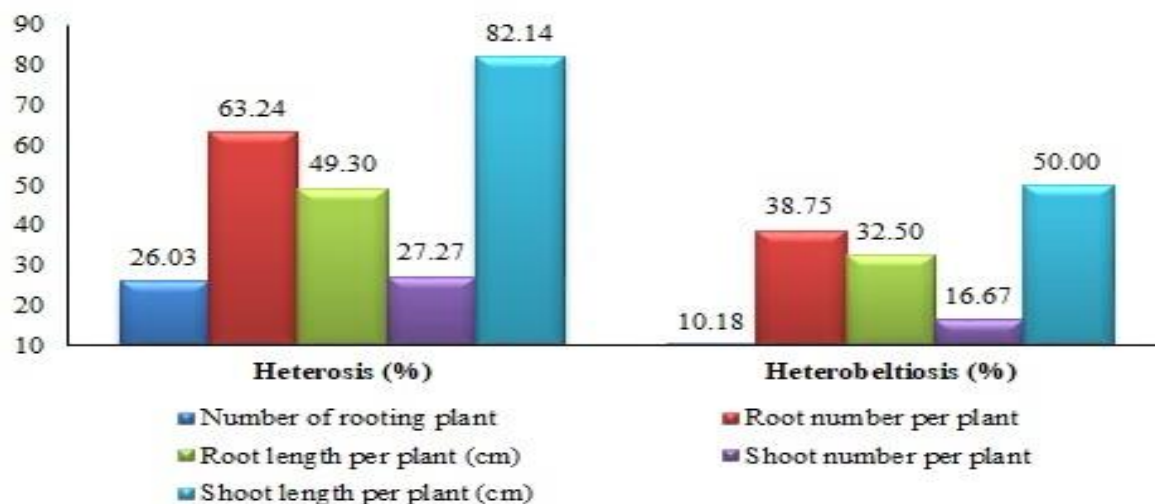
Sources	Degree of freedom	Number of roofing plant	Root number per plant (cm)	Root length per plant	Shoot number per plant	Shoot length per plant (cm)
Replication	2	93.78	14.77	1.77	1.33 *	0.45
Cultivars	2	657.44 *	253.45 *	40.78 *	1.33 *	70.78 *
Error	4	23.61	18.44	3.11	0.17	3.61
Total	8	199.61	76.28	12.19	0.75	19.61



**Table 2.** Values of investigated properties in term of rooting applications

Cultivars	Number of rooting plant (number)	Number of root per plant (number)	Root length per plant (cm)	Shoot number per plant	Shoot length per plant (cm)
Stoneville 468	26.88 b	19.22 b	9.89 b	4.00 b	7.11 b
Askabat 100	49.22 a	27.22 b	12.89 b	4.66 b	11.10 b
F <sub>1</sub> hybrid	54.88 a	37.56 a	17.22 a	5.33 a	16.77 a
CV (%)	9.70	15.65	12.80	10.20	15.98
LSD <sub>0.05</sub>	11.01	9.74	3.99	0.93	1.53

CV (%): Coefficient of variation, LSD: Least significant degree

**Figure 7.** Hybrid vigor (Heterosis and heterobeltiosis) values of F<sub>1</sub> hybrid

Also, Askabat 100 has produced 12.89 roots per plant. The F<sub>1</sub> hybrid has most number of roots than Askabat 100 and Stoneville 468 having minimum numbers of root (Table 2). Cotton has a rapidly growing taproot and the seedling can reach 20-25 cm deep without even growing above the ground. The final depth of the root system depends on soil moisture, aeration, temperature and variety, but is usually about 180-200 cm. Although cotton stem pieces are rooted, they cannot taproot form like a normal cotton plant.

### 3.4. Root Length in Plant

Differences of mean of root length of cultivars were found very important (Table 1). According to Table 2, means of root length of cultivars were differed. F<sub>1</sub> hybrid has development very long roots than Stoneville 468 and Askabat 100. While F<sub>1</sub> hybrids getting 17.22 cm root length, the Askabat 100 and Stoneville 468 cultivars had 12.89 cm and 9.89 cm root length, respectively. Root length is very important to reach water and nutrients in the soil. Furthermore, the number of roots is also important in terms of the plants holding in the soil and resisting wind effects.

### 3.5. Shoot Number Per Plant

In the study, where the average number of shoots varied between 4.0 and 5.33 (Table 2), and difference among genotypes was found to be significant (Table 1). While the F<sub>1</sub> hybrid is produce 5.33 shoot, Askabat 100 and Stoneville 468 have produced 4.66 and 4.00 shoots,

respectively. The high numbers of shoots were found important in terms of photosynthesis. Özyiğit [2] reported that shoot development was maximum 74.2 % in cotyledon nodes.

### 3.6. Shoot Length per Plant

Plant shoot lengths of genotypes were found to be significant (Table 1). The F<sub>1</sub> hybrid had the longest shoots, followed by Askabat 100 and Stoneville 468. This situation may have occurred due to genetic difference. Shoot length of F<sub>1</sub> cross was 16.77 cm, while shoot length of Askabat 100 and Stoneville 468 were 11.10 cm and 7.11 cm, respectively. Plant shoot length was found to be related to shoot number. Those with more plant shoots formed shorter shoots.

### 3.7. Hybrid Vigor (Heterosis and Heterobeltiosis)

The hybrid vigor of the F<sub>1</sub> hybrid for the traits examined is monitored from Figure 7. According to Figure 7, high heterosis and heterobeltiosis values were found to be significant. The highest heterosis and heterobeltiosis were observed in plant shoot length. It was followed by plant root number and plant root length. The importance of hybrid power of root number and length was found to be important in terms of clone production in cotton plant. The F<sub>1</sub> hybrid produced 50% more shoots, 38.75% more root count, 32.50% more root length and 10.18% more clones than its superior parent.

#### 4. CONCLUSIONS

Cotton plant is produced with seeds. Lots of studies have shown that cotton can also be produced by vegetative propagation methods. While cotton is propagated by grafting method [1, 6-7], Özyiğit [2] produced vegetative from internodes under in vitro conditions. In addition, Karaca et al. [5] had been successful in seedling with grafting. In addition, Karaca et al. [5] had been successful in seedling with grafting. In this study, another vegetative reproduction method has been experiment, which rooting. At the end of study, it was determined that not only cultivars belonging to *G. hirsutum* L but also *G. barbadense* L with their F1 hybrid had been produced by rooting. Moreover, F1 hybrid was found more important than their parents in term of investigated properties, showed more important heterotic effects (heterosis and heterobeltiosis). This may be due to heterozygous genetic makeup of the F1 hybrid. The results of study showed that rooting can be done on cotton in advanced plant form. On the other hand, the fact that F1 hybrid is superior to its parents in terms of root number and root length has shown that F1 crosses can be used as rootstock for clone production. Thus, quality seeds will be provided to farmers by producing clones of productive and high quality varieties on clone rootstock.

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## Çift Silindirli Su Soğutmalı Bir Motorun Biyel Kolu Yapısal Analizi

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**Anahtar Kelimeler**  
 Biyel kolu,  
 Dizel motor,  
 Yapısal analiz,  
 Ansys

**Öz:** Biyel kolu, taşıtlarda pistonun doğrusal hareketini krank milinin dairesel hareketine iletmek için kullanılır. Bu hareket iletimi sırasında çeşitli gerilme türlerine maruz kalan biyel kolunun malzeme seçimi ve tasarımı, biyel kolunun mukavemet değerlerini etkileyen önemli faktörlerdendir. Bu çalışmada, çift silindirli dizel bir motor olan TR-ERB modelinin biyel kolu için yapısal analiz testleri yapılmış ve bu modele alternatif bir tasarım geliştirilmiştir. İlk aşamada TR-ERB modeli için beş farklı malzeme üzerinden analizler gerçekleştirilmiş ve daha sonra en uygun bulunan malzeme için alternatif model tasarımı üzerinden analizler tekrarlanmıştır. Yapısal analiz sonucunda en iyi sonucu veren malzeme 42CrMo4 olup gerilme değeri 425 MPa'dır. Malzemedeki deformasyon değerleri incelendiğinde ise en az deformasyon değeri 42CrMo4 malzemesinde olup miktarı 0,0609'dır. Maksimum deformasyon değeri dökme demir malzemesinde 0,107'dir. Eşdeğer elastik gerilim miktarında en iyi değer AISI1050 malzemesinde elde edilmiştir.

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## Structural Analysis of a Two-cylinder, Water-Cooled Engine

**Keywords**  
 Connecting rod,  
 Diesel engine,  
 Structural analysis,  
 Ansys

**Abstract:** The connecting rod is used in vehicles to transmit the linear motion of the piston rod to the circular motion of the crankshaft. The selection and design of the connecting rod, which is subjected to various types of tension in this movement transfer, is one of the important factors affecting the strength of the connecting rod. With this study, analysis tests of the connecting rod of the TR-ERB model, which is a twin cylinder diesel engine, have been made and then an alternative design has been made for this model. In the first stage, analyses were made on five different materials for the TR-ERB model, then the analyses were repeated over the alternative model design for the material with the highest strength. As a result of the structural analysis, the material that gives the best result is 42CrMo4 and its stress value is 425 MPa. In terms of deformation values in the material, the least deformation value is in 42CrMo4 material and its amount is 0.0609. Its value at maximum deformation is 0.107 in cast iron material. The best value was obtained for the AISI1050 material in the equivalent amount of elastic tension.

### 1. GİRİŞ

Motor teknolojisinin gelişmesindeki en büyük etken şüphesiz ki Otto ve Dizel çevrimlerinin bulunmasıdır. Dizel motorları, silindir içerisine alınan havanın sıkıştırılıp, sıcaklığı ve basıncı artan yanma odası içerisindeki havanın üzerine yakıt püskürtülmesi ve yakıtın alev alması ve patlaması prensibiyle çalışır [1]. Yanma odasına gelen hava ve yakıtın yakılabilmesi için pistonun doğrusal bir ileri geri hareketi yapması gerekmektedir. Biyel kolunun motordaki ana fonksiyonu ise pistonun aldığı doğrusal hareketi krank miline dairesel hareket olarak

iletme [2]. Biyel kolu bu hareketi sırasında; piston başındaki gaz basıncından kaynaklı basma yüküne, piston hızındaki değişiklikler nedeniyle karşılıklı çekme ve basma kuvvetlerine, salınım hareketinden dolayı biyel gövdesinde eğilmeye ve büyük basma kuvvetlerinden dolayı burkulmaya maruz kalır. Biyel kolu eksenel çekme ve basma yüklerine ek olarak çok yönlü genlikli eğilme yüklemeleri, atalet kuvvetleri ve burkulma yüklerine de maruz kalır. Motorun normal çalışması durumunda 2000-3000 devir yapan biyel kolunun motor içerisinde hareket ederken oluşacak gerilmelere karşı da yeterince dayanıklı olması gerekmektedir [3].

İçten yanmalı motorlarda, motor parçalarının tasarım ve hesaplamalarında sonlu elemanlar metodu kullanılmaktadır. Sonlu elemanlar metodu, karmaşık mühendislik ve matematiksel modellerde kullanılan bir sayısal analiz yöntemidir [4]. İlk olarak uçak gövdelerinin analizlerinde kullanılan bu metot daha sonra yaygınlaşarak mühendislik problemleri ve uygulamaları için de kullanılmaya başlanmıştır. Sonlu elemanlar yöntemi özellikle kütle aktarımı, yapı statığı, ısı aktarımı ve elektriksel potansiyel problemlerinde kullanılır [5]. Sistem analiz edilmesi gereken parçayı sonlu eleman denilen küçük parçalara ayırarak problemin çözüleceği uzayı ayırıştırır [6]. Sınır değerlerinin bu formülasyonu sonucu cebirsel bir denklem sistemi elde edilmektedir. Bu denklemler daha sonra çeşitli metotlarla çözülmektedir [7].

Literatüre baktığımızda; Ali ve ark., biyel kolu üzerine farklı miktarda değişken yükler uygulanmasını etkilerini sonlu elemanlar yöntemiyle incelemişlerdir. Analiz sonuçlarını biyel kolu üzerindeki basma ve çekme yük dağılımlarını göre yapmışlardır [8]. Mohankumar ve ark., Ansys programında biyel koluna gelen yanma basıncının oluşturduğu kuvvetleri analiz etmişlerdir. Çalışmalarında, farklı malzemelerden yapılmış biyel kolunun analizleri yapılmıştır. Analizde emniyet faktörü, yorulma analizi ve deformasyon miktarları irdelenmiştir [9]. Al Hasan, Biyel kolu ve pimleri üzerine etkileyen kuvvetlerin analizi sonlu elemanlar yöntemi ile gerçekleştirmiştir. Çalışma sonucunda; biyel kolunun küçük ve büyük başında farklı malzemeler ile takviye edilmesi ile olumlu sonuçlar elde etmiştir [10]. Shenoy, deneysel çalışma sonucunda elde ettikleri yanma basıncına göre biyel kolu ve pimleri üzerine etkileyen kuvvetlerin etkilerini sonlu elemanlar analizi yapmıştır. Analizler iki aşamada gerçekleşmiştir. İlk bölümde statik yük altında FEA ve quasi-dinamik yük altında FEA analizleridir. Analizler sonucunda; biyel kolu üzerine etkileyen kuvvetler belirlenmiştir. İkinci kısımda ise biyel kolunun yorulma ömrü incelenmiştir. Sonuçta ise eC-70 çeliği diğer malzemeye göre %10 daha hafiftir [11]. Dale, toz metal teknolojisi, dövme ve dökme yönetimine göre imal edilen biyel kolunun mekanik analizi yapmıştır. Çalışma sonucunda; dövme yöntemi döküm yöntemine göre %25-33 daha fazla yorulma dayanımına sahip olduğunu saptamıştır. Malzeme özellikleri ve imalat tekniğinin biyel optimizasyonunda önemli olduğunu belirlemiştir [12]. Webster ve ark., biyel kolunun sonlu elemanlar yöntemi ile analiz yapmışlardır. Biyel kolunun üzerine etkileyen kuvvet miktarı deneysel çalışma ile belirlemişlerdir. Biyelin büyük ve küçük başlarındaki etkileyen kuvvet dağılımlarını incelemişlerdir [13].

Literatürdeki çalışmalar incelendiğinde, farklı malzemelerden üretilmiş ve farklı yükler altındaki biyel kolunun yapısal analizi incelenmiştir. Bu çalışmada ise farklı malzeme ve yüklere ek olarak, yeni bir biyel tasarımı yapılmıştır. Bu çalışmada; çift silindri dizel bir motor olan TR-ERB modelinin biyel kolu için yapısal analiz testleri yapılmış ve bu modele alternatif bir tasarım geliştirilmiştir. İlk aşamada TR-ERB modeli için beş farklı malzeme üzerinden analizler gerçekleştirilmiş ve daha

sonra en uygun bulunan malzeme için alternatif model tasarımı üzerinden analizler tekrarlanmıştır.

## 2. MATERYAL VE METOT

Biyel kolunun yapısal analizlerinin yapılabilmesi için öncelikle bir tasarım modeli belirlenmiştir. Bu çalışma kapsamında TR-ERB modeli biyel kolu ana tasarım modeli olarak kabul edilmiştir. Bu model üzerine beş farklı malzeme tanımı yapılarak, biyel kolunun statik analizleri yapılmıştır. Yapılan analiz sonuçlarına göre bu malzemeler içerisinde biyel kolu için en uygun olan malzeme belirlenmiştir.

### 2.1. Malzemeler

Piston ile krank milini birbirine bağlayan biyel kolu, piston kuvvetini kranka ileterek krankın dönüşünü sağlar. Bu hareket sırasında biyel kolu basma, çekme ve burkulma kuvvetlerine maruz kalır. Biyel kolu malzemesi seçilirken de tüm bu kuvvetler göz önüne alınmalı ve bu zorlamalara karşı dirençli malzemeler seçilmelidir. Bu özellikleri karşılaması amacıyla biyel kolu için aşağıdaki malzemelerin analizler yapılmıştır.

- 42CrMo4
- C70S6 Çelik
- 36MnVS4
- AISI 1050 Çeliği
- Gri Dökme Demir

Bu malzemelerin biyel kolunun istenilen özelliklerine göre değerlendirilmesini yaparak, biyel kolu için en uygun malzemeyi tanımlayabiliriz [13].

Tablo 1'de analizler için belirlenmiş olan beş farklı malzemenin özellikleri belirlenmiştir. Bu özelliklerin kendi aralarında kıyaslamaları yapılmıştır. En iyi sonuç AISI 4140 çeliği hem mukavemet değerleri açısından hem de maliyet açısından diğer malzemelere göre daha uygun bulunmuştur. Ancak değerlendirme için sadece bu tablo baz alınmamış ve analizlerden elde edilen sonuçlara da bakılmıştır.

**Tablo 1.** Analizi Yaptırılacak Malzemelerin Özellikleri Tablosu [15,16]

Özellik/ Malzeme	42CrMo4	C70S6 Çelik	36MnVS4	AISI 1050 Çeliği	Gri Dökme Demir
Yoğunluk	7850	7817	7800	7850	7200
Çekme-Akma Dayanımı	750 MPa	550 MPa	742 MPa	580 MPa	551.49 MPa
Basma-Akma Dayanımı	750 MPa	550 MPa	742 MPa	580 MPa	551.49 MPa
Çekme-Kopma Dayanımı	1110 MPa	900 MPa	998 MPa	460 MPa	861.7 MPa
Birim Hacim Fiyatı	0,639 Usd /kg	0,8 Usd/kg	0,9 Usd/kg	0,438 Usd/kg	0,648 Usd/kg

### 2.2. Mühendislik Hesapları ve Analizler

Biyel kolu tasarımında referans alınan TR-ERB markalı biyel kolunun ölçüleri baz alınarak Solidworks üzerinden tasarım tamamlanmıştır. Solidworks üzerinden modellenmiş olan biyel kolları ANSYS programına aktararak malzeme bilgileri ve uygulanan kuvvetler programda tanımlanarak statik analizleri yaptırılmıştır.

Analizler biyel kolunun maksimum yük ve basınç altındaki durumu yani 20°'lik konumu baz alınarak yapılmıştır.

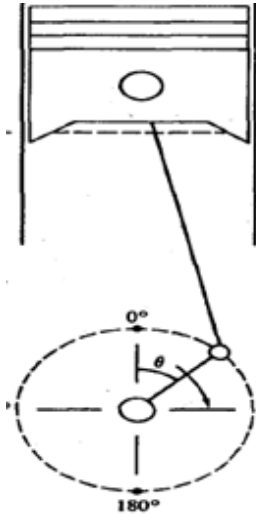
Bu çalışmada kullanılan dizel motorun maksimum basıncı katalog değerlerinden elde edilmiştir. Silindir içi basınç değeri 2,26 MPa' dır. Bu basınçtan yola çıkarak kuvvet hesabı yapılırsa;

$$\begin{aligned} \text{Piston Çapı} &= 77 \text{ mm} \\ F &= P * A = 2,26 * (\pi * 38,5^2) \\ F &= 10518,64 \text{ N} \end{aligned} \quad (1)$$

olarak bulunur. Pistona dik olarak etkiyen bu kuvvet biyel koluna 20° 'lik açı ile vurduğundan biyel üzerinden işlem yaparken bu kuvvetin bileşenleri dikkate alınır (Şekil 1).

$$\begin{aligned} F &= F^1 * \text{Cos}20 \\ 10518,64 &= F^1 * \text{Cos}20 \\ F^1 &= 11194,80 \text{ N} \end{aligned} \quad (2)$$

olarak hesaplanır. Bu kuvvet biyel kolu 20°'lik konumdayken biyeye etkiyen dik kuvveti bize gösterir. Biyel kolu için statik analiz yapılırken biyeye etkiyen kuvvet olarak denklem ikiden elde edilen kuvvet esas alınmıştır [17].



Şekil 1. Biyel Kolunun geometrisi

Analizi yaptırılacak biyel kolu Tablo 2'de verilen ölçüler doğrultusunda Solidworks üzerinden Şekil 2'de gözüktüğü gibi modellenmiştir.

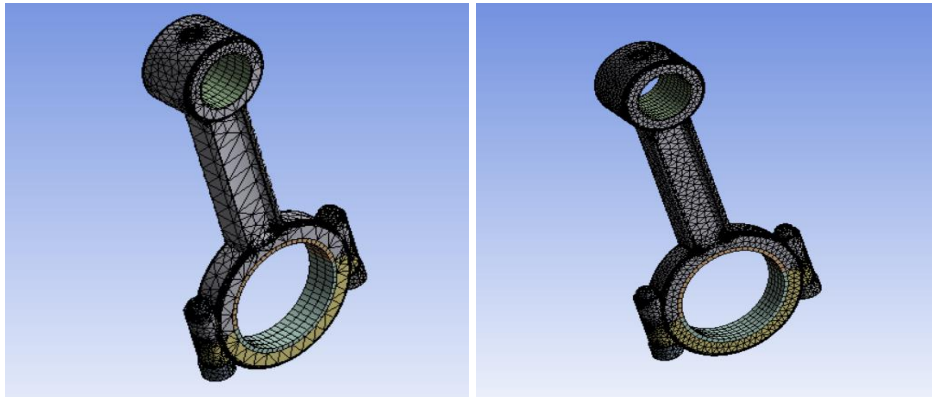
Tablo 2. Biyel Kolu Ölçüleri

Strok	68 mm
Küçük Biyel Başı İç Çapı	20 mm
Küçük Biyel Başı Dış Çapı	30 mm
Büyük Biyel Başı İç Çapı	40 mm
Büyük Biyel Başı Dış Çapı	52 mm
Burç İç Çapı	19 mm
Yatak İç Çapı	37 mm
Yağlama Deliği Çapı	4 mm
Profil Kesiti	16 mm
Profil Derinliği	3,6 mm



Şekil 2. Biyel Kolunun 3D Modellenmesi

Solidworks üzerinden çizilmiş olan model-1 datası Ansys workbench ortamına alınmış ve gerekli analizlerin yapılabilmesi için ağ modeli (mesh) örülmüştür. Programda default olarak tanımlı mesh modeli kullanıldığı zaman biyel kolu üzerine 7803 nodes ve 4242 eleman tanımlanmaktadır. Ancak biyel kolu gibi karmaşık bir geometri için bu düğüm ve eleman sayısı oldukça yetersizdir. Bu nedenle mesh modeli oluşturulurken "default mesh" modülü yerine "fine mesh" modülü kullanılmıştır. Bu modül üzerinde de iyileştirmeler yapılarak toplamda 555088 düğüm ve 390245 eleman elde edilmiştir. Bu eleman sayısı analizler için yeterli bulunmuştur.



Şekil 3. Model-1'in Default ve Fine Olarak Tanımlı Mesh Görünümleri



Biyel kolunun statik analizini yaptırılırken, biyelin krank miline bağlandığı yataklardan model sabitlenmiştir. Biyel kolu 20°'lik konumundayken yanma basıncı maksimumu durumda olduğu bilindiği için pistonu etkileyen kuvvetin düşey bileşeni hesaplanmış ve bu kuvvet biyele etki ettirilerek analiz yaptırılmıştır. Kuvvetin etki noktası biyel kolunun pistonu bağlandığı gajın piminin bulunduğu küçük biyel başı olarak seçilmiştir. Böylece biyel kolu dik konumdaymış gibi kabul edilerek analizler bu konumda yaptırılmıştır (Şekil 3).

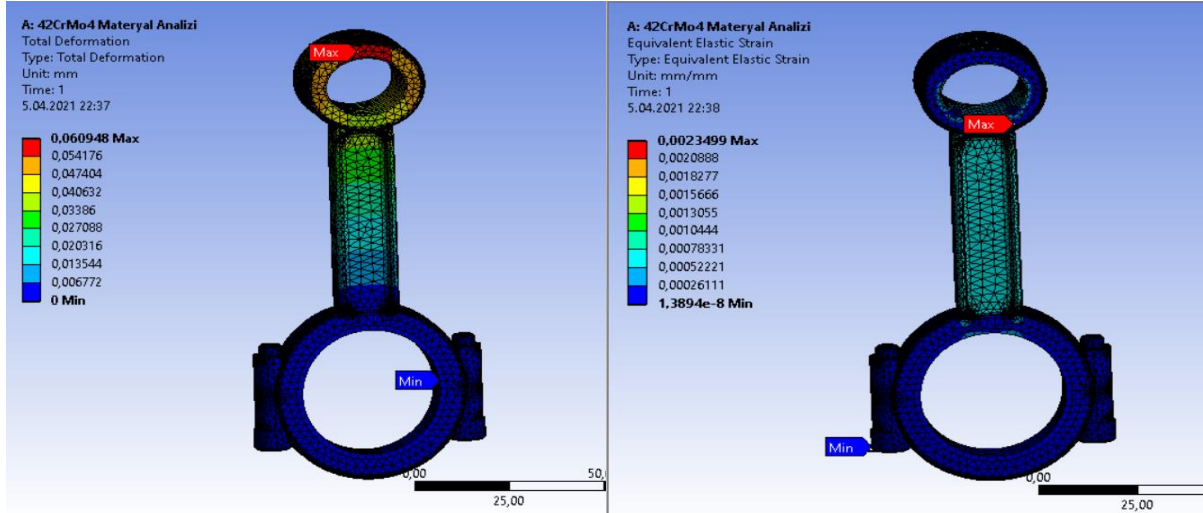
### 2.2.1. 42CrMo4 Malzemesi Analizleri

Model-1'de biyel malzemesi olarak 42CrMo4 çelik, yataklar için AISI 4340 tavlı çelik ve burç için 1023 karbon çeliği kullanılmıştır. Yatak ve burçların sürtünme etkisine karşı daha dayanıklı olması gerektiğinden sert malzemelerden seçilmiştir. Seçilen malzemelerin özellikleri Solidworks üzerinden belirlenip Ansys programına aktarılmıştır. Daha sonra her bir parça üzerine kendi malzemesinin tanımlaması

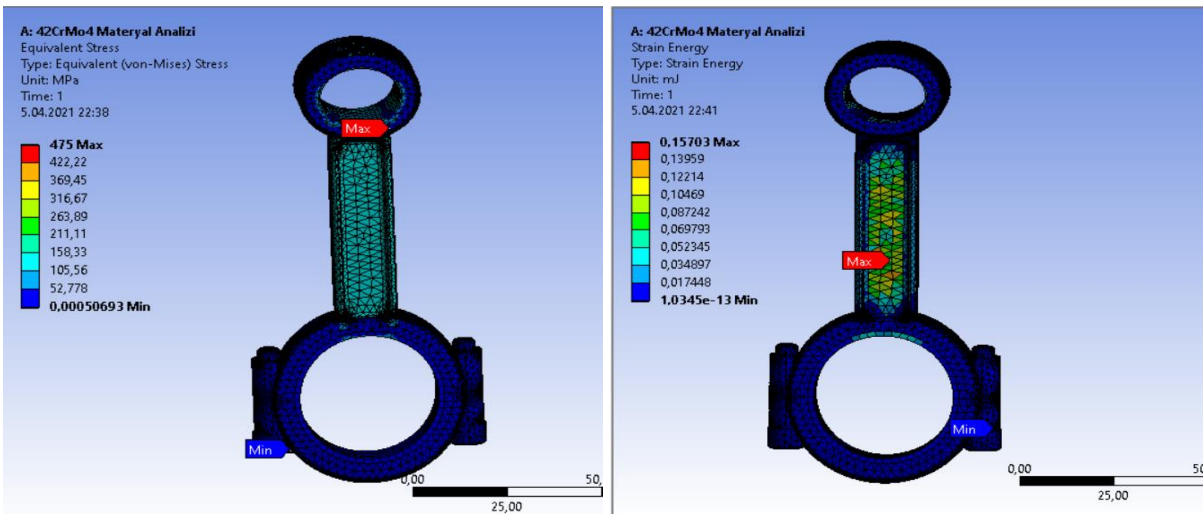
yapılmıştır. Analiz sonuçları Şekil 4 ve Şekil 5'te verilmiştir. Bu sonuçlara göre maksimum deformasyon bölgesi biyelin üst yağlama deliği ve çevresinde bulunmuştur. Maksimum eşdeğer gerilme ise biyel küçük başı ve kol uzunluğu arasında meydana gelmiştir.

### 2.2.2. C70S6 Çeliği Analizleri

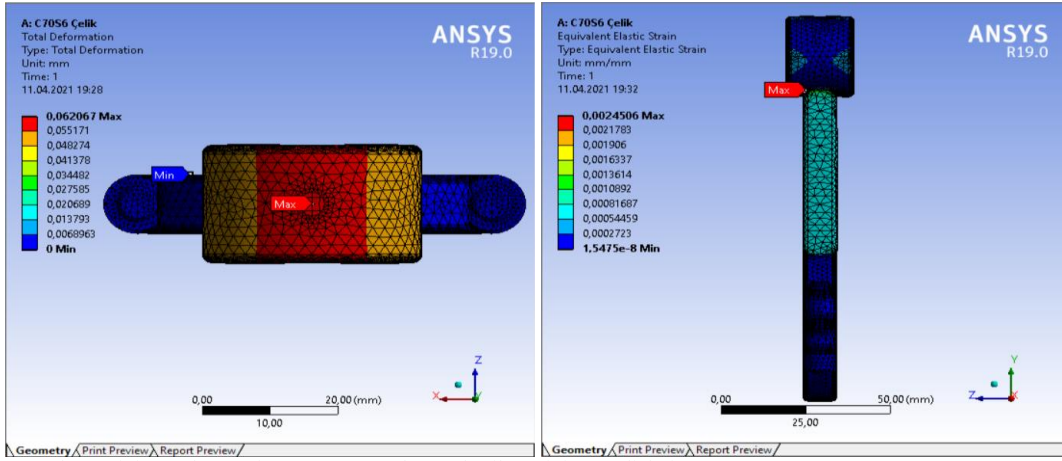
Analizlerde kullanılacak olan ikinci model için C70S6 çeliği kullanılmıştır. Yataklar için AISI 4340 tavlı çelik ve burç için 1023 karbon çeliği kullanılmıştır. Malzemelerin mekanik özellikleri Ansys üzerine aktarılmış ve model üzerinde gerekli tanımlamalar yapılmıştır. Malzeme özellikleri ve tanımlamalar yapıldıktan sonra parça model-1'de olduğu gibi alt başından sabitleniş ve biyelin küçük başından kuvvet etki ettirilmiştir. Analiz sonuçları Şekil 6 ve Şekil 7'de verilmiştir. Bu sonuçlara göre maksimum deformasyon bölgesi biyelin üst yağlama deliği ve çevresinde bulunmuştur. Maksimum eşdeğer gerilme ise biyel küçük başında meydana gelmiştir. Maksimum gerilme enerjisi biyel kol uzunluğunda görülmüştür.



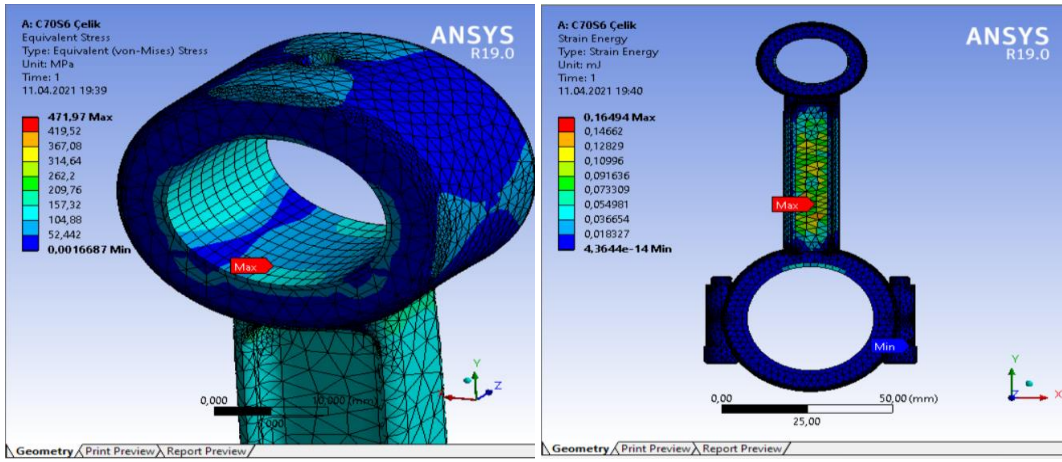
Şekil 4. 42CrMo4 Malzemesinin Toplam Deformasyon ve Eşdeğer Elastik Gerilme Analizleri



Şekil 5. 42CrMo4 Malzemesinin Eşdeğer Gerilme ve Gerilme Enerjisi Analizleri



Şekil 6. C70S6 Malzemesinin Toplam Deformasyon ve Eşdeğer Elastik Gerinim Analizleri

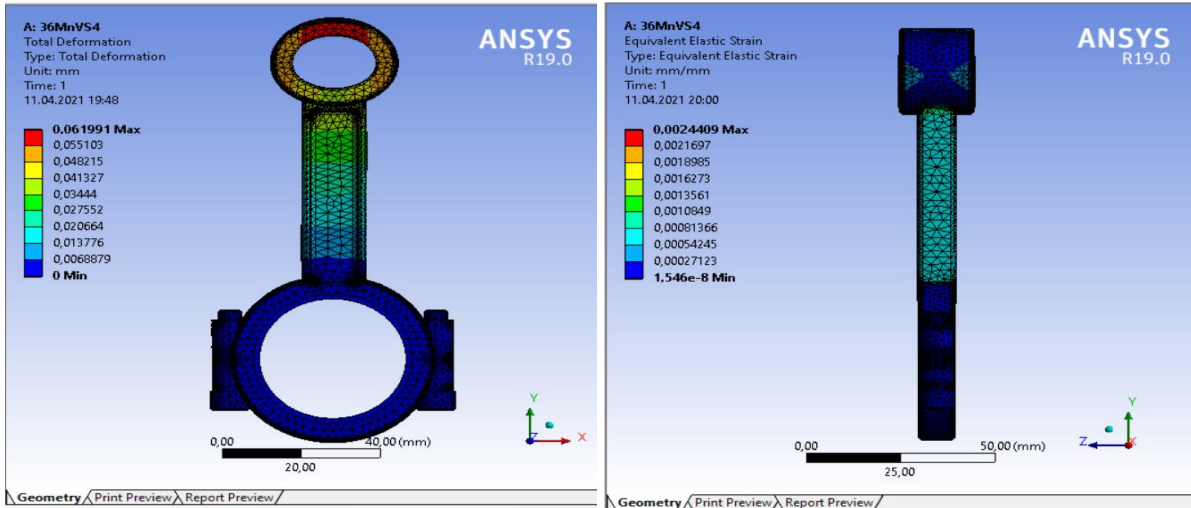


Şekil 7. 4C70S6 Malzemesinin Eşdeğer Gerilim ve Gerilme Enerjisi Analizleri

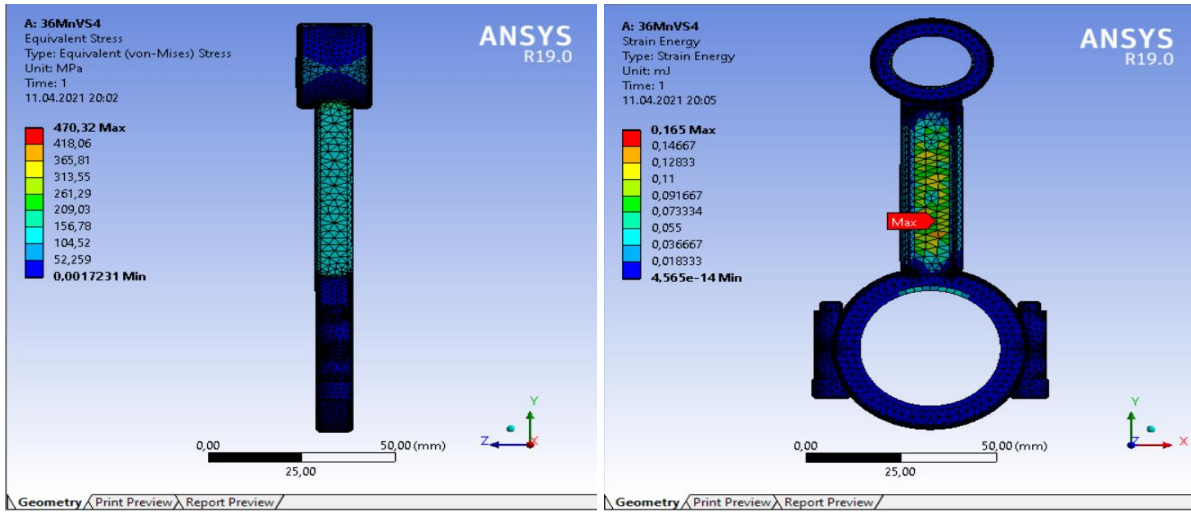
### 2.2.3 36MnVS4 Malzemesi Analizleri

Model III malzemesi olarak 36MnVS4 malzemesi seçilmiştir. Analiz sonuçlarında biyelin ana malzemesinin önemini ayırt edebilmek için biyel burç ve yataklarında tüm modellerde aynı malzemeler kullanılmıştır. Malzemenin özellikleri Ansys ortamına girildikten sonra malzeme tanımlamaları yapılmıştır. Model üzerinde yeniden mesh oluşturulup işlem

yüzeyleri elde edildikten sonra analiz sonucunda görülmek istenen sonuçlar programa tanımlanmıştır. Analiz sonuçları Şekil 8 ve Şekil 9'da gösterilmektedir. Analizler sonucunda maksimum deformasyon biyel üst yağlama deliği ve çevresinde görülmüştür. Maksimum gerilme enerjisi biyel kol uzunluğu bölgesinde görülmektedir.



Şekil 8. 36MnVS4 Malzemesinin Toplam Deformasyon ve Eşdeğer Elastik Gerinim Analizleri

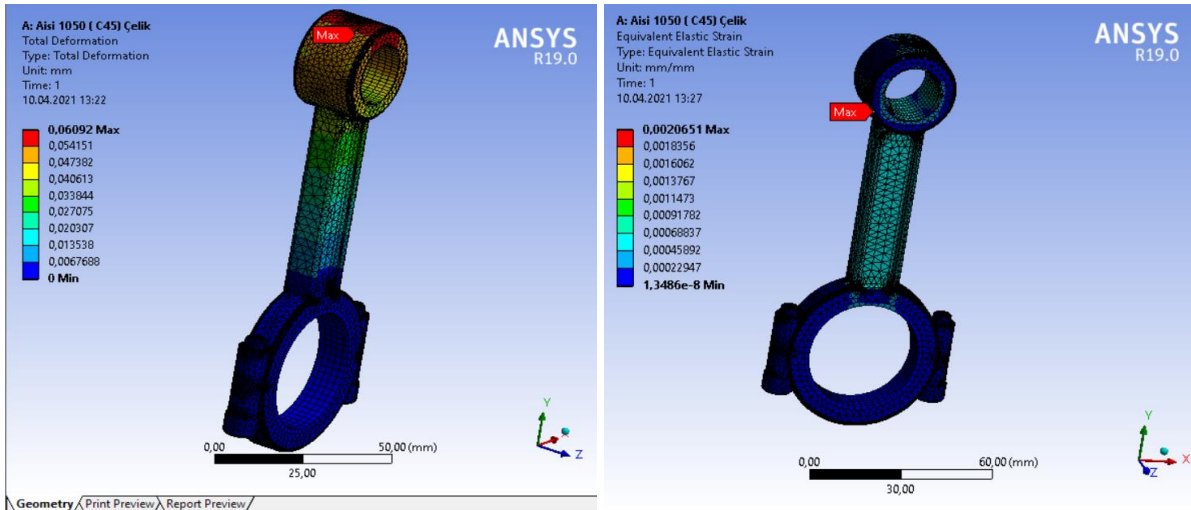


Şekil 9. 36MnVS4 Malzemesinin Eşdeğer Gerilim ve Gerilme Enerjisi Analizleri

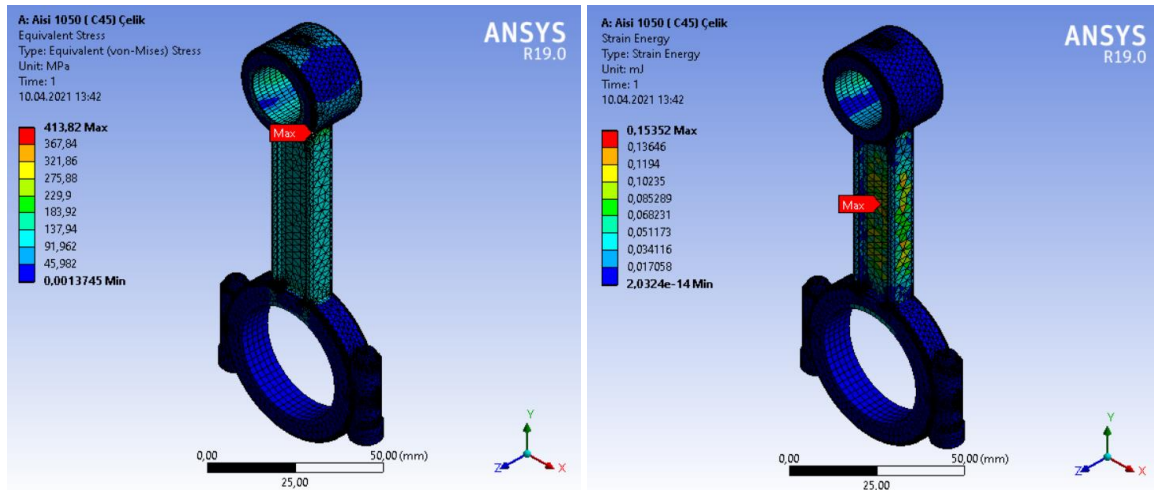
## 2.2.4. AISI 1050 Çeliği Analizleri

Model IV'de ölçüleri tanımlanmış olan biyel kolunun yatak, burç ve cıvata malzemeleri aynı kalırken biyelin malzemesi AISI 1050 çeliği olarak değiştirilmiştir. Ansys ortamına alınan tasarım modeline malzeme atamaları gerçekleştirilmiş ve default olarak tanımlı olan

mesh modeli fine olarak değiştirilmiş ve analizler yapılmıştır. Analizler sonucunda maksimum deformasyon biyel üst yağlama deliği ve çevresinde görülmektedir. Maksimum gerilme değeri ise biyel küçük başının bağlantı noktasında görülmektedir. Analiz sonuçları Şekil 10 ve 11'de görülmektedir.



