

**TOKAT GAZİOSMANPAŞA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ**

*Tokat Gaziosmanpasa University, Faculty of Agriculture*  
**TOKAT, TÜRKİYE**



***GAZİOSMANPAŞA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ DERGİSİ***

*Journal of Agricultural Faculty of Gaziosmanpasa*

*University*  
*(JAFAG)*

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**Yönetim Adresi/Administration Adress:**

*Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi Yayın Ofisi*

*Tokat Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi, 60240 Taşlıçiftlik Yerleşkesi – TOKAT Telefon: (356) 252 16  
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*Gaziosmanpasa University Journal of Agricultural Faculty Publishing Unit Tokat Gaziosmanpasa University,  
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**E-Posta /E-mail:**

[ziraatdergi@gop.edu.tr](mailto:ziraatdergi@gop.edu.tr)

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## AMAÇ VE KAPSAM

Gaziosmanpaşa Üniversitesi Ziraat Fakültesinin 1985 yılından beri hakemli ve bilimsel süreli yayınıdır. Tokat Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, Tarım bilimleri (tarım ekonomisi, zootekni, biyosistem mühendisliği, tarla bitkileri, su ürünleri mühendisliği, bahçe bitkileri, bitki koruma, toprak bilimi ve bitki besleme) alanındaki uluslararası bilimsel makaleleri Türkçe ve İngilizce olarak yayınlamayı amaçlamaktadır. Dergi yılda en az iki kez basılır. 2014 yılı itibarıyla senede 3 baskı yapılmıştır.

## YAYIN POLİTİKASI

Dergide yayınlanacak makaleler İngilizce yayınlanır. Makaleler incelenmek üzere dergiye sorumlu yazar tarafından sunulur. Sunulan makalelerin başka bir yerde yayınlanmamış olması gerekir. Telif Hakkı Devir Sözleşmesi Formu tüm yazarlar tarafından imzalanmış olmalıdır.

Dergimizde yayınlanacak makaleler araştırma ve yayın etiğine uygun olmak zorundadır. Etik kurul kararı gerektiren klinik ve deneysel hayvan çalışmaları için ayrı etik kurul onayı alınmış olmalı ve belgelendirilmelidir. Dergimize gönderilecek bilimsel yazılarda, ICMJE (International Committee of Medical Journal Editors) tavsiyeleri ile COPE (Committee on Publication Ethics)'un "Editör ve Yazarlar için Uluslararası Standartlar"ı dikkate alınmaktadır.

Dergiye sunulan makale, Dergi Sekreteryası tarafından yazım kuralları ve içerik açısından ön değerlendirmeye alınır. Dergide basılacak nitelikte bulunmayan makale yazara iade edilebilir. Uygun bulunanlar ise bilimsel açıdan değerlendirilmek üzere konusunda uzman hakemlere (maksimum 15 gün süre için) gönderilir. Hakem incelenmesinden sonra basıma uygun olmayan makaleler yazara bildirilir, makaleler iade edilmez. Hakem onayından geçenler içinde düzeltme yapılması istenen makaleler gerekli dokümanlarla yazara iletilir. Yazar gerekli düzeltmeleri en kısa sürede (maksimum 15 gün) tamamlayarak dergi e-posta adresine gönderir. Editörler kurulu nihai kararını vererek makaleyi uygun bulursa basım ünitesine gönderir. Basımına karar verilen ve düzeltme için yazara gönderilen eserde, ekleme veya çıkartma yapılamaz.

Bir yazarın derginin aynı sayısında ilk isim olarak, en fazla iki eseri basılabilir.

Yayınlanan makalelerin tüm sorumluluğu yazar(lar)ına aittir

## AIMS AND SCOPE

*Journal of Agricultural Faculty is scientific, peer reviewed journal and belonged to the Tokat Gaziosmanpaşa University Faculty of Agriculture since 1985. Journal of Agricultural Faculty of Tokat Gaziosmanpaşa University aims to publish the international scientific paper on agriculture sciences (agricultural economics, animal science, biosystems engineering, field crops, fisheries engineering, horticulture, plant protection, soil science and plant nutrition). The journal is published at least twice in a year. The journal was published three issues in a year at 2014 year.*

## PUBLISHING POLICY

*Manuscripts are published in English. The manuscripts are submitted to the journal from Turkey and the other countries for review by corresponding author. The manuscript submitted should not have been submitted and published in another journal*

*Manuscripts published in our journal must be appropriate to the research and publication ethics. Separate ethical board resolutions are needed for each clinical and experimental study on animals which requires ethical board decision. International Committee on Publication Ethics' (ICMJE) recommendations and Committee on Publication Ethics' (COPE) "International Standards for Editors and Auditors" should be taken into consideration for the scientific manuscripts sent to our Journal.*

*Submitted manuscript to the journal is considered to preliminary assessment by the Editorial Board of journal. The Editorial Board has the right to decline the manuscript in event the manuscript does not meet the journal publishing rules. Manuscripts that meet the basic requirements are numbered and sent to three referees, experts in particular field of science, to peer review process (for max. 15 days period). Then, if the referees do not find the manuscript for publication, the related manuscript are not returned to the author, manuscript are archived. After peer reviewing, if the referees find the manuscript for publication with requires revision and corrections, author is informed, and the referee's suggestions and the related documents are sent to the corresponding author. The author is sent the corrected and revised manuscript to the Editorial Board as soon as possible (max. 30 days). Then, Editorial Board takes the final decision (positive or negative) for publication of manuscript. For the content of the accepted manuscripts, no editing, changes, including addition or deletion, can be made.*

*Only two manuscripts of each author, as the first author for one of the manuscript, can be published in same issue of the journal.*

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## ETİK İLKELER VE YAYIN POLİTİKASI YAYIN ETİĞİ İLKELERİ

Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, yayın politikasında akademik ilke ve etik değerlere bağlıdır. Etik ilke ve değerlere ilişkin ulusal ve uluslararası standartlara uygun olarak yayın hayatını sürdürmektedir. Bu kapsamda, COPE (Committee on Publication Ethics) tarafından belirlenen standartlar ve YÖK "Bilimsel Araştırma ve Yayın Etiği Yönergesi"nde belirlenen esaslar dikkate alınmaktadır (<https://publicationethics.org/>, <https://www.yok.gov.tr/Sayfalar/Kurumsal/mevzuat/bilimsel-arastirma-ve-...>). Makale değerlendirme sürecinde kabul edilen araştırma ve yayın etiği standartlarına aykırılığı tespit edilen eserlerin yayın talebi reddedilir. Eserin yayınlanmasından sonra söz konusu aykırılığın tespit edilmesi halinde eser yayından kaldırılır. Hakemli dergide yayın ilkeleri ile ilgili tüm taraflardan (yazar, dergi editörü, hakem ve yayımcı kuruluşlar) beklenen genel etik davranışlar ve sorumluluklara ilişkin tanımlamalar aşağıda belirtilmektedir.

### Yazar(lar)ın Sorumlulukları

- Kaynakça listesi eksiksiz olmalıdır.
- İntihal ve sahte veriye yer verilmemelidir.
- Aynı araştırmanın birden fazla dergide yayımlanmasına teşebbüs edilmemelidir.
- Bilim araştırma ve yayın etiğine uymalıdır.
- Tüm yazarların araştırmaya katkısı bulunmalıdır.
- Makalede geçen tüm veriler gerçek ve orijinal olmalıdır.
- Tüm yazarlar hatalı makalenin geri çekilmesini ve hataların düzeltilmesini sağlamak zorundadır.

### Bilim araştırma ve yayın etiğine aykırı eylemler şunlardır:

- İntihal: Başkalarının fikirlerini, metotlarını, verilerini, uygulamalarını, yazılarını, şekillerini veya eserlerini sahiplerine bilimsel kurallara uygun biçimde atf yapmadan kısmen veya tamamen kendi eseriymiş gibi sunmak,
- Sahtecilik: Araştırmaya dayanmayan veriler üretmek, sunulan veya yayımlanan eseri gerçek olmayan verilere dayandırarak düzenlemek veya değiştirmek, bunları rapor etmek veya yayımlamak, yapılmamış bir araştırmayı yapılmış gibi göstermek,
- Çarpıtma: Araştırma kayıtları ve elde edilen verileri tahrif etmek, araştırmada kullanılmayan yöntem, cihaz ve materyalleri kullanılmış gibi göstermek, ilgili teori veya varsayımlara uydurmak için veriler ve/veya sonuçlarla oynamak, destek alınan kişi ve kuruluşların çıkarları doğrultusunda araştırma sonuçlarını tahrif etmek veya şekillendirmek,
- Tekrar yayım: Bir araştırmanın aynı sonuçlarını içeren birden fazla eseri ayrı eserler olarak sunmak,
- Dilimleme: Bir araştırmanın sonuçlarını araştırmanın bütünlüğünü bozacak şekilde, uygun olmayan biçimde parçalara ayırarak ve birbirine atf yapmadan çok sayıda yayın yaparak ayrı eserler olarak sunmak,
- Haksız yazarlık: Aktif katkısı olmayan kişileri yazarlar arasına dâhil etmek, aktif katkısı olan kişileri yazarlar arasına dâhil etmemek, yazar sıralamasını gereksiz ve uygun olmayan bir biçimde değiştirmek, aktif katkısı olanların isimlerini yayım sırasında veya sonraki baskılarda eserden çıkarmak, aktif katkısı olmadığı halde

## ETHICAL PRINCIPLES AND PUBLICATION POLICY

### PRINCIPLES OF PUBLICATION ETHICS

Journal of Agricultural Faculty of Gaziosmanpaşa University is committed to academic principles and ethical values in its editorial policy. It continues its publication life in accordance with national and international standards regarding ethical principles and values. In this context, the standards set by COPE (Committee on Publication Ethics) and the principles set in the Council of Higher Education "Scientific Research and Publication Ethics Directive" are taken into account (<https://publicationethics.org/>, <https://www.yok.gov.tr/Sayfalar/Kurumsal/mevzuat/bilimsel-arastirma-ve-...>). The publication request of the works that are found to be in violation of the research and publication ethics standards accepted in the manuscript evaluation process is rejected. If the said contradiction is detected after the publication of the work, the work is removed from the publication.

Author's responsibilities:

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- No plagiarism, no fraudulent data is allowed;
- It is forbidden to publish same research in more than one journal;
- Authors obliged to participate in peer review process;
- All authors have significantly contributed to the research;
- Statement that all data in manuscript are real and authentic;
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- Authors should ensure that any studies involving human or animal subjects conform to national, local and institutional laws and requirements.

The actions against science research and publication ethics include;

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- Distorting: Dealing with the records of research and the data obtained, showing the unused methods, devices and materials used in the research, playing with data and / or results to fit the relevant theory or assumptions, or falsifying or shaping the results of the research in the interests of the people and organizations supported.
- Slicing: Presenting the results of a research as separate works by disrupting the uniqueness of the research, by dissecting it inappropriately and making a large number of publications without reference to each other.
- Unfair writer: To include people who do not have active contribution among the authors, not to include the people who have active contribution among the writers, to change the ranking of the authors without any justification and in an inappropriate way, to remove the names of those who have active contributions from the

nüfuzunu kullanarak ismini yazarlar arasına dâhil ettirmek.

• Diğer etik ihlali türleri: Destek alınarak yürütülen araştırmaların yayınlarında destek veren kişi, kurum veya kuruluşlar ile onların araştırmadaki katkılarını açık bir biçimde belirtmemek, insan ve hayvanlar üzerinde yapılan araştırmalarda etik kurallara uymamak, yayınlarında hasta haklarına saygı göstermemek, hakem olarak incelemek üzere görevlendirildiği bir eserde yer alan bilgileri yayınlanmadan önce başkalarıyla paylaşmak, bilimsel araştırma için sağlanan veya ayrılan kaynakları, mekânları, imkânları ve cihazları amaç dışı kullanmak, tamamen dayanaksız, yersiz ve kasıtlı etik ihlali suçlamasında bulunmak (YÖK Bilimsel Araştırma ve Yayın Etiği Yönergesi, Madde 8).

#### **Hakemlerin Sorumlulukları**

•Hakemlik süreci, bilimsel akademik yayıncılığın başarısında önemli bir konumda bulunmaktadır. Hakemler bu sürecin sağlıklı yürütülebilmesi ve iyileştirilmesine gayret göstermelidir.  
•Hakemler araştırmayla, yazarlarla ve/veya araştırma fon sağlayıcılar ile çıkar çatışması/çakışması içerisinde olmamalıdır.  
•Değerlendirmeleri tarafsız olmalıdır.  
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#### **Editörün Sorumlulukları**

•Editörler bir makaleyi kabul etmek ya da reddetmek için tüm sorumluluğa ve yetkiye sahiptir.  
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•Hakemlerin ismini değerlendirme tamamlanana kadar saklı tutmalıdır.  
•Makalenin yayımlanmasından sonra herhangi bir araştırmacı tarafından bilimsel hata tespit edildiğinde ilgili düzeltme/düzeltilmelerin yayımlanmasını ya da geri çekilmesini desteklemelidir.

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•Yayıncılık etiğinin yayın kurulu tarafından izlenmesi/korunması,  
•Akademik kaydın bütünlüğünü korumak,  
•Etik standartlardan ödün vermemek,  
•Gerektiğinde düzeltmeleri, açıklamaları ve özürleri yayımlamak,  
•Okuyucunun dergide yayımlanan bir makalede önemli bir bilimsel hata ya da intihal, yinelenen makaleler gibi konularda herhangi bir uyarısı olduğu zaman ziraatderdi@gop.edu.tr adresine mail atarak editör kuruluna bildirebilir. Derginin bilimsel ve teknik yönden gelişmesi için bir fırsat olacağı bilinci ile, yapacağınız uyarılar/eleştiriler, editör kurulu tarafından memnuniyetle karşılanarak hızlı ve yapıcı bir şekilde iyileştirmelerimiz gerçekleştirilmektedir.

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## Using Paclobutrazol, Daminozide, Chlormequat, Propiconazole on Vegetative Growth and Flowering Control of Zinnia

Fazilet PARLAKOVA KARAGÖZ<sup>1,\*</sup> Atilla DURSUN<sup>2</sup>

<sup>1</sup>Atatürk University, Agriculture Faculty, Department of Horticulture, TR-25240 Erzurum

<sup>2</sup>Kyrgyz – Turkish Manas University, Faculty of Agriculture, Department of Horticulture and Agronomy, Bishkek, Kyrgyzstan

\*Corresponding author's email: f.parlakova@atauni.edu.tr

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**Abstract:** PGRs such as paclobutrazol, daminozide, chlormequat and propiconazole help to alter various growth characteristics of plants and they are used commonly in the modern production system of ornamental plants. The objective of this work was to evaluate the effects of the optimum dose, decreasing and increasing amounts of the optimum dose of paclobutrazol, daminozide, chlormequat and propiconazole used in the modern production system of zinnia (*Zinnia elegans* Jacq.) vegetative growth parameters and flowering in a greenhouse experiment. Plant height was reduced with the administration of 2 g L<sup>-1</sup> of daminozide (T<sub>4</sub>) at a ratio of 20.87% compared to the control group. The plants treated with 1.5 ml L<sup>-1</sup> of chlormequat (T<sub>9</sub>) had the highest main stem diameter, flower stalk diameter (4.50 mm) and side branch length (16.17 cm). It was determined that the highest side branch diameter (3.78 mm) and number of flower buds (4.67 per plant) were manifested with T<sub>4</sub>. The treatment of T<sub>4</sub> can be used to increase the number of flower buds of zinnias. The result showed that the use of 2 g L<sup>-1</sup> of daminozide (T<sub>4</sub>) reduced the internode length compared to the control group at a rate of 58.60%. In summary, it was concluded that the chemicals and all the doses used in the experiment did not affect the deterioration of the flower quality of zinnias. On the contrary, it was concluded that more compact plants can be obtained by providing height control especially by applying different doses of daminozide. In order to shorten the production period of zinnias, it may be advisable to apply 0.25 ml L<sup>-1</sup> of propiconazole.

**Keywords:** Plant growth retardants, *Zinnia elegans* Jacq., plant height, compact plant form

### Paclobutrazol, Daminozid, Klormequat, Propiconazole Zinnia'nın Vejetatif Büyümesi ve Çiçek Kontrolü Üzerinde Kullanılması

**Öz:** Paclobutrazol, daminozide, chlormequat ve propiconazole gibi PGR'ler bitkilerin çeşitli büyüme özelliklerini değiştirmeye yardımcı olur ve bunlar süs bitkilerinin modern üretim sisteminde yaygın olarak kullanılır. Bu çalışmanın amacı, bir sera denemesinde, zinnia (*Zinnia elegans* Jacq.) vejetatif büyüme parametreleri ve çiçeklenmesinin modern üretim sisteminde kullanılan paclobutrazol, daminozide, chlormequat ve propiconazole'nin optimum dozu, dozlarının azaltılması ve artırılması etkilerini değerlendirmektir. Bitki boyu, kontrol grubuna kıyasla %20.87 oranında 2 g L<sup>-1</sup> daminozid (T<sub>4</sub>) uygulanarak azaltılmıştır. 1.5 ml L<sup>-1</sup> chlormequat (T<sub>9</sub>) ile muamele edilen bitkiler, en yüksek ana sap çapına, çiçek sapı çapına (4.50 mm) ve yan dal uzunluğuna (16.17 cm) sahiptir. En yüksek yan dal çapının (3.78 mm) ve çiçek tomurcuklarının sayısının (bitki başına 4.67) T<sub>4</sub> ile ortaya konulduğu belirlenmiştir. T<sub>4</sub> uygulaması, zinnia'nın çiçek tomurcuklarının sayısını arttırmak için kullanılabilir. 2 g L<sup>-1</sup> daminozid (T<sub>4</sub>) kullanımının, internod uzunluğunu kontrol grubuna kıyasla % 58.60 oranında azalttığını sonuçlar göstermiştir. Özetle, denemede kullanılan kimyasalların ve tüm dozların zinnia'nın çiçek kalitesinin bozulmasını etkilemediği sonucuna varılmıştır. Aksine, özellikle farklı dozlarda daminozid uygulanarak bitki boyu yükseklik kontrolü sağlanarak daha kompakt bitkilerin elde edilebileceği sonucuna varılmıştır. Zinnia'nın üretim süresini kısaltmak için, 0.25 ml L<sup>-1</sup> propiconazole uygulanması tavsiye edilebilir.

**Anahtar kelimeler:** Bitki büyüme geciktiricileri, *Zinnia elegans* Jacq., bitki boyu, kompakt bitki formu

#### 1. Introduction

Due to its wide adaptability, diverse forms in terms of plant size and quick flowering, flower colors and shapes the *Zinnia violacea* Cav. (formerly the *Z. elegans* Jacq.) is the most widely grown one-year herbaceous plant and also the most important among the other zinnia species in terms of economic value (Burlec et al. 2019). Another advantage of zinnias is its cultivation as a potted plant as well as its attractiveness and marketing. The control of plant growth and reduction of plant height are very important factors in potted ornamental

plant production (Hadizadeh et al. 2010). Therefore, it is essential to reduce the height of the plant and at the same time keep the plant quality at a desired level. One of the important effects of plant growth retardants (PGRs) is the control of plant height. The advantage of using plant growth retarders in crop production is to improve the appearance of the plant by maintaining the shape and size of the plant relative to the size of the pot (Christopher & Lopez;2010Meijon et al. 2009).

In the modern production system of ornamental plants, the use of PGRs is encouraged and PGRs help to

alter various growth characteristics of plants. However, their excessive use is a threat to the environment. Also commercially acceptable PGR formulations may influence consumer acceptance because they contain synthetic growth regulators. The synthesis of ecologically safe formulations of PGRs and their use at optimum dosage will increase their acceptability by manufacturers and consumers. Hence, the specific objectives of this study were to optimize the different PGRs (paclobutrazol, daminozide, chlormequat and propiconazole) and their different concentrations for *Zinnia elegans* and to elucidate the effects of zinnia potted plants and 12 treatments for standardizing this practice as a best management strategy.

**2. Materials and Methods**

*Zinnia elegans* ‘GIALLA’ seeds (Tasaco Agriculture Industry and Trade Inc. Antalya, Turkey) were planted in plug trays (molded plastic propagation trays with 1.5 cm<sup>3</sup> inverted cone-shaped pockets), on 21 June 2018-2019. The trays were placed in a climate-controlled research greenhouse. Temperatures inside the greenhouse were determined as 15±2 °C at night and daytime temperatures were determined as 27±2 °C. Plant growth retardants (different levels of paclobutrazol, daminozide, chlormequat and

propiconazole) (Table 1) were applied to all groups, except the control group, twice in two different growth stages: (1) at the stage where both cotyledon leaves appear and (2) at the stage where two-thirds of the seedling leaves appear. The zinnia seedlings (drench on leaves, stem and in growth medium) were irrigated with different doses of the plant growth retardants during two different growth stages in the greenhouse.

On 16 July 2018-2019, all seedlings were transplanted from the plug trays into pots (400 cc; 10.5x8 cm) in the greenhouse. Day 14 after transplant, all groups were given chemical fertilizer 15-5-30+ME water soluble fertilizer 2.0 g L<sup>-1</sup> (FERTIGOLD 15-5-30+ME) with the irrigation water. The experiment was carried out as a factorial experiment based on a completely randomized design with four factors including chlormequat at 3 levels, daminozide at 4 levels, paclobutrazol at 3 levels and propiconazole at 2 levels and the control group with 13 treatments (Table 1), 3 replications, and each replication included 5 pots. In total, 210 pots were used in this experiment. Irrigation was applied as needed to maintain growth medium moisture. Growth of the zinnias was monitored daily in the greenhouse from June through September and consecutively two years (2018-2019).

**Table 1.** Content, commercial name of plant growth retardants used in the study and their treatments codes and levels

**Çizelge 1.** Çalışmada kullanılan bitki büyüme geciktiricilerinin içerikleri, ticari adları ve uygulama kodları ve seviyeleri

Treatmens no	Treatments code	Levels	Used plant growth retardant	Commercial name of plant growth retardant	Composition
1	T0	Control	Without plant growth retardant	-	-
2	T1	0.6 ml L <sup>-1</sup>	Paclobutrazol	Bonzi	4 g/l (0.39% w/w) Paclobutrazol
3	T2	0.9 ml L <sup>-1</sup>	Paclobutrazol	Bonzi	4 g/l (0.39% w/w) Paclobutrazol
4	T3	1.2 ml L <sup>-1</sup>	Paclobutrazol	Bonzi	4 g/l (0.39% w/w) Paclobutrazol
5	T4	2.0 g·L <sup>-1</sup>	Daminozide	Alar 85	85.14% w/w Daminozide
6	T5	4.0 g·L <sup>-1</sup>	Daminozide	Alar 85	85.14% w/w Daminozide
7	T6	6.0 g·L <sup>-1</sup>	Daminozide	Alar 85	85.14% w/w Daminozide
8	T7	8.0 g·L <sup>-1</sup>	Daminozide	Alar 85	85.14% w/w Daminozide
9	T8	1.0 ml L <sup>-1</sup>	Chlormequat	Cycocel	11.8% Chlormequat
10	T9	1.5 ml L <sup>-1</sup>	Chlormequat	Cycocel	11.8% Chlormequat
11	T10	2.0 ml L <sup>-1</sup>	Chlormequat	Cycocel	11.8% Chlormequat
12	T11	0.25 ml L <sup>-1</sup>	Propiconazole	Tilt 250E	250g/l Propiconazole
13	T12	0.55 ml L <sup>-1</sup>	Propiconazole	Tilt 250E	250g/l Propiconazole

Characteristics evaluated was the time until the first appearance of the first flower buds, plant height, main stem and flower diameter, leaf chlorophyll (using chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan)), stomatal conductance (using a porometer (Sc 1 Porometer, Decagon Devices Inc., WA, USA)), number of side branches, number of flower buds, flower stalk diameter, length of internodes, leaf area (using CI 202 Portable digital brand leaf area meter) and the fresh and dry weight of the plants. All data in the present study were processed by SPSS (Statistical Package for Social Sciences, Version 22.0) and the means were separated

by Duncan’s multiple range tests.

**3. Results and Discussion**

The effects of different levels of the plant growth retardants were significant for plant height at a 1% probability level. The mean comparison of data in different treatments (Table 2) showed that plants treated with 2 g L<sup>-1</sup> of daminozide (T<sub>4</sub>) had the lowest height (17.67 cm). Plant height was reduced in T<sub>4</sub> at a rate of 20.87% when compared to the control (T<sub>0</sub>) and T<sub>12</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> treatments were in the same statistical group with T<sub>4</sub>. PGRs are widely used in the control of plant



height of a large number of plants. Daminozide treatments are used for height control in floriculture; potted *Mussaenda* 'Queen Sirikit' (Cramer and Bridgen 1998), ornamental cabbage and kale (Gibson & Whipker 2001), *Calendula officinalis* L. (Hashemabadi et al. 2012) and potted 'Lilliput' *Zinnia elegans* Jacq. (Pinto et al. 2005). It has been reported that different concentrations of uniconazole decreased total plant height by more than 20% in 'Red Pigmy' and 'Golden

Emblem' cultivars (Whipker et al. 1995). Propiconazole (Tilt 250E) has effected the root growth of *Triticum durum* (Meksem et al. 2007) and increased the green leaf area and seed yield of grass (Rolston et al. 2004). Moreover, no previous studies using Propiconazole on ornamental plants as plant growth regulators have been encountered. In the present study, plant height was reduced in T<sub>7</sub> by a 11.20% ratio when compared to the control (T<sub>0</sub>).

**Table 2.** The effect of treatments on the some morphological characters of *Zinnia elegans* Jacq.

**Çizelge 2.** Uygulamaların *Zinnia elegans* Jacq.'in bazı morfolojik özellikleri üzerindeki etkisi

Treatment	Plant height (cm)	Main stem diameter (mm)	Side branches diameter (mm)	Flower stalk diameter (mm)	Side branch length (cm)	Number of side branches (number plant <sup>-1</sup> )	Number of flower buds (number plant <sup>-1</sup> )	Flower diameter (mm)
T <sub>0</sub>	22.33±0.2 a-d	5.77±0.7 b-e	3.13±0.2 a-d	3.45±0.2 bc	13.80±4.2 a-c	6.33±0.6 <sup>ns</sup>	2.33±0.6 bc	53.26±4.8 b
T <sub>1</sub>	22.77±1.2 a-d	5.97±1.0 a-e	3.05±0.5 a-d	3.66±0.4 bc	12.03±2.3 b-d	5.67±0.6	1.67±1.2 c	69.95±9.2 a
T <sub>2</sub>	24.20±1.7 ab	5.13±0.1 de	2.86±0.5 b-e	3.18±0.1 c	11.00±1.8 a-c	5.67±1.5	2.33±0.6 bc	73.73±1.8 a
T <sub>3</sub>	25.37±0.9 a	5.91±0.1 b-e	3.17±0.1 a-d	3.56±0.4 bc	14.07±0.3 b-d	6.33±0.6	2.00±0.0 bc	77.57±3.9 a
T <sub>4</sub>	17.67±2.4 e	6.23±1.0 a-d	3.78±0.4 a	3.55±0.1 bc	10.93±0.6 b-d	7.67±2.1	4.67±1.5 a	50.50±6.7 b
T <sub>5</sub>	20.43±2.9 c-e	5.04±0.4 e	2.35±0.4 de	3.63±0.5 bc	10.63±1.3 d	8.00±2.0	2.00±0.0 bc	44.22±7.8 b
T <sub>6</sub>	19.20±1.4 de	5.90±0.4 b-e	2.50±0.8 de	3.94±0.2 ab	8.50±3.3 cd	9.33±4.2	1.67±0.6 c	9.57±0.7 c
T <sub>7</sub>	19.80±1.1 de	6.31±0.9 a-c	3.45±0.4 ab	4.05±0.6 ab	11.73±0.8 b-d	7.33±1.5	3.67±1.5 ab	69.31±8.4 a
T <sub>8</sub>	22.87±0.9 b-d	5.46±0.4 c-e	3.38±0.3 a-c	3.96±0.7 bc	14.87±1.8 d	6.00±2.0	3.67±0.6 ab	69.56±4.6 a
T <sub>9</sub>	23.63±0.3 a-c	7.03±0.1 a	3.48±0.3 ab	4.50±0.1 a	16.17±2.8 a	7.00±1.0	3.67±1.5 ab	68.66±2.5 a
T <sub>10</sub>	21.37±2.7 a-d	5.46±0.4 ab	2.18±0.3 e	3.37±0.3 ab	9.47±0.3 ab	6.33±0.6	3.00±1.0 a-c	68.67±7.0 a
T <sub>11</sub>	22.80±1.3 a-d	6.87±0.5 c-e	2.55±0.7 c-e	3.34±0.3 bc	14.80±1.2 ab	5.67±0.6	2.67±1.5 bc	75.01±5.3 a
T <sub>12</sub>	19.83±3.8 de	5.35±0.3 e	2.46±0.6 de	3.84±0.6 a-c	9.73±2.4 d	6.00±1.0	1.33±0.6 c	66.10±7.4 a
Mean	21.71±2.7	5.84±0.8	2.95±0.6	3.69±0.5	12.13±2.9	6.72±1.8	2.67±1.3	61.24±18.6

Values are shown as mean ± standard error. Different letters in the same column indicate significant differences between means (P < 0.05) based on the Duncan's multiple range test.

The maximum main stem diameter was obtained with T<sub>9</sub> as 7.03 mm. T<sub>10</sub> was in the same statistical group with T<sub>9</sub>. Cycocel is a plant growth retardant that is widely used in reducing the growth of a large number of plants. In the present study, plants treated with 1.5 ml L<sup>-1</sup> of cycocel (T<sub>9</sub>) had the highest main stem diameter, flower stalk diameter (4.50 mm) and side branch length (16.17 cm). It was determined that the highest side branch diameter (3.78 mm) and number of flower buds (4.67 per plant) were in T<sub>4</sub>. T<sub>7</sub> and T<sub>9</sub> were in the same statistical group with T<sub>4</sub> (Table 2). The PGRs have been applied to alter various characteristics in ornamental plants including an increase in the number of flowers (Carey et al. 2013; Sajjad et al. 2017). Treatment with cycocel at concentrations of 2000, 4000 and 6000 ppm had no effect on the final height of *Arundina graminifolia* (Wanderley et al. 2014). It was determined that the plant growth regulators (ancymidol, benzyladenine, chlormequat chloride, daminozide, ethephon, paclobutrazol and uniconazole) can control stem elongation and can be effective in promoting branching by the application for kalanchoe plants (Currey & Erwin 2012). In addition, Cycocel can reduce the height of the flowering branches in the "Red Elite" geranium (Olivera & Browning 1993; Taherpazir & Hashemabadi 2016). As a result of our study, it was concluded that the treatment of T<sub>4</sub> can be used to increase the number of flower buds of zinnias.

No significant differences were observed in the number of side branches with the treatments. The mean comparison of data in different treatments showed that all the treatments except T<sub>6</sub> significantly increased flower diameter as compared to T<sub>0</sub>. The numerically highest flower diameter was determined in T<sub>3</sub> (Table 2). Although there was no statistically significant difference in flower diameter among different levels of plant growth retardants, results showed that the use of plant growth retardants increased flower diameter compared to the control. It is believed that the impact of growth retardant on flower diameters depends on the frequency of the use of growth retardant, environmental conditions, species sensitivity to growth retardants and methods (Pinto et al. 2005).

In the evaluation of zinnias, the longest internode length was determined in T<sub>0</sub> (31.35 mm), T<sub>3</sub> (33.47 mm) and T<sub>10</sub> (27.74 mm); the shortest internode length was observed in T<sub>4</sub> (12.98 mm) (Table 3). The result showed that the use of 2 g L<sup>-1</sup> of daminozide (T<sub>4</sub>) reduced the internode length compared to the control with at a ratio of 58.60%. However, no significant differences were observed in chlorophyll content (SPAD) among the treatments. Maximum stomatal conductance was obtained from the plants in T<sub>9</sub> (21.70 mmol (H<sub>2</sub>O) m<sup>-2</sup> s<sup>-1</sup>), T<sub>7</sub> (21.47 mmol (H<sub>2</sub>O) m<sup>-2</sup> s<sup>-1</sup>) and minimum stomatal conductance was observed in the plants of T<sub>5</sub> (9.57 mmol (H<sub>2</sub>O) m<sup>-2</sup> s<sup>-1</sup>) (Table 2). Leaf area is a key

feature in plant growth affected by growth retardants. In fact, photosynthesis increases in parallel with the amount of leaf area. In this study, leaf area increased with the treatment in T<sub>5</sub> (53.56 cm<sup>2</sup>) and decreased with T<sub>4</sub> (23.33 cm<sup>2</sup>). Leaf area increased in T<sub>5</sub> by 20.72% ratio compared to the control (T<sub>0</sub>) (Table 3). It is suggested that the reducing effect of growth retardants

on the leaf area is associated with the prevention of the synthesis of GA, the enhancement of ABA and the prevention of cell elongation (Taherpazir and Hashemabadi 2016). The fresh and dry weight of plants was not affected by the treatments in this study (Table 3).

