TOKAT GAZİOSMANPAŞA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ

Tokat Gaziosmanpasa University, Faculty of Agriculture
TOKAT, TÜRKİYE

GAZİOSMANPAŞA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ DERGİSİ

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AMAÇ VE KAPSAM

Gaziosmanpaşa Üniversitesi Ziraat Fakültesinin 1985 yılından beri hakemli v bilimse 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 itibariyle senede 3 baskı yapmıştır.

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 ayrı etik kurul onayı alınmış olmalı ve belgelendirilmelidir. Dergimize gönderilecek bilimsel yazılarda, ICMJE (International Committee of Medcial 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 1 5 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 yazarına 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 Gaziosmanpasa University Faculty of Agriculture since 1985. Journal of Agricultural Faculty of Tokat Gaziosmanpasa 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.

The author(s) are responsible for the content of the published manuscripts

ETİK İLKELER VE YAYIN POLİTİKASI

YAYIN ETİĞİ İLKELERİ

Gaziosmanpaşa ÜniversitesiZiraat 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 edilmemeli,
• 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 atıf yapmadan kısmen veya tamamen kendi eseriymiş gibi sunmak,
• Sahtecilik: Araştırmaya dayanmayan veriler üretmek, sunulan veya yayınlanan 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 atıf 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 gerekçesiz 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 Gaziosmanpasa 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:

The references list should be complete;
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;
All authors are obliged to provide retractions or corrections of mistakes,
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;

Plagiarism: Presenting others' ideas, methods, data, applications, writings, figures or works as if they were their own works, partly or completely, without referring to the scientific rules.
Fraud: to produce data that is not based on research, to organize or modify the work submitted or published on the basis of unreal data, to report or to publish them, to make a research that has not been done.
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 writ•er: 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.
•Değerlendirilen makaleler hakem tarafından gizli tutulmalıdır.

Editörün Sorumlulukları
•Editörler bir makaleyi kabul etmek ya da reddetmek için tüm sorumluluğa ve yetkiye sahiptir.
•Editörler kabul ettiği ya da reddettiği makaleler ile ilgili çıkar çatışması/çakışması içerisinde olmamalıdır.
•Sadece alana katkı sağlayacak makaleler kabul edilmelidir.
•Hakemlerin ismini değerlendirme tamamlanana kadar saklı tutmalıdır.
•Makalenin yayımlanmasından sonra herhangi bir araştırmacı tarafından bilimsel hata tespit edildiğinde ilgili düzeltme/düzeltmelerin yayımlanmasını ya da geri çekilmesini desteklemelidir.

Yayımcının Sorumlulukları
•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.

Değerlendirme Süreci
Yayımlanması için gönderilen eser, yayın ilkeleri doğrultusunda editör tarafından ön incelemeye alınır. Editör, dergide yayımlanabilecek nitelikte bulmadığı makaleleri hakemlere göndermeden yazara/yazarlara iade

work during publication or in later editions, and to use their influence even if there is no active contribution.

Other types of ethical violations: Not expressing the contributions of the persons, institutions or organizations that support them in the research, and their contributions in the research, Not to obey the ethical rules in human and animal research, to respect the rights of patients in their publications, To share the information contained in a work that he is commissioned to examine as an arbitrator with others, To use the sources, facilities and devices provided for scientific research out of their use purposes. To blame for a completely irrelevant, unwarranted and intentional violation of ethics (YÖK Scientific Research and Publication Ethics Directive, Article 8).

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To contribute to the decision-making process, and to assist in improving the quality of the published paper by reviewing the manuscript objectively.
Reviewers should have no conflict of interest with respect to the research, the authors and/or the research funders;
Judgments should be objective;
Reviewed manuscripts should be treated confidentially.

Editorial responsibilities:
Editors have complete responsibility and authority to reject/accept an manuscript;
Editors should have no conflict of interest with respect to manuscripts they reject/accept;
Only accept a paper when reasonably certain;
Preserve anonymity of reviewers.
No plagiarism, no fraudulent data.
When errors are found, promote publication of correction or retraction;
To act in a balanced, objective and fair way while carrying out their expected duties, without discrimination on grounds of gender, sexual orientation, religious or political beliefs, ethnic or geographical origin of the authors.

Duties of the Publisher
Monitoring/safeguarding publishing ethics by editorial board;
Guidelines for retracting manuscripts;
Maintain the integrity of the academic record;
Preclude business needs from compromising intellectual and ethical standards;
Always be willing to publish corrections, clarifications, retractions, and apologies when needed.
In an manuscript published in the journal, the reader can send an e-mail to ziraatdergi@gop.edu.tr when he has any warnings about important scientific error or plagiarism, recurring manuscripts. With the awareness that the journal will be an opportunity for the scientific and technical development of the journal, your warnings / criticisms are welcomed by the editorial board and our improvements are made quickly and constructively.

Evaluation Process
The submitted manuscript for publication is taken into consideration by the editor in accordance with the principles of publication. In case of finding not qualified to publish it in the journal, the editor has the right to make

kararı verme hakkına sahiptir. Ayrıca yazım kurallarına uymayan veya anlatım dili yetersiz olan makaleler, düzeltilmek üzere yazara/yazarlara iade edilir. Değerlendirmeye alınan makaleler, incelenmek üzere en az 2 hakeme gönderilir. Değerlendirmede çift yönlü kör hakemlik uygulaması esastır. Hakem değerlendirmesinden geçen makalelere ait düzeltmeler en kısa sürede sisteme yüklenmelidir.
Editör, hakem raporlarını ve/veya istenilen düzeltmelerin yeterli olup olmamasını dikkate alarak makalenin yayımlanıp yayımlanmamasına yönelik nihai karar vericidir. Makalenin yayımlanmasından önce makalede sayfa düzeni yapılarak son kontrol için yazarına gönderilir. Yazar makalenin son kontrolünü yaptıktan sonra ziraatdergi@gop.edu.tr mail adresine gönderir. Kontrolün düzgün yapılmaması sonucunda oluşabilecek baskı hataları yazarların sorumluluğundadır. İşlemi tamamlanan eserler kabul tarihi dikkate alınarak derginin yayınlanacak sayısında bulunması gereken makale limitleri dahilinde yayımlanır.

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Dergiye başvurusu yapılan makalelerin, hakemlik sürecine alınmadan önce intihal programı ile (iThenticate Plagiarism Detection Software) (http://www.ithenticate.com) taratılmış olması gerekmektedir. Tarama sonucunda Kaynaklar bölümü haricinde, benzerlik oranı %20 ve aşağı değeri taşıyan makaleler başvuruya kabul edilmektedir. Makale sisteme yüklendikten sonra iThenticate raporunun da ziraatdergi@gop.edu.tr mail adresine gönderilmesi süreç için gereklidir

Etik Kurul Onayı
Yazarlar yayımlatmak istedikleri makale ile ilgili olarak gerekli olan etik kurul onayını aldıkları kurumu ve onay numarasını Materyal ve Yöntem bölümünde mutlaka belirtmelidirler. Yayın kurulu gerekli gördüğünde "Etik Kurul Onay Belgesini" ayrıca isteyebilir. Makalenin etik kurul onayı gerektirip gerektirmediği aşağıda bildirilen kısımdan yazarlar ve alan editörleri tarafından mutlaka sorgulanması gerekmektedir.

Etik Kurul izni gerektiren araştırmalar aşağıdaki gibidir.
•Anket, mülakat, odak grup çalışması, gözlem, deney, görüşme teknikleri kullanılarak katılımcılardan veri toplanmasını gerektiren nitel ya da nicel yaklaşımlarla yürütülen her türlü araştırmalar
•İnsan ve hayvanların (materyal/veriler dahil) deneysel ya da diğer bilimsel amaçlarla kullanılması,
•İnsanlar üzerinde yapılan klinik araştırmalar,
•Hayvanlar üzerinde yapılan araştırmalar,
•Kişisel verilerin korunması kanunu gereğince retrospektif çalışmalar,
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•Başkalarına ait ölçek, anket, fotoğrafların kullanımı için sahiplerinden izin alınması ve belirtilmesi,
•Kullanılan fikir ve sanat eserleri için telif hakları düzenlemelerine uyulduğunun belirtilmesi.

a decision to return the manuscripts to the author / authors without sending to the referees. Papers should be written with fluent English without any grammatical and typographical errors. Manuscripts with any of those errors will be rejected and sent to the authors for corrections before submission and review. The journal uses double-blind system for peer-review; both reviewers and authors' identities remain anonymous. The paper will be peer-reviewed at least by two reviewers and one editor from the journal. The authors should upload the corrected manuscript with correction form and answers to the reviewers' comments immediately after receiving the comments. After the author has made the final check of the manuscript, he sends it to the ziraatdergi@gop.edu.tr e-mail address.
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Determination of Leaf and Stomata Characteristics of Some Late Blooming Almond (*Amygladus communis* L.) Cultivars and Their Hybrids Grown in Semi-arid Climate Conditions

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Abstract: This study was carried out on some domestic and foreign late blooming varieties and their genotypes obtained as a result of hybridization in the almond collection parcel of Harran University Faculty of Agriculture in Sanliurfa/Turkiye in the summer period of 2020. In the research, 5 different cultivars and 6 hybrids were examined in the orchard. In the study, 198 leaves were taken from 3 trees of each variety and 9 of each tree from 2 directions. It was planned and carried out according to the split plots experimental design in random blocks. North and South directions were taken into account when taking leaf samples. According to the findings, when the leaf characteristics were examined in general, the highest values in terms of leaf width, leaf length, petiole length and leaf area were found in 'Genotype-7' hybrids among cultivars and hybrids. As a result of stoma analysis on the lower surface of the leaves, the highest overall average (196.47 units mm^{-2}) was determined in Genotype-3, while the lowest (127.10 units/mm^2) was found in 'Ferragnes' variety. As a result of the analysis, when all the average values were taken into account, it was determined that the leaf area was 16.74 cm^2, the average stomata density was 153.51/mm^2 and the leaf area was 256975.74/leaf stomata. It is thought that the study will be descriptive for almond varieties and hybrids grown in the same ecology. This study was carried out in order to determine the effects of the adaptation of the almond varieties that are intensively grown in the Southeastern Anatolia Region and that bloom late, and some hybrids that are likely to be grown in the region in the future, on the morphological characteristics.

Keywords: Almond, genotype, leaf, leaf area, stomata density

Yarı Kurak İklim Koşullarında Yetiştirilen Bazı Geç Çiçek Açan Badem (*Amygladus communis* L.) Çeşitleri ve Hibritlerinin Yaprak ve Stoma Özelliklerinin Belirlenmesi

Öz: Bu çalışma, Şanlıurfa/Türkiye'de Harran Üniversitesi Ziraat Fakültesi badem toplama parselinde melezleme sonucu elde edilen bazı yerli ve yabancı geç çiçek açan çeşitler ve bunların genotipleri üzerinde 2020 yılı yaz döneminde yapılmıştır. Bahçede çeşit ve 6 melez incelenmiştir. Çalışmada her çeşitten 3 ağaç ve her ağacın 2 yönünden 9'ar olmak üzere 198 yaprak alınmıştır. Yaprak örnekleri alınırken kuzey ve güney yönleri dikkate alınmıştır. Deneme tesadüf bloklarında bölünmüş parseller deneme desenine göre planlanmış ve yürütülmüştür. Elde edilen bulgulara gore yaprak özellikleri genel olarak incelendiğinde, çeşitler ve melezler arasında yaprak genişliği, yaprak uzunluğu, yaprak sapı uzunluğu ve yaprak alanı açısından en yüksek değerler 'Tip-7' melezlerinde bulunmuştur. Yaprakların alt yüzeyinde yapılan stoma analizi sonucunda en yüksek toplam ortalama (196.47 adetmm-^{2}) Tip-3'te, en düşük (127.10 adet/mm^2) ise 'Ferragnes' çeşidinde bulunmuştur. Analiz sonucunda tüm ortalama değerler dikkate alındığında yaprak alanı 16.74 cm^2, ortalama stoma yoğunluğu 153.51/mm^2 ve yaprak alanı 256975.74/yaprak stoma olarak belirlenmiştir. Çalışmanın aynı ekolojide yetiştirilen badem çeşitleri ve melezleri için tanımlayıcı olacağı düşünülmektedir. Güneydoğu Anadolu Bölgesinde yoğun olarak yetiştirilen ve geç çiçek açan badem çeşitleri ile bölgede gelecekte yetiştirilmesi olası bazı hibritlerinin bölgeye adaptasyonunun morfolojik özelliklere etkisini belirlemek amacıyla bu çalışma yürütülmüştür.

Anahtar Kelimeler: Badem, genotip, stoma yoğunluğu, yaprak, yaprak alanı

1. Introduction

Almond is a hard-shelled fruit variety belonging to the *Prunus* L. genus of the *Rosaceae* family. It was named *Amygdalus communis* L. by Linnaeus in 1753. Its homeland is Central and Western Asia. Almond has spread from this region to China, Iran, India, Syria and Mediterranean countries. Almond is a temperate climate fruit known for its ability to tolerate heat and drought. The ecological determinant of this fruit species is that it blooms early with minimal chilling requirement (Ak et al., 2002; Martinez-Gomez et al., 2003; Kuden et al., 2014; Ak et al., 2020).

Stomata play an important role in gas exchange and transpiration and in the establishment of the

physiological balance in the plant. Stomata is important element that can reduce significant decreases in water potential, prevent embolism, and provide adaptation. It is important to determine the stomata parameters of plants grown in the same ecology (Kocacaliskan, 2008; Dickison, 2000; Escalona, 2013; Giday et al., 2013; Hopper et al., 2014; Boso et al., 2016; Sevik et al., 2017; Odabasioglu & Gursoz, 2019; Brodribb et al., 2020; McAdam et al., 2021; Lin et al, 2021).

According to the publications, the amount of stomata in the leaves varies according to the genotype, the shape of the leaves on the shoot and the location of the sample taken from the leaf (Rosselli, et al., 1989), the amount of stomata in the varieties with low sensitivity to cold is higher than the varieties with high sensitivity to cold, the amount of stomata in two different planes of the leaves (Çağlar & Tekin, 1999), there is a direct proportionality between the leaf size and the amount of stomata in the leaf (Demirkaya, 1999), small stoma sizes provide adaptation (Pinto et al., 2004) has been reported. Recent studies of trees suggest that the stomata regulate transpiration in a manner that optimizes the capacitive discharge of water from stem tissue, while at the same time avoiding excessive embolism (Ahmad & Prasad, 2012).

The aim of this study is to determine some leaf and stomata characteristics of some domestic and foreign late blooming cultivars and their genotypes. Thus, genotypes and their relationships with parents in terms of leaves and stomata were revealed. In this direction, it is aimed to determine the response of almond, which is an important fruit genotype, to leaf and stomata parameters in semi-arid conditions, depending on the varieties, with different statistical methods.

2. Materials and Methods

2.1. Materials

This study was carried out in Harran Plain, where is one of the main target sites of the Southeastern Anatolia Project (GAP) carried out in Southeast Turkey. Sanliurfa province is located in the Southeastern Anatolia Region of Turkey, on the Syrian border. The orchard from which the almond varieties were obtained had an elevation of 350 meters and located at 36° 88 latitude and 38° 92 '' longitude. Climate data from 1929 to 2021 are as follows. Highest temperature 46.8 ^{0}C (July); the lowest temperature was measured as -12.4^{0}C (February). The annual average precipitation in Sanliurfa is calculated as 462 mm. Annual average temperature is 18.6 ^{0}C, evaporation is 2048 mm, wind speed is 2.8 m/sec (Anonymous, 2022).

In this study, genotypes obtained by crossing ‘Ferragnes’, ‘Lauranne’, ‘Gulcan-1’ and ‘Gulcan-2’ with foreign origin varieties were used. Hybrid individuals were produced within the scope of TUBITAK TOVAG projects 108O388 and 113O963 (Acar et al. 2013, Acar et al. 2014). Six hybrid genotypes were used from some hybrid plants obtained from domestic and foreign almond varieties in this study area. These are: ‘Genotype-3’ (‘Lauranne’ x ‘Nurlu’), ‘Genotype-4’ (‘Gulcan-2’ x ‘Lauranne’), ‘Genotype-6’ (‘Gulcan-1’ x ‘Guara’), ‘Genotype-7’ (‘Gulcan-2’ x ‘Guara’), ‘Genotype-8’ (‘Gulcan-2’ x ‘Moncayo’), ‘Genotype-14’ (‘Gulcan-2’‘Penta’).These cultivars and genotypes that aged 5 years were grafted onto ‘GF-677’ rootstock. Plants are irrigated by drip irrigation method according to normal needs.

2.2. Methods

In the study, a total of 198 leaves were taken, 3 trees from each variety and 9 from each tree from 2 directions. The north and south directions of the trees were taken into account. The upper and lower surfaces of the leaves were completely painted with transparent nail polish and left to dry. Tape was attached to the dried nail polish leaves and then removed and attached to the slides. Thus, stomata were examined under the microscope (Las Leica DM1000) with 10X (objective)-10X (ocular) magnification (field of view 0.319 mm^2) (Bekisli, 2014).

Leaf aspect ratio was determined by dividing the leaf width value by the leaf length value. A total of 18 leaves, selected from 2 different directions of the selected cultivars and hybrids, were collected and the leaf lengths were measured with a ruler without including the petiole in the laboratory (Oraguzie et al., 1998; Kotobuki, 1996).Leaves were scanned to a computer and the Image-J software was used for leaf surface measurements (Kaya et al., 2003).

The number of stomata displayed in the 0.776 mm² field of view of the slide molds photographed under the microscope was determined by adapting it to the 1 mm² field (Kara & Ozeker, 1999; Bekisli, 2014; Dikmetas, 2017).The length and width of 10 stomata in the photographs of the stoma patterns were measured in the MShot-1.3.10 computer program and measured in µm (Bekisli, 2014).The stomata aspect ratio was obtained by dividing the stomata width by the stomata length. The wet weights of the leaf samples were determined, and the turgor weight was found by keeping them in petri dishes containing 100 mL of water for 24 hours. Afterwards, the samples were dried in an oven at 65-

70ºC and their dry weights were determined. Sanchez et al. (2004) determined the RLWC.

The data obtained from the experiment were analyzed by using the Minitab statistical software, and the differences between the cultivars were determined by the LSD (P≤0.05).Principal component analysis (PCA) and clustering analysis (Dendogram) were performed using the PAST 4.03 software.

3. Results and Discussion

3.1. Analysis of leaf parameters

The findings of some leaf characteristics of 11 different almond cultivars used as material in this study are given in Tables 1, 2, 3,4,5 and 6.

The highest length value (11.83 cm) in the leaves in the north direction was found in Genotype-7, while the lowest average was determined as 8.41 cm in Genotype-3. Other genotypes and cultivars ranged between these two values. When the averages of the leaves in the south direction were examined, the highest value was found in Genotype-7 with 12.72 cm, while the lowest value was determined in Gulcan-2 variety (Table 1). The difference between the means was found to be statistically significant.

Table 1. Leaf lengths of different almond varieties and genotypes (cm)
Çizelge 1.*Farklı badem çeşitlerinin yaprak uzunlukları (cm)*

Varieties and Genotypes	Leaflengths (cm) North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	11.20 ±1.236 ab	10.49 ±0.325 b-e	10.84
GUARA	10.64 ±1.484b-e	11.00 ±0.618bc	10.82
GULCAN-1	9.03 ±1.777gh	11.27 ± 0.700 b	10.15
GULCAN-2	8.70 ±1.705gh	8.76 ±1.046h	8.73
LAURANNE	9.70 ±1.192 d-g	9.74 ±1.053 e-g	9.72
GENOTYPE-3	8.41 ±0.321h	9.28 ±0.569f-h	8.84
GENOTYPE -4	9.46 ±0.958f-h	9.39 ±1.218f-h	9.42
GENOTYPE -6	10.41 ±1.109b-f	9.77 ±0.876ef	10.09
GENOTYPE -7	11.83 ±0.936a	12.72 ±1.005a	12.27
GENOTYPE -8	10.91 ±0.896a-d	9.49 ±0.566f-g	10.20
GENOTYPE -14	11.42 ±0.528 ab	10.71 ±0.520 b-d	11.06
Average	10.15	10.24	10.19
LSD*	1.137	0.813	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05).

The highest width value (3.41 cm) in the leaves in the north direction was found in Ferragnes, while the lowest value was determined as 2.49 cm in Genotype-3. Other genotypes and cultivars ranged between these two values. When the averages of the leaves in the south direction were examined, the highest value was found in Ferragnes with 3.63 cm, while the lowest value was determined in the Genotype-8 (Table 2).

Table 2. Leaf widths of different almond varieties and genotypes (cm)
Çizelge2.*Farklı badem çeşitlerinin yaprak genişlikleri (cm)*

Varieties and Genotypes	Leafwidth (cm) North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	3.41 ± 0.337 a	3.63 ± 0.239 a	3.52
GUARA	3.08 ± 0.097 a-d	3.50 ± 0.304 ab	3.29
GULCAN-1	2.11 ± 0.496 g	2.64 ± 0.245 d-f	2.37
GULCAN-2	2.58 ± 0.465 f	2.70 ± 0.415 d-f	2.64
LAURANNE	2.54 ± 0.200 f	2.97 ± 0.320 d	2.75
GENOTYPE-3	2.49 ± 0.214 f	2.73 ± 0.217 d-f	2.98
GENOTYPE -4	2.58 ± 0.286 f	2.61 ± 0.252 ef	2.61
GENOTYPE -6	2.59 ± 0.226 f	2.63 ± 0.234 d-f	2.59
GENOTYPE -7	3.03 ± 0.234 b-e	3.50 ± 0.180 ab	2.61
GENOTYPE -8	3.14 ± 0.409 ab	2.57 ± 0.234 f	3.26
GENOTYPE -14	3.12 ± 0.204 a-c	2.84 ± 0.142 de	2.85
Average	2.79	2.94	2.86
LSD*	0.346	0.345	-

*: Classification is made according to LSD 5%. The difference between the averages bearing different on the same column is statistically significant. (P <0.05)

The highest value in the leaf stem length in the northern direction was determined in Genotype-14. The lowest value was determined in Gulcan-1 cultivar. In the south direction, the highest value was observed in Genotype-7 with 2.34 cm, and the lowest value was observed in Genotype-3 with 1.3 cm (Table 3).

Table 3. Petiole lengths of different almond cultivars and genotypes (cm)
Çizelge 3.*Farklı badem çeşitleri ve çeşitlerinin yaprak sapı uzunlukları (cm)*

Varieties and Genotypes	Petiole lenghts (cm) North (Value ±Standard Deviation)	South (Value ±Standard Deviation)	Average
FERRAGNES	1.80 ± 0.346d	1.43 ± 0.229cd	1.62
GUARA	1.66 ± 0.328ef	1.56 ± 0.142cd	1.61
GULCAN-1	1.30 ± 0.415i	1.66 ± 0.371cd	1.48
GULCAN-2	1.57 ± 0.418h	1.36 ± 0.490 de	1.47
LAURANNE	1.67 ± 0.578ef	1.61 ± 0.464cd	1.64
GENOTYPE-3	1.34 ± 0.278h	1.30 ± 0.254e	1.32
GENOTYPE -4	1.69 ± 0.297e	1.59 ± 0.483cd	1.64
GENOTYPE -6	1.91 ± 0.395c	1.69 ± 0.333c	1.80
GENOTYPE -7	2.09 ± 0.407b	2.34 ± 0.312a	2.22
GENOTYPE -8	1.50 ± 0.269 g	1.60 ± 0.187cd	1.55
GENOTYPE -14	2.40 ± 0.259a	2.18 ± 0.253ab	2.29
Average	1.72	1.67	1.70
LSD*	0.033	0.325	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

The averages of the ratio of leaf width to leaf length were found to be statistically significant between cultivars and genotypes. The highest value (0.305 cm) in the leaves in the north direction was found in Ferragnes, while the lowest average was determined as 0.233 cm in Gulcan-1. Other genotypes and cultivars ranged between these two values. When the averages of the leaves in the south direction were examined, the highest value was found in Ferragnes with 0.346 cm, while the lowest value was determined in Gulcan-1 cultivar (Table 4).

Table 4. The leaf aspect ratio of different almond varieties and genotypes

Çizelge 4.*Farklı badem çeşitlerinin yaprak en/boy oranı*

Varieties and Genotypes	The leaf aspect ratio		
	North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	0.305 a	0.346 a	0.325
GUARA	0.289 a-c	0.318 ab	0.306
GULCAN-1	0.233 h	0.235 i	0.234
GULCAN-2	0.296 ab	0.308 bc	0.302
LAURANNE	0.262 d-g	0.304 bc	0.283
GENOTYPE-3	0.296 ab	0.295 b-d	0.295
GENOTYPE -4	0.273 b-f	0.278 c-f	0.275
GENOTYPE -6	0.249 e-g	0.270 d-h	0.259
GENOTYPE -7	0.256 e-g	0.275 d-g	0.265
GENOTYPE -8	0.288 a-d	0.270 d-h	0.279
GENOTYPE -14	0.273 b-f	0.266 d-h	0.270
Average	0.275	0.288	0.281
LSD*	0.026	0.031	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

When the averages of the leaf area were examined, the highest value (25.43 cm²) was found in Genotype-7 leaves in the north direction, while the lowest average was determined as 9.50 cm² in Gulcan-1. Other genotypes and cultivars ranged between these two values. When the averages of leaves in the south direction are examined, the highest value was determined as 20.70 cm² in Genotype-7, while the lowest value was determined in Genotype-4 hybrids (Table 5).

Table 5. Leaf area of different almond varieties and genotypes (cm²)

Çizelge 5.*Farklı badem çeşitlerinin yaprak alanları (cm²)*

Varieties and Genotypes	Leaf area (cm²)		
	North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	20,60 ± 3,197bc	20,27 ± 2,467ab	20,33
GUARA	20,17 ± 2,709b-d	19,33 ± 3,37a-c	19,75
GULCAN-1	9,51 ± 3,701j	14,95 ± 3,437e-g	12,23
GULCAN-2	11,05 ± 4,231ij	13,93 ± 4,888e-g	12,49
LAURANNE	17,11 ± 3,029d-g	15,75 ± 4,113d-f	16,43
GENOTYPE-3	16,49 ± 2,566e-h	14,26 ± 2,178e-g	15,37
GENOTYPE -4	13,39 ± 2,083hi	11,46 ± 1,507g	12,42
GENOTYPE -6	16,17 ± 2,887e-h	14,26 ± 1,994e-g	15,22
GENOTYPE -7	25,43 ± 2,918a	20,70 ± 2,174a	23,07
GENOTYPE -8	17,79 ± 3,064cd	13,37 ± 1,999fg	15,58
GENOTYPE -14	23,14 ± 1,481ab	19,28 ± 2,955a-d	21,21
Average	17,34	16,14	16,74
LSD*	3,377	3,533	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

When the averages of there lative water content of the leaves were examined, the highest value (75.21%) was found in the leaves in the north direction, while the lowest average was found in Genotype-14 as 41.42%. Other genotypes and cultivars ranged between these two values. When the averages of leaves in the south direction were examined, the highest value was found in Genotype-8 with 81.88%, while the lowest value was determined in Genotype-14 hybrids (Table 6).

Table 6. Relative water content of leaves (%)

Çizelge 6.*Yaprakların oransal su içeriği (%)*

Varieties and Genotypes	Relative water content (%)		
	North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	66,59 ± 14,546 a	78,00 ± 6,944 ab	72,29
GUARA	72,10 ± 15,752 a	72,83 ± 12,521 a-d	72,46
GULCAN-1	64,43 ± 21,282 a	61,11 ± 18,870 f	62,77
GULCAN-2	75,21 ± 16,280 a	66,12 ± 19,786 b-f	70,66
LAURANNE	67,59 ± 12,758 a	65,63 ± 16,651 b-f	66,61
GENOTYPE-3	63,08 ± 14,074 a	62,22 ± 13,611 ef	62,65
GENOTYPE -4	75,15 ± 10,028 a	67,90 ± 17,730 a-f	71,52
GENOTYPE -6	69,90 ± 16,898 a	72,61 ± 13,137 a-e	71,25
GENOTYPE -7	75,02 ± 9,011 a	77,85 ± 14,330 a-d	76,43
GENOTYPE -8	70,93 ± 15,267 a	81,88 ± 11,131 a	76,40
GENOTYPE -14	41,42 ± 12,211 b	49,60 ± 14,863 g	45,51
Average	67,40	68,70	68,05
LSD*	16,998	15,590	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

3.2. Analysis of stomata parameters

Stoma parameters are given in Tables 7,8,9 and 10. The highest amount of stomata at the tip of the leaves taken from the north was observed in Laurenne variety with 199, while the lowest value was determined in Genotype-4 with 109 units. The highest amount of stomata of the leaf taken from the middle was again in Lauranne variety, while the lowest number of stomata was seen in Ferragnes variety. In the leaves taken from the bottom part, the highest stomata amount was seen in Genotype-3, while the lowest stomata number was determined in Genotype-14 (Table 7).