Şekil 10. AISI 1050 Malzemesinin Toplam Deformasyon ve Eşdeğer Elastik Gerilim Analizleri



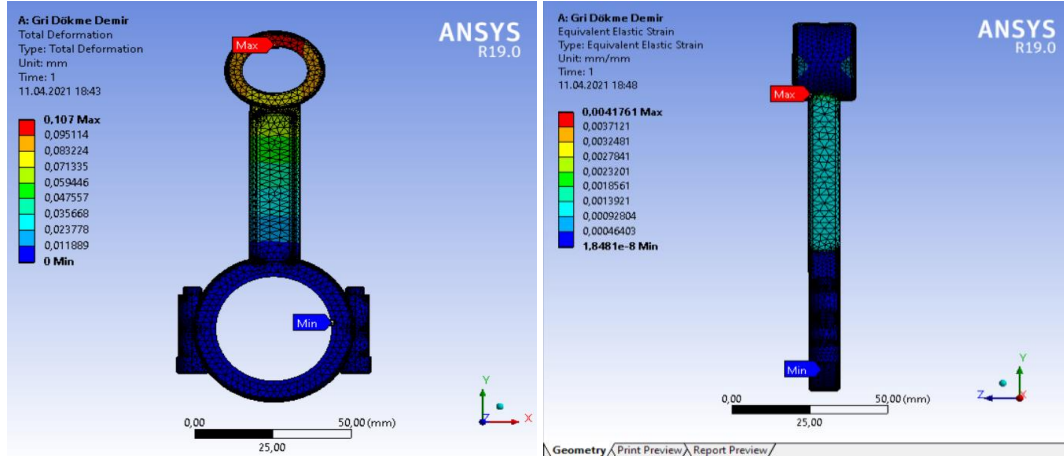
Şekil 11. AISI 1050 Malzemesinin Eşdeğer Gerilim ve Gerilme Enerjisi Analizleri



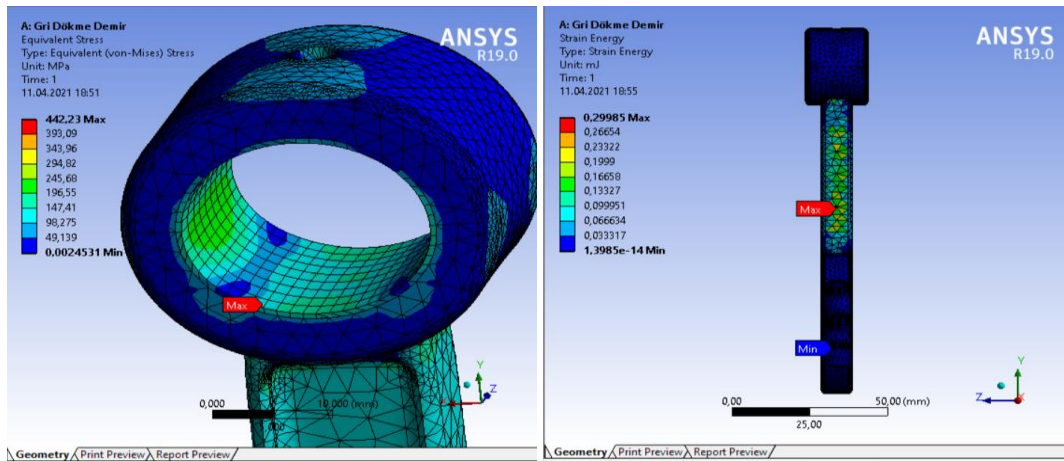
### 2.2.5. Gri Dökme Demir Malzeme Analizleri

Bu model için biyel malzemesi gri dökme demir olarak seçilmiştir. Biyel burç ve yatakları diğer modellerle aynı olacak şekilde yataklar için AISI 4340 tavlı çelik ve burç için 1023 karbon çeliği kullanılmıştır. Analiz sonuçları Şekil 12. ve Şekil 13.'te gösterilmiştir. Analiz

sonuçlarına göre maksimum gerinim biyel küçük başının bağlantı noktasında görülürken maksimum uzama enerjisi biyel kol uzunluğunda görülmüştür. Maksimum deformasyon diğer analizlerde de olduğu gibi biyelin üst yağlama deliği ve çevresinde görülmektedir.



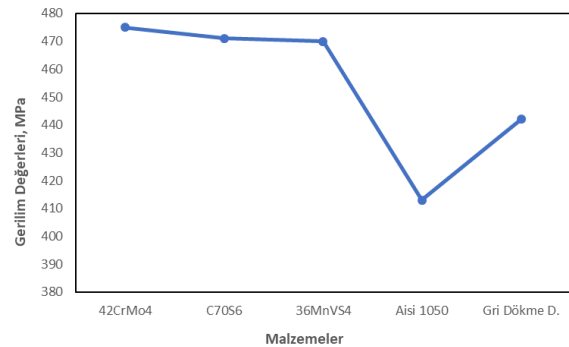
Şekil 12. Gri Dökme Demir Malzemesinin Toplam Deformasyon ve Eşdeğer Elastik Gerinim Analizleri



Şekil 13. Gri Dökme Demir Malzemesinin Eşdeğer Gerilim ve Gerilme Enerjisi Analizleri

### 3. BULGULAR

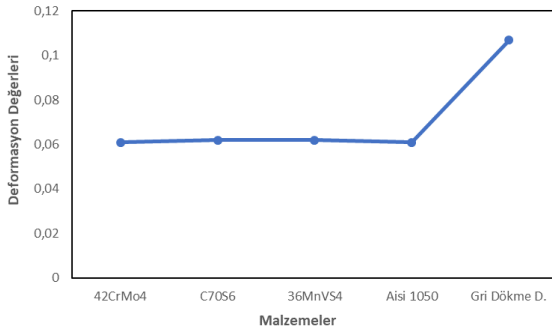
Bu çalışmada, iki silindirik bir dizel motora ait olan biyel kolunu beş farklı malzemesi için statik analizi Ansys programında yapılmıştır. Yapılan bu analiz sonucunda biyel kolu için en uygun biyel kol malzemesi tespit edilmiştir. Çalışma sonucunda tüm malzemeler için maksimum gerilme değerleri hesaplanmıştır. Şekil 14'te farklı malzemeler için analiz sonucunda elde edilmiş maksimum gerilme değerleri görülmektedir. Şekil incelendiğinde maksimum gerilme değeri 42CrMo4 malzemesinde elde edilmiştir. 42CrMo4 malzemesi için gerilme değeri 475 MPa'dır.



Şekil 14. Farklı malzemeler için analiz sonucu elde edilen maksimum gerilme değerleri

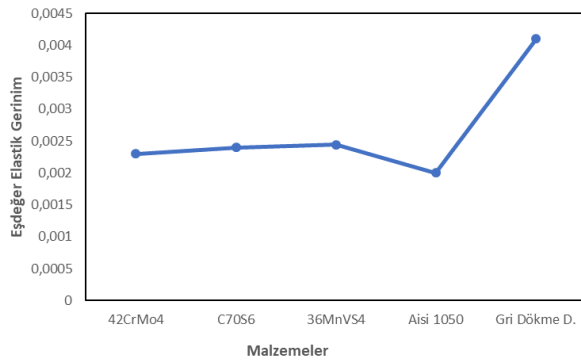
Şekil 15'te beş farklı malzeme için toplam deformasyon değerlerini gösteren grafik verilmiştir. Şekil incelendiğinde en düşük deformasyon değeri 42CrMo4 malzemesinde, en büyük deformasyon değeri ise gri dökme demir malzemesinde elde edilmiştir. Toplam

deformasyon maksimum ve minimum değerleri sırasıyla 0,107 ve 0,0609'dır.



Şekil 15. Farklı malzemeler için analiz sonucu elde edilen toplam deformasyon değerleri

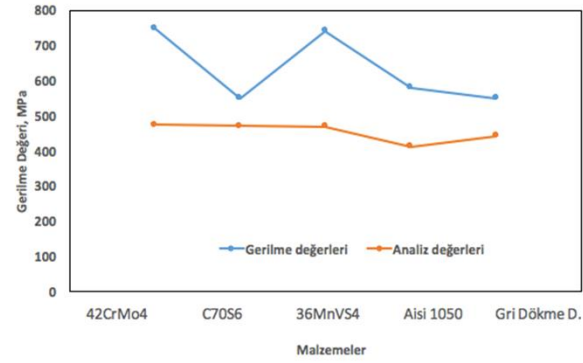
Şekil 16'da beş farklı malzeme için eş değer elastik gerinim değerleri verilmiştir. Şekil incelendiğinde en düşük deformasyon değeri AISI1050 malzemesinde, en büyük deformasyon değeri ise gri dökme demir malzemesinde elde edilmiştir. Toplam deformasyon maksimum ve minimum değerleri sırasıyla 0,0041 ve 0,002'dir.



Şekil 16. Farklı malzemeler için analiz sonucu elde edilen eş değer elastik gerinim

Çalışma sonucunda elde edilen beş farklı malzeme maksimum gerilme değerleri malzemelerin çekme değerlerine göre karşılaştırılmıştır. Tüm malzemelerin çekme değerleri analiz sonuçları ile elde edilen gerilme değerlerinden daha yüksektir (Şekil 17). Bu sonuçlara göre tüm malzemeler silindir içi basıncın etkilediği değeri taşıyabilmektedir. Fakat motorun farklı çalışma şartları esnasında kuvvetin statik olmaması dinamik kuvvetlerin olmasının biyel koluna gelene gerilme değerlerini yükseltmektedir. Bu nedenle kullanılan malzemeler için bir emniyet katsayısı gerekmektedir. Analizi yapılan malzemelerin emniyet katsayıları; 42CrMo4 çeliği için akma dayanımı 750 MPa ve analizden elde edilen oluşabilecek maksimum gerilme değeri 475 MPa olarak bulunmuştur. Bu durumda malzemenin emniyet katsayısı 1,57 olarak bulunur. C70S6 çeliği için akma dayanımı 742 MPa olarak bulunmuş olup, analizde elde edilen maksimum gerilme değeri 471 MPa'dır. Bu durumda C70S6 çeliği için hesaplanan emniyet katsayısı 1,06 olmaktadır. 36MnVS4 alaşımı için akma dayanımı 742 MPa olarak bulunmuştur. Analizlerden elde edilen maksimum gerilme değeri ise 470 MPa'dır. Bu durumda

malzemenin emniyet katsayısı 1,57 olarak bulunur. AISI 1050 çeliği için akma dayanımı 580 MPa olarak bulunmuştur. Analizlerden elde edilen maksimum gerilme ise 413 MPa'dır. Bu durumda malzemenin emniyet katsayısı 1,40 olarak bulunur. Gri dökme demir için akma gerilmesi 551 MPa olarak bulunmuştur. Malzemenin analiz sonuçlarına göre elde edilen maksimum gerilme ise 442 MPa'dır. Bu durumda elde edilen emniyet katsayısı 1,24'tür.



Şekil 17. Farklı malzemeler için çekme değerleri ve analiz değerlerini karşılaştırılması

Bu durumda gerilme değerleri üzerinden bir karşılaştırma yaparsak; 36MnVS4 alaşımı ve AISI 4140 çeliği emniyet katsayısı en yüksek olan malzeme olarak bulunurken, C70S6 çeliği en düşük emniyet katsayısına sahip malzeme olarak bulunmuştur.

Von-mises gerilme analizlerine göre değerlendirme yapacak olursak 42CrMo4 çeliğinin dayanım değerleri diğer malzemelere oranla daha yüksek çıkmıştır. Gri dökme demir ve AISI 1050 çeliğinin dayanım değerleri 413-442 MPa aralıklarındayken diğer malzemeler 470-475 MPa aralıklarına kadar mukavemetli bulunmaktadır. 42CrMo4 çelik, C70S6 çeliği ve 36MnVS4 çeliğinin değerleri birbirine çok yakın olarak çıkarken; AISI 1050 çeliği değerleri nispeten diğerlerinden daha düşük, gri dökme demirin değerleri ise daha yüksek çıkmıştır. Alaşım çelikleri olan malzemeler birbirlerine benzer özellikler göstermişlerdir.

#### 4. SONUÇLAR

Bu çalışmada, iki silindri bir dizel motora ait olan biyel kolunu beş farklı malzemesi için yapısal analizi Ansys programında yapılmıştır. Çalışma sonucunda biyel kolu için en uygun biyel kol malzemesi tespit edilmiştir. Analiz sonuçları malzemelerin von-mises gerilme değerleri ve sahip oldukları emniyet katsayılarına göre değerlendirilmiştir. Bu sonuçlara göre 42CrMo4 çeliği biyel tasarımımız için en uygun malzeme olarak bulunmuştur. Analiz sonuçlarına göre maksimum gerilme değeri 475 MPa ve emniyet katsayısı ise 1,57 olarak tespit edilmiştir. Biyelin üst kısmında bulunan yağlama deliği ve çevresi tüm analizlerde maksimum deformasyona uğrayan bölge olarak belirlenmiştir. Yağlama deliğinin varlığı biyel başının mukavemet açısından değer kaybetmesine neden olmuştur. Analizler biyel kolunun alt yataklarından sabit olarak



düşünülp yapılmasından dolayı biyelin kranka bağlandığı alt yataklar tüm analizlerde mukavemetli çıkmıştır. Daha sağlıklı bir analiz çalışması yürütmek için biyele dinamik analiz yaptırılıp, çeşitli yük şartları altında elde edilen mukavemet değerleri incelenerek daha verimli sonuçlar elde edilebilir.

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## Genetic Diversity Analysis of Some Upland Cotton (*Gossypium hirsutum* L.) Genotypes Using SSR Markers

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### Keywords

Genetic diversity, MAS, PIC, SSR, Upland cotton

**Abstract:** Cotton plant is an important crop cultivated under biotic and abiotic stress conditions worldwide. The best way to avoid the harmful effects of chemicals used to combat these stresses is to develop tolerant or resistant varieties in plant breeding programs. In the present study, some of Upland cotton varieties were screened with 20 polymorphic SSR primers, and their population structure and genetic diversity analysis were examined. 17 SSR primers amplified 99 alleles with a 5.82 allele per locus. The mean PIC value of the markers was 0.312. The highest PIC value (0.491) belongs to the Nau3736 SSR marker while Bnl1611 and Bnl3449 markers had the lowest PIC value (0.105). The Genetic Distance (GD) values of the markers varied between 0.26 and 1.09. The highest GD values were between Sure Grow 96 and Carmen, Sealand-542 and Siokra ¼, and between Sphinx V and Stoneville-453 cultivars. As a result, the genetically distant cultivars (Acala maxx, Carmen, Aleppo 40, Siokra ¼, and Tex) can be recommended to use as parents in Marker-assisted selection (MAS) technology to develop new cotton varieties which are resistant or tolerant to stress factors.

## SSR Markörleri Kullanılarak Bazı Upland Pamuk (*Gossypium hirsutum* L.) Genotiplerinin Genetik Çeşitlilik Analizlerinin Yapılması

### Anahtar Kelimeler

Genetik çeşitlilik, MAS, PIC, SSR, Upland pamuk

**Öz:** Pamuk bitkisi dünya genelinde biyotik ve abiyotik stres koşullarında yetiştirilen çok önemli bir tarla bitkisi. Bu stres koşullarıyla mücadelede kimyasal ilaçlarının olumsuz etkilerinden kaçınmanın en etkili yolu, bitki ıslah metodlarıyla biyotik ve abiyotik stres faktörlerine dayanıklı/tolerant yeni bitki çeşitlerini geliştirmektir. Bu çalışmamızda bazı upland pamuk genotipleri 20 adet polimorfik SSR primerleriyle taranmış, populasyon yapıları ve genetik çeşitlilik analizleri yapılmıştır. 17 SSR primeri lokus başına 5.82 allel olmak üzere totalde 99 allel üretmiştir. Markörlerin ortalama PIC değerleri 0.312 olmuştur. En yüksek PIC değeri (0.491)'ni Nau3736 SSR marköründe elde edilirken, en düşük PIC değeri (0.105) Bnl1611, Bnl3449 markörlerinde elde edilmiştir. Markörlerin genetik mesafe değerleri 0.26 ile 1.09 arasında değişmiştir. En yüksek genetik mesafe Sure Grow 96 ve Carmen, Sealand-542 ve Siokra ¼ ile Sphinx V ve Stoneville-453 arasında ölçülmüştür. Sonuç olarak, genetik olarak birbirinden uzak olan çeşitler (Acala maxx, Carmen, Aleppo 40, Siokra ¼, and Tex), stres faktörlerine karşı dirençli çeşit geliştirme teknolojisi olan Markör Destekli Seleksiyon (MAS) ıslah programına ebeveyn olarak kullanılabilirliği güçlü bir şekilde önerilmektedir.

### 1. INTRODUCTION

Cotton (*Gossypium spp.*) is an important crop as a source of natural fiber and oil. It covers 35% of the fiber used worldwide. There are about 50 species of cotton, and 45 of these are known to be diploids ( $2n = 2x = 26$ ), and 5 are allotetraploid ( $2n = 4x = 52$ ) [1]. The most common diploid ones are *G. arboreum* L. and *G. herbaceum* L. with

AA ( $2n = 2x = 26$ ) genome group, and the tetraploid ones are *G. hirsutum* L. and *G. barbadense* L. with AADD ( $2n = 4x = 52$ ) genome group [2].

Approximately there are 20 diseases, pests, and harmful stress factors that reduce the yield, quality, and restrict cotton cultivation worldwide. Particularly, more than 41% of the yield loss is due to biotic factors such as insects (15%), weeds (13%), and other harmful pathogens [3]. To

combat such stress factors, Cook. [4]. has proposed four possibilities: (i) breeding new tolerant or resistant varieties, (ii) developing a healthy root environment, (iii), increasing the quality of water, and (iv) protecting the plants from airborne threats. Developing tolerant/resistant genotypes against biotic and abiotic stress conditions are more environmentally safe compared to chemicals that pollute the atmosphere.

Corresponding to recent climate changes, the identification of desired alleles at QTL underlying tolerance/resistance to biotic and abiotic stresses is a primary breeding strategy for improving crop productivity and production under stress conditions [2;5]. The strategy involves germplasm screening, QTL mapping, and the development of DNA markers linked to QTL. Marker-assisted selection (MAS) in breeding programs has been applied to introduce the desired alleles into the genetic backgrounds of elite varieties [6].

Fundamental studies on quantitative traits associated with stress tolerance/resistance are necessarily required to apply marker-assisted selection to practical crop breeding. On the other hand, diversity studies may contribute to screening desired genotypes as parents for crossing but cannot directly contribute to breeding through marker-assisted selection. When a broad range of diverse germplasm and genome-wide DNA markers are used for the analysis, genome-wide association mapping using genotypic and phenotypic data is applicable to identify chromosomal regions involving QTL/genes conferring target traits [7; 8; 5; 9].

Genetic diversity comes from the allelic variation in the genome (insertion, deletions in DNA) and constitutes the basis of Marker-assisted breeding. Genetic diversity and the selection of parents play important roles in terms of the level of variation, and heterosis (hybrid vigor), hybrid strength, molecular breeding, and in obtaining cotton genotypes tolerant or resistant to diseases and pests, early with high efficiency. To achieve this, molecular markers that measure the genetic diversity at the DNA level must be employed [5; 9]. DNA markers can be classified as sequence-based (SNP, Single Nucleotide polymorphism), hybridization-based (RFLP, Restriction Fragment Length Polymorphism), and PCR-based markers (RAPD, Randomly Amplified Polymorphic DNA; AFLP, Amplified Fragment Length Polymorphism; SSR, Simple Sequences Repeats) [10]. Genetic markers are used for predicting the genetic diversity in wild and designed populations [11; 12; 13], Quantitative Trait Loci (QTL) and association mapping [7; 8; 14; 15; 16], pedigree analyses [17], heterotic group classification [18; 19], and for the protection of genotype rights [20].

The Simple Sequence Repeat (SSR) (Microsatellites or Short tandem repeat) molecular markers were used in the genetic diversity analyses because they have a high ability to show genetic differences between cotton genotypes, they are present in all eukaryotic cells, show uniform distribution throughout the genome, provide the opportunity to determine genetic diversity, are repeatable, allow working on low DNA samples amount, are cheap

and co-dominant, and give reliable results. SSR markers are in 1-4 to 1-6 nucleotide length [21; 22; 23; 24; 25]. Bertini et al. [26] reported that they obtained 66 alleles in total, with an average of 2.13 alleles per locus in her genetic diversity with 53 cotton varieties using 31 SSR markers. Liu et al. [27] reported that they obtained 165 polymorphic DNA fragments in a study using SSR markers on Asian cotton (*Gossypium arboreum* L.), and the genetic similarities of the accessions were between 0.58 and 0.87. The development of new varieties against biotic and abiotic stress factors increased the quality, germination, emergence, seedling, growing, and yield of cotton [28; 29; 30; 31].

Some important parameters must be known about the individuals that will be used as parents in developing new varieties with the breeding method. Genetic differences and the degree of these differences among the genetic materials that will be used in the breeding program have critical importance in variety breeding. Islam et al. [32] reported that a high rate of stability was observed in yield in some regions because of the use of germplasm sources as breeding materials, where genetic diversity is very small. Therefore, genotypes to be used in plant breeding should be analyzed to determine their kinship degrees and those have close kinship degree is not recommended to use in plant breeding program.

The present study was conducted to determine the genetic diversity between upland cotton (*Gossypium hirsutum* L.) germplasm and examine population structure using 20 SSR markers.

## 2. MATERIALS AND METHOD

### 2.1. Plant Materials

A total of seventeen upland (*G. hirsutum* L.) cotton genotypes belong to the AD<sub>1</sub> genome group collected from different countries (The USA, Syria, Turkey, Australia, Albania) used for genetic diversity analysis, in Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Department of Agricultural Biotechnology, in Kahramanmaraş City-Turkey.

### 2.2. DNA Extraction, and Visualization Genomic DNA

From the young leaves, 0.5 g amount picked up at the 3-4-leaf stage, from each genotype with sterile scissors. The samples were then washed with distilled water (dH<sub>2</sub>O) and ethanol and placed in plastic tissue bags in thermally insulated containers with -80°C dry ice during transport to the laboratory and were kept in the -80°C freezer until DNA isolation. The Genomic DNA isolation protocol Centyltrimethylammoniumbromide (CTAB) was developed by Doyle and Doyle. [33] was modified and used in this study.

### 2.3. SSR Amplification

Amplification with PCR and gel electrophoresis stage was performed according to Zhang and Stewart. [34]. The PCR protocol consisted of incubating at 94°C for 5 min, then 34

cycles of at 94°C for 1 min, at 60°C for 1 min and 72°C for 2 min; later at 72°C for 7 min. A reaction volume of 15 µL was used for each PCR process. In PCR reaction mixture, there were 0.75 µL dNTP (Conc.10 mM), 1.5 µL 10X PCR

buffer, 1 µL forward primer, 1 µL reverse primer, 0.5 µL Taq DNA polymerase enzyme (Conc.5 µL, 2 µL template DNA (Conc. 25ng/µL), 8.25 µL ddH<sub>2</sub>O (double-distilled) components [35].

**Table 1.** Information about Simple sequence repeat (SSR) used in this study

Primer Name	Species, Germplasm	Chromosome	Repeat motif	Publication
BNL1079	<i>G. hirsutum</i> L, Deltapine 90	AD_chr.18	(CA)11 (GT)11	Mei et al. [36].
BNL1604	<i>G. hirsutum</i> L, Deltapine 90	AD_chr.7-16	(AG)25	Mei et al. [36].
BNL3449	<i>G. hirsutum</i> L, Deltapine 90	AD_chr.18	(AC)15, (TC)6T (AC)15G(CA)2	Yu et al. [37].
BNL3479	<i>G. hirsutum</i> L, Deltapine 90	AD_chr.18	(AC)15(TC)6T(AC)15 G(CA)2	Yu et al. [37].
BNL2571	<i>G. hirsutum</i> L, Deltapine 90	AD_chr. 13-18	(AG)13, (TC)13	Yu et al. [37].
BNL1611	<i>G. hirsutum</i> L, Deltapine 90	AD_chr.19	(AG)12	Yu et al. [37].
CIR 0141	<i>G. hirsutum</i> L Guazuncho-2	AD_chr.07-16	(TG)30	Nguyen et al.[38].
CIR0253	<i>G. hirsutum</i> L, Guazuncho-2	AD_chr.5-19-22	(TC)15, (N)8(A C)5(N)7(CA)8	Nguyen et al. [38].
CIR0099	<i>G. hirsutum</i> L, Guazuncho-2	AD_chr.18-25	(GT)8	Nguyen et al. [38].
NAU3736	<i>Gossypium raimondii</i>	AD_chr.01-15	(GTA)6	Guo et al. [39].
NAU2714	<i>Gossypium raimondii</i>	AD_chr.6-25	(TTA)7	Guo et al. [39].
NAU4024	<i>Gossypium raimondii</i>	AD_chr.14	(GTC)6	Guo et al. [39].
NAU2761	<i>Gossypium raimondii</i>	AD_chr.02-17	(TTAA)6	Guo et al. [39].
NAU2173	<i>G. hirsutum</i> L, Xuzhou 142	AD_chr.14	AAG (17)	Han et al. [40].
JESPR0065	<i>G. hirsutum</i> L, Tamcot Sphinx	AD_chr.04-12	(GAA)25	Reddy et al. [41].
JESPR0153	<i>G. hirsutum</i> L, Tamcot Sphinx	AD_chr.13,18,20	(CTA)18	Reddy et al. [41].
JESPR0114	<i>G. hirsutum</i> L, Tamcot Sphinx	AD_chr.09-23	(GT)12	Reddy et al. [41].
MGHES16	<i>G. hirsutum</i> L, Acala Maxxa	AD_chr.11-21	(CT)10, (TCT)4	Qureshi et al. [42].
MGHES31	<i>G. hirsutum</i> L, Acala Maxxa	AD_chr.12-26	(CAT)9	Qureshi et al. [42].
NAU2251	<i>G. hirsutum</i> L, Xuzhou 142	AD_chr.12-26	AGA (8)	Han et al. [40].

In table 1., it is indicated the SSR markers properties were obtained from the varieties of *G. hirsutum* L. and *Gossypium raimondii* cotton species. Bnl1079, Bnl1604, Bnl3449, Bnl3479, Bnl2571, Bnl1611 SSR markers were obtained from *G. hirsutum* L. species's Deltapine 90 variety; Cir0141, Cir0253, Cir0099 from Guazuncho-2; Nau2173, Xuzhou 142, Jespr0065, Jespr00654, JesprR00654 from Tamcot Sphinx; Mghes16 and Mghes31 were obtained from Acala Maxxa variety. Nau3736, Nau2714, Nau4024, Nau2761, Nau2173 SSR markers were obtained from the cotton species, *Gossypium Raimond*. Besides, in table 1, the references, repeat motifs, and chromosomes that SSR markers used in this study were also displayed [43].

#### 2.4. Genetic diversity and phylogenetic analyses

Each SSR marker locus, amplified alleles were scored as either '1' (present) or '0' (absent). Each amplified allele of SSR was indicated with A, B, C and so on... letters. Analysis of genetic distance (GD) to obtain genetic dissimilarity matrix PopGENE 1.32 ver. software was used [44].

Genetic diversity was conducted for all SSR locus across germplasm individuals based on the number of alleles, polymorphic alleles numbers, percent of polymorphism, gene diversity [45], and the polymorphic information content (PCI) [46] was determined with PowrMarker 3.25 version program [47]. Genetic distances (GD) [48] were calculated, and the phylogenetic tree was built with the

distance matrix using UPGMA (Unweighted pair group method with arithmetic) data panel provided in PowerMarker 3.25 [47]. The phylogenetic tree was created in MEGA-X 10.1.7 version. PIC values show the number of alleles at each locus and the distinctive features of the markers through the relative frequencies of alleles in the population [49]. PIC calculation was made based on the formula given below.

$$PIC=1-\sum(P_i)^2 \quad (1)$$

In equation 1, the P-value is the frequency of the  $i^{\text{th}}$  allele of the total Upland cotton genotypes subjected to the analysis [50].

#### 2.5. Population Structure Analysis

The genetic structure of the subpopulation (*Q-matrix*) was analyzed with Bayesian Model-Based (MBB) analysis model provided in Structure 2.3.4 version software [51]. To calculate the Q matrix, the software was set up to run under 10,000 (Leng of burning periods)-100,000 (Number of reps after burning) Markov Chain Monte Carlo iteration after the burn-in and Number of the population (K) from 1 to 10 and number of iterations was 5. The average likelihood value L(K) was calculated for each K cross of all the runs. The number of populations is estimated by estimating  $\Delta K$  [52]. The results were transferred to the web-based "Structure Harvester" (<http://taylor0.biology.ucla.edu/structureHarvester/>) program.

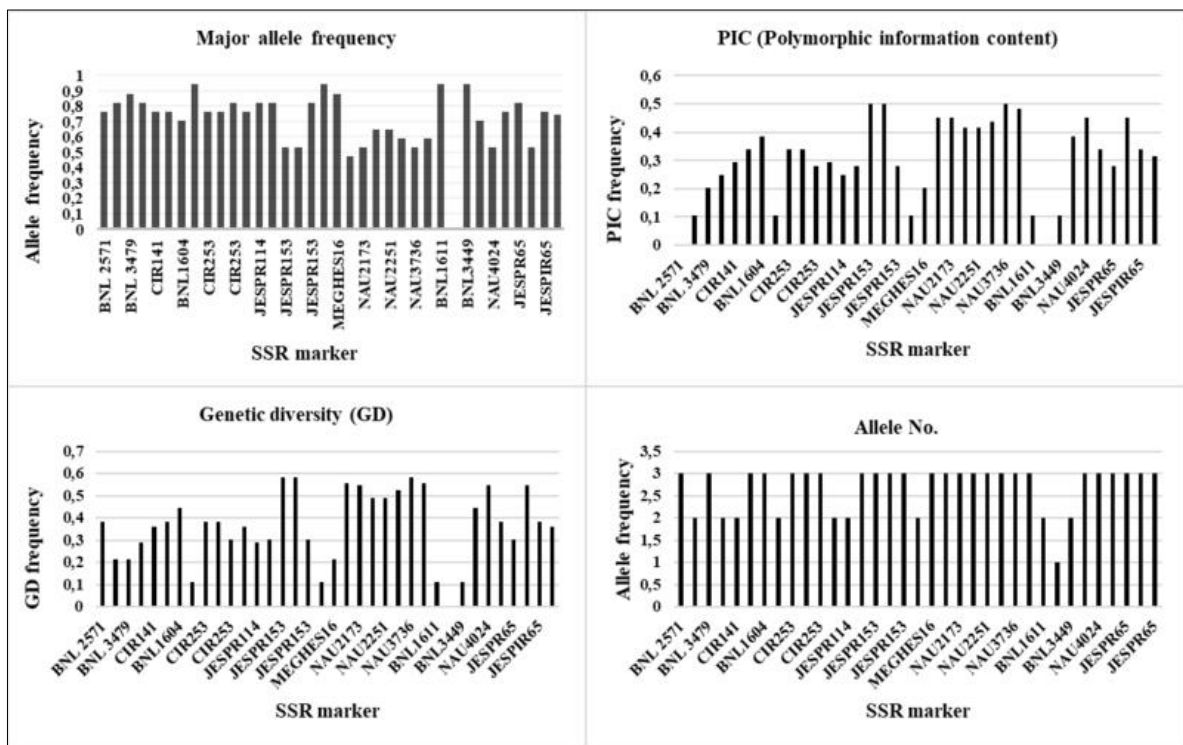


### 3. RESULTS and DISCUSSIONS

As a result of the amplification made using 20 polymorphic SSR primers, 99 alleles were produced. There was an average of 5.82 alleles per locus. Zhang et al. [53] and Lacape et al. [54] obtained 5.5 alleles per average locus that ranged between 2 and 26 per locus, which was similar to the results of the present study. Besides, Lacape et al. [54] also reported that the number of the alleles obtained per marker did not only stem from the diversity in germplasm,

but also depended on the marker type, the fragment separation technique used, and the resolution.

As given in figure 1, after scanning with 20 SSR markers [43] the cotton varieties belong to *G. hirsutum* L. 17 SSR markers were found as polymorphic. Polymorphic information content (PIC) ranged from 0.0-0.5 with an average of 0.312; Each SSR marker amplified at least 2 loci and Jespr SSR produced the most alleles. Genetic diversity among SSR markers differed from 0.0 to 0.6 (Figure 1).



**Figure 1.** Major allele frequency (MAF), Polymorphic information content (PIC), Genetic Diversity (GD), and Number of Alleles per SSR locus

**Table 2.** Analysis results of SSR primers used in this study

Primer Name	Forward Primer (5'-3')	Reverse Primer (3'-5')	Alleles Band size (bp)	Allele no.	PIC
BNL1604	AGAGGGAGTAAAGATTGGGG	TCCAGTCTTTTTGCCTTGG	120-50	6	0.362
BNL3449	AAGCTGTGGCTATGATGCCT	AGAGCAAAAACAATTACAAAAGC	180-150	2	0.105
BNL3479	AGTGGGTTGGACTTTCATGC	CACGGGCTTTTTTTTTTCA	350-200	5	0.216
BNL2571	TCGCTATCGCTCTGAAATCA	ATGCCACGGAATTAGCAAAC	400-320	3	0.345
CIR 0141	CGCACAAAGGAATAGAAG	ACCCAACATAAGGACTAAA	300-200	4	0.271
CIR0253	CCAACCAAGAAACCAG	GTAAGCATGGGCATT	150-50	11	0.256
NAU3736	CATGTGCATTTATCCTGTC	CCAAGTGAGAGGCATTTTCT	200-80	6	0.491
NAU2714	GCAGCCATTACAGAACATCA	TCATTGATCCATTGCTTCTG	300-200	6	0.384
NAU4024	ACAAGCATTTTCATGGACCT	AGAAGGATGATGCAAGAGG	200-150	3	0.449
NAU2751	GACAAGTTTTGGACCCACT	TTCATAGAGGGTTTTGCAT	350-50	2	0.345
NAU2173	GCCAAATAGGTCACACAAA	AGCGAGAAGGAGACAGAAAA	350-320	6	0.450
JESPR0065	CCACCCAATTTAAGAAGAAATTG	GGTTAGTGTATTAGGGTCGTTG	300-200	12	0.325
JESPR0153	GATTACCTTCATAGCCACTG	GAAAACATGAGCATCCTGTG	300-100	12	0.390
JESPR0114	GATTTAAGGTCTTTGATCCG	CAAGGGTTAGTAGGTGTGTATAC	300-100	4	0.248
MGHES16	ACCCAATACAACCCATTT	GCAGAGAAAAGGGACAGAGG	400-50	5	0.154
NAU2251	TTCTCCAGTAACCAACAAAGG	AAAATATCATCCCCGTCAAA	400-200	9	0.422
BNL1611	CAATGAACAAAAATGTAAGGG	TGGGCATTTAGCCATTTACC	100-50	3	0.105
			Mean=	Mean=	
			5.82	0.312	



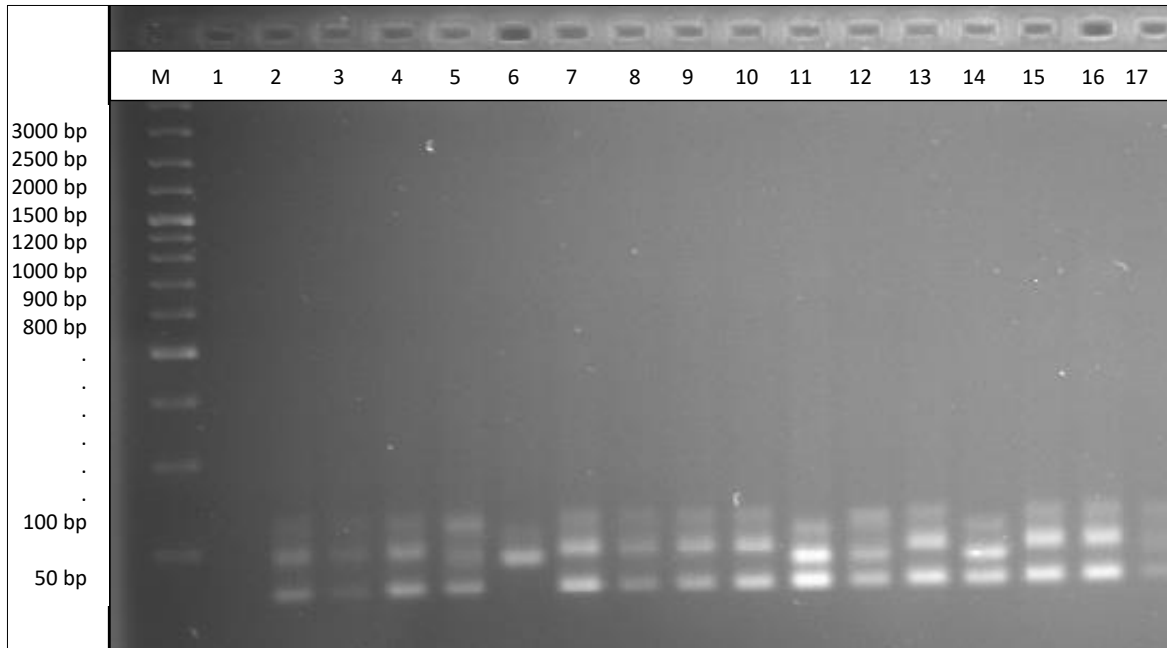
As indicated in table 2. the average Polymorphic Information Content (PIC) of the markers was 0.312. The PIC values of the markers changed between 0.105 and 0.491. The Nau3736 marker had the highest value with a PIC value of 0.491, and the Bnl1611 and Bnl3449 markers had the lowest value of 0.105. While the mean of alleles is 5.82, each of Jespr0065 and Jespr0153 SSR markers has amplified 12 loci (The highest alleles number) and both Bnl3449 and Nau2751 amplified 2 loci (2 alleles) markers. As a result of the amplification with 31 pairs of SSR primers, Bertini et al. [26] reported similar results with 2.13 alleles per average SSR locus, with PIC values ranging from 0.18-0.62 to 0-0.41 in 66 alleles. Seyoum et al. [35] propounded also close results such as PIC value ranged from 0.319 to 0.019 with 0.279 mean value, the number of alleles per locus ranged from 2 to 12 with 4.53 average.

Liu et al. [55] made amplification with 62 SSR primers and identified 139 alleles in 69 SSR locus as 2 alleles per locus, as well as 325 alleles as 5 alleles per locus; Lacape et al. [54] reported that they identified a total of 1128 alleles including an average of 5.61 alleles per locus because of

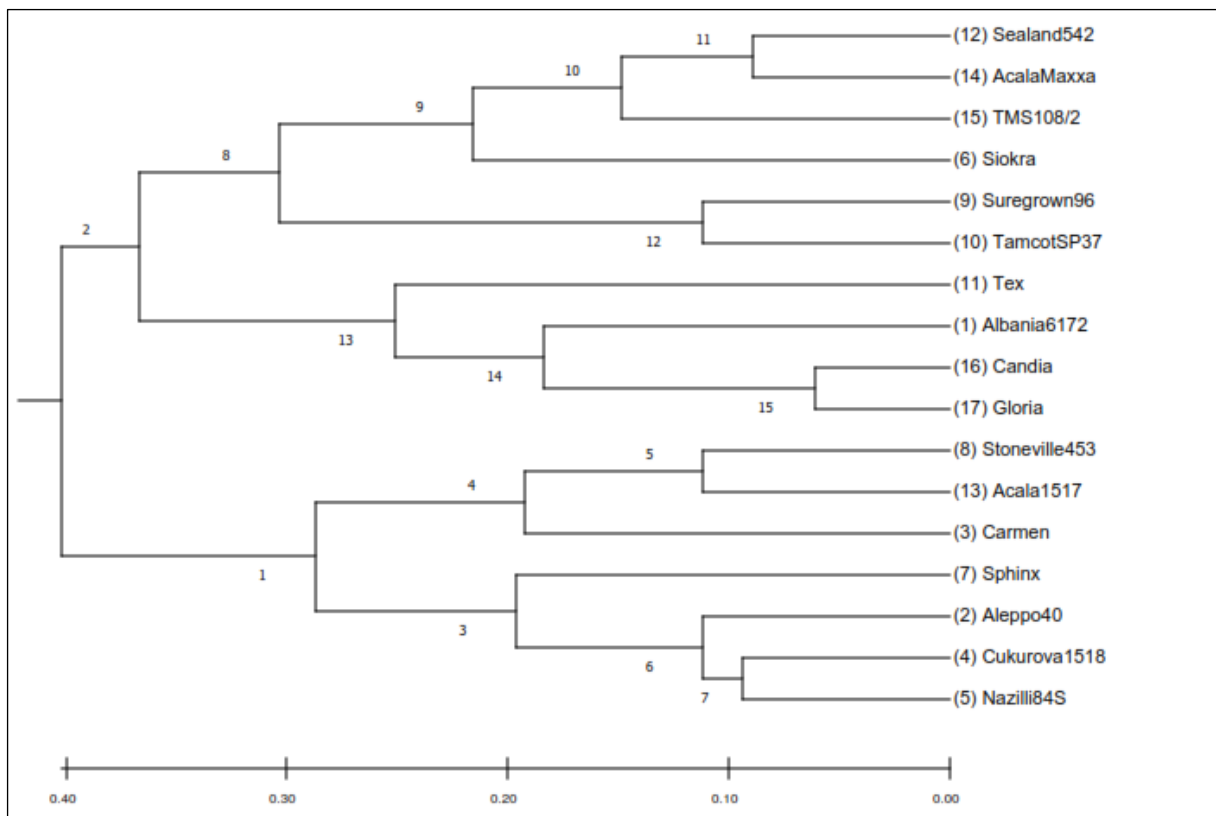
201 SSR markers using 47 wild *Gossypium* genotypes. The reason that Lacape et al. [54] had more alleles and higher average alleles per locus than us could be depended on their SSR amount and wild Cotton genotypes. Iqbal et al. [56] reported that they obtained 349 alleles with 50 primers, and detected polymorphism in 23 cotton genotypes using 49 primers. In these studies, it is considered that the reason for producing more alleles than in the present study is related to the excessive SSR primers used.

In figure 2, the Bnl1611 SSR marker produced 3 alleles with 100, 75, 50 bp between 100-50 bp fragment size. The Bnl1611 marker amplified the target locus in all genotypes except Gloria and produced at least 2 maximum 3 alleles in genotypes except the Nazilli 84s. The reason for not being able to produce bands in the Gloria genotype may be due to pipetting errors or absences of template DNA.

The gel image taken under UV light after Bnl1611 running through 1% agarose was given in Figure 2. Scoring fragments were performed in all genotypes except the Gloria variety.