**Table 3.** The effect of treatments on the some morphological characters of *Zinnia elegans* Jacq.

**Çizelge 3.** Uygulamaların *Zinnia elegans* Jacq.'in bazı morfolojik özellikleri üzerindeki etkisi

Treatments	Length of internodes (mm)	Chlorophyll content (SPAD)	Stomatal conductance (mmol (H <sub>2</sub> O) m <sup>-2</sup> s <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )	Plant fresh weight (g)	Plant dry weight (g)	Duration to first flower buds apperance (day)
T <sub>0</sub>	31.35±5.0 a***	41.92±0.7 <sup>ns</sup>	18.43±1.2 ab***	42.46±1.3 b-d***	28.26±6.2 <sup>ns</sup>	3.46±0.7 <sup>ns</sup>	45.33±7.5 ef***
T <sub>1</sub>	24.88±1.4 ab	41.32±2.2	14.07±3.7 b-d	39.92±0.2 b-d	28.64±3.2	3.67±0.7	51.00±10.5 c-e
T <sub>2</sub>	27.78±7.8 ab	43.79±1.5	13.57±2.3 b-d	29.52±1.1 e	26.74±4.8	3.80±0.6	47.33±1.5 ef
T <sub>3</sub>	33.47±2.3 a	41.90±0.9	14.23±2.1 b-d	38.76±0.5 b-d	32.99±2.7	4.43±0.7	43.33±1.5 ef
T <sub>4</sub>	12.98±4.5 d	45.53±1.6	15.37±3.3 bc	23.33±11.7 f	24.52±3.1	2.83±0.3	58.33±2.1 bc
T <sub>5</sub>	19.29±5.4 b-d	46.43±3.9	9.57±0.6 d	53.56±0.9 a	27.67±4	3.47±0.9	55.67±2.1 bd
T <sub>6</sub>	15.29±1.2 cd	45.00±1.3	15.80±2.8 bc	36.87±0.4 cd	22.50±7.1	2.86±0.8	59.33±3.1 b
T <sub>7</sub>	21.22±2.7 bc	45.33±1.0	21.47±1.3 a	43.14±0.7 bc	30.57±2.9	4.02±0.5	45.33±1.5 ef
T <sub>8</sub>	31.20±1.2 ab	44.77±1.8	14.33±2.2 b-d	37.70±4.4 b-d	29.40±4.6	3.91±0.6	49.33±0.6 a-c
T <sub>9</sub>	27.97±2.7 ab	43.97±3.9	21.70±6.2 a	44.22±0.4 b	35.62±3.4	4.52±0.5	50.67±2.1 c-d
T <sub>10</sub>	27.74±4.2 a	46.00±3.6	15.00±3.6 b-d	42.71±1.0 b-d	29.16±4.2	3.62±.4	55.33±4.2 d-f
T <sub>11</sub>	25.44±4.8 ab	45.17±1.8	11.30±1.7 cd	36.02±3.9 d	29.58±6.9	2.48±2.1	42.67±2.1 f
T <sub>12</sub>	21.87±8.1 bc	48.77±4.0	14.97±1.8 b-d	38.00±0.3 b-d	28.51±2.5	3.75±0.2	81.67±2.5 a
Mean	24.65±7.2	44.61±2.9	15.37±4.1	38.94±7.6	28.78±5.0	3.60±0.9	52.72±10.6

Control (T<sub>0</sub>); Paclobutrazol concentrations 0.6 (T<sub>1</sub>), 0.9 (T<sub>2</sub>) and 1.2 ml L<sup>-1</sup> (T<sub>3</sub>); Daminozide concentrations 2.0 (T<sub>4</sub>), 4.0 (T<sub>5</sub>), 6.0 (T<sub>6</sub>) and 8.0 g·L<sup>-1</sup> (T<sub>7</sub>); Chlormequat concentrations of 1.0 (T<sub>8</sub>), 1.5 (T<sub>9</sub>) and 2.0 ml L<sup>-1</sup> (T<sub>10</sub>) and Propiconazole concentrations 0.25 (T<sub>11</sub>) and 0.55 ml L<sup>-1</sup> (T<sub>12</sub>).

While the time until the first appearance of flower buds decreased with the use of 0.25 ml L<sup>-1</sup> of propiconazole (T<sub>11</sub>), it increased in T<sub>12</sub>. T<sub>0</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>7</sub> were in the same statistical group with T<sub>11</sub>. ‘Mustang’ geranium (*Pelargonium hortorum*) treated with paclobutrazol flowered earlier than the control plants (Cox 1991). Khaimov and Mizrahi (2006) and Cardoso and Habermann (2014) reported that PGRs have been applied to achieve early flowering in ornamental plants. The results of this study parallel the results of these researchers.

#### 4. Conclusion

In the present study, plant height was reduced with 8 g L<sup>-1</sup> of daminozide by 11.20% compared to the control. The plants treated with 1.5 ml L<sup>-1</sup> of cycocel had the highest main stem diameter, flower stalk diameter and side branch length. It was determined that the highest side branch diameter and number of flower buds were achieved with 2 g L<sup>-1</sup> and 8 g L<sup>-1</sup> daminozide. Leaf area increased with 4 g L<sup>-1</sup> of daminozide at a rate of 20.72% compared to the control. In summary, it was concluded that the chemicals and all the doses used in the experiment did not affect the deterioration of the flower quality of zinnias. On the contrary, it was concluded that more compact plants can be obtained by providing height control especially by administering different doses of daminozide. At the same time, it is estimated that potted zinnia plants which have been subjected to

height control are less likely to incur physical damage during transport. In addition, no signs of phytotoxicity were encountered in any of the treatments.

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## Comparison of Performance Characteristics of Agricultural Tractors

Mehmet Melih ÖZBAYER<sup>1</sup> Metin GÜNER<sup>2\*</sup>

<sup>1</sup>Republic of Turkey Ministry of Food, Agriculture and Livestock, Ankara,

<sup>2</sup>Ankara University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, Ankara

\*Corresponding author's email: metguner@gmail.com

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**Abstract:** In this study, the relationships between some parameters such as PTO (power take-off) power, engine speed, specific fuel consumption, travel speed, drawbar power, drawbar pull and tractor mass of agricultural tractors were compared. Test reports of tractors according to standard code 2 of Organization for Economic Cooperation and Development (OECD) obtained Nebraska University Tractor Test Laboratory reports were used as material. The statistical relationships between the parameters were investigated which obtained from the test reports. According to the results of regression analysis same results were found both at 85 per cent of the torque obtained in the torque corresponding to maximum power available at rated engine speed and at standard PTO speed for 2 WD and 4 WD tractors. The relations between PTO power and engine speed were found different in working conditions both at 85 per cent of the torque obtained in the torque corresponding to maximum power available at rated engine speed and at standard power take-off speed for 2 WD tractors. Besides, according to the results of regression analysis same results were found at a pull equal to 75 percent of the drawbar pull corresponding to maximum power at rated speed for 2 WD and 4 WD tractors. The relations between drawbar pull and specific fuel consumption were found different in working conditions at a drawbar pull equal to 75 per cent of the pull corresponding to maximum power at rated speed for 2 WD tractors. The overall efficiency ( $\eta$ ) of tractors was found on an average of 29.04.

**Key words:** Drawbar power, performance characteristics, power take-off power, test report, tractor

### Tarım Traktörlerinin Performans Karakteristiklerinin Karşılaştırılması

**Öz:** Bu çalışmada, tarım traktörlerinin PTO (PTO) gücü, motor devri, özgül yakıt tüketimi, ilerleme hızı, çeki gücü, çeki kuvveti ve traktör kütlesi gibi bazı parametreler arasındaki ilişkiler karşılaştırılmıştır. Nebraska Üniversitesi Traktör Test Laboratuvarı raporlarından elde edilen Ekonomik İşbirliği ve Kalkınma Teşkilatı (OECD) standart kod 2'ye göre traktörlerin test raporları materyal olarak kullanılmıştır. Test raporlarından elde edilen parametreler arasındaki istatistiksel ilişkiler araştırılmıştır. Regresyon analizi sonuçlarına göre, 2 WD ve 4 WD traktörler için hem nominal motor devrinde mevcut maksimum güce karşılık gelen torkta elde edilen torkun yüzde 85'inde hem de standart PTO hızında aynı sonuçlar bulundu. PTO gücü ile motor devri arasındaki ilişkiler, hem nominal motor devrinde mevcut maksimum güce karşılık gelen torkta elde edilen torkun yüzde 85'inde hem de 2 WD traktörler için standart PTO hızında çalışma koşullarında farklı bulunmuştur. Ayrıca regresyon analizi sonuçlarına göre 2 WD ve 4 WD traktörler için nominal hızda maksimum güce karşılık gelen çeki demiri çekişinin yüzde 75'ine eşit bir çekmede aynı sonuçlar bulunmuştur. Çeki kuvveti ile özgül yakıt tüketimi arasındaki ilişkiler, 2 WD traktörler için nominal hızda maksimum güce karşılık gelen çekmenin yüzde 75'ine eşit bir çeki kuvveti çalışma koşullarında farklı bulunmuştur. Traktörlerin toplam verimi ( $\eta$ ) ortalama 29.04 olarak bulunmuştur.

**Anahtar Kelimeler:** Çeki gücü, performans karakteristikleri, PTO gücü, test raporu, traktör

#### 1.Introduction

The meaning of the tractor (tracteur) is puller. Previously, tractors were used only in tow works. Later, in accordance with the developments in agriculture and agricultural machinery technique, the structure of the tractor changed significantly. Tractors are at least two axles, wheeled or tracked and are self-propelled. Tractors are used in agricultural and forestry for towing trailers, transporting tools and machines, and pulling or pushing. They are used to run and power the machines while they are moving or stationary (OECD, 2019). In agriculture, tractor is the most fuel-consuming machine. The research indicates that 20–55% of available tractor

power is lost in the process of interaction between tires and soil surface. Tire pressure and vertical wheel load are both easily managed parameters, which play a significant role in controlling the slip, the traction force and the fuel consumption of a tractor (Janulevicius and Damanauskas, 2015). A set of standard procedures to be applied by test stations to measure the performance characteristics of agricultural and forestry tractors is called the OECD Tractor Performance Test Code. Using these codes, it has made it possible to follow the same methods and compare the test results in tractor experiments conducted by research and test centers in different countries. Power takes off (PTO) and drawbar

performance tests are included in OECD standard Code 2 within the scope of the standard test code numbered one to ten for agricultural and forestry tractors (OECD, 2016). In addition, tractor performance was determined in order to enable the farmer to use the tractor efficiently and to trade properly (Taşbaş *et al.* 2003). Sumer *et al.* (1998) stated that there is a decrease in specific fuel consumption depending on the load applied to the power take off (PTO). Grisso *et al.* (2004), according to the test reports published by Nebraska Tractor Test Laboratory, stated that specific fuel consumption can be used to compare tractors with different working conditions and different sizes. Downs *et al.* (2006) examined the tests performed at Nebraska Tractor Test Laboratory and reported that the fuel efficiency will be half the fuel efficiency at full power position in 25% loading performed in the PTO test. Gil-Sierra *et al.* (2007) examined the partial loads at different engine speeds at six points according to OECD standard Code 2 and determined the corresponding fuel consumption values. Özgür (2009) found that the specific fuel consumption decreases as the power increases. The most decisive criterion in the selection of tractors is tractor performance. Drawbar power is preferred in comparison and evaluation of tractors. In this regard, it has been reported that the use of PTO performance data would be appropriate to evaluate the performance of agricultural tractors (Başer, 2008). Measured at 75% of the maximum drawbar pull, the power is suitable for heavy duty work such as primary tillage. Average fuel consumption at 75% and 50% loads of the drawbar pull at maximum power can represent tillage and planting operations, respectively, on farms producing grain (small grain). Similarly, the average fuel consumption at the 50% load test of the maximum power drawbar pull can give a good fuel consumption estimate for tractors used in growing crops (Grisso *et al.* 2014). Kabeel *et al.* (2010) studied theoretically and experimentally the performance of spot cooling of a tractor cabinet including a single internal heat source (tested body) by using vortex tube. Kumar (2019) observed that the maximum power output can be increased by the help of ballasting, the output power was found to be more in case of corresponding weight of 50 and 75% equivalent weight of iron ballast compared to liquid ballast.

The drawbar power- is directly proportional to the travel speed and the drawbar pulling force. Parameters affecting the drawbar power of a tractor; the characteristics of the engine, gear level, tires, drawbar, tow hook, ground structure, ground condition, angle of the ground with the horizontal, characteristics of the

fuel, tractor additional weights, extra loads coming from the towing equipment to the rear axle and being 2 WD or 4 WD (Ariöz and Güner, 2015). Kocher *et al.* (2017) has developed five different fuel consumption models, which include the parameters of drawbar power, travel speed and engine speed, which are a function of fuel consumption. He developed equations for each model and made statistical analyzes to calculate the estimated fuel consumption. The results obtained were evaluated, and fuel consumption was estimated by applying a single equation for each speed range tested. The aim of this study is to make statistical analysis of the PTO performance and drawbar performance values obtained from experiment reports based on OECD standard Code 2 of standard agricultural tractors and to evaluate the results obtained.

## 2. Materials and methods

In this study, the test reports of 418 agricultural tractors that were tested between 2004 and 2017 in Nebraska University Tractor Test Laboratory, which were tested according to OECD standard Code 2, were used (NTTL, 2018). Nebraska University Nebraska Tractor Testing Laboratory (NTTL) is the official tractor testing station for the Organization for Economic Cooperation and Development (OECD) in the USA. This independent laboratory is responsible for testing a representative tractor of each model sold in the state of Nebraska. It also tests tractors manufactured in the USA and sold in international markets. The laboratory publishes the results of all tests performed. The 418 tractors used in the research have an internal combustion (diesel) engine. The power of tractors at nominal engine speed varies between 45.50 kW and 356.41 kW. The power average is 142.21 kW. 370 tractors are two-wheel drive (2 WD), 48 are four-wheel drive (4 WD). Distribution of tractors according to their power at nominal engine speed is given in Table 1.

**Table 1.** Distribution of tractors according to their power at rated engine speed.

**Çizelge 1.** Traktörlerin nominal motor devrindeki güçlerine göre dağılımı.

Power at rated engine speed (P) (kW)	Number of tractors (pieces)	Percentage (%)
50>P	1	0
50≤P<100	152	36
100≤P<150	116	28
150≤P<200	67	16
200≤P<250	55	13
250≤P<300	7	2
300≤P	20	5

In this study, firstly, 418 tractors are grouped as 2 WD and 4 WD according to their technical specifications. After that statistical analysis of 2 WD and 4 WD groups were made. The results of the regression analysis and the variance analysis were evaluated. The overall efficiency ( $\eta$ ) of the tractors was calculated using the drawbar power, drawbar pull and specific fuel consumption data obtained from the experiment reports. Relationships between PTO power, engine speed, specific fuel consumption and specific energy parameters were determined at 85% of the torque at the maximum engine power obtained at the nominal engine speed, and at the standard PTO revolution (1000 rpm). The loading at 75% of the drawbar pull force may represent operation with primary tillage such as moldboard and disc plow, chisel, subsoil tool which usually require high power. Therefore, in the drawbar performance test at 75% of the pull at the rated engine speed and at the maximum power, the relationships between the drawbar power, pull force, engine speed, forward speed, specific fuel consumption and tractor mass parameters are determined. The overall efficiency of the tractors has been calculated from the following relation by making use of the draw power and fuel power (Sümer, 2005; Sabancı, 1997; Souza *et al.*, 1994).

$$\eta = 100 \frac{P_d}{P_f} \quad (1)$$

$$P_f = \frac{B_e \times H}{3600} \quad (2)$$

Where:  $\eta$  = Total tractor efficiency (%),  $P_d$ =Drawbar power (kW),  $P_f$ =Fuel power (kW),  $B_e$  =Fuel consumption (kg/h),  $H$  = Energy value of diesel fuel (=41870 kJ/kg)

Minitab 19 program was used to make statistical analysis in the study. The coefficient of determination ( $R^2$ ), F value showing the incompatibility test (lack of fit), P (probability) value showing the significance status and estimation equations were found. First order (linear) equation, second order (quadratic) equation and third order (cubic) equations were obtained as estimation equation. The coefficient of determination  $R^2$  shows how much percent of the independent variable explains the change in the dependent variable. It is the ratio of the disclosed change to the total change. The estimation equation is used to estimate the values of the

Y dependent variable from the values of the independent variable X. The probability level P value is found to determine whether the model fits the data correctly. The incompatibility test F (lack of fit) tells us whether a regression model is a weak or a strong model of data. When choosing, the highest  $R^2$ , the lowest F and the lowest P values are based on.

### 3. Results and discussion

#### 3.1. Relationship between engine speed and power of PTO at 85% of the torque for maximum engine power, rated engine speed and two WD tractors

When estimation equation, coefficient of determination  $R^2$ , incompatibility test F (lack of fit) values and probability level P (probability) values are examined,  $R^2$  values of first, second and third degree equations are very close to each other, lack of fit (F) values were found the same (Table 2). It can be said whether the model is meaningful by looking at the F value and the P value. P value was found to be significant with  $P < 0.001$  in the first, second and third degree equations. When we examine the regression values of the first degree equation, the coefficient of determination  $R^2 = 16.19\%$  was the lowest and  $P < 0.001$ . The significance of P here may be due to the large number of samples (DF = 370). The low coefficient of determination means that the estimation equation cannot be used, that is, the PTO power cannot be estimated by looking at the engine speed.  $R^2 = 16.19\%$  means that 16.19% of the total variation in the power take-off power variable can be explained, while 83.81% cannot be explained. The correlation coefficient between the PTO power and engine speed is  $R = 0.4$ , and it is desired that the R value be close to 1 in order for the relationship to be strong. The R value close to 1 indicates how much the data fits on a linear curve. As a result of the statistical analysis, the hypothesis of obtaining the PTO power value with the help of engine speed is insufficient. It is known that the power of the PTO increases up to the standard PTO speed or nominal engine speed, and starts to decrease after this speed. Power take off (PTO) power decreased as engine speed increased. In his study, Başer (2008) found that as the engine speed increased, the power of the PTO decreased.

**Table 2.** Regression analysis values of the relationship between engine speed and power of PTO at 85% of the torque for maximum engine power, rated engine speed and two WD tractors

**Çizelge 2.** Maksimum motor gücü, nominal motor devri ve 2 WD traktör için torkun %85'inde motor devri ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri

Regression Analysis: Power of PTO ( $P_p$ ) (kW) versus engine speed ( $n_m$ )(d/d)				
	((R <sup>2</sup> ) %)	(F)	P≤	The estimation equations
Linear	16.19	2.08	0.001	$P_p = 621.1 - 0.2343n_m$
Quadratic	16.40	2.08	0.001	$P_p = 2253 - 1.711n_m + 0.000334(n_m)^2$
Cubic	16.60	2.08	0.001	$P_p = 46418 - 61.33n_m + 0.02714(n_m)^2 - 0.000004(n_m)^3$

**3.2. Relationship between specific fuel consumption and power of PTO at 85% of the torque for maximum engine power, rated engine speed and two WD tractors**

The smallest R<sup>2</sup> and smallest P values were obtained in the first degree equation, the largest R<sup>2</sup> value and the smallest F value were obtained in the third degree estimation equation (Table 3). The first order equation was found to be significant with P <0.001. It would be appropriate to select the first order equation because the coefficient of determination R<sup>2</sup> and lack of fit (F) are close to each other and the P value is the lowest. The reason for the need for second and third degree equations in statistical

analysis is usually for raising R<sup>2</sup>. The specific fuel consumption decreased as the power of the drive shaft increased. Increasing drive shaft power and thus engine speed reduces specific fuel consumption. When the experiment reports are examined, it is seen that the lowest value of the specific fuel consumption is reached at the point where the maximum power is obtained. Özgür (2009) found in his study that the power decreased with increasing specific fuel consumption. Saral and Avcioglu (2002) reported that the specific fuel consumption depends very much on the structure of the engine, and it generally gets the lowest value below and close to the nominal speed .

**Table 3.** Regression analysis values of the relationship between specific fuel consumption and power of PTO at 85% of the torque for maximum engine power, rated engine speed and two WD tractors

**Çizelge 3.** Maksimum motor gücü, nominal motor devri ve iki WD traktör için torkun %85'inde özgül yakıt tüketimi ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus PTO power ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	40.00	6.41	0.001	$b_e = 0.3027 - 0.000343P_p$
Quadratic	41.24	6.30	0.006	$b_e = 0.3221 - 0.000698P_p + 0.000001(P_p)^2$
Cubic	42.39	6.19	0.007	$b_e = 0.3745 - 0.002135P_p + 0.000013(P_p)^2 - 0.000000(P_p)^3$

**3.3. Relationship between specific energy and power of PTO at 85% of the torque for maximum engine power, rated engine speed and two WD tractors**

When Table 4 is examined, the highest R<sup>2</sup> and the lowest F value are obtained in the third degree equation and the lowest P value is obtained in the first degree

equation. First degree equation was found to be significant with P <0.001. The highest R<sup>2</sup>, the lowest F and P values are taken into account when choosing. Here, the first degree equation can be chosen because the difference between R<sup>2</sup> and F values is small and the P value is the lowest. Specific energy increased as the PTO power increased.

**Table 4.** Regression analysis values of the relationship between specific energy and power of PTO at 85% of the torque for maximum engine power, rated engine speed and two WD tractors

**Çizelge 4.** Maksimum motor gücü, nominal motor devri ve 2WD traktörler için torkun %85'inde özgül enerji ve PTO gücü arasındaki ilişkinin regresyon analizi değerleri

Specific energy ( $E_s$ ) (kWh/L) versus PTO power ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	43.86	6.86	0.001	$E_s = 2.729 + 0.004170P_p$
Quadratic	44.23	6.84	0.120	$E_s = 2.606 + 0.006417P_p - 0.000009(P_p)^2$
Cubic	45.04	6.76	0.020	$E_s = 2.092 + 0.02049P_p - 0.000127(P_p)^2 + 0.000000(P_p)^3$

**3.4. Relationship between engine speed and power of PTO at the standard PTO speed for two WD tractors**

When Table 5 is examined, P values are the lowest in the first and third degree equations and are found to be significant with  $P < 0.001$ . The reason for the P value being  $P < 0.001$  may be due to the high number of samples ( $DF = 370$ ). However, the coefficient of specification  $R^2$  was obtained in the third highest equation and was found 26.41%. It is positive that p values are important, but low coefficients of determination is a negative situation. The low coefficient of determination means that the estimation

equation cannot be used, that is, the PTO power cannot be estimated by looking at the engine speed.  $R^2 = 21.06\%$  means that 21.06% of the total variation in the PTO power variable can be explained, while 78.94% cannot be explained. Looking at the P value, the relationship between engine speed and power take-off is important, but considering the coefficient of determination, the rate of estimation of power take-off by using engine speed is low. The hypothesis of obtaining the power of the PTO with the help of engine speed is insufficient. PTO power decreased as engine speed increased.

**Table 5.** Regression analysis values of the relationship between engine speed and power of PTO at the standard PTO speed for two WD tractors

**Çizelge 5.** İki WD traktör için standart PTO hızında motor devri ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri

PTO power ( $P_p$ ) (kW) versus engine speed ( $n_m$ ) (d/d)				
	( $R^2$ ) (%)	(F)	$P \leq$	The estimation equations
Linear	21.06	9.12	0.001	$P_p = 650.1 - 0.2588n_m$
Quadratic	22.14	9.07	0.025	$P_p = 2484 - 2.11n_m + 0.000466(n_m)^2$
Cubic	26.41	8.44	0.001	$P_p = 64073 - 95.51n_m + 0.04759(n_m)^2 - 0.000008(n_m)^3$

**3.5. Relationship between specific fuel consumption and power of PTO at the standard PTO speed for two WD tractors**

When Table 6 is examined, P values are found to be the lowest and significant with  $P < 0.001$  in the first and second order estimation equations. The degree of accuracy of the specific fuel consumption estimated, namely  $R^2 = 43.86\%$  in the first-order equation,  $R^2 = 46.89\%$  in the second-order equation and the highest  $R^2 = 47.22\%$  in the third-degree equation. Determination

coefficients are higher than previous data and the representation value of the model is 47.22%. In other words, 47.22% of the specific fuel consumption variation, which is the dependent variable, indicates that it is explained by the power of the PTO, which is the independent variable. Another value indicating the model's ability to represent, the lack of fit value was obtained in the lowest third order equation as  $F = 5.49$ . The specific fuel consumption decreased as the power of the PTO increased.

**Table 6.** Regression analysis values of the relationship between specific fuel consumption and power of PTO at the standard PTO speed for two WD tractors

**Çizelge 6.** İki WD traktör için standart PTO hızında özgül yakıt tüketimi ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus PTO power ( $P_p$ ) (kW)				
	( $R^2$ ) (%)	(F)	$P \leq$	The estimation equations
Linear	43.86	5.82	0.001	$b_e = 0.2691 - 0.000197P_p$
Quadratic	46.89	5.51	0.001	$b_e = 0.29 - 0.000506P_p + 0.000001(P_p)^2$
Cubic	47.22	5.49	0.133	$b_e = 0.3093 - 0.000931P_p + 0.000004(P_p)^2 - 0.000000(P_p)^3$

**3.6. Relationship between specific energy and power of PTO at the standard PTO speed for two WD tractors**

When Table 7 is examined, the lowest P values were obtained in the first and second degree equations and were found to be significant with  $P < 0.001$ . The highest  $R^2$  value was obtained in the third degree equation and

the lowest F value was obtained in the second and third degree equations. The highest  $R^2$  value is 48.21. It shows that 48.21% of the specific energy, which is the dependent variable, is explained by the independent variable the PTO power. Specific energy increased as the PTO power increased.



**Table 7.** Regression analysis values of the relationship between specific energy and power of PTO at the standard PTO speed for two WD tractors

**Çizelge 7.** İki WD traktör için standart PTO hızında özgül enerji ve PTO gücü arasındaki ilişkinin regresyon analizi değerleri

Specific energy ( $E_s$ ) (kWh/L) versus power of PTO ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	46.11	6.50	0.001	$E_s = 3.094 + 0.002827P_p$
Quadratic	48.03	6.00	0.001	$E_s = 2.861 + 0.006266P_p - 0.00011(P_p)^2$
Cubic	48.21	6.00	0.265	$E_s = 2.662 + 0.01065P_p - 0.00004(P_p)^2 + 0.000000(P_p)^3$

**3.7. Relationship between engine speed and power of PTO at 85% of the torque for maximum engine power, rated engine speed and four WD tractors**

The highest R<sup>2</sup> value and the lowest F value were found in the third degree equation (Table 8). The lowest P value was obtained in the first degree equation and it was found to be significant with P <0.001. When choosing, the highest R<sup>2</sup>, the lowest F and the lowest P values are taken into consideration. Since the difference between the R<sup>2</sup> and F values obtained here is small and the lowest P value is also in the first degree equation, the first degree equation can be selected as the estimation

equation. PTO power has increased as the engine speed has increased. In the analysis of the relationship between the engine speed and the PTO power in 85% of the torque at the maximum engine power obtained at nominal engine speed for two WD tractors, it was found that the PTO power decreased as the engine speed increased. However, in the same analysis for 4 WD tractors, it was found that as the engine speed increases, the power of the PTO increases. This may be due to the compression ratio, weight, gearbox, fuel equipment and motion transmission system differences of the tractors analyzed.

**Table 8.** Regression analysis values of the relationship between engine speed and power of PTO at 85% of the torque for maximum engine power, rated engine speed and four WD tractors

**Çizelge 8.** Maksimum motor gücü, nominal motor devri ve 4 WD traktör için torkun %85'inde motor devri ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri

PTO power ( $P_p$ ) (kW) versus engine speed ( $n_m$ ) (min <sup>-1</sup> )				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	29.17	4.39	0.001	$P_p = -2408 + 1.242n_m$
Quadratic	29.27	4.62	0.810	$P_p = -23230 + 20.82n_m - .0046(n_m)^2$
Cubic	35.90	4.29	0.043	$P_p = 18169928 - 25629n_m + 12.05(n_m)^2 - 0.00188(n_m)^3$

**3.8. Relationship between specific fuel consumption and power of PTO at 85% of the torque for maximum engine power, rated engine speed and four WD tractors**

The highest R<sup>2</sup> value and the lowest F value were found in the third degree equation (Table 9). The lowest P value was obtained in the first degree equation and it

is significant with P <0.001. The specific fuel consumption decreased as the power of PTO increased. However, after a certain PTO power, specific fuel consumption will begin to increase. It is seen in the test reports that the lowest value of the specific fuel consumption is reached at the point where the maximum power is obtained.

**Table 9.** Regression analysis values of the relationship between specific fuel consumption and power of PTO at 85% of the torque for maximum engine power, rated engine speed and four WD tractors

**Çizelge 9.** Maksimum motor gücü, nominal motor devri ve 4 WD traktör için torkun %85'inde özgül yakıt tüketimi ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus PTO power ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	35.19	24.97	0.001	$b_e = 0.3214 - 0.000253P_p$
Quadratic	36.07	12.69	0.436	$b_e = 0.2448 + 0.000377P_p - 0.000001(P_p)^2$
Cubic	39.45	9.56	0.124	$b_e = -0.5258 + 0.01026P_p - 0.000043(P_p)^2 + 0.000000(P_p)^3$

**3.9. Relationship between specific energy and power of PTO at 85% of the torque for maximum engine power, rated engine speed and four WD tractors**

When Table 10 is examined, the highest R<sup>2</sup> value and the lowest F value were found in the third degree

equation. The lowest P value was obtained in the first degree equation and it is significant with P <0.001. Specific energy increased as the PTO power increased. It is seen in the test reports that the specific energy value increases as the PTO power increases.

**Table 10.** Regression analysis values of the relationship between specific energy and power of PTO at 85% of the torque for maximum engine power, rated engine speed and four WD tractors

*Çizelge 10. Maksimum motor gücü, nominal motor devri ve 4 WD traktör için torkun %85'inde özgül enerji ve PTO gücü arasındaki ilişkinin regresyon analizi değerleri*

Specific energy ( $E_s$ ) (kWh/L) versus PTO power ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	38.17	27.16	0.001	$E_s = 2.431 + 0.003364P_p$
Quadratic	38.17	13.27	0.950	$E_s = 2.323 + 0.00423P_p - 0.000002(P_p)^2$
Cubic	40.45	9.51	0.212	$E_s = 19.95 - 0.2088P_p + 0.000846(P_p)^2 - 0.000001(P_p)^3$

**3.10. Relationship between engine speed and power of PTO at the standard PTO speed for four WD tractors**

When Table 11 is examined, the highest R<sup>2</sup> and the lowest F value are found in the third degree equation. The lowest P value was obtained in the first degree equation. P value in all equations is P > 0.001. Since the difference between the first order equation and the second order equation in the R<sup>2</sup> and F values is small and the lowest P value is in the first order equation, it is appropriate to choose the first order equation. PTO

power has increased as the engine speed has increased. In the analysis of the relationship between the engine speed and the PTO power at the standard PTO speed for two WD tractors, it was found that the PTO power decreases as the engine speed increases. However, in the same analysis for 4 WD tractors, it was found that the power of the PTO increased as the engine speed increased. This may be due to the compression ratio, weight, gearbox, fuel equipment and motion transmission system differences of the tractors analyzed.

**Table 11.** Regression analysis values of the relationship between engine speed and power of PTO at the standard PTO speed for four WD tractors

*Çizelge 11. Dört WD traktörler için standart PTO hızında motor devri ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri*

PTO power ( $P_p$ ) (kW) versus engine speed ( $n_m$ ) (min <sup>-1</sup> )				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	27.14	9.42	0.002	$P_p = -2172 + 1.134n_m$
Quadratic	29.94	9.71	0.290	$P_p = -128924 + 120n_m - 0.0281(n_m)^2$
Cubic	46.97	7.63	0.006	$P_p = 40773862 - 57636n_m + 27.16(n_m)^2 - 0.00426(n_m)^3$

**3.11. Relationship between specific fuel consumption and power of PTO at the standard PTO speed for four WD tractors**

When Table 12 is examined, the highest R<sup>2</sup> and the lowest F value are obtained in the third degree equation.

The lowest P value was obtained in the first degree equation and it was found to be significant with P <0.001. The specific fuel consumption decreased as the power of the PTO increased.

**Table 12.** Regression analysis values of the relationship between specific fuel consumption and power of PTO at the standard PTO speed for four WD tractors

*Çizelge 12. Dört WD traktör için standart PTO hızında özgül yakıt tüketimi ile PTO gücü arasındaki ilişkinin regresyon analizi değerleri*

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus PTO power ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	42.02	28.99	0.001	$b_e = 0.3348 - 0.000302P_p$
Quadratic	42.95	14.68	0.430	$b_e = 0.4597 - 0.001299P_p + 0.000002(P_p)^2$
Cubic	43.41	9.72	0.583	$b_e = -0.344 + 0.00828P_p - 0.00036(P_p)^2 + 0.000000(P_p)^3$

**3.12. Relationship between specific energy and power of PTO at the standard PTO speed for four WD tractors**

When Table 13 is examined, the highest R<sup>2</sup> and the lowest F value are found in the third degree equation.

The lowest P value was obtained in the first degree equation and P <0.001 was found significant. Specific energy increased as the PTO power increased. It is seen in the test reports that the specific energy increases as the PTO power increases.

**Table 13.** Regression analysis values of the relationship between specific energy and power of PTO at the standard PTO speed for four WD tractors

**Çizelge 13.** Dört WD traktör için standart PTO hızında PTO'nun özgül enerjisi ve gücü arasındaki ilişkinin regresyon analizi değerleri

Specific energy ( $E_s$ ) (kWh/L) versus PTO power ( $P_p$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	42.39	29.43	0.001	$E_s = 2.354 + 0.003632P_p$
Quadratic	42.91	14.66	0.553	$E_s = 1.231 + 0.0126P_p - 0.000017(P_p)^2$
Cubic	43.86	9.90	0.428	$E_s = 15.14 - 0.1532P_p + 0.000633(P_p)^2 - 0.000001(P_p)^3$

**3.13. Relationship between specific fuel consumption and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors**

The highest R<sup>2</sup> and the lowest F value were obtained in the third degree equation (Table 14). P values in all equations were found to be significant with P <0.001. The highest R<sup>2</sup> value is 54.91%. Accordingly, 54.91% of the specific

fuel consumption, which is the dependent variable, was explained by the drawbar power, which is the independent variable. It is appropriate to select the first order equation since the specification coefficient R<sup>2</sup>, F values are close to each other in all equation types and P values are the same. The specific fuel consumption decreased as the drawbar power increased.