While the highest number of stomata was seen in Laurenne variety with 216 pieces, the lowest value was determined in Guara variety with 106 pieces. In the leaves in the middle parts, the highest value was determined in Genotype-3, 213, and the lowest value was observed in the Ferragnes variety with 83. In the leaves taken from the bottom of the shoot, the highest value was seen in Genotype-3, while the lowest value was observed in Guara variety. These; leaf length (cm), petiole (pedicel) length (cm), leaf width (cm), leaf area, leaf width/leaf length ratio, leaf fresh weight, leaf dry weight, leaf relative moisture content, etc. features were examined (Table 7).

The highest value (34.46 µm) of stomata lengths at the tip of the leaves in the northern direction was found in Genotype-3, while the lowest average was determined as 26.7 µm in Genotype-14. Other genotypes and cultivars ranged between these two values. In cases of stomata width in the same direction, the highest value was found to be 22.26 µm in Genotype-6. The lowest value was found to be 12.47 µm in Genotype-14. Other cultivars and genotypes ranged between these two values (Table 8).

In the northern direction, the highest value (35.85

µm) of stomata lengths at the base of the existing leaves was found in Genotype-3, while the lowest average was determined as 28.08 µm in Genotype-14. Other cultivars and genotypes ranged between these two values. The highest stoma of the leaves in the northern direction was found at 22.65 µm Guara. The lowest value was determined in Genotype-14 with 14.06 µm (Table 8).

Table 7. Stomata density per unit area (pcs/mm^2)
Çizelge 7. *Birim alandaki stoma yoğunluğu (adet/ mm^2)*

Varieties and Genotypes	Stomata density per unit area(pcs/mm^2)								AVR.
	North (Value±Standard Deviation)				South (Value±Standard Deviation)				
	T**	M	B	Average	T	M	B	Average	
FERRAGNES	176,49	99,90	116,55	130,98 ± 40,282c-f	166,50	119,88	83,25	123,21 ± 41,724ef	127,10
GUARA	176,49	156,51	133,20	155,40 ±21,666a-d	106,56	123,21	93,24	107,67 ± 15,015 f	131,54
GULCAN-1	153,18	123,21	139,86	138,75 ± 15,015b-f	183,15	166,50	123,21	157,62 ± 30,94a-e	148,19
GULCAN-2	116,55	133,20	116,55	122,10 ± 9,612d-f	169,83	133,20	139,86	147,63 ± 19,512b-f	134,87
LAURANNE	199,80	189,81	143,19	177,60 ± 30,215ab	216,45	163,17	149,85	176,49 ± 35,241a-d	177,05
GENOTYPE-3	199,80	159,84	213,12	190,92 ± 27,727 a	193,14	199,80	213,12	202,02 a ± 10,173	196,47
GENOTYPE -4	109,89	149,85	129,87	129,87 ± 19,979 c-f	209,79	166,50	156,51	177,60 ± 28,321a-c	153,74
GENOTYPE -6	116,55	133,20	123,21	124,32 ± 8,380 d-f	139,86	123,21	163,17	142,08 ± 20,072b-f	133,20
GENOTYPE -7	153,18	173,16	169,83	165,39 ± 10,704a-c	159,84	149,85	99,90	136,53 ± 32,113b-f	150,96
GENOTYPE -8	189,81	136,53	133,20	153,18 ± 31,766 b-e	216,45	176,49	149,85	180,93 ± 33,521ab	167,06
GENOTYPE -14	109,89	116,55	93,24	106,56 ± 12,006f	116,55	83,25	139,86	180,93 ± 28,451ab	167,06
Average	176,49	142,86	137,53	152,290	180,82	145,85	137,53	113,220	153,51
LSD*	-	-	-	39,007	-	-	-	47,980	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05) **T: Tip of leaf M: Middle of leaf B: Bottom of leaf

Table 8. Stomata length (µm)
Çizelge 8. *Stoma boyu (µm)*

Varieties and Genotypes	Stomata lenght (µm)								AVR.
	North (Value±Standard Deviation)				South (Value±Standard Deviation)				
	T	M	B	AVR.	T	M	B	AVR.	
FERRAGNES	33,60± 1,541 a-c	31,76 ± 1,884 c-g	32,38 ± 4,958 a-g	32,58	33,60 ± 2,184 b	34,74 ± 2,327 ab	34,57 ± 2,682 b-d	34,30	33,44
GUARA	30,36± 4,164 d-g	30,92± 4,656 c-g	34,39 ± 4,173 a-d	31,89	27,85 ± 4,122 fg	32,99 ± 7,03 a-f	33,97 ± 2,409 b-d	31,60	31,74
GULCAN-1	26,72 ± 3,874 h	30,03 ± 2,959 e-g	35,5 ± 1,006 ab	30,75	27,45 ± 2,245 fg	29,25 ± 4,709 e-j	38,05 ± 3,007 a	31,58	31,16
GULCAN-2	32,03 ± 3,995 a-e	36,72 ± 1,663 a	35,85 ± 1,434 a	34,87	19,28 ± 3,311 h	34,81 ± 3,573 a	33,82 ± 2,524 bc	29,30	32,08
LAURANNE	30,29 ± 3,133 d-g	32,26 ± 3,701 c-f	31,30 ± 3,065 c-g	31,28	27,67 ± 4,239 fg	32,70 ± 3,702 a-h	34,56 ± 2,847 b-d	31,64	31,46
GENOTYPE-3	34,46 ± 2,803 a	33,59 ± 3,123 a-d	29,70 ± 1,877 gh	32,58	33,30 ± 3,311 b-d	32,34 ± 3,037 a-d	32,56 ± 1,862 cd	32,73	32,65
GENOTYPE -4	29,84 ± 2,658 e-h	28,88 ± 3,114 g	34,80 ± 3,712 a-c	31,17	28,88 ± 2,341 f	25,31 ± 3,277 k	35,32 ± 4,933 a-d	29,84	30,50
GENOTYPE -6	34,39 ± 3,078 ab	35,86 ± 1,872 ab	30,96 ± 2,871d-h	33,74	36,84 ± 3,814 a	33,17 ± 2,859 a-e	35,97 ± 1,983 a-c	35,33	34,53
GENOTYPE -7	31,36 ± 2,788 c-f	33,78 ± 3,244 a-c	33,53 ± 4,551 a-f	32,89	32,63 ± 3,411 b-e	34,29 ± 2,428 a-c	33,86 ± 6,94 b-d	33,59	33,24
GENOTYPE -8	32,70 ± 1,88a-d	33,19 ± 2,983 b-e	33,61 ± 1,521 a-e	33,17	33,53 ± 2,371 b-d	32,50 ± 2,65 a-i	37,11 ± 2,335 ab	34,38	33,77
GENOTYPE -14	26,70 ± 4,631 h	32,65 ± 3,618 b-f	28,08 ± 4,529 h	29,14	27,62 ± 2,139 fg	33,84 ± 1,645 a-d	32,37 ± 1,575 d	31,28	30,21
Average	31,13	32,69	32,74	32,19	28,97	32,36	34,74	32,02	32,10
LSD*									

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

Table 9. Stomata width (µm)
Çizelge 9. *Stoma eni (µm)*

Varieties and Genotypes	Stomata width (µm)								AVR.
	North (Value±Standard Deviation)				North (Value±Standard Deviation)				
	T	M	B	Avr.	T	M	B	Avr.	
FERRAGNES	19,03 ± 3,697 b-e	17,23 ± 1,126 d-h	18,92 ± 4,799 b-e	18,39	33,60 ± 1,761 b	19,09 ± 1,454 a-e	18,61 ± 2,421 c-g	23,77	21,08
GUARA	17,36 ± 3,129 c-g	19,05 ± 2,385 cd	22,65 ± 2,566 a	19,69	27,84 ± 4,38 f	18,47 ± 3,535 a-f	20,29 ± 1,609 b-e	22,20	20,94
GULCAN-1	14,59 ± 2,694 hi	14,83 ± 1,751 i	18,64 ± 2,969 b-g	16,02	27,44 ± 2,648 f	16,91 ± 3,968 b-i	25,36 ± 1,356 a	23,24	19,63
GULCAN-2	19,12 ± 2,424 b-d	22,76 ± 2,533 ab	18,34 ± 1,575 b-h	20,07	29,27 ± 2,379 f	21,07 ± 2,525 a	20,09 ± 1,849 b-e	23,48	21,77
LAURANNE	18,02 ± 2,602 c-f	18,11 ± 3,782 c-f	17,25 ± 2,185 c-i	17,79	27,67 ± 2,726 f	19,12 ± 3,288 a-d	20,71 ± 3,778 bc	22,50	20,14
GENOTYPE-3	12,47 ± 1,828 i	15,15 ± 2,135 hi	14,06 ± 1,723 k	18,91	27,62 ± 2,379 f	14,17 ± 2,133 hi	17,43 ± 1,533 g	23,41	21,16
GENOTYPE -4	20,68 ± 2,038ab	18,86 ± 3,107 c-e	17,20 ± 2,773 c-j	17,45	33,30 ± 2,137 b-d	18,42 ± 2,551 a-g	18,51 ± 2,714 c-g	20,77	19,11
GENOTYPE -6	16,64 ± 2,445 f-h	16,20 ± 1,591 f-i	19,50 ± 1,647 bc	21,45	28,88 ± 2,934 f	13,88 ± 2,227 hj	19,56 ± 1,855 c-g	26,33	23,89
GENOTYPE -7	22,26 ± 2,284 a	22,85 ± 1,701 a	19,24 ± 2,832 b-d	19,68	36,83 ± 2,222 a	20,12 ± 1,387 ab	22,03 ± 3,613 b	23,95	21,81
GENOTYPE -8	19,12 ± 6,088 b-d	19,93 ± 1,325 c	20,00 ± 2,308b	18,59	32,63 ± 1,035 b-e	19,84 ± 1,559 a-c	19,38 ± 1,825 c-g	24,18	21,38
GENOTYPE -14	19,20 ± 1,396 bc	17,8 ± 1,951 c-g	18,77 ± 2,648 b-f	13,89	33,53 ± 1,722 bc	16,98 ± 0,796 a-h	22,03 ± 2,703 b	19,74	16,81
Average	18,04	18,43	18,60	18,36	30,78	18,01	20,36	23,05	20,70
LSD(%5)*	2,244	2,605	2,331	-	2,285	3,588	2,214	-	

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

While the highest value (36.84 µm) of stomata lengths at the tip of the leaves in the south direction was found in Genotype-6, the lowest average was determined as 9.28 µm in Gulcan-2. Other genotypes and cultivars ranged between these two values. In cases of stomata width in the same direction, the highest value was found in Genotype-6, 36.83 µm. The lowest value was found to be 27.62 µm in Genotype-14. Other cultivars and genotypes ranged between these two values (Table 8).

Table 10. Stomata aspect ratio
Çizelge 10. *Stoma en/boy oranı*

Varieties and Genotypes	Stomata aspect ratio								
	North				South				AVR.
	T	M	B	Avr.	T	M	B	Ort.	
FERRAGNES	0,60 a	0,56 b	0,57 c	0,58	0,60 a	0,69 a	0,63 a	0,64	0,61
GUARA	0,57 b	0,55 b	0,89 a	0,67	0,54 b	0,56 b	0,53 b	0,54	0,61
GULCAN-1	0,49 c	0,52 c	0,52 c	0,51	0,59 a	0,57 b	0,66 a	0,61	0,56
GULCAN-2	0,59 a	0,62 a	0,51 d	0,57	0,63 a	0,58 b	0,59 b	0,60	0,59
LAURANNE	0,54 b	0,62 a	0,46 e	0,54	0,53 b	0,39 c	0,49 c	0,47	0,50
GENOTYPE-3	0,60 a	0,56 b	0,57 c	0,58	0,62 a	0,57 b	0,56 b	0,58	0,58
GENOTYPE -4	0,62 a	0,56 b	0,55 c	0,58	0,59 a	0,54 b	0,55 b	0,56	0,57
GENOTYPE -6	0,64 a	0,63 a	0,62 b	0,63	0,60 a	0,60 b	0,61 a	0,60	0,62
GENOTYPE -7	0,60 a	0,57 b	0,57 c	0,58	0,61 a	0,59 b	0,60 b	0,59	0,59
GENOTYPE -8	0,63 a	0,56 b	0,64 a	0,61	0,68 a	0,58 b	0,62 a	0,62	0,62
GENOTYPE -14	0,49 c	0,42 c	0,44 e	0,45	0,46 b	0,43 c	0,53 b	0,47	0,46
Average	0,58	3,77	0,58	1,64	0,59	0,55	0,58	0,57	1,11
LSD(%5)*	0,071	0,054	0,081	-	0,109	0,083	0,064	-	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

While the highest value (38.05 µm) of stomata lengths at the bottom of the leaves in the south direction was found in Gulcan-1, the lowest average was determined as 32.37 µm in Genotype-14. Again, in cases of stomata width in the same direction, the highest value was 25.36 µm in Gulcan 1. The lowest value was found to be 17.42 µm in Genotype-14. Other cultivars and genotypes ranged between these two values (Table 8). It was determined that the difference between the averages of the leaves taken from both the north and south directions in terms of stoma length and width was statistically significant.

The highest value in stomata width was detected in Genotype 6 (22.86 µm), and the lowest value was detected in Gulcan-1 variety with 14.83 µm. Other cultivars and genotypes ranged between these two values (Table 9).

The highest value (0.647 µm) of stomata aspect ratios at the tip of the leaves in the north direction was found in Genotype-6, while the lowest value was 0.495 µm in Genotype-14. In the stomata width/length conditions in the middle part in the same direction, the highest value was found in Genotype-6 as 0.635 µm. The lowest value was found to be 0.425 µm in Genotype-14. According to the width/length data at the bottom of the same direction, the highest value was found in Guara with 0.893 µm, while the smallest value was found in Lauranne with 0.491 µm. Other cultivars and genotypes ranged between these two values (Table 10).

The highest value for the stomata aspect ratio (0.684 µm) at the tip of the leaves of the shoots taken from the south was found in Genotype-8, while the lowest average was determined as 0.464 µm in Genotype-14. Again, the highest value was found in Ferragnes at 0.696 µm in stoma width/length conditions in the middle part in the same direction. The lowest value was found to be 0.392 µm in Genotype-14. According to the stomata width/length data at the bottom of the leaves of the same direction, the highest value was found in Gulcan-1 with 0.667 µm, while the smallest value was found in Lauranne with 0.495 µm. Other cultivars and genotypes ranged between these two values (Table 10).

3.3. Correlation of leaf and stomata parameters

When the number of stomata in the leaf of the cultivars and genotypes was examined, the highest stomata value was determined as 354334,26 in Genotype-14. The lowest stoma value was determined as 168452,63 in Gulcan-2 (Table 11).

Table 11. The number of stomata in the leaf of different almond cultivars and genotypes (pieces)
Çizelge 11.*Farklı badem çeşitleri ve çeşitlerinin yapraktaki stoma sayıları (adet)*

Varieties and Genotypes	Stomata Density in the Leaf		
	Leaf Area (mm^2)	Stomata Density ($pieces/mm^{2)}$	Total Stomata Density (pieces/leaf)
FERRAGNES	2033	127,10	258394,30
GUARA	1975	131,54	259791,50
GULCAN-1	1223	148,19	181236,37
GULCAN-2	1249	134,87	168452,63
LAURANNE	1643	177,05	290893,15
GENOTYPE-3	1537	196,47	301974,39
GENOTYPE -4	1242	153,74	190945,08
GENOTYPE -6	1522	133,20	202730,40
GENOTYPE -7	2307	150,96	348264,72
GENOTYPE -8	1558	167,06	260279,48
GENOTYPE -14	2121	167,06	354334,26
Average	1674	153,51	256975,74

As seen in Table 12, there is a significant positive correlation between leaf width and leaf area, genotypes and leaf length ($p \leq 0.01$). There is also a significant positive correlation between leaf area and leaf width ($p \leq 0.01$). There is a significant correlation between stoma width and stoma length ($p \leq 0.01$).In addition to stomata closure, which reduces transpiration rate per unit leaf area, branch reduction appears to be an effective mechanism that confers plants the ability to maintain water balance by reducing the total

transpiration area of the canopy and hence total plant water losses (Rood et al., 2000; Fischer & Polle, 2010; Ahmad & Prasad, 2012).

Table 12. Pearson correlation between genotypes, leaf and stomata sizes, RWC and stomata density
Çizelge 12.*Genotipler, yaprak ve stoma boyutları, YOSK ve stoma yoğunluğu arasındaki Pearson korelasyonu*

Leaf and stomata features	Genotypes	Leaf length	Leaf width	Leaf area	RWC	Stomata density	Stomata lenght
Leaf length	0.393**						
Leaf width	0.530**	0.654**					
Leaf area	0.574**	0.697**	0.696**				
RWC	-0.098	0.091	0.153	-0.012			
Stomata density	-0.413**	-0.222*	-0.323**	-0.425**	0.132		
Stomata lenght	-0.062	-0.034	-0.003	0.049	0.209*	0.014.	
Stomata width	-0.200*	-0.121	-0.035	-0.037	0.382**	0.081	0.782**

*: p≤0.05, **: p≤0.01

3.4. Cluster and principal component analyses

In our study, a dendogram tree was formed as a result of 11 different parameters examined in 11 different almond variety, and as a result, 4 different groups were formed in the dendogram graph (Figure 1). According to the dendogram, 'Lauranne' and 'Genotype-3' are in the first group. The second group includes 'Guara', 'Genotype-8' and 'Ferragnes'. The third group includes 'Genotype-7' and 'Genotype-14'. 'Gulcan-1', 'Genotype-4', 'Genotype-6' and 'Gulcan-2' are more similar to each other than the aforementioned features.

As seen in Figure 2, the 11 almond varieties used in the study, which have similar characteristics, are located in the different place. The main logic of these groupings is shown in Figure 2. The arrows in the graph show the values of the different parameters examined, that is, these values increase as you move towards the arrow direction.

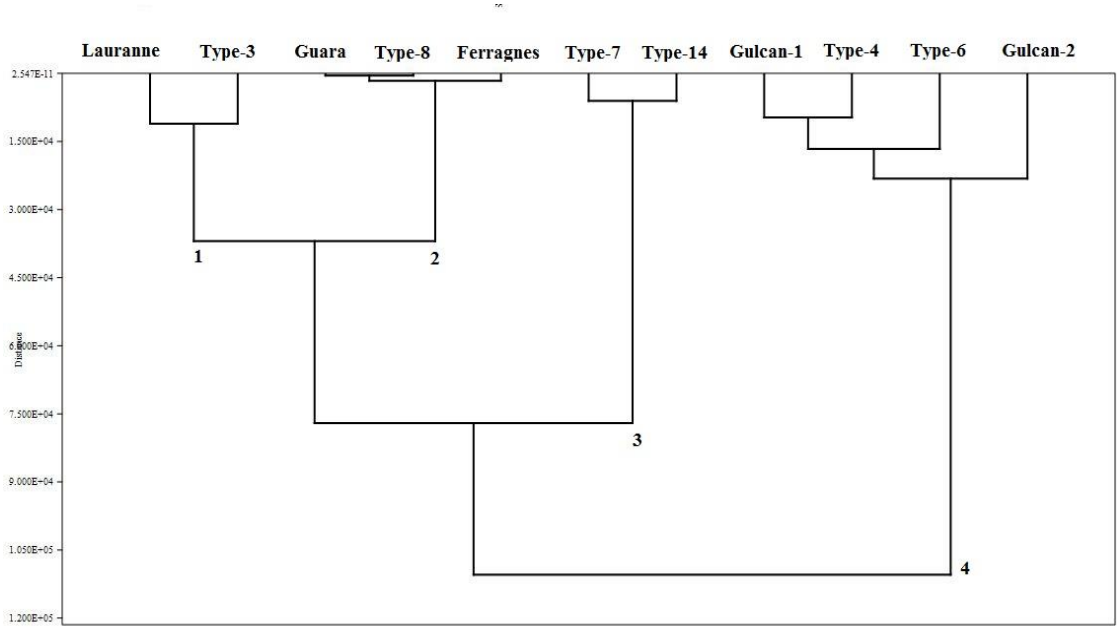

Figure 1. Comparative dendogram plot of some almond varieties
Şekil 1.*Bazı badem çeşitlerinin karşılaştırmalı dendogram grafiği*

Ordination of the 10 parameters given by PCA (Figure 2) indicates that the almond genotypes produced by PAST classification are markedly distinguishable and show a clear pattern of segregation on the ordination planes. The eigenvalues for the first two PCA axes are 0.175 and 0.590, respectively. The high eigenvalue for PCA axis 1 indicates that it explains the major variation in genotypes composition of almond groups. In this context, Figure 2 shows the clusters resulting from the classification of species and varieties. According to Figure 1 and Figure 2, it is revealed that the classification technique divides almond varieties into four different classes.

Under mild to moderate water deficits, stomata closure is one of the earliest plant responses, concomitant with the reduced water potential and turgor associated with even a small decrease in relative water content (Franco et al., 2006; Ahmad and Prasad, 2012). In this context, it is important that stoma measurements and physiological parameters to be made support each other. Changes in stomata morphology resulted in increased leaf water potential.

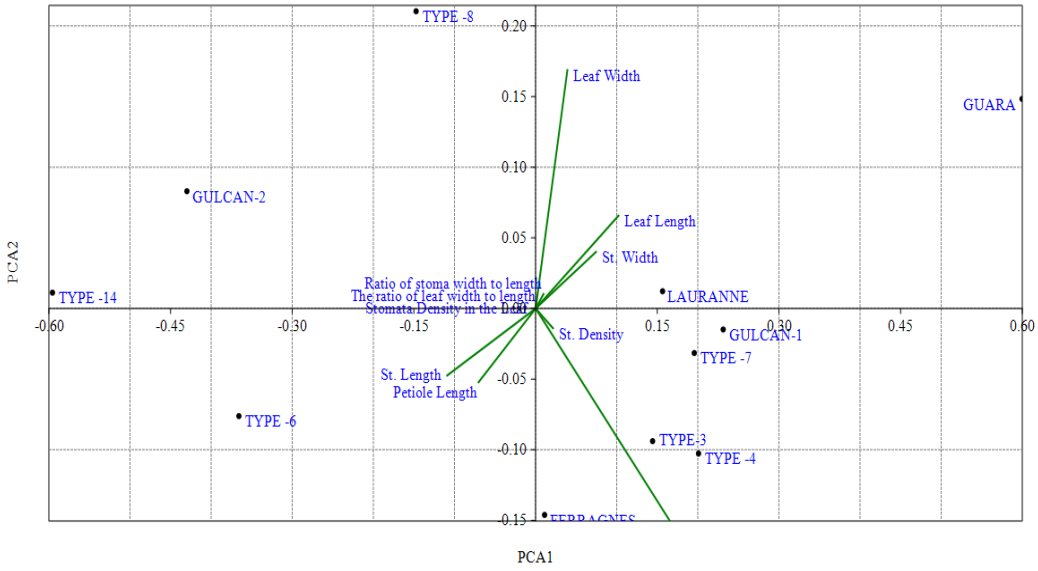

Figure 2. PCA plot of all measured variables for leaf parameters of some almond varieties
Şekil 2.*Bazı badem çeşitlerinin yaprak parametreleri için ölçülen tüm değişkenlerin PCA grafiği*

It was also found that a decrease in stomata conductivity was associated with an increase in leaf water potential in flooded bitter melon (Liao and Lin 1994).According to the PCA plot, 11 cultivars were found to be compatible with the relevant literature. It is recommended that the studies to be carried out in this direction should be supported by the aforementioned analyzes. On the other hand, Ashraf and Arfan (2005) did not find a significant relationship between stomata structure and water potential. However, according to Figure 2, there was a significant correlation between stomata density and RWC in 'Gulcan-1', 'Ferragnes', Genotype-3', 'Genotype-4' and 'Genotype-7' almond cultivars.

The study area has features reflecting the continental climate where semi-arid conditions are experienced. Sanliurfa has continental climate characteristics. Summers are very dry and hot, winters are rainy and relatively mild. The number of days with snow and frost is very few. For this reason, the difficulties of semi-arid climate are encountered in the cultivation of many fruit species. In this direction, it is important for a correct fruit growing to determine some differences of plant varieties and to determine the relationship between them. Photosynthesis rate, relative water content, leaf water content and stomata morphology of leaves change in regions where semi-arid conditions are experienced, such as in Sanliurfa. Ultimately, these conditions destabilize the membrane structure and permeability, protein structure and function, leading to cell death.

Almond is an important fruit species in regions with semi-arid climatic conditions. Leaf characteristics of different almond genotypes were found to be different due to the genotype effect and it was determined that stomata density could be affected by environmental conditions. A negative relationship between stoma frequency and stoma size was reported in the literature, and similar findings were obtained in the study. In Sanliurfa ecology, almonds see very high temperatures especially in June, July and August. For this reason, varieties or varieties/rootstock combinations that are resistant to heat and drought should be determined and grown accordingly. For this reason, in this study, newly hybridized cultivars were selected in the region and some leaves (leaf width, leaf length, petiole length, leaf area, leaf width-leaf length ratio) and stoma (number of stomata per unit leaf surface area) were selected under the conditions in the Harran University Almond Selection Plot.

Statistically significant differences were found between almond hybrids and cultivars in terms of all the characteristics examined. However, positive linear relationships were found between some leaf and stomata characteristics.

When the leaf characteristics were examined in general, the leaves of the ‘Genotype-7’ hybrid stood out among the cultivars and hybrids. In terms of leaf width, leaf length, petiole length and leaf area, the highest values were determined in ‘Genotype-7’ hybrids. When the petiole length was examined, it was determined that the longest petiole was in the leaves of the ‘Genotype-14’ hybrid.

4. Conclusion

The density and size of stomata in fruit trees vary according to species and variety, as well as the number

and sizes of stomata per unit surface area with the effect of environmental and internal factors. In fruit trees, stomata sizes decrease significantly with the increase in the number of stomata. As the stoma size decreases, water loss also decreases. As a result, stoma density; It has been stated that it varies according to the genotype of plant grown, the ecology of the cultivation, the cultural processes applied, the maturity level of the leaves, the location and location of the leaves.

Positive linear relationships were determined between leaf area and the other factors (leaf length, leaf width and pedicel length). A positive linear relationship was determined between stomata length and stomata width of almond cultivar and hybrid genotypes used in the study. However, the number of stomata in the leaf; differed independently of stomata width, stomata length and leaf characteristics. Accordingly, the number of stomata in unit leaf surface area, stomata width and stomata length can be a defining factor for almond cultivars and hybrids in a given ecology. Investigations on different directions of almond leaves showed that stomata characteristics could change depending on the direction. Findings supporting this view were found in almost all of the investigated almond genotypes. These plants, which have late flowering and self-fertility characteristics, are extremely important for semi-arid conditions.

It was concluded that the closure of stomata causes an increase in leaf water potential. It can be hypothesized that stomata closure slows down the transpiration rate and thus prevents leaf dehydration. It is recommended to evaluate the varieties that can tolerate these problems in regions with low precipitation.

In this study, cultivars and genotypes were grown under the same conditions and the cultivars were compared with each other. Leaf size is an indication that it should be grown in moist and irrigated areas. Plants with small leaf sizes should be preferred in arid areas. The obtained findings will shed light on future studies by evaluating the performance of cultivars and hybrids under regional conditions. It is important to continue the studies on the formation of these plants from late blooming and self-fertile individuals. Since it is a species that can be caught in late spring frosts during flowering and small fruiting periods, these studies should continue.