**Figure 2.** PCR gel image of BNL1611 (Locus) SSR primer. M=Marker, DNA ladder= Vivantis 10 bp (1% agarose 1 X TBE). 1=Gloria, 2=Aleppo-40, 3=Carmen, 4=Cukurova 1518,5=Albania-6172, 6=Nazilli 84S, 7=Siokra 1/4, 8=Sphinx V, 9=Stoneville-453, 10=Sure grow 96, 11=TamcotSP37, 12=Tex, 13=Sealand-542, 14=Acala 1517-95, 15=Acala Maxxa, 16=TMS 108/2, 17=Candia



**Figure 3.** Dendrogram based on Nei's [45] genetic distance: Method = UPGMA of *G. hirsutum* L. genotypes based on 20 Genome-Wide SSR markers

The results of UPGMA (Unweighted pair group method with arithmetic) analysis were shown in Figure 3. The genotypes are divided into two main clusters. 1<sup>st</sup> main cluster consists of 7 genotypes (Nazilli 84S, Çukurova 1518, Aleppo 40, Sphinx, Carmen, Acala 1517, Stonville 453), the 2<sup>nd</sup> cluster consists of 10 genotypes (Sealand 542, Acala maxxa, TMS 108/2, Siokra ¼, Sure grow 96, Tamcot SP 37, Tex, Albania 6172, Candia, Gloria). The 1<sup>st</sup> main cluster is divided into two subclusters and 2<sup>nd</sup> main cluster is also divided into two subclusters. Accordingly, 15 different subclusters emerged in total. In this respect, Sealand-542, Acala maxxa, TMS 108/2, Siokra ¼, Suregrow96, Tamcot, Tex, Albania-6172, Candia and Gloria were classified in a different cluster, in other words, they have pedigree relations, Stonville-453, Acala-1517, Carmen, Sphinx V, Aleppo-40, Çukurova 1518 and Nazilli 84S genotypes were classified in a different cluster. The first cluster was also divided into further subclusters, and Sealand-542, Acala maxxa, TMS 108/2, Siokra ¼, Sure grow 96, and Tamcot, were included in the first subcluster of this cluster while Albania-6172, Candia, Gloria, and Tex were included in the second subcluster.

Although Stoneville-453, Acala-1517, and Carmen varieties in Group 4 were collected from different geographical areas, there was a unity of origin among them. The Sphinx V, Aleppo-40, Çukurova 1518, and Nazilli 84S were classified in Group 3. Again, it was seen that the second subcluster of the second main cluster also may have common parents even though they were the genotypes obtained from different regions, as was the case in the first subcluster. Eminenur and Hancer. [57] conducted genetic diversity analysis with *G. hirsutum* L.

genotypes (Flash, BA119, ST506, Tamcot Sp21, Tamcot 22, Tamcot 94, Tamcot Camd-eS and Tamcot Sp23, Sphinx v, Stn468), and *G. barbadance* L. (Giza 70) variety, and reported that Giza 70 was included in a separate cluster and the remaining genotypes were included in a separate cluster.

In Figure 3, some genotypes classified in the same cluster have some similar characteristics. This may be clarified that they come from a common pedigree. For instance, Sealand-542 which is an interspecies hybrid (*G. hirsutum* L X *G. barbadance* L.), and Acala maxxa were classified in the same cluster. Nazilli 84S, which is a hybrid of Carolina Queen X 153-F and Çukurova 1518 are also among the closest relatives. It was seen that Sure Grow 96 and Tamcot SP37 genotypes, which were clustered in Group 12 that originated from the USA, and Candia and Gloria, two Australian originating varieties, were in Group 15.

SSR markers were distributed on 20 different chromosomes, and produced a total of 99 alleles ranging from 2-12 with a 5.82 average. Tyagi et al. [58] reported similar results in that they produced 546 alleles in 141 loci in genetic diversity study with 381 cotton accessions using 120 Genome-wide SSR markers. Bardak and Bolek. [59] reported similar results with the present study as they obtained a total of 173 alleles, including 3.93 alleles per locus, as a result of their study with 39 SSRs and 5 ISSRs using for screening 25 cotton genotypes.

According to Nei's [45] pair-wise comparison on the genetic distance between genotypes, and according to the

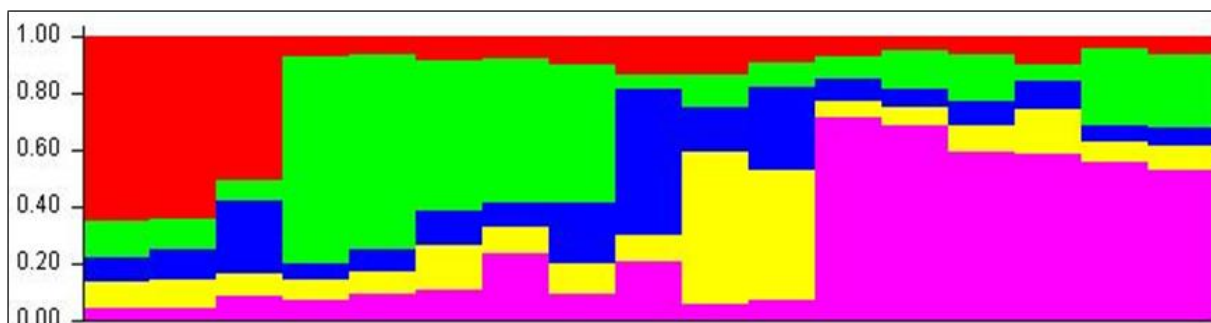
results of the Genetic Distance matrix, the highest GD (1.09) was measured at the highest score between Carmen and Sure Grow 96, Sealand-542 and Siokra ¼, and Sphinx V and Stoneville-453 genotypes. The lowest GD (0.26) was measured between Gloria and Carmen, and Tex and Stoneville-453 genotypes. The GD between the genotypes varied between 0.26 and 1.09, in this respect, it was seen that the GD between Cukurova 1518 and Gloria, Nazilli 84S and Siokra ¼, Tamcot SP37 and Cukurova 1518, TMS 108/2 and Çukurova 1518, Sure Grow 96 and Tex, Acala Maxxa and Tamcot SP37 and Acala-1517 and TMS 108/2 were the same. Bardak and Bolek. [59] also reported close results to this study that the lowest GD (0.04) was detected between Siokra ¼ and Nazilli 84S, and the highest GD (0.58) was between Erşan 92 cotton variety of the AD genome and *G. sturtianum* of the C genome group *Nandewareense* variety.

In the present study, the Genetic Distance (GD) between upland cotton genotypes ranged from 0.26 to 1.09. The

previous studies were reported Genetic distance between 0.06-0.34 [60], 0.06-0.38 [61]; 0.82-0.93 [56] and 0.19-0.36, [62] in upland cotton genotypes. These results are on the line with the previous genetic diversity studies.

Zhao et al. [63] have reported the highest genetic diversity in cotton from the USA followed by China; Chen and Du. [64] indicated the higher genetic diversity of introduced genotypes particularly obtained from China and USA countries than the domesticated genotypes.

The Structure analysis of population results revealed the highest population numbers took place at K:5 and this can be evaluated as cotton germplasm can be grouped into five subpopulations (figure 4). Five (5) different colors in Figure 4, and each color represents a population or genotype collected from the same geographical area.



**Figure 4.** Plot Q-Matrix shows the genotypic data analysis of cotton genotypes in Structure 2.3.3 ver. software. Each colored subsection bar represents the cotton genotypes group origin

These genotypes were obtained from the United States (Acala maxxa, Sure Grow 96, Stoneville-453, Tamcot SP 37, Teks, Sealand-542, Acala-1517, Siokra ¼, Sphinx V), Turkey (Çukurova 1518, TMS 108/2, Nazilli 84S), Albania (Albania-6172), Syria (Aleppo-40) and Australia (Carmen, Candia, Gloria). Abdurakhmonov et al. [65] reported that the germplasm materials they used were divided into three categories as native, Mexican, and African origin. Bardak et al. [66] reported that 48 cotton genotypes they used were divided into 3 different groups. Tyagi et al. [58] reported similar results as their 381 cotton genotypes were divided into 5 different groups. Genetically distant genotypes should be selected as parents as much as possible when breeding programs are carried out.

#### 4. CONCLUSIONS

As it is already known, the success of breeding depends on the selection of the right parents, which is the first and most important step in the strength and success of this process. Genetic distance between parents in a breeding program brings the possibility of allelic diversity and improved phenotypic values as well as a higher chance of hybrid vigor.

Therefore, exploring the genetic diversity, in other words, determining the genetic distance between genotypes plays a key role in the development of a new variety.

In the study, most SSR primers produced polymorphic bands. Some of them didn't produce.

The cotton varieties with far distance from each other such as Acala maxxa, Carmen, Aleppo 40, Siokra ¼, and Tex, (onsidering the high yield and quality) are highly recommended to use as parents in Marker-assisted selection (MAS) to develop new varieties.

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## Bingöl Yöresi Arıcılık İşletmelerinde (*Apis mellifera L.*) Nosema Hastalığının Araştırılması

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**Anahtar Kelimeler**  
 Bingöl,  
 Bal Arısı,  
 Nosema Hastalığı

**Öz:** Bu çalışmada Bingöl ili arı işletmelerinde Nosema hastalığı yaygınlığının belirlenmesi amaçlandı. Araştırmada Bingöl merkez ve diğer ilçelerde arıcılık yapan 123 adet arı işletmesi ziyaret edilerek toplam 1245 adet arı örneği toplandı. Örneklerin laboratuvar incelemeleri sonrası işletmelerden 26 (%21,13)'sı ve örneklerden 98 (%7,87)'i Nosema hastalığı yönünden pozitif bulundu. Araştırmada işletme bazında hastalık oranı en yüksek oranda merkez ilçedeki işletmelerde %24,52 görülürken en düşük oranın ise Karlıova ilçesindeki işletmelerde %13,33 tespit edildi. İşletmelerden alınan ve incelenen örneklerden ise en yüksek oran merkez ilçede %15,11 tespit edilirken en düşük oranın ise Karlıova %2,80 ve Yayladere %2,85 ilçelerinde saptandı.

## Investigation of Nosema Disease in Beekeeping Establishments (*Apis mellifera L.*) In Bingöl Region

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**Keywords**  
 Bingöl,  
 Honeybee,  
 Nosema Disease

**Abstract:** In this study, it is aimed to determine the prevalence of Nosema disease in bee establishments in Bingöl province. In the research, 1245 bee sample was collected by visiting 123 beekeeping establishments in the center of Bingöl and other towns. After laboratory examinations of the samples, 26 (21.13%) of the establishments and 98 (7.87%) of the samples were found to be positive for Nosema disease. In the study, the highest rate of the disease was found in the establishments in the central town (24.52%), while the lowest rate was found in the establishments in Karlıova town 13.33%. Among the samples taken from the establishments and examined, the highest rate was found in the central town 15.11%, while the lowest rate was found in Karlıova 2.8% and Yayladere 2.85%.

### 1. GİRİŞ

Arıcılık, dünyanın neredeyse her bölgesinde yapılan önemli tarımsal bir faaliyettir [1,2]. Yapılan arıcılık aktiviteleriyle bal, polen, arı zehri, arı sütü, bal mumu ve propolis gibi arı ürünleri ile gıda, eczacılık ve kozmetik sektörü gibi alanlarda kullanılan hammadde üretilmektedir [3,4]. Bunun yanında arılar meyve ağaçlarından ve bitkilerden nektar ve polen toplayarak besinsel ihtiyaçları tedarik ederken, diğer yandan da polinasyon ile ürün miktarını ve niteliğini artırarak tarımsal üretime önemli düzeyde katkı sağlamaktadır. Ayrıca yabancı floranın devamlılığını tozlaşma ile sağlamak sureti ile önemli katkıları olmaktadır [5,6]. Ülkemiz yüz ölçümü, topoğrafik yapısı, iklim ve bitki örtüsü açısından arıcılık için uygun bir coğrafyadır. Bu özelliğine rağmen diğer ülkelere oranla ülkemizde kovan

başına bal üretimi ise düşük düzeyde kalmakta olup bunda; teknik bilgi yetersizliği, bakım ve besleme noksanlığı, ana arı üretiminin istenilen seviyede olmaması, arı kışlatmalarındaki bilgisizlik ve en önemlisi de hastalık ve zararlılarının bilinmemesi, zamanında teşhis ve tedavinin yapılamaması gibi nedenlerin büyük payı vardır [7].

Arıcılık faaliyetlerinin daha verimli hale gelmesi, kovan başına elde edilen yıllık bal miktarında artışın sağlanması ve dünya çapında daha üst sıralara ulaşabilmesi için arıcılara bilimsel ve teknik yöntemlerle daha fazla bilgilendirmeler yapılması gerekmektedir. Ayrıca eş zamanlı olarak arı hastalıkları ile mücadele ve koloninin güçlendirilmesinde uygun yöntemlerin kullanılması sağlanmalıdır [8,9,10]. Son dönemlerde arıcılık faaliyetleri dünya genelinde olduğu gibi ülkemizde de tercih edilen üretim faaliyetleri arasına

girmiş olup her geçen gün arıcılığa olan ilgi artarak devam etmektedir [8].

Ülkemizin arıcılık için çok önemli bir potansiyele sahip olmasına rağmen beklenen verimin alınmamasında hastalıkların önemli bir yeri vardır [11]. Arıcılıkta kovanlarda bal verimini önemli düzeyde düşüren ve ayrıca kolonilerde büyük kayıplara neden olan birçok hastalık bulunmaktadır. Bu hastalıklar gerek yavru ve gerekse ergin arılarda görülmekte ve arı işletmelerine önemli düzeyde ekonomik açıdan zarar vermektedir [12,13]. Arılarda, bakteri, virüs, parazit ve mantarların sebep olduğu birçok hastalık bulunmaktadır. Bunlar; Amerikan Yavru Çürüklüğü, Avrupa Yavru Çürüklüğü, Arı Felci, Varroa, Nosema, Trake Akarı, Kireç Hastalığı, Taş Hastalığı, Tulumsu Yavru Çürüklüğü hastalıkları gibi kolonilerde önemli kayıplara neden olan hastalıklardır [11]. Nosema sporları, arıların bağırsak ve midelerinde hareket ederek gelişmeye devam eden ve arılarda ölümlere neden olan etkenler arasında en önemlileri olarak bilinmektedir. Hastalığın etkenleri *Nosema apis* ve *Nosema ceranae*'dir. Bu faktörler yetişkin arıların sindirim sistemine yerleşir ve burada Nosema hastalığının meydana gelmesine neden olur [14]. Nosema hastalığının teşhisi klinik bulgulara göre tespit edilebilse de kesin teşhis ancak laboratuvar yöntemleri ile yapılmaktadır [11,14,15].

Bingöl ve yöresi iklim ve bitki örtüsü, zengin endemik bitkileri içeren florası ile arıcılık için önemli bir merkez olarak bilinmektedir. İşletme sayısı ve arılı kovan sayısı bakımından önemli bir düzeyde olmasına rağmen bal üretimi bakımından beklenen başarı elde edilememektedir. Bunda birçok nedenin yanında arı hastalıklarının önemli bir etken olduğu gerçeğinden hareketle bu çalışmada, kolonilerde önemli kayıplara neden olan, bal verimi oranının düşmesinin de önemli bir etkeni olduğu düşünülen Nosema hastalığının araştırılması, düzeyinin belirlenmesi ve ortaya konulması amaçlanmıştır.

## 2. MATERYAL VE METOT

### 2.1. Materyal

Bu çalışmada Bingöl Merkez ve ilçelerinde arıcılık yapan ve Bingöl Arıcılar Birliğine kayıtlı bulunan 802 arı işletmesinin 123'ünden alınan 1245 arı örneği materyal olarak kullanıldı.

Çalışmada kullanılmak üzere arı örneklerini toplamak için Bingöl Merkez ve ilçelerindeki arı işletmelerine 2020 yılı ilkbahar, yaz ve sonbahar aylarında periyodik olarak gidildi. Gidilen işletmeler rastgele toplam işletmenin yaklaşık %10'unu oluşturacak şekilde belirlendi. İşletmelerden örnek alınan kovanlar işletmedeki toplam kovanın yaklaşık %5'ini oluşturacak şekilde olmasına dikkat edildi. Örnekler içerisinde %70'lik etil alkol bulunan 50 ml'lik falkon tüplere alındı. Alınan örneklerde ortalama 35-40 adet ergin arı olmasına dikkat edildi. Çalışma boyunca Merkez İlçe 53, Adaklı 5, Genç 15, Karlıova 15, Kiğı 10, Solhan 15, Yayladere 5 ve Yedisu 5 olmak üzere toplam 123

işletmeye gidildi ve bu işletmelerden toplam 1245 örnek toplandı.

### 2.2. Metot

İşletmelerden alınan örnekler Bingöl Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu laboratuvarına getirildi. Tüplerdeki arı örnekler 24 saat sonra havlu peçete üzerine konuldu ve arılardan alkolün uzaklaşması sağlandı. Arılardan alkol uzaklaştırıldıktan hemen sonra bisturi ve pens yardımı ile abdomenleri ayrıldı ve porselen havan içerisine konuldu ve üzerine 10-15 ml serum fizyolojik su ilave edilip tokmakla ezilerek homojen bir karışımın oluşması sağlandı [11].

#### 2.2.1. Natif İnceleme

Alınan örneklerden hazırlanan homojen karışımdan içerisinde herhangi bir partikül bulunmayan kısımdan bir miktar lam üzerine alındı ve üzerine lamel kapatılarak ışık mikroskopunda 40 objektif ile incelendi ve *Nosema* sporları arandı [11].

#### 2.2.2. Boyama Yöntemi İle İnceleme

Alınan örneklerden hazırlanan homojen karışımdan temiz bir lam üzerine 2-3 damla alındı, başka bir lamla yüzeye yayılması sağlandı ve kurutuldu. Kuruma işleminden sonra lamlar hafif alevden geçirilerek tespit edildi. Lamaların üzerine %1'lik safranin boyasından 2-3 damla damlatıldı ve alevde lamaların üzerindeki boyaların kaynarak lam yüzeyine yayılması sağlandı. Soğuma işleminden sonra lamlar musluk suyunda yıkandı. Daha sonra lam üzerine pipetle metilen mavisi boyası döküldü 20 dakika beklendi. Boya döküldü ve musluk suyunda yıkandı. Lamlar kurutuldu ve mikroskopta incelemeye hazır hale getirildi. Lamlar mikroskopta 100'lük immersiyon objektifi ile incelendi safranin ile boyanan *Nosema* sporları gözlemlendi. İncelemede *Malpighamoeba mellificae* kistleri ve mantar sporları metilen mavisi boyası ile boyandıklarından *Nosema* sporlarından ayırmaları sağlandı. Yapılan mikroskopik incelemede *Nosema* sporları gözlenen örnekler pozitif olarak değerlendirildi [11,16,17].

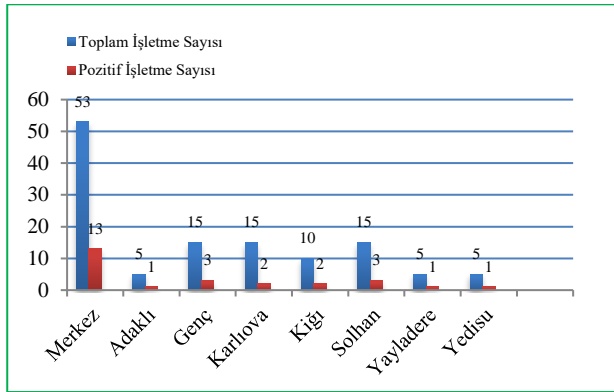
#### 2.2.3. İstatistiksel Değerlendirme

Bu çalışmada toplanan örneklerde incelemeler sonrası pozitif olan örneklerin oranı yüzde olarak değerlendirildi ve tabloda gösterildi [18].

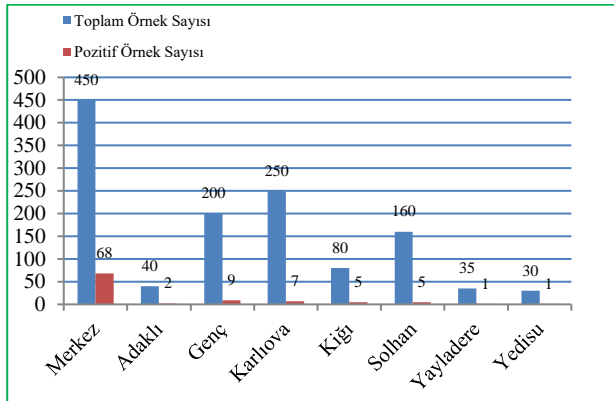
## 3. BULGULAR

Çalışmada işletmelerden toplanan örneklerden Bingöl merkezde 13, Adaklı ilçesinde 1, Genç ilçesinde 3, Karlıova ilçesinde 2, Kiğı ilçesinde 2, Solhan ilçesinde 3, Yayladere ilçesinde 1 ve Yedisu ilçesinde ise 1 işletme olmak üzere toplamda 26 arı işletmesi *Nosema* hastalığı yönünden pozitif olarak gözlemlendi (Tablo 1, Şekil 1). İlçelerdeki arı işletmelerinden alınan örneklerde ise; Merkez ilçede 68, Adaklı ilçesinde 2, Genç ilçesinde 9, Karlıova ilçesinde 7, Kiğı ilçesinde 5, Solhan ilçesinde 5, Yayladere ilçesinde 1 ve Yedisu ilçesinde 1

olmak üzere toplamda 98 örnek pozitif olarak tespit edildi (Tablo 1, Şekil 2).



Şekil 1. Arı işletmelerinin pozitiflik oranları



Şekil 2. Arı örneklerinin pozitiflik oranları

Tablo 1. Arı örneği alınan işletmelerin Nosema hastalığı yönünden pozitiflik düzeyi

Yerleşim Yeri	Toplam İşletme Sayısı	Örnek Alınan İşletme Sayısı	Pozitif İşletme Sayısı	%	Alınan Örnek Sayısı	Pozitif Örnek Sayısı	%
Merkez	356	53	13	24,52	450	68	15,11
Adaklı	39	5	1	20,00	40	2	5,00
Genç	99	15	3	20,00	200	9	4,50
Karlıova	75	15	2	13,33	250	7	2,8
Kiğı	79	10	2	20,00	80	5	6,25
Solhan	91	15	3	20,00	160	5	3,12
Yayladere	25	5	1	20,00	35	1	2,85
Yedisu	38	5	1	20,00	30	1	3,33
<b>Toplam</b>	<b>802</b>	<b>123</b>	<b>26</b>	<b>21,13</b>	<b>1245</b>	<b>98</b>	<b>7,87</b>

#### 4. SONUÇ

Dünya çapında geniş bir dağılıma sahip olan Nosema hastalığı ilk defa Zander tarafından 1909 yılında adlandırılmıştır [19]. *N. apis*, Nosema hastalığının tek etkeni olarak görülmekteyken, *N. ceranae*'nin 1996 yılında Asya bal arısı *Apis ceranae*'da hastalık oluşturduğu ortaya konulmuştur [20,21]. *N. ceranae*'nin Avrupa'da *A. mellifera*'da enfeksiyon oluşturduğu ve aynı zamanda uyum sağladığı bildirilmiştir. Böylelikle *N. ceranae*'nin dünyanın çoğu yerinde, batı bal arısı ırkları arasında geniş bir yayılıma sebep olduğu ve *N. apis*'in yerini aldığı belirtilmektedir [22].

Kısa bir süre sonra, *N. ceranae*'nin varlığına Amerika ve Asya'da rastlanıldığı doğrulanmıştır [23,24]. Öncelikle, Nosema hastalığına neden olan etmenin *N. apis* olduğu ve dünyanın her yerinde yayılım gösterdiği düşünülürken, *N. ceranae*'nin ise yalnızca Asya bal arısı

ırkı olan *Apis ceranae*'yı etkilediği söylenmekteydi [25,26]. Ancak son yıllardaki çalışmalar, *N. ceranae*'nin *Apis mellifera*'ya da bulaştığı ve böylece tüm Avrupa'dan Avustralya, Tayvan ve Kuzey Amerika'ya kadar yayıldığı bildirilmektedir [21,27,28].

Kanada'da yapılan bir çalışmada 2008, 2010 ve 2012 yıllarında toplanan bal arısı örneklerinde *Nosema* sporları saptanmış olup oransal olarak bakıldığında; 2008'de toplanan örneklerin %39'unda, 2010'da örneklerin %60'ında ve 2012'de ise örneklerin %45'inde karışık olarak *N. apis* ve *N. ceranae* sporları olduğu belirlenmiştir [29]. Bollan ve ark. [30] tarafından İskoçya'da yapılan bir çalışmada kolonilerde %70,4 oranında *N. ceranae* ve *N. apis* görüldüğü bildirilmektedir. Fries [22] tarafından İsveç'te yapılan bir çalışmada kolonilerin %17'inde *N. ceranae* ve *N. apis* birlikte görülürken %83'ünde ise sadece *N. apis*'e rastlanıldığı belirtilmektedir. Taric ve ark. [31] tarafında yapılan bir çalışmada Yugoslavya'nın kuzey bölgesinden toplanan 2439 arı örneğinin 630'u (%25,83) nosema sporları bakımından pozitif bulunmuştur. Ansari ve ark. [32] tarafından Suudi Arabistan'da yapılan bir çalışmada nosema hastalığının oranını %20,59 düzeyinde olduğu ve bu oranın ise %58'inin *N. ceranae* olduğu bildirilmiştir. Lotfi ve ark. [33] tarafında yapılan bir çalışmada İran'ın kuzeybatısındaki 294 arı kovanının 72'sinde Nosematosis tespit edilmiştir.

Ülkemizde nosema hastalığının ilk teşhisi 1986 yılında Uygur ve Girişgin [34] tarafından Türkiye Kalkınma Vakfı Arı Hastalıkları Laboratuvarında teşhisi yapılmıştır. Aydın ve ark. [12] yaptıkları çalışmada nosema sporlarının varlığını Balıkesir, Bursa ve Çanakkale illerinde araştırmışlar ve sonuçları sırasıyla Balıkesir %30, Bursa %25,8 ve Çanakkale'de %25 düzeyinde tespit ettiklerini bildirmişlerdir. Çakmak ve ark. [35] yaptıkları bir çalışmada Bursa'da 22 ayrı bölgedeki 217 arı kovanından alınan arı örneklerinde nosema sporlarının yaygınlık düzeyini %24 düzeyinde olduğunu saptamışlardır. Topçu ve Arslan [16] tarafında Kars ilinde yaptıkları çalışmada 8 lokasyon bölgesindeki toplam 50 arılıktaki yürüttükleri çalışmada 8 bölgenin 7 yerleşim yerinde %87,50 seviyesinde ve 50 örnekleme arılığın 20'sinde ise %40 düzeyinde *N. apis* sporlarına rastlamışlardır. Şimşek [36] tarafından yapılan çalışmada Elâzığ'daki arı işletmelerinden alınan örneklerde nosema hastalığının yaygınlığının %8,7 oranında olduğunu tespit edilmiştir. Doğaroğlu ve Sıralı [37] yaptıkları çalışmada Trakya'da nosema hastalığı yaygınlığının %6,5 düzeyinde olduğunu saptamışlardır. Ütük ve ark. [38] tarafından 2006-2010 yılları arasında Türkiye'nin 36 şehirlerinde Merkez Veteriner Kontrol ve Araştırma Enstitüsü, Arı hastalıkları Laboratuvarına gelen toplam 140 adet numune üzerinde yapılan çalışmada %4,28'inde nosema hastalığı, %2,85'inde hem varroa hem de nosema hastalığı, %56,42'sinde ise yalnızca varroa zararlısı tespit etmişlerdir. Büyük ve ark. [39] yaptıkları çalışmada Kırşehir ilinde 5 lokasyondan Merkez, Mucur, Akpınar, Boztepe ve Kaman olarak tanımlanan 5100 bal arı örneği incelenmişler çalışmada hastalığın görülme sıklığının Merkez bölgesinde %25, Boztepe ilçesinde %60, Mucur bölgesinde %23,07 ve

Akpınar bölgesinde %12,5 olduğunu tespit etmişlerdir. Zerek [40] Hatay yöresinde yaptıkları çalışmada İşletmelerin %45'inde ve kovanlarında %20'sinde *N. ceranae* enfeksiyonu saptanmıştır.

Yaptığımız bu çalışmada Bingöl merkez ve diğer ilçelerde Bingöl Arıcılar Birliğine kayıtlı arı işletmelerinden alınan örneklerde Nosema hastalığının %7,8 oranında olduğu tespit edildi. Sonucun [36-38] araştırmacıların bulgularıyla uyumlu iken, [12,16,22,29-33,35,39,40] araştırmacıların bulgularından ise düşük oranda olduğu gözlemlendi. Bu durumun merkez ilçede diğer ilçelere nazaran daha yüksek oranda gözlenmesinde; merkez ilçe arıcılarının çoğunluğunun gezginci arıcılık yapmaları ve dışarıdan gelen gezginci arıcıların çoğunlukla merkez ilçe meralarında konaklamalarının sebep olabileceği düşünülmektedir.

Yaptığımız bu çalışmada Bingöl merkez ve tüm ilçelerinde nosema hastalığına rastlanılmış olup nosema hastalığının en yüksek oranda Bingöl merkez ilçede rastlanılmıştır. Hastalığın yayılmasında gezginci arıcılığın önemli bir etken olduğu da dikkate alınarak gezginci arıcıların merkez ve diğer ilçe meralarında konaklamaları kontrollü bir şekilde sağlanmalıdır. Hastalık yönünden şüpheli işletmeler ile diğer arı işletmelerinin temasının engellenmesi, arıcıların bilgilendirilmesi ve koruyucu önlemlerin alınması noktasında çalışmalar yapılmalıdır.

Arıcılığın önemli problemlerinden olan arı hastalık ve zararlıları eğer zamanında fark edilip gerekli önlemler alınmazsa, arıcılığın gelişmesi için yapılan tüm gayret ve çabaları olumsuz yönde etkilemeye devam edecektir. Diğer yandan arıcıların kullandıkları ortak su kaynaklarında bölgede hastalığın yayılmasının önemli bir etkeni olabileceği düşünülmektedir. Bundan dolayı nosema hastalığının yaygınlığı üzerine yapılan çalışmalar yalnızca kolonilerdeki arılarda ve arılıkta değil aynı zamanda arı kolonilerinin kullandıkları su kaynaklarında da olmalıdır. Böylelikle hastalıkta olası tüm etkenlerin tespiti ve ortaya konması ile daha kalıcı önlemlerin alınmasına imkân sağlayacağından önemli görülmektedir.

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## Synthesis and Characterization of ZnO@Fe<sub>3</sub>O<sub>4</sub> Composite Nanostructures by Using Hydrothermal Synthesis Method

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### Keywords

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 ZnO@Fe<sub>3</sub>O<sub>4</sub>,  
 Morpho-  
 structural  
 characteriza-  
 tion,  
 Medical  
 imaging,  
 Magnetic and  
 optical  
 properties

**Abstract:** In this study, ZnO@Fe<sub>3</sub>O<sub>4</sub> composite nanostructures were synthesized using the hydrothermal method. X-ray diffraction analysis was performed for the structural characterization of nanostructures obtained with the addition of Fe<sub>3</sub>O<sub>4</sub> at different ratios, and no impurity peaks were found. Scanning electron microscope (SEM) and transmission electron microscope (TEM) were used for morphological imaging. It was understood that ZnO nanoparticles were decorated around Fe<sub>3</sub>O<sub>4</sub> in the morphology of nanostructures. Fe, Zn, and O peaks were detected in elemental analysis. Energy band gaps of ZnO@Fe<sub>3</sub>O<sub>4</sub> nanocomposite structures were obtained from absorbance data collected by use of UV-VIS spectrometer. The band gap values of nanostructures were calculated to be in the range of 2-2.1 eV. Magnetic properties were determined using a vibrating sample magnetometer (VSM), and the values of 3.76 emu/g and 7.96 emu/g were found depending on the Fe<sub>3</sub>O<sub>4</sub> content. Although these values show a limited ferromagnetic property, they are important in optoelectronic and medical imaging applications due to the advanced optical and electronic properties of ZnO.

## Hidrotermal yöntem kullanılarak ZnO@Fe<sub>3</sub>O<sub>4</sub> kompozit nanoyapıların sentezi ve karakterizasyonu

### Anahtar Kelimeler

Nanokompozit  
 ZnO@Fe<sub>3</sub>O<sub>4</sub>,  
 Morfo-yapısal  
 karakterizasyon,  
 Medikal  
 görüntüleme,  
 Manyetik ve  
 optik  
 özellikler

**Öz:** Bu çalışmada, ZnO@Fe<sub>3</sub>O<sub>4</sub> kompozit nanoyapılar hidrotermal yöntem kullanılarak sentezlenmiştir. Farklı oranlarda Fe<sub>3</sub>O<sub>4</sub> katkısıyla elde edilen nanoyapıların yapısal karakterizasyonu için X-ışını kırınım analizi gerçekleştirildi ve herhangi bir safsızlık pikine rastlanmadı. Morfolojik görüntüleme taramalı elektron mikroskobu (SEM) ve geçirimsiz elektron mikroskobu (TEM) kullanıldı. Nanoyapıların morfolojisinde Fe<sub>3</sub>O<sub>4</sub> etrafında ZnO nano taneciklerinin dekore edildiği anlaşılmıştır. Elemental analizde ise Fe, Zn ve O pikleri kaydedilmiştir. ZnO@Fe<sub>3</sub>O<sub>4</sub> nanokompozit yapıların bant aralığı enerjileri Uv-Vis spektromotresi aracılığıyla elde edilen absorbans datalarından elde edilmiştir. Nanoyapıların bant gap değerleri yaklaşık olarak 2-2.1 eV aralığında hesaplanmıştır. Manyetik özellikler ise Vibrating Sample Magnetometer (VSM) kullanılarak tespit edildi ve Fe<sub>3</sub>O<sub>4</sub> katkı oranına bağlı olarak 3.76 emu/g ile 7.96 emu/g değerleri bulundu. Bu değerler sınırlı bir ferromanyetik özellik göstermesine rağmen ZnO'nun sahip olduğu gelişmiş optik, elektronik özelliklerinden dolayı optoelektronik ve medikal görüntüleme uygulamalarında önem arz etmektedir.

### 1. INTRODUCTION

Nanostructures, which are the basic elements of nanoscience and nanotechnology, have come to the fore as important engineering materials in composite, semiconductor, photocatalytic, magnetic, metal oxide, and biomedical applications with their structural, physical, chemical, and biological properties. Metallic-based nanostructures have received intense attention

from both scientific and industrial community [1]. As a matter of fact, magnetic information storage has gained a place in a wide range of areas from industrial applications such as biomedicine and catalysis to new applications in photonics, imaging, and biomedical field [2]. Metallic or metal oxide nanostructures have been extensively studied in recent years due to their specific surface area and crystallinity. Metallic nanostructures stand out in technological product developments with their tunable and improvable properties such as melting

points, electrical and thermal conductivity, light absorption and scattering properties, optical sensitivity, photocatalytic activity, and wettability. Zinc oxide (ZnO), which is a metal oxide, is a biocompatible metal oxide resistant to corrosion and oxidation with its cheap raw material, high crystallinity, synthesis at low temperatures, high optical transmittance, and electrical conductivity. With these significant properties, it has become an important engineering material for optoelectronics, solar cells, detectors, biosensors, drug delivery, and biomedical applications [3]. Iron-based magnetic nanoparticles, especially  $\text{Fe}_3\text{O}_4$  as another important metal oxide, are important materials used in ceramics, catalysis, magnetic information storage, medical imaging, drug delivery, biomedical applications, and various electronic applications with their natural magnetic properties. Thus, thanks to the unique and compatible properties of their structures,  $\text{Fe}_3\text{O}_4$  and ZnO allow the production of a higher performance hybrid or composite nanostructure in future applications. In previous studies, sol-gel [4, 5], co-precipitation [6], deposition [7], wet milling [8], microwave assisted [9], and wet chemical [10] methods were used for the synthesis of ZnO/ $\text{Fe}_3\text{O}_4$  nanocomposites.

ZnO based nanostructures show good electric, optoelectronic, and catalytic properties [11-13].  $\text{Fe}_3\text{O}_4$  nanoparticles illustrates superparamagnetic and/or ferromagnetic properties depending on their size [14]. Decorating ZnO nanoparticles with  $\text{Fe}_3\text{O}_4$  give them magnetic characteristics which enhance the application range of the ZnO nanoparticles [6,10]. Therefore, particles electrical and electronically active ZnO nanoparticles with good magnetic properties can be used in different applications such as magnetic filtering, magnetic catalysis materials, etc [15]. Recently, the hydrothermal method has started to be used for producing nanosized hybrid materials. This method allows the synthesis of different hybrid structures under suitable pressure and temperature conditions without disturbing the chemical structure of the materials [16]. In the present study, the structural, morphological, optoelectronic, and magnetic properties of ZnO@ $\text{Fe}_3\text{O}_4$  nanostructures obtained in different ratios (1:1 and 1:2) using the hydrothermal method were investigated in detail. SEM (scanning electron microscopy) and TEM (transmission electron microscopy) were used in the structural analysis. EDS (energy dispersive spectra) and X-ray diffraction methods were used in the analysis of chemical composition. UV-vis spectroscopy was used to assess the energy band gaps. Vibrational sample magnetometry was used in the investigation of the magnetic characteristics.

## 2. MATERIALS AND METHODS

### 2.1. Materials and Measurements

Iron(II) sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , Sigma-Aldrich), zinc acetate ( $\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$ , Sigma-Aldrich) salts, and urea ( $\text{CO}(\text{NH}_2)_2$ , Sigma-Aldrich, PEG (polyethylene glycol; MW=4,000), NaOH (sodium hydroxide) were used for the synthesis of ZnO@ $\text{Fe}_3\text{O}_4$  nanostructures. X-

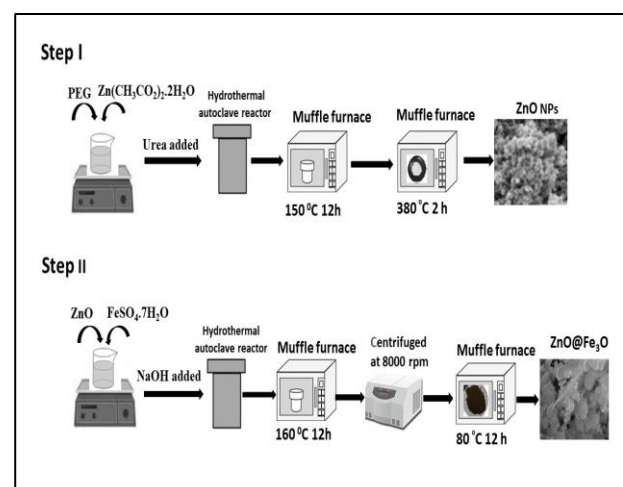
ray diffraction (XRD) patterns (RIGAKU miniflex600), scanning electron microscope (SEM-HitachiSU3500), high resolution transmission electron microscopy (HR-TEM, Joel 2100 F), energy dispersive x-ray spectroscopy (EDS, Oxford), UV-Vis spectrum (UV-1800 ENG240V), and vibrating sample magnetometer (Cryogenic Limited PPMS) were used for structural, morphological, elemental, optical, and magnetic characterizations, respectively.

### 2.2. Synthesis of ZnO Nanoparticles

After 1 g of PEG was dissolved in 20 mL of water, 2.19 g of  $\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$  salt was added. After dissolution, 0.5 M 20 mL urea solution was added dropwise. The resulting mixture was transferred to 50 mL capacity Teflon lined stainless steel autoclaves and then heated in an oven at 150 °C for 12 hours. After spontaneous cooling to room temperature, the precipitate was rinsed with distilled water and ethanol and dried at 80 °C for 12 hours. The resulting solid was annealed in a muffle furnace at 350 °C for 2 hours (with a temperature increase of 2 °C/min).

### 2.3. Synthesis of Magnetic ZnO@ $\text{Fe}_3\text{O}_4$ Composite Nanostructures

0.2 g of ZnO was dispersed in 20 mL of deionized water in an ultrasonic bath. In a separate beaker, 0.328 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was dissolved in 20 mL of deionized water. 1.6 g of NaOH was added to it, and it was mixed for 10 minutes. Then the prepared solution was poured onto ZnO. It was mixed in the ultrasonic bath for 5 more minutes, taken to 50 mL capacity Teflon autoclave coated with stainless steel, and kept at 150 °C for 12 hours. The resulting black precipitate was collected by centrifugation at 8000 rpm for 20 minutes by use of a centrifuge and washed with deionized water and absolute ethanol several times. The precipitate was kept at 80 °C for 12 hours ( $\text{ZnO}@\text{Fe}_3\text{O}_4$ -1:1). The same procedures were repeated for 0.656 g of  $\text{Fe}_3\text{O}_4 \cdot 7\text{H}_2\text{O}$  and 3.2 g of NaOH ( $\text{ZnO}@\text{Fe}_3\text{O}_4$ -1:2) [17-19]. Also, such process are illustrated schematically in Fig. 1.



**Figure 1.** Schematic representation of synthesized ZnO@ $\text{Fe}_3\text{O}_4$  nanostructures using hydrothermal method



### 3. RESULTS AND DISCUSSION

#### 3.1. Morpho-structural Characteristic of Nanostructures

The XRD graph of ZnO, Fe<sub>3</sub>O<sub>4</sub>, ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:1), and ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:2) nanostructures synthesized by the hydrothermal method is given in Figure 2. As can be understood from the XRD diffraction patterns obtained, peaks characteristic to the ZnO and Fe<sub>3</sub>O<sub>4</sub> structures were also observed in ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures with 1:1 and 1:2 weight ratios. In the ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:2) diffraction, the characteristic peaks became more evident with the increase of Fe<sub>3</sub>O<sub>4</sub> content. However, the ZnO peak intensity decreased. In addition, the lack of any impurity peak in the XRD diffraction patterns in these structures indicates that they have high crystallinity. The resulting XRD patterns were seen to be consistent with the ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures obtained by different methods in the literature, but they gave better results in terms of impurity [5,6], [20-22].