**Table 14.** Regression analysis values of the relationship between specific fuel consumption and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors

**Çizelge 14.** Maksimum motor gücü, nominal motor devri ve iki WD traktör için çeki demiri çekişinin %75'inde özgül yakıt tüketimi ve çeki çubuğu gücü arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus drawbar power ( $P_d$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	49.90	4.81	0.001	$b_e = 0.4021 - 0.000867P_d$
Quadratic	53.02	4.51	0.001	$b_e = 0.4494 - 0.002030P_d + 0.000006(P_d)^2$
Cubic	54.91	4.33	0.001	$b_e = 0.5399 - 0.005318P_d + 0.000042(P_d)^2 - 0.000000(P_d)^3$

**3.14. Relationship between engine speed and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors**

When Table 15 is examined, the highest R<sup>2</sup> and the lowest F value were obtained in the first and second

order equations. R<sup>2</sup> and F values were found close to each other in all equations. The lowest P value was obtained in the first degree equation and it was found to be significant with P <0.001. As the engine speed increases, the drawbar power decreases.

**Table 15.** Regression analysis values of the relationship between engine speed and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors

**Çizelge 15.** Maksimum motor gücü, nominal motor devri ve iki WD traktör için çeki demiri çekişinin %75'inde motor devri ve çeki gücü arasındaki ilişkinin regresyon analizi değerleri

Drawbar power ( $P_d$ ) (kW) versus engine speed ( $n_m$ ) (min <sup>-1</sup> )				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	17.99	2.21	0.001	$P_d = 520.2 - 0.1999n_m$
Quadratic	18.34	2.21	0.212	$P_d = 2189 - 1.703n_m + 0.000338(n_m)^2$
Cubic	18.43	2.22	0.522	$P_d = -23272 + 32.5n_m - 0.01496(n_m)^2 + 0.000002(n_m)^3$

**3.15. Relationship between tractor mass and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors**

When Table 16 is examined, the highest R<sup>2</sup> and the lowest F value are obtained in the third degree equation. P value was found to be significant with P < 0.001 in the first, second and third degree equations. The largest R<sup>2</sup>

= 89.25%. Considering the highest determination coefficient, 89.25% of the change in drawbar power can be explained by the tractor mass, while 10.75% cannot be explained. According to these results, a strong relationship can be mentioned between the tractor mass and the drawbar power. As the tractor mass increases, its drawbar power has increased.

**Table 16.** Regression analysis values of the relationship between tractor mass and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors

*Çizelge 16. Maksimum motor gücü, nominal motor devri ve iki WD traktör için çeki demiri çekişinin %75'inde traktör kütlesi ve çeki gücü arasındaki ilişkinin regresyon analizi değerleri*

Drawbar power ( $P_d$ ) (kW) versus tractor mass ( $m_t$ ) (kg)				
	(R <sup>2</sup> ) (%)	(F)	P ≤	The estimation equations
Linear	87.67	2.76	0.001	$P_d = -19.37 + 0.01263m_t$
Quadratic	88.93	2.43	0.001	$P_d = 21.6 + 0.002097m_t + 0.000001(m_t)^2$
Cubic	89.25	2.35	0.001	$P_d = 85.58 - 0.0229m_t + 0.000004(m_t)^2 - 0.000000(m_t)^3$

**3.16. Relationship between travel speed and drawbar pull at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors**

When Table 17 is examined, the highest R<sup>2</sup> and the lowest F value were found in the third degree equation.

The lowest P value was obtained in the first degree equation and it was found significant with P < 0.001. As the travel speed increases, the drawbar pull decreases. It is seen in the test reports that as the travel speed increases, the drawbar pull decreases.

**Table 17.** Regression analysis values of the relationship between travel speed and drawbar pull at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors

*Çizelge 17. Maksimum motor gücü, nominal motor devri ve iki WD traktör için çeki kuvvetinin %75'inde ilerleme hızı ve çeki kuvveti arasındaki ilişkinin regresyon analizi değerleri*

Drawbar pull ( $F_d$ ) (kN) versus travel speed ( $V_t$ ) (km/h)				
	(R <sup>2</sup> ) (%)	(F)	P ≤	The estimation equations
Linear	4.32	2.21	0.001	$F_d = 59.22 - 2.865V_t$
Quadratic	5.45	2.19	0.005	$F_d = -2.75 + 11.31V_t - 0.7984(V_t)^2$
Cubic	7.44	2.12	0.079	$F_d = -572.9 + 202.6V_t - 21.89(V_t)^2 + 0.7638(V_t)^3$

**3.17. Relationship between specific fuel consumption and drawbar pull at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors**

When Table 18 is analyzed, the highest R<sup>2</sup> = 10.75% and the lowest F = 2.44 value were obtained in the third degree equation, the lowest P value was obtained in the first and third degree equations and it was found significant with P < 0.001. It is a negative situation that the determination coefficients are low. The highest representation value of the model, R<sup>2</sup> = 10.75% and the other representation value, F = 2.44 were found in the third degree equation. The relationship between specific fuel consumption and drawbar pull is important. However, when looking at the highest coefficient of determination; While 10.75% of the change in specific fuel consumption can be explained by the drawbar pull, 89.25% means it cannot be explained. The hypothesis of

estimating specific fuel consumption is insufficient depending on the drawbar pull. The specific fuel consumption increased as the drawbar pull increased. However, when the test reports are examined, it is seen that the specific fuel consumption decreases as the drawbar pull increases. The reason for the different results of the analysis is that 4 WD tractors can be reached at a rated engine speed of 7.5 km / h, while 2 WD tractors can be reached at rated speeds above 7.5 km / h (9-10 km / h). In the analysis made for 75% of the drawbar pull from which the nominal engine speed was obtained for two WD tractors, it seems that the specific fuel consumption increases while the drawbar pull increases due to the fact that the distribution is not at 7.5 km / h, but at higher speeds. In addition, the specific fuel consumption may have increased because 2 WD tractors have less ability to hold onto the ground compared to 4 WD tractors. In the study of

Küçüksarıyıldız (2006), 2 WD found that with the increase of drawbar pull on a tractor, the specific fuel consumption decreased. He stated that the specific fuel consumption decreased and the effect of the drawbar

pull on the specific fuel consumption was important due to the fact that the increase in the drawbar pull increased the effective engine power.

**Table 18.** Regression analysis values of the relationship between specific fuel consumption and drawbar pull at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors

**Çizelge 18.** Maksimum motor gücü, nominal motor devri ve iki WD traktör için çeki kuvvetinin %75'inde özgül yakıt tüketimi ve çeki kuvveti arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus drawbar pull ( $F_d$ ) (kN)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	2.79	2.50	0.001	$b_e = 0.3149 + 0.000475F_d$
Quadratic	3.77	2.65	0.054	$b_e = 0.2892 + 0.001978F_d - 0.000019(F_d)^2$
Cubic	10.75	2.44	0.001	$b_e = 0.1117 + 0.01752F_d - 0.000426(F_d)^2 + 0.000003(F_d)^3$

**3.18. Relationship between travel speed and specific fuel consumption at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors**

When Table 19 is examined, the highest R<sup>2</sup> value and the lowest F value are obtained in the third degree equation. The lowest P value was obtained in the second degree equation and it was found to be significant with P < 0.001. It is a negative situation that the specification coefficients are low. Considering the highest (R<sup>2</sup> =

6.02%) determination coefficient, 6.02% of the change in specific fuel consumption can be explained by the travel speed, while 93.98% cannot be explained. The hypothesis of estimating specific fuel consumption is insufficient depending on the travel speed. However, the results of the analysis were found to be the same. Specific fuel consumption decreased as the travel speed decreased. When the test reports are examined, it is seen that the specific fuel consumption decreases as the speed of travel increases.

**Table 19.** Regression analysis values of the relationship between travel speed and specific fuel consumption at 75% of the drawbar pull for maximum engine power, rated engine speed and two WD tractors

**Çizelge 19.** Maksimum motor gücü, nominal motor devri ve iki WD traktör için çeki kuvvetinin %75'inde ilerleme hızı ve özgül yakıt tüketimi arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus travel speed ( $V_t$ ) (km/h)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	0.23	2.20	0.355	$b_e = 0.3482 - 0.001974V_t$
Quadratic	6.01	2.12	0.001	$b_e = 0.938 - 0.1399V_t + 0.007957(V_t)^2$
Cubic	6.02	2.05	0.861	$b_e = 1.04 - 0.1764V_t + 0.01226(V_t)^2 - 0.000167(V_t)^3$

**3.19. Relationship between travel speed and specific fuel consumption at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors**

The highest R<sup>2</sup> value was obtained in the third degree equation, the lowest F value and the lowest P value were obtained in the second degree equation (Table 20). P value is high in all equations and P > 0.001 was found. The highest determination coefficient is R<sup>2</sup> = 13.33%. While 13.33% of the change in specific fuel

consumption can be explained by the travel speed, 86.67% cannot be explained. The hypothesis of estimating specific fuel consumption is insufficient depending on the travel speed. However, the results of the analysis were found to be the same as the results of the experiment. The specific fuel consumption has decreased as the travel speed has increased. When the experiment reports are examined, it is seen that the specific fuel consumption decreases as the travel speed increases.

**Table 20.** Regression analysis values of the relationship between travel speed and specific fuel consumption at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors

**Çizelge 20.** Maksimum motor gücü, nominal motor devri ve dört WD traktör için çeki kuvvetinin %75'inde ilerleme hızı ve özgül yakıt tüketimi arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus travel speed ( $V_t$ ) (km/h)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	2.06	10.99	0.330	$b_e = 0.3257 - 0.004832V_t$
Quadratic	13.25	10.02	0.020	$b_e = 2.281 - 0.4584V_t + 0.02621(V_t)^2$
Cubic	13.33	10.38	0.850	$b_e = -0.26 + 0.419V_t - 0.0748(V_t)^2 + 0.00387(V_t)^3$

**3.20. Relationship between drawbar pull and specific fuel consumption at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors**

When Table 21 is examined, the highest R<sup>2</sup> value and the lowest F value are obtained in the third degree

equation. The lowest P value was found as P > 0.001 in the first degree equation. The specific fuel consumption decreased as the drawbar pull increased. When the test reports are examined, it is seen that the specific fuel consumption decreases as the drawbar pull increases.

**Table 21.** Regression analysis values of the relationship between drawbar pull and specific fuel consumption at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors

**Çizelge 21.** Maksimum motor gücü, nominal motor devri ve dört WD traktör için çeki kuvvetinin %75'inde çeki kuvveti ile özgül yakıt tüketimi arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus drawbar pull ( $F_d$ ) (kN)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	17.81	9.75	0.003	$b_e = 0.3237 - 0.000437F_d$
Quadratic	19.28	5.26	0.376	$b_e = 0.2632 + 0.0009F_d - 0.000007(F_d)^2$
Cubic	20.48	3.69	0.425	$b_e = -0.0257 + 0.01066F_d - 0.000114(F_d)^2 + 0.000000(F_d)^3$

**3.21. Relationship between travel speed and drawbar pull at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors**

The highest R<sup>2</sup> value and the lowest F value were obtained in the third degree equation and the lowest P

value was obtained in the second degree equation, and P < 0.001 was found significant (Table 22). As the travel speed increases, the drawbar pull decreases. When the test reports are examined, it can be seen that the drawbar pull decreases as the speed of travel.

**Table 22.** Regression analysis values of the relationship between travel speed and drawbar pull at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors

**Çizelge 22.** Maksimum motor gücü, nominal motor devri ve dört WD traktör için çeki kuvvetinin %75'inde ilerleme hızı ve çeki kuvveti arasındaki ilişkinin regresyon analizi değerleri

Drawbar pull ( $F_d$ ) (kN) versus travel speed ( $V_t$ ) (km/h)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	1.96	0.90	0.348	$F_d = 131.2 - 4.409V_t$
Quadratic	27.12	15.19	0.001	$F_d = -2659 + 643V_t - 37.4(V_t)^2$
Cubic	27.68	0.34	0.566	$F_d = 4006 - 1664V_t + 228.2(V_t)^2 - 10.17(V_t)^3$

**3.22. Relationship between tractor mass and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors**

While the highest R<sup>2</sup> value was obtained in the third degree equation, the lowest F value was obtained in the second degree equation and the lowest P value was obtained in the first degree equation (Table 23). P value was found to be significant with P < 0.001. The

coefficient of determination was found to be the highest R<sup>2</sup> = 82.27%. Considering the highest coefficient of determination, 82.27% of the change in drawbar power can be explained by the mass of the tractor, while 17.73% means it cannot be explained. According to these results, a strong relationship can be mentioned between the tractor mass and the drawbar power. As the tractor mass increases, its drawbar power has increased.

**Table 23.** Regression analysis values of the relationship between tractor mass and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors

**Çizelge 23.** Maksimum motor gücü, nominal motor devri ve dört WD traktör için çeki kuvvetinin %75'inde traktör kütlesi ve çeki gücü arasındaki ilişkinin regresyon analizi değerleri

Drawbar power ( $P_d$ ) (kW) versus tractor mass ( $m_t$ ) (kg)				
	(R <sup>2</sup> ) (%)	(F)	P≤	The estimation equations
Linear	81.56	4.73	0.001	$P_d = -83.25 + 0.01536m_t$
Quadratic	82.25	4.68	0.200	$P_d = -309.7 + 0.03762m_t - 0.000001(m_t)^2$
Cubic	82.27	4.83	0.812	$P_d = -20 - 0.0051m_t + 0.000002(m_t)^2 - 0.000000(m_t)^3$

**3.23. Relationship between engine speed and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors**

The highest R<sup>2</sup> value and the lowest P value were obtained in the third degree equation. P value was found

to be P> 0.001 in all equations (Table 24). The lowest F value was obtained in the first degree equation. As the engine speed increases, the drawbar power decreases. When the test reports are examined, it is seen that the drawbar power decreases as the engine speed increases.

**Table 24.** Regression analysis values of the relationship between engine speed and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors

**Çizelge 24.** Maksimum motor gücü, nominal motor devri ve dört WD traktör için çeki kuvvetinin %75'inde motor devri ve çeki gücü arasındaki ilişkinin regresyon analizi değerleri

Drawbar power ( $P_d$ ) (kW) versus engine speed ( $n_m$ ) ( $\text{min}^{-1}$ )				
	(R <sup>2</sup> ) (%)	(F)	P <sub>≤</sub>	The estimation equations
Linear	2.68	1.24	0.272	$P_d = 476.2 - 0.1195n_m$
Quadratic	19.78	5.54	0.007	$P_d = -7344 + 7.536n_m - 0.001868(n_m)^2$
Cubic	28.28	5.77	0.002	$P_d = 150617 - 219.5n_m + 0.1065(n_m)^2 - 0.000017(n_m)^3$

**3.24. Relationship between specific fuel consumption and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors**

The highest R<sup>2</sup> value and the lowest F value were obtained in the third degree equation and the lowest P

value was obtained in the first and second degree equations, and it was found significant with P = 0.001 (Table 25). The specific fuel consumption decreased as the drawbar power increased. When the test reports are examined, it is seen that the specific fuel consumption decreases as the drawbar power increases.

**Table 25.** Regression analysis values of the relationship between specific fuel consumption and drawbar power at 75% of the drawbar pull for maximum engine power, rated engine speed and four WD tractors

**Çizelge 25.** Maksimum motor gücü, nominal motor devri ve dört WD traktör için çeki kuvvetinin %75'inde özgül yakıt tüketimi ve çeki gücü arasındaki ilişkinin regresyon analizi değerleri

Specific fuel consumption ( $b_e$ ) (kg/kWh) versus drawbar power ( $P_d$ ) (kW)				
	(R <sup>2</sup> ) (%)	(F)	P <sub>≤</sub>	The estimation equations
Linear	23.43	13.77	0.001	$b_e = 0.3289 - 0.000205P_d$
Quadratic	28.45	8.75	0.001	$b_e = 0.2195 + 0.000792P_d - 0.000002(P_d)^2$
Cubic	29.53	3.00	0.002	$b_e = -0.0282 + 0.004247P_d - 0.000018(P_d)^2 + 0.000000(P_d)^3$

**3.2. Tractor overall efficiency**

Tractor overall efficiencies were calculated for all tractors analyzed in the study. The arithmetic mean of tractor overall efficiency ( $\eta$ ) was found as 29.04. When the tractor overall efficiency is analyzed, it is seen that there is an inverse proportion between the tractor overall efficiency and the specific fuel consumption. In general, specific fuel consumption decreases as the overall efficiency of the tractor increases. The inverse relationship between the overall efficiency of the tractor and the specific fuel consumption is an expected result.

According to the results of statistical analysis for both WD tractors at 85% of the maximum engine power torque at nominal engine speed and at the standard PTO speed, it was found that as the engine speed increases, the PTO power decreases. According to the results of the statistical analysis performed at 75% of the drawbar pull at the rated engine speed at maximum power for two WD tractors, it was found that the specific fuel consumption increased as the drawbar pull increased. The arithmetic mean of tractors overall efficiency ( $\eta$ ) was found as 29.04.

**4. Conclusions**

Statistical analysis for two WD tractors both at 85% of the torque at the maximum engine power at rated engine speed and for parameters at the standard PTO speed gave the same results. Statistical analysis for four WD tractors both at 85% of the torque at the maximum engine power at rated engine speed and for parameters at the standard PTO speed gave the same results.

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## Range Extension of European Hake from The Eastern Black Sea Coasts of Turkey

Rafet Çağrı ÖZTÜRK<sup>1</sup>, Uğur KARADURMUŞ<sup>2</sup>, Mehmet AYDIN<sup>3\*</sup>

<sup>1</sup>Department of Fisheries Technology Engineering, Sürmene Faculty of Marine Sciences, Karadeniz Technical University, Trabzon

<sup>2</sup>Department of Underwater Technology, Maritime Vocational School, Bandırma Onyedi Eylül University, Balıkesir

<sup>3</sup>Department of Fisheries Technology Engineering, Fatsa Faculty of Marine Sciences, Ordu University, Ordu

\*Corresponding author's email: maydin69@hotmail.com

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**Abstract:** Eight specimens of European hake, *Merluccius merluccius* (Linnaeus, 1758) were caught in 2021 by commercial gill net from the Eastern Black Sea coasts of Turkey. The total length of the specimens ranged from 12.3 to 22.3 cm. Mitochondrial gene regions of 16S rRNA and COI were analyzed to genetically characterize *M. merluccius* specimens. This record is the first confirmed report suggesting that *M. merluccius* has expanded its distribution range eastward in the Black Sea. The occurrence of the species at different times and in a wide depth range (20-92 m) strengthens our opinion that this species is adapted to the region. We highlight that the current status of environmental factors for a productive habitat may increase the biomass level of European hake in the long run in the Eastern Black Sea.

**Keywords:** Eastern Black Sea, First record, Expansion, Merlucciidae, Morphology, mtDNA

## Berlam Balığının Türkiye'nin Doğu Karadeniz Kıyılarında Dağılımının Genişlemesi

**Öz:** Sekiz adet Berlam, *Merluccius merluccius* (Linnaeus, 1758), 2021 yılında Doğu Karadeniz'in Türkiye kıyılarında Ordu'da ticari galsama ağları ile yakalanmıştır. Bireylerin toplam boyları 12,3 cm ile 22,3 cm arasında değişmektedir. Mitokondriyal DNA'nın 16S rRNA ve COI gen bölgeleri analiz edilerek *M. merluccius* genetik olarak karakterize edilmiştir. Bu kayıt, *M. merluccius*'un Karadeniz'deki dağılım alanını doğuya doğru genişlettiğini gösteren ilk doğrulanmış rapordur. Türün farklı zamanlarda ve geniş derinlik aralığında (20-92 m) ortaya çıkması, bu türün bölgeye uyum sağladığı kanaatimizi güçlendirmektedir. Verimli bir habitat için mevcut çevresel faktörlerin Doğu Karadeniz'de uzun vadede Avrupa Berlamı'nın biyokütle seviyelerini artırabileceğini vurguluyoruz.

**Anahtar Kelimeler:** Doğu Karadeniz, İlk kayıt, Genişleme, Merlucciidae, Morfoloji, MtDNA

### 1. Introduction

The European hake, *Merluccius merluccius* (Linnaeus, 1758), is a demersal and benthopelagic species that mainly inhabits muddy bottoms of shallow (30 m) and deep (1000 m) waters. Adults feed mainly on fish (small hakes, sardines, anchovies, pilchard), while juveniles feed on crustaceans (Preciado et al., 2008). The European hake is among the main target species of demersal fisheries in the Western and Eastern Mediterranean Sea (Gücü & Bingel, 2011). It is one of the most heavily exploited fish species on the west coast of Europe (Casey & Pereiro, 1995).

The genus *Merluccius* comprises 12 species widely distributed along the coasts of Europe, America, and Africa. Phylogenetic analysis based on mitochondrial and nuclear sequences indicates the presence of two distinct clades: American clade and Euro-African clade (Campo et al., 2007; Perez et al., 2021; Quinteiro et al., 2000). The Euro-African clade includes the European hake. *M. merluccius* is widely distributed over the

northeast Atlantic shelf (Arancibia, 2015), being more abundant from the British Isles to the south of Spain (Alvarez et al., 2004). Its range extends from Mauritania to off the western coast of the waters south of Iceland and Norway (Casey & Pereiro, 1995; International Council for the Exploration of the Sea [ICES], 2008). There is a limited number of available DNA sequences of *M. merluccius* throughout its distribution range.

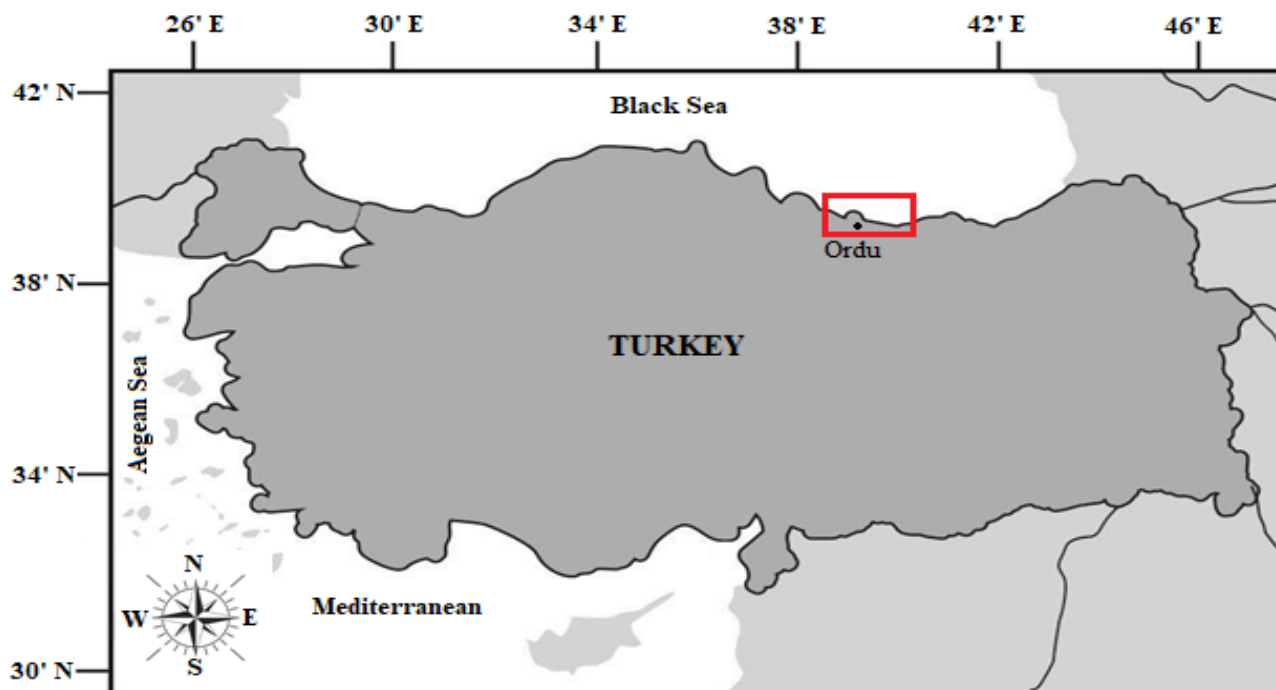
The first occurrence of *M. merluccius* in the Black Sea was mentioned in the marine fish checklist for the Black Sea by Bilecenoğlu et al. (2014), referring to Ninni (1923). Various studies (Geldiay, 1969; Svetovidov, 1986) claim that European hake was found in the Black Sea. Different researchers have reported that European hake is sparsely distributed in the Eastern Black Sea, referring to Casey and Pereiro (1995). Still, there is no evidence of its existence in the Eastern Black Sea. Fishermen and fishmongers in the region were also unable to recognize the species and stated seeing it for the first time. This paper aimed to highlight, for the first

time, the presence of European hake in the Eastern Black Sea.

## 2. Materials and Methods

Eight specimens of European hake were caught from the Eastern Black Sea coast, Fatsa, Ordu (41°02'21.61"N – 37°30'06.13"E) (Figure 1) between May and July in 2021. Specimens were obtained

between 20 – 92 m depth by a commercial whiting gill net (18 mm mesh size). Specimens were identified morphologically according to Fischer et al. (1987) and genetically based on mtDNA sequences. Total length (TL) and total weight (TW) were measured to nearest 0.1 cm and 0.01 g, respectively. Sex was determined macroscopically, according to Gunderson (1993).



**Figure 1.** Study area. The red frame indicates the capture site of European hake in the eastern Black Sea coasts of Turkey.

**Şekil.1.** Çalışma sahası. Kırmızı alan Türkiye'nin Doğu Karadeniz kıyılarında Berlam Balığının yakalandığı sahayı gösterir.

Sequence analysis of two mitochondrial gene regions, 16S rRNA and cytochrome c oxidase subunit I (COI), were performed to identify and characterize specimens genetically. Genomic DNA was isolated from the fin clips of eight specimens using the Wizard SV Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. Quantity and purity of DNA were assessed using nanodrop (NanoDrop 8000, Thermo Fisher). The 16S rRNA gene region was amplified with 16Sbr-H and 16Sar-L (Palumbi, 1996). The COI gene region was amplified with primers of Fish-F1 and Fish-F2 (Ward et al., 2005). PCR assay was performed in a total volume of 25 µl containing 12.5 µl 2X Master mix (HibriGen), 1µM of each primer (forward and reverse), 100 ng DNA, and ultrapure water. The thermal cycling condition was as follows: 95 °C for 3 min, followed by 35 cycles of 95 °C for 50 s, 54 °C for 45 s, and 72 °C for 45 s with a final extension step of 5 min at 72 °C. Amplicons were visualized on agarose gel and sequenced on ABI 3500

Genetic Analyzer (Thermo Fisher) with a Big Dye v.3.1 Terminator Cycle Sequencing Kit.

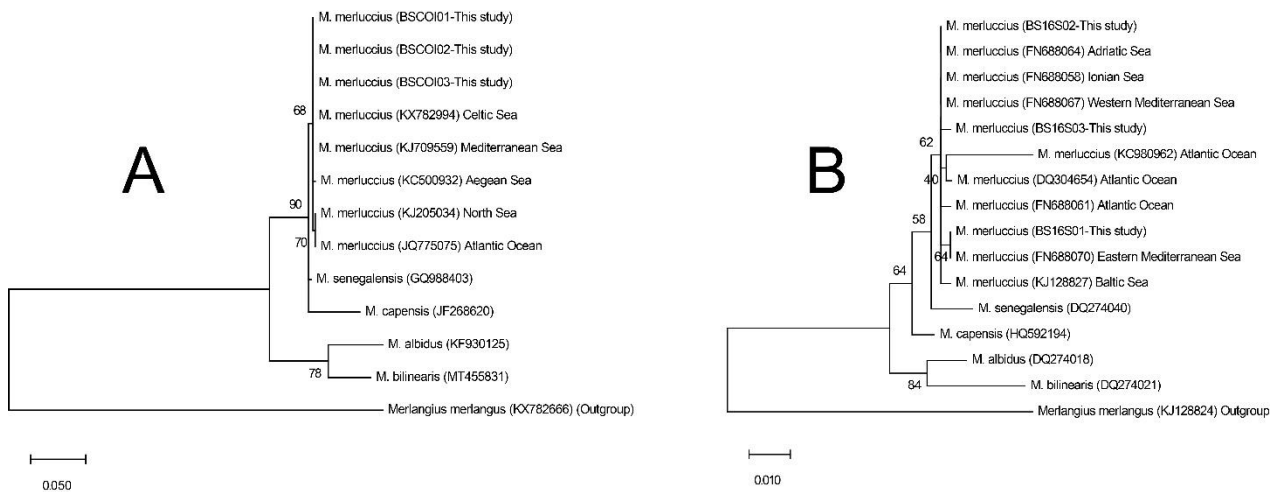
Raw sequences were trimmed and aligned in BioEdit (Hall, 1999) using the ClustalW algorithm (Thompson et al., 1994). Quality checked sequences were compared with reference sequences in the GenBank database (<https://www.ncbi.nlm.nih.gov>) using BLAST (Basic Local Alignment Search Tool), and species identification was performed by comparing sequence similarity. Phylogenetic relationships were inferred with a maximum likelihood tree using available COI and 16S rRNA sequences of *M. merluccius* with known geographic information. The reference COI sequences of *M. merluccius* (Accession numbers: KX782994, KJ709559, KJ205034, KC500932, JQ775075), *M. senegalensis* (GQ988403), *M. capensis* (JF286820), *M. albidus* (KF930125), *M. bilinearis* (MT455831) and 16S rRNA sequences of *M. merluccius* (FN688058, FN688061, FB688064, FN688067, FN688070, KC980962, DQ304654, KJ128827), *M. senegalensis*

(DQ274040), *M. capensis* (HQ592194), *M. albidus* (DQ274018), *M. bilinearis* (DQ274021) were retrieved from NCBI GenBank database. *Merlangius merlangus* was used as an outgroup. The appropriate model of sequence evolution for 16S rRNA and COI genes was determined based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) as Tamura-Nei (COI) and Kimura 2-parameter (16S rRNA). The robustness of the trees was tested with 1000 bootstrap replicates in Mega X (Kumar et al., 2018).

### 3. Results and Discussion

The generated partial COI and 16S rRNA sequences were 589 bp and 568 bp, respectively. A total of two haplotypes for COI and three haplotypes for 16S rRNA were identified from eight specimens. Comparison of

COI and 16S rRNA sequences against the GenBank database using BLAST gave a successful match with available *M. merluccius* sequences with pairwise sequence identity similarity of 100% for COI and 99.65% for 16S rRNA genes. Generated COI and 16S rRNA sequences were deposited in GenBank (Accession numbers: COI, MZ540345-MZ540346; 16S rRNA, MZ540342-MZ540344). The maximum likelihood tree generated with COI and 16S rRNA revealed a clear separation of *M. merluccius* from other *Merluccius* species, in harmony with previous reports (Campo et al., 2007; Perez et al., 2021; Quinteiro et al., 2000). The Black Sea specimens nested with reference *M. merluccius* sequences, yet there was no clear separation based on geographic origin (Figure 2).



**Figure 2.** Maximum likelihood tree constructed with the COI (A) and 16S rRNA sequences (B) of *Merluccius merluccius* along with reference sequences obtained from the NCBI GenBank database.

**Şekil 2.** *Merluccius merluccius*'un COI (A) ve 16S rRNA dizileri (B) ile birlikte NCBI GenBank veri tabanından elde edilen referans dizileri ile oluşturulan maksimum olasılık ağacı.

The TL and TW of the sampled specimens ranged between 12.3 – 22.3 cm and 12.63 – 81.86 g, respectively (Figure 3). Sampling and morphological details of the European hake specimens are given in Table 1. The presence of the European hake was previously reported from the Mediterranean Sea (Çiçek & Aşar, 2010; Özvarol, 2014; Sangun et al., 2007), the Aegean Sea (Gurbet et al., 2013; Soykan et al., 2015), and the Marmara Sea coasts (Daban et al., 2020; Gül et al., 2019) of Turkey. The only evidence suggesting the presence of *M. merluccius* existence in the Black Sea was claimed by Türker and Bal (2018). The researchers caught the European hake during the bottom trawl surveys conducted in the Western Black Sea (Zonguldak-Amasra). To obtain more reliable results from the studies on fish stocks, if possible, different

studies must be carried out separately for each fish species along with its length-weight relationships and updated within specific periods.

The Black Sea is a semi-enclosed basin connected to the Mediterranean Sea via the Bosphorus Strait and Dardanelles. In recent years, the physical, chemical, and biological properties of the Black Sea have changed significantly with the impact of global climate change. In this process, fish species that have settled in the Black Sea ecosystem have been in constant change/development (van der Voo, 1990). New species are settling in the Black Sea, and the first sightings and new geographical records are increasing day by day (Aydın, 2015; Aydın, 2017; Aydın, 2020; Aydın & Bodur, 2018; Aydın & Gül, 2021; Engin et al., 2015; Göktürk et al., 2012; Öztürk & Özbulut, 2016).