Different results have been obtained in studies on stomata morphology, leaf characteristics and the relationship between water content and arid conditions. This shows that it is necessary to carry out different studies on the subject. The data obtained in determining the morphological and physiological characteristics of the leaves are the values reached under field conditions. In this respect, it is important to make pomological analyzes of the varieties in the same ecology and to evaluate the plants in question as genetic material.

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Influence of Selected Heavy Metals on Cell Growth and Camphor Secretion in *Achillea gypsicola* Hub. Mor. *In vitro* Cell Cultures

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Abstract: The use of abiotic and biotic elicitors for increasing the accumulation of pharmaceutical active ingredients in plant tissues has gained an increasing interest worldwide. This study was intented to provide promoting accumulation of camphor and phenolic compound using cadmium chloride ($CdCl_2$) and silver nitrate ($AgNO_3$) in cell culture of *Achillea gypsicola*. Growing cells from 8-day-old cultures were treated with three concentrations (5, 25 and 50 µM) of $CdCl_2$ and $AgNO_3$, along with the control. The quantification of camphor and phenolic compound were performed using Headspace-GC-MS and spectrophotometer, respectively. The content of camphor and phenolic compound, cell number and cell dry weight were significantly affected by increasing doses of $AgNO_3$ and $CdCl_2$. The highest significant change in camphor content was observed in cell treated with 25 µM $CdCl_2$ and $AgNO_3$ with a 6.88 and 6.32 fold increase, respectively. The application of 50 µM $AgNO_3$ and $CdCl_2$, however, resulted in a rapid decine in all attributes studied, implying that culture of *A. gypsicola* is susceptible to elicitation by high concentrations of these elicitors. In conclusion, using $AgNO_3$ and $CdCl_2$ elicitors in cultured tissues of *A. gypsicola* would be of great importance to enhanced production of desired bioactive compounds of medicinal importance.

Keywords: Compositae, Elicitor, Natural products, Terpenoid

Achillea gypsicola Hub. Mor. *In vitro* Hücre Kültürlerinde Seçilmiş Ağır Metallerin Hücre Büyümesi ve Kamfor Birikimi Üzerindeki Etkisi

Öz: Farmasötik aktif bileşenlerin bitki dokularında birikimini arttırmak için abiyotik ve biyotik elisitörlerin kullanımı dünya çapında artan bir ilgi kazanmıştır. Bu çalışma, *Achillea gypsicola* hücre kültüründe kadmiyum klorür ($CdCl_2$) ve gümüş nitrat ($AgNO_3$) kullanılarak kamfor ve fenolik bileşik birikimini teşvik etmeyi amaçlamıştır. Sekiz günlük kültürlerden büyüyen hücreler, kontrol ile birlikte üç konsantrasyonda (5, 25 ve 50 uM) $CdCl_2$ ve $AgNO_3$ ile muamele edildi. Kamfor ve fenolik bileşik miktar tayini sırasıyla Headspace-GC-MS ve spektrofotometre kullanılarak yapıldı. Kamfor ve fenolik bileşik miktarı, hücre sayısı ve hücre kuru ağırlığı, artan $AgNO_3$ ve $CdCl_2$ dozlarından önemli ölçüde etkilenmiştir. Kamfor miktarındaki en önemli değişiklik, sırasıyla 6.88 ve 6.32 kat artışla 25 uM $CdCl_2$ ve $AgNO_3$ ile muamele edilen hücrede gözlendi. Bununla birlikte, 50 uM $AgNO_3$ ve $CdCl_2$'nin uygulanması, çalışılan tüm özelliklerde hızlı bir düşüşle sonuçlandı ve bu, *A. gypsicola*'nın süspansiyon kültürünün bu elisitörlerin yüksek konsantrasyonları tarafından ortaya çıkmaya duyarlı olduğunu ima etti. Sonuç olarak, *A. gypsicola*'nın kültürlenmiş dokularında $AgNO_3$ ve $CdCl_2$ elisitörlerinin kullanılması, tıbbi öneme sahip arzu edilen biyoaktif bileşiklerin üretiminin arttırılması için büyük önem taşıyacaktır.

Anahtar Kelimeler: Compositae, Elisitör, Doğal bileşikler, Terpenoid

1.Introduction

Plant secondary metabolites (SMs) have been used for centuries in traditional folk medicine around the world to meet many different needs due to their many biological activities. In accordance with, the qualitative and quantitative evaluations of medicinal plants mostly focusing on the enhancement of SM synthesis and accumulation have increased over the past decades (Açıkgöz, 2020a; Dağlioğlu et al., 2022). It has been well documented that, SMs accumulate in small amounts in specialized tissues of various plant organs in most plants presumably in a certain stage of growth and development (Bourgaud et al., 2001; Murthy et al., 2014). SMs from medicinal and aromatic plants were obtained from wild or cultivated plants, with the majority of commercial supply is obtained from collected wild plants throughout the world (Nosov, 2012). In concequence of this, their consistent production directly from wild and/or field grown plants to meet the commercial demand becomes a challenging task (Verma & Shukla, 2015; Ramirez-Estrada et al. 2016). The need, therefore, is evident to develop some

reliable and feasible approaches enabling the production of valuable bioactive compounds with sufficient amount and good quality (Matkowski, 2008; Jamwal et al., 2018). Cell and tissue cultures can offer several potential advantages over the extraction of compounds from wild and field grown plants, the principal ones being a rapid, continuous, sustainable and economical production of valuable bioctive compounds with high concentration and purity (Davies & Deroles, 2014). The potential benefits of plant cell and tissue culture techniques in producing these valuable phytochemicals for commercial use have long been recognized (Rao & Ravishankar, 2002; Smetanska, 2008; Srivastava et al., 2019). In spite of its common usage, however, plant cell and tissue cultures have a dilemma of low yield of valuable plant bioactive compounds of commercial importance (Zhao et al. 2005; Isah et al. 2018). Therefore, various strategies have been developed for stimulating the synthesis of SMs using plant cell and tissue culture (Açıkgöz et al., 2018a; Açıkgöz et al., 2018b; Halder et al., 2019).

The synthesis of biologically active chemicals frequently occurs in plants exposed to environmental stresses comprising diverse elicitors or signal molecules (Ramakrishna & Ravishankar, 2011; Yue et al., 2016). SM synthesis and accumulation can be stimulated by the treatment of elicitors, activating plant defense system and triggering stress response, to overcome the low yield in cell and tissue cultures (Georgiev et al., 2009; Thakur et al., 2019). A number of studies have shown promising results in that employing biotic and abiotic elicitors to plant tissues to initiate, stimulate or enhance the biosynthetic pathways leading to enhanced production of important bioactive compounds (Verma & Shukla, 2015; Isah et al., 2018).

Various elicitors, with biological and non-biological origin (heavy metal salts, yeast, silver nitrate, salicylic acid, cadmium chloride, and chitosan etc.) have been widely used to induce the synthesis and increase the accumulation of bioactive compounds *in vitro* culture (Açıkgöz et al., 2022). The use of abiotic elicitors with chemical and physical stimuli *in vitro* cultures has atracted less interest as compared to the biotic elicitors (Radman et al., 2003). It has been proven that heavy metal induced stress activates signaling pathways which influence the synthesis of certain plant metabolites (Srivastava et al., 2019; Batı Ay et al., 2022). Silver nitrate and cadmium chloride have been proven to be associated with the accumulation of SMs *in vitro* cultures (Cetin et al., 20014; Park et al., 2016).

As in many parts of the world, bioactive substances of medicinal and aromatic plants were predominantly obtained form the plants grown wild in Turkey, with well over 11 000 flowering plant species, about one third of them is endemic (Baser, 2002). Intensive collection of economically important medicinal plants from their natural habitats has recently threatened their existence in the wild throughout the country. The genus *Achillea*, a member of *Asteraceae* family, commonly known as yarrow is represented by 59 species in Anatolia, 31 of which are endemic to Turkey, with a long history of use in traditional folk medicine (Mohammadhosseini et al., 2017; Demirci et al., 2018). *A. gypsicola*, an endemic species to Turkey, is of considerable importance as a good source of camphor with well-known medicinal properties (Açıkgöz, 2019; Açıkgöz, 2020b).

To our knowledge, there is no published work related with effect of $CdCl_2$ and $AgNO_3$ elicitors on production of SMs in *A. gypsicola.* In view of this, the current study intented to study the effect of $AgNO_3$ and $CdCl_2$ elicitors on cell growth and accumulation of phenolic compounds and camphor in *A. gypsicola* cell suspension culture.

2. Materials and Methods

2.1. Preparation of cell suspension cultures

The seeds of *A. gypsicola* were collected from the wild plants present in its natural habitat in Central Anatolia of Turkey (40°73′ N, 34°47′E) and properly identified at Ordu University, Turkey. For surface sterilization, the seeds were subjected to 70% (v/v) ethanol for 2 min and 25% (v/v) NaOCl for 45 min. Afterwards, the seeds were rinsed 3-4 times using distilled water for removing ethanol traces. The seeds soaked in 200 µM KNO_3 solution for 48 h were cultured in MS medium containing 2 mg/l GA_3 in a growth chamber at 25 ºC and for 16/8 (L/D) photoperiod and sterile plantlets were grown (Açıkgöz et al. 2019). The stem segments of sterile plantlets were used to establish cell suspension cultures. The stem explants were transferred to B_5 medium enriched with 0.5 mg/l BAP + 0.5 mg/l NAA. The obtained calluses were agitated at 105 rpm on a rotary shaker at 25 ºC for 16/8 (L/D) photoperiod.

2.2. Elicitation process and extraction

Approximately, 2,5 g cell suspension cultures were placed in 250 mL Erlenmeyer flasks having liquid medium of 50 mL and four concentrations (0, 5, 25 and 50 µM) of $AgNO_3$ and $CdCl_2$ were added. The cell suspension cultures were collected at 8, 48 and 72 h after

elicitation. After aseptically filtered and washed using deionized water, harvested suspensions were placed in a deep-freezer (-20 ^{o}C) till to extraction. Before subjecting to chemical analysis, the suspensions were powdered using a mortar and pestle. The procedure explained by Açıkgöz, (2021) was employed to prepare ethanol extracts. Briefly, 10 ml 96% ethanol was added to 2 g suspensions and homogenized for 2 min. The resulting mixture was placed in water bath at 45 ^{o}C for 12 hours before centrifuging at 4000 rpm on a rotary shaker for 5 min. The supernatants were evaporated for complete dryness in a rotary evaporator at 75 ^{o}C. The dried extracts were dissolved in 1 ml methanol and used for further analysis.

2.3. Determination of phenolic compounds, camphor and cell growth

To determine the total phenolic contents, Folin-Ciocalteu reagent based spectrophotometric assay was used as explained by Slinkard and Singleton (1977). The standard curve of gallic acid solution was used and the absorbance was recorded at 765 nm. The total phenolic content was estimated as milligram gallic acid equivalent per gram of fresh cell weight (mg GAE/g fresh weight). Dimethylamino cinnamaldehyde (DMAC) chromogenic reagent was employed to determine total flavanol according to (Prior et al. (2010). The absorbance at 640 nm was monitored and total flavanol values were presented as milligram cathecin equivalent per gram of fresh cell wight (m CTE/g fresh weight). For determination of total flavonol contents, Neu solution was used as described by Dai et al., (1995). Briefly, 1% 2-aminoethyl diphenylborinate and methanol was added to the extracts. The absorbance of resulting mixture was displayed at 410 nm and presented as milligram rutin equivalent per gram of fresh cell wight (m RE/g fresh weight). The protocol of Qu et al. (2006) was performed to determine total anthocyanin using McIIvaine's buffer and absorbance was recorded at 570 nm. As an indicator of anthocyanin content, color value (CV) of the extract was estimated using the equation of CV = 0.1 × Absorbance × Dilution factor (CV g^{-1} FCW).

The content of camphor was quantified through a Headspace GC-MS integrated with Shimadzu QP2010 ultra and Shimadzu AOC-5000 plus auto sampler was used. The capillary separation was performed on an RTX-5M 30 m column. Before the analysis, the device was uploaded with camphor standard and the retention times and mass fragments of the solution were determined. The calibration curve was plotted and the content of camphor was given in µg g^{-1}. The working layouts of GC-MS were given as below; carrier gas of helium, 250 ^{o}C injection temperature, 0.5 ml injection volume, 70 eV ionization voltage, 100 ^{o}C temperature and 10 min heating period.

Growth parameters such as cell number, cell viability (%), and cell dry weight (g l^{-1}) were recorded in cell suspension cultures. The Nageotte Counting Chamber as given by Moroff et al. (1994) was used to determine the number cells in suspensions. In measuring cell viability, the trypan blue staining technique described by Laloue et al. (1980) was employed. The filtered cells stored in an oven at 55 ^{o}C for 48 h were used to estimate cell dry weight.

2.4. Design of experiment and data analysis

All treatments were performed in triplicates. Variance analysis of the data was carried out based on completely randomized design using Minitab 17 statistical software. The significance test among the treatment means was performed by Tukey test at the 5% level.

3. Results

$AgNO_3$ and $CdCl_2$ elicitors significantly affected the accumulation of phenolic compounds and camphor along with cell growth in general. Exposing *A. gypsicola* cell suspension cultures to different concentrations of $AgNO_3$ caused significant alterations in all parameters studied, except total phenolic content (Tables 1 and 2). The influence of culture time was, however, non-significant for any of the attributes evaluated. The interaction between $AgNO_3$ doses and culture periods was significant for total phenolic content, indicating that the effect of $AgNO_3$ doses differed depending on culture period.

Significant and regular increases corresponding to 5 and 25 µM $AgNO_3$ doses were observed in all parameters, but the additional dose of 50 µM $AgNO_3$ resulted in significant decreases. The maximum production of camphor (1.6711 µg g^{-1}) was recorded with the use of 25 µM $AgNO_3$, which is 6.32 times higher compared to the control culture. Similarly, treatment of 25 µM $AgNO_3$ produced 31.5%, 42.8%, 30.25%, and 3.05% increases in total flavanol, total anthocyanin, cell number and cell dry weight in comparison to the control culture, respectively.

Subjecting *A. gypsicola* cell suspension cultures to several concentrations of $CdCl_2$ created a significant influence on total phenolic, total flavanol, total flavonol, camphor content, total anthocyanin, cell dry weight and

cell number (Tables 3 and 4). Increasing concentrations of $CdCl_2$, on the contrary, created no significant changes in cell viability. Culture period did not show any significant effects on the attributes evaluated, whereas interaction effect between $CdCl_2$ doses and culture periods was significant for cell viability.

Table 1. Contents of phenolic compounds and camphor in cell suspension cultures of *A. gypsicola* treated with silver nitrate

Çizelge 1. *Gümüş nitrat ile muamele edilmiş A. gypsicola hücre süspansiyon kültürlerinde fenolik bileşikler ve kamfor içeriği.*

Silver nitrate doses (µM)	Culture periods (hours)			Mean
	8	48	72	
Total phenolic (mg g^{-1})				
0 (control)	0.6369 abc*	0.6382 ab	0.6418 ab	0.6389
5	0.6396 abc	0.6452 ab	0.6548 ab	0.6465
25	0.6774 a	0.6851 a	0.6925 a	0.6850
50	0.6659 a	0.6558 ab	0.6536 ab	0.6584
Mean	0.6549	0.6560	0.6606	
Total flavanol (mg g^{-1})				
0 (control)	0.0099	0.0111	0.0115	0.0108 b*
5	0.0117	0.0123	0.0123	0.0121 b
25	0.0136	0.0141	0.0150	0.0142 a
50	0.0111	0.0106	0.0111	0.0109 b
Mean	0.0441	0.0434	0.0448	
Total flavonol (mg g^{-1})				
0 (control)	0.0426	0.0436	0.0429	0.0430 b*
5	0.0448	0.0459	0.0476	0.0461 a
25	0.0437	0.0419	0.0458	0.0438 ab
50	0.0453	0.0422	0.0428	0.0434 b
Mean	0.0441	0.0434	0.0448	
Total anthocyanin (CV g^{-1})				
0 (control)	0.0053	0.0058	0.0055	0.0056 b*
5	0.0059	0.0062	0.0059	0.0060 a
25	0.0075	0.0080	0.0086	0.0080 a
50	0.0041	0.0039	0.0052	0.0044 c
Mean	0.0057	0.0059	0.0063	
Camphor (µg g^{-1})				
0 (control)	0.2561	0.2624	0.2740	0.2642 d*
5	0.3707	0.3768	0.3676	0.3717 c
25	1.6661	1.6710	1.6760	1.6711 a
50	0.8497	0.8349	0.8273	0.8373 b
Mean	0.7857	0.7863	0.7862	

* The lowercase letters represent significance at 0.05 level ($P<0.05$).

Table 2. Cell number, cell dry weight and cell viability in *A. gypsicola* cell suspension cultures treated with silver nitrate for various hours.

Çizelge 2. *Çeşitli saatler boyunca gümüş nitrat ile muamele edilmiş A. gypsicola hücre süspansiyon kültürlerinde hücre sayısı, hücre kuru ağırlığı ve hücre canlılığı.*

Silver nitrate doses (µM)	Culture periods (hours)			Mean
	8	48	72	
Cell number				
0 (control)	82.840	82.760	83.120	82.906 d*
5	92.920	93.240	94.200	93.453 b
25	106.300	108.640	109.000	107.980 a
50	84.640	84.500	85.640	84.927 c
Mean	91.675	92.285	92.990	
Cell dry weight (g l^{-1})				
0 (control)	9.2462	9.2452	9.2578	9.2497 d*
5	9.4612	9.4405	9.4427	9.4481 b
25	9.5192	9.5294	9.5461	9.5316 a
50	9.3378	9.3390	9.3443	9.3403 c
Mean	9.3911	9.3885	9.3977	
Cell viability (%)				
0 (control)	97.9733	97.7467	97.3467	97.6889 a*
5	97.5400	98.0667	98.1400	97.9156 a
25	98.2067	97.3467	98.2167	97.9233 a
50	95.6800	94.7000	95.3000	95.2267 b
Mean	97.3515	96.9650	97.2508	

* The lowercase letters represent significance at 0.05 level ($P<0.05$).

Table 3. Contents of phenolic compounds and camphor in cadmium chloride tretaed *A. gypsicola* cell suspension cultures.

Çizelge 3. *Kadmiyum klorür ile muamele edilmiş A. gypsicola hücre süspansiyon kültürlerinde fenolik bileşikler ve kamfor içeriği*

Cadmium chloride doses (µM)	Culture periods (hours) 8	48	72	Mean
Total phenolic (mg g^{-1})				
0	0.6369	0.6382	0.6418	0.6390 c*
5	0.6371	0.6388	0.6410	0.6390 c
25	0.6807	0.6870	0.6921	0.6866 a
50	0.6562	0.6503	0.6455	0.6507 b
Mean	0.6549	0.6410	0.6551	
Total flavanol (mg g^{-1})				
0	0.0099	0.0111	0.0115	0.0108 b*
5	0.0111	0.0113	0.0121	0.0115 b
25	0.0136	0.0140	0.0156	0.0144 a
50	0.0120	0.0118	0.0114	0.0117 b
Mean	0.0117	0.0121	0.0126	
Total flavonol (mg g^{-1})				
0	0.0426	0.0436	0.0429	0.0430 b*
5	0.0427	0.0429	0.0443	0.0433 b
25	0.0448	0.0459	0.0476	0.0461 a
50	0.0415	0.0431	0.0416	0.0421 b
Mean	0.0429	0.0438	0.0441	
Total anthocyanin (CV g^{-1})				
0	0.0053	0.0058	0.0055	0.0056 b*
5	0.0062	0.0045	0.0055	0.0054 b
25	0.0081	0.0078	0.0074	0.0078 a
50	0.0053	0.0043	0.0038	0.0045 b
Mean	0.0062	0.0056	0.0056	
Camphor (µg g^{-1})				
0	0.2561	0.2624	0.2740	0.2642 d*
5	0.4708	0.4757	0.4666	0.4710 c
25	1.7890	1.8154	1.8515	1.8186 a
50	0.9535	0.9488	0.9446	0.9490 b
Mean	0.8673	0.8755	0.8841	

* The lowercase letters represent significance at 0.05 level (P<0.05).

Table 4. Cell number, cell dry weight and cell viability in *A. gypsicola* cell suspension cultures treated with cadmium chloride for various hours.

Çizelge 4. *Çeşitli saatler boyunca kadmiyum klorür ile muamele edilmiş A. gypsicola hücre süspansiyon kültürlerinde hücre sayısı, hücre kuru ağırlığı ve hücre canlılığı.*

Cadmium chloride doses (µM)	Culture periods (hours) 8	48	72	Mean
Cell number				
0	82.800	82.700	83.100	82.867 c*
5	84.000	83.800	85.000	84.267 c
25	108.000	108.700	109.000	108.567 a
50	96.600	97.500	98.700	97.600 b
Mean	92.850	93.175	93.950	
Cell dry weight (g l^{-1})				
0	9.2462	9.2452	9.2578	9.2497 c*
5	9.3378	9.3390	9.3443	9.3403 c
25	9.5253	9.5294	9.5461	9.5336 a
50	9.4612	9.4405	9.4427	9.4481 b
Mean	9.3926	9.3896	9.3977	
Cell viability (%)				
0	97.9733 a*	97.7467 a	97.3467 a	97.6889
5	98.4200 a	98.3433 a	97.8267 a	98.1966
25	98.0500 a	98.2333 a	97.6600 a	97.9811
50	96.7567 a	94.5567 b	93.6767 b	94.9967
Mean	97.8000	97.2200	96.6275	

* The lowercase letters represent significance at 0.05 level (P<0.05).

The content of camphor reached its maximum of 1.8186 μg g^{-1} at with 25 μM $CdCl_2$, indicating a 6.88-fold increase compared to the starting culture. Treatment of 25 μM $CdCl_2$ produced 33.3%, 39.2%, and 31.0 increases in total flavanol, total anthocyanin and cell number compared with untreated culture as control, respectively. Similarly, suspension cultures treated with 25 μM $CdCl_2$ ended up with a 7.4 and 7.2-fold increases in total phenolic and total flavonol as compared to control. Similar to $AgNO_3$, a significant decrease in all parameters was noticed in suspension cultures treated with 50 μM $CdCl_2$.

4. Discussion

It has been well documented that heavy metals stimulate the biosynthesis of numerous bioactive compounds of economic importance in plant tissue culture (Kim et al. 2004; Zhao et al., 2010; Park et al., 2016; Srivastava et al., 2019; Zafar et al., 2020). For example, Ag+ has been proven to be associated with ethylene pathways which regulates the synthesis of SMs as a defense response of plant tissues (Pitta-Alvarez et al., 2000; Li et al., 2016). However, little information is available in scientific literature regarding heavy metal induced accumulation of SMs in Achillea species (Ghanati et al., 2014). There is no published study on the effects of $AgNO_3$ and $CdCl_2$ in *A. gypsicola* cell suspension culture.

In an attempt to increase the content of camphor, phenolic compounds and cell growth, we exposed cell suspension cultures to various levels of $AgNO_3$ and $CdCl_2$ for different exposure times. Our results indicated that both $AgNO_3$ and $CdCl_2$ could elicit cell growth and the production of phenolic compounds and camphor in *A. gypsicola* suspension culture. It is interesting to note that the effect of $AgNO_3$ and $CdCl_2$ on the content of camphor, phenolic compounds and cell growth were almost the same. The treatments of $AgNO_3$ and $CdCl_2$ up to 25 μM significantly enhanced the content of almost all phenolic compounds and camphor along with cell growth.

The effect of $AgNO_3$ and $CdCl_2$ was quite more obvious in camphor content in which 25 μM treatments of both elicitors resulted in more than 6-fold increase as compared to the initial culture. Regarding well-known medicinal properties of camphor, therefore, it sounds reasonable to conclude that both $AgNO_3$ and $CdCl_2$ would be effectively used as potent elicitors in suspension culture of *A. gypsicola*. The application of 50 μM $AgNO_3$ and $CdCl_2$, however, produced a rapid decline in all attributes studied, implying that cell suspension culture of *A. gypsicola* is susceptible to elicitation by high concentrations of $AgNO_3$ and $CdCl_2$. These data suggest that the stimulatory effects of $AgNO_3$ and $CdCl_2$ elicitors appear to be concenration dependent (Li et al., 2016). Among others, the concentration of elicitors is a key factor affecting the magnitude of the response and it varies depending on plant species, culture conditions and inoculation period (Isah et al., 2018;). Studying with cell suspension cultures of *Silybum marianum*, Ashtiani et al., (2010) indicated that Ag+ in low concentrations positively elicited silymarin production and cell growth, whereas a high dose of Ag+ showed inhibition. Similarly, Cetin et al., (2014) reported that treatment with $CdCl_2$ in 1.5 mM gave the lowest amounts of total phenolics, while application of 1.0 mM $CdCl_2$ produced the highest values in *Vitis vinifera* cell suspension culture.

In the present study, an increase in all phenolic compounds including anthocyanin was observed in cell suspension cultures treated with 5 and 25 μM $AgNO_3$ and $CdCl_2$. In an earlier study, however, we found that salicylic acid significantly enhanced synthesis of anthocyanin in *A. gypsicola* cell suspension culture, while methyl jasmonate resulted in a significant decrease (Açıkgöz et al., 2019). Furthermore, Ghanati et al., (2014) reported that treatment of *Achillea millefolium* with silver nanoparticles significantly increased camphor content, whereas the level of anthocyanin decreased. Based on these findings, it appears reasonable to conclude that different elicitors somehow produce metabolite-dependent responses. Moreover, elicitors do not function equally in all species and the response to an elicitor might be species-specific (Cai et al., 2012; Pitta-Alvarez et al., 2000).

Elicitor specificity and concentration, type of culture medium and time duration of elicitor exposure are among major regulating factors responsible for enhanced synthesis of SMs. In the current study, however, no significant variation was observed in the effect of culture period in any of the parameters in $AgNO_3$ and $CdCl_2$ treated cell suspensions. In a previous study, we found that culture period significantly affected total flavanol, camphor content and cell number in cell suspension culture of *A. gypsicola* treated with various doses of MeJA and SA (Açıkgöz, 2017; Açıkgöz et al., 2019). In a study, Namdeo et al. (2002) reported that suspension cultures of *Catharanthus roseus* elicited using *T. viride* for 48 h produced a 3-fold increase in ajmalicine content. A longer period of elicitor exposure, however, adversely affected ajmalicine production.

5. Conclusion

To our knowledge, this is the first instance of using $AgNO_3$ and $CdCl_2$ elicitors for enhancing camphor, phenolic compounds and cell growth in cell suspension cultures of *A. gypsicola*. The addition of $AgNO_3$ and $CdCl_2$ significantly increased the accumulation of camphor in cell suspension cultures. As compared to the starting culture, the addition of 25 µM $CdCl_2$ and $AgNO_3$ to cell suspension cultures brought about 6.88 and 6.32-fold increases in camphor content, respectively. The production of phenolic compounds and cell growth were also stimulated by $AgNO_3$ and $CdCl_2$ elicitors. Furthermore, culture period caused no significant variation in any of the attributes studied. The results of the current study revealed that $AgNO_3$ and $CdCl_2$ as elicitors could have a good potential in increasing the synthesis of phenolic compounds and camphor in *A. gypsicola* cell suspension culture.

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Evaluation of Soil Color and Soil Fertility Relations on Cultivated Semi-Arid Sloping Landscapes

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Abstract: Soil color is a critical property, providing important information on soil properties. Soil color highly spatially varies on cultivated semi-arid sloping landscapes, indicating differences in soil properties that affect soil fertility. This study evaluated the relationships between color variables (L*: soil brightness, a*: redness, and b*: yellowness) and some basic soil properties on air dry and wet (around field capacity) soil samples, in a semi-arid sloping landscape having been under wheat cultivation for a long time. The values of color variables and soil properties were graphed and relationships between them were modeled using most proper regression models. The soil properties were poorly related to values of a* and b*, while $CaCO_3$, sand, clay, and K contents and EC were highly significantly correlated with values of L*-wet (L*-values obtained on moist soil samples). Soil EC and $CaCO_3$ content can be safely predicted by L*-wet in the study area. Also, the L*-wet should be preferred over L*-dry in predicting soil properties from soil color components in the study area and similar soils.