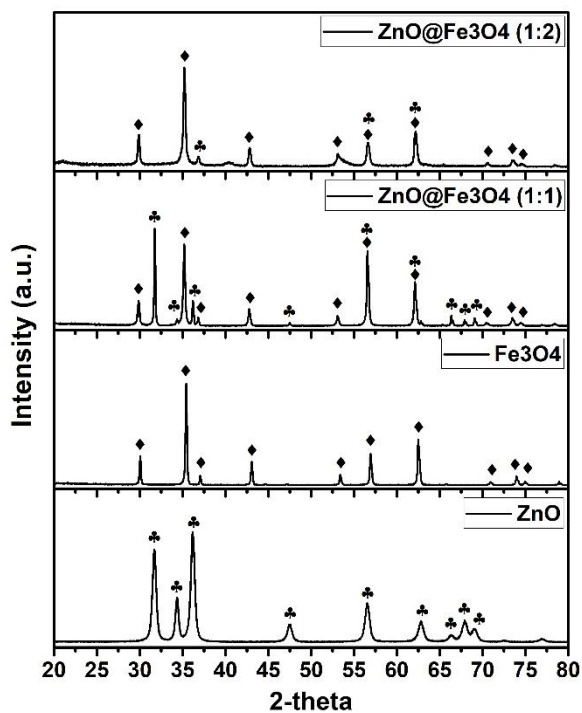


Figure 2. XRD spectra of ZnO, Fe<sub>3</sub>O<sub>4</sub>, and ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures

The synthesized SEM images of the ZnO nanostructures containing different weight ratios of Fe<sub>3</sub>O<sub>4</sub> are shown in Figure 3a and Figure 3b. As shown in the SEM images, the ZnO and Fe<sub>3</sub>O<sub>4</sub> structures were detected to have composite character. In addition, TEM analysis was carried out to check the nano dimensions of the composite structures. The presence of different types of structures was observed in the TEM analysis, as shown in Figure 4a and Figure 4b. It was understood that 10-20 nm irregular spherical ZnO NPs accumulated around Fe<sub>3</sub>O<sub>4</sub> structures with 50-100 nm amorphous branches [22].

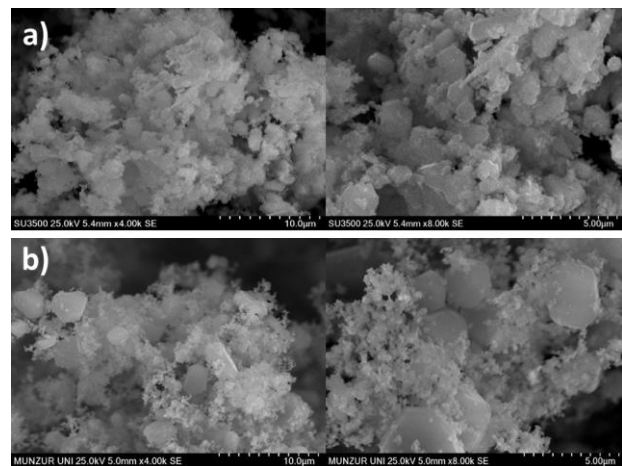


Figure 3. SEM photos of ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:1), ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:2)

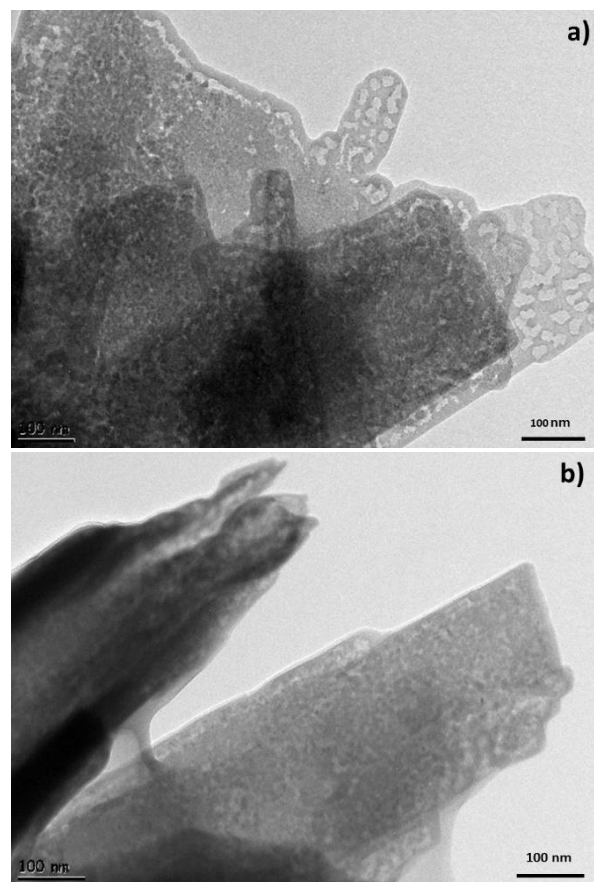
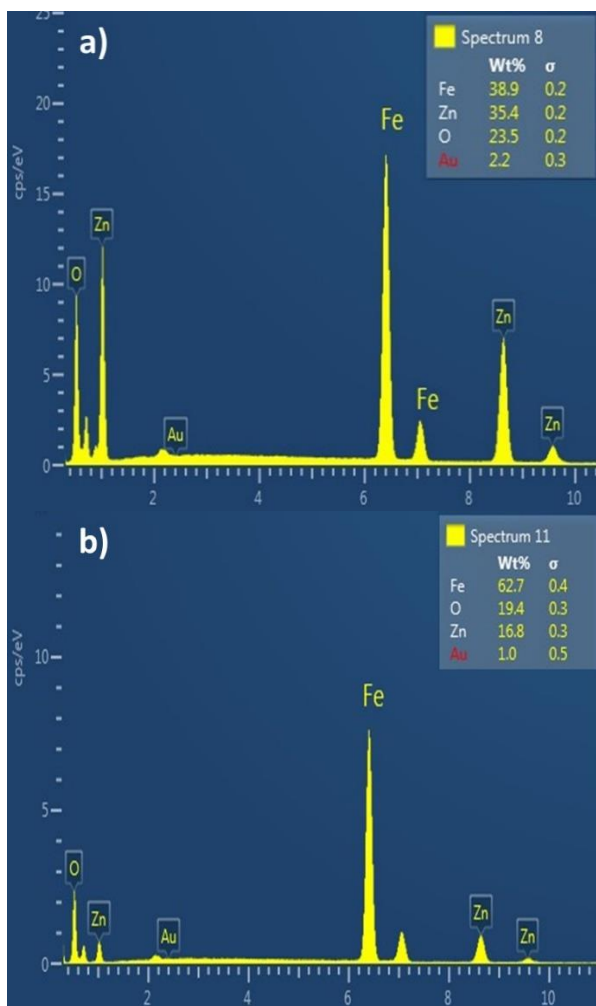


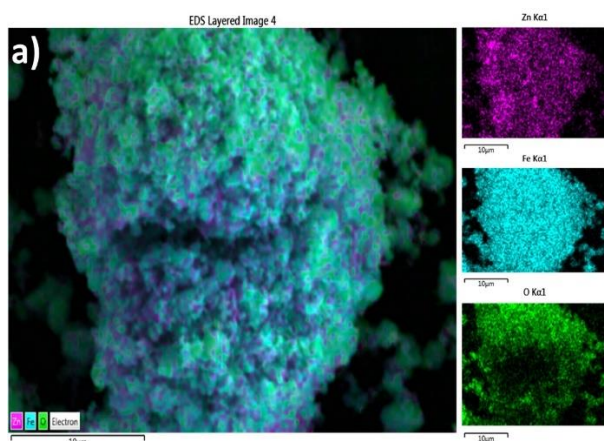
Figure 4. TEM photos of (a)-ZnO@Fe<sub>3</sub>O<sub>4</sub>(1:1), (b)-ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:2)

The EDS-spot and EDS-mapping results of the hydrothermally synthesized ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures are shown in Fig. 5 and Fig. 6, respectively. Fe, Zn, O were observed in the obtained EDS spectrum as shown in Figure 5a and Figure 5b. Before the SEM investigations Au sputtering was applied to the samples which is a standard procedure used to enhance the performance of the SEM. This is procedure cause a visible Au peak in the EDS spectra. Such a peak is not related to the structure of the nanoparticles. While the percentages of the Fe, Zn, and O elements in the ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:1) nanostructure were 38.9, 35.4, and 23.5, respectively, they were observed as 62.7, 16.8, and 19.4 in the ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:2) nanostructure. Addition of Fe<sub>3</sub>O<sub>4</sub> at different weight ratios nearly doubled the Fe

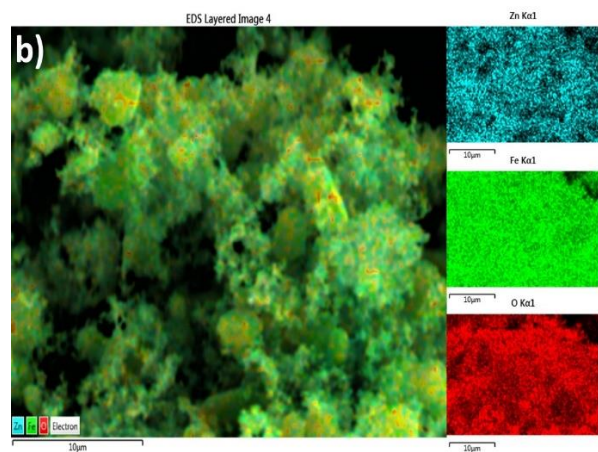
ratio in the structure. Moreover, in the EDS-mapping images given in Figure 6a and 6b, color intensities are presented based on the presence and content of the Fe, Zn, and O phases in the ZnO@Fe<sub>3</sub>O<sub>4</sub> composite structure.



**Figure 5.** EDS spectrum of (a)-ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:1), (b)-ZnO@Fe<sub>3</sub>O<sub>4</sub> (1:2)



**Figure 6.** (a) EDS-mapping spectrum of (a)-ZnO@Fe<sub>3</sub>O<sub>4</sub>(1:1) nanostructure



**Figure 6.** (b) EDS-mapping spectrum of ZnO@Fe<sub>3</sub>O<sub>4</sub>(1:2) nanostructure

### 3.2. Optical Properties of ZnO@Fe<sub>3</sub>O<sub>4</sub> Nanostructures

The optical absorbance data of the hydrothermally produced ZnO, Fe<sub>3</sub>O<sub>4</sub>, and ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures scanned in the range of 200 nm-800 nm are shown in Figure 7a,b,c,d. When the absorbance spectrum of the ZnO nanostructure was examined, no significant difference was observed. This can be attributed to the very small nanosize of ZnO nanoparticles [23]. In Figure 7b, the absorbance peak of the Fe<sub>3</sub>O<sub>4</sub> nanostructure is recorded as approximately 436 nm. These peaks are consistent with those obtained in the literature [17],[24]. The absorbance peaks of ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures were detected to be 235 nm, 325 nm, and 380 nm, respectively (Figure 7c-d). Furthermore, optical band gaps of the nanostructures were estimated from Einstein's energy equation (1) [17, 25-27].

$$E_g^{nano} = hc/\lambda \quad (1)$$

where  $E_g^{nano}$  is the energy band gap of the nanoparticles,  $c$  is the speed of light,  $h$  is Planck's constant, and  $\lambda$  is the cut off wavelength obtained from the absorption spectra. The direct energy band gap of the ZnO nanostructure in Figure 6a was obtained as 2.3 eV. In general, single crystal ZnO band gap is accepted as 3.34 eV. The value obtained in the present study was considerably lower than the value of 3.34 eV. The low values obtained in similar studies in the literature were attributed to the defects in the nanostructure [28]. The optical energy band gap of the Fe<sub>3</sub>O<sub>4</sub> nanostructure was found to be 2 eV, which is consistent with the literature [17, 29]. Moreover, as shown in Figure 7c-d, the energy band gap of ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures was determined around 2.1 eV.



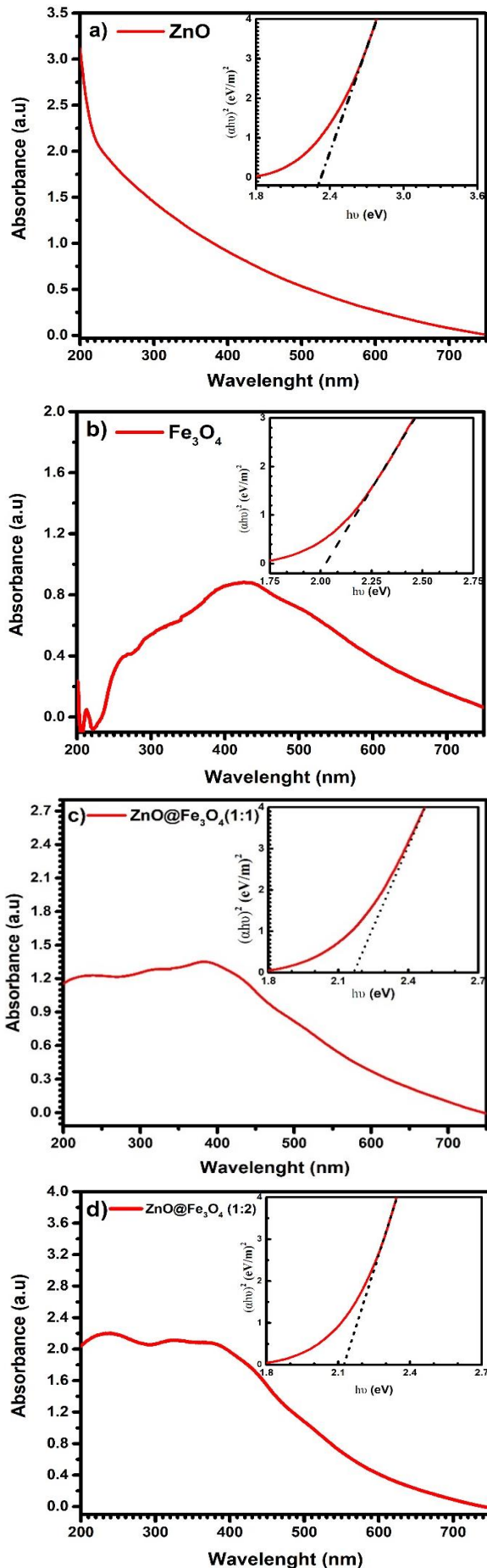


Figure 7. Absorbance and band gap characteristics of ZnO (a),  $\text{Fe}_3\text{O}_4$  (b),  $\text{ZnO@Fe}_3\text{O}_4$  -1:1 (c) and  $\text{ZnO@Fe}_3\text{O}_4$  -1:2 (d) nanostructures

### 3.3. Magnetic Properties of $\text{ZnO@Fe}_3\text{O}_4$ Nanostructures

Magnetic hysteresis curves of  $\text{ZnO@Fe}_3\text{O}_4$  (1:1) and  $\text{ZnO@Fe}_3\text{O}_4$  (1:2) nanocomposite structures are shown in Figure 8a and Figure 8b. While the magnetic saturation value of the nanostructure synthesized at a ratio of 1:1 was obtained as 3.76 emu/g, as shown in Figure 8a, that of the nanostructure synthesized at a ratio of 1:2 was obtained as 7.97 emu/g, as shown in Figure 8b. With the doubling of the  $\text{Fe}_3\text{O}_4$  content by weight ratio, that value approximately doubled as well. However, these values are lower than those found just for the  $\text{Fe}_3\text{O}_4$  nanostructures in the literature. In their magnetic saturation measurements of  $\text{Fe}_3\text{O}_4@Bi_2S_3$  nanoflowers synthesized by the hydrothermal method, Yetim et al., found a value of 89.8 emu/g for the  $\text{Fe}_3\text{O}_4$  nanostructure [17]. In a similar study, Karacam et al. reported the magnetic saturation values of the  $\text{Fe}_3\text{O}_4$  nanostructures as 87.7 emu/g and 90.7 emu/g [18]. The magnetic value of the ZnO additive obtained in this study decreased considerably. Sin et al. found the magnetic saturation value as 2.81 emu/g in the  $\text{ZnO@Fe}_3\text{O}_4$  nanostructure they synthesized with a facile surfactant-free method [30]. Długosz et al., reported the magnetic saturation value of the  $\text{Fe}_3\text{O}_4/\text{ZnO}$  nanostructure they synthesized with a microwave reactor as 9.5 emu/g [22]. This study is consistent with the values obtained in the literature. It is also known that the ZnO structure does not have good magnetic properties. However, it was revealed that a ferromagnetic property was shown through the addition of ZnO to the  $\text{Fe}_3\text{O}_4$  structure. These results are important especially in terms of the use of  $\text{ZnO@Fe}_3\text{O}_4$  nanostructures as contrast agents in medical imaging applications [31, 32].

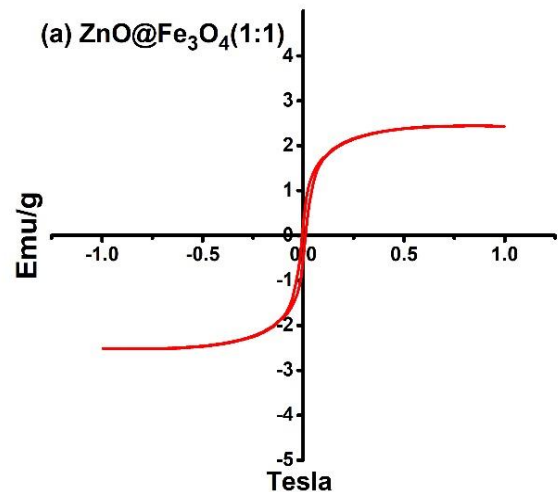


Figure 8. (a) Magnetic hysteresis of  $\text{ZnO@Fe}_3\text{O}_4$  -1:1 nanostructure

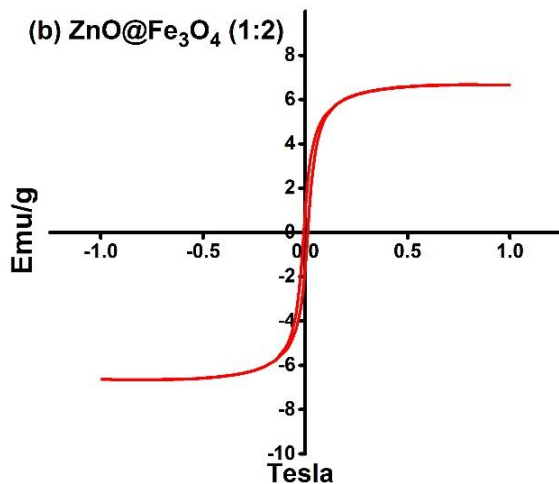


Figure 8. (b) Magnetic hysteresis of ZnO@Fe<sub>3</sub>O<sub>4</sub> -1:2 nanostructure

#### 4. CONCLUSIONS

This study investigated the structural, morphological, elemental, optical, and magnetic properties of ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures synthesized by the hydrothermal method. ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures were successfully produced by the hydrothermal method. The ZnO and Fe<sub>3</sub>O<sub>4</sub> structures were defined in the structural characterization, and no impurity peaks were found. In the morphological images, the presence of composite nanostructures was revealed by both SEM and TEM microscopy. The energy band gaps of the synthesized ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures were calculated as approximately 2.1 eV. This value is important in terms of being a potential nanomaterial in solar cell, photodetector, photocatalysis, sensor, supercapacitor, and optoelectronic applications, especially with its use in semiconductor technology. In the magnetic susceptibility measurements, the values of 3.76 emu/g and 7.97 emu/g were calculated for ZnO@Fe<sub>3</sub>O<sub>4</sub> nanostructures. Although these values show a limited ferromagnetic property, they may contribute to the medical imaging applications of ZnO additive with advanced optical properties.

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## A Macroanatomic Study on Coronary Arteries and its Branches in Southern Karaman Sheep: Corrosion Casting Technique and Latex Method

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### Keywords

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Southern  
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Coronary artery,  
Corrosion  
casting,  
Latex

**Abstract:** This study was carried out to reveal the coronary arteries and their branches that provide vascularization of the heart in Southern Karaman sheep. Eight Southern Karaman sheep were used in the study. The coronary arteries and their branches providing arterial vascularization of the heart were determined using latex injection and corrosion casting technique. Arteria coronaria sinistra and its branches were observed to be more dominant than arteria coronaria dextra. It was determined that after the origin of arteria coronaria sinistra, it splits into two main branches as ramus interventricularis paraconalis and ramus circumflexus sinister. It was determined that arteria coronaria dextra continued on its way as ramus circumflexus dexter after reaching the sulcus coronarius and gave branches to ensure the vascularization of the region tissues. This study aimed to reveal the coronary arteries and their branches in Southern Karaman of Turkey's domestic sheep breeds, and it is thought that it will contribute to new studies on this breed.

## Güney Karaman Koyunlarında Koroner Arterler ve Dalları Üzerine Makroanatomik Bir Çalışma: Korozyon Kast Tekniği ve Lateks Yöntemi

### Anahtar Kelimeler

Anatomi,  
Güney  
Karaman  
koyunu,  
Koroner  
arter,  
Korozyon  
kast,  
Lateks

**Öz:** Bu çalışma Güney Karaman koyununda kalbin vaskularizasyonunu sağlayan koroner arterler ve dallarının ortaya çıkarılması amacıyla yapıldı. Çalışmada 8 adet Güney Karaman ırkı koyun kullanıldı. Lateks enjeksiyon ve korozyon kast teknikleri kullanılarak kalbin arteriyel vaskularizasyonunu sağlayan koroner arterler ve dalları belirlendi. Arteria coronaria sinistra ve dallarının arteria coronaria dextra'ya göre daha dominant olduğu gözlemlendi. Arteria coronaria sinistra'nın orijininin sonra ramus interventricularis paraconalis ve ramus circumflexus sinister olarak 2 ana dala ayrıldığı belirlendi. Arteria coronaria dextra'nın ise sulcus coronarius'a vardikten sonra yoluna ramus circumflexus dexter olarak devam ettiği ve seyri esnasında bölge vaskularizasyonunu sağlamak üzere dallar verdiği tespit edildi. Bu çalışmada Türkiye'nin yerli koyun ırklarından biri olan Güney Karaman koyununda koroner arterler ve dalları ortaya konulmaya çalışılmış olup, çalışmanın bu ırk üzerine yapılacak olan yeni çalışmalara katkı sağlayacağı düşünülmektedir.

### 1. INTRODUCTION

The Southern Karaman sheep is a separate breed formed as a result of crossbreeding Karagül rams with Akkaraman and Dağlıç sheep brought by the nomads (Türkmen) who migrated from Türkistan to the Mediterranean during the Ottoman period. Southern Karaman Sheep is highly productive in terms of meat and milk and is a frequently preferred breed inbreeding [1,2].

The heart is vascularized by the arteria (a) coronaria dextra and arteria coronaria sinistra, which originate from the sinus aorta portion of the ascending aorta [3-7]. Arteria coronaria sinistra is more dominant in cattle [8], buffalo [9], sheep [10], and dog [11]. Arteria coronaria dextra is more dominant in goats [12], pigs [13], donkeys [14], and 90% of humans [15].

After arteria coronaria sinistra originates from the aorta, it reaches the sulcus coronarius by going downwards and slightly to the left between the truncus pulmonalis and



auricula sinistra [16,17,18]. At the level of this sulcus, it divides into two main branches: ramus circumflexus sinister and ramus interventricularis paraconalis [19,20]. The branches given by ramus interventricularis paraconalis are ramus coni arteriosi, ramus collateralis sinister proximalis, ramus collateralis sinister distalis, and rami septales during its course [21]. Ramus circumflexus sinister gives to feed atrium sinistrum ramus proximalis atri sinistri, ramus intermedius atri sinistri and ramus distalis atri sinistri [7] to feed ventriculus sinister ramus proximalis ventriculi sinistri, ramus marginis ventricularis sinistri and ramus distalis ventriculi sinistri [22].

Arteria coronaria dextra originates from the beginning of the aorta at the level of the valvula semilunaris dextra [7]. After reaching the sulcus coronarius, it continues as ramus circumflexus dexter [7,10,11]. During the course of the arteria coronaria dextra, it is also divided into branches as ramus proximalis atri dextri, ramus intermedius atri dextri, ramus distalis atri dextri, ramus coni arteriosi, ramus proximalis ventriculi dextri, ramus marginis ventricularis dextri, and ramus distalis ventriculi [6,7]. This study aimed to reveal the coronary arteries and their branches that provide arterial vascularization of the heart in Southern Karaman of Turkey's domestic sheep breeds.

## 2. MATERIAL AND METHOD

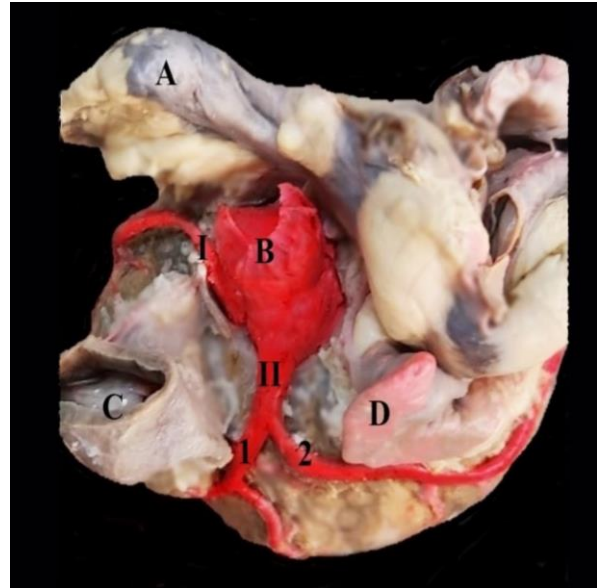
The present study was admitted by Atatürk University Local Ethics Committee (2021-23). In the study, eight Southern Karaman sheep were obtained from Konya Bahri Dağdaş International Agricultural Institute to be used in the study. The arteria carotis communis in the neck region of the sheep [23] were cut under xylazine HCl (0.2 mg/kg/IV) and ketamine HCl (2.2 mg/kg/IV) anesthetized. Then, their blood was drained, and coronary arteries and their branches were washed with 0.9% physiological saline. Afterward, a latex mixture colored with red acrylic dye was applied to the coronary arteries from the ascending aorta by the latex injection method [24]. They were kept in 10% formaldehyde solution for 72 hours for fixation. The dissected coronary arteries and their branches were named based on Nomina Anatomica Veterinaria [25] and photographed to illustrate vessels.

To create cast models of the coronary arteries, an acrylic solution prepared as 80% liquid (polymethylmetachrylate) and 20% powder (monomethylmetachrylate) was colored with red dye, which was injected from the ascending aorta. The tissues were kept in water overnight and then incubated in a 20% potassium hydroxide solution (KOH) solution at 37°C for 24 hours. The coronary arteries and their branches, which were cast and dissected, were photographed and named based on Nomina Anatomica Veterinaria [25].

## 3. RESULTS

The ascending aorta started from the ventriculus sinister. According to the study, it gave arteria coronaria dextra and arteria coronaria sinistra from the sinus aorta section to provide arterial vascularization of the heart immediately after its origin. It was observed that both main branches were partially intramyocardial (Figure 1, Figure 2a,c, and Figure 3a,b,c).

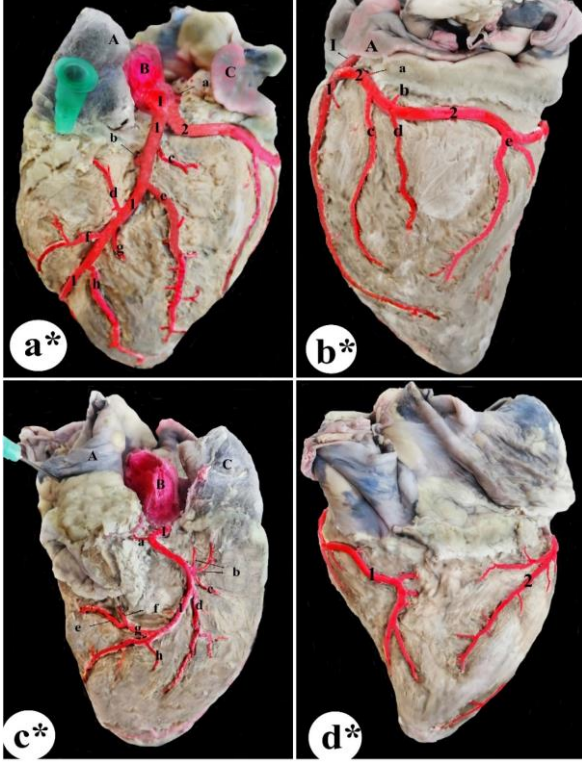
Compared to the arteria coronaria dextra, the arteria coronaria sinistra separated from the aorta ascendens in the section that overlaps the valvula semilunaris sinistra as a thicker root in all animals used in the study. The ramus proximalis atri sinistri of arteria coronaria sinistra fed the atrium sinistrum shortly after its formation from the aorta ascendens. After that, the arteria coronaria sinistra split into two more main branches, the ramus interventricularis paraconalis, and the ramus circumflexus sinister (Figure 1, Figure 2a, and Figure 3a,b,c).



**Figure 1.** Aorta ascendens and its branches in the heart of the South Karaman sheep, A: auricula dextra, B: aorta ascendens, C: truncus pulmonalis, D: auricula sinistra I: arteria coronaria dextra, II: arteria coronaria sinistra, 1: ramus interventricularis paraconalis, 2: ramus circumflexus sinister.

The ramus interventricularis paraconalis first proceeded subepicardially in the sulcus interventricularis paraconalis, caudoventrally towards the apex cordis, with the vena cordis magna. It was seen that it gave branches to facies atrialis before reaching incisura apicis cordis along its path. Ramus interventricularis paraconalis gives branches towards facies auricularis. These branches are from proximal to distal; proximal branch to ventriculus sinister, ramus collateralis proximalis, distal branch to ventriculus sinister and ramus collateralis distalis. After feeding the ventriculus sinister, the ramus collateralis proximalis spread to the proximal 1/3 of the ventriculus sinister. The ramus collateralis distalis was found to be dispersed, supplying the ventriculus sinister's middle and distal 1/3. Ramus septalis, the branch it gave to feed ramus coni arteriosi, and ventriculus dexter, the branch it gave to feed the

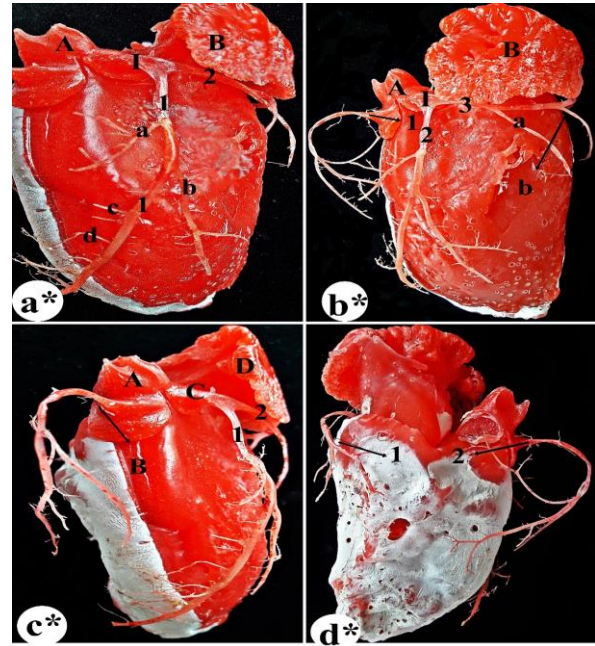
conus arteriosus region, were the branches that ramus interventricularis paraconalis gave respectively from proximal to distal from proximal to facies atrialis after its origin. In addition to these, it was determined that ramus interventricularis paraconalis gave many rami septales branches to feed the septum interventriculare during its course (Figure 2a and Figure 3a).



**Figure 2.** Branches of arteria coronaria sinistra and arteria coronaria dextra in the heart of South Karaman sheep with the latex method, a\*: ramus interventricularis paraconalis and its branches; A: truncus pulmonalis, B: aorta ascendens C: auricula sinistra I: arteria coronaria sinistra, a: ramus proximalis atrii sinistri, b: ramus septalis, c: proximal branch of ramus interventricularis paraconalis for ventriculus sinister, d: ramus coni arterio , e: ramus collateralis proximalis, f: branch of ramus interventricularis paraconalis to ventriculus dexter, g: distal branch of ramus interventricularis paraconalis to ventriculus sinister h: ramus collateralis distalis, 1: ramus interventricularis paraconalis, 2: ramus circumflexus. b\* ramus circumflexus sinister and its branches; A: auricula sinistra, I: arteria coronaria sinistra. a: ramus intermedius atrii sinistri, b: ramus distalis atrii sinistri, c: ramus proximalis ventriculi sinistri d: ramus marginis ventricularis sinistri,, e: ramus distalis ventriculi sinistri, 1: ramus interventricularis paraconalis, 2: ramus circumflexus sinister. c\*: arteria coronaria dextra and its branches; A: auricula dextra, B: aorta ascendens, C: truncus pulmonalis, I: arteria coronaria dextra, a: ramus proximalis atrii dextri, b: ramus coni arteriosi, c: ramus proximalis ventriculi dextri, d: ramus marginis ventricularis dextri, e: ramus distalis atrii dextri, f: ramus intermedius atrii dextri, g: ramus intermedius atrii dextri ve ramus distalis atrii dextri'nin ortak kökü, h: ramus proximalis ventriculi dextri. d\*: ramus interventricularis subsinuosus and ramus circumflexus dexter; 1: ramus interventricularis subsinuosus, 2: ramus circumflexus dexter.

Arteria circumflexus sinister, the other of the two main branches of the arteria coronaria sinistra, proceeded laterally on the heart's facies auricularis within the sulcus coronarius. It was observed that it provided ramus intermedius atrii sinistri to vascularize the atrium sinistrum along its dorsal surface during its course. It was discovered that it gave rise to the ramus proximalis ventriculi sinistri, a thicker branch that feeds the ventriculus sinister from the caudoventral direction.

After continuing its course for a while, it was determined that one of them originated from its dorsal side and gave ramus distalis atrium sinistri to feed the atrium sinistrum. The other one, which was thicker and separated from the ventral face, proceeded in the caudoventral direction and gave ramus marginis ventricularis sinistri to feed the ventriculus sinistri. The ramus marginis ventricularis sinistri originated shortly after the margo ventricularis and spread to the proximal 1/3 of the ventriculus sinistri, according to research. The ramus circumflexus sinister was observed to continue its route for a bit of time before splitting into two more branches that came from a single root on its ventral surface and proceeded in a caudoventral direction. These branches, which gradually spread up to the distal 1/3 of the ventriculus sinistri, were found to be ramus distalis ventriculi sinistri. After giving all these branches, ramus circumflexus sinister tended towards caudoventrally and continued on its way as ramus interventricularis subsinuosus in sulcus interventricularis subsinuosus. In the meantime, it was observed that it gave thin side branches to feed the tissues of the region (Figure 2b,d and Figure 3b,d).



**Figure 3.** Branches of arteria coronaria sinistra and arteria coronaria dextra in the heart of South Karaman sheep with Corrosion Casting Technique, a\*: ramus interventricularis paraconalis and its branches; A: aorta ascendens, B: auricula sinistra, I: arteria coronaria sinistra, a: ramus septalis, b: ramus collateralis proximalis, c: ramus coni arteriosi, d: branch of ramus interventricularis paraconalis to ventriculus dexter, 1: ramus interventricularis paraconalis, 2: ramus circumflexus sinister. b\* ramus circumflexus sinister and its branches; A: aorta ascendens, B: auricula sinistra, I:arteria coronaria sinistra., a: ramus proximalis ventriculi sinistri, b: ramus marginis ventricularis sinistri, 1: arteria coronaria dextra, 2: ramus interventricularis paraconalis, 3: ramus circumflexus sinister. c\*: arteria coronaria dextra and arteria coronaria sinistra; A: aorta ascendens, B: arteria coronaria dextra, C:arteria coronaria sinistra D: auricula sinistra 1: ramus interventricularis paraconalis, 2: ramus circumflexus sinister. d\*: ramus interventricularis subsinuosus and ramus circumflexus dexter; 1: ramus interventricularis subsinuosus, 2: ramus circumflexus dexter.Şekil, grafik, resim açıklamasında numaradan sonra nokta kullanılmalı ve bu noktaya kadar olan kısım (nokta dâhil) koyu yazılmalıdır. Açıklama kısımlarında metin Times New Roman, 8 punto, sola hizalı olarak yazılmalı ve görsel ile açıklama satırı arasında boşluk olmamalıdır. Görsel ile önceki paragraf arasında bir satır boşluk olmalıdır.

It was found that arteria coronaria dextra moved between the truncus pulmonalis and the auricula dextra, originating from the aorta ascendens at the level of the valvula semilunaris dextra. It was discovered that it inclined towards the facies atrialis and passed through the sulcus coronarius, under the subepicardial adipose tissue. It continued as ramus circumflexus dexter after reaching the sulcus coronarius. The ramus proximalis atri dextri was discovered to be the thin branch that arteria coronaria dextra gave towards the dorsocranial to feed the atrium dextrum. It continued over the ventral aspect of the arteria coronaria dextra. It continued towards the facies auricularis, showing ramus coni arteriosus, which is responsible for the arterial vascularization of the conus arteriosus region and separated with ramus proximalis ventriculi dextri in two branches in five of the materials used in the study. Three branches are separated from a single root as two branches. The ventriculus dexter was fed over the caudoventral side of the arteria coronaria dextra after the ramus proximalis ventriculi dextri was separated. This branch proceeded intramyocardially in the middle levels of the ventriculus dexter, parallel to the sulcus interventricularis paraconalis. The ramus marginis ventricularis dextri was found as the branch that separated from the caudoventral of the arteria coronaria dextra and proceeded parallel to the sulcus interventricularis subsinuosus to feed the ventriculus dexter. This branch proceeded across the middle 1/3 of the ventriculus dexter and had branching along the way. Ramus intermedius atri dextri and ramus distalis atri dextri were discovered at the level of margo ventricularis dexter to be branches that ramus circumflexus dexter gave towards dorsocranial to feed the atrium dextrum. From a common root, these two branches were split off. It was found that ramus distalis ventriculi dextri, which proceeds towards caudoventral on the ventral surface of ramus circumflexus dexter, is involved in the arterial vascularization of ventriculus dexter. Various numbers of thin lateral branches were separated from this branch in order to ensure the vascularization of the region during its course (Figure 2c,d and Figure 3c,d).

#### 4. DISCUSSION

The study was similar to the literature findings [6,7,10,17,26]. As a result, it was discovered that arteria coronaria dextra et sinistra originating from the aorta ascendens provides arterial vascularization of the heart in Southern Karaman sheep. Unlike our findings, Aksoy et al. [27] found that a separate third coronary artery accompanied the arterial vascularization of the heart. In contrast to research on buffalo, donkey, Malakan horse, and goat [9,14,28,29], arteria coronaria sinistra had a thicker layer than arteria coronaria dextra in our study. Similar to us in the most investigations in goat [30], Roe deer [19], and sheep [6,7], arteria coronaria sinistra was found to be thicker than arteria coronaria dextra. In the literature, it was observed that nine out of ten Awassi sheep [27], Hasak sheep [7], nine out of 14 Kıvrıkcık sheep [10], five out of ten Hemşin and Tuj sheep each [6], ramus proximalis atri sinistri was separated from arteria coronaria sinistra. In this study,

the literature findings of arteria coronaria sinistra [7,8,9,14,20,26,31,32] were similarly divided into ramus interventricularis paraconalis and ramus circumflexus sinister. Monfared et al. [18], on the other hand, found that, unlike our study findings, arteria coronaria sinistra was divided into three branches in 18.5% of cats.

Unlike our study findings, Doğruer and Özmen [10] reported that ramus interventricularis paraconalis terminated in 10 of the materials in Kıvrıkcık sheep, and Gürbüz and Aksoy [6] reported that it was terminated in facies auricularis in 6 of 10 Tuj and 7 of 10 Hemşin sheep. Similar to the literature [7,8], it was observed in the study that ramus interventricularis paraconalis ends by turning to facies atrialis before reaching incisura apicis cordis. Teke et al. [7] reported that, in accordance with our study findings, ramus collateralis sinister proximalis originates from the caudoventral of ramus interventricularis paraconalis and distributes to the upper 1/3 of the ventriculus sinister, while ramus collateralis sinister distalis originates from ramus interventricularis paraconalis in the caudoventral direction and it provides nutrition to the middle and distal 1/3 of the ventriculus sinister by taking it. Unlike the Hasak sheep [7], it was seen that a distal branch was separated from the ramus interventricularis paraconalis in the caudolateral direction to the ventriculus sinister after giving the ramus collateralis proximalis branch in the study. Despite the conclusions of the study, Aksoy ve Karadağ [17], Christensen and Campeti [31], and Gürbüz and Aksoy [6] revealed that in 5 Tuj and 4 Hemşin sheep, the ramus septalis was separated from the arteria coronaria sinistra. In contrast to the literature [7], the ramus coni arteriosus was identified as the second branch of the ramus interventricularis paraconalis, giving way to the facies atrialis after the ramus septalis.

In contrast to our findings, Gürbüz and Aksoy [6] found that ramus intermedius atri sinistri was lacking in one of the materials and two of the Hemşin sheep in his study on Tuj sheep, and that ramus distalis atri sinistri was responsible for vascularization of the region. Parallel to our study findings, in the literature [8,17], it was found that ramus proximalis ventriculi sinistri originates from the ventral aspect of the ramus circumflexus sinister. In addition, contrary to our findings, this artery sometimes originates from the angle between the ramus interventricularis paraconalis and the ramus circumflexus sinister in Kıvrıkcık sheep [10], in Tuj sheep [6] and Hemşin sheep [6]. According to Aksoy et al. [33], r. proximalis ventriculi sinistri was not found in two of five fox hearts. According to Doğruer and Özmen [10], the ramus marginis ventriculi sinistri is the second branch that separates from the ramus circumflexus sinister in the caudoventral direction, originates alone in 14 sheep, which is similar to our findings. Together with ramus distalis ventriculi sinistri in six sheep, which is contrary to our results. In addition to this, Gürbüz and Aksoy [6] stated that this artery started with ramus proximalis ventriculi sinistri in four Tuj and three Hemşin sheep, ramus distalis ventriculi sinistri in one Tuj and three Hemşin sheep, and in other materials the artery started from ramus circumflexus sinister as a



single root. Unlike the findings of the study on Hasak sheep [7], it was observed that ramus distalis ventriculi sinistri started in two branches. Similar to the study findings, it has been reported that ramus circumflexus sinister continues on its way as ramus interventricularis subsinuosus in sulcus interventricularis subsinuosus in cattle [8], sheep [10] and Hasak sheep after giving all these branches.

As reported in studies on alpaca [26], Hasak sheep [7] and Tuj and Hemşin breed sheep [6], arteria coronaria dextra originates from the aorta ascendens and proceeded between the truncus pulmonalis and auricula dextra, after reaching the sulcus coronarius, it continued on its way as ramus circumflexus dexter. Unlike our study findings, it has been reported in the literature [14,17] that arteria coronaria dextra is more dominant. Similar to the findings of the study [6,7,8], it has been reported in the literature that ramus proximalis atrii dextri, ramus coni arteriosi, ramus proximalis ventriculi dextri, ramus marginis ventricularis dextri originated from arteria coronaria dextra. Unlike the findings in the literature [6-8], Özüdoğru [7] and our study revealed that ramus intermedius atrii dextri originated from ramus circumflexus dexter instead of arteria coronaria dextra. Contrary to the study findings, ramus coni arteriosi originates from the aorta ascendens in Kıvrıkcık sheep [10] and dog [34], and in Zavot cattle [35] and Malakan horse [28], it has been reported to originate as a single branch from arteria coronaria dextra. In 5 of the materials studied, ramus coni arteriosi, ramus proximalis ventriculi dextri, and ramus proximalis ventriculi dextri came from a common root, similar to what was found in 3 Hasak sheep [7], 6 Hemşin sheep, and 4 Tuj sheep [6]. In Hasak breed sheep [7], Zavot breed cattle [35], and Malakan horses [28], it has been discovered that it originates in two branches from a single root separate from arteria coronaria dextra.