**Figure 3.** The sampled specimen of *Merluccius merluccius* with 22.3 cm TL, captured from the eastern Black Sea coast on July 18, 2021

**Şekil.3.** *Merluccius merluccius*'un Doğu Karadeniz kıyısından yakalanan 22,3 cm TL boya sahip örneği, 18 Temmuz 2021

**Table 1.** Capturing details, total length (cm) and total weight (g) of sampled *Merluccius merluccius* individuals by sex

**Çizelge 1.** Örneklenen *Merluccius merluccius* bireylerinin cinsiyete göre yakalama detayları, toplam uzunluk (cm) ve toplam ağırlıkları (g)

Capture date	Capturing depth (m)	Total length (cm)	Total weight (g)	Sex
03.05.2021	90	12.4	12.63	Male
28.05.2021	84	12.3	13.85	Male
13.06.2021	92	15.3	27.09	Female
22.06.2021	20	15.0	22.29	Female
09.07.2021	45	16.8	35.60	Female
09.07.2021	58	16.9	32.41	Female
14.07.2021	64	15.5	26.05	Male
18.07.2021	50	22.3	81.86	Female

Environmental factors are usually admitted as the main factors controlling the spatio-temporal distribution of fish populations (Planque et al., 2011). Fish distribution is affected by several biotic and abiotic factors, such as food availability and temperature and may be influenced at the same time by various variables and conditions (Zheng et al., 2002). The environmental conditions such as temperature (11.8 – 15.0 °C), chlorophyll-*a* (0.1 – 0.9 mg·m<sup>3</sup>) and food availability (crustaceans and small pelagic) might play a key role in the spatial distribution of the biomass of European hake as previously reported (de Pontual et al., 2015; Druon et al., 2015; Sion et al., 2019; Vasilakopoulos et al., 2014). Yalçın and Gurbet (2016) described that the higher *M. merluccius* abundances were found in deeper from 50 m with salinity >38.55 ‰ and water temperature ranging from 14.5 to 19 °C. Sakallı and Başusta (2018) reported that the mean annual surface water temperature has varied between 12 and 17 °C in the last 34 years in the

Black Sea, and the relative increase in average surface water temperature is predicted to be 5.1 °C at the end of this century. In the coastal waters of Turkey in the Black Sea, the Chl-*a* concentrations vary from 0.20 to 1.23 mg·L<sup>-1</sup> in the coastal waters and decrease in the open waters to 0.22 – 0.90 mg·L<sup>-1</sup> (Polat Beken et al., 2017). The potential of crustaceans and small pelagic fish (anchovy and sardine) in the Black Sea (Gücü et al., 2017) might support *M. merluccius* existence in the long term. The European hake stocks in the Mediterranean Sea suffer from the fishing pressure, with a fishing mortality rate that is on average five times higher than the target fishing mortality level (Food and Agriculture Organization [FAO], 2016). Despite its regional importance, exploitation due to fishing mortality is also present in the Aegean Sea (Gurbet et al., 2013) and the Sea of Marmara (Gül et al., 2019). All types of trawling (bottom and beam) are prohibited in the Eastern Black Sea (from Ordu to Turkish Georgian border) due to having a very narrow continental shelf. Gillnets, hand lines and deep-water cast nets that are less harmful to the benthic ecosystem are used for demersal fisheries (especially whiting) in the Eastern Black Sea (Karadurmuş et al., 2021). If the European hake forms a population in this area, it is thought that it will be less exposed to overfishing, unlike other areas (the Mediterranean Sea, the Aegean Sea, the Sea of Marmara, and even the Western Black Sea).

#### 4. Conclusion

The present individuals were obtained through fishing. This finding is the first confirmed report suggesting that *M. merluccius* has an eastward distribution range in the Black Sea. The occurrence of



the species at different times and in a wide depth range strengthens our opinion that this species has adapted to the region. We highlight that the current status of environmental factors for a productive habitat may increase the biomass level of European hake in the long run in the Eastern Black Sea. We estimate that if European hake adopts this region, it may create a population in the long term and become a sustainable fishery resource thanks to comparatively lower fishing pressure in the area. It should be noted that further studies are needed to understand the existence of female adults and juveniles.

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## Molecular Characterization of Cylindrical Inclusion Protein Gene Regions of Turkish *Zucchini yellow mosaic virus* (ZYMV) Isolates

Şerife TOPKAYA<sup>1\*</sup>  Filiz ERTUNÇ<sup>2</sup> 

<sup>1</sup>Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat

<sup>2</sup>Ankara University, Faculty of Agriculture, Department of Plant Protection, Ankara

\*Corresponding author's email: [serife.topkaya@gop.edu.tr](mailto:serife.topkaya@gop.edu.tr)

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**Abstract:** *Zucchini yellow mosaic virus* (ZYMV) is an economically important viral pathogen that causes intense mosaic symptoms and disfigurement in cucurbits. The aim of this study is to characterize ZYMV isolates obtained from Ankara, Antalya, Burdur, Konya, Karaman, Aksaray provinces of Turkey according to cylindrical inclusion (CI) protein sequences and to determine conserved areas on protein in Potyviruses. For this purpose, molecular studies and sequence analyzes were performed with primers specific to the CI protein region of collected cucurbit samples during 2019-2014 years. At the end of the study, the N terminus of Turkish ZYMV's CI protein 888 nucleotides long and 296 amino acids (aa) was amplified. Phylogenetic analysis of the nucleotide sequences of the CI region showed that the majority of isolates (40) belonged to a large molecular subgroup (A1) most common in Europe and the world, and three isolates (Y4, Y21, Y23) belonged to subgroup A5. Moreover, according to the coat protein nucleotide analysis, these three isolates (Y4, Y21, Y23) were grouped with the molecular subgroup A4, the group that emerged recently in Europe. The CI ZYMV nucleotide binding motif (NTBM) and RNA helicase activity site (five motifs) were conserved among isolates according to amino acid analysis.

**Keywords:** Cucurbites, cylindrical inclusion, helicase activity, Phylogenetic analysis, Sequence analyzes, ZYMV

### Türkiye ZYMV İzolatlarının CI Protein Gen Bölgelerinin Moleküler Karakterizasyonu

**Öz:** Kabak sarı mozaik virüsü (ZYMV), kabakgillerde yoğun mozaik semptomlarına ve şekil bozukluğuna neden olan ekonomik açıdan önemli bir viral etmendir. Bu çalışmanın amacı, Türkiye'de farklı illerden (Ankara, Antalya, Burdur, Konya, Karaman, Aksaray) elde edilen ZYMV izolatlarının silindirik inklüzyon (CI) protein dizilerine göre karakterize edilmesi ve Potyvürüslerde protein üzerinde korunan alanların belirlenmesidir. Bu amaçla CI protein bölgesine spesifik primerler ile moleküler çalışmalar ve dizi analizleri yapılmıştır. Çalışma sonunda, Türk ZYMV'nin CI proteini 888 nükleotid uzunluğunda ve 296 amino asit (aa) olan N uç kısmı çoğaltılmıştır. CI bölgesinin nükleotid dizilerinin filogenetik analizi, izolatların (40) çoğunluğunun Avrupa ve dünyada en yaygın olan büyük bir moleküler alt grubuna (A1) ve üç izolatın (Y4, Y21, Y23) A5 alt grubuna ait olduğunu göstermiştir. Ayrıca, kılıf proteini nükleotid analizine göre, bu üç izolat (Y4, Y21, Y23), Avrupa'da son zamanlarda ortaya çıkan grup olan A4 moleküler alt grubu ile gruplanmıştır. CI ZYMV nükleotid bağlama motifi (NTBM) ve RNA helikaz aktivite bölgesinin (beş motif) aa analizine göre izolatlar arasında korunduğu görülmüştür.

**Anahtar Kelimeler:** Filogenetik analiz, Sekans analizi, Kabakgiller,

#### 1. Introduction

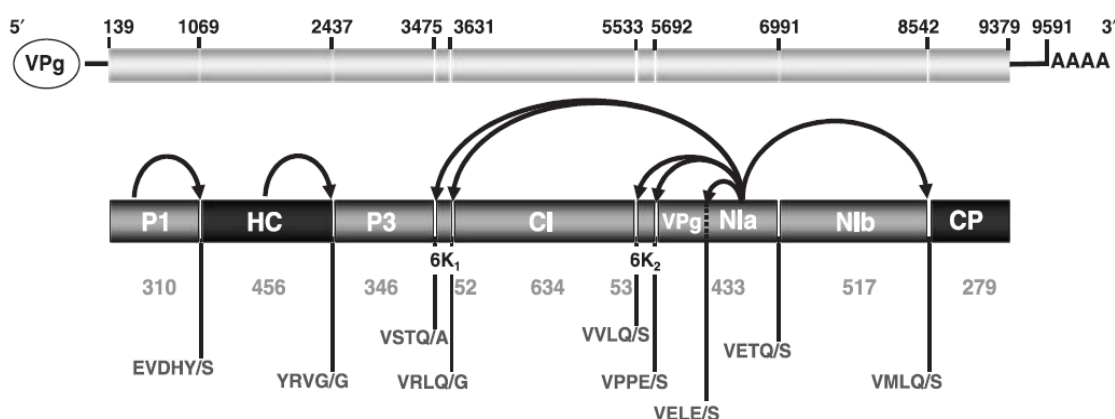
*Zucchini yellow mosaic virus* (ZYMV) causing intense mosaic symptoms and abnormalities in infected in cucurbit crops, causes significant economic losses and is included in the Potyviridae family, the Potyvirus genus (Choi et al., 2007; Desbiez & Lecoq, 1997; Hull, 2002). The genus *Potyvirus* is the largest genus of plant viruses. There are more than 200 species and more uncertain species in this group. Viruses belonging to this genus cause great economic losses by decreasing the quality and quantity of cucurbit products (melon, watermelon, cucumber, squash). The symptoms caused by ZYMV in plants are usually yellow mosaic on the leaves, severe deformation, swelling, significant shrinkage of the leaf blades, and severe stunting. In

fruits, it causes swellings such as tubers and causes deformities on them. Infected melon and watermelon fruits also develop misshapen and longitudinal deep cracks. Seed formation is significantly reduced in infected plants and seeds are often deformed. In the tropic regions, ZYMV is often found as a complex with PRSV-W and WMV. Like other strains of Potyviruses, ZYMV is transmitted non-persistently by aphid species such as *Aphis gossypii* and *Myzus persicae* (Desbiez & Lecoq, 1997).

ZYMV particles are a 750 nm long, filamentous polyprotein consisting of a positive-sense RNA molecule (Hull, 2002; Lisa & Lecoq, 1984). At the 5th end of the genome, there is the genome linked protein (VPg) and at the 3rd end there is the polyA tail. The

virus genome encodes 10 functional proteins (Glasa & Pittnerova, 2006; Revers et al., 1999, Urcuqui-Inchima et al., 2001). These are P1 protein, which acts as a protease in opening the genome, auxiliary component protein (helper component protein, HC-Pro), which plays a role in systemic transport of the virus by insects and suppresses gene silencing, cylindrical inclusion protein (CI) that plays a role in cell-to-cell transport and has RNA helicase activity; nuclear inclusion b protein (NIb), which plays a role in genome replication, and cover protein (CP), which plays a role in the systemic

transport of the virus from cell to cell. CI plays a role in different stages of viral infection. It has been reported that the CI protein plays a role in virus replication, cell-to-cell, and long-distance transport through interactions with the viral P3N-PIPO protein. CI protein acts as an avirulence factor in gene-for-gene interactions with dominant-resistance host genes and as a recessive-resistance overcoming factor (Sorel et al., 2014). Many viral and plant factors in plant cells have been shown to interact with this protein (Figure 1).



**Figure 1:** Schematic representation of the ZYMV-RNA genome and processing of proteins (Gal-On, 2007)

**Şekil 1:** ZYMV-RNA genomunun şematik gösterimi ve proteinlerin işlenmesi

In recent years, many studies on the biological and molecular variability of ZYMV have been published in the World (Coutts et al., 2011; Glasa et al., 2007; Maina et al., 2017; Novakova et al., 2014; Yakoubi et al., 2008). Most molecular studies have been based on the analysis of CP and/or partial NIb-CP sequences. It has also been reported that in the absence of the complete genomic sequence, the cylindrical inclusion (CI) coding region can be used for diagnostic and taxonomic purposes (Adams et al., 2005; Ha et al., 2008; Lee et al., 1997).

The presence of ZYMV in various studies has been reported in Turkey such as in Samsun (Şevik & Arlı-Sökmen, 2003), İzmir, Aydın, Manisa ve Balıkesir (Kaya & Erkan, 2011), Adana ve Mersin (Kamberoğlu et al., 2016), Kıbrıs (Helvacı et al., 2019, Nacar et al., 2021), Aksaray (Yeşil, 2019a), Yozgat (Yeşil, 2019b), Nevşehir (Yeşil 2020), Hatay (Sertkaya et al., 2004). In Turkey, there are molecular studies about CP region of ZYMV, but no information about the CI region (Özer et al., 2012; Topkaya et al., 2019; Topkaya, 2020; Yeşil & Ertunc, 2012; Yeşil, 2014). In this recent research, it is aimed the molecular characterization of CI region of ZYMV isolates collected from different provinces of

Turkey and to investigate the possibilities of use in phylogenetic classification.

## 2. Material method

### 2.1. RNA isolation

In this study, total RNA isolation studies from samples (Tablo 1) collected from Konya, Karaman, Aksaray, Burdur, Ankara, and Antalya provinces were performed by Astruc et al. (1996) according to the method proposed.

RT-PCR was performed using RNAs obtained after total RNA isolation from samples taken from different provinces and different cucurbit plants. In the two-step RT-PCR process, firstly, cDNA was obtained from RNAs obtained from RNA isolation by using random hexamer primer and MMLV-RT enzyme. In the second step, these cDNAs were used as templates and PCR was carried out with primers synthesized specifically for CI protein regions.

### 2.2. cDNA synthesis and RT-PCR methods.

Using 2 µl of total RNA for reverse transcription (RT), complementary DNA (cDNA) synthesis was performed in a 20 µl volume containing 4 µl 5X MMLV buffer (5X), 0,2 mM dNTP (25 mM), 1 µl random



hexamer primer (10 µmol), 0,25 µl RNase inhibitor µl distill water for 25°C for 10 min, followed by 42°C (10u/µl), 1 µl Reverse transcriptase (200u/µl) and 11.65 for one hour and 72°C for 10 min.

**Table 1:** The isolates used in the study, location, collected year, and symptoms

**Çizelge 1:** Çalışmada kullanılan izolatlar, toplanma yeri, toplanma yılı ve semptomlar

Province	District	Collected	Isolate	Host	Symptoms	
Ankara	Şereflikoçhisar	2011	Ş1	Melon	D	
			Ş3	Melon	D	
			Ş5	Melon	D	
			Ş8	Pumpkin	D	
			G1(m)	Squash	Mo	
	Gölbaşı	2011	G2	Squash	D, Mo	
			G3	Squash	D	
			Kz1	Squash	M,D	
	Kazan	2010	Ahm1	Squash	M, D	
			Ayaş	2013	AYS7	Squash
	Çubuk	2011	C5		Pumpkin	M, C
			C11	Pumpkin	D	
			C13	Pumpkin	D	
			C17	Melon	D	
			Be5	Squash	M	
			Be6	Squash	M	
			Be13 (m)	Squash	C	
	Beypazarı	2014	Be15	Squash	C;	
			Be18	Squash	M, Mo	
			Be22 (m)	Squash	Mo	
Kumluca			2012	K3 (m)	Squash	D
				K13	Squash	C, M, V
	K17	Squash		C, M, V		
	AS1	Squash		C, M, V		
	AS5	Squash		V		
Aksu	2012	AS6	Squash	V		
		AS8 (m)	Squash	D		
		AS11	Squash	C, M, V		
		Demre	2013	Demre	Cucumber	D
				2014	D14 (m)	Cucumber
Elmalı	2013	Y4	Melon	M		
		Y21(m)	Squash	Mo		
		Y23(m)	Squash	Mo		
Burdur	2014	E-7	Melon	D		
		Brd1	Pumpkin	M, D		
		Brd2	Pumpkin	M, D		
		Brd4	Pumpkin	M, D		
*Aksaray	Merkez	2010	AKS 2/5	Pumpkin	M,	
	Ortaköy	2009	AK 5/7	Pumpkin	M,D	
	Ortaköy	2009	AK 6/2	Pumpkin	M,	
*Karaman	Kazımkarabekir	2010	KAR15/1	Pumpkin	M	
			KAR12/4	Pumpkin	M,D	
*Konya	Merkez	2010	A 3/1	Pumpkin	M	
	Ereğli	2009	ER 2/8	Cucumber	M	
	Yunak	2010	YUN8/4	Pumpkin	M,D	
	Ereğli	2009	ER 6/8	Pumpkin	M,D	

M: Mosaic, D: Deformation, Mo: Mottling, V: vein banding, C: Clorosis

\*Provided by Dr. Serkan Yeşil

### 2.3. Phylogenetic analysis

Protein regions of 43 plants from different provinces and districts that gave positive results at the end of RT-PCR were studied. The PCR products with positive bands at the end of the RT-PCR process were sent for DNA sequence analysis by Sanger method. The data

obtained at the end of the sequencing were cleaned using the Chromas program and saved as a single file. Sequencing studies were carried out bidirectionally, and the obtained forward and reverse sequences were combined using the "CAP3 Sequence Assembly" computer program and consensus sequences were

obtained. Based on the results obtained, the amplified parts of the isolates were compared with the isolates previously reported in the NCBI gene bank, and similarity rates were determined. For this purpose, the data were sequenced using the MEGAX (Kumar et al., 2018) program and a phylogenetic tree was created. Phylogenetic analysis was obtained using the "Neighbors joining tree" analysis method, 1000 bootstrap and Kimura 2 parameters.

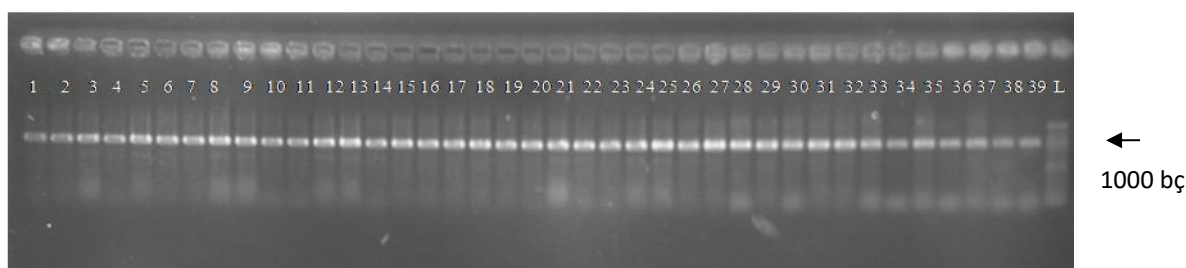
### 3. Results and Discussion

In the study, samples were taken collected from cucurbit plants showing virus symptoms from Ankara, Antalya, and surrounding provinces (Konya, Karaman, Aksaray, Burdur) where cucurbit cultivation is intensively performed. After the diagnosis of ZYMV

positive isolates with serological tests (Topkaya et al., 2019), sequence analysis of the cylindrical inclusion protein gene regions encoded by the positive ZYMV isolates was performed, and phylogenetic analysis was performed by comparing the protein gene regions of the ZYMV isolates available in the literature.

#### 3.1. RT-PCR results

In the amplification of the CI protein gene region of the ZYMV isolates used in the study, ZY-debCI-5' and ZY-milCI-3' primers, which amplify the partial region (N-terminal part) of the CI region (approximately 1000 bp), were used and at the end of the RT-PCR process, approximately 1000 bp bands were obtained in 1% agarose gel (Figure 2).



**Figure 2:** RT-PCR results of CI region

**Şekil 2:** CI bölgesinin RT-PCR sonuçları

L:100 bp ladder (NEB), K: Negatif kontrol, 1: C3, 2:C5, 3:C11, 4:C13, 5:C17, 6:C24, 7:BE5, 8:BE6, 9:BE7, 10:BE10, 11:BE13, 12:BE15, 13:BE18, 14:BE22, 15:BE26, 16:BE27, 17:S1, 18:S3, 19:S5, 20:S8, 21:G1, 22:G2, 23:G3, 24:KZ1, 25:AHM1, 26:AYS7, 27:K3, 28:K17, 29:K13, 31:AS5, 32:AS6, 33:AS8, 34:AS11, 35:H1M, 36 Y4, 37: BRD1, 38: BRD2, 39: AK 5/7

#### 2.4. Phylogenetic analysis

Sequence informations of the ZYMV CI protein region from Turkish ZYMV isolates were successfully obtained and submitted to the GenBank, with accession numbers (KP828388, KP828423, KP828389, KP828390, KP828391, KP828414, KP828427, KP828421, KP828422, KP828393, KP828394, KP828392, KP828401, KP828402, KP828403, KP828395, KP828396, KP828397, KP828398, KP828399, KP828400, KP828404, KP828405, KP828406, KP828410, KP828407, KP828408, KP828409, KP828418, KP828411, KP828412, KP828415, KP828416, KP828417, KP828420, KP828419, KP828413, KP828425, KP828426, KP828424)

Analyses were performed using 43 nucleotide sequences obtained in this work and 12 obtained from

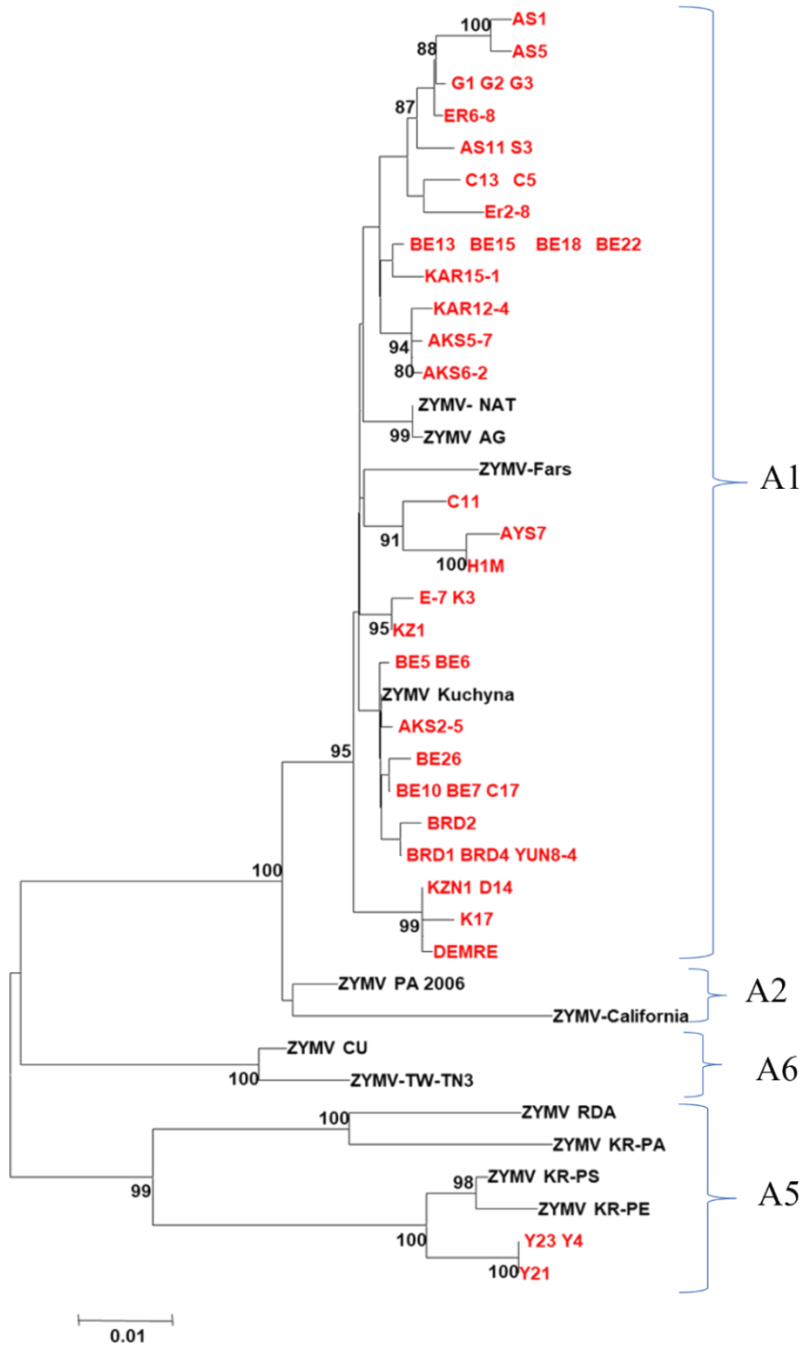
GenBank. The CI nucleotide sequences were translated to amino acids using MEGAX computer software programs. Phylogenetic trees were done by neighbor-joining (NJ) methods implemented in MEGAX (Kumar et al., 2018), with 1000 bootstrap replicates.

When we examine the phylogenetic tree obtained by the nucleotide sequence of the CI protein region, it is seen that most of the isolates we used in the study are in the A1 subgroup, which is common in the world and Europe. BE5, BE6, BE7, BE10, BRD1, BRD2, BRD4, C17, AKS2-5, BE26 and YUN8-4 isolates with Kuchyna, the Slovak isolate in the A1 group; C11, AYS7 and H1 isolates showed similar branching as ZYMV-Fars isolate. KZN1, D14, K17, DEMRE isolates were included in the A1 subgroup, but formed a different cluster from the reference and other isolates. AS1, AS5, G1, G2, G3, ER6-8, AS11, S3, C13, C5, ER2-8, KAR15-1, KAR12-4, AKS5-7, AKS6-2 isolates showed similar branching with ZYMV-AG and ZYMV-NAT isolate (Figure 3).

In the DAMBE program, isolates according to CI region, G1, 2, G3 isolates; BE13, BE15, BE18, BE22 isolates; BE5 and BE6 isolates; BE7, BE10, and C17 isolates; BRD1, BRD4 and YUN8-4 isolates; KZN1 and

D14 isolates showed similar base sequences among themselves. While Y23 and Y4 in the A5 subgroup showed similar base sequences, Y21 showed a different sequence from these two isolates (Figure 3). The genetic distance between Turkish ZYMV isolates and with

isolates from different parts of the world was 0.001-0.1 and genetic identity between groups was 90–99% (Table 2). Genetic identity indicated a very distant genetic relationship between the groups.



**Figure 3:** Phylogenetic tree of CI protein region

**Şekil 3.** CI protein bölgesinin filogenetik ağacı

The isolates obtained in this study are shown in red, and the isolates from the GenBank database are shown in black. Bootstrap values above 80% are shown.

**Table 2:** The genetic distance between Turkish ZYMV isolates and with isolates from different references.  
**Çizelge 2:** Türkiye ZYMV izolatları ile farklı referans izolatları arasındaki genetik uzaklık

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40			
1	DQ124239	ZYMV_Kuchyna																																									
2	EF062582	ZYMV-NAT	99																																								
3	EF062583	AG	99	10																																							
4	JN183062	ZYMV-Fars	99	98	98																																						
5	JQ716413	ZYMV_PA_2006	98	98	98	97																																					
6	L31350	ZYMV-FLORIDA	96	96	96	95	97																																				
7	AJ307036.2	ZYMV_CU	93	93	93	92	94	92																																			
8	AF127929.2	ZYMV-TW-TN3	93	92	92	92	93	91	99																																		
9	AB369279	ZYMV_RDA	90	91	91	90	91	88	92	91																																	
10	AY279000	ZYMV_KR-PS	91	91	91	90	91	89	92	91	92																																
11	AY278999	ZYMV_KR-PE	90	90	90	89	91	88	91	91	92	99																															
12	AY278998	ZYMV_KR-PA	90	90	90	89	91	88	91	91	96	92	92																														
13	AKS2-5		10	99	99	98	98	96	93	92	90	91	90	90																													
14	AKS5-7		99	99	99	98	98	95	93	92	90	91	90	90	99																												
15	AKS6-2		99	99	99	98	98	95	93	92	90	91	90	90	99	10																											
16	AS1		98	98	98	97	97	95	92	91	89	90	89	89	98	98																											
17	AS5		98	98	98	97	97	95	92	91	89	90	89	89	98	98	10																										
18	BE13_BE15_BE18_BE22		99	99	99	98	98	96	93	92	90	91	90	90	99	99	98	98																									
19	BE26		10	99	99	98	98	96	93	92	90	91	90	90	10	99	99	98	98																								
20	BE5_BE6		10	99	99	98	98	96	93	93	90	91	90	90	10	99	99	98	98	10																							
21	BRD2		10	99	99	98	98	96	93	92	90	91	90	90	99	99	99	98	98	99	99																						
22	C11		99	99	98	98	98	95	93	92	90	90	90	89	99	98	98	97	97	99	99	98																					
23	BE10_BE7_C17		10	99	99	98	98	96	93	93	91	91	90	90	10	99	99	98	98	99	10	10	99	99																			
24	C13_C5		99	98	98	98	95	93	92	90	90	90	90	90	99	99	99	98	98	99	99	98	99																				
25	KZNI_D14		99	99	99	98	98	95	93	93	90	90	90	90	99	99	99	98	98	99	99	99	99	98	99																		
26	AYS7		98	98	98	97	97	95	92	92	90	90	90	89	98	98	98	97	97	98	98	98	98	98	98	98																	
27	ER6-8		99	99	99	98	98	95	92	92	90	90	90	90	99	99	99	99	99	99	99	99	99	98	99	98																	
28	G1_G2_G3		99	99	98	98	98	95	92	92	90	90	90	90	99	99	99	99	99	99	99	99	99	99	98	99	98																
29	HIM		99	98	98	98	97	95	93	92	91	91	90	90	99	98	98	97	97	98	98	99	99	98	98	98	10																
30	K17		99	98	98	98	97	95	93	92	90	90	90	89	99	98	98	97	97	98	98	99	98	98	98	10	97																
31	E-7_K3		99	99	99	98	98	96	93	93	90	91	90	90	99	99	99	98	98	99	99	99	99	99	98	99	98	99															
32	KAR12-4		99	99	99	98	98	95	93	92	90	91	91	90	99	10	10	98	98	99	99	99	99	98	98	98	98	99															
33	KAR15-1		99	99	99	98	98	95	93	92	90	90	90	89	99	99	99	98	98	10	99	99	99	99	98	98	98	99															
34	KZI		99	99	99	98	98	96	93	93	91	91	90	90	99	99	99	98	98	99	99	99	99	99	98	98	98	99															
35	AS11_S3		99	99	99	98	98	95	92	92	90	90	90	90	99	99	99	99	99	99	99	99	99	98	98	98	97																
36	Y23_Y4		90	90	90	91	88	92	91	92	98	98	92	90	91	91	89	89	90	90	90	90	91	90	90	90	90	90															
37	BRDI_BRD4_YUN8-4		10	99	99	98	98	96	93	93	90	91	90	90	10	99	98	98	99	99	10	10	99	99	98	98	98	99															
38	DEMRE		99	99	98	98	98	95	93	93	90	90	90	90	99	98	98	97	97	99	99	99	98	98	98	10	98	98															
39	Y21		90	90	90	91	88	92	91	92	98	98	92	90	91	91	89	89	90	90	90	90	91	90	90	90	90	90															
40	Er2-8		98	98	98	97	97	94	93	92	90	90	90	90	98	98	98	98	99	98	98	98	98	98	98	97	99																

**Table 3:** Protein sequence comparisons of the CI protein region with reference isolates  
**Çizelge 3:** CI protein bölgesinin referans izolatları ile protein sekans karşılaştırmaları

#ZYMV_Kuchyna	HYRTTGKFLF	FTRNTAAFVA	NEIASSEGE	FLVRGAVGSG	KSTSLPAHLA	KGGKVLLEP	TRPLAENVSR	QLAGDFFPH	VTLRMKGLSC	FGSSNITVMT	[100]
#ZYMV_NAT	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_AG	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_Fars	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_PA_2006	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV-California	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_CU	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV-TW-TN3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_RDA	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_KR-FS	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_KR-PE	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#ZYMV_KR-PA	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AKS2-5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AKS5-7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AKS6-2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AS1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AS5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BE13_BE15_BE18_BE22	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BE26	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BE5_BE6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BRD2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#C11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BE10_BE7_C17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#C13_C5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#KZNI_D14	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AYS7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BR6-8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#G1_G2_G3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#HIM	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#K17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#E-7_K3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#KAR12-4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#KAR15-1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#KZ1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#AS11_S3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#Y23_Y4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#BRD1_BRD4_YUN8-4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#DEMRE	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#Y21	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]
#Er2-6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[100]

RNA helicase region

NTP binding motif



**Table 3:** Protein sequence comparisons of the CI protein region with reference isolates (Table 3. cont.)  
**Çizelge 3:** CI protein bölgesinin referans izolatları ile protein sekans karşılaştırmaları (Tablo 3 devamı)

#ZMYV_Kuchyna	KVSAIIPGRE CDEDTQFAVK VKTEDHLSFQ AFVGAQKTS NADMQHGNN IDIYVASINE VMLSKLLTE RQFSYTKVDG RTMQLGKTTI ETHGTSQKPH FVATNLIEN GVTLDVECVV DFGLKVAEL DSENRVRYN KKSYSI	[296]
#ZMYV_NAT	.....	[296]
#ZMYV_AG	.....	[296]
#ZMYV-Fars	.....	[296]
#ZMYV_FA_2006	.....H.....	[296]
#ZMYV-California	.....H.....	[296]
#ZMYV_CU	.....H.....	[296]
#ZMYV-TW-TN3	.....Y.....	[296]
#ZMYV_RDA	.....R.....	[296]
#ZMYV_RR-PS	R.....	[296]
#ZMYV_RR-PE	.....G.....	[296]
#ZMYV_RR-PA	.....R.....	[296]
#AKS2-5	.....	[296]
#AKS5-7	.....	[296]
#AKS6-2	.....	[296]
#AS1	.....I.....	[296]
#AS5	.....	[296]
#BE13_BE15_BE18_BE22	.....	[296]
#BE26	.....	[296]
#BE5_BE6	.....	[296]
#BRD2	.....	[296]
#C11	.....	[296]
#BE10_BE7_C17	.....	[296]
#C13_C5	.....	[296]
#KZNI_D14	.....	[296]
#AYS7	.....I.....	[296]
#ER6-8	.....	[296]
#G1_G2_G3	.....	[296]
#HLM	.....	[296]
#KL7	.....S.....	[296]
#E-7_K3	.....	[296]
#KAR12-4	.....	[296]
#KAR15-1	.....	[296]
#KZ1	.....	[296]
#AS11_S3	.....	[296]
#Y23_Y4	.....H.....	[296]
#BRDI_BRD4_YUN8-4	.....	[296]
#DEMRE	.....	[296]
#Y21	.....?.....	[296]
#ET2-8	.....V.....	[296]

RNA helicase regions

The protein sequence generated using the nucleotide sequence of the CI protein region and the protein comparison with reference isolates were made using the MEGA6 computer program. The results obtained are shown in Table 1. The 296 amino acid partial protein sequence consisting of 888 nucleotides of the CI protein region was compared with reference isolates selected from available sequences in the NCBI gene bank. When the amino acid regions were evaluated at the end of the comparisons, it was found that the CI protein region was not very variable and the previously reported NTP binding motif “GAVGSGKST” (26-35. aa) and RNA helicase function “VLLLEPTRPL”, “KVSAT”, “LVYV” and “VATNIIENGVTL” motifs appear to be preserved (Table 3).