Keywords: Soil color, Soil fertility, Soil water content, semi-arid sloping landscapes

Ekili Yarı-Kurak Eğimli Bir Arazide Toprak Rengi ve Toprak Verimliliği Arasındaki İlişkinin Değerlendirilmesi

Öz: Toprak rengi, toprak özellikleri hakkında önemli bilgiler sağlayan kritik bir özelliktir. Toprak rengi, ekili yarı kurak eğimli arazilerde mekansal olarak oldukça değişkendir ve bu, toprak verimliliğini etkileyen toprak özelliklerindeki farklılıkları gösterir. Bu çalışmada uzun süredir buğday ekimi yapılan yarı kurak eğimli bir arazide hava kuru ve ıslak (tarla kapasitesi civarında) toprak örneklerinde renk değişkenleri (L*: toprak parlaklığı, a*: kırmızılık ve b*: sarılık) ile bazı temel toprak özellikleri arasındaki ilişkileri değerlendirilmiştir. Renk değişkenlerinin değerleri ve toprak özellikleri grafik haline getirilmiş ve aralarındaki ilişkiler en uygun regresyon modelleri kullanılarak modellenmiştir. Toprak özellikleri a* ve b* değerleriyle zayıf bir şekilde ilişkiliyken, $CaCO_3$, kum, kil ve K içerikleri ve EC, L*-wet (nemli toprak numunelerinde elde edilen L* değerleri) değerleriyle yüksek oranda anlamlı bir şekilde ilişkili çıkmıştır. Toprak EC ve $CaCO_3$ içeriği, çalışma alanındaki L*-wet ile güvenle tahmin edilebilir. Ayrıca, çalışma alanındaki toprak rengi bileşenlerinden ve benzer topraklardan toprak özelliklerinin tahmin edilmesinde L*-wet, L*-dry yerine tercih edilmelidir.

Anahtar Kelimeler: Toprak rengi, Toprak verimliliği, Toprak su içeriği, yarı kurak eğimli alanlar

1. Introduction

Soil is one of the most important components of agriculture and can have a significant influence on crop yields and quality. Rapid and accurate description of soil properties is critical in environmental and agricultural nexus. Technological solutions developed such as synthesis of fertilizers, site-specific management systems, and mapping and modelling of physicochemical and morphological soil properties provide key information to make proper decisions for sustainable use of soil resources in harmony with other components of environment. However, these technologies are not accessible by majority of farmers as they are expensive and time consuming (Walter 2002).

Soil color, which is one of the most important soil morphological characteristics, can provide information on mineral composition and organic matter (Fang et al. 1999), water, and nutrient contents (Shen et al. 2006; Budak et al. 2018). Soil color is widely used in soil research as it can be determined easily at a low cost (Günal et al. 2008). The light soil color indicates high calcium carbonate content, which suggests low fertility compared to dark brown to black soils, which are rich in organic matter (Shields et al. 1968, Schulze et al. 1993; Bigham et al. 1978, Schwertmann 1993). Soil color can

also provide information on chemical processes as a function of the soil moisture regime (Ji et al. 2007).

Soil color components of a*, b*, and L* can be used as indicators for establishing important quantitative relationships between soil color and soil fertility or rather, between soil color and those soil properties affecting soil fertility. However, the color of soils does not usually have agronomic significance, since the same color can be due to different constituents and correspond to soils of different and uneven appearance and properties. The relationship between soil fertility and iron and its oxides, through color has been demonstrated by several studies. An analysis between the three fundamental quantities of the Munsell Chart (hue, value, and chroma) and the iron content showed that the soil iron content was positively correlated with the soil chroma (luminosity) (Courault et al. 1988). However, this correlation is much more important in the relationship between soil iron content and chroma. Organic matter can be estimated from the color of a soil. A study, which was conducuted on Anatolian soils also had a significant relationship between soil color and soil organic matter content (R^2 = 0.35) (Günal ve Erşahin 2006). However, the influence of organic matter on the soil color depends on the nature of the organic matter, i.e. the ratio between humic acids and fulvic acids (Shields et al. 1968). Moreover, it is accepted that light colored soils are considered less fertile than dark colored soils because they are poor in organic matter. The amount of $CaCO_3$ in the soil influences its color. Courault et al. (1988) reported that correlation coefficient between soil luminosity and the amount of $CaCO_3$ was far greater than those with the other two soil color quantities (soil redness and soil yellowing). Also, soil reflectivity measured at a wet condition (e.g., around field capacity) on a soil sample may be different from the one measured on dry condition (e.g., at air dry water content) of the same soil sample, resulting in different correlation magnitudes between a soil property (e.g., pH) and a color component (e.g., a*).

The objectives of this study were 1) to evaluate correlation between some principal soil basic soil properties and soil color components of a*, b*, and L* at wet (around field capacity) and dry conditions (at air dry water content) on an approximately 100-ha sloping landscape, showing color contrasts, under dryland wheat production, and 2) to evaluate differences between correlation coefficients at dry and wet conditions for the purpose of determining the proper soil water state for modeling soil fertility color relations in the study are.

2. Materials and Methods

2.1. Study Area

The study was conducted on a sloping approximately 100-ha cultivated landscape, 20 km from Çankırı city center (near the Çankırı-Ankara Road and on the agricultural land of Aşağı Pelitözü). Rainfed wheat has been grown in the study area for over 70 years using the conventional tillage practices (Fig. 1). Soil sampling was conducted after two weeks wheat was harvested. The elevation of study area is between 514 and 805 m. The study area is located in the Bozkır formation, which is an Upper Miocene geological formation. White colored gypsum is the dominant lithology and there is clay and marl (green-gray in color) as an intermediate bands with gypsum (Sarp 2010). The study area has a semi-arid climate; mean annual temperature is 11.3°C, and the mean annual precipitation is 440.8 mm. Soil moisture regime is Xeric. Mean soil temperature at 50 cm depth in the center of Çankırı is 14.7°C, thus, soil temperature regime is classified as Mesic (Sünal, 2018).

2.2 Soil sampling

Soil samples were taken at 155 sampling sites from approximately 0-30 cm soil depth (depending on the tillage depth at the sampling site). The sampling sites were discriminated to achieve condition that there were at least three representative samples from each area with a difference in color. The color variability of the study area could be observed with the naked eye Fig.1). For each sampling site, the position was recorded using a GPS. The soil samples were then dried in a room, passed through a 2mm sieve and prepared for analysis.

Figure 1. Location of study area
Şekil 1.*Çalışma alanının konumu*

2.3 Methods

2.3.1 Determination of soil color with a spectrometer

The spectrophotometer or simply spectrometer gives numerical values L*, a*, b* using the XYZ system defined by the International Commission on Illumination (CIE). The principle of this system consists in translating the color of an object into a point having X, Y, and Z coordinates (trichromatic values) in the CIELAB color space. Having been used widely since 1976, this system is a benchmark standard for measuring the color objectively. The numerical values of L*, a*, and b* designate the three quantities, which characterize a color. L* describes the brightness, which goes from L* equal to 0 for black to L* equal to 100 for maximum brightness. The two parameters a* and b* express the deviation of the color from that of a gray surface of the same lightness. The point a* varies between red (values are positive in the range of red) and green (values are negative in the range of green). Point b* varies between yellow (values are positive in the yellow range) and blue (negative values are in the blue range).

2.3.2 Analyses of basic soil properties

Analyses of 155 soil samples taken from the field were carried out in Soil Laboratory at Forestry Faculty of Çankırı Karatekin University. The soil variables analyzed, and the methods used are given in the Table 1.

Table 1. Soil variables and the methods used for their analysis
Çizelge 1. *Toprak değişkenlerinde kullanılan analiz yöntemleri*

Soil property	Methods/device	Reference
Soil color	Colorimeter	
Soil texture	Mechanical analysis	Gee and Bouder 1986
Available potassium content and available sodium content	Using a flame photometer	Kacar 1994
Field capacity	Pressure chambers	Cassel and Nielsen 1986
Wilting point	Pressure chambers	Cassel and Nielsen 1986
Available water capacity	Difference between field capacity and wilting point	Cassel and Nielsen 1986
Electrical conductivity	With an EC electrode in 1 / 2.5 soil-water suspension	Rhoades *et al.*1999
Soil reaction (pH)	With a pH electrode in 1 / 2.5 soil-water suspension	Rhoades *et al.* 1999
Organic matter content	Walkley-Black method	Nelson and Sommers 1982
$CaCO_3$ content	Scheibler calcimeter	Çağlar 1958
Available P content	Olsen method	Olsen *et al.*1954
Aggregate stability index	Wet sieving	Kemper and Rosenau 1986

2.3.3 Statistical Analysis

The descriptive statistics for soil properties and color components were calculated. The values for L*, a*, b* were graphed against the values of soil properties, and the relationship was modelled using the most proper regression model (linear, exponential, and polynomial). The performance of regression models was evaluated by coefficient of determination (R^2) and the relative mean square error. Some outliers in data sets of some soil variables were trimmed to decrease skewness of data and to improve model fit. In this regards, 10 data points for EC, 8 for sand content, 5 for of $CaCO_3$ content, 9 for clay content, and 10 for K content were trimmed. In statistical tests, the null hypothesis was rejected at 5% level of significance, unless stated otherwise.

3. Results and Discussion

Descriptive statistics of the soil properties of the study area are given in Table 2. Majority of soils are clay. Sand content varies between 11.2% and 43.7%, with a mean of 25.7%, and clay content varies between 40.5% and 69.7% with a mean of 54.59%. A soil attribute with a CV>40% is considered highly, between 15% and 40% is moderately, and <15% little variable (Mulla and McBratney 2000). A soil variable with a skewness (S) smaller than $|0.5|$ is considered slightly skewed and deemed normally distributed, between $|0.50|$ and $|1.0|$ moderately skewed and greater than $|1.0|$ is considered strongly skewed (Webster 2001). Kurtosis measures weighting of the tails relative to the middle of the distribution (Kleinbaum et al., 2013). Standardized kurtosis for a standard normal distribution is 3, and this value is often subtracted from the calculated value. The resulting statistic can be negative for flat distributions with short tails, approximately zero for a normal distribution, and it is positive for distributions with large tails (Kleinbaum et al., 2013).

Majority of the soil properties are moderately variable. Soil pH exhibited lowest and EC exhibited highest variability. Both of the variables had highly right-skewed distribution. Soil textural components behaved inconsistently in variability, kurtosis, and skewness. The greatest variably among soil textural components occurred for sand and lowest for clay content. The variability of clay content was far lower than those for sand and silt contents.

The mean of $CaCO_3$ content of soils was 17.12%. Soil $CaCO_3$ content had moderately variable, slightly negatively kurtotic, and slightly right-skewed distribution. Organic matter (OM) content of soils ranged from 0.62% to 2.95% with a mean of 2.19% (Table 2). The values of OM content were moderately variable, slightly positively kurtotic, and strongly left-skewed, suggesting presence of some relatively extremely low OM-valued localities in the study area.

Distributions for P and K contents are highly inconsistent in kurtosis, skewness, and coefficient of variation (CV) (Table 2). Witling point and FC had similar variability, while their values for kurtosis and skewness were highly inconsistent. Field capacity of the study soils varied between 19.54% and 42.06%, with a mean of 30.56% and WP 5.56% and 20.59%, with a mean of 15.43%; mean values for both of the variables are in accord with clay soils (Koorevaar et al., 1983). Aggregate stability values in the study area vary between 0.327 and 0.611, with a mean of 0.492 (Table 2), which indicates that the soil are moderately resistance to erosion. Values for AS showed a slightly variable, slightly left-skewed, and slightly positively kurtotic distribution.

Descriptive statistics for soil color components are given in Table 3. Most prominent difference between values obtained on moist and air-dry samples occurred for L*. The values for all three components (a*, b*, and L*) showed a slight variability in wet and dry cases.

The relationship between soil properties and the two parameters a* (soil redness) and b* (soil yellowness) were not adequately strong. The highest correlation coefficient was obtained between brightness (L*) for moist samples (L*-wet) and soil properties in all the cases. Relationships between L* and silt, OM, and P contents and soil pH, FC, WP, and aggregate stability index were not significant. Therefore, relationships between those soil properties and L* were not modeled.

Table 2 Descriptive statistics for properties of study soils
Çizelge 2 *Çalışma alanı topraklarının tanımsal istatistikleri*

Soil property	N	Min.	Max.	Mean	SD	S	K	CV (%)
pH (1:2.5)	155	6.80	7.69	7.15	0.23	1.70	1.49	3.21
EC (µS cm^{-1})	144	2.49	2630	472.1	521.31	3.16	9.31	110.43
Sand (%)	144	11.2	43.7	25,7	7,52	0.20	-0,68	29,28
Clay (%)	144	40.5	69.7	54.59	6.13	0.13	-0.25	11.23
Silt (%)	155	5.45	47.05	20.18	5.61	0.96	3.00	27.80
$CaCO_3$ (%)	150	4.65	32.76	17.12	6.22	0.38	-0.34	36.32
OM (%)	155	0.62	2.95	2.19	0.54	-1.10	0.64	24.65
K (mg/kg)	144	13.51	65.1	38.94	12.98	- 0.008	-0.92	33.33
P (mg/kg)	155	0.123	2.082	0.245	0.209	6.161	44.993	85.23
FC (%)	125	19.54	42.06	30.56	4.53	0.21	-0.126	14.85
WP (%)	93	5.56	20.59	15.43	2.36	-1,02	2.75	15.34
AS	155	0.327	0.611	0.492	0.055	-0.211	0.204	11.16

N: Number of samples, EC: Electrical Conductivity, OM: Organic Matter, N: Nitrogen, Na: Sodium, K: Potassium, P: Available Phosphorus, FC: Field capacity, WP: Wilting point, SA: Aggregate stability, Min: Minimum, Max: Maximum, SD: Standard deviation, S: Skewness, K: Kurtosis, CV: Coefficient of variation.

Table 3 Descriptive statistics of soil color variables obtained with air dry and wet (around field capacity) samples
Çizelge 3 *Hava kuru ve ıslak (tarla kapasitesi civarında) numunelerle elde edilen toprak rengi değişkenlerinin tanımlayıcı istatistikleri*

SMS	CC	N	Minimum	Maximum	Mean	SD	CV%
Dry	L*	155	36.13	63.26	47.85	5.92	12.38
	a*	155	2.86	7.86	5.19	0.86	16.61
	b*	155	11.05	19.93	14.68	1.50	10.22
Wet	L*	155	28.82	50.48	37.12	4.32	11.65
	a*	155	4.15	7.99	6.23	0.83	13.26
	b*	155	11.42	21.74	15.58	1.81	11.59

SMS: Soil moisture status, CC: color component, N: Number of samples, L: Brightness, a+: Redness, b+: Yellowness, SD: Standard deviation, CV: Coefficient of variation.

Values for L* obtained with air dry soil samples (L*-dry) associated less strongly to soil properties compared to those obtained for L*-wet in all cases. A significant negative correlation occurred between sand content and L*-wet in study soils (Fig. 2). The relationship was explained best by an exponential regression equation. Guo et al. (2012) found a stronger relationship between soil brightness and sand content (r = 0.76) compared one obtained in this study.

Contrary to sand content, a stronger and positive correlation occurred between the values of L* and clay content. This is consistent with the study by Li et al. (2001) who reported that sandy soils had lower reflectance than clay soils. In this study, no significant correlation was found between OM content and values of L* in either cases, which would be attributed to high clay content of the study soils, which be offsetting the effect of organic matter on soil reflectance.

An exponential relationship was found between potassium (K) content and L*-values in both cases (wet and dry cases) (Figure 4). There is an inherent relationship between K content and clay content. However, contrary to clay content, K content was negatively associated with L*, which is highly interesting. Also, similarly to clay content, the relationship between L*-wet and K content is a little stronger than the relationship between L*-dry and K content.

A third-degree polynomial regression equation successfully described the relationship between L* and EC of soils. Expectedly, increased EC resulted in increased L* values. The relationship is stronger in the wet soils. The influence of EC on L* is more pronounced between the range 700 and 2500 mS cm^{-1} in both cases (Fig. 5).

A positive association occurred between $CaCO_3$ content and L* values and a linear regression equation could successfully describe this raltionship (Fig. 6). Also, degree of association occurred between L* and CaCO3 was the greatest compared to those occurred between L* and other soil properties. The results obtained in this study agree to those reported by Courault et al. (1988).

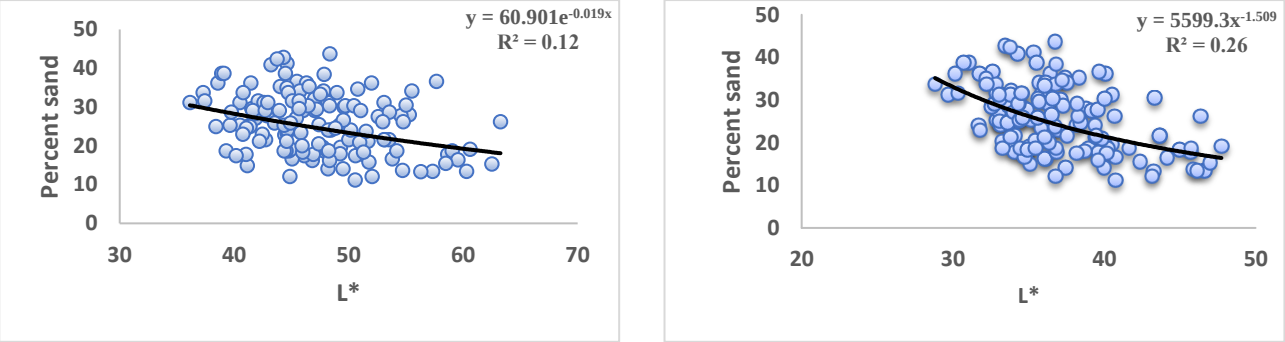

Figure 2. Relationship between sand content and L*: In dry soil (left) and Wet soil (right)
Şekil 2. *Kum içeriği ve L*: arasındaki ilişki Kuru toprakta (solda) ve Islak toprakta (sağda)*

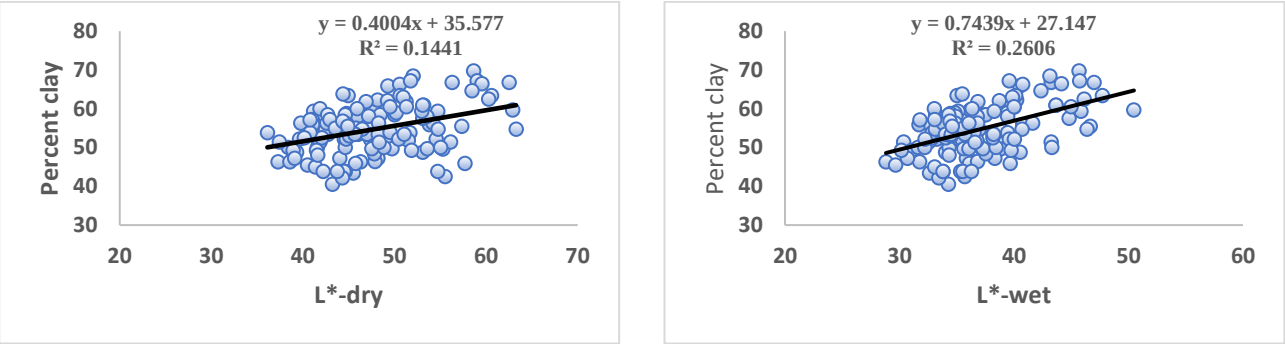

Figure 3 Relationship between clay content and L*: In air-dry soil (left) and wet (around field capacity) soil (right)
Şekil 3. *Kil içeriği ve L*: arasındaki ilişki Hava-kuru toprakta (solda) ve ıslak (tarla kapasitesi civarında) toprakta (sağda)*

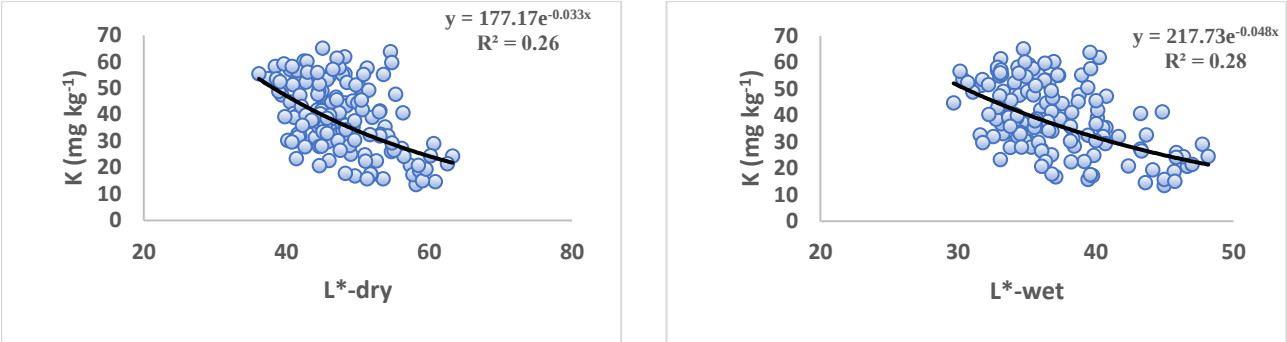

Figure 4. Relationship between potassium content and L*: In air-dry soils (left) and wet soils (right)
Şekil 4. *Potasyum içeriği ile L*: arasındaki ilişki Hava-kuru topraklarda (solda) ve ıslak topraklarda (sağda)*

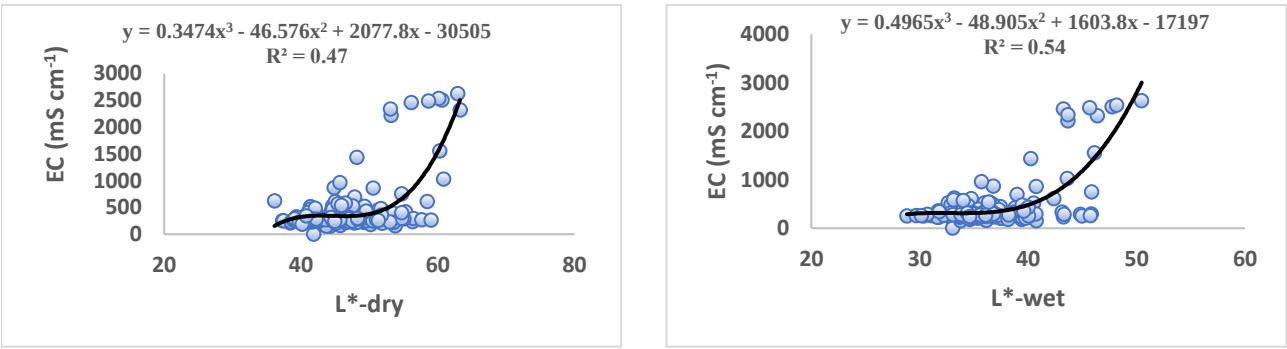

Figure 5 Relationship between EC and L*: In air-dry soils (left) and wet soils (right)
Şekil 5*. EC ve L* arasındaki ilişki: Hava-kuru topraklarda (solda) ve ıslak topraklarda (sağda)*

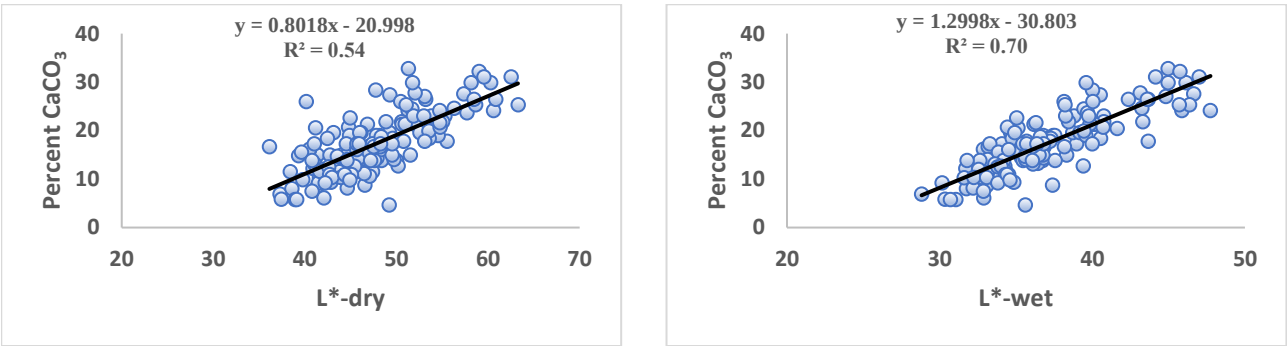

Figure 6 Relationship between lime $CaCO_3$ and L*: In air-dry soils (left) and wet soils (right)
Şekil 6. *Kireç $CaCO_3$ ve L*: arasındaki ilişki Hava-kuru topraklarda (solda) ve ıslak topraklarda (sağda)*

4. Conclusions

Limited number of soil attributes were adequately correlated with soil color component of L*. Most of the soil attributes were correlated to color components of a* and b* either insignificantly or significantly but weakly. The L*-values obtained on wet soil samples (L*-wet) were correlated more strongly with soil properties in all the cases. Clay, sand, K, and $CaCO_3$ contents and EC were significantly and adequately strongly associated to values for L*-wet and L*-dry. The most strong association occurred between CaCO3 content and L*-wet. A linear regression equation could successfully describe the relationship between $CaCO_3$ and L* in both cases. Also, a relatively high association occurred between EC and L*-wet. A three-degree polynomial regression equation could successfully model the relationship between the two attributes. Soil textural components of sand and clay content were significantly moderately correlated with both of L*-wet and L*-dry. Similarly, to soil textural components soil K content was moderately significantly correlated with L* in both cases. The L*-wet can be used to predict soils rich in $CaCO_3$ high in EC. Also, that high $CaCO_3$ content and EC restrict the growth of many crops, an idea can be drawn rapidly and easily on the local areas with lighter color that rich in $CaCO_3$ and high in EC. It was concluded that the measurement of L*-wet should be preferred over L*-dry in modeling soil color-soil properties relations.

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Molecular Determination of Some Important Viruses Causing Infection in Potato Fields in Turkey

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Abstract: Potato is one of the most important agricultural crops worldwide and known to be susceptible to more than 40 viruses in nature. In this research,298 leaf samples collected in 2020 from potato fields in Afyon, Nevşehir and Bolu provinces were used to determine viruses affecting potato production in the region. The leaves of potato plants showing virus symptoms (mosaic, deformation, yellowing, curling) were subjected to RT-PCR using virus-specific primers, in order to detect the presence of Potato leafroll virus (PLRV), Potato virus A (PVA), Potato virus X (PVX) and Potato virus M (PVM). As a result of the study, one or more viruses were detected in 46 (15.43%) of the 298 leaf samples tested. A total of 43 samples were infected with PLRV (14.42%) and 3 samples were infected with PVX (1.%). It was determined that the most common virus in plant samples collected from Afyon, Nevşehir, and Bolu Central regions was PLRV followed by PVX. PVA and PVM were not detected in the samples. The sequences of some positive isolates of PLRV were obtained and used along with the isolates registered in the GenBank to reveal phylogenetic relationships among them. PLRV isolates from Turkey were classified in Group 2 along with isolates from Serbia, Canada, and Pakistan based on phylogenetic analyses. As a result, only 5 PLRV isolates were studied in this study. Further studies are planned with more isolates and other protein regions. RT-PCR products obtained with PVX did not give good results in RT-PCR, and it is planned to work with different primers in the future.