## 5. CONCLUSION

In the study, arterial vascularization of the heart was also provided by arteria coronaria dextra et sinistra and its branches in South Karaman sheep, but unlike arteria coronaria dextra et sinistra and its branches reported in the literature, in South Karaman sheep, it has been revealed that;

-Arteria coronaria sinistra is more dominant than arteria coronaria dextra,

-Ramus coni arteriosi is the second branch that separates from ramus interventricularis paraconalis,

-Ramus distalis ventriculi sinistri originates from ramus circumflexus sinister in two branches,

-Ramus intermedius atrii dextri originates from ramus circumflexus dexter.

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## Power Generation Variation Analysis of Solar Panels Coated With TiO<sub>2</sub>

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### Anahtar Kelimeler

Renewable energy, Solar panels, Pollution, Photocatalytic materials, Titanium dioxide

**Abstract:** Solar energy, which is an inexhaustible, clean and easily accessible energy source, can be converted into electrical energy with the help of photovoltaic (PV) panels. Environmental factors such as dust and dirt cause pollution of PV panels and decrease the efficiency of energy conversion. One of the methods used to reduce the negative effect of dirt on panel efficiency is to coat the surface of the panels with photocatalytic materials. Oxygen and nanoparticles are formed on photocatalytic surfaces with the help of ultraviolet rays in sunlight. These particles form a chemical reaction between the coating and the surface, breaking down and destroying the dirt on the surface. In this study, the effect of titanium dioxide (TiO<sub>2</sub>) as a photocatalytic material on the efficiency of solar panels was investigated. Experimental studies were carried out in Bingöl city using two 285 W polycrystalline solar panels. One of the panel surfaces is coated with TiO<sub>2</sub> and no treatment has been applied to the surface of the other panel. When the measured data were analyzed, it was seen that while the powers of the two panels were almost the same at the beginning, with the contamination of the panels, the power obtained from the TiO<sub>2</sub> coated panel was up to 19% higher. In addition, it was observed that the excess power produced as a result of cleaning the PV panels after rainy days decreased again.

## TiO<sub>2</sub> ile Kaplanan Güneş Panellerinin Güç Üretim Değişimi Analizi

### Keywords

Yenilenebilir enerji, Güneş panelleri, Kirlenme, Fotokatalitik malzemeler, Titanyum dioksit

**Öz:** Tükenmeyen, temiz ve kolay erişilebilen bir enerji kaynağı olan güneş enerjisi fotovoltaiik (PV) paneller yardımıyla elektrik enerjisine dönüştürülebilmektedir. Toz ve kir gibi çevresel faktörler PV panellerin kirlenmesine ve enerji dönüşüm verimlerinin düşmesine neden olmaktadır. Kirin panel verimi üzerindeki olumsuz etkisini azaltmak için kullanılan yöntemlerden biri panellerin yüzeyini fotokatalitik malzemelerle kaplamaktır. Fotokatalitik yüzeylerde güneş ışığındaki ultraviyole ışınları yardımıyla oksijen ve nano partiküller oluşur. Bu partiküller, kaplama ile yüzey arasında kimyasal bir reaksiyon oluşturarak yüzeydeki kirleri parçalar ve yok eder. Bu çalışmada, fotokatalitik malzeme olarak titanyum dioksitin (TiO<sub>2</sub>) güneş panellerinin verimi üzerine etkisi incelenmiştir. Deneysel çalışmalar Bingöl İlinde iki adet 285 W polikristal güneş paneli kullanılarak yapılmıştır. Panel yüzeylerinden biri TiO<sub>2</sub> ile kaplanmış ve diğer panelin yüzeyine herhangi bir işlem uygulanmamıştır. Ölçülen veriler incelendiğinde, başlangıçta iki panelin güçleri hemen hemen aynıken panellerin kirlenmesiyle birlikte TiO<sub>2</sub> kaplanmış panelden elde edilen gücün %19 a kadar daha fazla olduğu görülmüştür. Ayrıca yağmurlu günlerden sonra PV panellerin temizlenmesi sonucunda üretilen fazla gücün tekrar azaldığı görülmüştür.

### 1. INTRODUCTION

Population growth, industrialization, and the increase in electric powered devices raise the energy demand of countries. As a result of the use of fossil fuels, carbon

dioxide is released into the atmosphere. The increasing amount of carbon dioxide causes the greenhouse effect in the atmosphere and thus the average temperature of the world rises. An increase of 1 °C in temperature may lead to visible changes in the world's climate zones. Increases up to 3 °C may cause melting of polar ice caps,

rising of seas, drying up of lakes and agricultural drought [1,2]. In addition, many negative factors such as limited fossil energy resources, sudden price changes, foreign dependency in energy causing various political and economic problems make it necessary to turn to renewable energy resources. When the data of the rates of electricity generation from renewable energy sources of the Organization for Economic Cooperation and Development (OECD) countries between 1991 and 2017 are examined, it is observed that there is a continuous increase [3]. In Turkey, the amount of energy production from renewable energy sources has increased every year and today, half of the total installed power is renewable energy sources. As of the end of September 2019, the distribution of installed power by resources; 31.4 percent hydraulic energy, 28.6 percent natural gas, 22.4 percent coal, 8.1 percent wind, 6.2 percent solar, 1.6 percent geothermal and 1.7 percent is other sources [4].

In order to meet the increasing energy demand, the use of solar energy, which is eternal and clean energy, is becoming widespread. Compared to the damage done to the environment while producing electricity from fossil fuels, the production of electricity from solar energy with photovoltaic panels is an extremely clean and environmentally friendly method.

The panels absorb the sunlight falling on them and generate direct current depending on the efficiency of the cells. There are solar cells on solar panels proportional to the power of the panel. Panel efficiency is the ratio of the amount of electrical energy produced to the solar energy coming to the panel surface. There are many factors that affect the efficiency of the panel. Cell type, quality and design, and the quality of glass and other components affect the efficiency of the panel. In addition, factors such as temperature, amount of radiation, shading, angle of incidence of light also determine the amount of power to be produced in the panel.

The installation cost of photovoltaic systems is higher than other electricity generation systems. With the long-term use of the system, this cost can be compensated by spreading over the years. In addition, high efficiency of the system is also important for tolerating the cost.

Due to being outdoors, the surface of the panels is contaminated with particles such as dust, chemical dirt, leaves and bird droppings over time. Due to these dirt accumulated on the panel surface, less sunlight falls on the solar cells inside the panels. Depending on the decrease in the amount of light falling on the cells, the electrical energy produced by the panels also decreases [5]. There have been many studies examining the effect of dirt on panel efficiency [6-9].

The surfaces of the panels in the regions with low rainfall are dustier. Where there is excessive dust, a yield loss of 14% occurs. If the angle of the panels with the horizontal axis is greater than 16 degrees, it can be assumed that the panel surface has been cleaned with rain water. Contamination under these conditions is assumed to cause a yield loss of 6%. However, if the

angle of inclination is less than 16 degrees or if there is little rainfall in the region, and if more dirt accumulates on the panel surfaces due to dusting caused by agricultural and industrial activities in the region, this rate is greater than 6%. In a PV system whose surface is cleaned periodically, dust losses are around 5% [10,11].

In this study, the change of panel efficiency in the case of application of photocatalytic materials to solar panels under the conditions of climate, air pollution level, etc. of Bingöl has been revealed. There is no such study in the literature for this region before. In addition, the data obtained sets a precedent for other settlements with similar conditions.

## 2. MATERIALS AND METHOD

Wind is one of the factors affecting panel efficiency. Wind speed and intensity change the power produced by the panel. The temperature of the panel cells is taken into account in examining the efficiency of solar panels. The formula used for the relationship between temperature and wind is given in Eq.-1 [12].

$$T_c = T_a + \omega \times \left( \frac{0,32}{8,91 + 2 \times V_f} \right) \times I_T \quad (1)$$

Here,  $T_c$  is the panel cell temperature,  $T_a$  is the atmosphere temperature,  $\omega$  is the assembly coefficient,  $V_f$  is the wind speed, and  $I_T$  is the instantaneous radiation.

Another factor affecting panel efficiency is temperature. As the temperature increases, the output voltage of the panel decreases and the current increases a little. The output power of PV panels varies directly with the amount of radiation and inversely with the panel temperature. In other words, if the panel temperature increases, the panel power decreases. Many studies [13-17] and postgraduate theses [18-20] have been carried out both to examine the negative effects of temperature on panel efficiency and to eliminate these negative effects. The variation of panel power depending on temperature is given in Eq.-2 and Eq.-3 [21].

$$T_C = T_{ORT} + 0,0256 \times G \quad (2)$$

$$P_{PV-OUT} = P_{N-PV} \times \left( \frac{G}{G_{REF}} \right) \times [1 + K_T (T_C - T_{REF})] \quad (3)$$

Here;  $P_{PV-OUT}$  PV cell output power (W),  $P_{N-PV}$  nominal power (W) of PV cell under reference conditions,  $G$  solar radiation (W/m<sup>2</sup>),  $G_{REF}$  solar radiation ( $G_{REF}=1000$  W/m<sup>2</sup>) under reference conditions,  $K_T$  maximum power temperature coefficient ( $K_T=-3.7 \times 10^{-3}$  (1/°C) for mono and poly-crystalline Si),  $T_C$  PV cell temperature (°C),  $T_{ORT}$  ambient temperature (°C),  $T_{REF}$  PV cell temperature under reference conditions ( $T_{REF}=25$  °C) ) represents.

Equation-2 and Equation-3 show that temperature has a direct effect on panel power. If calculations are made using these equations, it will be seen that the panel output power will be 221.695 W at 30 °C ambient



temperature and 226.32 W at 25 °C ambient temperature. This difference for each panel will reach a significant value in large power solar power plants.

### 2.1. Photocatalysis Phenomenon and Photocatalytic Materials

Photocatalysis and photosynthesis can be compared to each other. By absorbing the sun's rays falling on chlorophyll in plants, it converts water and CO<sub>2</sub> into oxygen and glucose. Similarly, the photocatalyst absorbs the sun's rays by forming radicals (oxide, peroxide and hydroxyl radicals), which are strongly oxidizing species on its surface, and allows the formation of harmless species such as water and CO<sub>2</sub> by breaking down harmful organic molecules. Photocatalysis and photosynthesis events, which are compared in this way, are simply shown in Figure 1 [22].

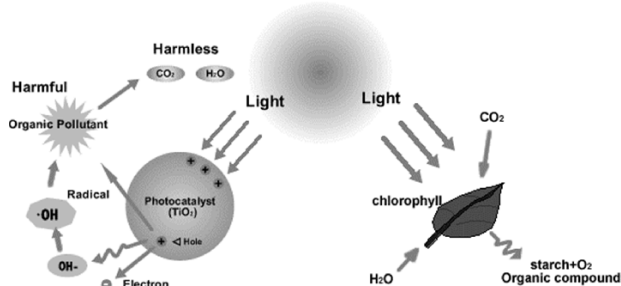


Figure 1. Photocatalysis process as compared to photosynthesis [23]

Recently, panels are coated with nano coating method. With this method, the panel surface is covered with a layer containing particles such as nano-sized titanium dioxide (TiO<sub>2</sub>), silicon dioxide (SiO<sub>2</sub>), etc. Thus, many different superior properties such as self-cleaning, easy-to-clean, antimicrobial, non-abrasive, scratch-proof and corrosion-proof are given to the material [24,25].

Coating methods are implemented by dipping, spinning, spray, plasma and chemical vapor precipitation methods [24]. Surfaces coated with these methods are called hydrophobic (water-repelling), hydrophilic (water-attracting) or photocatalytic surfaces. The water droplet falling on the surface does not spread on hydrophobic surfaces, but takes the shape of a sphere, while it spreads on hydrophilic surfaces.

TiO<sub>2</sub> is a semiconductor material and has three crystal structures: brookite, anatase and rutile. In photocatalytic applications, TiO<sub>2</sub> in the anatase crystal structure is mostly used.

Thin films containing TiO<sub>2</sub> have many advanced features such as self-cleaning at nanoscale, enabling the creation of antibacterial surfaces, preventing odors, non-fogging, etc., due to their photocatalytic properties and very good hydrophilic surface. Thanks to these superior properties, the use of TiO<sub>2</sub> films has become quite common recently. On the surfaces on which these films are coated, organic dirt adhered to the surface is broken down with the help of UV light, and thanks to the hydrophilic feature, water molecules flow on the surface in the form of a thin film and the fragmented dirt is

easily removed from the surface. This allows the surface to be self-cleaning.

### 2.2. Experimental Study

In experimental system, two polycrystalline solar panels of the same power (285 W) were placed in the construction at the same angle and an experimental application was made in the Central District of Bingöl (Latitude: 38,8851578 Longitude: 40,5072665 Altitude: 1.126 m). Photocatalytic surface consisting of titanium dioxide (TiO<sub>2</sub>) material is coated on one of the panels. No treatment has been applied to the surface of the other panel. As of 19.09.2021, the short circuit current and open circuit voltages of the panels were measured at approximately the same time every day, and the measurement results were recorded (Figure 2 and Figure 3). With the help of these measurements, the power produced by the solar panel covered with self-cleaning material (TiO<sub>2</sub>) and the power produced by the normal panel were compared.



Figure 2. Experimental system



Figure 3. Measurement of short circuit current and open circuit voltage

The short-circuit current and open-circuit voltages of the panels were measured with a measuring instrument every day and the measurement results were recorded (Annex: 1).

When the first measured data on 19.09.2021 are analyzed, it is seen that the power difference between the two panels is 6.64 watts and the percentage difference is 2.26%. However, in the measurements made on 22.09.2021, it is seen that the difference increases up to 10.2 watts and up to 19.74% as a percentage, depending on the contamination of the panels. After it rained on September 24, 2021, it was determined that the power produced on September 25 was close to each other again. With the rain, the power difference produced as a result



of cleaning the panels decreased. Again, when the data in the table is examined, it is seen that the power produced on cloudy days and in shady conditions decreases considerably. Based on the measurements made, the average power difference produced between the two panels in the measurements made on September 19-31 was calculated as 5.39%, 4.09% on October 1-31 and 3.18% on November 1-30.

### 3. RESULTS AND DISCUSSION

Panel powers for each month are shown in Annex-1 as a table and in Figure 4 graphically. When the measured data are examined, the power difference of the two panels was very close to each other at first, but in the following days, the difference approached 20% due to the pollution of the panels. In addition, it was observed that the power difference produced as a result of cleaning the panels after rainy days decreased again.

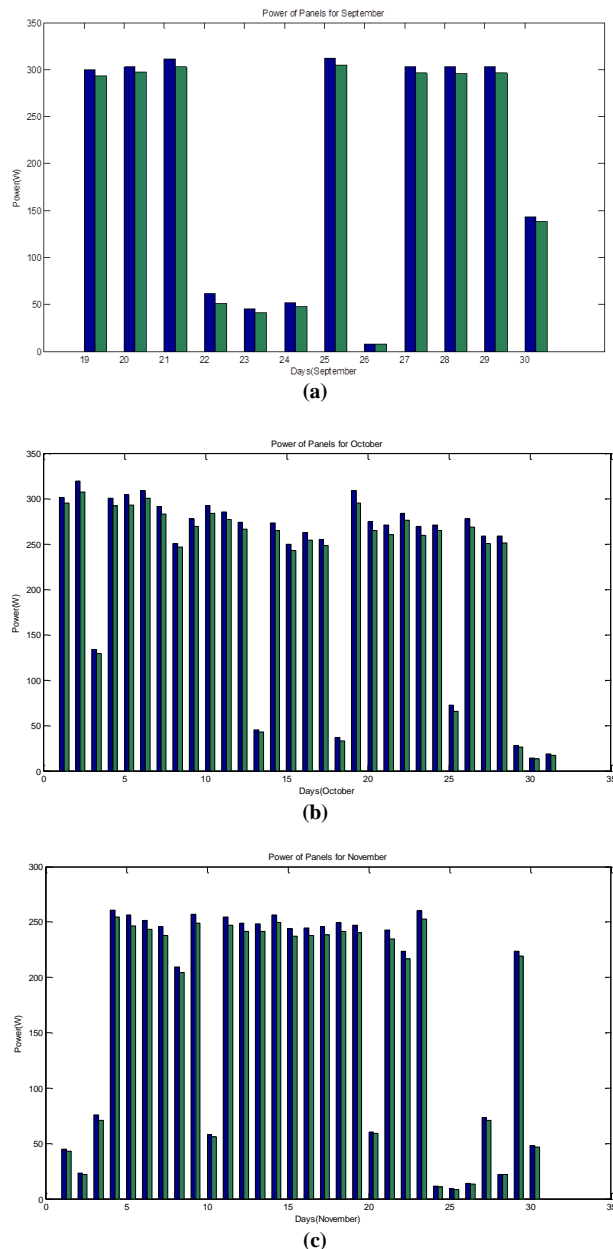


Figure 4. Power of panels (a) September (b) October (c) November

The power produced by the photocatalytic coated panel is greater than the power of the other panel under all conditions. In other words, it is seen in the measurements that the power produced by the photocatalytic coated panel is slightly higher than the power produced by the normal panel, both in sunny days, rainy weather and shady conditions, whether there is dirt or not. Because the light falling on the panel is less reflected and absorbed better thanks to the photocatalytic coating.

In addition, in September and October, panel powers were measured over 300 W on sunny days, but a maximum of 260 W was recorded in November. Although there is less pollution in November, the measurement of less power value can be explained by the temperature and wind factor.

Considering all the measured values, it is seen that the panels coated with photocatalytic material in the autumn season in Bingöl show a better efficiency than the normal panels. Although the percentage difference in yield increases to larger values on a daily basis, the seasonal average is around 4%. When the yield amount is compared on a monthly basis, the highest yield rate was 5.39% in September, while it decreased gradually in other months.

### 4. CONCLUSION

The obtained results show the effects of pollution, temperature and wind on panel efficiency. In addition, when the panel surface is covered with  $TiO_2$ , it is seen that the efficiency of the panels increases to a certain extent. The amount of change in yield was presented as daily, monthly and autumn season averages. Considering that the pollution is less in the fall and more in the summer, it is estimated that the production difference between the two panels will be higher in the summer months. In addition, Bingöl is a relatively underdeveloped city in terms of population and industrial development. It is predicted that in similar cities with this feature, the yield increase in  $TiO_2$  coated panels will be the same, while this difference will be higher in crowded and industrially developed cities.

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**ANNEX: POWER MEASUREMENT RESULTS OF PANELS****Table 1.** Panel power values measured in September

No	Date	Hour	Photocatalytic Panel			Normal Panel			Power Diff. (W)	Per (%)	Explanation
			Current (A)	Voltage (V)	Power (W)	Current (A)	Voltage (V)	Power (W)			
1	19.09.2021	12:41	9.04	33.2	300.1	8.84	33.2	293.49	6.64	2.26	Sunny-27 °C
2	20.09.2021	11:42	8.82	34.3	302.5	8.65	34.4	297.56	4.97	1.67	Sunny-27 °C
3	21.09.2021	12:40	9.19	33.9	311.5	8.92	33.9	302.39	9.15	3.03	Sunny-29 °C
4	22.09.2021	12:40	1.82	34	61.88	1.52	34	51.68	10.20	19.74	Cloudy-28 °C
5	23.09.2021	12:25	1.35	34	45.9	1.21	34	41.14	4.76	11.57	Cloudy-24 °C
6	24.09.2021	12:55	1.47	35.6	52.33	1.35	35.6	48.06	4.27	8.89	Rainy-13 °C
7	25.09.2021	11:45	9.08	34.4	312.4	8.87	34.4	305.13	7.22	2.37	Sunny-18 °C
8	26.09.2021	16:50	0.26	32.5	8.45	0.25	32.5	8.125	0.33	4.00	Sunny-20 °C
9	27.09.2021	12:55	9.04	33.6	303.7	8.82	33.6	296.35	7.39	2.49	Sunny-26 °C
10	28.09.2021	12:30	8.92	34	303.3	8.69	34	295.46	7.82	2.65	Sunny-26 °C
11	29.09.2021	12:55	8.71	34.8	303.1	8.47	35	296.45	6.66	2.25	Sunny-25 °C
12	30.09.2021	12:15	4.11	34.9	143.4	3.96	34.9	138.2	5.24	3.79	Cloudy-22 °C
<b>Average Difference</b>									<b>5.39</b>		

**Table 2.** Panel power values measured in October

No	Date	Hour	Photocatalytic Panel			Normal Panel			Power Diff. (W)	Per (%)	Explanation
			Current (A)	Voltage (V)	Power (W)	Current (A)	Voltage (V)	Power (W)			
1	01.10.2021	12:10	8.70	34.60	301.02	8.48	34.80	295.10	5.92	2.00	Sunny-23 °C
2	02.10.2021	12:55	9.20	34.70	319.24	8.81	34.90	307.47	11.77	3.83	Sunny-22 °C
3	03.10.2021	14:02	4.12	32.50	133.90	3.97	32.50	129.03	4.88	3.78	Cloudy-20 °C
4	04.10.2021	12:00	8.68	34.60	300.33	8.44	34.60	292.02	8.30	2.84	Sunny-19 °C
5	05.10.2021	12:40	8.82	34.50	304.29	8.49	34.50	292.91	11.39	3.89	Sunny-18 °C
6	06.10.2021	12:30	8.83	35.00	309.05	8.56	35.10	300.46	8.59	2.86	Sunny-16 °C
7	07.10.2021	12:55	8.50	34.30	291.55	8.22	34.40	282.77	8.78	3.11	Sunny-17 °C
8	08.10.2021	10:00	7.26	34.50	250.47	7.13	34.60	246.70	3.77	1.53	Sunny-17 °C
9	09.10.2021	13:25	8.15	34.10	277.92	7.88	34.20	269.50	8.42	3.12	Sunny-21 °C
10	10.10.2021	12:40	8.56	34.10	291.90	8.29	34.20	283.52	8.38	2.96	Sunny-22 °C
11	11.10.2021	12:30	8.55	33.40	285.57	8.29	33.40	276.89	8.68	3.14	Sunny-22 °C
12	12.10.2021	12:50	8.16	33.60	274.18	7.90	33.70	266.23	7.95	2.98	Sunny-22 °C
13	13.10.2021	12:00	1.32	33.90	44.75	1.25	34.00	42.50	2.25	5.29	Cloudy-24 °C
14	14.10.2021	12:00	8.12	33.60	272.83	7.85	33.70	264.55	8.29	3.13	Sunny-24 °C
15	15.10.2021	11:45	7.27	34.30	249.36	7.05	34.40	242.52	6.84	2.82	Sunny-25 °C
16	16.10.2021	12:00	7.72	34.00	262.48	7.46	34.10	254.39	8.09	3.18	Sunny-23 °C
17	17.10.2021	11:00	7.36	34.60	254.66	7.16	34.70	248.45	6.20	2.50	Sunny-21 °C
18	18.10.2021	11:45	1.06	34.50	36.57	0.96	34.60	33.22	3.35	10.10	Cloudy-16 °C
19	19.10.2021	12:30	8.77	35.20	308.70	8.36	35.30	295.11	13.60	4.61	Sunny-18 °C
20	20.10.2021	12:50	8.03	34.20	274.63	7.75	34.20	265.05	9.58	3.61	Sunny-19 °C
21	21.10.2021	13:10	7.87	34.40	270.73	7.57	34.40	260.41	10.32	3.96	Sunny-20 °C
22	22.10.2021	11:50	8.30	34.20	283.86	8.05	34.30	276.12	7.75	2.80	Sunny-19 °C
23	23.10.2021	13:00	7.73	34.80	269.00	7.44	34.90	259.66	9.35	3.60	Sunny-21 °C
24	24.10.2021	11:00	7.78	34.80	270.74	7.58	34.90	264.54	6.20	2.34	Sunny-19 °C
25	25.10.2021	12:35	2.02	35.70	72.11	1.82	35.80	65.16	6.96	10.68	Cloudy-14 °C
26	26.10.2021	13:00	7.72	36.00	277.92	7.44	36.10	268.58	9.34	3.48	Sunny-17 °C
27	27.10.2021	12:40	7.43	34.80	258.56	7.18	34.90	250.58	7.98	3.19	Sunny-16 °C
28	28.10.2021	12:30	7.40	35.00	259.00	7.15	35.10	250.97	8.03	3.20	Sunny-15 °C
29	29.10.2021	11:50	0.79	35.30	27.89	0.74	35.30	26.12	1.77	6.76	Rainy-10 °C
30	30.10.2021	12:40	0.41	34.60	14.19	0.38	34.70	13.19	1.00	7.58	Rainy-8 °C
31	31.10.2021	13:40	0.53	34.60	18.34	0.49	34.70	17.00	1.34	7.85	Rainy-11 °C
<b>Average Difference</b>									<b>4.09</b>		



**Table 3.** Panel power values measured in November

No	Date	Hour	Photocatalytic Panel			Normal Panel			Power Diff. (W)	Per (%)	Explanation
			Current (A)	Voltage (V)	Power (W)	Current (A)	Voltage (V)	Power (W)			
1	01.11.2021	16:15	1.28	34.80	44.54	1.23	34.80	42.80	1.74	4.07	Cloudy-13 °C
2	02.11.2021	12:40	0.67	35.00	23.45	0.62	35.10	21.76	1.69	7.76	Rainy-11 °C
3	03.11.2021	11:20	2.14	35.40	75.76	2.00	35.50	71.00	4.76	6.70	Cloudy-13 °C
4	04.11.2021	12:20	7.52	34.70	260.94	7.33	34.70	254.35	6.59	2.59	Sunny-21 °C
5	05.11.2021	11:10	7.32	35.00	256.20	7.02	35.10	246.40	9.80	3.98	Sunny-16 °C
6	06.11.2021	13:25	7.18	35.00	251.30	6.95	35.00	243.25	8.05	3.31	Sunny-20 °C
7	07.11.2021	13:00	7.03	35.00	246.05	6.80	35.00	238.00	8.05	3.38	Sunny-19 °C
8	08.11.2021	13:40	6.02	34.80	209.50	5.88	34.80	204.62	4.87	2.38	Sunny-19 °C
9	09.11.2021	12:30	7.41	34.70	257.13	7.17	34.70	248.80	8.33	3.35	Sunny-15 °C
10	10.11.2021	12:10	1.63	35.60	58.03	1.56	35.70	55.69	2.34	4.19	Cloudy-11 °C
11	11.11.2021	12:30	7.07	36.00	254.52	6.85	36.10	247.29	7.24	2.93	Sunny-13 °C
12	12.11.2021	11:15	7.01	35.50	248.86	6.79	35.60	241.72	7.13	2.95	Sunny-12 °C
13	13.11.2021	12:00	7.03	35.30	248.16	6.82	35.40	241.43	6.73	2.79	Sunny-14 °C
14	14.11.2021	12:00	7.29	35.20	256.61	7.07	35.30	249.57	7.04	2.82	Sunny-14 °C
15	15.11.2021	11:50	6.72	36.30	243.94	6.51	36.40	236.96	6.97	2.94	Sunny-13 °C
16	16.11.2021	11:30	6.95	35.20	244.64	6.73	35.30	237.57	7.07	2.98	Sunny-13 °C
17	17.11.2021	11:40	6.97	35.30	246.04	6.74	35.40	238.60	7.44	3.12	Sunny-13 °C
18	18.11.2021	12:15	7.05	35.40	249.57	6.83	35.40	241.78	7.79	3.22	Sunny-13 °C
19	19.11.2021	11:35	7.02	35.20	247.10	6.81	35.30	240.39	6.71	2.79	Sunny-12 °C
20	20.11.2021	12:35	1.64	36.70	60.19	1.61	36.80	59.25	0.94	1.59	Rainy-6 °C
21	21.11.2021	11:40	6.79	35.80	243.08	6.54	35.90	234.79	8.30	3.53	Sunny -12 °C
22	22.11.2021	13:30	6.26	35.70	223.48	6.07	35.70	216.70	6.78	3.13	Sunny -13 °C
23	23.11.2021	12:30	7.30	35.60	259.88	7.08	35.70	252.76	7.12	2.82	Sunny -14 °C
24	24.11.2021	11:20	0.32	34.80	11.14	0.31	34.90	10.82	0.32	2.93	Cloudy -6 °C
25	25.11.2021	12:10	0.26	34.40	8.88	0.25	34.50	8.63	0.25	2.90	Rainy -7 °C
26	26.11.2021	11:30	0.39	35.10	13.62	0.38	35.20	13.38	0.24	1.82	Rainy -6 °C
27	27.11.2021	12:30	2.01	36.39	73.14	1.95	36.43	71.04	2.11	2.96	Cloudy -8 °C
28	28.11.2021	12:35	0.62	35.50	22.01	0.61	35.60	21.72	0.29	1.35	Cloudy -8 °C
29	29.11.2021	12:40	6.20	36.10	223.82	6.06	36.20	219.37	4.45	2.03	Sunny -13 °C
30	30.11.2021	12:40	1.32	36.10	47.65	1.29	36.20	46.70	0.95	2.04	Cloudy-11 °C
<b>Average Difference</b>									<b>3.18</b>		



## Investigation of Biogas Production Potential from Livestock Manure by Anaerobic Digestion in Bingöl Province

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### Keywords

Alternative energy, Anaerobic digestion, Organic waste, Animal feces, Energy of household appliances

**Abstract:** Biogas is one of the sustained alternative energy sources. The annual biogas potential of Bingöl province was calculated according to the data of bovine, ovine and poultry animals between 2015-2020 years which were obtained from the Turkish Statistical Institute (TSI). In addition, liquid and available manure amount, biogas potential, electrical energy and heat energy amounts were determined according to the animal data of the districts in Bingöl province for 2020. It is estimated that the biogas energy potential between 2015-2020 years with 39.02 million m<sup>3</sup> will be the most in 2018, with 36.1 m<sup>3</sup> the least in 2019 and 36.5 million m<sup>3</sup> in 2020. In 2020, the biogas energy potential of the districts were found among; 263 thousand m<sup>3</sup>-12.65 million m<sup>3</sup>. It is clear that in Bingöl province in 2020 from a total number of 5694302 livestock, 865202 of which are bovine and ovine that approximately 754 thousand tons of utilizable manure, 36.5 million m<sup>3</sup> biogas, 171.4 GWh electrical energy and 171449\*10<sup>6</sup> kcal m<sup>-3</sup> heat energy can be obtained. A facility to be established in Bingöl will enable to increase the use of renewable energy sources, decrease methane gas emissions and meet energy needs of farmers or householders.

## Bingöl İli Hayvancılık Gübresinden Anaerobik Çürütme ile Biyogaz Üretim Potansiyelinin Araştırılması

### Anahtar Kelimeler

Alternatif enerji, Anaerobik çürütme, Organik atık, Hayvan gübresi, Ev aletleri enerjisi

**Öz:** Biyogaz, sürekliliği olan alternatif enerji kaynaklarından biridir. Türkiye İstatistik Kurumu (TÜİK)'nden alınan Bingöl iline ait 2015-2020 yılları arasındaki büyükbaş, küçükbaş ve kanatlı hayvan sayıları verilerine göre yıllık biyogaz potansiyeli hesaplanmıştır. Ayrıca, Bingöl ilinde bulunan ilçelerin 2020 yılı hayvan verilerine göre yaş ve kullanılabilir gübre miktarı, biyogaz potansiyeli, elektrik enerjisi ve ısı enerjisi miktarları belirlenmiştir. Elde edilen verilere göre, 2015-2020 yılları arasında biyogaz enerjisi potansiyelinin 39.02 milyon m<sup>3</sup> ile en fazla 2018 yılında, 36.1 m<sup>3</sup> ile en az 2019 yılında, 2020 yılında ise 36.5 milyon m<sup>3</sup> olabileceği görülmüştür. 2020 yılında Bingöl ilçelerindeki biyogaz enerjisi potansiyeli 263 bin m<sup>3</sup> - 12.65 milyon m<sup>3</sup> arasında değişmiştir. 2020 yılı içerisinde Bingöl ilindeki 865202'si büyükbaş ve küçükbaş olmak üzere toplam 5694302 hayvandan, yaklaşık 754 bin ton kullanılabilir gübre, 36.5 milyon m<sup>3</sup> biyogaz, 171.4 GWh elektrik enerjisi ve 171449\*10<sup>6</sup> kcal m<sup>-3</sup> ısı enerjisi elde edilebilecektir. Bingöl'de kurulacak biyogaz enerji üretim tesisleri, yenilenebilir enerji kaynaklarının kullanımının artırılmasına, metan gazı emisyonlarının azaltılmasına, çiftçilerin veya hanelerin bazı enerji ihtiyaçlarının karşılanmasına olanak sağlayacaktır.

## 1. INTRODUCTION

Depending on population growth and industrial development, energy demand is constantly increasing. The current amount of energy has become unable to meet this demand. This situation causes a continuous increase in energy prices. In addition, due to limited fossil fuel reserves, there will be difficulties in meeting energy needs in the coming years. Therefore, the demand for new and renewable energy sources has been increasing rapidly in the world as well as in Turkey in recent years, and many studies have been carried out seriously. Since using of renewable energy sources, there has been less damage to the nature and the use of fossil fuels is reduced [1, 2].

Global warming, which is one of the serious problems of nowadays, will have negative effects on our world in the future. Global warming and climate change occur with the increase of greenhouse gases (carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrogen oxide (NO) and water vapor (H<sub>2</sub>O)). The most serious reason for climate change is the uncontrolled increase in the amount of CO<sub>2</sub> in nature. Population growth have led to, the decrease in green areas, the increase in fossil fuel-powered motor vehicles and consequently increase in the amount of CO<sub>2</sub> released into the nature. This problem make policies eager to take an action against CO<sub>2</sub> amount globally. Renewable energy sources play the most important role in reducing the CO<sub>2</sub> emissions [3, 4]. The energy obtained from organic wastes is more advantageous than renewable energy sources such as solar, wind and geothermal. Because these wastes has continuity. Therefore, this energy source will be one of the most common renewable energy sources in the future. Biogas production from organic solid wastes is obtained by anaerobic digestion (AD), which is one of the most widely used and traditional biochemical methods, as shown in Figure 1. In addition, the conversion of AD organic wastes to biogas is the most preferred biotechnological method [5, 6, 7].



Figure 1. Waste recycling technologies [7].

Anaerobic digestion is a biological process in which anaerobic bacteria decompose organic matter and produce biogas in conditions with little or no oxygen. During the AD, nutrients are retained and digestion residues are made suitable to become an organic fertilizer that can replace mineral fertilizers that require fossil energy [8]. The main by-product of AD is the digested solid fraction, which is rich in nitrogen (N) content and can be used as fertilizer. As a result of AD process, the significant methane emission caused by the uncontrolled decomposition of organic waste into the atmosphere will be stopped and the emission of methane gas, which is 25 times more effective in retaining heat in the atmosphere than carbon dioxide, will be trapped [9, 10]. Anaerobic digestion is possible in principle at temperatures between 3 °C and about 70 °C. The differentiation generally varies between three temperature ranges respectively: the psychrophilic temperature is below 20 °C, the mesophilic temperature is

between 20 °C and 40 °C, and the thermophilic temperature is above 40 °C [11-13].

Since the interest in biogas potential obtained from animal manure has increased in recent years, biogas potential has been investigated for many provinces in Turkey as well. These provinces and studies are following as; Adana [14, 15], Ankara [16], Bitlis [17], Burdur [18], Bursa [19], Elazığ [20], Erzincan [21], Hatay [22], Isparta [23], Kahramanmaraş [24], Kayseri [25], Muş [26], Tekirdağ [27], Yozgat [28]. Organic matter which is consisted in animal manure are fermented and digested by microorganisms (bacteria, protozoa and archaea) under anaerobic conditions and produce CH<sub>4</sub> in 3 stages. Organic wastes (carbohydrates, proteins and fats) undergo fermentation by micro-organisms and decompose into hydrogen, carbon dioxide, acetic acid, butyric acid, propionic acid, various alcohols and other compounds. In the second stage H<sub>2</sub>, CO<sub>2</sub> and acetic acid are reacted by acetogenic bacteria. And in the final stage, methane and carbon dioxide gases are formed by methanogenic bacteria [12].

Biogas energy is a variable renewable that can be used to replace fossil fuels in energy and heat generation and can also be converted into transportation fuel after its purification. It is rich in methane and carbon dioxide can replace natural gas as a raw material for the production of other biochemicals [29, 30]. In addition, it has been preferred as one of the most energy productive and environmentally advantageous technologies for bioenergy production [31].

Biogas is a fuel gas which containing 50-70% methane (CH<sub>4</sub>), 25-50% carbon dioxide (CO<sub>2</sub>), 1-5% hydrogen (H<sub>2</sub>), 0.3-3% nitrogen (N<sub>2</sub>), traces of ammonia (NH<sub>3</sub>) and hydrogen sulfide (H<sub>2</sub>S) [32-34]. It can be produced from raw materials such as agricultural wastes, animal manure, municipal wastes, plant materials, sewages, green wastes or food wastes. And it is a renewable energy source and in mostly exerts a very small carbon footprint. It can be also used as a fuel to generate heat and electricity, or it can be injected into the gas grid as biomethane. This energy production creates more employment and livelihood in rural areas. It also plays a major role in reducing important greenhouse gases such as CO<sub>2</sub> and CH<sub>4</sub> [35]. By converting these two gases into biogas production instead of dispersing them into the atmosphere, the energy we need will be met without harming the environment, thus contributing to the reduction of global warming. The use of biogas energy may become advantageous when designing sustainable energy solutions in industrial applications, farms and households as seen in developing countries [31, 36].

The production of biogas is a process of gas formation as a result of biological decomposition of organic materials in anaerobic conditions. In a biogas production facility, livestock manure is diluted with water and transferred to an on-off tank. AD is carried out by bacteria, protozoa and archaea (microbiological flora) which are in different amount and in the structure of animal manure. Biogas is formed 10-15 days after the filling of the tank and this

production continues for about 60 days and then decreases. As a result of AD, gases accumulate in the upper part of the tank and organic fertilizers in the lower parts [12, 16].

In this study, the biogas potential that can be obtained from livestock manure in Bingöl province and its districts according to 2020 datas were determined. In addition, the biogas potential of Bingöl province the years between 2015-2020 was also calculated. The data of this study were obtained from Bingöl Provincial Directorate of Agriculture Forestry and Turkish Statistical Institute [38, 39]. By determining the number of animals in the provinces and districts, the amount of livestock manure that can be obtained according to the animal type was calculated. Hence the annual biogas and electricity potential of Bingöl province and energy savings were calculated theoretically.

## 2. MATERIALS AND METHODS

### 2.1. Geographical Characteristics of Bingöl Province

This study includes the borders of Bingöl province, which is located between 41-20° and 39-56° east longitudes and 39-31° and 36-28° north latitudes. Bingöl province, is located in the Eastern Anatolia Region of Turkey, has a total population of 281768, 165867 of which are in the central district, according to 2020 TSI datas. And that means 67.7% of the population lives in central district [40]. It has an area of 8004 km<sup>2</sup>, of which 3 million 137 thousand 710 decares (approximately 42% of the surface area) covers pastureland and 300 thousand decares of agricultural land [39]. As seen in Figure 2, Bingöl province consists of 8 districts in total namely; Adaklı, Genç, Karlıova, Kiğı, Center, Solhan, Yayladere and Yedisu. Livelihood of the province is animal husbandry. Locals earn a good income from the beekeeping sector with the sale of livestock and dairy products.



Figure 2. Geographic map of Bingöl province [40].

### 2.2. Animal Data

The animal data of Bingöl province for the year 2020 which is obtained from the Ministry of Agriculture and Forestry are given in Table 1. The number of animals was determined, based on this informations. The total number of ovine make up sheep with 73.4% and cattle make up with 99.95% of

bovine. According to TSI datas, in 2020 when animal husbandry was the highest numbers, the number of bovine decreased compared to the previous year, while the number of ovine and poultry increased compared to the previous years (Table 1). As it is clear that in Table 1, poultry constitute the majority of the number of animals in the province [39].

Table 1. The total number of livestock in Bingöl province between 2015–2020 [38, 39].

Years	Bovine	Ovine	Poultry	Total
2015	166695	817548	904404	1888647
2016	165849	859668	1832055	2857572
2017	182892	783043	2624772	3590707
2018	184670	792160	2582922	3559752
2019	197689	635603	2847660	3680952
2020	144550	720652	4829100	5694302

The district where sheep and goats breeding is carried out the most in 2020 is Karlıova with 348375 ovine numbers as mentioned in Figure 3. Karlıova is followed by Solhan with 147291 sheep and then Central districts with 101198 sheep and goats. The majority of bovine breeding is carried out in Bingöl central (60087), Karlıova (20986) and Solhan (19984) districts, respectively (Figure 3). Since the majority of poultry are in the Central district, it is considered the place where animal husbandry is most engaged in terms of the total number of bovine, ovine and poultry, with approximately 4.7 million animals [38]. Poultry breeding is more preferred in areas close to transit centers where transportation is easy, rather than rural areas [41]. Therefore, the number of poultry in the central district of Bingöl has a considerably higher number than other districts. It is also reported that the reason for the huge differences in the number of total animals by years is due to the grants given by the Ministry of Agriculture and Forestry in the relevant years [39]. Animal husbandry is carried out the least in Yayladere district with a total number of 8845 animals, including bovine, ovine and poultry. It is considered that the reason Kiğı, Yayladere and Yedisu total animal assets are less than other districts is to be proportional to the number of populations they have. In addition, the fact that there is such a great number of sheep and goats in Karlıova district can be shown among the results that the geographical structure of the district is more suitable for these type of animal breeding and the improvement projects carried out by the public [42].

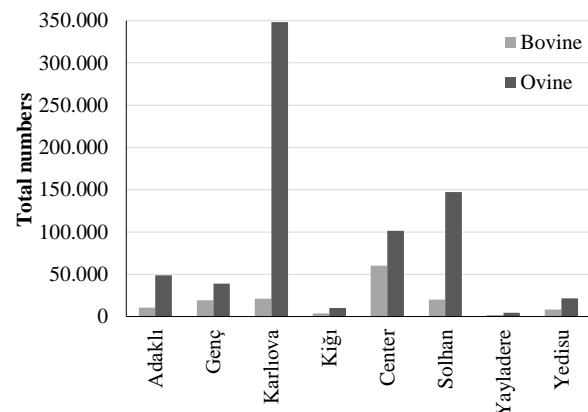


Figure 3. Ruminants distribution by districts in 2020.