According to different researchers, the ZYMV CP region is divided into three main groups (Desbiez et al., 2002; Ha et al., 2008; Zhao et al., 2003). Firstly, ZYMV isolate were classified into two main groups based on the analyses of partial nt sequences of CP gene by Desbiez et al. (2002). Later Zhao et al. (2003) and Ha et al. (2008) reported three groups: I, worldwide; II, containing isolates only from Asia; and III, containing isolates only from China.

In previous studies, Lee et al. (1997) compared the CI protein gene regions of 14 ZYMV Singapore isolates and found five more conserved regions in addition to the sequence of the nucleotide binding (GAVGSGKST) motive, which is seen as the membrane binding component of RNA helicase complexes. In addition, as a result of the phylogenetic tree they made with the sequences of the CI and CP gene regions, similar branches were found, and they showed that the phylogenetic relationship between Potyviruses could be determined using the CI gene region. Researchers have suggested that the CI gene region can be used as an alternative approach in the evolution studies of Potyviruses.

The sequences of the (CP) and (CI) proteins of ZYMV isolates from Austria, Germany, Italy and Slovenia have been reported. As a result of DNA sequence comparison of 30 ZYMV isolates from different geographical regions around the world, it was seen that the Austrian isolates showed a high level of similarity with the Slovenian and Hungarian isolates. The isolates from Germany and Italy turned out to be distantly related and clustered with isolates from other parts of the world. The results of the study showed that a specific isolate can spread rapidly to geographically adjacent areas, but may not be related to isolates found

in other neighboring countries (Pfosser & Baumann, 2002)

#### 4. Conclusion

This is the first report of CI protein sequence information of the ZYMV in Turkey. Analysis with the CI region yielded results similar to the results obtained from the CP region and showed that the CI region could be used to classify ZYMV isolates. This study helps us to further understand the genetic diversity of ZYMV isolates infecting cucurbit plants collected from different provinces of Turkey.

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## Total phenolics, total flavonoids and antioxidant activity of *Moringa oleifera* grown in different locations of Giresun-Türkiye

Burhan ÖZTÜRK<sup>1</sup>, Malik Aarsal KÖSE<sup>1</sup>, Umut ATEŞ<sup>1</sup>, Tarık YARILGAÇ<sup>1</sup>

<sup>a</sup> Ordu University, Faculty of Agriculture, Department of Horticulture, Ordu-Türkiye  
\*Corresponding author's email: [umutates.es@gmail.com](mailto:umutates.es@gmail.com)

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**Abstract:** In this study, nitrogen (N), protein, total phenolics, total flavonoids and antioxidant activity were investigated in dried samples of the *Moringa oleifera* plant grown in Bulancak, Çamoluk, Espiye, Şebinkarahisar and Tirebolu districts of Giresun province in Türkiye. The analyses were performed by DPPH and FRAP methods for antioxidant activity. In the study, the plants grown in Bulancak location had significantly higher nitrogen, protein and antioxidant activity than other growing regions. On the contrary, significantly lower nitrogen and protein were measured in plants grown in Espiye and Tirebolu. The total phenolics and total flavonoids were determined the highest in plants grown in Espiye and Tirebolu locations, the lowest values in Çamoluk and Şebinkarahisar locations. According to the antioxidant activity tests, the antioxidant activity of the plants grown in Espiye location was significantly higher than in other growing regions. The lowest antioxidant activity was measured in Şebinkarahisar and Çamoluk locations. As a result, it was revealed that region conducted the cultivation affects on the protein and bioactive compounds of the *Moringa oleifera* plant. Espiye, Bulancak and Tirebolu locations can be recommended for cultivation.

**Keywords:** Antioxidant, DPPH, flavonoids, *Moringa oleifera*, phenolics, protein

### Giresun-Türkiye'nin farklı lokasyonlarında yetiştirilen *Moringa oleifera*'nin toplam fenolikler, toplam flavonoidler ve antioksidan aktiviteleri

**Öz:** Bu çalışmada Giresun iline bağlı Bulancak, Çamoluk, Espiye, Şebinkarahisar ve Tirebolu ilçelerinde yetiştirilen *Moringa oleifera* bitkisinin kurutulmuş örneklerinde azot (N), protein, toplam fenol, toplam flavonoid ve antioksidan aktivitesi incelenmiştir. Antioksidan aktivitesi için DPPH ve FRAP testlerine göre analizler yapılmıştır. Araştırmada, Bulancak ilçesinde yetiştiriciliği yapılan bitkilerin, diğer yetiştiricilik bölgelerine göre önemli derecede daha yüksek azot, protein ve antioksidan aktivitesine sahip olduğu tespit edilmiştir. Espiye ve Tirebolu'da yetişen bitkilerde önemli derecede daha düşük azot ve protein ölçülmüştür. Toplam fenol ve toplam flavonoid bakımından, Espiye ve Tirebolu'da yetişen bitkilerde en yüksek; Çamoluk ve Şebinkarahisar'da yetişen bitkilerde en düşük değerler belirlenmiştir. Antioksidan aktivite testlerine göre, Espiye'de yetişen bitkilerin antioksidan aktivitesinin diğer yetiştiricilik bölgelerine kıyasla önemli derecede daha yüksek olduğu saptanmıştır. Aksine en düşük antioksidan aktivitesi, Şebinkarahisar ve Çamoluk lokasyonlarında ölçülmüştür. Sonuç olarak, yetiştiricilik yapılan bölgenin *Moringa oleifera* bitkisinin protein ve biyoaktif bileşikleri üzerine etkisinin olduğu açığa çıkarılmıştır. Yetiştiricilik için Espiye, Bulancak ve Tirebolu lokasyonları tavsiye edilebilir.

**Anahtar Kelimeler:** Antioksidan, DPPH, fenol, flavonoid, *Moringa oleifera*, protein

### 1. Introduction

In the rapidly developing world, people's changing living conditions and eating habits and the increasing interest in healthy nutrition constantly increase the interest in products with different tastes, consumption possibilities, and rich nutritional content. One of these products is *Moringa oleifera*. *Moringa oleifera* is the most well-known and cultivated species of the *Moringa* genus in the world. *Moringa oleifera*, whose homeland is the north of India, is grown in countries such as Indonesia, Sri Lanka, Malaysia, Philippines, Mexico, South and Central America, Africa and Middle East. Although it grows well in tropical and subtropical climates, especially in sandy soils, its lack of soil selectivity, formation of pile roots, high adaptability to

cold and high drought tolerance has caused *Moringa oleifera* to be cultivated and spread in more expansive areas around the world in recent years (Anwar et al., 2007). The wide spread of the *Moringa oleifera* plant has caused it to gain different local names in many regions. These are brief; Mulangay, Drumstick tree, Mlonge, Kelor, Horseradish tree, Marango, Benzolive, Saijihan and Sajna (Fahey, 2005).

*Moringa oleifera* is described as a miracle tree, due to its rich content of bioactive compounds, mineral and protein content. Especially the leaves contain high levels of vitamin C, vitamin B<sub>6</sub>, provitamin A, vitamin E, beta carotene, protein, potassium, phosphorus, sodium, sulfur, zinc, copper, iron, amino acids, calcium and magnesium. These make a significant contribution

to antioxidant capacity (Bharali et al., 2003; Anwar et al., 2007; Lea, 2010; Moyo et al., 2011). Due to the rich nutritional content of its seeds, roots and leaves, its use as a raw material in the pharmaceutical, food, dye and animal feeding industries has become widespread. Its seeds are used in oil, perfume and hair care, and its wood is used in lumbering. The leaves are used in daily nutrition, salads and animal nutrition. In addition, it is consumed in daily diets because it has a high nutritional content and gives a feeling of satiety when consumed. Also, its leaves are dried and used to increase the shelf life of foods due to its anti-bacterial properties and being a source of disinfectants and natural antioxidants. It is also reported to be used as a dietary product because it gives a feeling of satiety (Nellis, 1996; Siddhuraju & Becker, 2003; Fahey, 2005; Anjorin et al., 2010; Khalafalla et al. 2010; Mishra et al. 2011).

This study aimed to determine the protein, total phenolics, total flavonoids and antioxidant capacity of *Moringa oleifera* grown in different districts (Bulancak, Camoluk, Espiye, Şebinkarahisar and Tirebolu) of Giresun province of Türkiye.

## 2. Materials and Methods

### 2.1.Plant materials

*Moringa oleifera* (cv. PKM-1), which constitutes the plant material of the research, was grown from seed. When the plants reached 10-15 cm in size, they were planted by hand in the first week of June 2019, with 1.0 m row spacing and 0.8 cm row spacing in different districts of the Giresun province of Turkey (Bulancak, Camoluk, Espiye, Şebinkarahisar and Tirebolu). 90 days after planting, which they were about 120 cm high, the stem and branches of the plant were cut by hand from the plant, then stems and leaves were separated with hand, and sun-dried at 30 °C for 3-5 days. Only leaves were used in the analyses. The samples obtained from each cultivation region were ground into powder by milling using a grinder (Waring, UK).

**Table 1.** Climate data of study areas

**Çizelge 1.** Çalışma alanlarının iklim verileri

Climate data	Bulancak	Çamoluk	Espiye	Şebinkarahisar	Tirebolu
Mean. temperature (°C)	24.9	21.4	24.3	24.8	24.2
Max. temperature (°C)	26.0	26.7	25.4	26.3	26.0
Min. temperature (°C)	18.5	12.4	19.1	12.0	18.7
Rainfall (mm)	13.8	8.3	18.4	3.6	20.6
Relative humidity (%)	73.0	54.0	78.5	55.2	79.0

### 2.2.Climate date

Climate data were presented in Table 1.

### 2.3.Nitrogen and protein content

Nitrogen was detected according to the Kjeldahl distillation method (Bremner, 1965). The protein content of *Moringa oleifera* was calculated from the nitrogen content (N=6.25) as determined by the Kjeldahl method.

### 2.4. Total phenolics, total flavonoids, antioxidant activity

A suspension of 5% *Moringa oleifera* leaves was prepared in pure water and stirred at 45°C for 8 hours. The samples were then filtered on Whatman filter paper 1. The filtrates were used to determine total phenolics, total flavonoids, and antioxidant activity (Ozturk et al., 2019).

Spectrophotometric measurements for total phenolics, total flavonoids and antioxidant activity were performed at the UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolics were determined following the method described by Singleton and Rossi (1965) and were expressed as g GAE (gallic acid equivalent) kg<sup>-1</sup> drying weight (dw). Total flavonoids were measured according to the method described by Chang et al. (2002) and were expressed as g QE (quercetin equivalent) kg<sup>-1</sup> dw. The antioxidant activity of *Moringa oleifera* was determined according to two different procedures of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Aglar et al., 2017) and Ferric Ions (Fe<sup>+3</sup>) Reducing Antioxidant Power (FRAP) (Benzie & Strain, 1996), and the results were expressed in mmol Trolox equivalent (TE) kg<sup>-1</sup> dw.

### 2.5. Statistical analysis

Whether the data was typically distributed was checked by Kolmogorov-Smirnov Test. Levene's test confirmed homogeneity control of the group variances. After the variance analysis of the data, Tukey's multiple-comparison test was used to check whether there were significant differences between treatments. The statistical analyses were performed by using SAS software (SAS 9.1 version, USA).

## 3. Results and Discussion

### 3.1. Nitrogen and protein

The nitrogen content was between 1.53% and 2.80%; the protein content varied between 9.54% and 17.50%. The nitrogen and protein contents of samples among locations were significant differences. The nitrogen and protein content of samples in Bulancak location were

significantly higher than other locations. Again, significantly higher nitrogen and protein were determined in plants grown in Şebinkarahisar compared to Espiye and Tirebolu. Whereas, similar levels of nitrogen and protein were measured from Çamoluk, Espiye and Tirebolu locations. The protein and nitrogen levels were changed from 9.54% to 16.04% and from 1.53% to 2.57%, respectively. Also, Tirebolu locations samples for nitrogen (1.53%) and protein (9.54%) were the lowest among all locations (Table 2).

Proteins are organic compounds formed by the combination of many amino acids and provide the necessary energy for the body to repair, strengthen and regenerate tissues damaged in human health, regulate metabolism, strengthen the immune system (Çetiner & Bilek, 2018). Therefore, consumers' interest in foods with high protein content is increasing day by day. In our study, it was observed that the nitrogen and, accordingly, the protein content varied according to the locations.

**Table 2.** Protein and nitrogen content of *Moringa oleifera* grown in different locations

**Çizelge 2.** Farklı lokasyonlarda yetiştirilen *Moringa oleifera*'nin protein ve azot içeriği

Locations	N (%)	Protein (%)
Bulancak	2.80 a	17.50 a
Çamoluk	2.57 bc	16.04 bc
Espiye	2.51 c	15.67 c
Şebinkarahisar	2.67 b	16.69 b
Tirebolu	1.53 c	9.54 c

Means in columns with the same letter do not differ according to Tukey's test at  $P < 0.05$ .

It was determined that the plants samples Bulancak location had a higher content compared to other growing regions. As a matter of fact, it has been reported in studies that genetic and climatic factors (temperature, lighting, etc.) and cultural practices such as irrigation and fertilization may affect protein content (Makinde, 2013; Sarwar et al., 2018; Sarwar et al., 2020). According to Gidamis et al. (2003) the protein content of *Moringa oleifera* leaves grown in Tanzania was 33.12%; Moyo et al. (2011) 30.30% in South Africa; Nouala (2006) West Africa 23.27%; Sanchez et al. (2006) found it to be 26.7% in Nicaragua. It has been observed that the reported values are higher than our findings. This difference is because the climates where the studies are carried out are within the natural cultivation areas of *Moringa oleifera*; The reason may be that our locations have a lower temperature compared to the climate in which the research was conducted.

### 3.2. Total phenolics, total flavonoids, antioxidant activity

In terms of bioactive compounds, significant differences were seen between locations. Total phenolics were between 68.23 and 92.30 g GAE kg<sup>-1</sup>

dw; total flavonoids content ranged from 37.75 to 70.29 g QE kg<sup>-1</sup> dw. Similar levels of total phenolics and total flavonoids were measured in Espiye and Tirebolu locations, significantly higher than in other locations. However, samples of Çamoluk and Şebinkarahisar locations had similar levels of lower total phenolics and total flavonoids than samples of Espiye, Tirebolu and Bulancak locations (Table 3).

*Moringa oleifera* contains high amounts of polyphenols, flavonoids, ascorbic acid and phenolic acids. Due to its rich nutritional content, it has been used as a therapeutic in traditional medicine from past to present (Ma et al., 2020). However, it has been reported that this rich content may differ according to geographical location and environmental conditions (Panwar & Mathur, 2020). Indeed, in a study conducted by Saini et al. (2014), *Moringa oleifera* plants grown in Pakistan contain higher bioactive compounds than those grown in India, Thailand, Nicaragua, and the USA. The total phenolic content of *Moringa oleifera* leaves grown in Mexico was between 241.3 and 468.4 µg GAE/mL; it has been reported that the total flavonoids content is between 107.9 and 316.3 µL RE/mL (Coz-Bolanos et al., 2018).

**Table 3.** Total phenolics, total flavonoids and antioxidant activities of *Moringa oleifera* grown in different locations

**Çizelge 3.** Farklı lokasyonlarda yetiştirilen *Moringa oleifera*'nin toplam fenolikler, toplam flavonoidler ve antioksidan aktiviteleri

Locations	Phytochemical characteristics			
	Total phenolics (g GAE kg <sup>-1</sup> dw)	Total flavonoids (g QE kg <sup>-1</sup> dw)	FRAP (mmol TE kg <sup>-1</sup> dw)	DPPH (mmol TE kg <sup>-1</sup> dw)
Bulancak	77.78 b	63.22 b	40.83 b	21.60 a
Çamoluk	71.51 c	41.68 c	31.15 c	13.62 c
Espiye	92.30 a	70.29 a	47.40 a	23.78 a
Şebinkarahisar	68.23 c	37.75 c	26.41 d	12.20 c
Tirebolu	89.32 a	67.35 a	37.52 b	16.78 b

Means in columns with the same letter do not differ according to Tukey's test at  $P < 0.05$ .

Gómez-Martínez et al. (2020) in 3 different regions in Spain, and Siddhuraju and Becker (2003) in their research in Nicaragua determined that the regions where cultivation is carried out have a significant effect on antioxidant activity. Similarly, in our study, differences in total phenolics, total flavonoids and antioxidant activity were detected between growing regions. It can be stated that this difference is caused by climatic differences such as light, temperature and humidity in the cultivation region. As a matter of fact, in our research, it was observed that low altitude growing regions (locations of Espiye, Bulancak and Tirebolu) had higher bioactive compounds compared to high altitude regions such as Çamoluk and Şebinkarahisar locations.

#### 4. Conclusion

As a result, it has been determined that the *Moringa oleifera* plants grown in different districts of the Giresun province of Türkiye have differences in protein, total phenolics, total flavonoids and antioxidant activity, and plants grown in the Black Sea coastal climate with relatively subtropical climate conditions at low altitude contain higher bioactive compounds. Espiye, Bulancak and Tirebolu locations can be recommended for cultivation. There is a need for more detailed studies on adaptation, yield and nutrient content in different climate conditions of Türkiye.

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## Determination of Estrus in Cattle with Artificial Neural Networks Using Mobility and Environmental Data

Adil Koray YILDIZ<sup>1\*</sup>, Mehmet Metin ÖZGÜVEN<sup>2</sup>

<sup>1</sup>Yozgat Bozok University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, Yozgat

<sup>2</sup>Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Biosystems Engineering, Tokat

\*Corresponding author's email: [adilkorayyildiz@gmail.com](mailto:adilkorayyildiz@gmail.com)

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**Abstract:** Detection of estrus with high accuracy directly affects the possibility of cows becoming pregnant and so also milk production. Most milk is obtained in the early lactation period, after calving. Animals in estrus are more active than others. This mobility can be measured by a testing device called "pedometer." Estrus can be estimated using detected movement changes with artificial neural networks (ANN) models. This study aims to create and assess the effectiveness of a neural network model to estimate estrus in cattle by using movement and environmental data. Movement data of 78 cattle, which showed 184 estruses have been captured along with climatic data during a seven-month period at a private agricultural organization. Data such as cow age, lactation number and number of days elapsed from estrus were also taken into account and evaluated. ANN models were compared with accuracy, precision and F-scores. Two-layer classification networks were tested for feed-forward neural network model. Optimal inputs to the neural network model were found to be motion data, motion data of the previous period, the number of days after the previous estrus, temperature and humidity. Two-layer network with 37 for the first layer and 40 neurons in the second layer has been the most successful model with a 0.1775 F - score. The study has shown that the accuracy of estrus prediction is increased by evaluating movement data along with climate data.

**Keywords:** Dairy cattle, estrus detection, artificial neural networks

### Hareketlilik ve Çevre Verileri Kullanılarak Yapay Sinir Ağları ile Sığırlarda Kızgınlık Tespiti

**Öz:** Kızgınlığın yüksek doğrulukla tespiti, ineklerin gebe kalma olasılığını ve dolayısıyla süt üretimini doğrudan etkiler. Sütün çoğu, doğumdan sonra erken laktasyon döneminde elde edilir. Kızgınlık dönemindeki hayvanlar diğerlerinden daha aktiftir. Bu hareketlilik, "pedometre" adı verilen bir test cihazı ile ölçülebilir. Yapay sinir ağları (YSA) modelleri ile tespit edilen hareket değişiklikleri kullanılarak kızgınlık tahmin edilebilir. Bu çalışma, hareket ve çevresel verileri kullanarak sığırlarda kızgınlığı tahmin etmek için bir sinir ağı modelinin etkinliğini oluşturmayı ve değerlendirmeyi amaçlamaktadır. Özel bir tarım kuruluşunda yedi aylık dönemde 184 kızgınlık gösteren 78 büyükbaş hayvanın hareket verisi ve çalışma dönemindeki iklim verisi elde edilmiştir. İnek yaşı, laktasyon sayısı ve kızgınlıktan sonra geçen gün sayısı gibi veriler de dikkate alınmış ve değerlendirilmiştir. YSA modelleri doğruluk, kesinlik ve F-skorumla karşılaştırılmıştır. İki katmanlı sınıflandırma ağları, ileri beslemeli sinir ağı modeli için test edilmiştir. Sinir ağı modeline en uygun girdilerin hareket verileri, önceki döneme ait hareket verileri, bir önceki kızgınlıktan sonraki gün sayısı, sıcaklık ve nem olduğu anlaşılmıştır. Birinci katmanda 37 ve ikinci katmanda 40 nöron bulunan iki katmanlı ağ, 0,1775 F-skoru ile en başarılı model olmuştur. Çalışma, iklim verileriyle birlikte hareket verilerinin değerlendirilerek kızgınlık tahmininin doğruluğunun arttığını göstermiştir.

**Anahtar Kelimeler:** Süt Sığır; Kızgınlık Tespiti, Yapay Sinir Ağları

#### 1. Introduction

The use of sensors, big data, artificial intelligence and machine learning is making a significant contribution to animal farmers reducing production costs, increasing productivity, improving animal welfare and raising more animals per hectare (Neethirajan, 2020). Estrus detection is one of the most important factors affecting the reproductive performance of dairy herds (Senger, 1994). Inadequate or inaccurate determination of estrus in time leads to delayed insemination, decreased pregnancy rate and prolongation of birth interval (Özgülven, 2018). For this reason, automatic systems have been developed for

high accuracy detection of estrus (Dulyala et al., 2014). Detection of estrus with high accuracy directly affects the possibility of cows becoming pregnant. The high rate of the breeding ability of cows primarily increases the production of calves. It also affects the milk production. Most milk is obtained in the early lactation period, after calving. Controlling reproduction allows for longer periods and higher milk production (Daniel, 2006). Additionally, as the number of heifers cannot be provided due to the increase in the calving interval, so the herd will age and gets smaller. The success of the selection will be adversely affected. Thus, the

determination of estrus should be accurate and non-estrus animals should not be perceived as in estrus.

The rate of detection of estrus is between 43% and 52% and this value can reach up to 80%. Factors such as air temperature, milk yield and some diseases affect the determination of estrus (Orman, 2011). In the summer, conception rates may be half that of colder months (Peralta et al., 2005). In some enterprises, 5%-30% of animals are inseminated even though they are not in estrus (Tömek, 2007). In their study, Shahriar et al. (2016), obtained 82-100% estrus accuracy in pasture areas. The estrus period is more difficult than other species in the cows due to the short and variable estrus period (Demirci, 2007). Many methods are used in the determination of estrus. Direct research by observing (inspection), determining by using the search bulls, placed instruments in the sacrum region, the measurement of electrical resistance of vaginal mucosa, determination of hormone (progesterone, estrogen) in blood and milk, rectal examination and determining by pedometer are methods for detecting estrus (Firk et al., 2002; Demirci, 2007). It is also possible to perform estrus detection with electronic devices such as video cameras, mounting detectors, temperature sensors and hormonal fragrance sensors (Williamson et al., 2006). The most common method of estrus detection is done by cow-keeper observation. Specialized personnel are needed in this method (Saribay & Erdem, 2008). Automatic detection of estrus by using mobility is also possible. In the literature, the results of estrus detection by sensors and cameras range from 51% to 86% (Roelofs et al. 2005). Animals in the estrus are more mobile than other animals. This mobility is measured by the step-counting devices called “pedometer” that are attached above the dew claws of the cows.

Today, artificial intelligence methods are an effective solution for complex and time-consuming problems. Artificial intelligence is a research area that examines the mental functions of people in decision-making processes by using computer models and formulates. Artificial intelligence methods can be used in order to determination of estrus (Mitchell et al., 1996; De Mol et al., 1999; Firk et al., 2002; Brunassi et al, 2010; Yin et al., 2013; Shahriar et al., 2016; Thanh et al, 2018). Artificial Neural Network (ANN), which is one of the effective and widespread methods of artificial intelligence, can be used in the classification of animal and herd behavior (Nadimi et al., 2012). It is thought that ANN models can determine the estrus status by detecting changes in animal behavior (Krieter

et al., 2005). But, although animals have general behavior determined, they can react differently in most cases (Hulsen, 2012). Shelter conditions, climatic conditions, and animal population density can affect mobility and consequently estrus prediction.

Walking activity and behavioral measures of estrus are affected by many individual and environmental factors such as lactation-related social interactions, housing, age, genetics and physiological aspects (Galina & Orihuela, 2007; Aungier et al., 2012). It is thought that accuracy will be increased by evaluating environmental conditions and animal data as input in the ANN model. Thus, this study establishes an ANN model that will determine the estrus period in cows by using motion and environmental data and to investigate its effectiveness. In addition to the movement information obtained using the pedometers, the data of the climate and animals were included in the study. Different input sets have been evaluated using ANN models in different topologies and thus it is aimed to determine the most suitable model and data types.

## 2. Materials and Methods

Observations made for the study were conducted in a commercial dairy cattle farm in Tokat province. Using the Dairy Plan (GEA Westfalia Surge) herd management software, all kinds of veterinary records such as birth and calving dates, vaccine and drug histories, as well as data on lactation numbers and milk yields were recorded. To monitor estrus, 78 healthy animals of Holstein cows, which were in the first three lactation periods were observed. The study was approved by the Animal Experiments Local Ethics Committee of Tokat Gaziosmanpaşa University (2012-HADYEK-028). Climatic data on barns and farm were recorded during the study period. To measure and record the climate data, iMetos 300 climatic measuring station (Metos, Hassfurt) was established in the open area in the middle of the barns. iMetos 100 climatic measuring stations (Metos, Hassfurt) were established inside the barns. Stations were used to measure humidity, temperature and dew point values. Measurements were made for every minute and recorded in the database using an internet connection. Then, hourly averages of the data from the database were obtained and recorded for the training of ANN models. The humidity of the air affects the sensation of temperature. Thus, to define the comfort and stress conditions, the Temperature Humidity Index (THI) was used to show the effect of humidity on temperature (Garcia, 2006). THI was calculated using Equation 1.

$$THI = s + (0.36)c + 41.2 \quad (1)$$

In equality,  $s$  is the hourly average dry thermometer temperature in °C, and  $c$  is the dew point. The wireless pedometer system (Robolab MOO, Konya) was used to track the movement of the cows. The system consists of pedometers attached to the wrists of cows, interconnection hubs and central server. Pedometers hourly send data to the interconnection hubs in the barns with wireless data transfer. The interconnection hubs collect the data from pedometers then transmit the central server, where the received data are stored in the database. The motion tracking system calculated the number of movements with the values read from the acceleration sensor in the pedometers and evaluated this number as motion data. Movement data were recorded hourly, such as climate data. In the dataset, the outputs were set to be [1,0] if the cow is in estrus and [0,1] if it is not.

All data were divided into three sets: 10% test, 10% validation and 80% training. While training the networks with the training set, performance was evaluated with the validation set for each epoch of training. “The validation error” is recorded if the performance value obtained from the validation set was worse than the previous iteration. If the training gives a certain number of validation errors, the training was terminated. So, the network was prevented over-learning. The success of the final network was evaluated using the test set that was not used in the training process. All inputs were normalized between -1 and +1 before being used in ANN training. Min-max method was used for normalization (Equation 2).

$$P_n = 2(P - P_{min}) \div (P_{max} - P_{min}) - 1 \quad (2)$$

In Equation 2,  $P$  is the actual input.  $P_{min}$  represent the smallest value and  $P_{max}$  represent the maximum value of the input variables. The result of the equation  $P_n$  means the normalized value.

The ANN model structure was selected as hierarchical feed forward multilayer network, which was reported to be successful (Nadimi et al., 2012). The output layers of the investigated ANN models were determined as two neurons to express the estrus condition.

### 2.1. Investigation of Input Types

To examine the effects of inputs on the model and determine the most appropriate inputs, input sets were arranged in different combinations of input types. For this, the input data were divided into three basic groups. The movement-related group has three motion

data for the last hour, one hour before and two hours before. The climate group has the average temperature, humidity and THI data calculated for the last hour. In the animal information group; The number of lactations, the age of the animal (in days) and the number of days after estrus was obtained. Using these input types, three basic input groups were prepared in different combinations with 3 for motion data, 3 for climate data and 7 for animal data. Based on the movement group as the master group, 96 different input clusters were created because of the combinations of these groups. These clusters were tested with a network, which has 15 neurons in the single-hidden layer. The input cluster that gave the best result was selected as the optimum input.

### 2.2. Determination of ANN Topology

It was stated that finding optimal topology for ANN could be a time-consuming process depending on the computer processing power and problem complexity (Madadlou et al., 2009). Chegini et al. (2008) reported that the two-layer ANN was more successful. Thus, two-layer models were investigated in the study. More than two hidden layers or many more neurons in the layers are not recommended as it will directly increase the processing (Alpaydm, 2010). The number of neurons in the hidden layer was increased in certain amounts and their performance was evaluated to find the optimal number of neurons. The network with the lowest error was selected because of these tests.

The method that Chegini et al. (2008) used in their study, was applied to determine the most appropriate two hidden layered networks: The second layer was increased to the end for each neuron increase in the first layer. So that, all possible conditions were tried sequentially. The first number of neurons was 1-1 for the first and second layers, respectively. Neurons were increased by three to 52-52. The best training algorithm for classification problems is the scaled conjugate gradient (SCG) backpropagation (Moller, 1993; Bishop, 1995). Thus, each network, which was generated by this method was trained using the SCG backpropagation algorithm. The  $\sigma$  parameter, which is the weight change between cycles of the algorithm, was determined as  $5.0e-5$  and the  $\mu$  that adjusts instability, as  $5.0e-7$ . The terminating limit of the training algorithm was arranged as 1000 epochs. Validation was performed in all trainings. 6 validation errors received consecutively was chosen as the termination criterion of training. The target performance criterion was selected as zero. Another



termination parameter, the smallest gradient value of the error function was determined as 1e-66.

The hyperbolic sigmoid tangent function, which was reported for the best performance in previous studies, has been used as a transfer function in the hidden layer neurons of the ANN models (Alpaydin, 2010; Bishop, 1995). The "Softmax" transfer function was used in the output layer of the networks. This function, which mathematical expression is given in Equation 3, takes the ANN outputs as probability associated with classes.

$$f(n_i) = \exp(n_i) \div \sum_{j=1}^s \exp(n_j) \tag{3}$$

In the equation,  $S$  gives the number of the class,  $j$  is the index.  $i$  is the index of the output. The outputs of the Softmax function are between 0 and 1. The sum of one output set is equal to 1. The function identifies classes by making the value of the highest output neuron as 1 and the others as 0.

### 2.3. Comparison of ANN Models

To demonstrate success in the classification networks, it is necessary to show whether the classification has been done correctly. There are two kinds of correct estimates for the estrus classification: First is " True Positive" when the cow is in the estrus class also predicted as estrus. And the second is " True Negative" when cows are not in the estrus class also predicted as not estrus. Accuracy of the classification models is calculated by dividing the sum of positive and negative trues by the all-estimates sum. Although accuracy is accepted as a general criterion, it is insufficient to demonstrate the success of the classification model. The number of negative classes (for this problem, the animals, which were not in the estrus state) can be much more than the positive. As a result of this excessive, the estimation of the negative trues raises the accuracy rate. The model may be supposed as successful even if the prediction of positive class, which is valuable for the problem is not in the desired success. "Sensitivity," "Precision" and "F-Score" calculations were used to evaluate the performance of classification networks and to make their comparison objective. Sensitivity is an indicator of how many estruses are predicted by the model. Precision is an indication of how many predictions are predicted correctly. Because of the importance of the positive class (estrus), both the sensitivity and precision should be high for a successful classification model.

F-score was used to compare the success of ANN models. The F-score, which is given in Equation 4 is a sufficient criterion for evaluating sensitivity and precision.

$$F = 2 \times \frac{p \times s}{p + s} \tag{4}$$

In the equation,  $P$  defines precision.  $S$  defines sensitivity. And result gives the F-score between 0 and 1. If the both two values are large, the F-score approaches 1. The reduction of at least one reduces the F-score pretty. Accuracy, sensitivity, Precision and F-score calculations were made with the test set before separation from the data. Then, using the most successful networks, the same calculations were made for all data and confusion matrices were created.