Keywords: PVA, PVM, PVX, PLRV, RT-PCR

Türkiye'de Patates Alanlarında Enfeksiyon Oluşturan Bazı Önemli Viral Etmenlerin Moleküler Olarak Belirlenmesi ve Filogenetik Analizi

Öz: Patates, dünyada en önemli tarımsal ürünler arasında yer alır ve doğal koşullarda 40'dan fazla virüs türüne hasssas bir bitki türüdür. Bu araştırmada, Afyon, Nevşehir ve Bolu illerindeki patates tarlalarından 2020 yılında toplanan 298 yaprak örneği, bölgede patates üretimini etkileyen virüslerin tespiti için kullanılmıştır. Virüs simptomu (mozaik, şekil bozukluğu, sararma, yapraklarda kıvrılma) gösteren patates bitkilerinin yaprakları, Potato leafroll virus (PLRV), Potato virus A (PVA), Potato virus X (PVX) ve Potato virus M (PVM)'nin varlığını tespit etmek amacıyla, virüs-spesifik primerler kullanılarak RT-PCR testine tabi tutulmuştur. Test edilen 298 tane yaprak örneğinin 46'sında (%15.43), bir veya birden fazla virüs tespit edilmiştir. Çalışma sonucunda, test edilen örneklerin 43'si PLRV (%14.42), 3'ü PVX (%1) ile enfekteli bulunmuştur. Afyon, Nevşehir ve Bolu illerinden toplanan bitki örneklerinde en yaygın görülen virüsün PLRV, ardından PVX olduğu belirlendi. Örneklerde PVA ve PVM tespit edilmedi. PLRV ile enfekteli olan bazı pozitif izolatların elde edilen sekans verilerinin GenBank'a kayıtlı izolatlarla karşılaştırılması yapılarak filogenetik ilişkileri belirlenmiştir. Filogenetik analize göre, Türkiye PLRV izolatları Sırbistan, Kanada ve Pakistan izolatları ile birlikte Grup 2'de kümelenmiştir. Sonuç olarak, bu çalışmada sadece 5 PLRV izolatı ile çalışılmıştır. İleryen çalışmalarda daha fazla sayıda izolat ile ve diğer protein bölgeleri ile çalışmalar yapılması planlanmaktadır. PVX ile elde edilen RT-PCR ürünleri RT-PCR'da iyi sonuç vermemiş olup, ileride farklı primerler ile çalışılması planlanmaktadır.

Anahtar Kelimeler: PVA, PVM, PVX, PLRV, RT-PCR

1. Introduction

The second most popular food source in people's plant-based diets, after grains, is the potato (*Solanum tuberosum L.*). Today, potato is farmed across Turkey (Çalışkan et al., 2020). In production, imported varieties from nations including the Netherlands, Germany, France, USA, and England are employed in place of native varieties in Turkey, one of the world's major producers of potatoes (Çalışkan et al., 2010). As a result, Turkey has a current account deficit and is dependent on imported seeds. Turkey imports the 500,000 tons of seed potatoes needed for cultivating potatoes from other nations (Onaran, 2014). Turkey is ranked 16th globally in terms of production in 2020 (FAOStat, 2020). Turkey produced 45 50000 tons of potatoes in 2018, and 5200000 tons of potatoes in 2020 (FAOStat, 2020). More than 60% of the nation's production of potatoes, which play a significant role in both food and industry in Turkey, is concentrated in the provinces of Niğde, Nevşehir, İzmir, Bolu, and Afyon

(Yılmaz et al., 2006).

One of the earliest viral diseases examined on potato plants is Potato leafroll virus (PLRV), the culprit that causes Leafroll virus disease in potato fields. PLRV is a member of the *Luteoviridae family* and the *Polerovirus genus.* It has isometric particles with a diameter of 24 nm and single-strand positive RNA (ssRNA) within (Peters, 1970; Hooker, 1986).In nations where potatoes are grown, the potato virus X (PVX) is a relatively prevalent virus. It is transferred and propagated via contact with ill and healthy plants in outdoor settings, as well as by people, animals, or tools and equipment. It has filamentous particles.

The Potato virus M (PVM), which causes crop losses of up to 40% in several European nations, also contains filamentous particles. *Myzus persicae* and *Aphis nasturtii* are significant vectors of the virus, which is *non-persistently* spread by aphids in field circumstances. According to a survey on Potato virus X (PVX), Potato virus S(PVS), PVA, Potato virus Y(PVY), and PLRV that was undertaken in 23 places throughout Turkey, the incidence of PVS was 93.13%, followed by PVX (81.12), PVY+PVA (39.59%), and 32.08% PLRV (Özbayram and Yorgancı, 1989). A survey investigation that was carried out in the provinces of Afyon and Bolu in 2003–2004 employed DAS–ELISA and PCR techniques, and the outcomes were verified by experiments using mechanical inoculation on test plants.

According to Güner and Yorgancı (2009), the most prevalent viruses are PVY and PVS, which are present at rates of 20.46% in Afyon and 13.06% in Bolu. This study examined the prevalence and incidence of PLRV, PVX, and PVM in potato-growing regions in the Turkish provinces of Afyon, Bolu, and Nevşehir.

2. Materials and Methods

2.1 Materials

The surveys conducted to potato growing areas in Afyon, Nevşehir and Bolu provinces in 2020 within the scope of the 1002 TÜBİTAK project. During the surveys, 298 plants showing signs of the virus such as mosaic, deformation, yellowing, curling were collected (Figure 1). Leaf samples stored in the freezer at -20 °C were used in molecular studies.

The primers used for PLRV, PVA, PVM, and PVX are given in Table 3.1.

Figure 1: Infected plants collected during surveys (potato leafroll virus)
Resim 1: Surveyler sırasında toplanan enfekteli bitkiler(patates yaprak kıvırcıklık virüsü)

Table 1. Primers, their polarities and the expected fragment sizes (Peker, 2007; Meena et al. 2017).
Tablo 1. *Primerler, polariteleri ve beklenen ürün büyüklüğü*

Virus	Primer Sequences	Target Gene Region	Polarite	Expected Fragment Size	Annealing Temperature
PVA	5'-GACACTACCAATGCTCAAAG-3' 5'-CTCTTCTGAAGGTGTGACTAT-3'	Coat protein	Sense Antisense	560 bp	62 ºC
PVM	5'-ATCTGAAATAGTGAGTATGGG-3' 5'-GCCACCTTGGTTACGTGCTT-3'	Coat protein	Sense Antisense	408 bp	56 °C
PVX	5'-TAGCACAACACAGGCCACAG-3' 5'-GGCAGCATTCATTTCAGCTTC-3'	Coat protein	Sense Antisense	562 bp	62°C
PLRV	5'- CGCGCTAACAGAGTTCAGCC-3' 5'-GCAATGGGGGTCCAACTCAT-3'	Coat protein	Sense Antisense	336 bp	62°C

2.2 Method

2.2.1. Isolation of total RNA

In the study, RNA isolation process were carried out using the leaf samples collected from potato production areas of Afyon, Bolu and Nevşehir provinces. RNA isolation was performed according to the method of Astruc et al. (1996) given as follows.

❖ Samples were diluted in 1:2(w/) with extraction buffer solution [(100 mM Tris-HCl (pH.8.0), 50 mM EDTA (pH. 7.0), 5 mM NaCl, 10 mM 2-mercapto-ethanol (1/1000)] v) was diluted and crushed.

❖ After taking 1 ml of the plant sap into an eppendorf tube, the samples were centrifuged at 4,000 rpm for 3 minutes, then 50 µl of Sodium Dodecyl Sulfate (SDS) (20%) was added to the pellet and mixed by vortex.

❖ The tubes were then incubated in heating blocks at 65ºC for 30 minutes.

❖ 250 µl of potassium acetate (5M) was added to the tubes, kept in ice for 20 minutes, and then centrifuged at 13,000 rpm for 15 minutes.

❖ The supernatant was divided into two parts, 500 µl of it was placed in newly prepared microcentrifuge tubes and stored at -70ºC. The remaining 500 µl of supernatant was placed in the newly prepared microcentrifuge tubes, 500 µl of 96% Ethanol was added, and it was mixed with vortex.

❖ Then, 50 µl of sodium acetate (3M) was added to the tubes and the samples were mixed again and kept at -70 ºC overnight.

❖ The next day, the samples were centrifuged at 14,000 rpm for 15 minutes, and the liquid part was removed.

❖ Microcentrifuge tubes were inverted and dried on filter paper for 5 minutes and washed by adding of 1 ml of ethanol (70%) to the pellet.

❖ In order to precipitate RNAs, the tubes were centrifuged at 13,000 rpm for 5 minutes, the ethanol in the tube was discarded, and the microcentrifuge tubes were left to dry at room temperature.

❖ Total RNAs obtained from the samples were reconstituted with 50 µl of distilled water and stored at -20ºC until use.

2.2.2. Complementary DNA (cDNA) synthesis

Utilizing RNAs acquired from the total RNA isolation technique, complementary DNA (cDNA) synthesis was done. In microcentrifuge tubes for cDNA synthesis, 2 µl total RNA, 1 µl a random hexamer primer (5'-d (NNNNNN)-3'N = G, A, T, or C) (10 mol), and 7µl of distilled water were mixed. The mixture was put on ice for three minutes after 5 minutes at 65 °C of incubation.

To each tube was filled to a capacity of 20 by adding 5X MMLV buffer (5X), dNTP (25 mM), 0.5 random hexamer primer (10 mol), 0.25 RNAse inhibitor (10 U/l), 0.25 reverse transcriptase (Thermo Scientific), and distilled water. The tubes were then incubated at 25 °C for 5 minutes, 42 °C for 60 minutes, and 72 °C for 10 minutes to produce cDNA.

2.2.3. Polymerase Chain Reaction (PCR)

Using the cDNA templates obtained in the first stage and virus-specific primers, PCR procedures were conducted. At this point, 2µlcDNA, 5µl of 5X Green GoTaq® Flexi Buffer from Promega, 0.2 µl dNTP, 0.5 µl forward primer and 0.5µl of reverse primer each with 100 pmol, 1.5 µl MgCl2 (25 mM), 0.25µlTaq polymerase enzyme from Promega, and 0.25 µl Dimethyl sulfoxside (DMSO). The PCR themocycler was filled with the tubes.

The reaction took place at 94 degrees Celsius for 2 minutes for initial denaturation, 1 minute for denaturation during the cycle, and 30 seconds at 56 degrees Celsius (for PVM) for primer annealing. (62°C for other viruses), 2 minutes at 72°C for synthesis completion (primary extension) and 35 cycles of amplification were used. Finally, the reaction was completed by incubating the samples at 72°C for 10 minutes (final extension).

2.2.4. Agarose gel electrophoresis

Tris-borate-EDTA (TBE) buffer was used to prepare a 1.2% agarose gel for the electrophoresis technique examination of the PCR products that were obtained. Then, 7µlethidium bromide stock solution (10 mg/ml) was added to it. For one hour, the samples were electrophoresed at 100 V. The PCR products were evaluated in the gel imaging device after electrophoresis, and pictures of the products in the gel were taken.

2.2.5. Phylogenetic analysis of Potato leaf roll virus isolates

Five PLRV isolates' RT-PCR products that tested positive at the end were sent for sequencing analysis using the Sanger method in order to conduct phylogenetic investigations. The MEGA10 program was used to evaluate the raw data that was received from the sequencing (Kumar et al., 2018) Following that, BLAST was used to match the sequencing data to those of the reference isolates listed in the National Center for Biotechnology Information (NCBI) (Basic

Local Alignment Search Tool). By using the neighbor joining method, a phylogenetic tree was constructed, and the degree of relatedness was compared.

3. Results

3.1 Results of RT-PCR

Total RNAs were extracted from the 298 leaf samples that had previously been collected in order to identify the existence of viral agents in the potato production regions of the provinces of Afyon, Bolu, and Nevşehir. The extracted RNA samples were used to create cDNA using a hexamer primer in order to detect PLRV, PVM, and PVX. Then, using virus-specific primers, the RT-PCR technique was used.

As a result of RT-PCR performed with primers specific to PLRV, a band of the expected size (336 bp) was obtained in 43 leaf samples (Figure 2).

As a result of RT-PCR performed with PVX-specific primers, a band of expected size (562 bp) was obtained in 3 leaf samples (Figure 3).

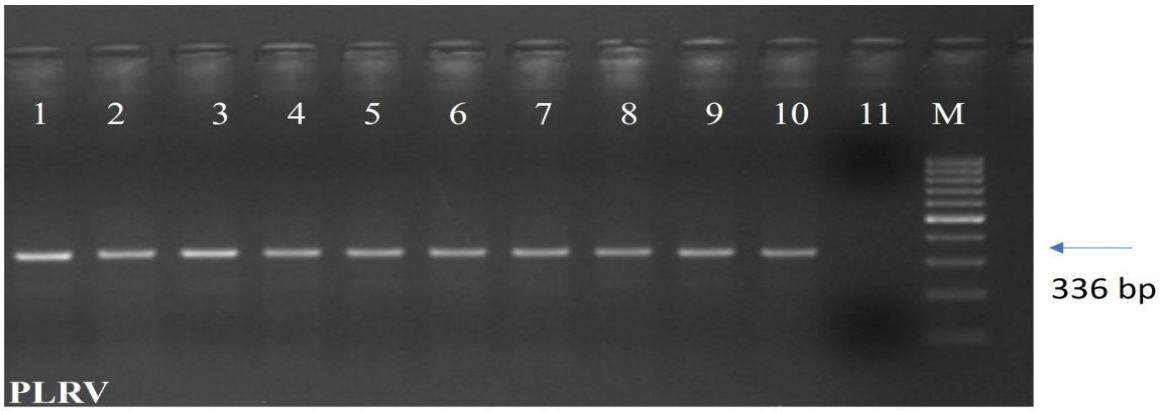

Figure 2. The RT-PCR results of some isolates were obtained with PLRV specific primers. M: 100 bp DNA Ladder (Fermentas), 1: Ş7-1, 2: Ş7-2, 3:Ş7-3, 4: Ş74, 5:Ş7-5, 6: Sa11-7, 7: Ş7-9, 8: Ş7-16, 9: NP22, 10: NP68, 11: Negative control.

***Şekil 2**. PLRV spesifik primerler ile elde edilen bazı izolatlara ait RT-PCR sonuçları M: 100 bp DNA markör (Fermentas), 1: Ş7-1, 2: Ş7-2, 3:Ş7-3, 4: Ş74, 5:Ş7-5, 6: Sa11-7, 7: Ş7-9, 8: Ş7-16, 9: NP22, 10: NP68, 11: Negatif kontrol.*

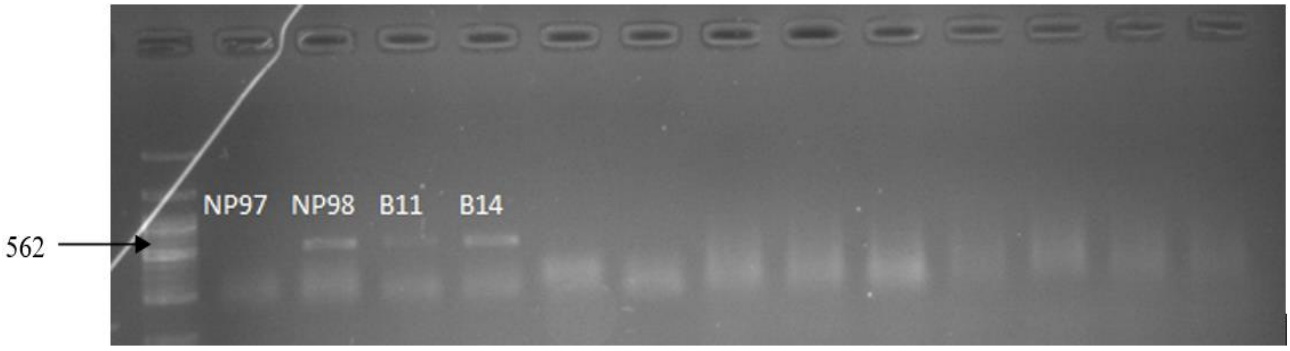

Figure 3. The RT-PCR results of some isolates obtained with PVX-specific primer.M: 100 bp DNA Ladder (Fermentas)

***Şekil 3.** PVX'e özgü primer ile elde edilen bazı izolatların RT-PCR sonuçları.M: 100 bp DNA markör (Fermentas)*

Table 2. The presences of PVM, PVX and PLRV in leaf samples taken from potato production areas of Bolu, Nevşehir and Afyon in the survey studies carried out in 2020.

***Table 2.** 2020 yılında yapılan sürvey çalışmalarında Bolu, Nevşehir ve Afyon patates üretim alanlarından alınan yaprak örneklerinde PVM, PVX ve PLRV varlığı.*

			Number of infected samples (Infection rate, %)			
Province	Number of samples tested	Number of samples infected	PLRV	PVX	PVM	PVA
Bolu	89	14	14 (15.73%)	-	-	-
Nevşehir	98	12	11 (11.22%)	1 (1.02%)	-	-
Afyon	111	20	18 (16.21%)	2 (1.80%)	-	-
Total	298	46	43 (14.42%)	3 (2.82%)	-	-

3.2. Phylogenetic relationships among Potato leaf roll virus isolates

The sequences of the partial coat protein region of five PLRV isolates (two from Nevsehir and three from Afyon) consisting of 337 nucleotides were obtained. The isolates obtained in this study were registered in the NCBI with the following accession numbers: Sa11-7: OP824688, S7-9: OP824689, S7-16: OP824690, NP22: OP824691, NP68: OP824692. BLAST analysis of the sequences of PLRV isolates showed 99-100% nucleotide and 100% amino acide identities with the reference isolates. The table of BLAST analysis of Turkish PLRV isolates is shown in table 4.5. Phylogenetic tree was constructed using the UPGMA method implemented in MEGA X (Kumar et al., 2018) based on the comparison of nucleotide sequences (Figure 4).

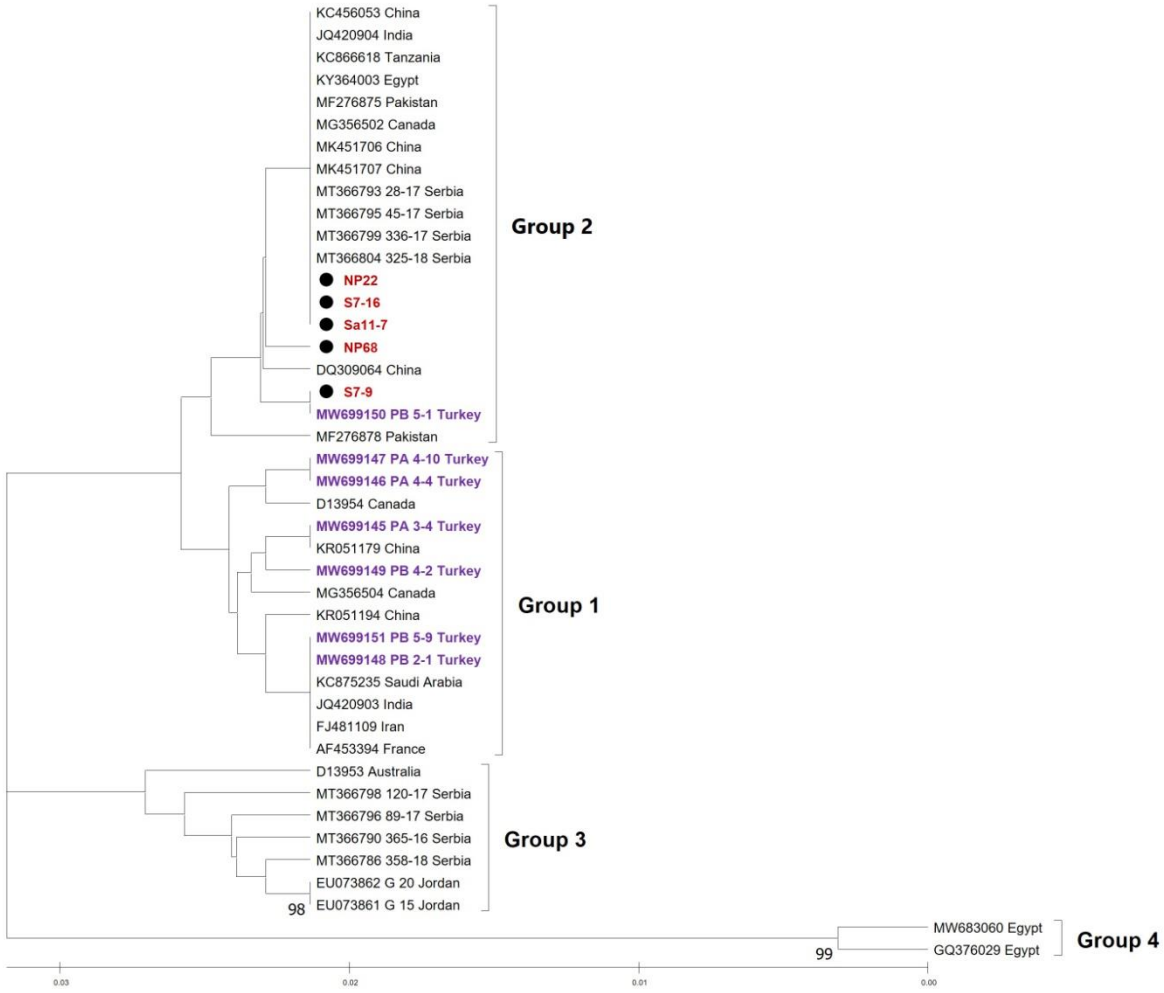

Figure 4. Phylogenetic tree constructed with PLRV isolates using UPGMA method. The evolutionary history was inferred using the UPGMA method The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Kumar et. Al. 2018). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. There were a total of 325 positions in the final dataset.

Şekil 4*. UPGMA yöntemi kullanılarak PLRV izolatları ile oluşturulan filogenetik ağaç. Evrimsel tarih, UPGMA yöntemi kullanılarak çıkarılmıştır. En uygun ağaç gösterilmiştir. Önyükleme testinde (1000 kopya) ilişkili taksonların bir arada kümelendiği kopya ağaçların yüzdesi, dalların yanında gösterilir (Kumar et. Al. 2018). Ağaç, filogenetik ağacı anlamak için kullanılan evrimsel mesafelerle aynı birimlerde dal uzunlukları ile ölçeğe göre çizilmiştir. Nihai veri setinde toplam 325 pozisyon vardır.*

4. Discussion and Conclusion

The viruses that reduce productivity in many cultivated plants around the world cannot be directly controlled, just as other infections. The majority of plant virus management and control techniques focus more on prevention than on therapy. As a result, in viral infections, the accurate implementation of a disease control strategy depends on the early and

precise diagnosis of the pathogen and the application of sensitive procedures. Serological and molecular methods are employed to produce more accurate results in diagnosis (Erkan et al., 2011).

A variety of symptoms, including yellowing, a reduction in leaf size, stunting, curling and rolling of the top leaves, mosaic, vein clearing/banding, necrotic lesions, deformities and cracks in tubers, and crescent-shaped cavities in the tuber eyes, are all signs of potato viruses. Similar symptoms were observed in plants during surveys done in the provinces of Bolu, Nevşehir, and Afyon in 2021. By using the RT-PCR method, potato leaf samples from the provinces of Bolu, Nevşehir, and Afyon were tested for the presence of several viruses in this study.

The possibility that viruses that spread from year to year through tubers were present in the potato tuber seeds used for planting in these locations is one explanation for this. According to Çıtır and Özbayram (1982), an average of 95% of the seed tubers used by farmers are contaminated with at least one virus. Viruses also enter Turkey via imported seed tubers. Furthermore, Bostan and Haliloğlu (2004) discovered the identical circumstance for the seed tubers that the producers had obtained from the businesses in the areas where potato farming is widespread.

The absence of effective, reliable, and affordable virus testing methods in tubers before they are imported or utilized for seed purposes may be the primary cause of this issue. The major source of virus infection is the infected tuber, and the virus is subsequently transmitted throughout the year via vectors or mechanical mechanisms (Bostan et al., 2006). By using the RT-PCR technology, the presence of PLRV, PVA, PVM, and PVX viral agents was examined in this study's potato production regions of the Central Districts of Bolu, Nevşehir, and Afyon provinces. As a consequence, 180 out of 300 leaf samples collected from potato growing locations had one or more virus species found in them.

The most prevalent virus in plant leaf samples from the provinces of Bolu, Nevşehir, and Afyon was identified as PLRV with single infections. PLRV, PVM, and PVX were found to be the most prevalent viruses in Bolu, Nevşehir, and Afyon, respectively. Two samples from Afyon and one from Nevşehir each had one PVX-infected sample.

The most prevalent potato viruses, according to a study carried out in Erzurum by Yardımcı and Bostan (1999), were PLRV (42.2%), PVX (38.3%), and PVY (7%). Infections of PVX+PLRV (6.29%), PVX+PVY (2.96%), and PLRV+PVY were also found to be mixed. In samples lacking virus symptoms, the DAS ELISA approach was used to identify PVY, PLRV, and PVY+PVS infections. In contrast to our study, this one in Erzurum found that PVY was the virus with the lowest prevalence, whereas PLRV had the highest prevalence.

In this investigation, it was also possible to find combination infections of the aforementioned viral agents. In contrast to our research, it was found that PLRV (14.42%) was the most prevalent virus to infect potatoes, whereas PVM (5.61%) and PVX (1.02%) were found to be present at low levels. In one investigation, DAS-ELISA was used to check samples from field studies for the presence of PVX, PVS, PVY, PVA, PLRV, and PVM.

PVX, PVY, PVS, and PLRV were found in the samples to cause single infections, while PVY+PVS, PVY+PLRV, PVY+PVS+PLRV, PVS+PVA, and PVY+PVA were shown to induce mixed infections. In addition to positive results, the test also produced some negative outcomes. In contrast to our findings, viruses were identified to produce both combined infections (PVA+PVM) and single infections (PVA, PLRV, PVM, and PVX) in the samples (Güner, 2007).

In the phylogenetic tree, the isolates formed 4 different groups. Similarly, Ristic et al. (2021) reported that PLRV isolates were divided into 4 groups in the phylogenetic tree they made with CP sequence data. Within the scope of the study, newly obtained PLRV isolates from Turkey (Purple colored) were included in Group 2 together with isolates from Serbia, Canada, and Pakistan. On the other hand, PLRV Tokat isolates (Red colored) obtained in the master thesis made by Engür (2020), were included in group 1 together with isolates from China, Canada, India, Iran, and France. In the phylogenetic tree, while the isolates NP22, S7-16 and Sa11-7 clustered with isolates from Serbia, Canada, and Pakistan, the isolate NP68 clustered with China isolate in different branches. the isolate S7-9 clustered with the previously reported Tokat isolate (MW699150: PB5-1) (Figure 4.7).

Among the positive PCR products obtained as a result of RT-PCR studies, five samples for PLRV (S7-9, NP68, NP22, S7-16, Sa11- 7) were selected and sent to the sequence for analysis. As a result of the sequence analysis, the results were obtained from the 5 PLRV isolates and continued to be studied with these isolates. RT-PCR products obtained with PVX did not give good results in the RT-PCR, and it is planned to

work with different primers in the future.

Potatoes are an important food source in Turkey as well as in the whole world. These viruses, which are seen in potato production areas, cause significant yield loss in tubers, along with green parts infections, and continue their continuity with seeds to be used in the future. As with other disease agents and harmful insects, viruses cannot be combated with chemical means. For this reason, special attention should be paid to the fact that the tubers to be used as seeds are certified and free from viral factors. The farmers who produce potatoes in Turkey should be educated on this issue and their awareness should be raised. For the control of virus agents such as PLRV that can be transmitted persistently by aphids, aphids and other vector insects carrying these diseases should be combated. In addition, in order to take precautions against PVY, PVS, and PVX transported by mechanical means, attention should be paid to the cleanliness of the tools and equipment used during the harvest and care should be taken not to injure the plants during the green period.

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Population development and Parasitoids of *Acanthiophilus helianthi* (Rossi) (Diptera: Tephritidae) On Five Different Safflower Varieties in Van, Turkey

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Abstract: *Acanthiophilus helianthi* (Rossi, 1794) (Diptera: Tephritidae) also known as safflower fly, is an oligophagous species that cause significant damage to safflower plants Safflower, *Carthamus tinctorius* (L.) (Asterales: Asteraceae), which is among its hosts, is an important energy plant that is drought resistant and has high adaptability. This study investigated the population growth of *A. helianthi* on five different safflower varieties (Asol, Ayaz, Balcı, Dinçer and Göktürk) in Van Yuzuncu Yıl University, Faculty of Agriculture for two years (2019-2020) in Van province. Result of the study, it was determined that the pest showed a similar population change in both years, the adult individuals were seen at the end of June - mid-August, and their pupae from this date to the beginning of September. It was seen that the insect density was statistically different between the varieties at different sampling dates and the highest adult density was found in variety of Asol pupae in both years, and in Göktürk variety. During sampling with sweep net, 4 parasitoids, *Ormyrus* sp., *M. annulatus*, *E. acroptilae* and *Bracon* sp., were detected and it was understood in the literature that they were parasitoids of *A. helianthi*.