The average accepted values of calculation the biogas energy potential are given in Table 2 below [20, 43]. It is accepted that approximately 1:3 of the manure obtained from animal wastes are destroyed by mixing with nature in the pastures, and only 2:3 is usable [20]. In the calculation; equations given in between Eq. 1-11 below are used [14-28].

**Table 2.** Acceptances for biogas energy potential [16, 37, 43, 44].

Animal Species	Annual Manure (tons an <sup>-1</sup> )	Biogas obtained from 1 tons of manure (m <sup>3</sup> )
Bovine	3.6 t	33 m <sup>3</sup>
Ovine	0.7 t	58 m <sup>3</sup>
Poultry	0.022 t	78 m <sup>3</sup>

Annual amount of liquid manure that animals can produce;

$$M_b = n * 3.6 \text{ tons an.}^{-1} \quad (1)$$

$$M_o = n * 0.7 \text{ tons an.}^{-1} \quad (2)$$

$$M_p = n * 0.022 \text{ tons an.}^{-1} \quad (3)$$

$$M_t = M_b + M_o + M_p \text{ tons an.}^{-1} \quad (4)$$

In the equations;  $n$ : Number of animals,  $M_b$ : Amount of liquid manure obtained from bovine animals,  $M_o$ : Amount of liquid manure obtained from ovine animals,  $M_p$ : Amount of liquid manure obtained from poultry,  $M_t$ : It expresses the total amount of liquid manure. Usable amount of liquid manure. ( $M_u$ );

$$M_u = M_t * 2 * 3^{-1} \text{ tons an.}^{-1} \quad (5)$$

Potential biogas energy ( $PB_e$ );

$$PB_{eb} = M_b * 2 * 3^{-1} * 33 \text{ m}^3 \text{ an.}^{-1} \quad (6)$$

$$PB_{eo} = M_o * 2 * 3^{-1} * 58 \text{ m}^3 \text{ an.}^{-1} \quad (7)$$

$$PB_{ep} = M_p * 2 * 3^{-1} * 78 \text{ m}^3 \text{ an.}^{-1} \quad (8)$$

$$PB_{et} = PB_{eb} + PB_{eo} + PB_{ep} \text{ m}^3 \text{ an.}^{-1} \quad (9)$$

In the equations given above;  $PB_{eb}$ : Biogas energy obtained from bovine,  $PB_{eo}$ : Biogas energy obtained from ovine,  $PB_{ep}$ : It refers to biogas energy obtained from poultry and  $PB_{et}$ : Total potential biogas energy.

Total potential electrical energy ( $PEE_t$ );

$$PEE_t = PB_{et} * 4.70 \text{ kWh an.}^{-1} \quad (10)$$

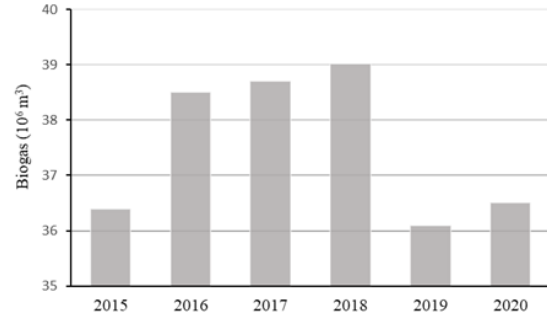
Total potential thermal energy ( $TPT_e$ ) is between values given below [14-28];

$$TPT_e = PB_{et} * 4700 \text{ kcal m}^{-3} - 5700 \text{ kcal m}^{-3} \text{ (approximately)} \quad (11)$$

### 3. RESULTS AND DISCUSSION

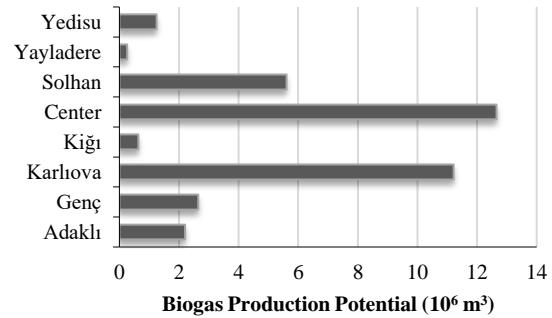
#### 3.1. Biogas Energy

The biogas potential according to the amount of manure that can be obtained from bovine, ovine and poultry between 2015-2020 in Bingöl province is given in Figure 4. It is seen in Figure 4 that the biogas production potential between these years could be at most in 2018 with 39.02 million m<sup>3</sup> and at least in 2019 with 36.1 million m<sup>3</sup>.



**Figure 4.** Distribution of biogas production potential of Bingöl province by years.

In 2020, approximately 36.5 million m<sup>3</sup> of biogas will be obtained from a total of 5694302 animal population. In Figure 5 below is given, the percentage (%) potential distribution of biogas that can be produced in the central and the other districts of Bingöl province in 2020. The majority of biogas energy in Bingöl province, 34.68% can be obtained from the Central, 30.73% Karlıova and 15.39% Solhan districts (Figure 5). The minimum biogas production potential in the province can be obtained in Yayladere district with 0.72% (Figure 5).



**Figure 5.** Distribution of biogas production potential by districts in 2020.

The Central district with a total number of 4665425 animals, including 60087 bovine, 101198 ovine and 4504140 poultry is the highest in number of animals. According to datas 12650.7\*10<sup>3</sup> m<sup>3</sup> of biogas energy is generated from 257.5\*10<sup>3</sup> tons of usable manure per year that can be obtained from the central district. In addition, the obtained biogas energy is equivalent to 59458.4\*10<sup>3</sup> kWh electrical energy or 59.5\*10<sup>9</sup> kcal thermal energy (Table 3).

**Table 3.** Theoretical biogas potential produced by animal species in the province and districts of Bingöl in 2020.

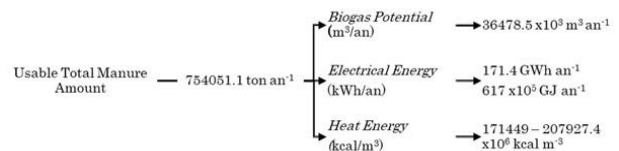
Districts	Num. of Animals	Usable Manure Amount	Biogas Potential	Electrical Energy	Heat Energy
	Head	10 <sup>3</sup> t an <sup>-1</sup>	10 <sup>3</sup> m <sup>3</sup> an <sup>-1</sup>	10 <sup>3</sup> kWh an <sup>-1</sup>	10 <sup>9</sup> kcal m <sup>-3</sup>
Adaklı	111356	48.5	2206.2	10369.2	10.4
Genç	107918	65.4	2647.4	12442.8	12.4
Karlıova	473047	214.5	11210	52687.3	52.7
Kiğı	80516	14.4	637.1	2994.7	2.9
Center	4665425	257.5	12650.7	59458.4	59.5
Solhan	204877	117.2	5612.4	26378.4	26.4
Yayladere	8845	6.4	262.9	1236	1.2
Yedisu	42318	30.1	1251.4	5881.9	5.9
<b>Total</b>	<b>5694302</b>	<b>754</b>	<b>36478.1</b>	<b>171448.7</b>	<b>171.4</b>

### 3.2. Energy Conversions

Bingöl with its all districts have a potential of 754051.1 tons liquid manure production per year from bovine, ovine and poultry. The comparison with other fuel types of 36478.5\*10<sup>3</sup> m<sup>3</sup> biogas which can be obtained from liquid manure in 2020 was made based on the following assumptions. According to the assumptions stated below, approximately 171.4 GWh of electrical energy and 171449-207927.4 x10<sup>6</sup> kcal m<sup>-3</sup> of heat energy are revealed from 36478.5\*10<sup>3</sup> m<sup>3</sup> of biogas (Figure 6). The energy equivalents obtained from 1 m<sup>3</sup> biogas are given in Table 4 below.

**Table 4.** The energy equivalents obtained from 1 m<sup>3</sup> of biogas [16, 20, 37, 43-46]:

<b>1 m<sup>3</sup> biogas</b>	4.70 kWh electrical energy
	4.700 – 5.700 kcal m <sup>-3</sup> heat energy
	0.43 kg butane gas
	12.3 kg turd
	3.47 kg wood
	0.8 L gasoline
	0.63 L gas oil
	1.46 kg charcoal energy

**Figure 6.** Energy conversions according to the total amount of biogas in 2020.

### 3.3. Electrical Energy

According to our calculations a house of 4 people consumes approximately 21.3 kWh electrical energy per day and 72.4 kWh per week, as shown in Table 5 below. And this consumption reaches about 7774.5 kWh per year. The electrical energy that can be obtained from animal manure in Bingöl province is 171448.7\*10<sup>3</sup> kWh year<sup>-1</sup> according to 2020 datas. This amount of electrical energy that can be obtained annually from biogas energy in Bingöl, has the power to meet the electricity approximate of 22052 households.

According to the January 2021 residential tariffs, 1 kWh of electricity is 0.7102 TL (Turkish lira) taxes included [47]. Accordingly, the annual electricity bill of a household is approximately 5521.4 TL. Thanks to biogas energy, Bingöl province has the potential to meet 121.7 million TL the electricity bill of per year.

**Table 5.** Electricity consumption of a household per week.

Household Appliances	P	Num.	DWT	DC	NWU	WC
	Watt-hour	1-10	Hours	Watt-hour	1-10	Watt-hour
Refrigerator (A+)	46	1	24	1104	7	7728
Dishwasher (A+)	1800	1	2	3600	5	18000
Wash. Machine (A+)	800	1	2	1600	3	4800
LCD TV (A+)	65	1	5	325	7	2275
Iron	2600	1	2	5200	2	10400
Vacumm Cleaner	2000	1	2	4000	3	12000
Bakery	2500	1	1.5	3750	2	7500
Led Lamp	12	5	5	300	7	2100
Kettle	2200	1	0.2	440	7	3080
Toast Machine	2000	1	0.2	400	3	1200
Microwave Oven	800	1	0.1	80	7	560
Laptop	90	1	3	270	7	1890
Hair Dryer	1300	1	0.2	260	3	780
Other (Charging etc.)	10	1	2	20	7	140
<b>Total</b>				<b>21349</b>		<b>72453</b>

P: Power, DWT: Daily working time, DC:Daily consumption, NWU: Number of weekly use, WC: Weekly consumption

#### 4. CONCLUSIONS

Global warming, which has been caused by the accumulation of greenhouse gases in the atmosphere in recent years, has become the biggest problem of nowadays [48, 49]. This will cause serious drought and water problems in the future. In order to meet the needs of the increasing the world population, the demand for agriculture and industry will increase and the number of animals will need to increase as well. This will accelerate global warming by increasing the amount of methane and carbon dioxide gas day by day. In order to reduce global warming, the use of alternative energy sources should be risen. In particular, studies on biogas energy should be focused. Moreover, continuity should be ensured in the source of raw materials in biogas plants.

Biogas is an alternative energy source to meet the increasing energy needs in the world as well as in Turkey. Biogas production will reduce the use of fossil fuels and reduce CO<sub>2</sub> emissions. Biogas is a combustible gas that is produced from waste materials under anaerobic conditions by AD method, similar to natural gas and mostly consisting of CH<sub>4</sub> and CO<sub>2</sub>.

In this study, the amount of manure that can be obtained from bovine, ovine and poultry in Bingöl province and accordingly the biogas production potential was determined. According to 2015-2020 year data, depending on the total number of animals in Bingöl province, the highest biogas production was obtained in 2018 with 39.02 million m<sup>3</sup>. As can be seen in 2020 data, the amount of manure that can be obtained from total of 5694302 animals (bovine, ovine and poultry) in Bingöl has the potential to produce approximately 36.5 million m<sup>3</sup> of biogas energy. The total biogas production potential of the province is max. as following respectively; Central district (12.6 million m<sup>3</sup>), Karlıova (11.2 million m<sup>3</sup>) and Solhan (5.6 million m<sup>3</sup>). The min. biogas production potential has been determined in Yayladere with approximately 263 thousand m<sup>3</sup>. According to 2020 data (bovine, ovine and poultry) in Bingöl province, 36.5 million m<sup>3</sup> of biogas can be produced with 754051.1 tons of annually usable manure. In addition, this 36.5 million m<sup>3</sup> of biogas has been determined that it is equivalent to 171.4 GWh of electrical energy, 171449 million kcal m<sup>-3</sup> of heat energy, 126.6 kg of wood and 29.2 million liters of gasoline.

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## Investigation of *In Vivo* Effects of Carbon Tetrachloride (CCl<sub>4</sub>) and Quercetin on Some Metabolic Enzyme Activities in Rat Erythrocyte

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### Keywords

Carbon tetrachloride, Quercetin, Glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, Glutathione reductase, Glutathion S-transferaz.

**Abstract:** In the study; the purpose was to investigate the *in vivo* impact of carbon tetrachloride (CCl<sub>4</sub>) and quercetin (Qu) on activities of important metabolic enzymes such as Glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), glutathione reductase (GR) and glutathione S-transferase (GST) in rat erythrocytes. At the experimental stage, rats were divided into 4 groups. 1.Group (Control): Pure olive oil at a dose determined according to their body weight (1mL/kg) was given to the rats in this group, 2.Group (CCl<sub>4</sub>: 1.0 mL/kg (ip)(1:1), 3.Group (Ku: 25) mg/kg (ip), 4.Group (CCl<sub>4</sub>(1.0 ml/kg (ip)+ Ku (25 mg/kg (ip) was injected. The study was continued for 3 days. The results revealed that the activities of; G6PD (p<0.01), 6PGD (p<0.01), GR(p<0.001) and GST (p>0.05) enzyme activities were decreased in the CCl<sub>4</sub> group compared to the control group. It was determined that enzyme activities were higher in CCl<sub>4</sub>+Qu applied groups compared to CCl<sub>4</sub> group. The application of Qu caused an increase in the enzyme activity value. This can be accepted as an indication that the inhibition caused by CCl<sub>4</sub> has disappeared. Consequently; It is thought that Qu may be effective in preventing oxidative damage due to CCl<sub>4</sub> administration.

## Sıçan Eritrositlerinde Karbon Tetraklorürün(CCl<sub>4</sub>) ve Kuersetinin Bazı Metabolik Enzim Aktiviteleri Üzerine *İn Vivo* Etkisinin İncelenmesi

### Anahtar Kelimeler

Karbon tetraklorür, Kuersetin, Glukoz 6-Fosfat Dehidrogenaz, 6-fosfogluconat dehidrogenaz, Glutasyon redüktaz, Glutasyon S-transferaz.

**Öz:** Çalışmada; Sıçan eritrositlerinde karbon tetraklorür (CCl<sub>4</sub>) ve Kuersetinin(Ku)'in, Glukoz 6-fosfat dehidrogenaz (G6PD), 6-fosfogluconat dehidrogenaz (6PGD), glutasyon redüktaz (GR) ve glutasyon S-transferaz (GST) gibi önemli metabolik enzim aktiviteleri üzerine *in vivo* etkilerinin incelenmesi hedeflendi. Deneysel aşamada sıçanlar 4 gruba ayrıldı. 1.Grup(Kontrol): Bu gruptaki ratlara vücut ağırlıklarına göre belirlenen dozda(1 mL/kg)saf zeytin yağı, 2.Grup(CCl<sub>4</sub>: 1.0 mL/kg (i.p.)(1:1), 3.Grup(Ku: 25 mg/kg (i.p),4.Grup(CCl<sub>4</sub>(1.0 ml/kg (i.p.)+ Ku (25 mg/kg (i.p.) enjekte edildi.Çalışma 3 gün sürdürüldü. Sonuçlar değerlendirildiğinde; G6PD (p<0,01), 6PGD (p<0,01) GR (p<0,001) ve GST (p>0,05) enzim aktiviteleri kontrol grubuna oranla CCl<sub>4</sub> grubunda azalmıştır. CCl<sub>4</sub>+Ku uygulanan gruplarda ise enzim aktivitelerinin CCl<sub>4</sub> grubuna oranla yüksek olduğu belirlenmiştir. Ku uygulanması, enzim aktivite değerinin yükselmesine neden olmuştur. Buda CCl<sub>4</sub>'ün neden olduğu inhibisyonun ortadan kalktığıının göstergesi olarak kabul edilebilir. Sonuç olarak, CCl<sub>4</sub> uygulamasına bağlı olarak ortaya çıkan oksidatif hasarı önlemede Ku'nın etkili olabileceği düşünülmektedir.

## 1. INTRODUCTION

Carbon tetrachloride (CCl<sub>4</sub>) is a colorless, non-flammable, quickly evaporating, fragrant dense liquid, and is widely used in the production of petroleum products, varnishes, lacquers, resin solvents and organic compounds [1]. It is frequently used in dry cleaning, firefighting, grain disinfection and insect control [2]. Biologically inactive CCl<sub>4</sub> is transformed into reactive toxic metabolites for their activation and takes part in target cells [3,4,5]. CCl<sub>4</sub> exerts its toxic effect with the formation of a free radical, the trichloromethyl radical. The peroxy radical, which is formed as a result of the combination of this radical with oxygen, is a strong lipid peroxidation initiator that plays a role as the primary mechanism in the formation of cell damage by disrupting the cell membrane structure [3,6,7,8]. Malondialdehyde (MDA), which is formed as the end product of lipid peroxidation, is frequently used in the determination of oxidative damage [9]. In CCl<sub>4</sub> poisoning; analogous to oxidative damage as an experimental model [10] (Figure 1).

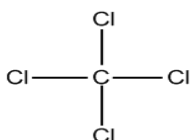


Figure 1. Molecular structure of carbon tetrachloride (CCl<sub>4</sub>) [11].

Quercetin (Qu) is one of the most widely studied bioflavonoids in the flavonols class [12] and is one of the most common flavonoids found and many dietary sources [13]. Qu, occurs mainly in the leaves and other parts of the plant in the form of glycosides or aglycones formed by the attachment of one or more sugar groups to phenolic groups by glycosidic bonds. Qu has powerful anti-oxidative and cytoprotective impact on oxidant-induced endothelial cell apoptosis due to its chemical structure [12]. Qu, which is included in various herbal teas today; It is used in biochemistry, food chemistry, paint chemistry, medical chemistry, paint industry and cosmetics [14,15]. Qu, prevents oxidative damage and cell death by prohibit lipid peroxidation and scavenging free oxygen radicals [16,17]. Qu, exerts its possible biological properties through its antioxidant activity [18] (Figure 2).

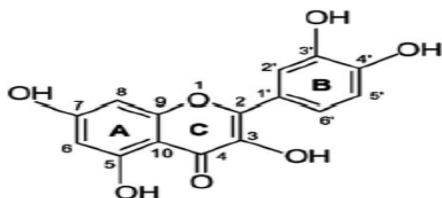


Figure 2. The structure of quercetin [19].

G6PD and 6PGD are known as metabolic enzymes that catalyze the first and third steps of the pentose phosphate pathway. As an agent that protects cells against oxidative stress, NADPH is known as an important molecule for biochemical processes such as reducing biosynthesis reactions [20,21]. NADPH is the most important molecule that reduces the disulfide form of glutathione,

as well as being considered as a cellular reducer, which is significant for nitric oxide biosynthesis, detoxification of xenobiotics and pharmaceuticals [22-24]. GR preserve the cell counter the harmful effects of these molecules by reducing oxidized molecules thanks to the -SH groups in its construction. In addition, membrane proteins such as GSH, the reduced form of GR, hemoglobin and spectrin, and the preserve of thiol groups of various enzymes, DNA and protein synthesis, detoxification of xenobiotics and some metabolic end products, and transport of amino acids [25,26]. The GR enzyme catalyzes the regeneration reaction of glutathione in the presence of NADPH, thereby maintaining the intracellular redox balance [27]. The binding reactions of GSH to endogenous and exogenous hydrophobic electrophiles are carried out by GST isoenzymes. GSTs can non-catalytically bind many exogenous and endogenous compounds such as fatty acids, hormones, flavonoids, bilirubin and xenobiotics [28,29]. The purpose of the presented study was to investigate the *in vivo* effects of CCl<sub>4</sub> and Qu treatments on some metabolic enzymes G6PD, 6PGD, GR and GST activities in rat erythrocytes.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

The chemicals used in the study were purchased from Sigma and Merck.

### 2.2. Experimental Design

Twenty-eight Wistar albino male rats weighing 200-300 g were used. They were fed ad-libitum with standard laboratory chow and water at a constant temperature of 20 ± 2°C and twelve (12 hours) light-dark cycle (light; 07:00-19:00, dark 19:00-07:00). The rats were randomly divided into 4 groups of 7 in each group. The study was approved by the Bingol University Experimental Animal Ethics Committee. (BUHADEK: 4.10.2018-2018/08-08/01).

1. Group (Control): Pure olive oil i.p. given through in a dose determined according to the body weight of the rats (1 mL/kg) was given to the rats in this group [30].

2. Group (CCl<sub>4</sub>): The rats in this group were mixed with 1 mL/kg of CCl<sub>4</sub> in olive oil at a ratio of 1:1 and administered i.p. was applied as. [31].

3. Group (Qu): The rats in this group were administered i.p. at a dose of 25 mg/kg by using the stock solution prepared in dimethyl sulfoxide (DMSO) [32].

4. Group (CCl<sub>4</sub> + Qu): The rats in this group were administered 1 mL/kg CCl<sub>4</sub> (1:1 in olive oil) + 25 mg/kg Qu (using the stock solution prepared in DMSO) i.p. At the end of the 3rd day, blood samples were taken from the rats under anesthesia.

### 2.3. Preparation of Hemolysate

Blood samples were taken into EDTA tubes. Fresh blood samples were taken into EDTA tubes. Afterwards, plasma and leukocytes were eliminated by centrifugation for 15 minutes (2500 xg). Red cells packed with KCl solution (0.16 M) were washed three times. Blood

samples were centrifuged at (2500 xg) and supernatants were discarded. Erythrocytes were hemolyzed using 5-fold distilled water. Samples were centrifuged at +4°C (10000 xg) for 30 minutes to eliminate cell membranes and insoluble molecules. The supernatant was saved for analysis [33].

## 2.4. Determination of Enzyme Activities

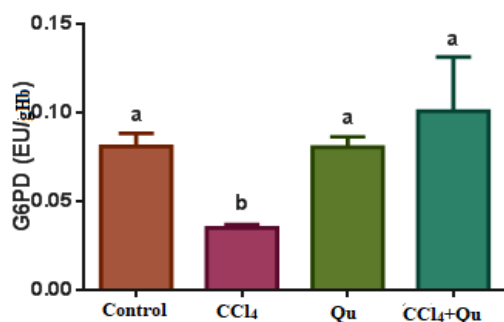
G6PD and 6PGD enzyme activity was measured spectrophotometrically according to the Beutler method at 340 nm. This method is based on the principle that NADPHs in the reaction medium absorb light at 340 nm. In reactions catalyzed by both G6PD and 6PGD, enzymes react with NADP<sup>+</sup> as a co-substrate and convert to NADPH. This increase is followed spectrophotometrically, the amount of absorption per minute is found and converted into enzyme units [34-36]. The method proposed by Carlberg and Mannervik was used for the measurement of GR enzyme activity. This method depends on the reduction of NADPH in the reaction catalyzed by the GR enzyme. This decrease was monitored by spectrophotometry at 340 nm to determine enzyme activity [37] and the activity of the GST enzyme was determined according to the Habig method. This method is based on the conversion of 1-chloro-2,4-dinitrobenzene (CDNB) to S-glutathione dinitrobenzene (DNB-SG). The product of this reaction, DNB-SG, shows maximum absorbance at 340 nm [38].

## 3. STATISTICAL ANALYSIS

Statistical evaluation was made using the SPSS 20 program. Data were expressed as mean  $\pm$  SD. The Kruskal Wallis test followed by the Mann Whitney U test was used to define the diverse among the groups. The diverse among the groups was remarkable important when  $p < 0.05$ .

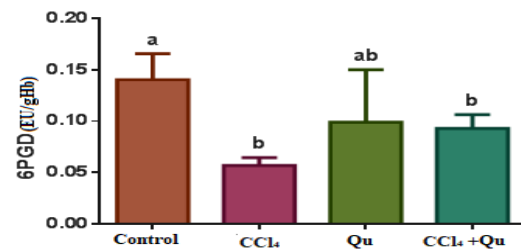
## 4. RESULTS AND DISCUSSION

According to the results of the current study; G6PD enzyme activity was statistically lower in the CCl<sub>4</sub> group compared to the control group ( $p < 0.01$ ). Qu applied group; G6PD enzyme activity was found close to the control group. In the CCl<sub>4</sub>+Qu group, the G6PD enzyme activity was discovered to be higher than the CCl<sub>4</sub>-treated group (Figure 3)



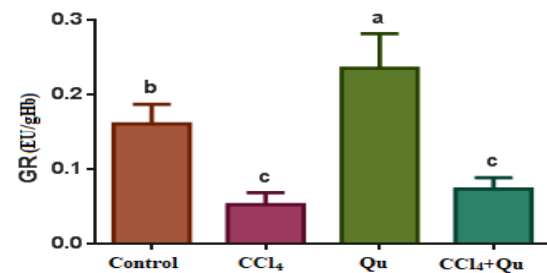
**Figure 3.** The in vivo effect of CCl<sub>4</sub> and Qu on rat erythrocyte G6PD enzyme activity (a,b differs between groups with different letters) ( $p < 0.01$ ).

When the 6PGD enzyme activity was assessed, a statistically significant reduction was observed in the CCl<sub>4</sub> treated group compared to the control group ( $p < 0.01$ ). The 6PGD enzyme activity was found to be higher in the Qu-treated group than in the CCl<sub>4</sub> group. Enzyme activity rose in CCl<sub>4</sub>+Qu group compared to CCl<sub>4</sub> group. These results show that Qu reduces the inhibitory effects of CCl<sub>4</sub> on 6PGD enzyme activity (Figure 4).



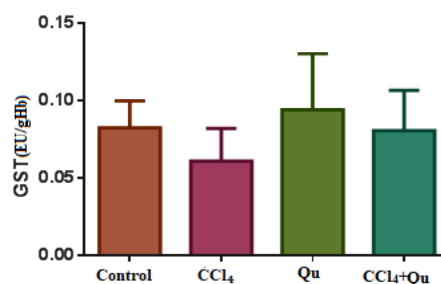
**Figure 4.** In vivo effect of CCl<sub>4</sub> and Qu on rat erythrocyte 6PGD enzyme activity (a,b, difference between groups with different letters) ( $p < 0,01$ ).

When the GR enzyme activity is evaluated; it was observed that the enzyme activity was reduced in the CCl<sub>4</sub> applied group compared to the control group ( $p < 0,001$ ). In the CCl<sub>4</sub> +Qu applied group, it was identified that the GR enzyme activity was increased compared to the CCl<sub>4</sub> group (Figure 5).



**Figure 5.** In vivo effect of CCl<sub>4</sub> and Qu on rat erythrocyte GR enzyme activity (a,b,c there is difference between groups with different letters) ( $p < 0,001$ ).

When the GST enzyme activity was assessed; it was determined that there was a decrease in the CCl<sub>4</sub> group compared to the control group, and a partial increase was observed in the CCl<sub>4</sub>+Qu group compared to the CCl<sub>4</sub> group. No statistical differences were discovered between the groups ( $p > 0,05$ ) (Figure 6).



**Figure 6.** In vivo effect of CCl<sub>4</sub> and Qu on rat erythrocyte GST enzyme activity ( $p > 0,05$ ).

When the results of this study are evaluated in general; G6PD, 6PGD and GR enzyme activities are inhibited by CCl<sub>4</sub>. As a result of prevention of G6PD and 6PGD enzyme activities, cells cannot produce enough NADPH in the pentose phosphate pathway to detoxify endogenous and exogenous oxidized molecules. At the same time, inhibition of GR and GST enzyme activities may cause disruption in intracellular GSH metabolism, thus rendering cells vulnerable to oxidative stress. In the study; G6PD enzyme activity was decreased in CCl<sub>4</sub> group compared to control group (p<0.01). Enzyme activity in the Qu group was discovered to be close to the control group. In the CCl<sub>4</sub>+Qu group, the enzyme activity was discovered to be higher than in the CCl<sub>4</sub> group. This suggests that quercetin may have a decreasing effect on the inhibition of CCl<sub>4</sub> in G6PD enzyme activity. When 6PGD enzyme activity is evaluated; The decrease in enzyme activity (p<0.01) in the group treated with CCl<sub>4</sub> compared to the control group can be considered as an indicator of inhibition of the 6PGD enzyme by CCl<sub>4</sub> application. The rise in the enzyme activity value in the CCl<sub>4</sub>+Qu applied group can be considered as an indication that the inhibition effect is partially eliminated. While it was observed that GR enzyme activity reduction statistically important (p<0.001) in the carbon tetrachloride group compared to the control group, it was concluded that the enzyme activity increased partially in the CCl<sub>4</sub> +Qu administered group. When the GST enzyme activity was assessed, a partial reduction was determined in the CCl<sub>4</sub> group compared to the control group. It was seen that the increases or decreases between the groups were not statistically significant. In the literature review, no study was found that examined the effects of CCl<sub>4</sub> and Qu on metabolic enzymes in rat erythrocytes. In a different study, it was determined that carbon tetrachloride (CCl<sub>4</sub>) intoxication caused a decrease in enzyme activities such as NADPH-cytochrome c reductase, NADH-cytochrome reductase, coumarin hydroxylase, 7-ethoxy coumarin-O-deethylase, UDP-glucuronyl transferase and glutathione-S-transferase [39]. In a different study by Sheweita et al., it was determined that CCl<sub>4</sub> application caused inhibition of GST enzyme activity [40]. In a study by Elbe et al., the antioxidative effects of Qu in CCl<sub>4</sub>-caused cardiac detriment were histologically and biochemically investigated. While SOD activity were remarkably declined in CCl<sub>4</sub> administered group, Qu administration caused considerable rise in SOD activity. In CCl<sub>4</sub> toxicity, it was concluded that the use of Qu could provide beneficial effects owing to its antioxidative properties [41]. In a different study investigating the antioxidant defense system in tissue damage caused by CCl<sub>4</sub> and ethyl alcohol in goose liver and kidneys, which are among the important antioxidant and metabolic enzymes due to damage to the liver and kidneys, glutathione peroxidase(GSH-Px), catalase (CAT), glucose-6-phosphate dehydrogenase ( G6PD ) activities were decreased [42]. In a different study by Jeon et al., it was reported that carbon tetra chloride administration caused a decrease in the activities of important metabolic enzymes glutathione S-transferase and glutathione reductase in the rat liver [43]. In a different study researchers evaluated the effects of CCl<sub>4</sub>

and Qu treatment. They demonstrated that Qu isolated from *Delonix elata* L. plant has significant prophylactic effects on liver function markers AST, ALT, ALP, serum bilirubin and total protein, as well as antioxidant enzymes SOD, CAT, GPx and GST [44]. Literature also stated that after CCl<sub>4</sub> exposure, antioxidant enzyme activities such as SOD, CAT and GPx decreased significantly in the rat kidney, CCl<sub>4</sub> exposure caused oxidative stress by inhibiting antioxidant enzyme activities [45]. There are many other studies revealing various drugs and substances inhibit activities of metabolic enzymes [46-48].

## 5. CONCLUSION

When the results of this study are evaluated in general; G6PD, 6PGD and GR enzyme activities are inhibited by CCl<sub>4</sub>. In the CCl<sub>4</sub>+Qu applied groups, the enzyme activities were higher than the CCl<sub>4</sub> group. While the GST enzyme activity decreased in the CCl<sub>4</sub>-administered group, it partially increased in the Qu-treated group. These results indicated that Qu reduces the inhibition impact of CCl<sub>4</sub> on G6PD, 6PGD and GR enzyme activities. Accordingly, it is thought that Qu may be effective in preventing oxidative damage due to CCl<sub>4</sub> administration. At the same time, there is a need for further studies in which different Quercetin dosages will be used.

## CONFLICTS OF INTEREST

There is no conflict between the authors.

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## Determination of Some Yield Features of Foreign-Origin Alfalfa Cultivars (*Medicago sativa* L.) in Bingöl Conditions

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**Abstract:** This study was carried out to determine the some yield features of foreign origin alfalfa cultivars in Bingöl ecological conditions. In the research, a total of 15 alfalfa cultivars, 12 of them are from foreign-origin (Iside, Osjecka 99, Ezzelina, Diane, Prosementi, Queen, Emiliana, Riviere, Vicentina, Banat vs, La Torre, Sabrina and Escorial) and 3 of them are standard (Elçi, Bilensoy 80 and Basbag) cultivars were used as plant material. In the research; plant height, number of stems, stem thickness, green forage yield and dry matter yield were investigated. Average plant height was 97.6 cm, number of stems was 15.8, stem thickness was 2.80 mm, forage yield was 11018 kg da<sup>-1</sup> and dry matter yield was 3228 kg da<sup>-1</sup>. In line with these parameters examined, it has concluded that Ezzelina and Emiliana cultivars showed superior characteristics in Bingöl province.

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## Bingöl Koşullarında Yurtdışı Kaynaklı Yonca Çeşitlerinin (*Medicago sativa* L.) Bazı Verim Özelliklerinin Belirlenmesi

### Anahtar Kelimeler

Yonca,  
Sap sayısı,  
Sap çapı,  
Yeşil ot verimi,  
Kuru ot verimi

**Öz:** Bu çalışma, Bingöl ekolojik koşullarında bazı yabancı orijinli yonca çeşitlerinin ot verimini belirlemek amacıyla yürütülmüştür. Araştırmada 12'si yabancı-orijinli (Iside, Osjecka 99, Ezzelina, Diane, Prosementi, Queen, Emiliana, Riviere, Vicentina, Banat vs, La Torre, Sabrina ve Escorial) ve 3'ü de standart çeşit (Elçi, Bilensoy 80 ve Başbağ) olmak üzere toplam 15 yonca çeşidi bitkisel materyal olarak kullanılmıştır. Araştırmada; bitki boyu, sap sayısı, sap çapı, yeşil ot verimi ve kuru ot verimi incelenmiştir. Ortalama bitki boyu 97,6 cm, sap sayısı 15,8, sap çapı 2,80 mm, yeşil ot verimi 11018 kg da<sup>-1</sup> ve kuru ot verimi 3228 kg da<sup>-1</sup> olarak tespit edilmiştir. İncelenen bu parametreler doğrultusunda Bingöl ilinde Ezzelina ve Emiliana çeşitlerinin üstün özellikler gösterdiği sonucuna varılmıştır.

### 1. INTRODUCTION

Fodder (forage) crops farming has great importance in developed countries, and these countries allocate a significant portion of their agricultural land to forage crop farming. For example, the ratio allocated to forage crops in the total field land are 49.8% in Australia, 36.5% in Germany, 31.4% in the Netherlands; 25.8% in France, 25.4% in England and 23.0% in the USA [1].

Alfalfa has large cultivation areas in many countries of the world and is defined as the queen of forage crops because have a high adaptability, long life, can be cut many times in a vegetation period, high yield, high

nutritional value, so alfalfa is distinguished from other forage crops. Alfalfa is rich in protein, as well as vitamins such as carotene (provitamin A) and tocopherol (vitamin E). With the help of deep root, alfalfa easily benefits from water and plant nutrients found in deep areas that other plants could not use. Alfalfa leaves a nitrogen-rich soil for the next plant in its root nodosities [2].

Alfalfa naturally found in almost every region of Turkey. Cultivation of alfalfa has become increasingly common in recent years. This situation makes it necessary to research and adapt new varieties for our country besides existing alfalfa varieties. In forage

crops and especially alfalfa because mentioned reasons, it has become compulsory to develop new varieties. To increase the number of registered varieties by conducting regional adaptation studies especially after the enactment of the Pasture Law No. 4342, in Turkey important developments have taken place in terms of agriculture and forage crops [3].

In the previous studies; plant height was 59.8-76.3 cm in the first year and 90.0-121.3 cm in the second year of the Elci cultivar of alfalfa. In the first and second years of the study, the ztotal green forage yield was 6284-9159 kg da<sup>-1</sup> and 7538-10715 kg da<sup>-1</sup> [4].

In a three-year study examining the yield characteristics of 8 alfalfa cultivars in Ankara conditions; it has been reported that the green forage yield was determined as 7907-11140 kg da<sup>-1</sup> and dry matter yield was determined as 2619-3615 kg da<sup>-1</sup> [5].

In order to determine the suitable alfalfa varieties for the Eastern Anatolia region, 16 different alfalfa varieties have compared from aspect of yield and quality features in Bingöl conditions. The study has conducted in randomized block design with three replications during three years between 2014 and 2016. According to the results of the research, plant height values of alfalfa cultivars were 41.9-54.7 cm, forage yields were 2735-3591 kg da<sup>-1</sup> and dry matter yields were 924-1227 kg da<sup>-1</sup> [6].

The study has conducted in Bornova and Odemiş ecological conditions 2006 and 2007 years with four different alfalfa genotypes, it has found that there were significant differences from the aspect of genotypes between locations. The researchers determined that the forage yield was 9769-12235 kg da<sup>-1</sup>, dry matter yield was 2078-2862 kg da<sup>-1</sup>, plant height was 77.1-86.8 cm, main stem was 13.14-15.57, the main stem thickness was 3.57-3.99 mm in Odemiş condition and the forage yield was 9260-11832 kg da<sup>-1</sup>, dry matter yield was 1984-2601 kg da<sup>-1</sup>, plant height was 70.8-76.1 cm, main stem was 12.74-14.22 and main stem thickness was 2.84-3.12 mm in Bornova conditions [7].

The research conducted in order to determine the yield and quality characteristics of some perennial warm season legume forage crops in Adana region in the irrigated conditions the Nimet variety of alfalfa was used, the average plant height was 69.2 cm, the green forage yield was 5094.5 kg da<sup>-1</sup> and dry matter yield was 1135 kg da<sup>-1</sup> [8].

The aim of this study is to determine some yield features of foreign-origin alfalfa cultivars in Bingöl ecological conditions.

## 2. MATERIAL AND METHOD

### 2.1. Material

This research has conducted in Bingöl University Genc Vocational School's Application and Research Area between 2019 and 2021, under watery conditions and for two years. Genc is a town and district of Bingöl Province in the Eastern Anatolia Region of Turkey. Totally 15 alfalfa cultivars have been used in the study, 12 of which were of foreign origin (Iside, Osjecka 99, Ezzelina, Diane, Prosementi, Queen, Emiliana, Riviera vicentina, Banat vs, La Torre, Sabrina and Escorial) and the remaining 3 were used as standard (Elci, Bilensoy 80 and Basbag) and native ones.

When look at the data of climate, monthly average temperature value was 13.8-14.2 °C, total precipitation was 839.2-668 mm and relative humidity was 51.9-48.5% for 2020 and 2021. It has observed that the average temperature values for the years 2021 and 2022 are above the long-years average, while the precipitation amounts and relative humidity values are below the long-years averages.

Analysis of soil samples taken from 10 different points and 0-30 cm depth of the research location has carried out in "Bingöl University Faculty of Agriculture, Soil Science and Nutrition Department Laboratory". The soil structure of the research location was found to be sandy, clayey and loamy (clay rate 19.11%, silt rate 16.71% and sand rate 64.18%), pH level neutral (7.10), salt-free, less limy, organic matter and available phosphorus ratios low, potassium content sufficient determined.

### 2.2. Method

The field experiment has carried out in August 2019 following the soil preparation made in July. The research will be set up with 3 replications according to the randomized blocks trial pattern. In the experiment, parcel lengths were 5 m, the distance between rows was 20 cm and 6 rows were planted in each parcel. 3 kg of seed has be used per decare in planting. Fertilizer was given directly to the decare on the basis of pure substance before planting. Irrigation of the experiment has done by sprinkler irrigation.

The plant height (cm), the distance between the first bud until the soil surface was measured with a ruler. The stem thickness (cm) of the first plants that form flower bud has measured with a caliper between the second and third node in 10 plants. For determining the number of main stems, 10 plants have selected in each parcel. The stems emerging of the determined 10 plant roots have counted, the average number of stems was determined by taking the average per cultivar.

Green forage yield (kg da<sup>-1</sup>) located 1 row further from the top of the parcel and 1 row from the bottom of the parcel, and 0.5 m from the edges has removed from the



parcel. The remaining area was harvested by cutting and the green forage yield obtained from each parcel was weighed and the values were obtained were converted to yield per decare. A sample of 0.5 kg of forage obtained from each parcel have randomly taken and dried in a drying chamber at 70 °C for 48 hours in drying chamber. Then it has been kept in drying chamber for 24 hours and then weighed so the dry herbage weight has been found then the obtained dry matter has been converted to yield per decare.

The data that have been obtained have been evaluated by variance analysis with the help of the JMP statistical package program (a software belonging to the SAS program), and the differences of the groups have been compared with the Tukey test.

### 3. RESULTS AND DISCUSSION

#### 3.1. Plant Height (cm)

Two-year averages of plant heights of foreign origin alfalfa cultivars and groupings of these averages have given in Table 1. As seen in Table 1, the difference between the plant heights of alfalfa cultivars was significant in terms of years and cultivars ( $P \leq 0.01$ ), in terms of year x cultivar interaction was determined to be insignificant. In terms of cultivars, the highest plant height cultivar is Emiliana (106.2 cm), it has seen that other cultivars except Basbag and Sabrina cultivars are in the same group statistically. The lowest plant height was determined in Basbag (87.6 cm) and Sabrina (90.8 cm) cultivars. In terms of years, the average plant height (100.9 cm) determined in 2021 higher than the average plant height determined in 2020 (94.4 cm).

**Table 1.** Plant heights of alfalfa cultivars (cm)

Cultivars Name	2020	2021	Mean
Banat vs	98.7 <sup>ns</sup>	99.3	99.0 abc**
Basbag	86.6	88.7	87.6 c
Bilensoy 80	96.7	103.3	100.0 abc
Diane	92.1	101.9	97.0 abc
Elci	93.8	106.2	100.0 abc
Emiliana	108.8	103.5	106.2 a
Escorial	87.4	98.6	93.0 abc
Ezzelina	97.4	109.8	103.6 ab
Iside	87.4	105.7	96.6 abc
La Torre	91.2	97.4	94.3 abc
Osjecka 99	90.8	97.4	94.1 abc
Prosementi	99.9	103.9	101.9 abc
Queen	93.5	102.3	97.9 abc
Riviera vicentina	104.7	100.7	102.7 ab
Sabrina	86.2	95.3	90.8 bc
Mean	94.4 B**	100.9 A	97.6

CV(%): 7.50%, \*\*( $P \leq 0.01$ ), ns: none significant

When look at the previous studies on the plant height in alfalfa; it has been reported that it is detected between 79-100 cm in Bornova conditions [9], 57.70-79.40 cm in Northern Cyprus conditions [10], 74.8-86.8 cm in Izmir conditions [11], 85.10-93.20 cm in Tokat-Kazova conditions [12], 66.61-101.28 cm in Antalya conditions [13], 80.33-103.22 cm in Goller Region [14], 76.3-90 cm in Yozgat ecological conditions [15] and 59.3-109.3 cm in Bingöl conditions [16]. It is seen that the 87.6-106.2 cm alfalfa height obtained in this study is close and similar to the plant heights obtained in these studies.