The designing, training and testing of ANN models, statistical calculations, tables and graphs for the evaluation of the results were made with MATLAB software and NNTool plugin.

## 3. Results and Discussion

### 3.1. Climatic observations

If THI is above 80, it causes cows to be in a condition called "High Stress." The situation between 70 and 80 is called "Low Stress." Stress status affects animal mobility (Sönmez et al., 2005).

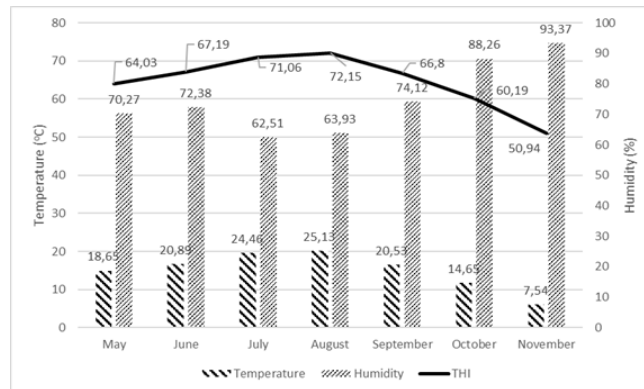


Figure 1. Average of temperature, humidity THI values

Şekil 1. Sıcaklık, nem ve SNI değerlerinin ortalaması

Figure 1 shows the average temperature, humidity and THI values for the months observed. In June, July, August and September, the average of THI increased to over 70. If THI values are evaluated momentarily during these months, it can be mentioned that the animals were in a stress state most of the time.

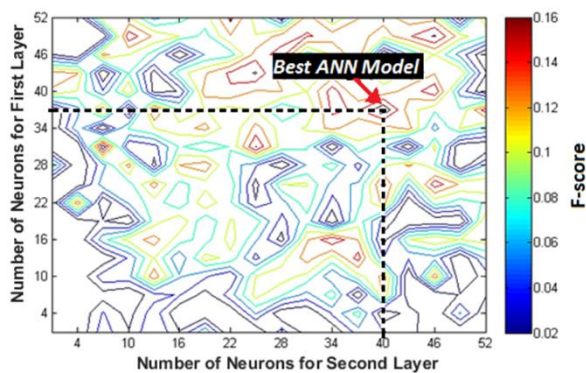
### 3.2. The Optimal inputs types for ANN

Between May and November, 184 estruses were reported for 78 cows during a seven-month observation period. The 96 different input clusters previously identified were trained with ANN models with 15 neurons in the single hidden layer and with the same

characteristics (transfer function, initial weight values, etc.). The input clusters were compared for the F-score. The movement data, one-hour previous motion data, the number of days after the estrus, temperature and humidity averages were chosen as the most successful inputs. A total of 87685 lines, corresponding to 80% of the 109605-row data generated by these inputs, were used in the training of networks. The rest was used as two equal parts (10960 lines for each) for validation and testing.

### 3.3. ANN Model Trainings

The number of neurons increased three for every step, in the range of 1-1 to 52-52 in order to test the two-layer networks. The graph in Figure 2 shows the F-score change according to the number of neurons in the layers.



**Figure 2.** F-score change according to neuron numbers for two-layer ANN

**Şekil 2.** İki katmanlı YSA için nöron sayılarına göre F-skoru değişimi

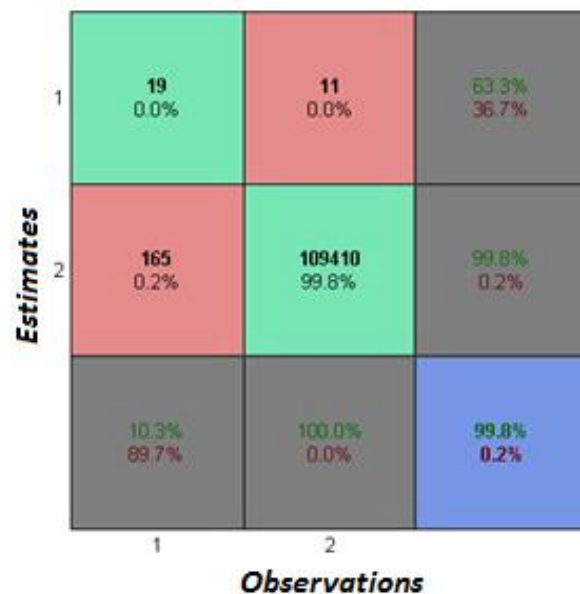
As shown in Figure 2, the most successful network with a 0.1775 F-score was the network with 37 neurons in the first layer and 40 in the second. The training was terminated with validation error warnings. The 6th validation error was reached at the 63rd epoch. The confusion matrix of the network for all data is given in Figure 3.

### 4. Conclusion

In this study, the most suitable inputs for ANN training were investigated firstly. The movement data, the movement data of the previous period, the number of days after the previous estrus, temperature and humidity were found as optimal inputs. The F-score of this input cluster was calculated as 0.1706, which is the highest among other input clusters.

Cows have been stressed for some days due to temperature and humidity. This situation, which directly affected their mobility, was due to the climate effect as indicated by Sönmez et al. (2005). The F-score was calculated as 0.1488 by subtracting the temperature and humidity values from this input set. It was seen that environmental factors were important for determining estrus with ANN. Also, it was more

successful to use temperature and humidity as separate inputs instead of THI alone. F-score was 0.1020 for the input cluster, which climatic data were only THI. Another type of input that affects success is the number of days after estrus. The F-score was reduced to 0.0995 when this input was removed from the input. The number of days after estrus had a significant effect on the F-score. The movement data with the previous period, increase the success of the estimate due to its ability to show the sudden increase in the movement.



**Figure 3.** Confusion matrix of the most successful two-layer network. ("1" indicates estrus and, "2" indicates non-estrus class)

**Şekil 3.** En başarılı iki katmanlı ağın hata matrisi. ("1" kızgınlığı ve "2" kızgınlık olmayan sınıfı belirtir)

After finding the most suitable inputs for ANN, then the best model topology was studied using this input cluster. According to the F-score, the best result was obtained by an ANN model, which has 37 neurons in the first layer, and 40 neurons in the second layer, with a value of 0.1775. Its sensitivity was obtained as 0.1032 and precision as 0.6334. Its accuracy was 0.9983. The accuracy of all models was over 0.99. The reason for this was that the negative predicted number of negative classes increased the accuracy rate because the number of negative classes was much greater than the positive class.

Movement data, which were essential for estrus estimation used for ANN training in the current system, were a numerical value produced by motion sensors. Although these data were related to the movements of animals, it did not specify the exact number of steps. Previous studies have shown good results with the number of steps (Krieter, 2005; Nadimi et al., 2012). It is thought that more successful results will be obtained by an ANN model where direct step information is taken, including environmental and animal data.

Besides the evaluation of environmental data, another important point in this study is the use of hourly data. In many herd-management systems, reading movement data from the pedometer is performed at specific times and places, such as milking. With the help of wireless data transfer, hourly data were collected independently of location, so that estrus estimation could be made earlier.

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## Changes in the Physicochemical Properties Caused by Irrigation at the Pre-Harvest Cluster Drop Period in 'Tombul' Hazelnut Cultivar

Yaşar AKÇIN<sup>1</sup> , Saim Zeki BOSTAN<sup>2\*</sup> 

<sup>1</sup>Nuriye Halit Çebi Special Education Vocational High School, Ordu,

<sup>2</sup>Ordu University, Faculty of Agriculture, Department of Horticulture, Ordu

\*Corresponding author's email: szbostan@hotmail.com

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**Abstract:** This research was carried out to evaluate the changes of physicochemical contents during storage of 'Tombul' hazelnut cultivar irrigated at the pre-harvest cluster drop period, which is critical for cluster drops, in a conventional rain-fed 'Tombul' hazelnut orchard in Giresun province (Turkey) in 2015 and 2016. The experiment was designed as randomized plots with 3 replications. The plants (multi-stemmed bush) were irrigated with drip irrigation on 16 July (46.08 mm/60 cm), 26 July (44.68 mm/60 cm), 30 July (43.68 mm/60 cm) and 06 August (44.08 mm/60 cm). The dried in-shell hazelnuts were grouped as irrigated and unirrigated (control) samples. 12 kg of in-shell hazelnuts were used in each replication. The hazelnuts were stored in mesh bags under laboratory conditions (20-22 °C and 70-80% relative humidity) for 12 months. The results showed that moisture, ash, oil, and palmitic acid values were significantly affected by the irrigation application. The lowest moisture and ash values, and the highest oil and palmitic acid values were obtained from the samples of irrigated plants. On the other hand, the changes in protein, rancidity, peroxide and vitamin E, and other fatty acids, except for the palmitic acid, were not significant. In conclusion, it can be said that supplementary irrigation in the pre-harvest period has a significant effect on some physicochemical changes during storage in hazelnuts. According to these results, supplementary irrigation can be recommended for hazelnut storage quality in case of insufficient precipitation during the last cluster drop period.

**Keywords:** *Corylus avellana*, Cluster drop, Irrigation, Physicochemical, Storage

## 'Tombul' Fındık Çeşidinde Hasat Önü Çotanak Döküm Periyodundaki Sulamanın Depolama Süresince Meydana Getirdiği Fizikokimyasal Değişimler

**Öz:** Bu araştırma, Giresun ilinde geleneksel olarak yağmurla beslenen 'Tombul' fındık çeşidi ile tesis edilmiş bir bahçede, çotanak dökümleri için kritik olan son döküm döneminde uygulanan damla sulamanın depolanma sırasında fizikokimyasal içeriklerindeki değişimlerin belirlenmesi amacıyla 2015 ve 2016 yıllarında yürütülmüştür. Deneme tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak tasarlanmıştır. Ocaklardaki bitkiler (çok gövdeli çalı) 16 Temmuz (46.08 mm/60 cm), 26 Temmuz (44.68 mm/60 cm), 30 Temmuz (43.68 mm/60 cm) ve 06 Ağustos (44.08 mm/60 cm) tarihlerinde damla sulama ile sulanmıştır. Kurutulmuş kabuklu fındıklar sulu ve susuz olarak gruplandırılmıştır. Her tekerrürde 12 kg kabuklu fındık kullanılmıştır. Fındıklar, 12 ay boyunca laboratuvar koşullarında (20-22 °C ve %70-80 bağıl nem) file çuvallarda muhafaza edilmiştir. Sonuçlar, nem, kül, yağ ve palmitik asit değerlerinin ilave sulama uygulamasından önemli ölçüde etkilendiğini göstermiştir. En düşük nem ve kül değerleri ile en yüksek yağ ve palmitik asit değerleri sulanan bitki örneklerinden elde edilmiştir. Öte yandan, protein, ransidite, peroksit ve E vitamini ile palmitik asit dışındaki diğer yağ asitlerindeki değişimler önemli bulunmamıştır. Sonuç olarak, fındıkta depolama sırasında bazı fizikokimyasal değişiklikler üzerinde hasat öncesi dönemde ilave sulamanın önemli bir etkiye sahip olduğu söylenebilir. Bu sonuçlara göre, son çotanak döküm döneminde yetersiz yağış olması durumunda fındıkta depolama kalitesi için ilave sulama önerilebilir.

**Anahtar Kelimeler:** *Corylus avellana*, Çotanak dökümü, Sulama, Fizikokimyasal, Depolama

### 1. Introduction

In the hazelnut production areas especially Trabzon, Giresun and Ordu provinces in Turkey, climatic conditions can vary greatly in the region due to the land structure. One of the most important reasons for the yield fluctuations in hazelnut production from year to year is adverse climatic conditions (Bostan, 2009).

In the Eastern Black Sea region of Turkey, hazelnut production takes place in very hilly areas. Since the hazelnut root system has an exposed structure, it is very sensitive to drought, especially in sloping areas. Yield is

significantly affected by precipitation, especially between May and July. For this reason, irrigation in the critical period at least in areas where natural conditions, water resources and irrigation systems allow can be an effective solution in dry years (Tonkaz & Bostan, 2010; Bostan & Tonkaz, 2013).

Hazelnut trees should be irrigated, especially in regions with limited rainfall and years, and on soils with low water capacity, due to its positive effects on productivity. Hazelnut is generally accepted as a water stress sensitive species due to its low stomatal regulation

capacity. The water potential in the growing season affects the vegetative and generative activities and causes a decrease in quality and yield. At a time when different vegetative and generative developments (end of leaf area development, nut set, nut development, flower bud initiation and differentiation) overlap between June and August, abundant water is an important factor in overcoming developmental competition (Bignami et al., 2009; Bignami et al., 2011; Cristofori et al., 2014; Cristofori et al., 2019). Since irrigation during the development period has a very important effect on physiological characteristics and nut quality, long annual data on irrigation applications are needed for precise results (Dias et al., 2005).

The fact that the topography of the hazelnut growing areas in the first standard hazelnut region in Turkey is very rugged and inclined can cause significant changes in yield and pomological characteristics even within the cultivar as well as among the cultivars. As a matter of fact, it has been stated that the technological properties of hazelnut varieties grown in the Central and Eastern Black Sea Region of Turkey show significant changes according to the varieties, regions and years (Şahin et al., 1990).

It has been stated that the hot and dry months of June and July in the Central and Eastern Black Sea Region cause an increase in pre-harvest cluster drop in hazelnuts, a negative relationship is observed between the sunny days in July and hazelnut production, while the number of rainy days in the same month has a positive and significant effect on the yield (Bostan, 2005; Bostan & Tonkaz, 2013). Modern irrigation techniques should be applied to ensure increased plant water consumption due to temperature increases in the region (Tonkaz & Bostan, 2016). Among ecological factors, the drought especially in July (pre-harvest) and the falling below 60% of relative humidity of the air in this period cause low kernel percent and an increase in the cluster drop (Okay et al., 1986). Considering that the daily and monthly total precipitation values between June and August gradually decrease, it can be said that the average temperature and total precipitation values have a more critical effect on the ratio of cluster drop in the pre-harvest period where most of the cluster drop occur (Top & Bostan, 2020). On the other hand, Milosevic & Milosevic (2012) stated that lack of water is an important factor on the severity of the cluster drop, in other words, low yield; Mingeau et al. (1994) also stated that 15-20% water restriction during the kernel development period doubled the pre-harvest fruit drop and the empty fruit rate.

There are significant differences in crude protein, fat, fiber, ash content and energy among hazelnut varieties, which are a good source of energy and rich in protein (Ozdemir & Akinci, 2004). Hazelnut has a high level of chemical components that are important for nutrition and health. The high fat content is believed to result in reduced shelf life and rancidity, but the possible adverse effect on fruit storability can be mitigated by the presence of phenolics and the low level of total unsaturated fatty acids (Cristofori et al., 2008). The direct effect of malate and other organic acids on the taste and quality of hazelnuts is very low, on the contrary, sugar, lipid, linoleic acid and tocopherol contents are important parameters to be considered in the preservation and quality evaluation of hazelnut varieties (Botta et al., 1994).

Determination of sources of quality losses in hazelnut and preventive measures is important to achieve and maintain high quality products. Microbial, chemical/biochemical changes contribute to the shelf life of the hazelnut and its products. Due to insufficient/inadequate harvest, drying and storage methods and conditions, mould activities bring about significant quality losses. Improvement of the harvest, post-harvest and processing stages may improve the quality, but the best quality can only be attained if the whole production and processing line is designed and operated for that (Özdemir 1998). As well as during storage (Ghirardello et al., 2013), pre-storage growing conditions and some treatments also affect the shelf quality of hazelnuts (Kaya et al., 2005; Turan & İslam, 2016; Koç Güler et al., 2017a; Turan & İslam, 2018; Turan, 2019; Turan & Karaosmanoğlu, 2019).

While it is possible to come across many studies on the effect of irrigation on yield and quality in other hazelnut growing countries, these studies in Turkey are still in their infancy. No studies were found on the effect of irrigation on storage quality and shelf life. In this study, physicochemical changes during storage of 'Tombul' hazelnut cultivar irrigated at last cluster drop period which is the critical period were investigated. The cultivar 'Tombul' is currently one of the most important commercial cultivars in Turkey and in the world.

## **2. Material and Methods**

### **2.1. Plant materials and experimental site**

This study was carried out in Giresun province, which is an important hazelnut production region in Turkey, in 2015 and 2016. There is no irrigation in the hazelnut orchards in the region and the water needs of

the plants are conventionally met by rain. However, especially in recent years, due to the increase in temperatures in the region, additional irrigation in hazelnut farming has become inevitable for the need for plant water consumption (Tonkaz & Bostan, 2016).

The main cultivar 'Tombul' hazelnut (cv) in an orchard, in the Barça village (Location:40.87222338441944°, Latitude:40.872222900390625°, Longitude:38.44194412231445°) was used in the study. The altitude of the research orchard is 110 meters, the slope is about 60% and the distance between the ocaks (multi-stemmed bush) 4 meters. Excess stems were cut in 2014 dormant period, with 5 stems in each ocak. Soil pH of the trial area was determined as 5.79-6.37 in 2015 and 5.86-6.28 in 2016. This pH value is among the most suitable values for hazelnut cultivation and is slightly acidic.

In the study, drip irrigation was performed on 16 July (46.08 mm/60 cm), 26 July (44.68 mm/60 cm), 30 July (43.68 mm/60 cm) and 06 August (44.08 mm/60 cm), in the third nut and kernel development period (Bostan, 1998).

## 2.2. Preparation of samples:

Harvesting of clusters in the experimental area was done by hand picking from tree branches. In 2015, the hazelnuts collected on August 15 were separated from their husks with a husker machine the next day. The hazelnuts were laid in a single row on the concrete floor and dried in the sun for 7 days. In 2016, the harvest was made on August 8 and the hazelnuts were dried in the sun for 5 days. The drying process was terminated when the moisture content fell below 10% in-shell hazelnuts and below 5% in kernels. The dried in-shell hazelnuts were grouped as irrigated and unirrigated samples. 12 kg of in-shell hazelnuts were used in each replication. The first analyses (beginning) were made 2 days after the samples were placed in the laboratory. After the first analysis, the nuts were stored in mesh bags under laboratory conditions (20-22 °C and 70-80% relative humidity) for 12 months.

Moisture, ash, protein and fat analyses were performed twice, at the beginning of storage and at the 12th month; rancid, peroxide, vitamin E and fatty acids composition analyses were also performed 3 times, at the beginning of storage, at the 6th month and at the 12th month.

## 2.3. Analyse Methods:

**Moisture content (%)**:  $3 \pm 0.01$  g of each sample

ground in the blender was weighed with a balance with an accuracy of 0.01 g. The weighed samples were kept in an oven at  $105 \pm 2^\circ\text{C}$  until they reached a constant weight. Then it was cooled in a desiccator and weighed (AOAC, 2000a).

Calculation:

$$\text{Moisture (\%)} = (A_0 - A_1) \times 100 / A_0 \quad (1)$$

$A_0$ : Beginning weight of sample (g)

$A_1$ : Dry weight of sample (g)

**Ash content (%)**:  $3 \pm 0.01$  g of each sample ground in the blender was weighed with a balance with an accuracy of 0.01 g. The samples placed in the crucibles were kept in an oven at  $105 \pm 2^\circ\text{C}$  until they reached a constant weight. It was then cooled in a desiccator and immediately burned in the incinerator at  $530^\circ\text{C}$  for 8 hours. After incineration, it was cooled in a desiccator and weighed. (AOAC, 2000b).

Calculation:

$$\text{Ash (\%)} = A_0 \times 100 / A_1 \quad (2)$$

$A_0$ : Ash weight (g)

$A_1$ : Dry weight of sample (g)

**Protein content (%)**: For crude protein analysis, 0.5 g of each sample was weighed and placed in kjeldahl tubes. A tablet ( $\text{K}_2\text{SO}_4:\text{CuSO}_4$ ) was placed in the tube as a catalyst, and 12 ml of concentrated sulfuric acid was added and burned in a protein device (Gerhardt Vap40) incinerator for 1 hour at  $420^\circ\text{C}$  until the color became completely clear. After the gas escape was finished, the balloon was cooled down to about  $40^\circ\text{C}$ . The sample, which was placed in the distillation unit after the incineration process, was distilled with boric acid (3%  $\text{H}_3\text{BO}_3$ ) and sodium hydroxide (33%) solutions. Then, the collected distillate was titrated with 0.2 N hydrochloric acid solution. The amount of protein was calculated according to the formula below (AOAC, 2000c):

$$\text{Protein (\%)} = V \times S \times N \times 100 \times 5.30 / m \quad (3)$$

V: HCl spent for titration (ml)

m: Sample weight (g)

S: 0.014

N: Normality of HCl solution

**Fat content (%)**: Fat content was determined using the soxhlet device (Anonymous, 2000). The glass containers of the device were brought to a constant weight by drying in an oven, and the beakers to be filled with n-hexane were tared after drying. The temperature of the device is adjusted to  $130^\circ\text{C}$ , which is the appropriate temperature for n-hexane. 5 g of the ground hazelnut kernels were weighed on a 0.001 g sensitive balance and put into the cartridge. The cartridges are placed in the Soxhlet extraction device. 60 ml of n-



hexane was placed in each beaker. The first stage (immersion) of the device took 30 minutes and the second stage (washing) took 150 minutes. The last stage (recovery) was completed in 30 minutes. After the recovery was completed, the samples were put in an oven at 105±2°C. It was kept in the oven for one hour. The samples taken from the oven were weighed on the balance after cooling in the desiccator. After taking the total weight of the beaker, the % crude oil was calculated with the following formula:

$$\text{Fat (\%)} = (A2 - A1) / m \times 100 \quad (4)$$

A1: Weight of beaker brought to constant weighing (g),

A2: Total amount in beaker at last weighing (g),

m: Sample weight (g)

**Rancidity value (h):** Rancid value was determined in 743 Rancimat device from Metrohm according to (Anonymous, 1997) using 2.50±0.01 g of oil obtained from hazelnut kernel samples by cold pressing. All samples were examined under constant airflow (20 L h<sup>-1</sup>) at five different temperatures (100, 110, 120, 130 and 140°C). Induction times were obtained automatically with the device software with an accuracy of 0.005.

**Peroxide value (%):** Peroxide value was calculated by potentiometric titration method (Anonymous, 1990). Acetic acid/Isooctane was used as 3/2 (v/v), potassium iodide solution as saturated (14 g/10 ml purified water), starch solution as 1% and sodium thiosulfate solution as 0.01 N.

2-3 ml of sample was taken into the beaker and 100 ml of acetic acid/isooctane (3/2) solution was placed on it and the oil was dissolved. 0.2 ml of potassium iodide was added and kept in the dark for 5 minutes, then 50 ml of distilled water was added. At the end of the period, 75 ml of water and 1 ml of starch were added and titrated with sodium thiosulfate.

$$\text{Peroxide value} = V \times N / P \quad (5)$$

V: Spent sodium thiosulfate (ml)

N: Normality of sodium thiosulfate (0.01 N)

P: Sample amount

**Vitamin E (α-tocopherol) (%):** Hazelnut oil was obtained by pressing the hazelnut kernels in the cold press oil extraction device. The obtained extract was dissolved in 2 ml of heptane:tetrahydrofuran (THF) (95:5 v/v) before injection and passed through a 45µm filter. Analyzes were performed using the Agilent HPLC system (1260 Infinity). α-tocopherol was identified with a DAD detector at a wavelength of 292 nm. Phenomenex Luna silica column (250 x 4.6 mm i.d., 5 µm in particle size) was used for separation. The mobile phase (heptane: THF, 95:5) was passed through

the column with isocratic flow at a flow rate of 1.2 ml/min at 25°C. The separation process was completed in 20 minutes. The results were calculated from standard curves prepared using standard substances and expressed in µg tocopherol/g dry matter (Balz et al., 1992).

**Fatty acid composition (%):** The oil of the hazelnut kernels was obtained by pressing in a cold press oil extraction device. 40 mg of hazelnut oil samples were taken and dissolved with 4 ml of hexane. It was vortexed for 60 seconds by adding 3 ml of 2 M KOH (prepared in methanol). After the phases were separated, 1 ml of the upper phase (hexane phase) was taken into a GC vial and analyzed under the following conditions (Sushchik et al., 2003): Oven program

Beginning temperature: 120°C with 2°C/min heating rate to 180°C, then 4°C/min to 200°C, then 7°C/min to 230°C and left for 0.71 min. The furnace program used in gas chromatography is given in Table 1.

**Table 1.** Oven program used in gas chromatography  
**Çizelge 1.** Gaz kromatografisinde kullanılan fırın programı

Beginning temperature (°C)	Standby time (min)	Temperature rise rate (°C/min)	End temperature (°C)
120	2	2/min	180
180	0	4/min	200
200	3	0	200

GC terms

Device: Perkin Elmer Clarus 500 model

Column: Restek RTX 2330 (30 mx0.25 mm, 0.25 µm film thickness)

Injection volume: 1 µl

Injection port temperature: 250°C

FID terms

Detector temperature: 250°C

Dry air: 450 ml/min

Hydrogen: 45 ml/min

A mixture of methyl esters of 37 fatty acids was used as a standard for the identification of fatty acids. By comparing the chromatograms obtained from the samples and the chromatograms obtained from the standards, fatty acid types and relative % amounts were determined.

Carrier gas: Helium (1 ml/min)

Split ratio: 50/

#### 2.4. Statistical Analysis:

Experimental design was arranged in randomized plots with 3 replications and 6 ocaks (multi-stemmed bush) in each replication. Analysis of variance was

performed to determine the variation of the investigated parameters according to the storage time. Statistical analyses were made in the SAS JMP 13.2.0 statistical package program and LSD test was applied to determine the differences between the means.

### 3. Results and Discussion

**Moisture content:** In the first year, moisture change was found to be significant during storage and according to irrigation. While the moisture increased regularly during the storage period, it was determined that the irrigated samples were lower than the control samples.

In the second year, the interaction of storage and sample was found to be significant for moisture content change. The moisture content increased during storage in both sample groups, as in the first year, and at the end of storage, it was found to be higher in the control samples (4.20%) than the irrigated samples (3.88%). The moisture content of all samples remained below 5% in both years, and there was a difference between years in terms of the moisture content change according to the samples. According to the samples and storage time, the moisture content showed similar changes in both years (Table 2).

**Table 2.** Moisture, ash, protein and fat contents of 'Tombul' hazelnut kernels

**Çizelge 2.** 'Tombul' iç fındıklarının nem, kül, protein ve yağ içerikleri

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
<b>2015</b>				
<b>Treatment</b>				
Control (C)	4.03±0.74a**	2.20±0.32a*	16.42±0.95	58.73±1.50b**
Irrigation (I)	3.72±0.82b	2.07±0.34b	16.16±0.85	60.67±2.09a
LSD <sub>0.05</sub>	0.20	0.11	ns	1.29
<b>Storage (months)</b>				
0	3.13±0.38c**	2.39±0.18a**	16.50±1.07	59.55±1.92
6	3.67±0.22b	2.25±0.05a	16.52±0.87	59.38±1.63
12	4.83±0.36a	1.75±0.26b	15.84±0.57	60.17±2.57
LSD <sub>0.05</sub>	0.24	0.14	ns	ns
<b>Treatment * Storage</b>				
C*0	3.37±0.32	2.46±0.13	17.02±1.25	58.67±1.48
C*6	3.83±0.13	2.27±0.05	16.30±0.59	58.37±0.89
C*12	4.90±0.51	1.87±0.33	15.93±0.64	59.17±2.07
I*0	2.90±0.30	2.33±0.20	15.99±0.54	60.43±2.01
I*6	3.50±0.15	2.23±0.04	16.75±1.10	60.40±1.60
I*12	4.75±0.12	1.64±0.11	15.76±0.53	61.17±2.80
LSD <sub>0.05</sub>	ns	ns	ns	ns
<b>2016</b>				
<b>Treatment</b>				
Control (C)	4.00±0.42	2.31±0.25	15.88±1.04	59.77±1.40b**
Irrigation (I)	4.08±0.64	2.26±0.30	16.23±0.69	60.94±1.28a
LSD <sub>0.05</sub>	ns	ns	ns	0.85
<b>Storage (months)</b>				
0	3.40±0.10c**	2.52±0.17a**	15.95±1.27	61.08±1.53a*
6	4.17±0.19b	2.27±0.25b	16.03±0.71	59.74±1.16b
12	4.55±0.34a	2.07±0.18c	16.18±0.59	60.24±1.42ab
LSD <sub>0.05</sub>	0.11	1.16	ns	1.05
<b>Treatment * Storage</b>				
C*0	3.45±0.11d**	2.42±0.13ab*	15.20±1.21b*	60.92±1.17
C*6	4.30±0.18b	2.37±0.34bc	16.30±0.93a	58.94±1.06
C*12	4.26±0.11b	2.15±0.19d	16.15±0.70ab	59.44±1.29
I*0	3.35±0.14d	2.63±0.06a	16.71±0.86a	61.23±1.93
I*6	4.04±0.06c	2.17±0.08cd	15.76±0.25ab	60.55±0.54
I*12	4.85±0.13a	2.15±0.18d	16.21±0.53a	61.04±1.11
LSD <sub>0.05</sub>	0.15	0.22	0.95	ns

\*: P<0.05, \*\*: P<0.01

**Ash content:** In the present study, ash change during storage and according to irrigation was significant in the first year. While the ash decreased regularly during storage, the ash content of the irrigated samples was lower than the control. Storage and sample interaction were found to be significant for ash content change in

the second year. In both sample groups, as in the first year, the ash content decreased during storage and was less at the end of storage in irrigated samples (2.26%) than control samples (2.31%). According to the samples and storage time, the ash content showed similar changes in both years (Table 2).



**Protein content:** The protein content changes according to storage time and samples were found to be insignificant in the first year. In the second-year storage and sample interaction were found to be significant for protein content change (Table 2). The highest protein value was observed with irrigated samples (16.23%), while the lowest value was observed with control samples (15.88%).

**Fat content:** The fat content was significantly affected by the samples in both years, and it was determined more in irrigated samples. The change according to the storage time was significant only in the second year, and fat content significantly increased during storage. At the end of storage, it was found to be higher in the irrigated samples (60.94%) than the control samples (59.77%). In the interaction, the fat ratio change was insignificant in both years (Table 2).

**Rancidity value:** Rancidity value was significantly affected by storage time only in the first year, and the value after 12 months of storage was lower than the beginning value. There was no significant difference in rancidity value by samples (Table 3 and 4).

**Peroxide value:** The change in peroxide value according to application, storage and interaction were similar in both years, only the change according to storage time was significant, and the value increased after 12 months of storage (Table 3 and 4). The effect of irrigation on the peroxide content of the samples during storage was insignificant. While the peroxide value was determined lower in the second year samples, increased from zero to  $0.47 \text{ meqO}_2\text{kg}^{-1}$  in the first year and from  $0.26 \text{ meqO}_2\text{kg}^{-1}$  to  $1.64 \text{ meqO}_2\text{kg}^{-1}$  in the second year after 12 months of storage.

**Vitamin E ( $\alpha$ -tocopherol) amount:** The amount of vitamin E changed similarly to the rancidity value and was significantly affected by the storage time, only in the first year (Table 3 and 4). The amount of vitamin E was slightly higher in hazelnuts irrigated in both years. The value at the end of storage was also determined less than the beginning value.

**Fatty acid composition:** A total of 13 fatty acids were examined in the study. Among them, heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and linolenic acid (C18:3n3) were not significantly affected by irrigation and storage time (Tables 2 and 3). The ones with the highest ratio of 13 fatty acids were oleic acid (C18:1n9c), linoleic acid (C18:2n6c), palmitic acid (C16:0) and stearic acid (C18:0), respectively. Of the major fatty acids, only palmitic acid (C16:0) was significantly affected by irrigation application and

storage time in the first year (Table 3 and 4). While palmitic acid was found to be higher in irrigated samples, the value at the end of storage was higher than the beginning value. Irrigation application and storage time did not significantly affect the change in the content of the other major fatty acids.

Since no similar research could be found except for research in which only the change of oleic acid was examined during storage in irrigated and non-irrigated hazelnuts (Bignami et al., 2009), the results of this study could not be directly compared with other studies in terms of the physicochemical changes during storage in irrigated and unirrigated hazelnut. Therefore, our results could be compared with the results of some different applications and especially storage studies in hazelnut.

Moisture and water vapor in different parts of the hazelnut can cause physical changes as well as make differences in flavor or texture and even encourage microbial activity (Özdemir, 1998). During blending, care should be taken to ensure that the hazelnut dries well, and that the moisture content does not exceed 12% in-shell hazelnuts and 6% in hazelnut kernels. Thus, hazelnuts can be stored for a long time (Okay et al., 1986). In the other studies, it was stated that the kernel moisture content was nearly stable during storage (Ghirardello et al., 2013); not significant according to the storage times (in 'Tombul' cultivar separated by hand) (Akar & Bostan, 2018); decreased slightly during storage (Kaya et al., 2005); decreased significantly during storage (Koyuncu et al., 2005; Koç Güler et al., 2017a; Turan & Karaosmanoğlu, 2019); showed a tendency to decrease with fluctuation, and the highest values were determined at 12 months and then again decreased (Turan & İslam, 2018).

Our findings are in compliance with the results of the moisture content reaching the maximum in the 12th month in other studies. As can be understood from other results, moisture content can vary considerably according to many factors such as variety, year, storage conditions, and storage material of samples, whether the stored samples are shelled or kernel, storage period, pre-harvest applications.