Keywords: *Acanthiophilus helianthi*, parasitoids, population development, safflower

Acanthiophilus helianthi (Rossi) (Diptera: Tephritidae)'nin Van (Türkiye) İli'nde Beş Farklı Aspir Çeşidinde Popülasyon Gelişimi ve Parazitoitleri

Öz: *Acantiophilus helianthi* (Rossi, 1794) (Diptera: Tephritidae) Aspir sineği olarak bilinen aspir bitkilerinde önemli zararlara neden olan oligofag bir türdür. Konukçuları arasında bulunan aspir, *Carthamus tinctorius* (L.) (Asterales: Asteraceae) bitkisi, kuraklığa dayanıklı ve adaptasyon yeteneği yüksek önemli bir enerji bitkisidir. Bu çalışma Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi deneme alanlarında iki yıl boyunca (2019-2020) Van ilinde *A. helianthi*'nin farklı aspir çeşitleri üstünde (Asol, Ayaz, Balcı, Dinçer ve Göktürk) populasyon gelişimi incelenmiştir. Çalışma sonucunda zararlının her iki yılda da benzer bir populasyon değişimi gösterdiği, ergin bireylerin haziran sonu - ağustos ortası arasında ve bu tarihten eylül başına kadar pupalarının görüldüğü belirlenmiştir. Farklı örnekleme tarihlerindeki çeşitler arasında böcek yoğunluğunun istatistiksel olarak farklı olduğu ve her iki yılda da en yüksek ergin yoğunluğu Asol çeşidinde, pupada ise Göktürk çeşidinde olduğu görülmüştür. Atrapla örnekleme esnasında 4 parazitoit tür *Ormyrus* sp. (Hymenoptera: Ormyridae), *Microdontomerus annulatus* (Spinola, 1808) (Hymenoptera: Torymidae), *Eurytoma acroptilae* (Zerova, 1986) (Hymenoptera: Eurytomidae) ve *Bracon* sp. (Hymenoptera: Braconidae) elde edilmiş ve bunların literatürde *Acantiophilus helianthi*'nin parazitoitleri olduğu anlaşılmıştır.

Anahtar Kelimeler: *Acanthiophilus helianthi*, aspir, parazitoitler, populasyon gelişimi

1. Introduction

Safflower is a plant species of the genus *Carthamus*, belonging to the Asteraceae family. There are 25 safflower species determined in the world. (Singh et al., 2006). Safflower *Carthamus tinctorius* (L., 1753) (Asterales: Asteraceae), which is grown today, was cultured from *Carthamus lanatus* (Saffron thistle) (L., 1753) (Asterales: Asteraceae) and *Carthamus oxyacantha* (Wild safflower) (L., 1753) (Asterales: Asteraceae) (Taşlıgil & Şahin, 2016). Safflower, which was brought to Turkey by immigrants from Bulgaria in the first years of the Turkey Republic, was first registered as Yenice in 1931, followed by Dinçer in 1977 and Remzibey-05 in 2005. Among these three registered varieties, it is traditionally grown in a few provinces such as Dinçer and Remzibey-05, Balıkesir, Eskişehir, Isparta and Konya (Kayaçetin et al., 2012). Safflower, a hot and drought region plant, is an annual plant grown for its oilseeds. It is about 80 – 120 cm tall, very branched, and in the form of a shrub. The narrow and long leaves are dark green, with saw-toothed edges, and thorny in some species (Kayaçetin et al., 2012). Safflower flowers consist of yellow, red, orange, or a mixture of these colors. Although the color of the flowers changes according to the variety, these features also add market value to the safflower. Safflower seeds

are white or cream, and some may have dark stripes (Ekin, 2005). Today, there are more than 200 varieties, and their oil ratio varies between 38% and 71.7% (Taşlıgil & Şahin, 2016).

Safflower is susceptible to many fungal, bacterial, and viral diseases, some of which can cause significant damage (Singh & Nimbkar, 2006), and fungal diseases are the most common among them. When the safflower plant is irrigated, diseases become much more common than those grown with rain and cause significant damage to the product (Nimbkar, 2008 & Mirshekari et al., 2013). Many pests cause product economic losses in the safflower plant (Nimbkar, 2008; Saeidi & Adam, 2011 & Esfahani et al., 2012, Lotfalizadeh & Gharali 2014). These are *Acanthiophilus helianthi*, *Chaetorellia carthami*, *Terellia luteola*, *Urophora mauritanica* (Diptera: Tephritidae), *Uroleucon carthami*, *U. jaceae* (Hemiptera: Aphididae), *Thrips* sp. (Thysanoptera: Thripidae), whitefly, *Agrotis* sp., *Helicoverpa* sp., *Heliothis peltigera*, *Spodoptera littoralis*, *S. exigua* (Lepidoptera: Noctuidae), *Empoasca decipiens* (Hemiptera: Cicadellidae), *Oxycarneus pallens* (Hemiptera: Lygaeidae), *Tetranychus urtica* (Acari: Tetranychidae), *Tropinota* (*Epicometis*) *hirta* (Coleoptera:Scarabeidae). *Acanthiophilus helianthi* (Safflower fly), is a key pest of safflower which causes substantial yield losses in every season (Hand & Ro, 2018; Khuhro et al., 2021). It is a harmful species that feeds on various plant species belonging to the Cardueae (Asteraceae) family. Although the safflower fly *A. helianthi* is an oligophagous pest, it is one of the major pests limiting crop production in many countries (Talpur et al., 1995; Sabzailian et al., 2010; Saeidi & Adam, 2011; Damkacı, 2013; Basheer et al., 2014; Riaz et al., 2014). In addition to the oligophag harmful safflower, it also causes damage to 24 wild plants belonging to the Compositae family. It is the larvae that do the damage. After damage, the seeds usually dry before they are fully developed and take on a brown color. As a result of the damage, the oil rate in the seeds decreases, and their germination power is lost (Şengonca, 1983).

As a result of the researches, it has been observed that there are very few studies on the determination of the species that cause damage to the safflower plant. In this study, the population development of *A. helianthi*, which causes severe damage to *C. tinctorius* which has an important place among energy plants, was investigated. In the study, Turkey's most commercially preferred safflower varieties considered to host plants for safflower fly. As a result of the study, parasitoids of *A. helianthi*, the density of adults and pupae and the effects of cultivars on them were determined.

2. Materials and Method

In this study, population growth of *Acantiophilus helianthi* on five different safflower plants (Asol, Ayaz, Balcı, Dinçer and Göktürk) was investigated in field conditions in 25 March-30 September 2019 and 2020. The study area consists of a total of 25 parcels, 5 for each variety. The parcels were prepared with a length of 5 m x 5 m, leaving a 2 m gap between the parcels. The row spacing was 25 cm and 5 cm intervals were formed on the rows. Each plot is arranged in 12 rows. The field experiment was prepared according to the randomized plot design in the experimental areas of Van Yuzuncu Yıl University, Faculty of Agriculture, and was carried out along two years as five replications.

2.1. Density of adult and pupa

Adults and parasitoids of *A. helianthi* (Figure 1) were collected by sweep net at weekly (50 sweep net in each plot, a total of 250 sweep net for one variety) just after the plants started to form the flower bed. Weekly observations were made from the date the plants started to form the flower head, and every flower head that was damaged from the moment the sign of *A. helianthi* was seen (Figure 2) was recorded by making pupa counts under the binocular (Figure 2).

After the plants started to develop, all insects collected with sweep net every week were transferred to jars (kill bottle) containing ethyl acetate. After it was sorted and counted in the laboratory, the necessary information was written and left in eppendorf tubes. The scraping process was carried out for 2 years in a row, a total of 16 samples were collected. Photographs of the obtained insect species were taken under binoculars. Hymenoptera were identified by Hossein Lotfalizadeh (Iranian Research Institute of Plant Protection), Diptera Saeed Mohamadzade Namin (Islamic Azad University, Braconidae were identified by Konstantin Samartsev (Zoological Institute RAS, St. Petersburg).

2.2 Evaluation of data

The difference in the number of adults and pupae collected from different cultivars by weekly sampling was tested using the Twosex MSChart (Chi, 2021) program according to the Bootstrap (Paired, 100,000 B) technique (Chi & Liu 1985; Chi, 1988). Graphs related to the data were prepared using SigmaPlot (ver. 12) and MS Excel package programs.

Figure 1. *Acanthiophilus helianthi* (Kına E. Original).
Şekil 1. *Acanthiophilus helianthi (Kına E. Orijinal).*

Figure 2. Damage pattern and pupa appearance of *Acanthiophilus helianthi* in safflower (Kına E. Original).
Şekil 2. *Acanthiophilus helianthi'nin aspirde zarar şekli ve pupa görüntüsü (Kına E. Orijinal)*

3. Result and Discussion

It was determined that *Acantiophilus helianthi* formed a population with different densities on five different safflower cultivars discussed in the study.

3.1. Population density of *Acanthiophilus helianthi* on five different safflower varieties

The densities of adults and pupae of the pest on safflower varieties at the weekly were determined with the samples made in the experimental area in 2019 and 2020. The results are shown in (Figure 3) according to the years. Adult and pupa densities of the pest determined on cultivars at each sampling were compared statistically, and the results are given in Table 1. The climate data of both years in which the study carried out are shown in (Figure 4). In the first year of sampling, adult individuals were found on June 30 in all varieties (Table 1). When the samples collected on this date were compared according to their densities on different cultivars, it was seen that they were not statistically different ($P>0.05$) the average densities varied between 0.60-1.40 individuals/sweep net (Table 1). Adult densities were found to be statistically different in all samples except the densities determined on August 4 and July 7 in the samples made until the harvest period after this date ($P<0.05$).

The first pupae were found on Asol, Ayaz, and Göktürk cultivars on August 17 (Table 1). It was determined that pupa densities were not statistically different in all samples except the densities determined on August 24 in the samples made until the harvest period after this date ($P>0.05$) (Table 1). According to the data, the highest pupa density was found in Asol cultivar (4.40 individuals/sweep net) on August 24, and statistically, differences were found between cultivars.

Adult individuals were seen on 29 June in the second year samplings. When the samples collected on this date were compared according to the densities of being on different varieties, it was seen that they were statistically different in all samples except the densities determined

on August 3 (P<0.05) (Table 1). When we look at the pupa density data of the second year, the inflorescences were found on August 17 in other cultivars, except for Balcı and Dinçer, as in the first year. (P<0.05). Averages densities rate were changed from 0.40-4.80 individuals/ Sweep net. It was determined that pupa densities were not statistically different in all samples except the densities determined on August 24 in the samples made until the harvest period after this date (P>0.05) (Table 1). When looking the data, it was found that Balcı cultivar (2.60 individuals/ Sweep net) on 24 August was lower than other cultivars on average. It was determined that the averages were in the range of 1.40-1.60 on September 8, close to the harvest period, and there was no difference between the varieties (P>0.05 Table 1).

It was observed that there were fluctuations in adult density in all varieties during the sampling dates for two years (Figure 3). Until August 17, when the last individual was seen, the highest adult was found in the Asol variety for both years compared to other types, followed by the Balcı and Göktürk variety (Figure 3 and Table 1). When we look at the 2019 data, the pupa density showed a rapid increase on August 17-24, followed a stable course on August 31 and September 8, showed a rapid decrease until September 15, and ended entirely in the following week (Figure 3). It was observed that the adult density followed a similar course to the first year in the second year but was higher in terms of the number of individuals (Figure 3). The highest values were obtained from the Asol variety for both years, followed by the Göktürk ve Balcı variety (Figure 3 and Table 1).

Table 1. The adult and pupa density of *Acanthiophilus helianthi* on five different safflower cultivars in 2019 and 2020 (Mean ± SE)

Çizelge 1. *Acanthiophilus helianthi'nin 2019 ve 2020 yıllarında beş farklı aspir çeşidinde ergin ve pupa yoğunluğu* (Ortalama ± SH)

2019	**N**	**ASOL**	**AYAZ**	**BALCI**	**DİNÇER**	**GÖKTÜRK**	**P**
Adult 30 June	5	0.60 ± 0.36	0.60 ±0.36	1.00 ± 0.28	1.40 ± 0.22	0.80 ± 0.33	> 0.05
7 July	5	0.80 ± 0.52	1.40 ±0.46	0.60 ± 0.35	0.20 ± 0.18	0.60 ± 0.22	> 0.05
14 July	5	1.20 ± 0.33 b	0.60 ±0.36 b	2.60 ± 0.22 a		0.20 ± 0.18 b	**< 0.05**
21 July	5	1.00 ± 0.28 bc	1.40 ±0.35 bc	1.80 ± 0.33 ab	2.20 ± 0.33 a	0.80 ± 0.17 c	**< 0.05**
28 July	5	2.40 ± 0.22 a	0.40 ±0.22 c	1.40 ± 0.22 b	0.80 ± 0.33 bc	0.20 ± 0.18 c	**< 0.05**
4 August	5	0.20 ± 0.18			0.40 ± 0.36	0.60 ± 0.22	> 0.05
11 August	5	1.60 ± 0.36 a				0.20 ± 0.18 b	**< 0.05**
Total	7	1.11 ± 0.25 a	0.63 ± 0.20 ab	1.06 ± 0.33 ab	0.71 ± 0.29 ab	0.48 ± 0.09	**< 0.05**
Pupae 17 August	5	2.40 ± 1.04	1.20 ± 0.71			0.40 ± 0.36	> 0.05
24 August	5	4.40 ± 1.28 ab	1.40 ± 0.22 b	2.20 ± 0.52 ab	2.00 ± 0.69 ab	2.80 ± 0.99 ab	**< 0.05**
31 August	5	3.00 ± 0.80	3.40 ± 0.88	2.60 ± 0.60	2.20 ± 0.66	4.20 ± 1.42	> 0.05
8 September	5	3.00 ± 0.80	1.40 ± 0.22	1.40 ± 0.22	1.60 ± 0.36	1.20 ± 0.18	> 0.05
Total	4	3.20 ± 0.37 a	2.55 ± 0.46 ab	1.85 ± 0.54 b	1.60 ± 0.46 b	3.40 ± 0.88 ab	**< 0.05**
2020							
Adult 29 June	5	2.20 ± 0.33 a	0.60 ± 0.21 b	0.60 ± 0.35 b	0.20 ± 0.17 b	0.60 ± 0.22 b	**< 0.05**
6 July	5	0.60 ± 0.36 b	0.60 ± 0.22 b	1.40 ± 0.61 ab	0.40 ± 0.22 b	1.40 ± 0.22 a	**< 0.05**
13 July	5	2.20 ± 0.33 a	0.40 ± 0.18 b	0.40 ± 0.22 b	0.40 ± 0.22 b	0.80 ± 0.18 b	**< 0.05**
20 July	5	0.40 ± 0.35 b	1.00 ± 0.28 bc	1.20 ± 0.18 b	1.40 ± 0.22 b	2.20 ± 0.18 a	**< 0.05**
27 July	5	0.60 ± 0.22 b	0.80 ± 0.33 b	0.80 ± 0.17 b	2.60 ± 0.53 a	0.80 ± 0.17 b	**< 0.05**
3 August	5	0.80 ± 0.18	1.20 ± 0.33	1.20 ± 0.17	0.60 ± 0.36	0.80 ± 0.34	> 0.05
10 August	5	0.60 ± 0.22 bc	1.00 ± 0.00 ab	1.40 ± 0.22 a	0.60 ± 0.22 bc	0.20 ± 0.18 c	**< 0.05**
Total	7	1.08 ± 0.27	0.88 ± 0.08	0.79 ± 0.13	0.88 ±0.29	1.05 ± 0.20	> 0.05
Pupae 17 August	5	1.40 ± 0.53	1.40 ± 0.53			0.40 ± 0.35	> 0.05
24 August	5	4.20 ± 0.33 ab	3.20 ± 0.33 ab	2.60 ± 0.45 b	3.40 ± 0.45 a	4.60 ± 0.66 a	**< 0.05**
31 August	5	2.80 ± 0.86	3.20 ± 0.82	2.20 ± 0.52	2.80 ± 0.90	4.80 ± 1.24	> 0.05
8 September	5	1.40 ± 0.21	1.60 ± 0.35	1.40 ± 0.21	1.60 ± 0.35	1.40 ± 0.21	> 0.05
Total	4	2.45 ± 0.58	2.35 ± 0.43	1.55 ± 0.50	1.95 ± 0.65	2.80 ± 0.97	> 0.05

In population sampling of *A. helianthi* on different safflower cultivars, *Ormyrus* sp., *Microdontomerus annulatus*, *Eurytoma acroptilae* and *Bracon* sp. including parasitoids species were found (Figure 7). It has been reported that these species feed on the larvae and pupae of *A. helianthi* (Saeidi et al., 2011; Lotfalizadeh & Gharali 2014). Images of 4 parasitoids *Ormyrus* sp., *M. annulatus*, *E. acroptilae* and *Bracon* sp. on different safflower varieties of *A. helianthi* (Figure 5).

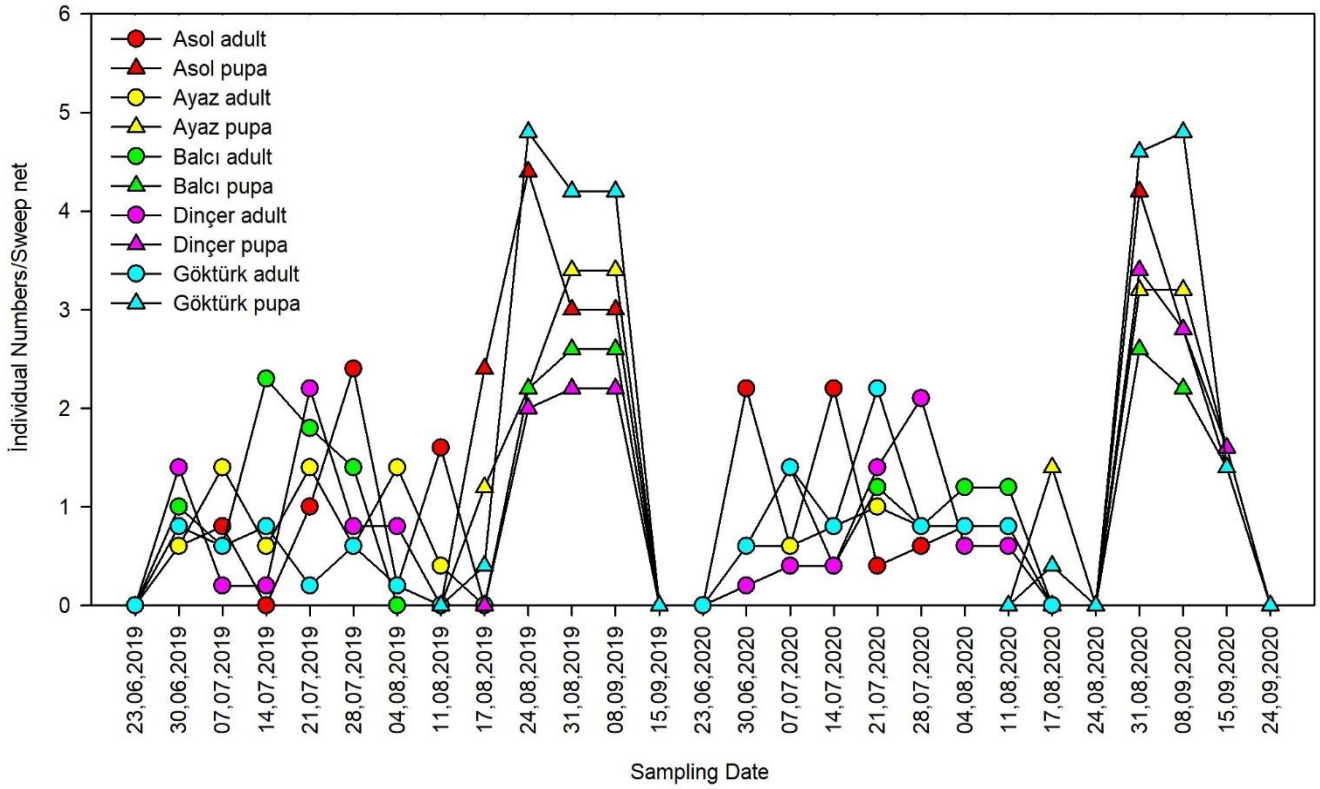


Figure 3. Adult and pupa densities of *Acanthiophilus helianthi* in 2019 and 2020 (number of adult individuals/ Sweep net, pupa number/plate).
Şekil 3. *Acanthiophilus helianthi'nin 2019 ve 2020'deki ergin ve pupa yoğunlukları (ergin birey sayısı/atrap, pupa sayısı/plaka).*

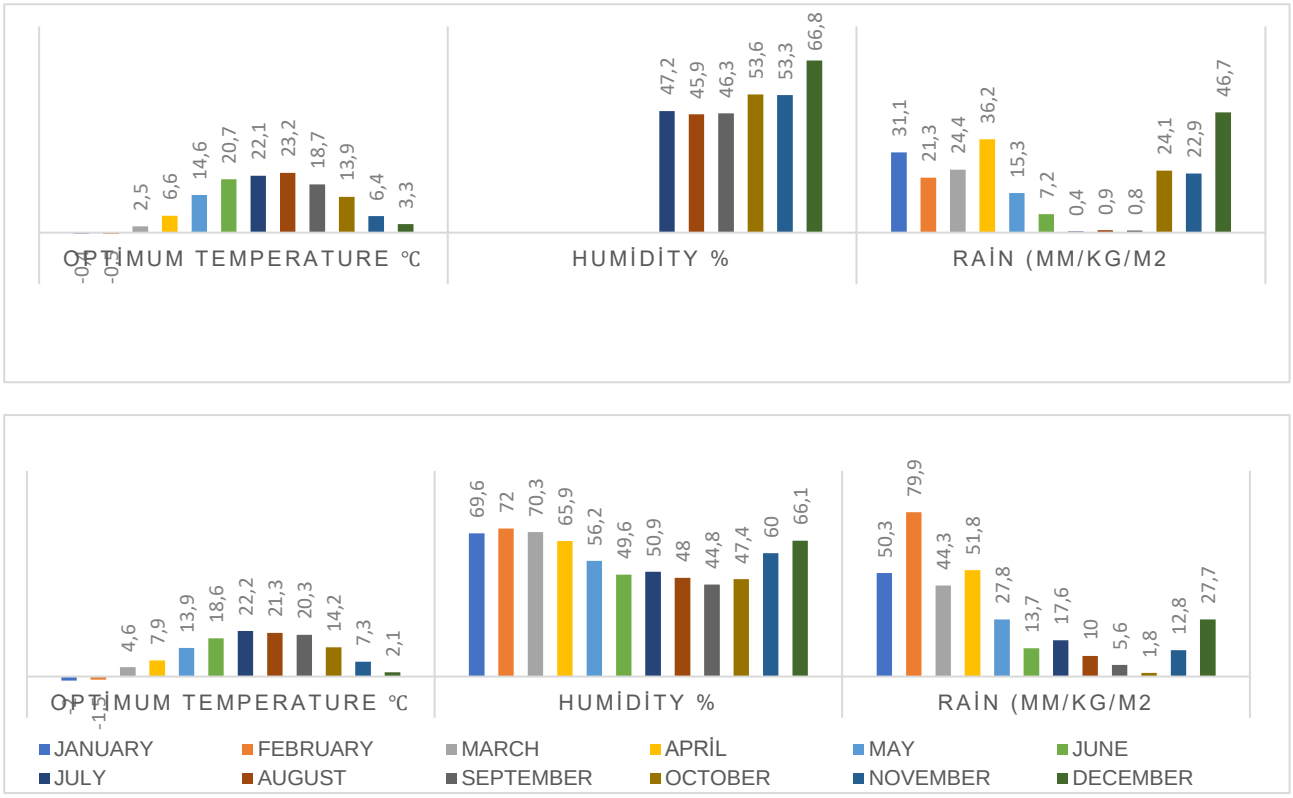
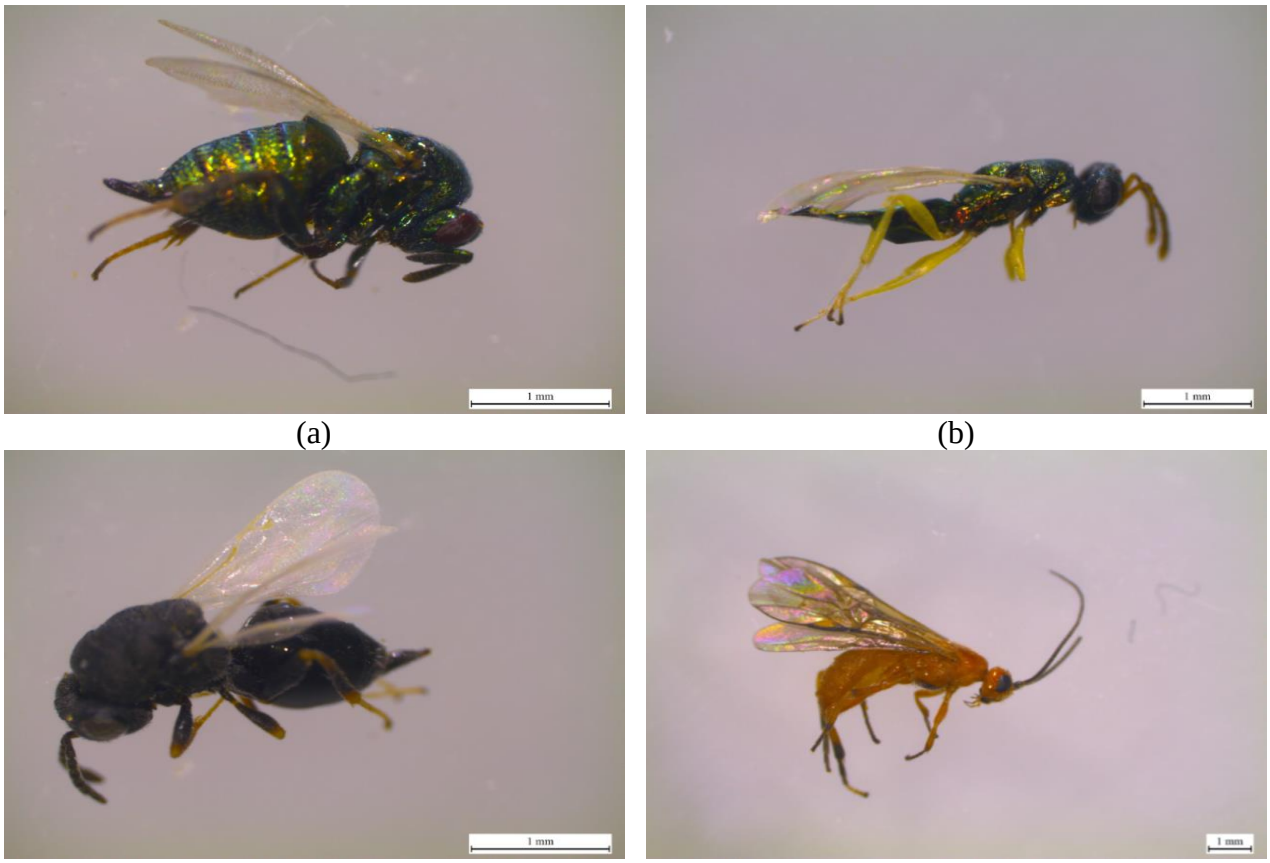


Figure 4. Climate data of Van province in 2019 and 2020.
Şekil 4. *Van ili 2019 ve 2020 iklim verileri.*



(a) (b)

(c) (d)

Figure 5. (a) *Ormyrus* sp. (b) *Microdontomerus annulatus* (c) *Eurytoma acroptilae* (d) *Bracon* sp.
Şekil 5. (a) *Ormyrus* sp. (b) *Microdontomerus annulatus* (c) *Eurytoma acroptilae* (d) *Bracon* sp.