#### 3.2. Stem Number

Two-year averages of plant heights of foreign origin alfalfa cultivars and groupings of these averages have given in Table 2. As seen in Table 2, the difference between the stem number of alfalfa cultivars was determined to be insignificant in terms of year, cultivar and year x cultivar interaction. The average number of stems of the cultivars is between 14.9-18.3 and the average is 16.1 in the first year, between 12.3-17.9 and the average is 15.4 in the second year. As the average of two years, it changed between 13.8-18.1 and the average two years were obtained as 15.8.

**Table 2.** The stem number of alfalfa cultivar (number)

Cultivars	2020	2021	Mean
Banat vs	15.3 <sup>ns</sup>	16.4	15.8 <sup>ns</sup>
Basbag	17.2	16.0	16.6
Bilensoy 80	16.3	14.1	15.2
Diane	16.2	16.2	16.2
Elci	15.1	15.0	15.1
Emiliana	14.9	14.5	14.7
Escorial	16.7	17.7	17.2
Ezzelina	15.2	14.7	14.9
Iside	16.8	14.2	15.5
La Torre	16.7	15.1	15.9
Osjecka 99	18.3	17.9	18.1
Prosementi	16.6	16.1	16.4
Queen	16.2	16.1	16.1
Riviera vicentina	15.2	12.3	13.8
Sabrina	15.2	15.3	15.3
Mean	<b>16.1<sup>ns</sup></b>	<b>15.4</b>	<b>15.8</b>

CV(%): 19.4%, ns: none significant

Regarding to previous studies in alfalfas stem number, Soya and Kavut [17] have determined between 12.1-12.4 in Bornova conditions, Kir ve Soya [11] have presented the between 11.3-18.5 in Izmir conditions, Mohammed [18] has found 10.6-25.8 in Ankara and Konya conditions, Demiroglu et al. [19] have found 11.3-11.9 in Bornova and Odemis conditions and Kavut et al. [7] have determined between 12.9-14.9 in Izmir conditions. In this study the average number of stems has obtained between 13.8-18.1 is similar to results that mentioned in above researches.

### 3.3. Stem Thickness (mm)

Two-year averages of stem thickness of foreign origin alfalfa cultivars and groupings of these averages have given in Table 3. As seen in Table 3, the difference between the stem thicknesses of alfalfa cultivars is significant in terms of year x cultivar and cultivar ( $P \leq 0.01$ ), in terms of year x cultivar interaction was determined to be insignificant.

**Table 3.** The stem thickness of alfalfa cultivars (mm)

Cultivars	2020	2021	Mean
Banat vs	2.76 <sup>ns</sup>	3.28	3.02 ab**
Basbag	1.98	2.52	2.25 c
Bilensoy 80	2.15	3.10	2.63 bc
Diane	2.55	3.08	2.82 abc
Elci	2.36	3.11	2.73 abc
Emiliana	3.34	3.28	3.31 a
Escorial	2.46	2.64	2.55 bc
Ezzelina	2.78	2.96	2.87 abc
Iside	2.56	3.05	2.81 abc
La Torre	2.33	2.78	2.56 bc
Osjecka 99	2.60	3.53	3.06 ab
Prosementi	2.87	2.74	2.81 abc
Queen	2.67	2.74	2.70 abc
Riviera vicentina	3.13	3.14	3.13 ab
Sabrina	2.44	3.07	2.76 abc
Mean	<b>2.60 B**</b>	<b>3.00 A</b>	<b>2.80</b>

CV (%): 11.6%, \*\*:  $P \leq 0.01$ , ns: none significant

In terms of cultivars, it has determined that the highest stem thickness was in Emiliana (3.31 mm), the lowest stem thickness was in Basbag (2.25 mm). Banat vs, Diane, Elci, Ezzelina, Iside, Osjecka 99, Queen, Riviera vicentina and Sabrina cultivars are also in the group with the highest value. In terms of years, it was determined that the 2.60 mm average stem thickness obtained in 2020 was statistically lower than the 3.00 mm average stem thickness obtained in 2021.

Regarding to previous studies on stem thickness in alfalfa; Seker [20] have found 2.78-2.89 mm in Erzurum ecological conditions, Soya and Kavut [17] have reported 2.78-2.90 mm in Bornova ecological conditions, Tongel and Ayan [21] reported that stem thickness varied between 2.93-3.27 mm on 19 alfalfa cultivars in Samsun ecological conditions, Dumlu et al. [22] reported that the stem thickness varies between 2.81-3.04 mm in Erzurum conditions. We found the stem thickness between 2.25-3.31 mm in this study that is similar to results that mentioned in above researches.

**Table 4.** The forage yield of alfalfa cultivars (kg da<sup>-1</sup>)

Cultivars Name	2020	2021	Mean
Banat vs	10150 <sup>ns</sup>	10337	10243 ab*
Basbag	10664	8748	9706 b
Bilensoy 80	10782	12014	11398 ab
Diane	11811	11673	11742 ab
Elci	10246	11977	11111 ab
Emiliana	10742	12052	11397 ab
Escorial	8761	10697	9729 b
Ezzelina	11878	16261	14069 a
Iside	9033	11453	10243 ab
La Torre	9673	9574	9624 b
Osjecka 99	10373	11588	10981 ab
Prosementi	13187	11662	12424 ab
Queen	9972	12173	11072 ab
Riviera vicentina	11836	11563	11700 ab
Sabrina	9372	10298	9835 b
Mean	10565 B*	11471 A	11018

CV: 17.8%, \*: P≤0.05, ns: none significant

The forage yield was obtained in the study is similar with forage yields of Kusvuran et al. [10] research that has obtained 10828 kg da<sup>-1</sup> in Northern Cyprus ecological conditions. In addition, Gokalp et al. [23] have determined 11150-13905 kg da<sup>-1</sup> in Tokat-Kazova conditions. However, the forage yield was obtained in the study higher than the Basbag et al. [24] results that is between 3672,13-6153,38 kg da<sup>-1</sup> in irrigated conditions in Diyarbakir. Also Yilmaz et al. [25] have reported 1297-1771 kg da<sup>-1</sup> in Kahramanmaras conditions. The yield obtained in alfalfa cultivation is largely related to the number of harvests. The number of harvests is higher in warm regions and less in cold regions. The difference in the number of harvests causes the forage yield obtained in alfalfa cultivation to

### 3.4. Green Forage Yield (kg da<sup>-1</sup>)

Two-year averages of forage yield of foreign origin alfalfa cultivars and groupings of these averages have given in Table 4. As seen in Table 4, the difference between the forage yields of alfalfa cultivars is significant in terms of year and cultivar (P≤0.05), in terms of year x cultivar interaction was determined to be insignificant. In terms of cultivars, it has seen that the highest forage yield cultivars was in Ezzelina cultivar (14069 kg). Banat vs, Bilensoy 80, Emiliana, Diane, Elci, Osjecka 99, Prosementi, Riviera vicentina, Iside and Queen cultivars are also in the highest value group. The lowest forage yield was determined in Basbag, Sabrina, La Torre and Escorial cultivars. In terms of years, it has seen that the average forage yield (11471 kg) in 2021 is higher than the forage yield (10565 kg) in 2020.

be different. Therefore, different forage yields can be obtained in different ecological regions.

### 3.5. Dry Matter Yield (kg da<sup>-1</sup>)

Two-year averages of dry matter yield of foreign origin alfalfa cultivars and groupings of these averages have given in Table 5. As seen in Table 5, the difference between the dry matter yields of alfalfa cultivars is significant only for cultivars (P≤0.05), in terms of year and year x cultivar interaction was determined to be insignificant. In terms of cultivars, it has seen that the highest dry matter yield has obtained from Ezzelina cultivar. Banat vs, Bilensoy 80, Emiliana, Diane, Elci, Osjecka 99, Prosementi, Riviera vicentina, Sabrina, Iside and Queen are also among the highest value

groups. The lowest dry matter yield was determined in Basbag, La Torre and Escorial cultivars.

**Table 5.** The dry matter yield of alfalfa cultivars (kg da<sup>-1</sup>)

Cultivars	2020	2021	Mean
Banat vs	3012 <sup>ns</sup>	3074	3043 ab *
Basbag	3076	2633	2855 b
Bilensoy 80	3180	3283	3232 ab
Diane	3327	3171	3249 ab
Elci	3143	3398	3270 ab
Emiliana	3194	3450	3322 ab
Escorial	2709	2974	2841 b
Ezzelina	3535	4376	3955 a
Iside	2954	3085	3020 ab
La Torre	3038	2854	2946 b
Oşjecka 99	3260	3310	3285 ab
Prosementi	3857	3510	3684 ab
Queen	2913	3495	3204 ab
Riviera vicentina	3446	3501	3474 ab
Sabrina	3031	3063	3047 ab
Mean	<b>3178<sup>ns</sup></b>	<b>3279</b>	<b>3228</b>

CV: 15.1%, \*: P<0.05, ns: none significant

The dry matter yields have obtained in current study is similar to the dry matter yields in previous researches of alfalfa that have presented below. Altınok et al. [5] obtained 3.214 kg da<sup>-1</sup> in Ankara conditions, Avci et al. [26] obtained 2094.0-2230.0 kg da<sup>-1</sup> in Konya and Ankara conditions and Yüksel et al. [27] obtained 2845-3339 kg da<sup>-1</sup> and 2032-2617 kg da<sup>-1</sup> in Ankara and Isparta locations, respectively. However, the dry matter yields in the study have found to be higher than the dry matter yields of 1104.7-1333.5 kg da<sup>-1</sup> in Erzurum ecological conditions [20], 2031-2710 kg da<sup>-1</sup> in Odemis and Bornova conditions [7], 1143-2183 kg da<sup>-1</sup> in the Goller [28] and 1122.7-1396.9 kg da<sup>-1</sup> in Erzurum ecological conditions [22]. The dry matter yield has directly related to the forage yield obtained. In areas where forage yield is high, dry matter yield has generally also obtained higher.

#### 4. CONCLUSIONS

The plant heights of alfalfa cultivars varied between 87.6-106.2 cm and the average was 97.6 cm, the number of stems varied between 13.8-18.1 and the average was 15.8, stem thickness varied between 2.25-3.31 mm and the average was 2.80 mm, the forage yield varied between 9706-14069 kg da<sup>-1</sup> and the average was 11018 kg da<sup>-1</sup>, the dry matter yield varied between 2841-3955 kg da<sup>-1</sup> and the average was obtained as 3228 kg da<sup>-1</sup>. Statistically, the highest plant height and stem thickness have obtained from Emiliana, while the highest forage yield and dry matter yield have obtained from Ezzelina. Emiliana and Ezzelina cultivars gave higher values than both standard and foreign cultivars. The number of stems of the cultivars did not differ statistically. In line with these parameters examined, it

has concluded that Ezzelina and Emiliana cultivars showed superior characteristics in Bingöl province.

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## Simulation of Disturbance Observer-Based Bone Tissue Change Prediction Approach for Orthopedic Drills

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 Bone  
 Drilling

**Abstract:** Orthopedic drills are currently used for various operations in surgical fields such as orthopedics, ear, nose, and throat surgery. The path that orthopedic drills travel through the tissue is controlled manually by surgeons, and manual control leads to the risk of damaging areas such as nerves and tissues. In our study, an innovative approach is presented against existing drill designs and breakthrough detection problems. In the proposed model, the change in the load torque and the change in friction force caused by the tissue change in the drilling path are considered as a disturbance effect, and a disturbance observer has been developed that allows these disturbances to be observed. Observation of the disturbance effects allows the perception of the hardness of tissue change during drilling since it gives the change of load torque changes and friction coefficient, which cannot be measured under normal operation. The performance of the proposed approach has been proven by simulation study.

## Ortopedik Matkaplar İçin Bozucu Gözlemci Tabanlı Kemik Doku Değişim Tahmin Yaklaşımı Benzetimi

**Anahtar  
 Kelimeler**  
 Ortopedik  
 Matkap,  
 Bozucu  
 Gözlemci,  
 Kemik  
 Delme

**Öz:** Günümüzde ortopedi, kulak burun boğaz gibi cerrahi alanlarda çeşitli operasyonlarda ortopedik matkaplar kullanılmaktadır. Ortopedik matkapların doku içerisindeki kat ettiği yol manuel olarak cerrahlar tarafından kontrol edilmektedir ve manuel kontrol sinir, doku gibi bölgelerde hasar oluşturma riskine yol açmaktadır. Çalışmamızda mevcut matkap tasarımlarına ve sorunlarına karşı yenilikçi bir model sunulmaktadır. Önerilen modelde yük momentindeki değişim ve matkap ucundaki doku değişikliğinden kaynaklanan sürtünme kuvveti değişimi bozucu etki olarak ele alınmış, bu bozucu etkilerin gözlemlenmesine olanak sağlayan bir bozucu gözlemci geliştirilmiştir. Bozucu etkilerinin gözlemlenmesi, normal şartlarda ölçülemeyen yük moment değişimlerinin ve sürtünme katsayısının değişimini verdiğinden dolayı, delme esnasında doku değişiminin algılanmasına olanak sağlamaktadır. Önerilen yöntemi başarımlı benzetim çalışmaları ile kanıtlanmıştır.

### 1. INTRODUCTION

Bone drilling is used primarily in orthopedic surgery, but also in many surgical interventions such as thoracic surgery, plastic surgery, otolaryngology [1]. During surgery, implants such as screws, nails, wires and plates are used to fix the bone as a result of post-traumatic fractures in the body [2,3]. Cylindrical holes are drilled in the bone with the appropriate drill bit attached to orthopedic drills and implants are placed in these holes [1]. One of the risks in the usage of surgical drills is the potential of the drill bit to damage nerve, vessel and muscle tissues during the drilling process [4,5]. The

rapidly rotating drill wraps around the surrounding tissues uncontrollably or the drill bit's inability to stop its movement immediately after exiting the second cortex of the bone is the reason for the potential of surgical drills to damage the tissues [4,6,7]. Breakthrough detection can be defined as the detection of the thrust force when the drill bit exits the second cortex [4,8]. During orthopedic surgical procedures, the force applied by the surgeon for the advancement of the drill bit varies since changing tissue hardness. The factors that affect the performance of the drilling process includes surgeon's dexterity, 'feel piercing' [9], phonological 'audible piercing' [10,11]. The drilling force perceived by the surgeon is a relative

concept. The drill bit penetration rate, the health/condition of the bone, and the drill type used in the operation affect the drilling force.

There are three types of approaches to detect the breakthrough in bone drilling in the literature. One of these approaches is the Computer Assisted Orthopedic Surgery approach in orthopedic surgery operations, which is based on the combination of medical imaging, skeletal-muscular system and the positioning of the surgical instrument in 3D space, and semi-robotic systems [12]. The second approach is the studies to integrate breakthrough detection sensors as force, vibration, acoustic, and torque sensors into the existing orthopedic drills [13–17]. Due to the cost-effectiveness of robotic and imaging systems, as well as the mounting and cost disadvantages of various sensors integrated into orthopedic drills, there has been a third approach for estimating tissue changes during drilling with real-time system dynamics analysis in recent years. In this approach, there are some studies to predict tissue change by analyzing the effects of changes in system dynamics on the closed-loop signal by using closed-loop signals used in drill control [4]. In orthopedic drills where direct current motors are used, the change of load torque and friction force indirectly causes a change in the closed-loop error signal, and this change allows the estimation of tissue change with a trained artificial intelligence-based breakthrough detection algorithm [4,7].

When the common literature was examined, no study was found to detect tissue change during a surgical drilling by estimating the disturbance with the disturbance observer. In this study, a new bone tissue change detection approach based on observing the change of the disturbance observer-based load torque and friction coefficient is presented. The mathematical model of the Brushless Direct Current Motor (BLDC) used in the orthopedic drill was constructed, then the quantitative value of the disturbance effect was observed with a closed-loop Proportional Integral Derivative (PID) controller, which will minimize the difference between the actual model and the model with disturbance applied. In the simulation studies, it has been proven that the change of the load torque and friction coefficient given to the system and whose waveforms are known, can be observed quantitatively with the developed disturbance observer.

## 2. MATERIAL AND METHOD

The disturbance observer-based torque and friction observer approach has been developed with the BLDC mathematical model, which is widely used in orthopedic drills. The change of load torque and friction gives meaningful information about the state of the drilling according to the hardness of the cortical and spongy bone types [4,13]. MATLAB/Simulink was preferred for simulation based on drill motor parameters [18], with the purpose of making real-time analysis, creating the mathematical model of the disturbance observer-based system.

### 2.1. Brushless Direct Current Motor and Its Mathematical Model

In the study, a brushless direct current motor (BLDC) was used due to its features such as high efficiency at low power, silent operation, low maintenance and low cost, absence of electrical arcs and minimum electrical losses. The following assumptions have been made in order to make the mathematical modeling of BLDC [19,20].

- All stator resistances and inductances are assumed to be equal and constant.
- The motor is not saturated.
- Eddy current and hysteresis effects in the magnetic materials of the machine have an insignificant effect on the rotational current.
- Semiconductors in the power switching circuit are ideal.
- All phases have the same Back-EMF waveform.
- Iron losses are negligible.

The flexibility of the rotor and shaft is assumed to be zero. A viscous friction model is used in the simulated model [21]. The relationship between the input voltage of a three-phase, star-connected BLDC and the phase currents and Back-EMF can be expressed mathematically as shown below.

$$V_{ab} = R(i_a - i_b) + L \frac{d}{dt}(i_a - i_b) + e_a + e_b \quad (1)$$

$$V_{bc} = R(i_b - i_c) + L \frac{d}{dt}(i_b - i_c) + e_b + e_c \quad (2)$$

$$V_{ca} = R(i_c - i_a) + L \frac{d}{dt}(i_c - i_a) + e_c + e_a \quad (3)$$

In the equations 1, 2 and 3,  $V$  represents the phase-to-phase voltage,  $R$  represents the resistance of each phase,  $L$  represents the inductance of each phase, and the Back-EMF voltages are represented as  $e_a$ ,  $e_b$ , and  $e_c$ , respectively. Electromechanical behavior of the motor according to the law of motion can be stated as;

$$T_e = B\dot{\theta} + J\ddot{\theta} + T_L \quad (4)$$

In equation 4,  $T_e$  represents electrical torque,  $\dot{\theta}$  represent mechanical speed,  $B$  represents viscous friction coefficient,  $J$  represents rotor inertia,  $T_L$  represents load torque. It is also expressed in the relations given in Equations 5 and 6 between voltages and currents:

$$V_{ab} + V_{bc} + V_{ca} = 0 \quad (5)$$

$$i_a + i_b + i_c = 0 \quad (6)$$

Based on Equations 5 and 6, only two voltage values are needed for modeling and when expressed as in Equations 7 and 8;

$$2V_{ab} + V_{bc} = 3Ri_a + 3L \frac{d}{dt}i_a + 2e_a - e_b - e_c \quad (7)$$

$$-V_{ab} + V_{bc} = 3Ri_b + 3L \frac{d}{dt}i_b + 2e_b - e_a - e_c \quad (8)$$

The produced electrical torque by the three-phase, star-connected BLDC is given in Equation 9.

$$T_e = (e_a i_a + e_b i_b + e_c i_c) / \dot{\theta}_m \quad (9)$$

The mathematical equations of trapezoidal Back-EMF voltages are expressed as in Equations 10, 11 and 12:

$$e_a = (k_e / 2\dot{\theta}_m) \text{Trapezoidal}(\theta_e) \quad (10)$$

$$e_b = (k_e / 2\dot{\theta}_m) \text{Trapezoidal}(\theta_e - 2\pi/3) \quad (11)$$

$$e_c = (k_e / 2\dot{\theta}_m) \text{Trapezoidal}(\theta_e - 4\pi/3) \quad (12)$$

Here,  $k_e$  and  $\theta_e$  represent the back-EMF constant and the electric angle, respectively. Also,  $P$  stands for the number of poles. Moreover, trapezoidal ( $\theta_e$ ) is a function defined as a trapezoidal wave, and a period of this function is as in Equation 13.

$$\text{Trapezoidal}(\theta_e) = \begin{cases} 1 & 0 \leq \theta_e \leq 2\pi/3 \\ 1 - \frac{6}{\pi(\theta_e - 2\pi/3)} & 2\pi/3 \leq \theta_e \leq \pi \\ -1 & \pi \leq \theta_e \leq 5\pi/3 \\ 1 - \frac{6}{\pi(\theta_e - 5\pi/3)} & 5\pi/3 \leq \theta_e \leq 2\pi \end{cases} \quad (13)$$

When equations 10, 11 and 12 are used in Equation 9, the expression of  $T_e$  is obtained as in Equation 14.

$$T_e = \frac{k_e}{2} \left[ \text{Trapezoidal}(\theta_e) i_a + \text{Trapezoidal}(\theta_e - \frac{2\pi}{3}) i_b + \text{Trapezoidal}(\theta_e - \frac{4\pi}{3}) i_c \right] \quad (14)$$

When the electrical torque equation expressed as  $T_e$  in Equation 4 is arranged, the speed  $w$  of the rotor of the motor is given in equations 15a and 16.

$$T_e = J \frac{dw}{dt} + Bw + T_L \quad (15a)$$

$$T_e - T_L = J \frac{dw}{dt} + Bw \quad (15b)$$

$$w(s) = \frac{T_e - T_L}{Js + B} \quad (16)$$

The electrical and mechanical BLDC parameters which are used in the simulation study are given in Table 1 [18].

**Table 1.** Motor parameters used in the simulation study [18]

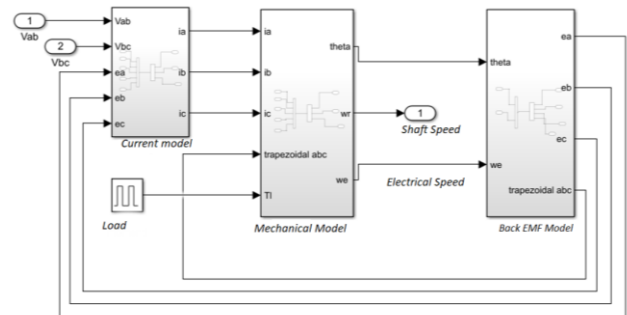
$R(\Omega)$	$L(H)$	$K_{emf}$ ( $V.s/rad$ )	$J$ ( $Nms^2/rad$ )	$B$ ( $Nms/rad$ )
2.875	0.0085	175	0.0008	0.0001

## 2.2. Implementation of Disturbance Observer Design

The systems in which the effects of unknown or uncertain disturbance effects on the system can be observed without the use of external additional detectors are called disturbance observers [22,23]. Disturbance observers are used to observe immeasurable states and variables which are commonly used in force control applications, robotic manipulators, and many systems process control system [22,24,25].

There are several advantages of using an observer-based method for estimating state of the drilling in bone drilling operation. Observer is a system, which is used to reconstruct the states of a dynamic system by using input signal measurements. They are used in many applications such as feedback control, system stability monitoring and fault detection [26]. The disturbance observer was developed in the late 1980s as a new type of observer for the control of mechatronic systems, and they are used to predict the unknown inputs of the system, unlike state observers which are used to reconstruct the unknown states of a system [27]. Disturbance observers increase the stability and performance of the control system by predicting unknown disturbances affecting the control systems. Also, a disturbance observer can be used in a control system to reduce the number of costly detectors needed [28].

In this study, MATLAB/Simulink was preferred for simulation, in order to carry out real-time simulations, creating a mathematical model of the BLDC and disturbance observer-based system, and interpreting the simulations according to motor parameters. The block diagram of the simulated BLDC model is given in Figure 1. The disturbance observer used for torque estimation in brushed direct current motors (DC), which was reported with research work by Lee and Ahn [27], has been adapted for BLDC and determined bone tissue changing in an orthopedic drill with observing the change in friction coefficient and load torque in the current study.



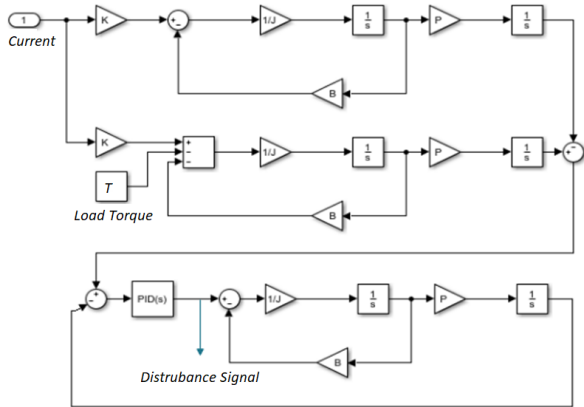
**Figure 1.** The simulation diagram of Brushless DC motor.

## 2.3. Detection of Changes in Load Torque and Friction Coefficient by Using Disturbance Observer

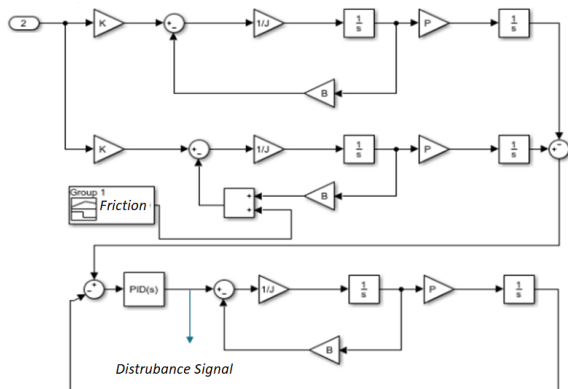
In this section, during the bone drilling process, the effect of the variation of the load torque and friction coefficient during the bicortical drilling path is modeled by using MATLAB/Simulink. While performing the simulation study, two models expressing the effects of either varying load torque or varying friction coefficient have been constructed. Considering the change in the load torque firstly; the effect of the electrical torque obtained from the mechanical model of the motor on the motor speed and position together with the load torque defined as  $T_e$ ,  $T_L$  is taken as basis in the design of the disturbance observer. Simulink model of disturbance observer based load torque change detection is shown in Figure 2. A square waveform with 2Nm amplitude load torque,  $T_L$  has been applied to BLDC model to mimic the real drilling path. The variation of the friction coefficient has been assumed



zero for the first model as shown in Figure 3. The models mentioned in Figure 2 and Figure 3 consist of three sub-models in which the upper model represents the mathematical model without any disturbance, the middle model represents the model encountered with disturbance, the lower model represents the disturbance observer which produces the quantity of disturbance with the difference of ideal model output and disturbance applied model output. A PID controller that processes the rotor position difference at the outputs of the two models is used [27]. The coefficients of the PID controller determined by the MATLAB/Simulink PID Tuning Toolbox with the obtained transfer function were found as 0.0081, 0.0014 and 0.0044 for P, I and D, respectively. Secondly, the variation of the friction coefficient is determined for with friction observer. Depending on the amount of friction, the heat generated during the bone drilling process may cause thermal injuries around the hole drilled between the drill bit and the bone[29,30]. As shown in Figure 3, a friction pattern which mimics the friction of the drilling path has been applied as friction variation.



**Figure 2.** The model of disturbance observer based load torque change detection



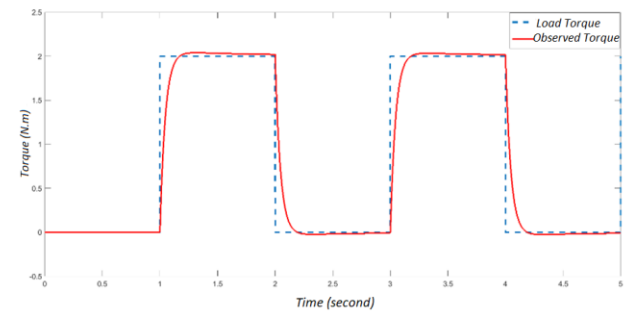
**Figure 3.** The model of disturbance observer-based friction coefficient variation detection

### 3. RESULTS AND DISCUSSIONS

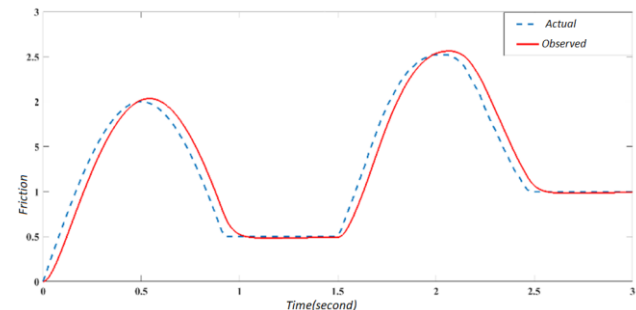
The proposed models have been constructed in Simulink for simulation works. Since the hardness of a cortical bone changes according to the drilling path as mentioned in the work of Torun and Öztürk [4], we have simulated the change of load torque and friction coefficient as drilling one hole on bicortical bone. A detailed discussion

about bicortical drilling path could be found with further reading of recent literature works[4,5,7,8,11,16,31]. For estimating the load torque, it is assumed that one-hole drilling is performed within 5 seconds. Drill bit contacts the first cortical layer at 1st second, then exits the first cortical layer and contacts the sponge layer at 2nd second, finally breakthrough occurs when drill bit exists the second cortical layer at 4th second. The actual load torque which was applied to model, and the observed value of the load torque are shown in Figure 4.

For estimating the friction coefficient between the drill bit and bone tissue a similar scenario has been assumed. Friction varies according to drill bit geometry, drill speed, and the hardness of drilled tissue as mentioned in [32,33]. In this work, it has been tried to form a synthetic friction coefficient waveform for the bicortical drilling path with knowledge of recent literature[4,6,31] which focuses on the changes in the dynamic of the drilling for bicortical bone. Drill bit contacts with the first cortical layer at zero instant. Friction increases as the drill bit travels through the first layer then decreases when it reaches the spongy bone. The friction value is lower in the sponge bone since the hardness of the sponge layer was lower than the cortical layer. The drill bit contacts the second cortical entry at the 1.5 second. Finally, breakthrough occurs as the drill bit exits the second cortical layer at 2.5 second. The synthetic wave form of friction coefficient and observed with disturbance observer have been shown in Figure 5.



**Figure 4.** Applied synthetic load torque versus observed load torque with disturbance observer



**Figure 5.** Applied synthetic friction versus observed friction with disturbance observer.

Observed load torque is close to the applied load torque at the steady-state, however, the sudden changes in the load torque cause the difference in the transient response of the disturbance observer. The rise time, delay time, and settling time have been measured as 140ms, 65 ms and

183 ms, respectively. The observed friction coefficient lags the synthetic friction coefficient with a 120 ms delay as shown in Figure 5. Since the objective of the study was to obtain the change of the tissue being drilled, it is important to observe the change of the load torque and friction with a reasonable time duration rather than obtaining the actual value of load torque and friction coefficient. Simulations show that change of load torque and friction could be obtained lower than 200ms later after the change of the drilled tissue. 200ms corresponds to the 0.2 mm drilling path with a drilling feed rate of 1mm/second (60mm/minutes) which is one of the nominal feed rate for bone drilling [33]. The 0.2 mm drilling path length can be regarded as a breakthrough error whose acceptable range lies within 1-2mm [34] to avoid damaging vital organs when drilling cortical bone.

As a result of the simulations, it was seen that the signals of the changing load torque value and the friction coefficient in the model were in a form that is similar to the signals at the observer output. This confirms that the orthopedic surgical drill, whose design will be developed in real-time, will be capable of detecting tissue changes in the transition through the bone layers during surgery and will be sufficiently sensitive and accurate for the detection of breakthrough.

#### 4. CONCLUSION

In this study, disturbance observer, which observes the changes in load torque that is directly proportional to the thrust force generated during the bone drilling process and the change in viscous friction coefficient is designed in the Matlab/Simulink simulation environment. As a result of the simulations that have been carried out in the MATLAB/Simulink environment, it has been observed that the signals of the changing torque value and friction coefficient in the model are in a form that is similar to the signals at the observer output. This specifies that the orthopedic surgical drill is capable of detecting tissue changes as it passes through the layers of bone during surgery and has sufficient sensitivity and accuracy for breakthrough detection. Real-time implementation of the proposed approach on sheep-femur drilling with BLDC based drill will be performed in future works.

#### ACKNOWLEDGMENTS

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## Structural Resistance of a Reinforced Concrete Building under Earthquake and Wind Loads in Isparta and Burdur Region

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### Keywords

Irregularity,  
Earthquake,  
Wind

**Abstract:** It has been clearly seen from building experiences and studies that lateral load effects such as earthquake load and wind load make building irregularities more obvious. Hence, it is of great importance to evaluate the regularity of the structural systems in accordance with the conditions determined by the building regulations. In this paper, irregularity effects were investigated according to Turkish Building Earthquake Regulation (TBEC) 2018. A reference building which have vertical setback irregularity were analyzed under earthquake and wind loads. Etabs software were used for structural analyses. Response spectrum method were used for the seismic analysis and TS498 for Wind load analysis. Effective relative storey drift, A1 –Torsional irregularity and B2 – Soft Storey Irregularity are comparatively investigated and Irregularity values were determined.

## Isparta ve Burdur Bölgesi'ndeki Betonarme Binanın Deprem ve Rüzgar Yükleri Altındaki Yapısal Dayanımı

### Anahtar Kelimeler

Düzensizlik,  
Deprem,  
Rüzgar

**Öz:** Deneyimler ve yapılan çalışmalar, deprem ve rüzgar yükleri gibi yanal yüklerin yapılarda bariz bir şekilde düzensizliğe neden olduğunu göstermektedir. Bu nedenle yapının düzensizlik açısından şartlara uygun olarak değerlendirilmesi büyük önem taşımaktadır. Bu çalışmada 2018 TBDY'ne (Türkiye Bina Deprem Yönetmeliği) göre yapıların düzensizlik etkileri araştırılmıştır. Düşey düzensizliğe sahip bir yapı deprem ve rüzgar yükü altında analiz edilmiştir. Sismik analiz için tepki spektrum metodu ve rüzgar yükü analizi için TS498 kullanılmıştır. Etkin görel kat ötelemesi, A1-Burulma düzensizliği ve B2-Yumuşak kat düzensizliği karşılaştırmalı olarak incelenmiş ve düzensizlik değerleri belirlenmiştir.

### 1. INTRODUCTION

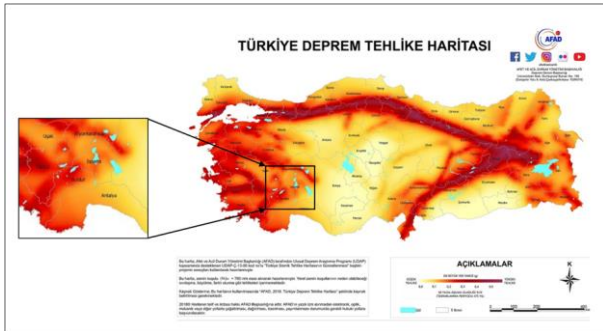
Wind and earthquakes, coupled with aging and vulnerable buildings, pose the potential for damage and loss of life and property. Both hazards can wreak catastrophic damage to buildings. Winds and earthquakes cause the majority of property loss in the world from all natural disasters. Although an seismic hazard may be more significant than the other, the rapid population growth, highrise buildings and irregularity buildings have greatly increased the potential of exposure to multiple hazards[1]. The effect of wind and earthquake on structures may vary depending on wind

load and seismic activity of the region, location and distance from the sea [2].

Turkey, one of the most seismically active countries of the world in terms of its geological features, is situated on an active area surrounded by active faults [3]. In the last century, destructive earthquakes have occurred along the North Anatolian Fault Zone (NAFZ) and East Anatolian fault lines where there are two active faults [4]. Earthquakes can not be prevented by human activity, these events occur as a result of a natural ground movement. Earthquake is one of the natural disasters that has caused the most destruction and loss of life for centuries [5] [6].



Many earthquakes occur every day in our country, where is about 92% of areas and about 95% of the population is on the earthquake zone. Therefore, all structures must be constructed against earthquake [7]. Although most of these earthquakes are of small intensity, sometimes large earthquakes occur. Such earthquakes cause loss of life and property and damage to structures. Some of these damages are irreparable depending on the current condition of the building and the nature of the earthquake [8]. However, with the necessary precautions, some of the negative consequences caused by earthquakes can be prevented or reduced [5]. The earthquake map of Turkey and the studied area in the study are given in Figure 1.



**Figure 1.** The earthquake map of Turkey and the studied area in the study [9]

Due to the shape formed by the fault lines in and around Isparta, this region is called the Isparta triangle or Isparta bend. The western edge of the Isparta Angle consists of the Fethiye–Burdur Fault Zone. The city of Isparta is located at the top of the triangle, below the point where the Burdur-Fethiye Zone intersects with the Akşehir Fault. Isparta and its surroundings are extremely sensitive against earthquakes. Isparta and its surroundings are extremely sensitive against earthquakes [8]. The earthquakes that occurred in Isparta and Burdur provinces are given in Table 1.

**Table 1.** The historical earthquakes that occurred in Isparta and Burdur [10]

Date	Region	Fault	$M_s$	$I_0$
End of 4th century BC	Dinar	?	?	?
88 BC	Dinar	?	?	IX-XI
AD 53	Dinar	?	?	VII-X
Beginning of 6th century AD	Isparta	?	?	VIII
Middle of 7th century AD	Isparta	?	?	IX-XI
AD 641–668	Isparta	?	?	VIII-X
1875	Dinar	Balkan Fault (20 km rupture)	?	IX-X
1889	Isparta	?	?	?
3 Oct. 1914	Burdur	Burdur Fault (23 km rupture)	7.1	IX
7 Aug. 1925	Dinar	Baklan Fault	6.0	VIII-IX
1933	Dinar	Baklan Fault	5.8	VIII
12 May 1971	Burdur	Burdur Fault	6.2	IX
1 Oct. 1995	Dinar	Dinar Fault (10 km rupture)	6.1	IX

Developments in structural materials and design technology in civil engineering have led to designs that satisfy strength requirements but are often flexible. This flexibility can cause unfavorable vibrations when the structure is subjected to wind or earthquake loads. These vibrations may lead to serious structural damage and affect the comfort of the occupants. Dynamics of buildings greatly depends on the characteristics of the external excitation as well as the physical properties of the building in terms of generalized masses, frequencies, and damping. Wind loads are characterized by low frequencies while earthquakes usually contain higher frequency load components [1].

On the other hand, besides the earthquake load, the wind load is also one of the important lateral loads that should be taken into account during the design phase of the building. Wind is a natural phenomenon that changes momentarily. Therefore, it is quite difficult to predict its properties such as intensity and direction. Analyzing how winds affect the structure dynamically and statically is a complex process [11].

In some cases, wind loads can be more critical to the carrier system and irreparable damage to the structure can occur if not taken into account during the design process. Wind load can cause serious damage especially in buildings which are high-rise and low cross-sectional areas [12]. With the increase in population, the number of high-rise buildings has increased considerably [13]. With the construction of high-rise buildings, the irregularity of the structure and its effects, which emerged as a result of wrong design, calculation, and application, started to become more evident.

Damages occurring in buildings after earthquake and wind loads may be caused by not taking into account irregularities such as A1 (Torsionally irregularity), A2 (Slab discontinuity), A3 (Existence of protrusions in the plan) and B1 (Strength irregularity between adjacent floors) and B2 (Soft story irregularity) during the design phase [9].

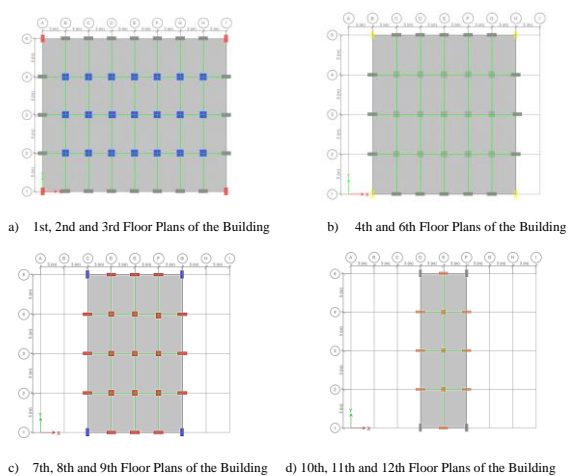
There have been many studies completed about seismic behavior of irregular structures. Demir et al. (2010) investigated horizontal seismic forces and torsional moments in different sites, the for the multiple storey structures [14]. Işık et al. (2018) examined the state of irregularity by the A3 plan in the TDBY of 2018[15]. Ilerisoy (2019) investigated vertical structural irregularities which are often inevitable due to building requirements and architectural imperatives, and having a major impact on building costs [16]. Ilgun et al. (2017) researched on A1 irregularity status in different spectral acceleration coefficients on reinforced concrete structures [17]. Keleş et al. (2021) and Uyan et al. (2021) investigated structural system safeties of 3, 5 and 7 storey existing reinforced concrete buildings having soft story irregularity according to TBEC-2018[18] [19]. Usta et al. (2022) analyzed regular and setback models of 5 storey, 9 storey, and 13 storey RC frames considered and the seismic risk effect over buildings that have vertical irregularity are investigated [20].

As seen, many studies have been conducted to investigate the effect of earthquake and wind. But there are less studies in which the structure is examined comparatively in terms of earthquake and wind load. In this study, it has been analyzed how earthquake and wind load that may occur in the Isparta and Burdur regions of Turkey will affect the performance of the structure which is located in these areas.

In the present context of study an reinforced concrete building is taken into consideration and the analysis is done as per the Turkish Standard. This building does not represent a particular real structure that has been built or proposed. However, the dimensions, general layout and other characteristics have been selected to be representative of a building for which the have vertical setback irregularity.

## 2. MATERIAL and METHOD

The aim in study is investigate the effects of earthquakes and wind loads. The building is considered to be located in Isparta and Burdur cities. For this purpose, a 12-storey reinforced concrete building was approached. The reinforced concrete building, having vertical setback irregularity. The building was dealt with the help of a ETABS software program [21]. Analysis and Irregularity controls of building were made under the earthquake and wind loads. The storey plans of the building are shown in Figure 2.a, Figure 2.b, Figure 2.c and Figure 2.d.