Ash content of hazelnut kernels, which mainly reflects the content of inorganic elements (Fan et al., 2020), changes significantly according to cultivars, years, and locations (Şahin et al., 1990). Akar & Bostan (2018) stated that the change in ash content of 'Tombul' hazelnut cultivar during 9 months of storage was insignificant. In the present study, the ash content decreased gradually during storage, which can be explained by different storage conditions.

**Table 3.** Rancidity, peroxide, vitamin E, and fatty acid compositions of 'Tombul' hazelnut kernels in 2015  
*Cizelge 3. 2015 yılında 'Tombul' ic findıklarının ransimat, peroksid, E vitamini ve yağ asidi bileşimleri*

Parameters	Treatments				Storage periods (months)				Treatment * Storage period								
	Control		Irrigation		0		12		C*0		C*12		I*0		I*12		LSD <sub>0,05</sub>
	Control	Irrigation	LSD <sub>0,05</sub>	0	12	LSD <sub>0,05</sub>	C*0	C*12	I*0	I*12	LSD <sub>0,05</sub>						
<b>Rancidity</b>	4.52±0.71	4.99±0.85	ns	5.08±0.49a*	4.42±0.93b	0.63	4.78±0.53	4.26±0.81	5.39±0.14	4.59±1.09	ns						
<b>Peroxide</b>	0.35±0.46	0.12±0.35	ns	0.00±0.00b**	0.47±0.47a	0.27	0.00±0.00	0.70±0.42	0.00±0.00	0.23±0.48	ns						
<b>Vitamin E</b>	393.71±56.30	396.60±61.91	ns	439.30±49.44**	351.01±17.64	32.25	429.76±60.90	357.66±12.10	448.84±38.09	344.36±20.78	ns						
<b>C14:0</b>	0.03±0.00	0.03±0.00	ns	0.03±0.00	0.03±0.00	ns	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	ns						
<b>C16:0</b>	5.14±0.16b**	5.34±0.11a	0.11	5.18±0.17b*	5.30±0.15a	0.11	5.05±0.15	5.22±0.13	5.31±0.07	5.38±0.14	ns						
<b>C16:1</b>	0.12±0.02	0.13±0.02	ns	0.11±0.01b**	0.14±0.01	0.01	0.11±0.01	0.14±0.01	0.12±0.01	0.14±0.01	ns						
<b>C17:0</b>	0.05±0.01	0.05±0.01	ns	0.05±0.01	0.05±0.01	ns	0.05±0.00	0.05±0.01	0.04±0.01	0.05±0.01	ns						
<b>C17:1</b>	0.06±0.01	0.07±0.01	ns	0.07±0.01	0.06±0.01	ns	0.07±0.01a**	0.05±0.01b	0.06±0.01ab	0.07±0.00a	0.01						
<b>C18:0</b>	2.56±0.09	2.68±0.27	ns	2.69±0.23	2.56±0.17	ns	2.58±0.05	2.55±0.12	2.81±0.28	2.56±0.21	ns						
<b>C18:1n9nc</b>	82.81±1.51	83.55±1.05	ns	83.30±1.62	83.06±1.01	ns	82.93±1.91	82.69±1.14	83.67±1.34	83.44±0.79	ns						
<b>C18:2n6c</b>	8.90±1.40	7.84±1.14	ns	8.28±1.71	8.45±0.97	ns	8.89±1.82	8.91±1.00	7.68±1.50	8.00±0.75	ns						
<b>C18:3n3</b>	0.14±0.01	0.14±0.03	ns	0.13±0.02	0.15±0.02	ns	0.13±0.01	0.15±0.02	0.14±0.03	0.15±0.02	ns						
<b>C18:3n6</b>	0.09±0.04	0.09±0.03	ns	0.06±0.01b**	0.12±0.01a	0.01	0.06±0.01c*	0.13±0.01a	0.06±0.01c	0.11±0.01b	0.01						
<b>C20:0</b>	0.06±0.05	0.05±0.04	ns	0.10±0.01a**	0.02±0.02b	0.01	0.10±0.01	0.03±0.03	0.09±0.02	0.01±0.00	ns						
<b>C20:3n6</b>	0.01±0.01b*	0.02±0.02a	0.004	0.00±0.00b**	0.03±0.01a	0.004	0.00±0.00c*	0.02±0.00b	0.00±0.00c	0.03±0.01a	0.006						
<b>C23:0</b>	0.01±0.02	0.02±0.01	ns	0.00±0.00b**	0.03±0.02a	0.01	0.00±0.00	0.03±0.02	0.00±0.00	0.02±0.01	ns						

\*, P<0.05, \*\*, P<0.01

**Table 4.** Rancidity, peroxide, vitamin E and fatty acid compositions of 'Tombul' hazelnut kernels in 2016  
*Cizelge 4. 2016 yılında 'Tombul' ic findıklarının ransimat, peroksid, E vitamini ve yağ asidi bileşimleri*

Parameters	Treatments				Storage periods (months)				Treatment * Storage period								
	Control		Irrigation		0		12		C*0		C*12		I*0		I*12		LSD <sub>0,05</sub>
	Control	Irrigation	LSD <sub>0,05</sub>	0	12	LSD <sub>0,05</sub>	C*0	C*12	I*0	I*12	LSD <sub>0,05</sub>						
<b>Rancidity</b>	4.73±0.51	5.09±0.38	ns	4.97±0.48	4.86±0.49	ns	4.86±0.56	4.60±0.47	5.07±0.42	5.11±0.38	ns						
<b>Peroxide</b>	0.86±0.71	1.04±1.02	ns	0.26±0.38b**	1.64±0.61a	0.42	0.32±0.49	1.40±0.40	0.20±0.24	1.88±0.72	ns						
<b>Vitamin E</b>	331.04±57.76	354.40±48.24	ns	362.02±49.34	323.43±52.10	ns	343.07±57.03	319.01±61.16	380.97±35.24	327.84±46.75	ns						
<b>C14:0</b>	0.03±0.01	0.03±0.00	ns	0.02±0.00*	0.03±0.00	0.001	0.02±0.01b*	0.03±0.00a	0.03±0.00a	0.03±0.00a	0.004						
<b>C16:0</b>	5.29±0.17	5.30±0.11	ns	5.25±0.14	5.34±0.13	ns	5.26±0.17	5.32±0.18	5.24±0.12	5.36±0.05	ns						
<b>C16:1</b>	0.14±0.01	0.14±0.01	ns	0.14±0.01	0.14±0.01	ns	0.14±0.01	0.14±0.00	0.14±0.01	0.13±0.01	ns						
<b>C17:0</b>	0.05±0.01	0.05±0.01	ns	0.05±0.00	0.05±0.01	ns	0.05±0.01	0.05±0.01	0.05±0.00	0.05±0.01	ns						
<b>C17:1</b>	0.08±0.01	0.07±0.01	ns	0.07±0.00b**	0.08±0.01a	0.005	0.07±0.01	0.08±0.01	0.07±0.00	0.08±0.01	ns						
<b>C18:0</b>	2.63±0.24	2.67±0.10	ns	2.67±0.22	2.63±0.13	ns	2.66±0.31	2.60±0.16	2.68±0.12	2.66±0.09	ns						
<b>C18:1n9nc</b>	83.25±1.76	83.87±0.52	ns	83.69±1.31	83.43±1.35	ns	83.36±1.85	83.14±1.83	84.02±0.31	83.73±0.67	ns						
<b>C18:2n6c</b>	8.11±1.47	7.44±0.50	ns	7.66±1.05	7.90±1.24	ns	7.99±1.44	8.24±1.64	7.33±0.34	7.56±0.63	ns						
<b>C18:3n3</b>	0.16±0.01	0.16±0.01	ns	0.16±0.01	0.16±0.01	ns	0.17±0.01	0.16±0.01	0.16±0.01	0.15±0.01	ns						
<b>C18:3n6</b>	0.12±0.01	0.13±0.01	ns	0.13±0.01a**	0.12±0.01b	0.008	0.13±0.01	0.12±0.01	0.13±0.01	0.13±0.01	ns						
<b>C20:0</b>	0.08±0.01	0.08±0.01	ns	0.09±0.01	0.08±0.01	ns	0.09±0.01	0.08±0.00	0.09±0.02	0.08±0.01	ns						
<b>C20:3n6</b>	0.02±0.01	0.03±0.01	ns	0.03±0.01	0.02±0.01	ns	0.03±0.01	0.02±0.01	0.03±0.01	0.03±0.01	ns						
<b>C23:0</b>	0.02±0.01	0.02±0.01	ns	0.03±0.01a**	0.01±0.00b	0.002	0.03±0.01	0.01±0.01	0.03±0.00	0.01±0.00	ns						

\*, P<0.05, \*\*, P<0.01

The protein content fluctuated as an increase-decrease in the control samples, and as a decrease-increase in the irrigated samples during storage. Similarly, Çakırmelikoğlu & Çalışkan (1993) stated that protein content did not show a one-sided change during storage; Bostan & Koç Güler (2016) stated that the crude protein content fluctuated as decreased- increased in in-shell hazelnuts stored for 12 months; Koç Güler et al. (2017a) stated that during the storage period, crude protein contents exhibited fluctuated variations; Akar & Bostan (2018) stated that the crude protein increased with storage in all samples; Turan & İslam (2018) stated that the protein content was increased with fluctuation during the storage for 24 months; Turan & Karaosmanoğlu (2019) stated that protein content significantly increased during storage, but the increase was not constant; Turan (2019) stated that the protein content showed fluctuation at the 12th month, but generally increased and decreased again at the end of the storage period. These fluctuations in protein content during storage may be related to changes in moisture content.

Contrary to Bignami et al. (2009) and Külahçılar et al. (2018), the effect of irrigation on fat content was found to be significant in the second year of the present study. This is thought to be due to the difference in irrigation treatments. On the other hand, in the other studies also found that the fat content did not change significantly during storage (Çakırmelikoğlu & Çalışkan, 1993; Ghirardello et al., 2013; Bostan & Koç Güler, 2016), increased at the end of storage (Ağar et al., 1995; Koyuncu, 2004; Koyuncu et al., 2005; Akar & Bostan, 2018); increased at the end with irregular change during storage (Koç Güler et al., 2017a; Turan & Karaosmanoğlu, 2019), and no statistically significant change during storage (Turan & İslam, 2018). Our results were similar to the results of some other studies in that the fat content increased during storage.

Fat is the predominant component in hazelnuts, and resistance to oxidation of lipids (rancidity) is generally associated with shelf life (Ghirardello et al. 2013). Similar to the others (Momchilova et al., 2017; Turan & İslam, 2018; Turan & Karaosmanoğlu, 2019; Turan, 2019), the rancidity value decreased during storage in our study.

The peroxide value, which is an important parameter in determining the storage life of hazelnuts, emerges at the end of the oxidation of unsaturated fatty acids. Peroxides cause secondary oxidation products, and these products can make hazelnuts unusable by

negatively affecting color, crispness, flavor and, odor (Demirci Ercoşkun, 2009). It is stated that bitter tastes cannot be perceived until peroxide values above 2.0 are reached in hazelnuts (Çakırmelikoğlu & Çalışkan, 1993). In the present study, peroxide value increased after 12 months of storage. Also in other studies, there are findings that the peroxide value increases at the end of storage (Ebrahim et al., 1994; Çetin et al., 2000; Demirci Ercoşkun, 2009; Santis et al., 2009; Ghirardello et al., 2013; Bostan & Koç Güler, 2016; Koç Güler et al., 2017a; Akar & Bostan, 2018; Turan, 2018; Turan & İslam, 2018; Karaosmanoğlu & Üstün, 2019; Turan, 2019; Turan & İslam, 2019).

Hazelnut oil is an excellent source of vitamin E and the 'Tombul' cultivar has the highest value. In this respect, there are significant differences between the lipids of different cultivars (Alasalvar et al., 2009). As a result of a previous study on the same cultivar, it was determined that the vitamin E content was higher in the samples of irrigated plants (Bostan, 2020). Although insignificant, the value was found to be higher in the samples of irrigated plants in this study. Moreover, it was stated that the tocopherols amount gradually decreased and that trend was slightly stronger at 20°C than at 4°C as well as for nuts in the shell than the kernels (Momchilova et al., 2017). Also, Koç Güler et al. (2017a) determined that the vitamin E content of hazelnut kernels stored in vacuum packages (18 months of storage at 20 °C, at 55-60% RH) decreased with significant but unstable changes during storage. As in other studies, vitamin E content decreased at the end of storage in our study.

The main saturated fatty acid in fresh hazelnuts is palmitic acid, followed by stearic acid. The most unsaturated fatty acids are oleic acid and linoleic acid, and oleic and linoleic acids are very important for Turkish hazelnut varieties (Ağar et al., 1995). It was observed that a higher acidity (oleic acid %) was detected after three months storage in shelled nuts of the untreated control (0% ETc) whereas similar values were observed after six and ten months in control and irrigation treatments and stated that this situation changed depending on the variety, year and interaction (Bignami et al., 2009). In our study, the change of oleic acid according to the storage period and treatments was almost similarly insignificant. The researchers stated that except for palmitoleic acid and linolenic acid, the changes in other fatty acids were not significant (Koç Güler et al., 2017b); palmitic and linoleic acid contents during storage were changed significantly, palmitic acid was higher than at the beginning of storage, and no

significant differences were found for other fatty acids (Koyuncu, 2004); in 'Tombul' cultivar, palmitic acid contents increased at the end of storage but this was insignificant (Koyuncu et al., 2005); palmitic acid, stearic acid, oleic acid significantly increased during storage in the 'Tombul' cultivar (Karaosmanoğlu & Üstün, 2019); fatty acids have not been changed during hazelnut storage up to 12 months (Momchilova et al., 2017); the palmitic and stearic acids increased at the end of the storage time, while the oleic, linoleic, linolenic decreased (Ghirardello et al., 2013); while oleic acid content decreased with fluctuation during storage, linoleic acid, palmitic and stearic acid increased with fluctuation (Turan, 2018; Turan & İslam, 2019). In our study, the results of the change of palmitic acid during storage agree with the literature findings.

#### 4. Conclusions

In this study, physicochemical changes during storage for 12 months in hazelnuts which irrigated at the pre-harvest period, when the cluster drops were the highest especially due to drought were investigated. In conclusion, it can be said that the variation of moisture and ash values according to the application was significant and lower in irrigated samples; the variation of oil content according to the application was significant and higher in irrigated samples; the changes in protein, rancidity, peroxide and vitamin E according to the application were not significant; one of the major fatty acids, only the change of palmitic acid according to the application was significant and it was higher in irrigated samples; the other composition was not significantly affected in general, and this situation may differ from year to year.

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## Decision-Making Process for Vegetable Production: The Case of Bafra Plain, Turkey

Nur İlkey ABACI<sup>1\*</sup> Kürşat DEMİRYÜREK<sup>1</sup>

<sup>1</sup>Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Economic, 55139, Samsun

\*Corresponding author's email: [ilkaysonmez55@gmail.com](mailto:ilkaysonmez55@gmail.com)

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**Abstract:** The purpose of this study is to reveal the decision-making process for vegetable production. Semi-structured interviews were conducted with selected vegetable growers, using the theoretical sampling method in Bafra plain of Samsun province, Turkey. The quantitative data were analyzed through descriptive statistics and qualitative data on the decision-making process were analyzed through content analysis. The vegetable growers generally make decision in two stages: thinking and implementation. In the thinking stage, they evaluate encouraging factors (experience, product characteristics, the availability of resources (land, labor, capital, and managerial skills), the social environment, positive trends in product prices, a desire to produce, rural dependence, customer demands and a desire to earn money) and the restricting factors (debts, pests, and diseases, the cost of products, weather conditions, unsuccessful experiences, negative perceptions towards specific products, the neighborhood of the land, lack of a sustainable contract with commissioners and limited terms for leasing land). In the decision-thinking stage, the decision-makers evaluate what decisions they want to make and then implement production plans. In the thinking stage, the growers need data on factors affecting production patterns. In economic theories, the farmer is believed to be only a rational entity that tries to maximize profits. However, this research shows that farmers' economic decisions are not always taken as rational but also behavioral. The research results showed that not only the price but also human behavior should be taken into consideration in the decision-making process.

**Keyword:** Decision, Decision-Making Process, Product Pattern, Qualitative Data

### Sebze Üretimine Karar Verme Süreci: Bafra Ovası Örneği, Türkiye

**Öz:** Bu çalışmanın amacı, sebze üretimine karar verme sürecini ortaya koymaktır. Samsun ili Bafra ovasında teorik örnekleme yöntemi kullanılarak seçilen sebze yetiştiricileri ile yarı yapılandırılmış görüşmeler yapılmıştır. Nicel veriler betimsel istatistiklerle, karar verme sürecine ilişkin nitel veriler ise içerik analiziyle çözümlenmiştir. Sebze yetiştiricileri genellikle iki aşamada üretim kararı vermektedir: düşünme ve uygulama. Düşünme aşamasında, teşvik edici faktörleri (deneyim, ürün özellikleri, kaynakların mevcudiyeti (arazi, emek, sermaye ve yönetim becerileri), sosyal çevre, ürün fiyatlarındaki olumlu eğilimler, üretme arzusu, kırsala bağımlılık, müşteri talepleri ve para kazanma arzusu) ve kısıtlayıcı faktörleri (borçlar, zararlılar ve hastalıklar, ürünlerin maliyeti, hava koşulları, başarısız deneyimler, belirli ürünlere yönelik olumsuz algılar, arazi komşuluğu, komisyoncularla sürdürülebilir bir sözleşmenin olmaması ve arazi kiralama için sınırlı koşullar) değerlendirmektedirler. Karar verme aşamasında, karar vericiler hangi kararları almak istediklerini değerlendirmektedir ve ardından üretim planlarını uygulamaktadır. Düşünme aşamasında yetiştiriciler üretim modellerini etkileyen faktörler hakkında verilere ihtiyaç duymaktadırlar. İktisat teorilerinde çiftçi, yalnızca karını maksimize etmeye çalışan rasyonel bir varlıktır ancak bu araştırma, çiftçilerin ekonomik kararlarının tamamen rasyonel olmadığını aynı zamanda davranışsal da olduğunu göstermektedir. Araştırma sonuçları, karar verme sürecinde sadece fiyatın değil, insan davranışlarının da dikkate alınması gerektiğini göstermektedir.

**Anahtar Kelimeler:** Karar, Karar Verme Süreci, Ürün Deseni, Kalitatif Veri

#### 1. Introduction

People have to consume nutrients in order to survive. Agriculture is necessary to produce food resources. For a smooth food supply for present and future generations, agriculture needs to be sustainable (ul Haq et al., 2020). Therefore, careful decisions should be made for agricultural production. However, the decision-making environment in agriculture has a complex structure. Many researchers believe that farmers take into account mostly economic factors (such as the price of products, input prices, the cost of products, capital status, etc.) in agricultural activities. Öhlmer et al. (1998) proposed to include the case of a farmer's agricultural decision-making in the

agricultural decision process due to farmers socio-cultural and personality characteristics.

Decision-making means choosing the most suitable one among the available alternatives. Farmers may prefer products that they have never grown regardless of the land conditions and the suitability of the operating characteristics, because of their personality traits. Farmers do not just decide on new technologies or practices emerging in the agricultural sector; rather, they decide which product will be the most suitable crops for their farm properties and which product will be produced. In addition, depending on the

characteristics of the season they can produce a wide variety of vegetables.

"The process" refers to the stages of the event, not to the outcome of any event, until the event takes place. Therefore, when the decision-making process is mentioned, this refers to the stages that are passed until the decision event is realized. However, most research on the decision-making process focuses not only on the process itself, but also on the final decision as a result of the process (Alorcon et al., 2013; McDonald et al., 2016).

Generally, some studies on decision-making are particularly concerned with the role of women farmers in farm management and how decisions are made within the family (Tsegaye et al., 2012; Sarma and Payeng, 2012; Kutlar et al., 2013; Sucharita and Bishnoi, 2016; Chayal et al., 2017; Mittal et al., 2018). Several studies have been dedicated to determining the factors that affect farmers' land consolidation decisions, agricultural insurance decisions, production branch preferences, input usage decisions and product production decisions (Aydın et al., 2016; Günden 2016). However, the researchers have failed to determine the processes that the farmers go through until they make the decision and the factors that are affected (restricting and encouraging) during the stages of the process. Furthermore, most of researchers in Turkey have focused on the decision and the results of

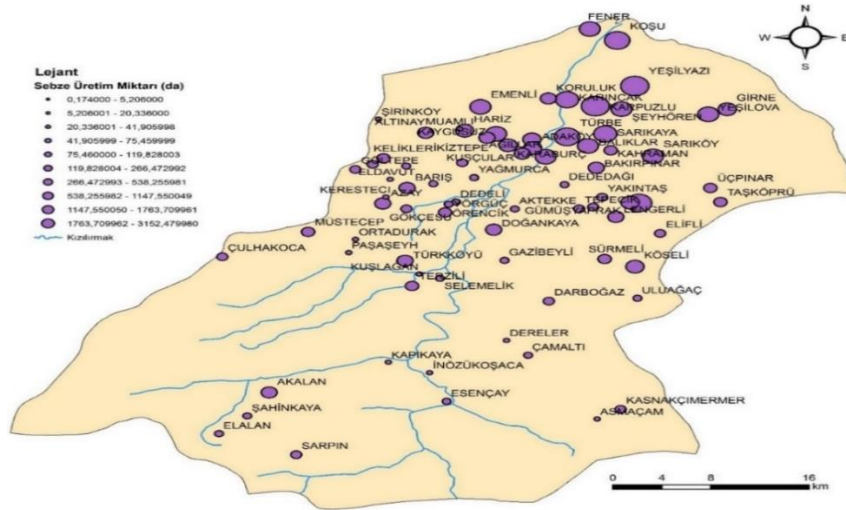
the decision rather than determining the types of decision-making of farmers.

Consequently, the researchers examining the decision-making process of farmers are limited. However, the decision-making behavior of farmers and the results of their decisions are closely related to the success of their businesses. To help farmers make better decisions, the decision-making process needs to be thoroughly understood. Therefore, the aim of this study is to reveal the decision-making process of the product pattern of farmers especially in the field of vegetable growing, where the number of varieties is high.

## 2. Materials and Methods

### 2.1. Research area and data collection

Samsun is one of the provinces that has the highest vegetable production in Turkey with 1.162.037 ton (TSI, 2017). The Bafra district, which was selected for the research area, is the county that produces the most vegetables in the province of Samsun. In 2017, 539,017 tonnes of vegetables were produced from 151,626 decares of land. 34,587 ton of this production included roots and tuberous vegetables (TSI, 2017). Therefore, this research aims to determine how vegetable growers in the Bafra district of Samsun decide among various products.



**Figure 1.** Map of villages included in the scope of sampling  
*Şekil 1. Örnekleme kapsamına alınan köylerin haritası*

The vegetable farmers in 81 villages of Bafra district constitute the sampling unit of the research. A map was created by using the ArcGIS 9.3 program to select the villages that can represent the research area

(Figure 1). The amount of vegetable production produced in all villages on the map has been visualized with the help of this program and the the villages



where the production was intense were selected in accordance with the purpose.

Semi-structured interviews were conducted using the theoretical sampling method. Theoretical sampling is a concept that was introduced by Glaser and Strauss in 1967. When the researcher collects data, the resulting concepts and processes can decide that they have reached an adequate number of data sources when they start to repeat each other (Yildirim and Simsek, 2013). According to the theoretical sampling method, 20 face to face in-depth interviews in Bafra region were found to be sufficient. The farmers should be recorded in order to ensure that the content analysis was correctly implemented. An ethics certificate was obtained from the Social Sciences Ethics Committee of Ondokuz Mayıs University (Decision No: OMU KAEK 2015/340) to record the interviews. In-depth interviews were performed with up to two farmers per day. In addition, necessary care was taken to create an environment where farmers could express themselves comfortably, and notes were carefully taken into the interview notebook according to the code of ethics.

## 2.2. Data analysis methods

The analysis of the data was implemented into two stages. In the first stage, the socio-economic characteristics of vegetable growers were examined, and in the second stage, the farmers' decision-making process was analyzed.

The socio-economic characteristics of the vegetable growers was analyzed by descriptive statistics. The analysis was made with the SPSS 21.0 package program. The qualitative data analysis was then used to reveal the decision-making process. The qualitative data obtained in-depth interviews with farmers are shown in Figure 2. First of all, digital voice recordings were transcribed. Secondly, the notes and voice records during the semi-structured interviews were combined and meaningful data were obtained. Then the coding phase was started. Coding stages, ensured defining the concepts and theme words provided a better understanding of the analysis. Coding is the process of naming meaningful sections (words, sentences, paragraphs, etc.) among the data obtained. The coding process requires sections to be subsections, examined, compared and correlated (Yildirim and Simsek, 2013). The concept is the meaning given to meaningful sections and events in the data. Concepts form the basic analysis units in content analysis. Theme (category) is the classification of the concepts obtained in the content analysis under a specific theme.

The categories or themes are more abstract and general than the concepts obtained in the content analysis (Collins, 1999). As a result of examining the concepts, the relationships with each other were revealed and these relations were explained with a higher-level theme. NVIVO package program was used for coding qualitative data and creating figures.

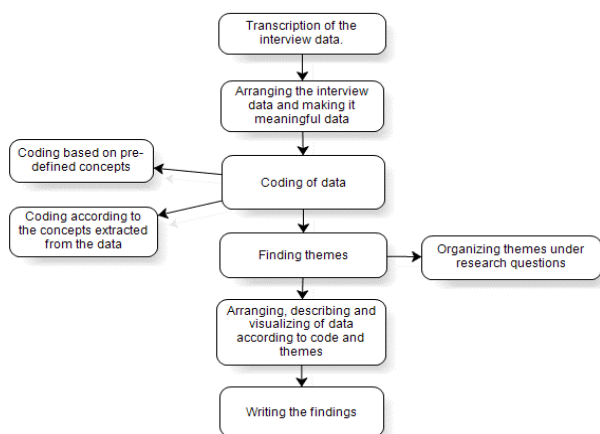
## 3. Results

### 3.1. The socio-economic characteristics of farmers

The average age of the farmers interviewed was 43 (between 32 and 57). When farmers' education distributions were examined; it was determined that 55% were primary school, 20% a middle school and 15% were high school graduates. The interviewed farmers had an average of 24 years (3 to 47 years) of agricultural experience. It was determined that 5% of the farmers owned between 1 and 49 decares, 40% of them 50 and 99 decares and 55% of them more than 100 decares. In the research, only the farmers who produce vegetables (50%) and those who carry out other agricultural business activities besides vegetable farming (50%) are equals. While half on the farmers produce only vegetables, the other half carry out other agricultural activities in addition to vegetable production. However, the business activity was mainly done with vegetable production. It was determined that 55% of the farmers had also non-agricultural income from trade or other source of revenues. In addition, it was determined that 70% of the farmers kept records abouted their sowing dates, yields, expenses and inputs of their products by taking notes on the calendar or by writing on their agendas. 70% of farmers were members of agricultural organizations such as vegetable producers association and agricultural credit cooperatives. It was seen that important decisions within the enterprise were made by the head of the male household, mostly (50%) in consultation with their families. In addition, 35% of them made their decisions with expert advice, while 15% made solely on their own decisions.

The farmers produced at least 3 and at most 11 kinds of products and with an average of 6 kinds of products. Farmers allocated 73% of their land to field crops and 27% to vegetable production in the summer period. The vegetable farmer preferred to produce watermelon with a rate of 59% in summer. Watermelon is followed by red pepper (19%), melon (17%), tomato (2%) and other vegetable products, respectively. The top three field crops farmers grown

the most were listed as 57% paddy, 21% wheat, and 13% corn. As winter crop, farmers produced vegetables on 96% of their lands and field crops on 4%. Farmers cultivated cauliflower on 42% of their land, red cabbage on 21%, white cabbage on 19%, broccoli on 13% and other vegetable crops on 5% as winter vegetables. Silage corn was the most grown product as field crop grown for winter.



**Figure 2.** The steps followed in the content analysis  
**Şekil 2.** İçerik analizinde izlenen adımlar

**3.2. An overview of the decision making process of farmers in the research area**

The production patterns of the farmers were shaped according to their habits. However, the production decisions of farmers were made through a quite complicated, dynamic and challenging process. Farmers have to take into account all possible risks that they may encounter before planting the seeds, and must act accordingly. Every production’s dynamic is different. Even if experience, habit and desire to make money seem to be at the forefront, motivation and the psychological state drive farmers to think for their decisions. Especially for farmers who produce vegetables, internal drift, in other words, the desire to act according to their feelings can be quite high. Research conducted by behavioral economists also confirms this conclusion. According to behavioral economists, the economic behavior of people, psychological and sociological factors should be included in economic data because people can reject material inferences due to various psychological reasons such as uncertainty, risk, fear of losing, and desire to gain reputation (Can, 2012). The most important determinants of the decision-making process for farmers in the research area were brokers, input dealers, and other farmers. They were very influential in the thinking phase. Farmers mainly advised and

obtained information from these people. They considered the demand for products from which pesticides are produced, the number of seed/seedling orders from seed and seedling dealers, and the products and prices made by the brokers in Bafra plain and other provinces. In planning the production, farmers generally decide the pattern for the next two years. This is because of crop rotation requirements.

Other factors were the duration for renting contract and the landowners possible interference with the product pattern. Farmers have been following a different product that has not been in production patterns for years. In the first year of growing a different product, if a farmer gets less than he expected then he replaces it, and may abandon their previous decision. There were many varieties of vegetable products, including early and late species. Some farmers keep records and follow the previous years’ information about what kind of vegetables on which periods and which varieties of species grown according to this situation. The interviewed farmers did not take into account the risks such as weather conditions because they had already accepted due to their belief (i.e. fatalism by Rogers (1995)). When the farmers decided, they did not consider the habits or prices of the previous year. Particularly, they consider which crops, how much area other farmers will produce in the following year and the decision on how much of the chosen products they decided to grow rather than how they choose the products. The behavioral characteristics and the size of the enterprises, affect the decision-making strategies. As a result of the analysis between the decision-making styles of the farmers and the size of the enterprises, this relation is confirmed statistically significant (Abaci, 2018). Small-scale agricultural enterprises decided to grow the products sold at a high price in the previous period to earn a high income, in other words, they considered about the prices of the previous year. This is confirmed by Cobbweb theorem. However, farmers who had large-scale enterprises generally apply an inverse strategy compared to small enterprises. They think that a product with a higher price in the previous year will be produced by other farmers and will suffer from production surplus, therefore they either reduce production of the product or give up the product altogether. Particularly, they consider which crops, how much area other farmers will produce in the following year. At the same time, the decision on how much of the chosen products they decided to grow, rather than how they choose the products.

### 3.3. Stages of the decision-making process of farmers

In this section, farmer decision making processes was modeled and the affecting factors on decision making were presented. Interview data were coded for the modeling and the decision-making process. Codes are grouped into main categories. Figure 3 shows the process of decision-making in Bafra district consists of two stages: (1) Thinking Stage and (2) Action Stage. Farmers assessed production patterns for the following year while evaluating their conformity to the products they intended to produce in general. This happens through thinking, which is a result of the mental activities. Therefore, the first stage is the "decision-

thinking stage." If farmers think that they can overcome the constraints, they start to prepare their minds into the next stage. This part of the process is expressed as the stage in which the farmers implement their product choices.

The stage in which farmers apply their product pattern decisions is the stage in which they prepare the soil for the product of their choice and make seedling/seed orders. The farmers then plant the crops and receive the results of their decisions at harvest time. Therefore, the most important decision stage is the thinking stage. In this stage, farmers make all their calculations about the products they will produce and make their decisions accordingly. After making their decisions, they can not undo them.

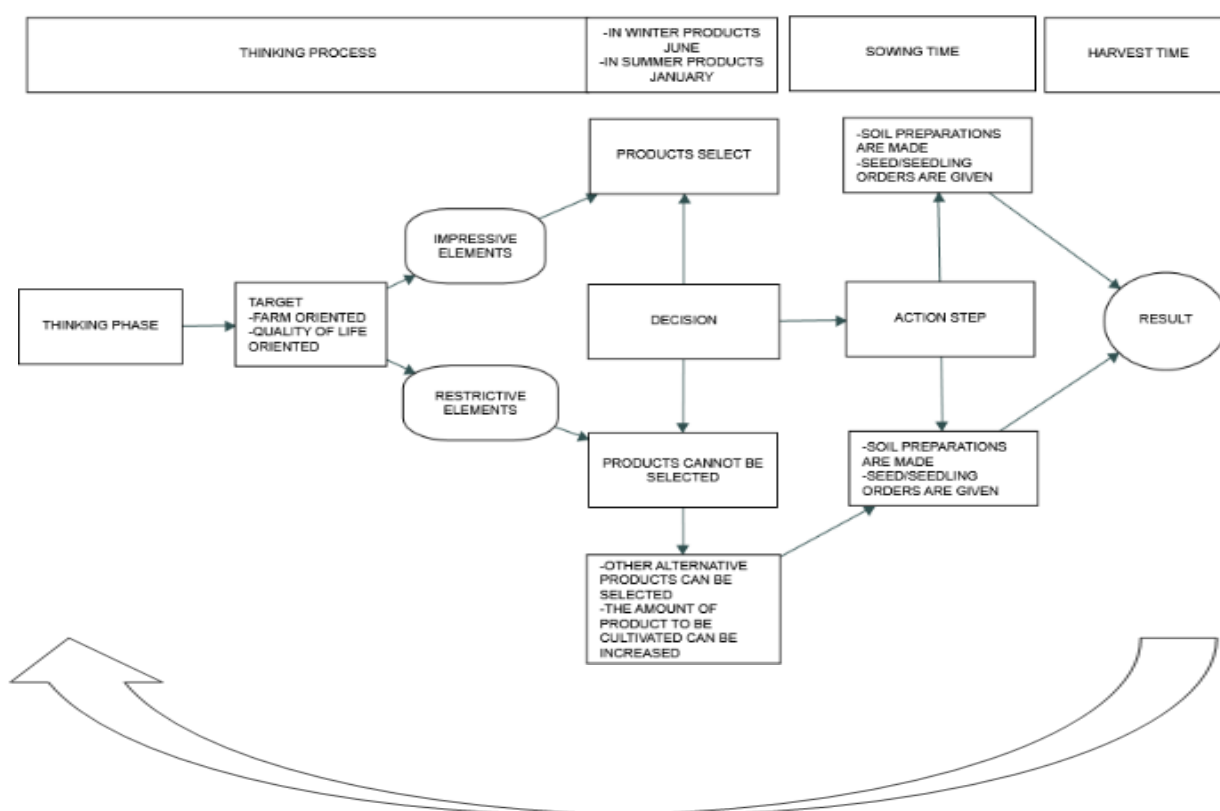


Figure 3. Farmers' decision-making process (Abaci, 2018)

Şekil 3. Çiftçilerin karar verme süreci

### 3.4. Decision thinking stage

In the decision thinking stage, decision-makers evaluate what decisions they want to implement and perform production planning. They consider various elements in the evaluation process. Some of these elements are encouraging farmers while others restrict. For this reason, these elements are described in detail in order to understand what the farmers are experiencing during the first stage of the decision-making process. It should also be noted that the

findings obtained in this section may differ depending on the research area. For this study, the research area is a plain, and the farmers who perform their production in this plain have not mentioned certain factors such as irrigation availability and organizational infrastructure because they do not have problems with these factors. Therefore, these factors are not included in the constraints of the study. It should be stated that the farmers in the research area start to make research about the products that they will produce in the

following season from the production period to the end of the harvest. During the interviews, the farmers were asked how they chose the products they included in the

product designs, and the factors that motivate and restrict farmers are categorized according to this data (Table 1).