It has not any research found on population growth, variety preference, and determination of parasitoids on Asol, Ayaz, Balcı, Dinçer, and Göktürk cultivars of *A. helianthi*. However, it has been observed that there are studies examining the population fluctuations of *A. helianthi* on different safflower cultivars (Al-Ali et al., 1977; Dusty et al., 2013). It was determined that the first adults of *A. helianthi* became active on 29-30 June and it has been different densities on five varieties until 10-11 August in safflower plants that started to be planted in April. It was determined that pupae started to appear on August 17, when the adult densities reached zero individuals, and continued until September 8. The observations of adults at the time when the flower heads begin to form and the density of pupae in the period when the harvest time support this information. *Ormyrus* sp., *M. annulatus*, *E. acroptilae* and *Bracon* sp. it was observed that the densities of the parasitoids increased at the time of pupae, and the number of individuals and the time of emergence differed over five species. Lotfalizadeh & Gharali (2014), determined Hymenopterous parasitoids of safflower seed pests in Iran. These arae *Pronotalia carlinarum* (Szelényi & Erdos 1951) (Hymenoptera: Eulophidae), *Aprostocetus* sp. (Hymenoptera: Eulophidae), *E. acroptilae*, *Sycophila submutica* (Thomson,1876) (Hymenoptera: Eurytomidae), *Ormyrus gratiusus* (Förster,1832), *Ormyrus orientalis* (Walker,1835) (Hymenoptera: Ormyridae), *Pteromalus albipennis* (Walker,1835), *Colotrechnus viridis* (Masi, 1921) (Hymenoptera: Pteromalidae), *M. annulatus*, *Adontomerus crassipes* (Boucek, 1982) (Hymenoptera: Torymidae), *Bracon luteator* (Spinola,1808), *B. brevicornis* (Wesmael,1838) ve *B. hebetor* (Say, 1836) (Hymenoptera: Braconidae) many parasitoids have been identified. When compared, it was determined that all 4 species identified in our study were the same. In another study conducted by Saeidi et al. (2011), *B. hebetor*, *B. luteator*, *C. viridis*, *M. annulatus*, *O. orientalis*, *E. acroptilae*, *P. carlinarum*, *Pteromalus* sp. (Hymenoptera: Pteromalidae) species were found to be related to *A. helianthi*. When compared with our study, it was observed that the detrmined species were common. In a survey study for the determination of pupa parasitoids was conducted in Iran and were determined *Antistrophoplex conthurnatus*, *Microdontomenus annulatus*, *Bracon hebetor*, *B. luteator*, *Pronotalia carlinarum*, *Ormyrus orientalis*, *Colotrechnus viridis*,

Pteromalus sp., *Eurytoma acroptilae* and *Isocolus tinctorious* as pupal parasitoids of *A. helianthi.* It has been determined that *M. annulatus* play an active role in pupa density of *A. helianthi* and creates differences in parasitism rate among species. The presence of the same parasitoids and their densities at different rates support our study (Saeidi et al., 2016).

Considering the adult densities, the highest values were determined in the Asol variety in both years, and the lowest values in Göktürk for the first year and Balcı varieties for the second year. The highest value in pupa densities was found in the Göktürk variety in both years and the lowest values were obtained from the Dinçer variety in the first year and the Balcı variety in the second year. When the results obtained with a study similar to this study were compared, it was determined that adults and pupae were encountered in the weekly samplings between 19 June and 15 July and that they formed different densities on seven cultivars (Goldasht, Padideh, Zarghan, Varamin, PI, Acataria, Mec163) showed similarity with our study (Dustiy et al., 2013). In another study carried out on the Gina variety of safflower, it was determined that adults emerged between 12 May and 31 July, and 79 adults were reached in 80 days. It has been concluded that the safflower fly is the larvae that cause the damage that it spends its life on the flower bed until it reaches adult (Al-Ali et al., 1977). When evaluated together with our study, it was observed that the adults emerged much earlier, and similarly, the damage symptoms caused by the larvae in the seeds were observed in all cultivars.

4. Results

As a result of two years of observation and data, it has been observed that *A. helianthi* has grown in the fields of five safflower cultivars planted. During sampling with sweep net, 4 parasitoids, *Ormyrus* sp., *M. annulatus*, *E. acroptilae* and *Bracon* sp., were detected and it was understood in the literature that they were the pupal parasitoids of *A. helianthi*. At the same time, it is thought that the color diversity of the flower heads in orange, red and yellow tones, the differences in the vegetative development of the plants depending on the climate and the density of the parasitoids may have affected the population density of *A. helianthi*. It is foreseen that these results will provide some information to the producers who will produce safflower in the future, and in addition to this information, more detailed studies will be an important step in minimizing product losses.

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The Effect of Microbial Transglutaminase (MTGase) Enzyme on Physical, Sensorial and Nutritional Properties of Atlantic Salmon (*Salmo salar* Linnaeus, 1758) Meatballs

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Abstract: In this research, fish balls were produced from Atlantic salmon (*Salmo salar* Linnaeus, 1758) with the addition of MTGase (0.60%), which is included in the seafood menus of catering companies, hotels, restaurants, and other ready-made food establishments in our country and especially around the world. In order not to risk food safety for such businesses where time and hygiene are very important, nutritional, physical, and sensory analyzes were carried out in fish balls to investigate the adequacy/applicability of the combined effect of short time and low temperature (4±1ºC; 3 hours) for MTGase enzyme activation. After the production and activation processes, the meatballs were baked in the oven at 180 ºC for 20 minutes. While the crude protein and moisture content of MTGase added group (B) was higher than the control (A) group ($p<0.05$), the energy content of group A was higher than group B ($p<0.05$). The pH values of groups A and B were determined as 7.40 and 7.32, respectively ($p<0.05$). It was also revealed that the brightness (L*) value increased with the use of MTGase. MTGase activation of short-term and low temperature combination (3 hours at 4±1ºC) did not show any difference in terms of TPA parameters. In terms of appearance and general acceptability parameters, the scores of group B found higher than group A. In addition to that, odor, texture, spice, onion, and garlic taste variables of group B found more acceptable.

Keywords: Atlantic salmon, catering, meatballs, microbial transglutaminase (MTGase), color, sensorial analysis

Atlantik Somonu (*Salmo salar* Linnaeus, 1758) Köftelerinin Fiziksel, Duyusal ve Besinsel Özellikleri Üzerine Mikrobiyal Transglutaminaz (MTGaz) Enziminin Etkisi

Öz: Bu çalışmada, ülkemizde ve özellikle dünya genelinde catering firmaları, oteller, restoranlar ve diğer hazır yiyecek işletmelerinin su ürünleri menülerinde yer alan Atlantik somonundan (*Salmo salar* Linnaeus, 1758), MTGaz ilavesi (%0.60) ile balık köftesi üretimi gerçekleştirilmiştir. Zamanın ve hijyenin oldukça önemli olduğu bu tür işletmeler için gıda güvenliğini riske atmamak adına kısa süre ve düşük sıcaklığın (4±1ºC; 3 saat) kombine etkisinin MTGaz enzimi aktivasyonu için yeterliliğinin/uygulanabilirliğinin araştırılması amacıyla balık köftelerinde besinsel, fiziksel ve duyusal analizler gerçekleştirilmiştir. Üretim ve aktivasyon süreçlerinin ardından köfteler fırında 180 ºC'de 20 dakika pişirilmiştir. MTGaz ilaveli grubun (B) ham protein ve nem içeriği kontrol (A) grubundan yüksek tespit edilirken ($p<0.05$), A grubunun enerji içeriği ise B grubuna oranla daha yüksek belirlenmiştir ($p<0.05$). A ve B gruplarının pH değerleri sırasıyla 7.40 ve 7.32 olarak tespit edilmiştir ($p<0.05$). Araştırmada, parlaklık (L*) değerinin MTGaz kullanımı ile arttığı da ortaya koyulmuştur. TPA sonuçlarına göre; MTGaz ilavesi sonrasında pişirme öncesi düşük sıcaklıkta kısa süreli (4±1ºC'de 3 saat) aktivasyon işleminin Atlantik somon köftesinin tekstüründe önemli değişikliklere neden olmadığı belirlenmiştir. Görünüş ve genel duyusal değerlendirme parametreleri bakımından B grubu daha yüksek puanlanmıştır. Ayrıca koku, tekstür, baharat, soğan ve sarımsak aroma değişkenlik parametreleri B grubunda daha beğenilir bulunmuştur.

Anahtar Kelimeler: Atlantik somonu, catering, köfte, mikrobiyal transglutaminaz (MTGaz), renk, duyusal analiz

1. Introduction

Consumers around the world are supplied with aquaculture products through fishing and aquaculture. In the aquaculture sector, Atlantic salmon (*Salmo salar*) is the most widely farmed fish species. Approximately 90 million 500 thousand tons of fish were obtained in 2020, of which 84 million tons were obtained from farms through controlled aquaculture. The farmed salmon farming industry, which started in the 1960s, has grown significantly over the past decades, and today about 70% of the salmon produced worldwide comes from aquaculture. In 2020, more than 2,600,000 tons of farmed

salmon were farmed worldwide, while only about 550,000 tons of natural salmon were caught (Statista, 2022a). Norway has the largest share in Atlantic salmon exports, while France, Singapore and Portugal are the leading importing countries (Statista, 2022b).

Türkiye is a country where the aquaculture sector is developing rapidly, and the export of aquaculture products has gained momentum in recent years. Trout, sea bass and sea bream are the most farmed and exported fish species in Türkiye. In addition, Türkiye imports a significant amount of Atlantic salmon from Norway (FAO, 2020). Between the years of 2014 and 2019, the average annual consumption of seafood per capita worldwide increased from 19.9 kilograms to 20.5 kilograms, but in 2020, seafood consumption fell to its lowest levels in recent years at 19.8 kg per capita (Statista, 2022c). In Türkiye, aquaculture consumption is quite below the world average. The main reasons for the low consumption in Türkiye are the popularity of seafood products and the prejudice of consumers against aquaculture fish. Additionally, the fact that fresh fish is more prominent in fish consumption and the lack of demand for processed seafood products.

While many product types such as canned, smoked, marinated, surimi, new generation packaged products, fish sausages, salami, burgers, etc. have found a place in foreign marketplaces, those products have not reached the deserved position in Turkish markets. The basis for increasing fish consumption in Türkiye can be realizable such as ensuring that children consume fish at least twice a week. Since the eating and drinking habits acquired in childhood will affect the whole life, the individual will continue this habit in later ages. It is essential that catering companies show the necessary sensitivity and plan their menus to include seafood 1 - 2 times a week so that children or young people who spend a large part of their day outside due to school and adults who are actively working can benefit from seafood at least at lunchtime. However, it is also known that seafood can become a risky food as it is very sensitive to spoilage due to its structure. It requires maximum attention, especially during preparation for mass consumption. In addition, there are quite high fluctuations in prices depending on the season. At this point, processed seafood products provide a solution to an important problem both in terms of industrialization and quality safety and cost fluctuations. For this reason, it is especially important to fillet large fish such as salmon, remove the bones, form to minced meat and use them as meatball raw material in the sector.

One of the processed seafood products is fish meatballs or burgers. In its simplest definition, it is a product containing additives such as onion, breadcrumbs, salt, black pepper, garlic, red pepper, egg, etc. added to fish minced meat; in addition to the above-mentioned in the industrial sense; It may also contain potato starch, milk powder, enzyme, phosphate, carrageenan, preservative, and stabilizing agents, etc. (Can, 2012; Öksüztepe et al., 2010; Ulusoy et al., 2017). Transglutaminase enzyme is an enzyme used in meatball, burger, and pate production. Transglutaminase (TGase) initiates the formation of covalent bonds between glutamine and lysine residues in proteins. The addition of microbial transglutaminase (MTGase) can improve the thermal stability of meat proteins by imparting desirable properties to the reconstituted products during heating (Başaran et al., 2010). The best pH range for the enzymatic activity of MTGase is from 5 to 8. However, some enzymatic activity can be maintained at very low (~ 4) and very high (~ 9) pH levels (Motoki and Seguro, 1998). The optimum temperature for enzymatic activity is 50°C. However, it has also been reported that MTGase is active at 10°C and maintains some activity even at temperatures just above freezing (Motoki and Seguro, 1998). In the literature, there are studies on traditional and industrial meatballs produced from different fish species with different additives (Altan, 2020; Ehsani et al., 2020; Gökoğlu, 1994; Kaba et al., 2012; Keser and İzci, 2020; Kılınççeker, 2014; Kılınççeker and Karahan, 2019; Mattje et al., 2019; Özpolat and Çoban, 2012; Sugitha et al., 2019; Yanar and Fenercioğlu, 1999). In a previous study, it was determined that minced meat obtained from breeder-sized rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) can be preserved for up to 24 days in a quality manner by packing in modified atmosphere (MA) with 75%CO_2 + 25%N_2 combination (Kocatepe et al., 2016). Various studies have shown that sausages, meatballs, and burgers produced from fish with the addition of MTGase are highly appreciated and help to increase fish consumption. For example, Cavenaghi-Altemio et al. (2018) surveyed the "propensity to purchase" and "willingness to reconsume" of MTGase-added sausages produced from catfish. While 66% of the respondents answered, "I would definitely buy it", 23% answered "I would very likely buy it again". Another researcher (Altan, 2020) had a survey group that

were consisted of children between the ages of 8 and 15, who do not prefer to consume fish due to the presence of bones, skin, or its characteristic smell, or whose fish consumption rate is relatively low. After children were tried the trout burger patties produced with MTGase, they were reported that the "taste / enjoying of the product" and "wanting to consume the product again" criteria scores as more than 90%. According to the literature, it is obvious that various products such as fish sausages, burger patties, etc. with MTGase additives, which is a processed fish product, will contribute significantly to increase the fish consumption of the society, especially children.

In this study, it was aimed to determine the effect of activation of meatballs obtained from imported Atlantic salmon minced meat at 4±1ºC for 3 hours after the addition of MTGase on the nutritional, physical and sensory properties of meatballs.

2. Materials and Methods

2.1. Material

In this study, 5 Atlantic salmon (*Salmo salar* Linnaeus, 1758) (between 4 and 5 kg) were obtained from Samsun Fish Market. The fish were quickly transported to Sinop University, Faculty of Fisheries, Fisheries Processing and Quality Control Laboratory in Styrofoam boxes in ice.

2.2. Preparation of Fish Cakes

After the fish are brought to the laboratory, cleaned, filleted and the remaining skin on the fillets is removed. The bones were removed from the fillets and minced into minced meat by passing through a Mateka RR-K30D brand/model cutter (5000 r./min.) for 90 seconds. Approximately 2 kgs of minced fish were separated to create of two-groups. All additives were added to the minced meat and passed through the cutter for another 30 seconds to ensure complete mixing of the meatball mixture. Meatball mixture: 70% minced fish, 15% grated onion, 7.5% breadcrumbs, 5% egg, 1.30% salt, 0.85% garlic, 0.25% crushed red pepper and 0.20% black pepper. *Group A (control):* Atlantic salmon meatballs without MTGase; *Group B:* Atlantic salmon meatballs with 0.60% MTGase enzyme addition. The groups were shaped as 5cm x 4cm x 1cm (length x width x thickness) and after resting at 4±1ºC for 3 hours, they were cooked in Ulubaş/UEO-TT/1400W brand/model oven at 180 ºC for 20 minutes. The study was carried out in 2 replicates and 3 parallels.

2.3. Analyses

Crude protein, crude lipid, crude ash, moisture, energy content, pH, water activity (aw), color analysis, texture profile analysis (TPA) and sensory analyses were performed. The analyses were carried out on cooked meatball samples.

2.3.1 Proximate Composition Analysis

Dry matter, crude protein and crude ash analyses of the groups were carried out according to AOAC (1995) and crude lipid analysis was carried out in Gerhardt SE-416 brand/model fully automatic soxtherm oil extraction device according to AOAC (2000). Atwater method was used for energy calculation of fish balls (Falch et al., 2010). According to this method:

$$Carbohydrate\ value = 100 - (Water + Lipid + Protein + Ash) \quad (1)$$

$$Energy\ (kcal) = (Lipid*9) + (Protein*4) + (Carbohydrate*4) \quad (2)$$

2.3.2. Physical analysis

The pH of the fish balls was measured with a digital pH meter of brand/model as Werkstatten 82362. 2 g of homogenized fish meat was homogenized in 20 ml of pure water (1:10 ratio) for 1 minute and the pH meter was immersed in this solution for 1 minute (Curran et al., 1981).

LabShift Novasina brand/model automatic water activity device was used to determine the water activity (a_w). Measurements were carried out according to Horwitz et al. (1980) by placing approximately 5 g of sample in the measuring cup at 25ºC.

Konica Minolta/CR-A 33a, brand/model colorimeter (CIE, 1976) was used for color measurement of fish balls. The samples were placed in transparent plastic petri plates with a diameter of 9 cm and the measurements were carried out in those petri plates. The *a value indicates redness or greenness, *b value indicates yellowness or blueness and *L value indicates the degree of brightness between *0* (black) and *100* (white).

TPA (texture profile analysis) analysis was performed within the scope of mechanical analysis. Brookfield CT3 Texture Analyzer brand/model device was used. For TPA analysis, 1cm x 1cm x 1 cm (length x width x thickness) standard size samples were taken from cooked salmon meatballs cooled to room temperature. The device was set

to 60% deformation target, 0.05 N trigger sensitivity, 0.50 mm/sec test speed, 2 mm/sec pre-test speed, 20 points/sec data transfer rate and 50 kg cell load. A 12-mm-diameter cylindrical probe was used in the device. Among the TPA parameters; hardness (N), adhesiveness (mJ), resilience, cohesiveness, gumminess (N), chewiness (N.mm) and springiness (mm) values were measured (Anonymous, 2018).

All analyses were performed with 2 replicates 3 parallel (n=6).

2.3.3. Sensory analysis

As sensory analysis form of meatballs, the hedonic scale created by Pons et al. (2006) was modified according to the product and converted into a 5-point scoring system and odor, texture, appearance, spice, onion, garlic, general taste, and general rating parameters were asked to panelists. Cooked fish meatball evaluations were carried out by 20 panelists experienced in the field of aquaculture (with 2 replicates). Meatball samples were placed on 10 cm diameter white foam plates separately for each panelist and panelists were not informed about the meatball contents. In the scoring scale of cooked meatballs; 5 points were evaluated as "very good", 4 points as "good", 3 points as "medium", 2 points as "consumable" and 1 point as “very bad”. A score of "3" on the raw trout scale and a score of "2" on the cooked trout scale was considered as the limit of consumability.

2.3.4. Statistical Analysis

Proximate composition, physical, color and TPA data were expressed as mean ± standard error (se) and homogeneity of variances was tested at $p<0.05$ significance level. The *t-test* was used to compare the means of the analysis results of the groups. Statistical analyses were performed using MINITAB (Version 17.1) software. Within the scope of sensory analysis, paired sample t-test was used to compare the panelists' opinions on the differences between the groups using IBM SPSS (Version 21.4) software.

3. Results and Discussion

3.1. Proximate Composition of Fish Meatballs

Total crude protein, crude lipid, crude oil, crude ash, moisture, carbohydrate and energy contents of group A and B are given in Table 1.

While the crude protein and moisture content of the MTGase-added group (B) was higher than that of group A (control) ($p<0.05$), the energy content of group A was higher than that of group B ($p<0.05$). Altan (2020) was reported that MTGase is preventing the moisture loss of trout burger patties due to structure enhancement and therefore, protein losses were seen at minimum levels. Although there was no statistical difference, the slightly higher crude lipid content of group A caused an increase in energy content. In terms of other nutritional analyses (crude lipid, crude ash, and carbohydrate), there was no difference between the groups ($p>0.05$).

Salmon is a fish species preferred by consumers due to its high lipid content. As it is known, lipids are one of the most important components affecting the taste of food/meal. In this study, no oil was added to the meatball batter considering the crude lipid content of salmon. Atlantic salmon has a higher lipid content compared to many natural fish species and aquaculture fish. In a study comparing the proximate composition of large rainbow trout burger patties with and without MTGase addition, it was reported that crude lipid content of patties decreased, crude protein content increased, and moisture content increased with 0.5% MTGase addition (Altan, 2020). In the same study, it was reported that the energy content of trout patties decreased with the addition of MTGase. In our study, the energy content of group A was found to be 209.33 kcal/100g and 194.18 kcal/100g in group B with MTGase addition and this value was higher than the energy content reported by Altan (2020). In the aforementioned study, 5% oil was added to the meatball mixture. Additives added to the meatball mixture affect the nutritional content of the final product.

Palmeira et al. (2014) reported that the crude protein content of trout meatballs increased with the addition of MTGase, similar with our study. In a study investigating the gel holding capacity of mackerel meat under high pressure with the addition of MTGase, it was reported that the protein solubility of the enzyme added group was lower (Gómez-Guillén et al., 2005). Similarly, Cardoso et al. (2011) reported that MTGase prevented protein loss in fish meat and the highest protein ratio was in the enzyme group. The use of MTGase reduces protein solubility in meatballs and enriches the final product in terms of protein content.

It is clear that the use of MTGase did not make a significant difference in the crude ash content of the groups. Similar with our study, Cardosa et al. (2011)

reported that the effect of MTGase supplementation on the crude ash value of minced sea bass fish was insignificant. MTGase enzyme is used in meat industry for different product development purposes. Uran et al. (2013) reported that the addition of 0.5% - 1% TGase enzyme to chicken breast meat caused an increase in protein and ash values and a decrease in lipid content, similar with our study.

Fish meat is a food with a very low carbohydrate content. Various researchers were reported the carbohydrate content of Atlantic salmon as 0.19g/100g (Şengör et al., 2013) and 1.72% (Kocatepe et al., 2022). In the production of Atlantic salmon meatballs, the additives added to the meatball batter increased the carbohydrate content of the final product up to 7.87 g/100g. This increase was seen lower in the group with MTGase addition ($p>0.05$).

Table 1. Proximate composition and energy content of Atlantic salmon meatballs
Çizelge 1. *Atlantik somonu köftelerinin besin kompozisyonu ve enerji içeriği*

	Group A (control)	***Group B (0.60% MTGase)***
Crude protein (%)	14.80 ± 0.07^{B}	15.19 ± 0.03^{A}
Crude lipid (%)	13.18 ± 0.58^{A}	11.77 ± 0.22^{A}
Moisture (%)	61.71 ± 0.12^{B}	63.82 ± 0.04^{A}
Crude ash (%)	2.42 ± 0.15^{A}	2.34 ± 0.08^{A}
Carbohydrate (%)	7.87 ± 0.57^{A}	6.86 ± 0.33^{A}
Energy (kcal/100 g)	209.33 ± 3.06^{A}	194.18 ± 1.01^{B}

Mean (n=6) ± std. error
A, B: (→) : The difference between groups with different letters is significant (p≤0.05).

3.1. Physical Properties of Fish Meatballs

Physical analysis is one of the main factors affecting the preferability of food for the consumer. While some of the physical properties provide information about the composition of the food, some of them are important in the development of pre-consumption perception. Especially the color of the food directly addresses consumer perception. Table 2 shows the pH, aw, L*, a* and b* color analysis results of Atlantic salmon meatballs.

The pH value of Atlantic salmon varies between 6.32 – 6.58 (Wang et al., 2003) and 6.13 – 6.20 (Duun & Rustad, 2008). The pH value of group A (without enzyme addition) was 7.40, while the pH content of the group with MTGase (B) was found as 7.32 ($p<0.05$). Alkaline substances used in the meatball ingredients affected the pH content of the final product. It was reported by Motoki and Seguro (1998) that MTGase activity continues between pH 4 and 9. For MTGase activation, the meatballs were rested at 4±1ºC for 3 hours and then cooked. Gaspar and Goes-Favoni (2015) reported that the addition of MTGase increased the pH level, and this was due to the high isoelectric point of MTGase itself. Karina and Setiadi (2020) reported that adding 1 – 1.5% MTGase to halibut fillets are directly increased the pH level sharply. In our study, the pH content of group A without enzyme addition was higher than group B. This difference between the studies is thought to be due to the enzyme ratio and the content of meatball ingredients used in our study.

Table 2. Physical properties of Atlantic salmon meatballs
Çizelge 2. *Atlantik somonu köftelerinin fiziksel özellikleri*

	Group A (control)	***Group B (0.60% MTGase)***
pH	7.40 ± 0.01^{A}	7.32 ± 0.00^{B}
A_w	0.95 ± 0.00^{B}	0.96 ± 0.00^{A}
Color analysis		
L	74.34 ± 0.94^{B}	76.08 ± 0.12^{A}
*a**	6.28 ± 0.16^{A}	5.37 ± 0.22^{B}
*b**	22.78 ± 0.07^{A}	22.01 ± 0.25^{B}

Mean (n=6) ± std. error
A, B: (→) : The difference between groups with different letters is significant (p≤0.05).

Water activity (a_w), which is a measure of the amount of moisture in foods, is directly related to the moisture content of the food. The aw content of fish meat varies between 0.97 – 0.99. With the addition of enzyme, it was also aimed to get a product with a higher water content. As mentioned before, the moisture content of group B containing enzyme was higher than group A. Similarly, water activity was also higher than the group without enzyme ($p<0.05$). It is obvious that some water is removed from the meatball content with the effect of cooking, but despite this, the aw content of the meatballs is 0.95 and above.

The color of food is a feature that gives a lot of information to the consumer about the content and general characteristics of the product and reflects the deterioration parameters of the food. Since color perception can vary from person to person, color results obtained with laboratory devices are more reliable. L* is expressed as brightness value. As seen in Table 2, the L* value of the enzyme-added group was found higher ($p<0.05$) than control group. Similar with our study, it has been reported by different researchers that the brightness value increases with the use of MTGase due to the catalyzing the cross-linking reaction of myosin leading to the formation of

protein intra- and inter- molecular covalent bonds, and it is resulting with formation of opaque gels (Karayannakidis et al., 2008; Ramirez et al., 2000). +a* value expresses reddish color while +b* color expresses yellow color. Both +a* and +b* values of group B were found lower ($p<0.05$). Cavenaghi-Altemio et al. (2018), were examined the color quality effect of MTGase addition at different ratios on catfish sausage and they stated that +a* and +b* values of low enzyme concentrations were higher, while these values decreased with enzyme increase.

The results of texture profile analysis (TPA) of Atlantic salmon meatballs are shown in Table 3. Brookfield Laboratories, which specializes in texture and has carried out very important studies in this field, defines TPA parameters in its manual as follows: Hardness is the deformation response to the analysis probe; adhesiveness is the tendency of the food to adhere to the teeth during the first bite; resilience is the effort to return to its initial state after the food is compressed in the first cycle; cohesiveness is the resistance of the food to a second deformation; gumminess is the force required to chew solid food to make it ready for swallowing; and springiness is the flexibility of chewing at the first moment in the mouth (Brookfield Inc., 2018). TPA analysis is a kind of laboratory simulation of the chewing force applied by the jaw muscles and teeth when the food is taken into the mouth. For this reason, properties of foods such as hardness, resilience, cohesiveness, etc. are measured by texture analysis and give clear results.

When the TPA findings of the groups were examined, no difference was detected in any factor ($p>0.05$) except for the resilience ($p<0.05$). Although no statistical difference was detected, the springiness values of group B were found increased. Similarly with our research, Altan (2020) reported that the springiness of cooked trout burger patties produced with MTGase was found higher in the groups with 0.5% and 1% enzyme addition compared to the control group. According to the TPA results of the study, it was determined that short-term (3 hours at 4±1ºC) resting at low temperature before cooking after the addition of MTGase did not cause significant changes in the texture properties of Atlantic salmon meatballs. This may be related to the fact that 3 hours of resting at 4±1ºC was not sufficient for the activation of the enzyme. Tokay et al. (2022) reported that short setting time (4 hours) was not efficient for the enzyme to activate and restructure fish meat. It can be suggested that the enzyme activation time and temperature should be increased for future studies such as meatballs/burgers/pate.