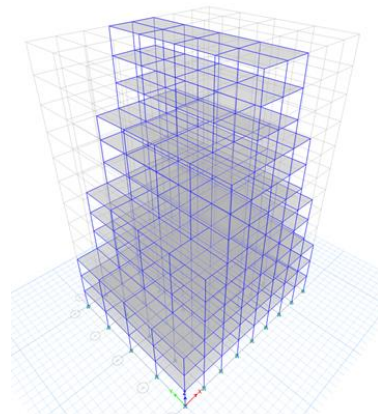
**Figure 2.** Floor plans of the building

The storey heights in the building are the same on each storey and are taken as 3 meter. Columns were designed in different sizes depending on the storey to catch optimum design and economy. Columns were chosen as 50x100 cm, 45x100 cm, 40x100 cm, 35x100 cm, 120x50 cm, 120x45 cm, 120x40 cm, 120x35 cm, 90x90 cm, 80x80 cm, 70x70 cm and 60x60 cm. Beams were chosen as 50x60 and 60x60. All slabs in the reinforced concrete building were formed with a thickness of 20 cm after analyzed according to TS 500/2000.

The dead load on the floors in the reinforced concrete building was  $G=3.5 \text{ kN (m}^2\text{)-1}$  and the live load was

taken as  $Q=2 \text{ kN (m}^2\text{)-1}$ . The dead load on the attic in the reinforced, concrete building was  $G=5 \text{ kN (m}^2\text{)-1}$  and the live load was taken as  $Q = 1.5 \text{ kN (m}^2\text{)-1}$  the modulus of elasticity was  $E=34000 \text{ MPa}$ ,

In order to represent other available buildings, the ground floor height was considered as 3 m. The building is unsymmetrical in Z direction. The distance between the axes of the building is designed 6 m intervals and the base-column joint area is defined as a fixed-support. The 3D view of the referenced building is given in Figure 3.



**Figure 3.** 3D View of the referenced building

The seismic design and examination of the structure was carried out according to TS 500. For this reason, the building importance coefficient, the structural system behavior coefficient, and the excess strength coefficient were taken as  $(I)=1$ ,  $(R)=8$ , and  $(D)=3$ , respectively. Thus, the performance of a reinforced concrete structure with irregularity has been determined as a result of wind and earthquake load effects.

### 2.1. Earthquake Analysis and Definitions

Determining the earthquake behavior of the modeled reinforced concrete structure, the coordinates of the building were taken into consideration, and according the Turkey Building Earthquake Regulation 2018, the acceleration records were determined by selecting Central Düzce from the Disaster and Emergency Management Centre (DEMC) Earthquake Zones Map. Accordingly, the earthquake load coefficients used in the analyzes for the DD-2 (earthquake ground-motion level, probability of exceedance of which is 10% in 50 years) earthquake level Isparta and Burdur provinces are given in Table 2. In addition, the horizontal elastic design spectrum of Isparta and Burdur provinces defined in ETABS are given in figure 4 and figure 5, respectively.

**Table 2.** Earthquake Load Coefficients for Isparta and Burdur Provinces

	$S_s$	$S_1$	$S_{D5}$	$S_{D1}$	Soil Class
<b>ISPARTA</b>	0.612	0.148	0.551	0.118	ZB
<b>BURDUR</b>	0.980	0.226	0.882	0.181	ZB

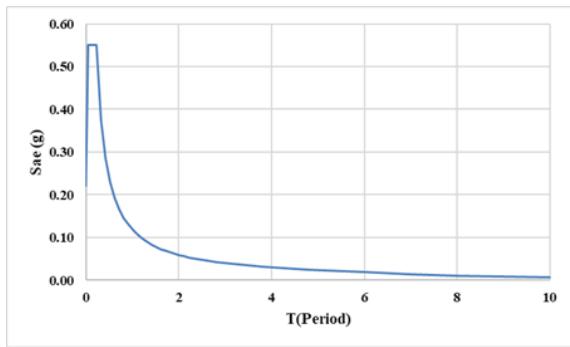


Figure 4. Horizontal elastic design spectrum of Isparta

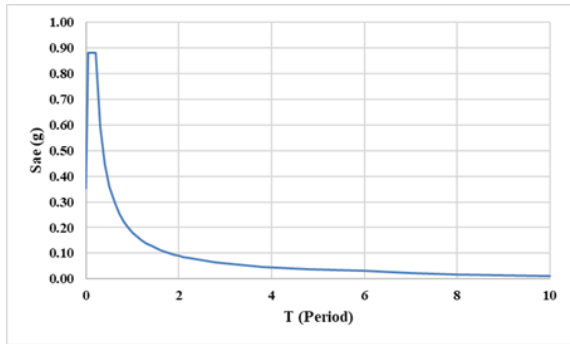


Figure 5. Horizontal elastic design spectrum of Burdur

In the 2018 Turkey Building Earthquake Regulation, there is a separate section for irregularity control of buildings. This section will be considered for irregularity checks as to the structure in the study.

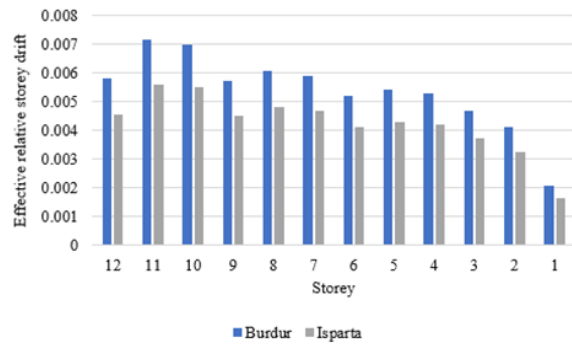
To make a comparison between different cities, the analysis of building structure was performed by using ETABS software program for the elastic response of spectra data of Turkish standards codes. Natural vibration period for x and y direction is obtained from mode 1 and mode 2 respectively. The first mode ( $T_1=0.975s$ ) mode indicates the elastic state of the structure. The bending vibration is typical and parallel to the long span of the structure.

### 2.1.1. Effective relative storey drift

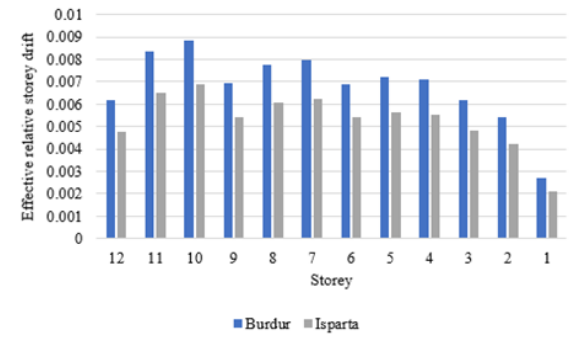
The Effective relative storey drift calculation is an important factor to control the stiffness of the structures during the design phase. An equation and a limit value are specified in the 2018 Turkish building earthquake code for the Effective relative storey drift control of the structures at the design stage. This equation and the limit value are shown in equation 1.

$$\lambda \frac{\delta_{i_{max}}^{(x)}}{h_i} \leq 0.008\kappa \quad (1)$$

For the Effective relative storey drift calculation, the fundamental vibration period of the building was determined for the provinces of Isparta and Burdur in the direction of the earthquake. The coefficients  $\lambda_x$  and  $\lambda_y$  were calculated for the fundamental period and then the effective relative storey drift ratios were found by using equation 1. Effective relative storey drift analysis for earthquake load was made according to Equation 1. The results obtained are given in Figure 6.



a) Effective relative storey drift for the X-direction



b) Effective relative storey drift for the Y-direction

Figure 6. Effective relative storey drift for the Isparta and Burdur according to earthquake load

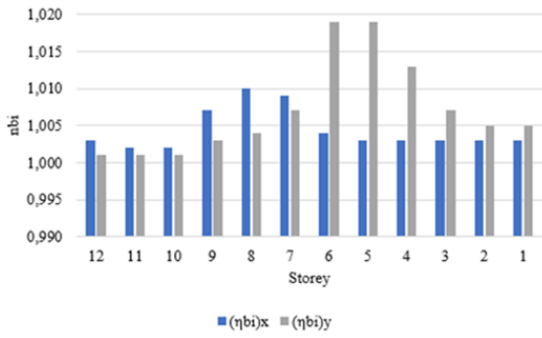
As a result of the earthquake force applied in the X direction, the maximum effective relative storey drift ratio was observed at the 11th floor and its value was calculated as 0.005610 for the Isparta. For Burdur, the maximum effective relative storey drift ratio was observed at the same floor and its value was calculated as 0.007176. The maximum effective relative storey drift ratio in the Y direction for the province of Isparta was observed at the 10th floor and its value was calculated as 0.006858. The maximum effective relative storey drift ratio for the province of Burdur was calculated as 0.008794 at the same floor. Obtained findings were examined. As a result of the examination, it was determined that it was within acceptable limits in terms of TBEC 2018.

### 2.1.2. A1 –Torsional irregularity

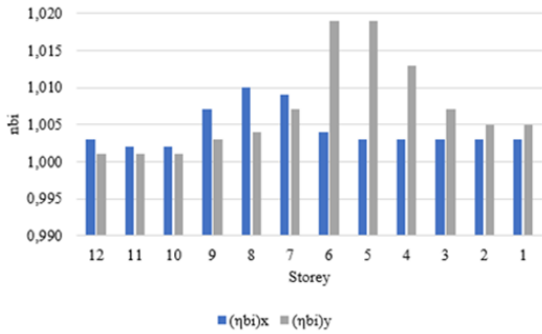
A1-Torsional irregularity is seen frequently in structures. In order to control the torsional irregularity, the torsional irregularity coefficient must be calculated. Torsional irregularity is the ratio of maximum interstorey drift value to the average interstorey drift value at each storey and given in equation 2. Torsional irregularity occurs when  $\eta_b$  value is between 1,20 and 2,00 in a structure.

$$\eta_{bi} = \frac{\Delta_{i_{max}}}{\Delta_{i_{ort}}} > 1.2 \quad (2)$$

The Torsional irregularity values obtained from the calculations are given in the Figure 7 according to the cities and X and Y directions.



a) A1-Torsional irregularities for Isparta in the X -Y direction



b) A1-Torsional irregularities for Burdur in the X -Y direction  
**Figure 7.** A1-Torsional irregularity

As the figure 7 is examined, it is seen that the data obtained from the structure as a result of the analysis remain within the specified limit values in terms of A1-Torsion irregularities control.

It can be said that the model structure provides sufficient safety against torsional irregularity under the acceleration values in the study.

**2.1.3. B2 – Soft storey irregularity**

With respect to the TBEC 2018, the soft-storey irregularity plays an important role when choosing the seismic-analysis method different from the other modern countries’ codes. According to the regulation, if the average relative displacement rate of 5% horizontal force eccentricity on a floor is more than 2.0 value, B2-Soft Storey irregularity case occurs in the building. The soft-storey conditions from the TSC 2018 are expressed with Equations (3) and (4):

$$\eta_{ki} = \frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}} > 2.0 \tag{3}$$

$$\eta_{ki} = \frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}} > 2.0 \tag{4}$$

The soft storey irregularity values obtained from the calculations are given in the Table 3 according to the cities and X and Y directions.

**Table 3.** B2- Soft storey irregularity

a) B2- Soft Storey Irregularity for Isparta City in the X -Y direction

Storey	H <sub>i</sub> (m)	X Direction				Y Direction			
		(Δ <sub>i</sub> ) <sub>ort</sub> (m)	(Δ <sub>i</sub> ) <sub>ort</sub> /H <sub>i</sub>	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$	(Δ <sub>i</sub> ) <sub>ort</sub> (m)	(Δ <sub>i</sub> ) <sub>ort</sub> /H <sub>i</sub>	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$
12	3	0.041	0.014	-	0.812	0.043	0.014	-	0.734
11	3	0.051	0.017	1.231	1.017	0.059	0.020	1.362	0.946
10	3	0.050	0.017	0.983	1.213	0.062	0.021	1.057	1.272
9	3	0.041	0.014	0.824	0.936	0.049	0.016	0.786	0.893
8	3	0.044	0.015	1.068	1.020	0.055	0.018	1.120	0.970
7	3	0.043	0.014	0.981	1.128	0.057	0.019	1.031	1.135
6	3	0.038	0.013	0.887	0.968	0.050	0.017	0.881	0.958
5	3	0.039	0.013	1.033	1.029	0.052	0.017	1.044	1.024
4	3	0.038	0.013	0.972	1.143	0.051	0.017	0.977	1.160
3	3	0.033	0.011	0.875	1.138	0.044	0.015	0.862	1.139
2	3	0.029	0.010	0.879	1.980	0.039	0.013	0.878	2.001
1	3	0.015	0.005	0.505	-	0.019	0.006	0.500	-



## b) B2- Soft Storey Irregularity for Burdur City in the X -Y direction

Storey	$H_i$ (m)	X Direction				Y Direction			
		$(\Delta_i)_{ort}$ (m)	$(\Delta_i)_{ort}/H_i$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$	$(\Delta_i)_{ort}$ (m)	$(\Delta_i)_{ort}/H_i$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$
12	3	0.064	0.021	-	0.815	0.067	0.022	-	0.737
11	3	0.078	0.026	1.228	1.022	0.091	0.030	1.357	0.950
10	3	0.077	0.026	0.978	1.219	0.096	0.032	1.053	1.274
9	3	0.063	0.021	0.820	0.937	0.075	0.025	0.785	0.892
8	3	0.067	0.022	1.068	1.020	0.084	0.028	1.122	0.971
7	3	0.066	0.022	0.980	1.129	0.087	0.029	1.030	1.135
6	3	0.058	0.019	0.886	0.970	0.076	0.025	0.881	0.958
5	3	0.060	0.020	1.031	1.030	0.080	0.027	1.044	1.026
4	3	0.058	0.019	0.971	1.141	0.078	0.026	0.975	1.161
3	3	0.051	0.017	0.876	1.134	0.067	0.022	0.862	1.137
2	3	0.045	0.015	0.882	1.977	0.059	0.020	0.880	1.998
1	3	0.023	0.008	0.506	-	0.030	0.010	0.501	-

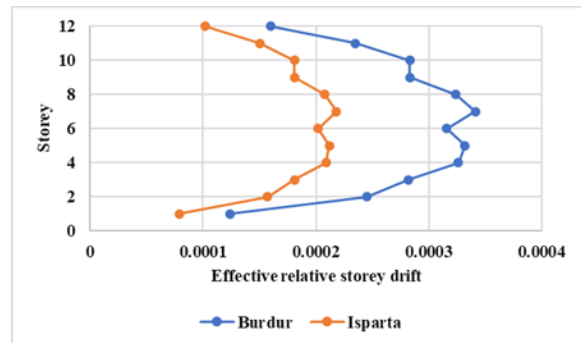
As the table 3 is examined, it is seen that the data obtained from the model structure as a result of the analysis. It is seen that B2-Soft Storey irregularity was observed only in Isparta Y direction.

## 2.2. Wind Analysis and Definitions

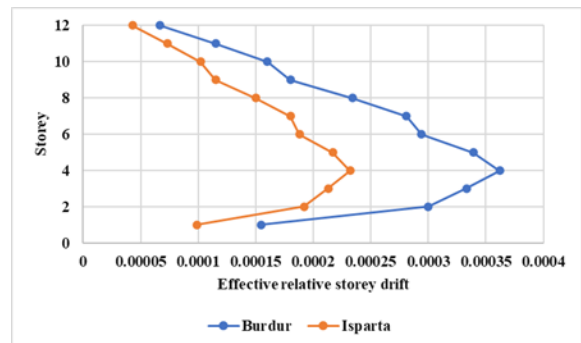
As mentioned before, in cases where the wind load is not taken into account in the design of the structures, permanent and high damages may occur in the structures. For this reason, the behavior of the model structure under the influence of wind load, which is discussed in the article, is also examined. The design of wind load was calculated based on TS498. Although Eurocode is generally used in wind analysis, TS498 was chosen in this study. The ETABS software program is selected to perform analysis and Wind analysis was carried out for the model structure of Isparta and Burdur Cities. The wind speed was determined as 40 m/s and 50 m/s, respectively based on the Republic of Turkey Ministry of Environment and Urbanization Meteorology. The land regions in which the structures are located were selected as the 2nd region also based on TS498

### 2.2.1. Effective relative storey drift

Analyzes were made for the Isparta and Burdur model structures and the effective relative storey drift values were calculated according to the wind loads. Effective relative storey drift analysis for wind load was made according to Equation 1. The results obtained are given in Figure 8.



a) Effective relative storey drift ratio in the X direction



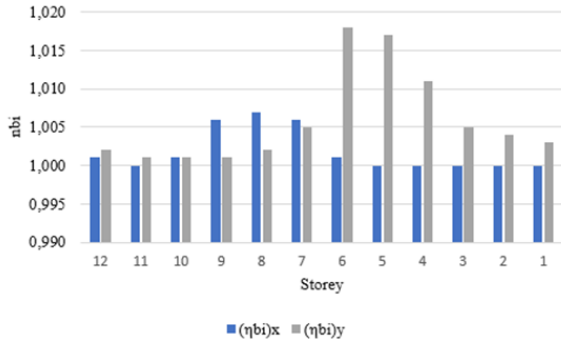
b) Effective relative storey drift ratio in the Y direction

**Figure 8.** Effective relative storey drift ratio in Isparta and Burdur provinces according to wind load

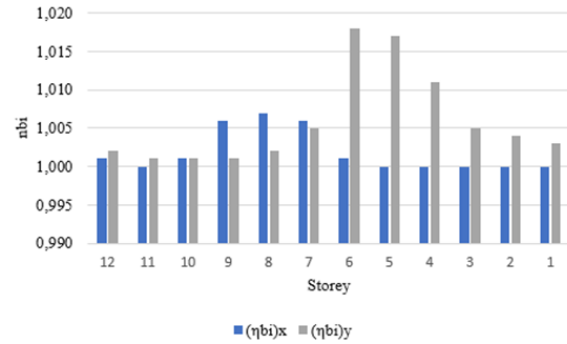
For Isparta X direction, the maximum effective relative storey drift ratio was calculated as 0.000218 at the 7th storey, according to the wind load. For Burdur province, the maximum effective relative storey drift ratio was calculated as 0.000341 at the 7th Storey. For Isparta Y direction, the maximum effective relative storey drift ratio was calculated as 0.000232 at the 4th storey. The maximum effective relative storey drift ratio for the province of Burdur was calculated as 0.000362 at the 4th storey. As the results of the analysis are evaluated in generally, it is seen that the effective relative storey drift for both cities is within the TBEC specified limits.

### 2.2.2. A1 – Torsional irregularity

Equation 2 was used to analyze the A1-torsion irregularity case in the Isparta and Burdur Cities according to the wind loads and the obtained values are given in Figure 9.



a) A1-Torsional irregularities for Isparta in the X -Y direction



b) A1-Torsional irregularities for Burdur in the X -Y direction  
Figure 9. A1-Torsional irregularity

As the figure 9 is examined, it is seen that the data obtained from the structure as a result of the analysis remain within the specified limit values in terms of A1-Torsion irregularities control. It can be said that the model structure provides sufficient security against torsional irregularity under the wind loads values in the study.

### 2.2.3. B2- Soft storey irregularity

Equation 3 and 4 were used to analyze the B2-Soft Storey irregularity case in the Isparta and Burdur Cities according to the wind loads and the obtained values are given in Table 4.

Table 4. B2-Soft Storey Irregularity

a) B2- Soft Storey Irregularity for Isparta City in the X -Y direction

Storey	$H_i$ (m)	X Direction				Y Direction			
		$(\Delta_i)_{ort}$ (m)	$(\Delta_i)_{ort}/H_i$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$	$(\Delta_i)_{ort}$ (m)	$(\Delta_i)_{ort}/H_i$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$
12	3	0.003	0.001	-	0.678	0.001	0.0004	-	0.582
11	3	0.005	0.002	1.474	0.832	0.002	0.001	1.719	0.719
10	3	0.005	0.002	1.202	1.002	0.003	0.001	1.391	0.887
9	3	0.005	0.002	0.998	0.875	0.003	0.001	1.127	0.770
8	3	0.006	0.002	1.142	0.949	0.004	0.001	1.299	0.836
7	3	0.007	0.002	1.053	1.072	0.005	0.002	1.196	0.966
6	3	0.006	0.002	0.932	0.953	0.006	0.002	1.035	0.867
5	3	0.006	0.002	1.049	1.018	0.006	0.002	1.153	0.930
4	3	0.006	0.002	0.983	1.155	0.007	0.002	1.075	1.083
3	3	0.005	0.002	0.866	1.153	0.006	0.002	0.923	1.104
2	3	0.005	0.002	0.867	1.975	0.006	0.002	0.906	1.943
1	3	0.002	0.001	0.506	-	0.003	0.001	0.515	-

## b) B2- Soft Storey Irregularity for Burdur City in the X -Y direction

Kat	H <sub>i</sub> (m)	X Doğrultusu				Y Doğrultusu			
		( $\Delta_i$ ) <sub>ort</sub> (m)	( $\Delta_i$ ) <sub>ort</sub> /H <sub>i</sub>	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$	( $\Delta_i$ ) <sub>ort</sub> (m)	( $\Delta_i$ ) <sub>ort</sub> /H <sub>i</sub>	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i+1}/H_{i+1})_{ort}}$	$\frac{(\Delta_i/H_i)_{ort}}{(\Delta_{i-1}/H_{i-1})_{ort}}$
12	3	0.005	0.002	-	0.678	0.002	0.001	-	0.581
11	3	0.007	0.002	1.475	0.832	0.003	0.001	1.720	0.718
10	3	0.008	0.003	1.201	1.002	0.005	0.002	1.392	0.890
9	3	0.008	0.003	0.998	0.875	0.005	0.002	1.123	0.769
8	3	0.010	0.003	1.143	0.951	0.007	0.002	1.301	0.835
7	3	0.010	0.003	1.052	1.072	0.008	0.003	1.197	0.965
6	3	0.009	0.003	0.933	0.952	0.009	0.003	1.036	0.867
5	3	0.010	0.003	1.051	1.018	0.010	0.003	1.153	0.930
4	3	0.010	0.003	0.982	1.156	0.011	0.004	1.075	1.084
3	3	0.008	0.003	0.865	1.153	0.010	0.003	0.923	1.106
2	3	0.007	0.002	0.868	1.978	0.009	0.003	0.904	1.940
1	3	0.004	0.001	0.505	-	0.005	0.002	0.516	-

As the table 4 is examined, it is seen that the data obtained from the model structure as a result of the analysis. It can be said that the model structure provides sufficient security against Soft Storey irregularity under the wind loads values in the study.

### 3. RESULTS

It is important to state that, even if the interstorey drift ratios in buildings may be relatively small with no significant risk issues for the main force resisting system of the structure, nonstructural systems represent a high percentage of loss exposure of buildings to earthquakes [1]. Hence, all risk issues, all extra additional loads in structures must be carefully considered.

In this study, the vertical setback irregularity, formed because of the various design reasons was investigated. A reference building were compared in term of city, earthquake and wind load. The analyses were done using the ETABS structural analysis program. In this context, in a building model a vertical setback irregularity was deliberately formed.

It is stated in the 2018 TBEC that all structures to be built in earthquake zones should be designed by taking into account the irregularity conditions and evaluating within certain limits. In this paper, the building model with setback irregularity was discussed. The structure was examined according to the located in two different cities, Isparta and Burdur, and exposed to earthquake and wind loads. For this, ETABS structural analysis program has been used to evaluate the results by the referenced building. During examination of the result analyses, torsional irregularity, soft storey irregularity, and effective relative storey drift conditions occurring in the building were examined and compared.

According to the results obtained from the response spektrum analysis, it is seen that the effective relative storey drift and A1-torsion irregularity in both cities meet the regulation limit values. In B2-Soft Storey irregularity control, the value obtained in Isparta Y

direction for the 2nd floor was found to be higher than the limit values of the regulation. In addition, it is seen that the effective relative storey drift values are higher in Burdur city than Isparta city. The reason for this is that the earthquake accelerations of Burdur city are higher compared to Isparta.

Besides the earthquake analysis, the results obtained from the wind load analysis, which is the second most important horizontal load acting on the structure, were compared in terms of torsional irregularity, soft story irregularity and effective relative storey drift. According to the results obtained, it is seen that the effective relative storey drift, torsional irregularity and soft storey irregularity meet the regulation limit values for both cities according to the X and Y directions.

As the values obtained as a result of response spectrum and wind analysis were compared, the values obtained in the wind load analysis were lower than the values obtained from the earthquake analysis.

The acceleration values that change according to the location and coordinates of the cities and the annual wind average have been effective on this situation. For this reason, it is possible to obtain more critical results in studies to be carried out for different regions and different heights, unlike the results obtained from this study.

As a result, It seems that reinforced concrete buildings designed for earthquake and wind are safe under moderate earthquake loads. Nevertheless, it is important to, even if the interstorey drift ratios in buildings may be relatively small with no significant apparent issues for the main force resisting system of the structure, nonstructural systems may represent a high percentage of loss exposure of buildings to earthquakes and winds. Hence, it is necessary to design by considering the wind load, especially in some regions in our country and appropriate regular design techniques are should recommended for the reduction under wind and earthquake loads.

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## Polymer-Based Transfection Agents Used in CRISPR-CAS9 System

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**Abstract:** Genome editing is a method used to make desired changes in the target gene. Today, various methods are used for genome-editing studies; among them, one of the most widely used methods is the clustered, regularly interspaced short palindromic repeats (CRISPR). CRISPR-associated (Cas) genes and their corresponding CRISPR sequences constitute CRISPR-Cas systems. Due to its simplicity, it is likely that the CRISPR–Cas system could be used effectively in ex vivo gene therapy studies in humans. If this happens, the importance of CRISPR carrier systems will gradually increase. Viral and non-viral systems are used as delivery modalities in genome-editing studies. It has been proven that nanoparticles are the most promising tools for gene therapy due to their adjustable size, surface, shape, and biological behaviours. The polymeric carrier system has become the main non-viral substitute for gene delivery due to its reduced immunogenicity and pathogenicity. In this review, information about current studies related to polymeric carriers used in non-viral CRISPR delivery systems is presented.

## CRISPR-CAS9 Sisteminde Kullanılan Polimer Bazlı Transfeksiyon Ajanları

**Anahtar  
 Kelimeler**  
 CRISPR,  
 Viral olmayan  
 Transfeksiyon  
 ajanları,  
 Polimerik  
 Trasnfeksiyon  
 Ajanları,

**Öz:** Genom düzenleme, hedef gende istenilen değişiklikleri yapmak için kullanılan bir yöntemdir. Günümüzde genom düzenleme çalışmaları için çeşitli yöntemler kullanılmaktadır; bunlar arasında en yaygın kullanılan yöntemlerden biri kümelenmiş, düzenli aralıklarla yerleştirilmiş kısa palindromik tekrarlardır (CRISPR). CRISPR ile ilişkili (Cas) genleri ve bunlara karşılık gelen CRISPR dizileri, CRISPR-Cas sistemlerini oluşturur. Basitliği nedeniyle, CRISPR-Cas sisteminin insanlarda *ex vivo* gen terapisi çalışmalarında etkili bir şekilde kullanılmaya başlanmıştır ve CRISPR taşıyıcı sistemlerin önemi giderek artmaktadır. Genom düzenleme çalışmalarında dağıtım yöntemleri olarak viral ve viral olmayan sistemler kullanılmaktadır. Nanopartiküllerin ayarlanabilir boyutları, yüzeyleri, şekilleri ve biyolojik davranışları nedeniyle gen terapisi için en umut verici araçlar olduğu kanıtlanmıştır. Polimerik taşıyıcı sistem, azaltılmış immünojenisitesi ve patojenitesi nedeniyle gen aktarımı için viral olmayan ana ikame haline gelmiştir. Bu derlemede, viral olmayan CRISPR dağıtım sistemlerinde kullanılan polimerik taşıyıcılarla ilgili güncel çalışmalar hakkında bilgiler sunulmaktadır.

### 1. INTRODUCTION

The field of genome modification includes techniques used to obtain centered modifications inside an organism's DNA. Until today, the rapid transition of gene editing into clinical practice has occurred thanks to the emergence of

programmable nucleases that allow scientists to perform gene editing in numerous cell types. Today, existing editing approaches include zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered, regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated

nuclease 9 (Cas9). ZFN is a targetable DNA fragmentation protein that can be used to cleave DNA sequences at any site. On the other hand, TALENs can cause a double-strand break in the target nucleotide sequence, which leads to genome modification by triggering the DNA damage response pathway [1]. From 2002 to 2011, respectively, ZFNs and TALENs have been mainly used in genome editing in animal and human cells

and plants; however, during this time, they have also exhibited some disadvantages that prevent their usage in certain cases. For example, ZFNs show limited specificity and can cause off-target mutations [2]. In addition, vector design for ZFNs and TALENs takes time and effort to perform [3]. Table 1 presents a comparison of the TALEN, ZFN, and CRISPR-Cas9 genome editing techniques.

**Table 1.** Comparison of the TALEN, ZFN, and CRISPR-Cas9 genome editing techniques [4]

	CRISPR/Cas9	ZFN	TALEN
Molecular elements	Single-stranded gRNA+Cas9 endonuclease	ZFN protein+FokI endonuclease	RVD of the TALE protein sequenced repeat region+FokI endonuclease
Recognition site and specificity	<ul style="list-style-type: none"> <li>Usually 20 bp directory sequence + PAM sequence</li> <li>Tolerate spatial/consecutive multiple mismatches</li> </ul>	<ul style="list-style-type: none"> <li>Each ZFN protein recognizes 3 bp DNA</li> <li>Tolerate a small number of positional mismatch</li> </ul>	<ul style="list-style-type: none"> <li>Each TALE protein unit recognizes a single base pair of DNA.</li> <li>Tolerate a small number of positional mismatch</li> </ul>
Challenges and restrictions	<ul style="list-style-type: none"> <li>A PAM sequence must come before the targeted area.</li> <li>Utilizing oligo synthesis and standard cloning procedures</li> </ul>	<ul style="list-style-type: none"> <li>Difficult to target regions with poor G nucleotide content</li> <li>Requires significant protein engineering</li> </ul>	<ul style="list-style-type: none"> <li>5' targeted base should be one T nucleotide for each TALEN monomer</li> <li>Difficult and complex cloning methods</li> </ul>
Advantages	Targeting multiple genes	Small protein size and suitable for viral vector	High recognition specificity and no need to link between repeats
Transfection difficulties	The widely used SpCas9 is medium in size and may cause packaging issues for some viral vectors, but smaller orthologs are available	Since ZFN expression elements are small, they are simple to use in a variety of viral vectors.	Due to the large size of functional components, it is difficult.

Due to the reasons mentioned above, the use of CRISPR-Cas9 in genome editing has been widely preferred since 2013. The CRISPR-Cas9 method is an RNA-guided endonuclease that can specifically target DNA sequences through nucleotide base pairing. This system is highly specific, versatile, and simple. It has become a valuable tool in biological research for both gene function and gene editing because of these properties [5]. With the reporting of the first CRISPR structure by Ishino in 1987, it has reached the present day as a mechanism used by many bacteria and archaea to protect them from virus invasion [6, 7]. In 2002, after observing the existence of several similar structures in different archaea and bacteria, Jansen proposed the acronym for CRISPR [8].

Finally, the approach was first applied to mammalian cells in 2013 and since then, the CRISPR-Cas system has occupied an important place in genome-editing research [9]. CRISPR-Cas structures are categorized from type I to type VI [10]. The type I system is characterized by the formation of Cas3, which is a protein with both DNase and helicase activities that can be used to degrade targets. The type II CRISPR-Cas system uses Cas1, Cas2, Cas9, and a fourth protein (either Csn2 or Cas4) [11]. The type III-A CRISPR-Cas system is an adaptive immune system guided by prokaryotic RNA, which uses Csm (a protein-RNA complex that can perform transcription-dependent immunity against foreign DNA) [12].

The type II CRISPR-Cas system is derived from *Streptococcus pyogenes* and consists of three parts: CRISPR RNA (crRNA), trans-activating crRNA

(tracrRNA), and a Cas9 protein [13]. Cas9 is an enzyme that uses CRISPR sequences to recognize and separate specific DNA strands; thus, the Cas9 enzyme and CRISPR sequencing can be used together to edit genes in organisms and form the basis of CRISPR-Cas9 technology [14]. The regulation process has a huge range of applications, including the improvement of biotechnology products, basic organic studies, and disease treatment [15].

In general, Cas proteins obtained from bacteria have two nuclease domains and work together with single-guide RNA (sgRNA) to create a double-strand break in the target DNA sequence. sgRNA is the component that directs the Cas protein to the target DNA since it contains crRNA and tracrRNA and has a short sequence complementary to the target DNA sequence [9]. Another critical component is the protospacer adjacent motif (PAM), which is located in the target DNA sequence and guides the determination of the cut side of the Cas protein. This system has made it possible to perform applications such as mutation deletion, gene suppression and activation in the target region [14].

By applying this method in the field of genetic engineering, the structure of DNA can be changed precisely in areas where the genome is cut. Moreover, to recognize and repair damaged DNA, this method can be used in the field of genetic engineering by making use of the mechanism of cells. The successful application of the CRISPR-Cas9 system is based on the correction of the cut ends formed by the double-strand break in the DNA as a

result of the cutting created in the desired region of the genome/gene by non-homologous end-joining or a homologous recombination repair mechanism. By modifying the components of the system, the desired and designed genome/gene change can be realized. Also, through linking the engineered sgRNA sequence to the target DNA region by base pairing, the Cas protein recognizes the cleavage site and cuts both strands of the chain from three nucleotides near the PAM by way of nuclease activity, and subsequently, endogenous DNA repair is initiated. After this step, if the knockout of the target gene is aimed, the double-strand break is repaired by the non-homologous repair mechanism that is prone to error. Insertion mutations that occur during this time cause the loss of gene products.

### 1.1. CRISPR–Cas9 Carrier Systems

There are several obstacles to the practical application of the CRISPR–Cas9 Carrier Systems. These obstacles can be summarised as follows:

1. The stability of the CRISPR–Cas9 system against serum nucleases is very low.
2. The CRISPR–Cas9 system can stimulate the innate immune system.
3. This system may interact nonspecifically with non-target cells and serum proteins.
4. The CRISPR–Cas9 system can be easily cleaned by the kidney system.
5. It is difficult to enter target tissues through blood vessels
6. Degradation products of carriers can be toxic
7. Requires different approaches for different cell line [16-21].

To overcome these problems, new carrier systems have been introduced over time. Viral vectors (e.g., lentivirus, adeno-associated virus, and adenovirus) and physical strategies (e.g., microinjection, electroporation and osmocytosis-induced transduction, mechanical cell deformation, and hydrodynamic injection) are the most widely used delivery system strategies [22-25]. Viral vectors are widely used in CRISPR–Cas9 delivery, and viral carrier systems provide high efficiency in genome editing. However, viral carrier systems also show some basic limitations such as the risk of cancer development, the fact that it is a very long and difficult process to produce on a large scale and their propensity for immune-system stimulation [26, 27].

Physical carriers such as electroporation and microinjection have high degrees of transfection efficiency, but they are not suitable as methods to be adopted for *in vivo* purposes [28-30]. Instead, a hydrodynamic application strategy can be used for *in vivo* experiments; however, it causes various problems such as hepatomegaly, hypertension, and heart dysfunction [31, 32]. In recent years, many studies have been conducted on the development of nanoparticle systems to deliver the CRISPR–Cas9 system to target cells. By overcoming the aforementioned obstacles through the synthesis of advanced nanocarriers, the gene-delivery efficiency can be significantly increased [33-35]. Polymers exhibit

significant potential due to their low carcinogenicity or immunogenicity, reduced restrictions with respect to load size, protection of cargo molecules, and creation of nanoformulations with the CRISPR–Cas9 system [36]. In addition, various modifications made on polymers can enhance their circulation time, the controlled release of cargo molecules and cell- or tissue-specific delivery capacities [37, 38]. This review focuses on polymeric nanocarrier systems used in the context of the CRISPR–Cas9 system.

### 1.2. CRISPR–Cas9 Polymeric Nanocarrier Systems

Polymeric nanoparticles are widely used for the transport of diverse types of nucleic acids [39]. Polymeric carriers can be synthesized from synthetic monomers [e.g., poly(lactic-co-glycolic) acid (PLGA) or polycaprolactone] or natural monomers (i.e., sugars such as chitosan) (Glass et al., 2018). Polymer can protect the cargo molecule from degradation and can increase specific release by functionalizing the cargo molecule with targeting moieties that will enable it to bind to surface receptors of the target cell [12]. However, there are few examples of polymeric carriers reported to date in the literature regarding the CRISPR–Cas9 delivery system. Nanoparticle systems can be used to transport all three forms of CRISPR–Cas9 components (i.e., Cas9 protein/sgRNA, Cas9 mRNA/sgRNA, and Cas9/sgRNA plasmid DNA) to desired cells.

In the literature, cationic polymers such as polyethyleneimine (PEI) have been studied for the transport of CRISPR–Cas9 systems. These polymers are highly positively charged, which can protect DNA from physical and chemical factors. In addition, it presents a high endosomal escape capability to liberate plasmid. So far, polymers such as PLGA, poly(amidoamine), PEI, and chitosan have been tested for delivery of the CRISPR–Cas9 plasmid to target cells, and they have demonstrated good genome-editing potential. They have high positive charges, can interact strongly with negatively charged nucleic acids, and can effectively escape from the endosome due to a strong proton-sponge effect; all of these ensure that it exhibits a high degree of gene-transfection efficiency [40]. In the research conducted by Ryu et al. [38], it was shown that commercial jet-PEI and 25-kDa branched PEI polymer-based DNA transfection reagent successfully delivered plasmid-encoding Cas9/sgRNA. However, the use of these nanocarriers has been restricted due to their high cytotoxicity [38]. Both the transfection and toxicity efficiency of PEI increase in parallel with its molecular weight. Therefore, low-molecular-weight PEI (LMWPEI) has been tested in CRISPR–Cas9 plasmid delivery since its low transfection efficiency is tolerable. Moreover, various modifications have been made to LMWPEI in order to improve its properties. Lino et al. [41] functionalized the LMWPEI polymer with polyethylene glycol (PEG) and cholesterol (CHOL) to obtain a lipopolymer. They showed notably that CHOL increased the permeability through the membrane and PEG increased the hemocompatibility and stability of the lipopolymer. This lipopolymer was then modified with specific aptamers for targeted delivery of

the CRISPR–Cas9 system [41]. Li et al. [34] developed a polymeric ‘core-shell’ complex with LMWPEI coated with RGD-R8-PEG-HA copolymer, which is a copolymer containing RGD-R8 peptide. In this arrangement, the peptide RGD-R8 had a significant effect on an integrin receptor that was overexpressed in tumours and tumour blood vessels and which gave the polymer tumour-targeting ability. In addition, Li et al. [42] showed that the anionic HA (hyaluronic acid) fragment reversed the cationic charge of the fluorinated LMWPEI and the PEG fragment made the complex more stable, thereby it reduced nonspecific interactions with the human body. In their research, Liu et al. [43] modified LMWPEI by fluorination. They revealed that the polymer subsequently became both hydrophilic and hydrophobic after fluorination, and nanoparticles with good resistance to the lipid bilayer were easily absorbed by cells, while escaping from endosomes also occurred [43]. Zhang et al. [44] assessed the *in vitro* transmission of the CRISPR–Cas9 system mediated by the cationic polymer PEI–cyclodextrin (PC) in HeLa cells. Their study showed that these nanocomplexes produced with Cas9/sgRNA plasmids were positively charged and could facilitate proper cell uptake and transfection. Ultimately, the efficient packaging and concentration of the plasmid encoding Cas9 and sgRNA in PC was realized. This study also emphasized that although it was similar to high-molecular-weight PEI in terms of structure, PC showed lower cytotoxicity, which allowed PC to function at high dose levels or repeated transfections [44]. Kang et al. [45], covalently linked conjugated branched PEI (bPEI) to the Cas9, then combined this grouping with sgRNA. This nanocomplex targeting the *mecA* gene was successfully transferred to *Staphylococcus aureus* despite the bacteria's thick cell wall. In addition, it was found that the efficiency of the polymer-conjugated Cas9 was relatively higher compared to the efficiency of genome editing of standard lipid-based formulations or natural Cas9 complexes [45].

PBAE has features such as low toxicity, high water solubility, good biocompatibility, pH sensitivity, and rapid drug release at acidic pH values [46]. A new nanostructure consisting of poly( $\beta$ -amino ester) (PBAE) was designed by Zhu et al. [46] to transfer CRISPR/short-hairpin RNA (shRNA) to HPV16 transgenic mice. E6 and E7 human papillomavirus (HPV) are oncoproteins that play an essential role in the development of cervical cancer and drug resistance. Carboxylated branched poly( $\beta$ -amino ester) nanoparticles produced by Rui et al. [45] ensured the efficient and rapid delivery of Cas9/sgRNA to the target cell cytoplasm and escaped from endosomes. This system demonstrated *in vitro* knockout and knockin efficiency results as 75% and 4%, respectively [47].

Xu et al. [48] showed a type of cationic CHOL-assisted PLGA nanoparticles (CLAN) consisting of PEG-PLGA and BHEM-CHOL (N,N-bis(2-hydroxyethyl)-N-methyl-N-(2-cholesteryloxycarbonyl aminoethyl) ammoniumbromide). While PEG was used to support the long-term circulation of the nanoparticles, cationic lipids were used to neutralize the negative charge of the

polymer. These scholars also designed a CLAN library with different PEG densities and surface loads to produce the appropriate CLAN and then tested macrophage uptake *in vivo* to screen for the ideal option. Their results showed that nanoparticles with the highest surface loading and relatively low PEG density had the best uptake by macrophages [48]. PEGylation is widely used with the reticuloendothelial system to reduce opsonization and clearance. It can also be applied as a strategy for CRISPR–Cas9 delivery [49].

Chitosan is a positively charged, nontoxic and biodegradable polymer. Chitosan, as a widely used polymer for gene delivery systems, also exhibits potential for the delivery of the CRISPR/Cas9 system [43]. Zhang et al. [50] combined chitosan with mPEG for CRISPR–Cas9 delivery and showed that this nanocomplex protected DNA from nuclease nebulization and digestion.

## 2. CONCLUSION

Genome-editing studies are a field whose scope and importance are increasing with new and variable methods emerging. Especially given its simplicity and applicability, genome editing with CRISPR is likely to be a much more frequently used method in the future. The use of polymers is an increasingly common way by which the vectors used in genome editing are delivered. A wide range of nanocarriers has already been applied as non-viral delivery systems in human gene therapy. Advancements in materials science and nano-ecology allow the emergence of synthetic vectors with optimum physicochemical properties and tissue/cell targeting capabilities. Polymeric *non-viral* vectors have advantages such as the avoidance of potential toxicity and immunogenicity, good reproducibility, and adherence to good manufacturing practices.

New application strategies are being developed for the delivery of CRISPR–Cas9 components into the cell efficiently. Among these, cationic polymers are susceptible to modifications that allow targeting as well as basic properties such as low immunogenicity and carcinogenicity, and cargo protection. In conclusion, it can be said that the application of polymers in CRISPR–Cas9 delivery is an interesting area that warrants future research.

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