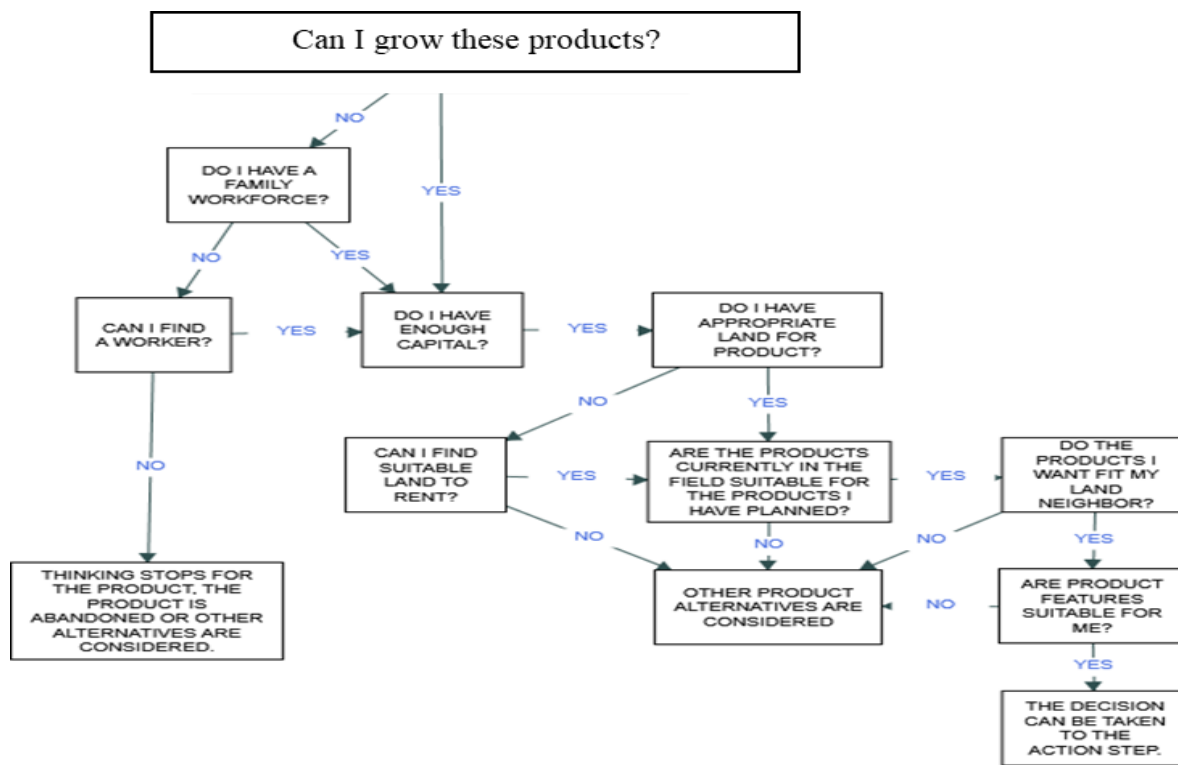


Figure 4. The decision thinking process of farmers (Abaci, 2018)

Şekil 4. Kararı düşünme süreci

Table 1. Factors affecting farmers in the thinking phase

Çizelge 1. Çiftçileri düşünme aşamasında etkileyen faktörler

Encouraging elements (+)	Restrictive elements (-)
<b>Agricultural experience</b>	<b>Debts</b>
<b>Source presence</b>	<b>Diseases and pests</b>
Land, labour, capital, managerial skills	
<b>Positive trends in product prices</b>	<b>Costs of products</b>
<b>Product features</b>	<b>Weather conditions</b>
Resistance to diseases	Climate change
Varieties with short harvest times	
Ease of harvesting,	
Products that require less labour and workforce	
Ease of marketing	
Products giving two crops in one year	
<b>Social environment</b>	<b>Failed experiences</b>
Final decision-makers (brokers, dealers selling pesticides, seedlings and seed sellers, other farmers)	
<b>The desire to produce and love for the village</b>	<b>Negative perception against the product (Intuitions)</b>
<b>Customer requests</b>	<b>Lease period of land.</b>
<b>The desire to make money</b>	<b>Neighbourliness</b>
	<b>The lack of a fixed broker.</b>

### 3.6. The decision implementation stage

If there is no restrictions, the decision-makers will arrange the planting date. During this time, they prepare the soil and make seedling/seed orders. The farmers who have limited cash have difficulty in orders from dealers. The farmers who order late will have to sow the field late. If such constraints occur, farmers can sometimes abandon their decisions to produce

certain products and may prefer to increase the area for other products. In this way, the decision-making process can continue after the decision stage. If farmers considered to be negatively affected by the products, they can decide not to wait for harvest time. Instead, they try to grow a suitable vegetable variety as a second crop. At the same time, farmers continue the

process by evaluating the decision they made at the time of planting and harvesting.

#### 4. Conclusion

Since some farmers see only vegetable production as a risk in their business, they carry out another income-generating activity alongside vegetables. Variability in vegetable prices, potential plant disease risks, the lack of trust to input dealers, worker dependency and expenses, conservation, and marketing problems are critical very challenging factors for farmers who earn their livelihood only from vegetable production.

In Bafra district, the decision-making process of vegetable producers consists of the process of thinking and applying stages. The product pattern decision is taken at the end of the thinking process, when farmers consider the factors that are encouraging or restrictive. If the encouraging elements are stronger, farmers consider the other features necessary for the product. At the end of the thinking process, the final decision is made. Afterward, the farmers implement the decision. Finally, the farmer obtains the results of the decision at the time of harvest.

In the decision-making process of farmers, actors (brokers, and input sellers, are of great importance and considered as determinants of farmers' decisions. Therefore, the correct orientation by actors will contribute to the diversification and quality of production. Informing these actors will help farmers to obtain the most accurate information before the decision stage, and enable the extension staff to apply the right strategy.

In this study, it is more important to understand how farmers decide on the land size of the products, rather than how they decide on their production patterns. To manage their land as best as possible, farmers should be able to distribute the products they decide to produce and to pay attention to practices such as crop rotation (alternative). Therefore, agricultural advisers should inform farmers about this issue.

This study found that the vegetable farmers in the research area do not act according to the concept of perfect rationality propounded by classic economic theories in their decision-making process, rather they act according to their interests and often make behavioral decisions according to their feelings.

In addition, this study implies that the decisions of farmers cannot be defined as right and wrong. This situation can only be determined when individuals begin to get results after implementing their decisions.

Thus, it is not pragmatic to determine the factors that affect the decisions of farmers. Therefore, decision support systems should be developed to enable farmers to make their decisions most appropriately.

The encouraging and restrictive factors deciding on the production models have a potential impact on the improvement of consultancy services. For this reason, it can be ensured that the decision support systems with these variables are developed and presented to the use of farmers/consultants so that farmers can decide on the optimum production pattern. The limitation in establishing this support system is the difficulty in determining psychological variables. When deciding on a subject, variables such as emotional state of people, influence of the environment in which they grow and live, decision-making styles, and personality traits (attitudes, values they have, etc.) are difficult to identify but extremely important. However, it is thought that this difficulty will be overcome with the expert team of researchers in psychology and sociology.

#### Conflict of Interest

The authors declare that they have no conflict of interests.

#### Acknowledgements

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## Possibilities of Using Poultry By-product Meal Instead of Fish Meal as An Alternative Protein Source in Rainbow Trout (*Oncorhynchus mykiss*, W.) Feeds: Growth Performance and Unit Production Cost

Murat BİLGÜVEN<sup>1</sup>

Mersin University, Faculty of Aquaculture, Mersin

Corresponding author's email: [mbilguven@mersin.edu.tr](mailto:mbilguven@mersin.edu.tr)

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**Abstract :** The way to grow cheaper fish is to use protein sources that can be an alternative to fish meal that is considered on the most expensive feed ingredient in fish feeds. For this purpose, poultry by-product meal (PBM) which is locally sourced and cheaper than fish meal, was used to replace 25, 50, 75 and 100 % of fish meal protein in rainbow trout (*Oncorhynchus mykiss*) poultry by-product meal was not included in the control feed. Feeds in this investigation were prepared as similar protein (43% CP) and digestible energy (13.28 MJ. DE kg<sup>-1</sup>) levels. A total of 300 rainbow trout with 50.6±1.35 g of average initial weight were used in the investigation. All the diets including the control were fed the rainbow trout in triplicate for 70 days. At the end of the trial, the difference between feeds I, II, II, IV and feed V was significant in terms of average live weight gain (ALWG) and feed consumption throughout the investigation (P<0.05). No difference was observed in terms of feed conversion ratio (FCR) and protein conversion ratio (PER). However, considering the cost of producing 1 kg of fish, it is obvious that although the cheapest fish is obtained from the group fed with IV and V feeds (P<0.05), it will take weeks for the fish in these groups to reach the average live weight of groups I, II and III. As a result, it was concluded that poultry by-product meal could be used instead of 75% of fish meal protein in trout feeds in terms of a normal fish development process.

**Keywords:** Rainbow trout, fish meal, poultry by-product meal, growth performance, fattening cost

## Gökkuşacağı Alabalığı (*Oncorhynchus mykiss*, W.) Yemlerinde Alternatif Protein Kaynağı Olarak Balık Unu Yerine Tavuk Unu Kullanma Olanakları: Büyüme Performansı ve Birim Balık Maliyeti

**Öz:** Daha ucuz balık yetiştirilmenin temel yolu, balık yemlerinde önemli bir yeri olan balık unu yerine alternatif olabilecek protein kaynaklarının kullanılmasıdır. Bu amaçla çalışmada gökkuşacağı alabalığı (*Oncorhynchus mykiss*) yemlerinde hem yerli üretimimiz hem de balık ununa oranla daha ucuz olan tavuk unu, balık ununun bir kısmı ya da tamamı (balık unu proteininin % 25, 50, 75 ve 100'ü) yerine kullanılmıştır. Kontrol yeminde ise tavuk unu yer almamıştır. Deneme yemleri benzeri protein (%43 HP) ve enerji (13.28 MJ SE.kg<sup>-1</sup>) içeriğine sahip olarak hazırlanmıştır. Ortalama 50,6±1.35 g olan 300 adet balığın kullanıldığı deneme 10 hafta sürmüştür. Deneme sonunda, I, II, II, IV nolu yemler ile V nolu yem arasında besi boyunca ortalama canlı ağırlık artışı (OCA) ve yem tüketimi açısından bulunan farklılık önemli olmuştur (P<0.05). Yemden yararlanma oranı (YYO) ve proteinden yararlanma oranı (PYO) söz konusu olduğunda ise farklılık gözlenmemiştir. Ancak 1 kg balık üretme maliyetine bakıldığında en ucuz balığın IV ve V nolu yemler ile beslenen gruplardan elde edilmesine (P<0.05) karşın bu gruplardaki balıkların I, II ve III nolu grupların ortalama canlı ağırlığına ulaşmasının haftalar alacağı aşıkardır. Sonuç olarak normal bir balık gelişimi süreci bakımından tavuk ununun alabalık yemlerinde balık unu proteininin % 75'i yerine kadar kullanılabileceği sonucuna varılmıştır.

**Anahtar Kelimeler:** Balık unu, Gökkuşacağı alabalığı, tavuk unu, büyüme performansı, besi maliyeti

### 1.Introduction

Adequate and balanced nutrition is a serious concern, especially in countries where the population increasing rate is high and the social income balance is not good, and this unfortunately will be a predominant issue the future. In this respect, it is an important issue to make the animal protein sources for human consumption economically is a trend topic producing globally. Fish consumption, on the other hand, is taken into account in terms of both healthy nutrition and the potential to be obtained cheaply. Protein is the most

essential nutrient in fish feeds. Fish meal is widely used in aquafeeds specifically for farmed carnivorous fish species. However, despite the increasing demand for fish meal, the shrinkage observed in production every year and the price fluctuation accordingly, due to the different quality caused by the fish it is produced and the production methods, alternative protein sources have been sought.

Poultry by-product (PBM) meal is a feed ingredient that is similar to fish meal in terms of protein digestibility (94% and 98; Hardy, 2000). It can be said



that the use of PBM in fish feeds will increase gradually, since it is domestically produced and close to fish meal in terms of nutrients. Methionine level is higher in PBM than fish meal. In terms of other essential amino acids, the values contained in PBM are very close and balanced to that of fish meal. The high crude fat (CF) content of PBM is also remarkable in terms of salmon and trout feeds, which have high energy content. Although PBM has a good place as a protein source in fish feeds, its quality varies depending on the essential amino acid profile it contains (Davies et al., 1991). In general, PBM contains 55-65% CP, 14-30% CF, 12-21% ash, and the average nutrient content is presented in Table 1 with fish meal (FM), meat meal (MM), meat-bone meal (MBM), blood (BM) meal and hydrolyzed feather meal (HFM) comparatively.

Along with the prohibitions that started in the European Union (EU) countries, within the scope of the harmonization framework with EU laws, important restrictions have been introduced on the inclusion of

animal protein sources in the feed of especially terrestrial farm animals. This is due to the prevention of the spread of certain diseases in recent times. There is no such problem for PBM, in which high temperatures are used in its production. (Özaslan, 2004). However, the extruded pellet making process also requires high temperature application.

Since fish meal, which is widely used in fish feeds, is unfortunately not produced enough in Turkey, a large part of the need is met through imports. However, chicken production, chicken meat consumption per capita and, accordingly, PBM production are increasing year by year in our country. In this context, the use of animal protein in feeds used in chicken production is prohibited within the framework of the European Union (EU). If this practice, which is called a feed ban in Turkey within the scope of harmonization with the European Union, comes into effect, its impact on poultry meat integration will also have significant and serious economic dimensions.

**Table 1.** Average Nutrient Composition of Different Animal Protein Sources, % (Bilgüven, 2002).

**Çizelge 1.** Farklı Hayvansal Protein Kaynaklarının Ortalama Besin Madde Bileşimi, % (Bilgüven, 2002).

Ingredient	PBM	FM	MM	MBM	BM	HFM
Moisture	7.0	8.0	6.0	7.0	9.0	7.0
Digestible Energy (DE), kcal/kg	3916	3567	3311	3233	3599	3882
Crude Protein	58.7	60.0	51.4	50.4	85.6	84.9
Crude Fat	13.1	10.0	9.1	9.7	1.3	2.9
Calcium	3.51	6.25	8.85	10.3	0.48	0.25
Phosphorus	1.81	0.59	4.44	5.1	0.24	0.65
Sulfur	0.52	0.12	0.47	0.25	0.34	1.47
Arginine	3.77	4.0	3.6	3.49	3.57	7.05
Histidine	1.01	1.4	0.96	0.96	5.14	0.99
Isoleucine	2.38	2.6	1.75	1.64	0.9	4.06
Leucine	4.0	4.8	3.19	3.06	10.91	6.94
Lysine	2.89	1.5	3.23	2.9	7.4	2.32
Methionine	1.06	2.4	0.7	0.65	0.87	0.55
Phenylalanine	1.84	2.5	1.81	1.7	5.85	3.05
Threonine	1.94	0.7	1.64	1.65	3.62	3.97
Tryptophan	0.46	0.3	0.34	0.3	1.04	0.52
Valine	2.89	4.5	2.52	2.45	7.48	6.48

In a study conducted by Yiğit et al. (2006) with Black Sea turbot (*Psetta maotica*) fry (30 g), It was concluded that instead of fish meal, poultry by-product meal can be used at a rate of 25% instead of fish meal protein without any negative effect on growth and feed intake.

Yanik and Aras (1999) reported that 25-50% of poultry by-product meal can be substituted for fish meal in trout feeds.

Yones and Metwall (2015) fed juvenile Nile tilapias (*Oreochromis niloticus*) with feeds used 50, 75 and 100% PBM instead of fish meal and containing %30 CP. PBM was not included in the control feed. No difference

was found in terms of final live weight, body weight gain, feed conversion ratio (FCR) and protein efficiency ratio (PER) and specific growth rate, so researchers have reported that up to 100% PBM can be used in the feed of juvenile Nile tilapia.

Chaklader et al. (2020) in their study conducted with an average of 3.58±0.01g Asian sea bass (*Lates calcarifer*) were fed in recirculated sea water tanks for 6 weeks with two different feeds containing 48% CP and 13% CF, in which PBM was not included or 100% contained. At the end of the trial, the final live weight and specific growth rate (SGR) and feed conversion



ratio (FCR) were significantly lower in the group fed with PBM substituted for whole fish meal ( $P < 0.05$ ).

Fish meal is widely and intensively used in the feeds of carnivorous fish and is the most important factor in the cost of fish feeds due to its importation and international exchange rate fluctuations. Poultry by-product meal, on the other hand, is a domestic product whose production is gradually increasing due to the increase in chicken consumption. Therefore, in this trial, it was investigated how much of the PBM can be used as a substitute for fish meal and how it affects the 1 kg feed and fish production cost.

## 2. Materials and Methods

The trial was carried out in a private trout farm in Mersin Elvanlı region. Trial chambers formed with 1 cm mesh stretching on a plastic pipe skeleton of 1x1x1m dimensions were placed in the pool allocated for the trial. 25 fish with an average weight of  $50.6 \pm 1.35$  g were stocked in each compartment and each of the experimental groups was arranged in 3 parallels.

The feeds to be used in the research were made in the feed application unit of Mersin University Faculty of Fisheries. Poultry by-product meal protein was used to replace 0, 25, 50, 75 and 100% of dietary fish meal protein in the experimental feeds, respectively and all the feeds were prepared as isocaloric (13.28 MJ DE kg<sup>-1</sup>) and isonitrogenic (43% CP) and were pelleted in 2 mm diameter and 6 mm length. The feed ingredients to be used in the feed production process were first ground to medium fineness (max. 0.2 mm). Trial feeds were pressed through discs. After feed production, trial feed samples were analyzed for nutrient content. The structure and nutrient analysis of the feeds used in the study are presented in Tables 2 and 3.

Fish were fed twice a day at saturation (*Ad libitum*) level and weighed every two weeks during the experiment. For this purpose, the fish were starved the day before. After these processes, the feeds were also weighed, and the amount of feed consumed in the said period was calculated. Dead fish were recorded regularly, and these individuals were taken into account when calculating feed conversion. Feeding was done slowly and carefully, and it was assumed that all the feeds given were consumed. The trial was completed in 70 days. In the weekly measurements of the water brought to the trial area through open channels, the water temperature was found to be between 12.4-14.8 °C throughout the experiment and the pH was measured as 7.8.

**Table 2.** Content of Trial Feeds, %

**Cizelge 2.** Deneme Yemlerinin İçeriği, %

Ingredient	Trial Feeds				
	I	II	III	IV	V
Fish Meal	36.0	27.00	18.00	9.00	0
Poultry By-product Meal	0.00	9.00	18.00	27.00	36.00
Meat-Bone Meal	5.00	5.00	5.00	5.00	5.00
Soybean Meal	20.00	20.00	20.00	20.00	20.00
Corn Gluten	11.00	11.00	10.00	10.00	10.00
Wheat Middlings	23.75	24.35	26.05	26.50	27.05
Red Pepper Powder	1.00	1.00	1.00	1.00	1.00
Fish Oil	2.00	1.50	1.00	0.50	0.00
Vitamin Mix. <sup>a</sup>	0.25	0.25	0.25	0.25	0.25
Mineral Mix <sup>b</sup>	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.10	0.10	0.10	0.15	0.20
L-Lysine	0.15	0.15	0.15	0.15	0.15
Pellet Binder	0.5	0.40	0.20	0.20	0.20
<b>TOTAL</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>

<sup>a</sup>Vitamin Mixture (1 kg): Vitamin A: 8.000.000 IU, Vitamin D<sub>3</sub>:800.000 IU, Vitamin E: 80.000 mg, Vitamin K<sub>3</sub>:4.800 mg, Vitamin B<sub>1</sub>:8.000 mg, Vitamin B<sub>2</sub>:12.000 mg, Vitamin B<sub>6</sub>:8.000 mg, Vitamin B<sub>12</sub>:20 mg, Vitamin C: 80.000 mg, Niacine: 80.000 mg, Pantothenic acid: 20.000 mg, Folic acid: 2.400 mg, Biotin: 200 mg, Inositol: 120.000 mg.

<sup>b</sup>Mineral Mixture (1 kg): Ca: 672.000 mg, Mg: 16.000, Mn: 24.000 mg, Zn: 32.000 mg, Fe: 24.000 mg, Cu: 2.000 mg, Co: 800 mg, I: 400 mg, Se: 80 mg.

AOAC (1995) standard analysis methods were applied to determine the nutrient composition of the feed and feed ingredients used in the research. Chloroform:methanol extraction (2:1, v:v) was used for crude oil analysis (Bligh & Dyer, 1959).

At the end of the experiment, live weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and feed and fish production cost were calculated. PER, which expresses the ratio between the protein consumed and the weight gain of the fish, was calculated according to the following formula (Howe et al., 1965):

$$PER = \frac{\text{Live Weight Gain (g)}}{\text{Consumed Protein with the diet (g)}} \quad (1)$$

The determined PER value takes into account the crude protein in the feed. In this case, errors that may occur as a result of these changes in fish feeds, which may have different moisture content, can also be corrected.

Protein efficiency rate (PER) reveals how the protein consumed with feed in a certain period is reflected in the live weight gain of fish. The higher this figure, the higher the protein's evaluation. In other words, as the weight gain and feed conversion ratio increase, the PER value also increases. (Steffens, 1989)

**Table 3.** Nutrient Composition of Trial Feeds, %**Çizelge 3.** Deneme Yemlerinin Bileşimi, %

Trial Feeds	Moisture	Ash	Crude Protein	Crude Fat	Crude Cellulose	Nitr.Free Extract	Digestible Energy (MJ kg <sup>-1</sup> )
I	8.57	9.95	43.43	8.08	2.61	27.36	13.22
II	9.56	9.34	42.88	8.13	2.88	27.21	13.26
III	8.15	8.73	43.49	7.80	2.84	28.99	13.28
IV	8.08	8.11	43.38	7.67	2.95	29.81	13.30
V	9.01	7.50	43.51	7.45	3.08	29.45	13.32

The wholesale prices in 2022 of the feed ingredients used in trial feeds was determined, then the feed price was calculated by multiplying the feed price with the feed conversion ratio. While expressing the unit fish cost, only feed ingredient prices were taken into account and no other inputs were calculated. The research was carried out in accordance with the "Random Blocks Trial Design" and the SPSS (V.26) program (Anonymous, 2021) was used in the statistical evaluation of the findings collected during the study (Turan, 1995). The groups were compared at the 1% and 5% significance level. (Steel & Torrie, 1981). There is no need for an ethics committee permission report, since no attempt was made on the fish during the experiment.

All fish handling procedures complied with Turkish Ethical guidelines for animal care (No. 28141) set by the Ministry of Food, Agriculture and Livestock, and this study was carried out with the approval of the Mersin University Animal Experiments Ethics Committee (HADYEK) dated 08.02.2021 and number of decisions 06.

### 3.Results and Discussions

At the end of the experiment, average live weight gain (ALWG), feed conversion ratio (FCR), protein efficiency ratio (PER) values were determined as growth parameters. The results obtained from this study was summarized in Table 4 and Figure 1..

The best average live weight gain of  $77.9 \pm 6.10$  g was obtained from the group fed with feed no. I. This was followed by Groups II, III, IV and V respectively, and The difference between the first 4 groups (I, II, III, IV) and the last group (V) was found to be significant ( $P < 0.05$ ). Group V fish consuming the fishmeal-free feed showed the lowest average body weight gain.

In the experiment conducted by Sevgili and Ertürk (1992) to investigate the most appropriate rate of addition to rainbow trout feeds, the researchers stated that there was no difference in live weight gain between the trout fed with feeds containing up to 20% of PBM and the control group without PBM and also reported that up to 80% of the feed protein can be obtained from this source and attention should be paid to the amino

acid levels in PBM.

Gümüş and Aydın (2013) conducted a study with carp (*Cyprinus carpio*) fry with a initial weight of 0.39 g PBM was included in the feeds used at the rate of 25, 50, 75 and 100 %, and it was not used in the control feed. Fish were fed diets containing 34% CP, 9% CF and 15 MJ DE kg<sup>-1</sup> for 13 weeks. Methionine and lysine have also been added to the feeds in order to provide a better essential amino acid profile. In parallel with the increase in PBM in trial feeds, a decrease in protein utilization and specific growth rate (SGR) was recorded. ( $P < 0.05$ ). No significant difference was observed in body composition.

Wang et al. (2006) used 30 and 50% of PBM instead of fish meal in *Nibea miichthioides* feeds grown in floating net cages and did not find any significance between the groups in terms of final live weight, specific growth rate, feed conversion rate and feed consumption in their research lasted for 8 weeks.

In another study in which 14.1% and 70.2% PBM and hydrolyzed feather meal were substituted for fish meal, Gouveia (1992) reported that body weight gain, feed conversion, specific growth (SGR) and protein efficiency (PER) ratio increased compared to the control group and concluded that chicken meal as a protein source can be included in rainbow trout feeds at a level of 80%.

Findings from the trial showed that PBM protein can be used as a substitute for up to 75% of fish meal protein and is in agreement with the findings obtained from various studies on the subject (Gouveia, 1992; Sevgili & Ertürk 2004; Wang et al., 2006, Gümüş & Aydın 2013). Moreover, Yones and Metwall (2015) stated in their study that PBM can replace 100% of fish meal.

In terms of protein efficiency ratio, the difference between Groups I, II and Groups III, IV, V was significant. ( $P < 0.05$ ). The difference between III, IV and V groups for PER of the was similar. In general, PER value rised up due to the increase in live weight gain and decreased in parallel with the increase in PBM protein in trial feeds. This result is in line with the findings of Gümüş and Aydın (2013), who determined that the special growth and protein efficiency rates decreased significantly due to the increase in the ratio of PBM in the feed. This can be explained by the fact that the biological value of protein in PBM is lower than that of fish meal, despite the addition of synthetic methionine and lysine to feeds containing PBM. Similarly, although I. Group consumed the most feed with  $100.80 \pm 4.70$  g during the fattening period, the best feed conversion ratio was found in Group I and the worst feed conversion

ratio in Group V. In other words, although the fish in Group V consumed proportionally more than the fish in the other groups, the body weight gain was not similar.

The lowest cost feed was the feed of Group V with 9.15 TL due to the decrease in PBM, the cost of feed increased and reached 14.55 TL in feed without PBM. In this respect, the lowest cost of 1 kg of fish production (considering only feed input) was in Group V. However, the average body weights of the fish in this group were followed approximately 30 days behind the average body weights of I, II and III. In this case, a choice who the producer has to make between the gain to be made from the difference in feed cost and the interest income that the gain from the fish sales will provide during the compensation period (ie. the time required for the live weight of the fish consuming the cheapest feed to reach the live weight of the fish consuming the most expensive feed), will be correct.

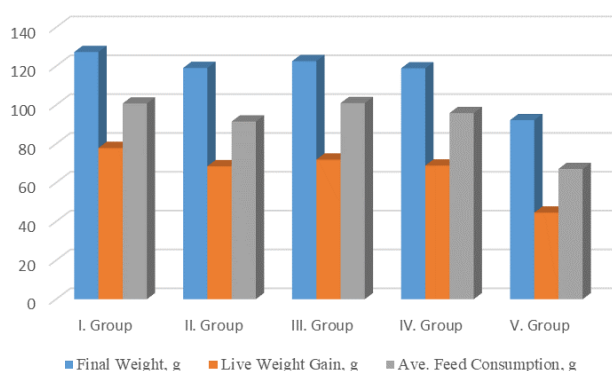
As a result of the exchange rate fluctuations in the price of fish meal in our country, the decrease in the amount of fish to be processed into fishmeal in the world, and the increase in the demand for fishmeal as a result of the expansion of the livestock sector in which fishmeal is used in parallel with the population increase, fish feed is the most expensive feed all over the world. Therefore, studies investigating the possibilities of using feed ingredients, which can be an alternative to fish meal, in feeds maintain their popularity. In parallel with this situation, the fact that chicken meat is cheaper than red meat in our country has enabled the poultry industry to develop rapidly and to be among the few countries in the world in this regard. Therefore, the production of poultry slaughterhouse residue flour has increased from year to year and the use of animal protein sources in farm animal feeds is prohibited or restricted, It also reveals the necessity of using this product more frequently and intensities in fish feeds.

**Table 4.** Results of The Experiment.

**Çizelge 4.** Deneme Sonuçları.

Items	Trial Groups				
	I	II	III	IV	V
Trial Period, day	70	70	70	70	70
Total Fish Number	25	25	25	25	25
Survival Rate, %	84	88	80	92	76
Initial Weight, g	49.40±1.21	50.60±1.40	51.40±1.85	50.10±1.33	51.60±0.69
Final Weight, g	127.30±6.93 <sup>a</sup>	119.11±2.05 <sup>a</sup>	122.60±5.52 <sup>a</sup>	118.90±7.31 <sup>a</sup>	96.22±8.12 <sup>b</sup>
Average Live Weight Gain, g	77.90±6.10 <sup>a</sup>	68.51±3.23 <sup>a</sup>	71.19±6.03 <sup>a</sup>	68.85±7.81 <sup>a</sup>	44.62±8.75 <sup>b</sup>
Average Feed Consumption, g	100.80±4.70 <sup>a*</sup>	91.50±3.43 <sup>a</sup>	100.98±6.67 <sup>a</sup>	95.86±8.90 <sup>a</sup>	67.09±6.62 <sup>b</sup>
Feed Conversion Rate (FCR)	1.29±0.06	1.34±0.04	1.42±0.03	1.40±0.09	1.48±0.12
Protein Efficiency Rate (PER)	1.78±0.07 <sup>a</sup>	1.75±0.05 <sup>a</sup>	1.62±0.04 <sup>bc</sup>	1.65±0.03 <sup>bc</sup>	1.52±0.15 <sup>c</sup>
Feed Cost for 1 kg, ₺	14.55	13.17	11.84	10.47	9.15
Fish Cost for 1 kg, ₺	18.84±0.84 <sup>a</sup>	17.57±0.54 <sup>a</sup>	16.83±0.39 <sup>ab</sup>	14.61±0.94 <sup>bc</sup>	13.87±1.60 <sup>c</sup>

\* The difference between averages with different letters is significant (P<0.05)



**Figure 1.** Final Live Weight, Total Live Weight Gain and Feed Consumption At The End of The Trial, g

**Şekil 1.** Deneme Sonunda Canlı Ağırlık, Toplam Canlı Ağırlık Artışı ve Yem Tüketimi, g

Although the biological value and digestibility of the protein of PBM is lower than that of fish meal, it is a

domestic feed ingredient that can be easily used instead of fish meal if its deficiencies are eliminated. Feeds containing PBM can be produced cheaper than the feeds in which fish meal is used at standard levels, and this type of feed has the potential to be easily used in qualitative restricted feeding techniques used in the planning of regular fish sales of the enterprises. In other words, fish producers who can make their own feed, will be able to make year-round sales planning, that is, weight gain control, by obtaining lower weight fish groups at a lower cost, at the rate that they increase PBM in feeds. As it is known, quantitative restricted feeding technique can increase cannibalism due to the fact that trout are carnivorous fish.

Fish deaths occurred after the weighing periods and no relationship was found with the feed groups.

#### 4. Conclusion

It is thought that this study, which aims to use PBM as a substitute for fishmeal protein, will contribute to reducing the demand for fishmeal, which is mostly met by imports. This study, which deals with poultry by-product meal, which is one of the important and domestic feed ingredient of our country, will bring up new research topics to investigate the possibilities of using PBM not only in trout feeds but also in other fish feeds. It is hoped that this trial will provide new recommendations for reducing, at least partially, the dependence on fishmeal.

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