Table 3. Texture profile analysis (TPA) characteristics of Atlantic salmon meatballs

Çizelge 3. *Atlantik somonu köftelerinin tekstür profil analizi (TPA) bulguları*

	Group A (control)	***Group B (0.60% MTGase)***
Hardness (N)	20.15 ± 1.62^{A}	20.13 ± 0.32^{A}
Adhesiveness(mJ)	0.55 ± 0.05^{A}	0.55 ± 0.55^{A}
Resilience	0.07 ± 0.00^{A}	0.05 ± 0.01^{B}
Cohesiveness	0.26 ± 0.00^{A}	0.26 ± 0.02^{A}
Gumminess (N)	5.30 ± 0.38^{A}	5.24 ± 0.56^{A}
Chewiness (N.mm)	46.9 ± 11.9^{A}	46.85 ± 13.25^{A}
Springiness (mm)	9.98 ± 0.39^{A}	10.24 ± 0.07^{A}

Mean (n=6) ± std. error

A, B: (→) : The difference between groups with different letters is significant (p≤0.05).

3.2. Sensorial Characteristics of Fish Meatballs

In the sensorial analysis results (Table 4), which was conducted to determine whether the panelists' ratings of the groups on the 5-point hedonic scale differed or not, it was observed that the differences between the mean ratings were observed between the variables of General Taste ($p= 0.000<0.05$); Appearance ($p= 0.021<0.05$) and General acceptability ($p= 0.000<0.05$).

Table 4. Paired sample t-tests statistics of sensory analyses

Çizelge 4. *Duyusal analiz verilerinin eşleştirilmiş örneklem t-testi sonuçları*

		Average	Panelist	Std. Deviation	Mean of Std. Error
Pair 1	**Odor A**	4,25	20	0,71635	0,16018
	Odor B	4,35	20	0,74516	0,16662
Pair 2	**Texture A**	4,20	20	0,52315	0,11698
	Texture B	4,40	20	0,68056	0,15218
Pair 3	**Spice taste A**	3,85	20	0,74516	0,16662
	Spice taste B	4,25	20	0,63867	0,14281
Pair 4	**Onion taste A**	3,65	20	0,81273	0,18173
	Onion taste B	3,95	20	0,99868	0,22331
Pair 5	**Garlic taste A**	3,75	20	1,01955	0,22798
	Garlic taste B	4,10	20	0,85224	0,19057
Pair 6	**General taste A**	3,80	20	0,76777	0,17168
	General taste B	4,58	20	0,67424	0,15077
Pair 7	**Appearance A**	3,95	20	0,68633	0,15347
	Appearance B	4,45	20	0,68633	0,15347
Pair 8	**General acceptability A**	3,93	20	0,73045	0,16333
	General acceptability B	4,70	20	0,47016	0,10513

Table 5. Paired sample t-tests of sensory analyses, paired differences
Çizelge 5. *Duyusal analizlerin eşleştirilmiş örneklem t-testi farklılıkları*

		Average	Std. Deviation	Std. Error	95% Confidence interval		*t*	df	Sig. (2-tailed)
				Average	Low	High			
Pair 1	**Odor A - Odor B**	-,10000	,55251	,12354	-,35858	,15858	-,809	19	,428
Pair 2	**Texture A - Texture B**	-,20000	,76777	,17168	-,55933	,15933	-1,165	19	,258
Pair 3	**Spice taste A – Spice taste B**	-,40000	,99472	,22243	-,86554	,06554	-1,798	19	,088
Pair 4	**Onion taste A – Onion taste B**	-,30000	1,08094	,24170	-,80589	,20589	-1,241	19	,230
Pair 5	**Garlic taste A – Garlic taste B**	-,35000	1,18210	,26433	-,90324	,20324	-1,324	19	,201
Pair 6	**Gen. taste A - Gen. taste B**	-,77500	,73404	,16414	-1,11854	-,43146	-4,722	19	**,000**
Pair 7	**Appearance A - Appearance B**	-,50000	,88852	,19868	-,91584	-,08416	-2,517	19	**,021**
Pair 8	**Gen. accept. A - Gen. accept. B**	-,77500	,76906	,17197	-1,13493	-,41507	-4,507	19	**,000**

There was no difference between the other variables (p>0.05). When the mean scores of the variables in which differences were observed are examined, it is possible to say that the mean scores of Group B in the general taste parameter are higher than Group A (4.57>3.80) and likewise, Group B is evaluated by the panelists with higher scores in the parameters of Appearance (4.45>3.95) and General acceptability (4.70>3.9250) compared to Group A.

On the other hand, when the average scores given by the panelists to the products were examined in general terms, it was found that Group B had higher average scores than Group A in terms of odor, texture, spice, onion, and garlic taste variables (Table 4 and 5). According to these results, Group B was found to be more acceptable by the panelists than Group A.

4. Conclusion

When the results of the research are evaluated, it can be said that although a relatively low amount (0.60%) of MTGase was used, it significantly protected the product quality compared to the control (A) group by reducing crude protein and moisture loss. In the control (A) group, the total energy amount increased due to the slightly higher crude lipid content. When the pH and aw differences between the groups are examined, even though statistical differences were observed in both analyses, the differences are not of any practical significance. When the color changes between the groups were examined, it was found that the use of enzyme increased the brightness (L*) while decreasing the redness (a*) and yellowness (b*) values. In terms of texture profile analysis (TPA) findings, the groups showed very similar results to each other. The main reason for this similarity is clearly seen to be the reverse synergistic effect of low temperature and limited time (3 h at 4±1ºC) on enzyme activation. It is possible to say that group B containing MTGase had higher scores than the control (A) group in terms of appearance, general taste, and general acceptability parameters, as well as higher scores than the control (A) group in terms of odor, texture, spice, onion, and garlic variables. According to these findings, group B containing MTGase achieved a higher level of appreciation by the panelists than the control (A) group.

To evaluate the research as a whole, although the MTGase enzyme showed low activity due to low temperature/time application and could not be effective on textural properties, it is still managed to provide preservation in an important component of the nutritional composition such as protein, and to preserve the color properties to a great extent and to gather all the appreciation in terms of all other sensory criteria. Considering that the biggest goal of the sector is to gain customer appreciation and that consumer preferences are based on product appearance, it can be said that Group B was very successful in providing the most important criteria for consumers (color, taste, general appearance, etc.). In future research, it may be recommended to develop new products with MTGase additives by increasing the activation temperature and duration slightly more without risking food safety.

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Effect of Altitude and Location on Compositions and Antioxidant Activity of Laurel Cherry (*Prunus Laurocerasus* L.)

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Abstract: The compositional and antioxidant properties of cherry laurel *(Prunus laurocerasus* L.) fruit, which grow in two different locations (Trabzon and Rize) and altitudes, were investigated. The results indicated that antioxidant activity, total phenolic content, citric acid, sugars and phenolic compounds were affected by location and altitude. While fruits of Trabzon province have higher composition content than those of Rize province, fruits collected at low altitudes in both regions were found to have superior properties. Total phenolic content in fruits from Trabzon and Rize provinces increased from 21.90 to 23.32 and from 16.84 to 18.91 mg gallic acid equivalent / 100 g dry weight (DW), β-carotene increased from 5.19 to 6.75 and from 4.16 to 5.61 mg / kg DW, and total sugar increased from 81.68 to 131.99 and from 86.44 to 99.58 mg / g DW when altitude decrease from 351 to 49 m and 316 to 14 m, respectively. Chlorogenic acid (1404.46-7358.63 mg / kg DW) and rutin hydrate (1491.05-2712.91 mg / kg DW) were major phenolic compounds in all samples.

Keywords: Altitude, Antioxidant activity, Cherry laurel, Phenolic composition, Location

Karayemiş Meyvesinin (*Prunus Laurocerasus* L.) Bileşimi ve Antioksidan Aktivitesi Üzerine Yükseklik ve Konum Etkisi

Öz: Bu çalışma kapsamında Trabzon ve Rize bölgelerinin farklı yüksekliklerinde yetiştirilen karayemiş (*Prunus laurocerasus* L.) meyvesinin bileşim ve antioksidan özellikleri incelenmiştir. Elde edilen sonuçlara göre meyvenin toplam fenolik madde miktarı, bireysel fenolik bileşenleri, şeker, sitrik asit içeriği ve antioksidan aktivitesinin yetiştirildiği yer ve rakımdan oldukça etkilendiği belirlenmiştir. Trabzon bölgesinde yetiştirilen meyve Rize bölgesinde yetiştirilene kıyasla daha yüksek bileşen miktarına sahip iken, en yüksek bileşen kompozisyonu her iki bölgenin düşük rakımında yetiştirilen meyvelerinde belirlenmiştir Trabzon ve Rize bölgelerinde rakımın sırası ile 351 den 49 m'ye ve 316'dan 14 m'ye düşmesi ile toplam fenolik içeriğinin 21.90 dan 23.32'ye ve 16.84'den 18.91 mg gallik asit miktarı eşdeğeri / 100 g kuru madde (KM)'ye yükseldiği, β-karoten miktarının 5.19'dan 6.75'e ve 4.16'dan 5.61 mg / kg KM'ye yükseldiği, toplam şeker miktarının ise 81.68'den 131.99'a ve 86.44'den 99.58 mg / g KM'ye yükseldiği belirlenmiştir Klorojenik asit (1404.46-7358.63 mg /kg KM) ve rutin (1491.05-2712.91 mg / kg KM) tüm örneklerde baskın fenolik bileşik olarak belirlenmiştir.

Anahtar Kelimeler: Antioksidan aktivite, Bölge, Fenolik bileşim, Karayemiş, Yükseklik

1. Introduction

Cherry laurel (*Prunus laurocerasus* L.), belongs to *Rosaceae* family and Prunus genus, generally grows wildly in Europe, Asia, the Balkans, Iran and the East Black Sea Region of Turkey. The fruit is used in various products such as jam, pekmez (a boiled fruit juice), marmalade and fruit juice as well as fresh or dried consumption (Alasalvar et al., 2005; Ozturk et al., 2017). In addition, cherry laurel has been also used for medicinal purposes in Turkey for many years due to its positive effects on health (Alasalvar et al., 2005). Cherry laurel is used in many countries as a traditional medicine to treat disorders abdominal pain, cough, bronchitis, stomach ulcers, hemorrhoids and eczema nausea as well as strengthening of bones, reduction of kidney stones, the establishment of acid-base balance in bloodstream (Ozturk et al., 2017). Diets with a high amount of fruits and vegetables are recommended to reduce the risk of various chronic diseases like cancer, coronary heart disease, and cardiovascular disease (Mphahlele et al., 2014). Recently, many researchers have been focused on elucidating mechanisms of bioactive compounds in fruits. These studies contribute to understanding the effect of fruit genotype and growth conditions on compositional properties (Crespo et al., 2010). Although the biosynthesis and accumulation of phytochemicals in fruits (mainly phenolic compounds) can be endogenously (such as genotype) controlled during developmental differentiation, the differences in the amount and variety of them emerge with the effect of many exogenous factors. The main exogenous factors are as follows: growing conditions (light, temperature,

irrigation, altitude, etc.), pre-harvest environmental conditions, level of maturity and storage conditions (Alasalvar et al., 2005; Crespo et al., 2010; Topalovic and Mikulic-Petkovsek, 2010; Mphahlele et al., 2014).
In literature, there are a lot of studies on different properties of cherry laurel such as chemical composition, antioxidant activity (Alasalvar et al., 2005; Celik et al., 2011;Yıldız et al., 2014), bioactive contents (Celik et al., 2011; Yıldız et al., 2014) and change in fruit quality at different storage temperatures (Ozturk et al., 2017). However, no study has been conducted on the changes in composition and antioxidant capacity of fruit which grow in different locations and altitudes. This study aims to investigate the fruit composition (phenolic content, β-carotene, organic acids and sugars) and antioxidant activity of cherry laurel grown in different locations and altitudes.

2. Material and methods

2.1. Samples preparation

The samples were collected from two different districts of Trabzon and Rize provinces of the East Black Sea Region located in the northeastern part of Turkey. At least 5 kg of fruit from 6 tree per locations collected on August 2018 from Trabzon Low (TL; 1° 0' 9.70" N latitude, 39° 43' 0.34"E longitude and altitude is 49 meters,), Trabzon High (TH; 40° 48' 51.19" N latitude 39° 36' 38.37" E longitude and altitude is 351 meters), Rize Low (RL; 41° 1' 31.84"N latitude 40° 31' 3.59"E longitude and altitude is 14 meters) and Rize High (RH; 1° 2' 53.53"N latitude 40° 53' 56.61"E longitude and altitude is 316 meters). Samples were packed in ice in an insulated container and transferred to the laboratory in the cold chain. Upon arrival to the laboratory, samples were homogenized with a Warring blender (Model HGB2WTS3, Connecticut, USA) after pre-treatments such as manual sorting, washing, removing of seeds by hand and used for analysis.

2.2. Total phenolic content and antioxidant capacity

The extraction was performed according to the method described by Veberic et al. (2009). Total phenolic contents were determined by the Folin–Ciocalteau procedure described by Krayjalyte et al. (2013) with minor modifications. In brief, 1 mL of 0.2 N Folin–Ciocalteau regent and 400 µL of distilled water were added to 100 µL extract. After 3 min incubation, 1 mL of Na_2CO_3 solution (7%) was added and incubated for 90 min at room temperature in dark. At the end of incubation time, the absorbance of the sample was measured at 725 nm using an UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolic content was calculated by a five-point calibration curve prepared by gallic acid and results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry weight (DW) samples.

The radical scavenging capacity of samples against DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined according to the method of Brand-William et al. (2005) with minor modifications. In brief, extract (100 µL) was mixed with 1.9 mL of DPPH methanolic solution (1mg/mL) and incubated for 60 min in the dark at room temperature. After the incubation period, absorbances were measured at 520 nm. A calibration curve was constructed by measuring the absorbance of five concentrations of trolox. Results were expressed in µmol trolox / 100g DW.

The ABTS (2.2'-azinobis [3-ethylbenzthiazoline-6-sulphonic acid]) antioxidant activity was determined according to Re et al. (1999). In brief, The ABTS•+ was prepared by mixing an ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate in dark for 16h. The stock solution was diluted with ethanol to an absorbance of 0.7000 ± 0.020 at 734 nm. A total of 200 µL extract was added to the ABTS radical solution (3.8 mL). The absorbance at 734 nm was measured after incubation for 60 min at room temperature. A calibration curve was constructed by measuring the absorbance of five concentrations of trolox. Results were expressed in µmol trolox / 100g DW.

2.3. HPLC analyses

Shimadzu HPLC system integrated with an autosampler (DGU2A 5R) including a column oven (CTO-10AS VP), a degasser system (DGU2A 5R), a gradient pump (LC-20AR), 175 a diode-array detector (DAD, SPD-M20A), a refractive index detector (RID-10A), and a 176 software package for system control and data acquisition (LC solution) was used for the following analyses. The analysis was performed in triplicate for each sample and results were expressed as mean ± SD

2.3.1. β-carotene analysis

The β-carotene extraction of samples was carried out according to the method of Sadler et al. (1990) with slight modifications. Obtained extract (20 µL) was injected into HPLC system, separations were carried out according to method of Barba et al. (2006). Isocratic elution was used on inertsil ODS-2 column (250x4.6mm, 5µm, GL Sciences INC., Tokyo, Japan) for separation at the flow rate of 1 mL/min, column

temperature at 32°C, with mobile phase consisting of methanol: acetone (70:30 v / v). Quantification was performed by using a calibration curve prepared with the five concentrations of β-carotene standard solutions and results were expressed as mg β-carotene / g DW.

2.3.2. Organic acid and Sugar

For extraction, 5 g of fruit puree was diluted fivefold with ultra-pure water and the mixture was homogenized with Ultra Turrax T18 (Ika Staufen, Germany) at 13000 rpm for 1 min. After centrifugation (25155 g) for 20 min at 4°C, the supernatant was filtered through 0.45 µm nylon (Lubitech, Songjiang, China) syringe filter. The filtrate was used in sugar and organic acid analyses.

Organic acids were determined with HPLC system using Rezex ROA column (300 x 7.8; Phenomennex, Torrance, CA), UV dedector (210 nm for malic and citric acids, 245 nm for ascorbic acid), according to the method described by Sturm et al. (2003). The flow rate was 0.5 mL/min, the mobile phase was 0.0025 M sulphuric acid, column temperature was 55°C and isocratic flow was used for elution. For quantification, calibration curve that was prepared with malic acid, citric acid and ascorbic acid standards at five different concentrations was used. The results were expressed as mg organic acid / g DW.

Sugar and sorbitol analyses were performed according to HPLC method of Sturm et al. (2003) with slight modifications. A refractive index (RI) detector was used for determinations of sugar and sorbitol contents. Isocratic elution was used on Rezex RCM-monosaccharide column (300 x 7.8; Phenomennex, Torrance, CA) separation at the flow rate of 0.6 mL/min, column temperature at 80°C, with mobile phase consisting of ultra-pure water (100%). Quantification was performed by using a calibration curve prepared with the five concentrations of a mixture consisting of glucose, fructose, sucrose and sorbitol and results were expressed as mg / g DW.

2.3.3. Phenolic compounds

The extraction of phenolic compounds was carried out according to the method of Kim and Lee (2002).Separation of phenolic compounds was performed according to the method of Campbell and Padilla-Zakour (2013) with minor modifications. Aliquots (20 µL) of each extract were injected into HPLC system consists of Inertsil ODS-3 column (250 nm x 4.6mm, 5µm: GL, Sciences INC., Tokyo, Japan). A linear solvent gradient was composed of a binary mobile-phase system with solvent A, 0.1% phosphoric acid in ultrapure water, and solvent B, 0.1% phosphoric acid in HPLC-grade acetonitrile at a flow rate of 1 mL/min and column temperature of 25°C. Elution program was applied for 55 min as follows: 92% A at 0 min, 89% A at 4 min, 65% A at 25 min, 40% A-60% B at 30 min, 40% A-60% B at 40 min, 65% A-35% B at 45 min, 89% A at 50 min, 92% A at 55 min. Phenolic compounds were detected by using the DAD and data collection was monitored at 280, 320 and 360 nm. The identification of phenolic compounds was done by comparing the retention time of authentic standards. The calibration curve prepared by using five different concentrations of phenolic standards mixture was used for the quantification. Results were expressed as mg phenolic compound / kg DW.

2.4.Statistical analysis

The effect of different locations and altitudes on the fruit quality parameter was analyzed by analysis of variance (ANOVA). Duncan's multiple-comparison test was used as a tool for comparisons of means at a level of $p < 0.05$ using the SPSS package program version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Total phenolic content and antioxidant capacity

The total phenolic contents of four cherry laurel samples are presented in Table 1. Total phenolic contents were varied from 16.84 to 23.32 mg GAE / 100 g DW. An increase in the total phenolic content of both locations was noted with lower altitude. The phenolic contents of the fruits grown at low and high altitudes were in the range of 18.91 (RL)-23.32 (TL) mg GAE / 100 g DW and 16.84 (RH)-21.90 (TH) mg GAE / 100 g DW respectively. Contradictory results in total phenolic contents of cherry laurel are available in the literature about the total phenolic contents of cherry laurel. Karabegović et al. (2014) found higher total phenolic content (36.2 to 46.3 mg GAE / g DW) than those reported (1.094 mg GAE / 100 g DW) by Karahalil and Şahin (2011). This may be due to geographical factors and analytical differences. The amount of phenolic substances in the fruit is governed by a few factors such as temperature, growing location, light intensity, altitude and age of tree (Mphahlele et al., 2014; Karabegović et al., 2014; Coklar, 2017). Total phenolic contents of cherry laurel samples collected from both altitudes of Trabzon province higher than those from Rize province ($p < 0.05$). This may be related to the difference in the annual rainfall amounts of two

provinces, therefore, there will be a difference in exposing time to sunlight. According to the Turkish State Meteorological Service report for 2017 year, Rize province takes approximately 1.5 times higher rainfall than that of Trabzon province. (Turkish State Meteorological Service, 2018). This indicates that Trabzon province receives more sunshine than that of Rize province. In this context, Pereira at al. (2006) reported that the sun exposed (high light intensity) berries have more phenolic content than those growing in the shade (low light intensity). Additionally, as shown in Figure 1, the color of the fruits from Trabzon province at both altitudes is darker than the fruits from Rize province. It is well known that the phenolic constituents contribute to the color in fruits (Topalovic and Mikulic-Petkovsek, 2010).

Figure 1. Cherry laurel samples from different location and altitudes; Trabzon High (TH), Trabzon Low (TL), Rize High (RH), Rize Low (RL)
Şekil 1. *Farklı bölge ve yüksekliklerde yetiştirilen karayemiş örnekler; Trabzon Yüksek (TY), Trabzon Düşük (TD), Rize Yüksek (RY), Rize Düşük (RD)*

Antioxidant activities determined by DPPH and ABTS of the samples are shown in Table 1. DPPH scavenging activities of the samples from Trabzon and Rize provinces were varied from 23.09 (TH) to 24.60 (TL) µmol trolox / 100 g DW and 19.71 (RH) to 22.12 (RL) µmol trolox / 100 g DW, respectively. There was a statistically significant difference in DPPH radical scavenging activity of the samples from the standpoint of harvesting province and altitude ($p < 0.05$). The fruits collected from Trabzon province at high and low altitudes have higher ABTS values (14.22 and 14.42 µmol trolox / 100 g DW, respectively) than those collected from Rize province (11.54-11.81 µmol trolox / 100 g DW, respectively). ABTS radical scavenging activities of Trabzon samples were higher than those of Rize province, while there was no significant change ($p > 0.05$) with the changes in altitudes. Mditshwa et al. (2013) reported that there were significant differences in DPPH antioxidant activity and total phenolic content of pomegranate fruit grown increased as altitude decreased. In accordance with our findings, Coklar (2017) found that the DPPH antioxidant activity of Eksikara grape decreased from 53.86 to 23.34 mmol TE / kg DW with altitude increased from 1000 to 1500 m. After approximate conversion of fresh weight (FW) to DW, antioxidant activity results of cherry laurel fruit are in accordance with previous studies who reported that DPPH scavenging activity of the fruit was in the range of 14.0 and 43.54 µmol trolox / 100 g FW (Celik et al., 2011; Yıldız et al., 2014; Ozturk et al., 2015). However, in a previous study, considerably higher values, which changed from 17.56 to 23.21 µmol trolox /g FW, were reported by ABTS assay (Ozturk et al., 2017).

There was a strong correlation between the total phenolic content and antioxidant capacity by DPPH ($r= 0.941$) and ABTS ($r= 0.944$), respectively. The phytochemicals like phenolic compounds, ascorbic acid, β-carotene, etc are known for their high antioxidant activity potential) (Mphahlele et al., 2014; Ozturk et al., 2017; Coklar , 2017).

3.2. HPLC analysis

3.2.1. β-carotene content

β-carotene contents of four cherry laurel samples are presented in Table 1. The β-carotene contents of the samples collected at low and high altitudes were in the range of 5.61 (RL) – 6.75 (TL) mg / kg DW and 4.16 (RH) -5.19 (TH) mg / kg DW, respectively. In accordance with the observations for the total phenolic content, cherry laurel fruit growing at low altitude conditions contain higher amounts of β-carotene than those growing at higher altitude conditions ($p < 0.05$). Similarly, cherry laurel fruit collected from Trabzon province has more β-carotene compared to those collected from Rize province. It is thought that the time of exposing sunlight and the amount of rainfall have an

impact on beta carotene content as discussed in the phenolic substance change. This result agrees with that of reported by Macar and Macar (2018) who concluded that the carotenoid content of Polygonum cognatum plant was 1.5 times higher from low altitude, compared with the higher one.

Previous studies have indicated that cherry laurel fruit is a good source of carotenoid with the total carotenoid concentration ranged from 206 to 274 mg 100 g FW (Alasalvar et al., 2005; Celik et al., 2011; Yıldız et al., 2014). In this study, only β-carotene content was determined and the total amount of carotenoid was expected to be higher. However, the differences between the findings may be explained by the environmental factors, plant varieties, age of tree, maturity level of fruit and post-harvest conditions or storage (Karabegović et al. 2014) as well as the differences in analytical techniques. It is well known that the β-carotene acts as an antioxidant by quenching free radicals and singlet oxygen. In this regard, as shown in Table 1, the higher the amount of beta carotene, the higher the DPPH antioxidant activity was found. There was a positive correlation between the DPPH scavenging activity and β-carotene (r=0. 882). A similar result was reported by Celik et al. (2011) who reported a positive correlation between the DPPH antioxidant activity and total carotenoids (r= 0.843).

Table 1. Antioxidant (DPPH and ABTS) capacity, total phenolic and β-carotene contents of cherry laurel samples grown in different locations and altitudes

Çizelge 1. *Farklı bölge ve yüksekliklerde yetiştirilen karayemiş örneklerinin, antioksidan (DPPH ve ABTS) kapasiteleri, toplam fenolik ve β-karoten içeriği*

Samples	Antioxidant Properties			
	Total Phenolic content (mg GAE/ 100 g DW)	DPPH (µmol trolox / 100 gDW)	ABTS (µmol trolox / 100 g DW)	β-carotene (mg /kg DW)
TH	21.90± 0.04c	23.09±0.08bc	14.22± 0.12b	5.19± 0.44b
TL	23.32± 0.04d	24.60±0.02c	14.42± 0.02b	6.75± 0.02c
RH	16.84± 0.40a	19.71±1.23a	11.54± 0.07a	4.16± 0.17a
RL	18.91± 0.21b	22.12±0.05b	11.81± 1.02a	5.61± 0.08b

Means with different letters in the column for each position and cherry laurel samples are significantly different ($p < 0.05$), Trabzon High (TH), Trabzon Low (TL), Rize High (RH), Rize Low (RL), Dry Weight (DW)

Table 2. Organic acids (mg g DW-1), sugar and sorbitol (mg g DW-1) contents of cherry laurel samples grown in different locations and altitudes

Çizelge 2. *Farklı bölge ve yüksekliklerde yetiştirilen karayemiş örneklerinin organic asit (mg g KM-1), şeker ve sorbitol (mg g KM-1) içeriği*

Samples	Organic Acids (mg /g DW)			Sugars and Sorbitol (mg / g DW)			
	Malic acid	Citric acid	Ascorbic acid	Glucose	Fructose	Sorbitol	Total Sugar
TH	124.72 ± 0.13c	26.93 ± 0.98c	0.46 ± 0.00c	36.87 ± 3.48a	30.54 ± 1.73a	14.26 ± 0.30b	81.68 ± 1.44a
TL	117.01 ±2.71 b	27.16 ± 1.03c	0.43 ± 0.00a	51.75 ± 0.51b	47.32 ± 0.01c	32.93 ± 0.08d	131.99 ± 0.60d
RH	119.86 ± 1.02bc	12.97 ± 0.57a	0.45 ± 0.00b	38.68 ± 0.10a	35.82 ± 0.18b	11.94 ± 0.12a	86.44 ± 0.10b
RL	89.93 ± 0.32a	20.56 ± 1.81b	0.43 ± 0.00a	38.34 ± 0.14a	38.19 ± 0.12b	23.05 ± 0.07c	99.58 ± 0.02c

Means with different letters in the column for each position and cherry laurel samples are significantly different ($p < 0.05$), Trabzon High (TH), Trabzon Low (TL), Rize High (RH), Rize Low (RL), Dry Weight (DW)

3.2.2. Organic acids and sugar

The contents of malic acid, citric acid and ascorbic acids in cherry laurel fruits grown at two different locations and altitudes are presented in Table 2. The major organic acid was malic acid (89.93-124.72 mg / g DW), followed by citric acid (12.97-27.16 mg / g DW) and ascorbic acid in small concentrations (0.43-0.46 mg / g DW) in cherry laurel fruit collected from Trabzon and Rize provinces. Malic acid content of fruit collected from Trabzon and Rize provinces decreased from 124.72 to 117.01 mg / g DW and from 119.86 to 89.93 mg / g DW, respectively, with the decrease in altitude ($p < 0.05$). The cherry laurel fruit collected from Trabzon at high altitude (TH) had the highest malic acid content (124.72 mg / g DW), whilst the lowest content was detected in that of Rize at low altitude (RL) (89.93 mg / g DW). Conversely, the amount of citric acid increased in Rize samples from 12.97 to 20.56 mg / g DW ($p < 0.05$) with a decrease in altitude. The highest citric acid content was detected in the TL sample (27.16 mg g DW-1), while the lowest citric acid content was detected in RH (12.97 mg / g DW). Amount of ascorbic acid in the sample from both location was decreased from 0.46 to 0.43 mg / g DW and 0.45 to 0.43 mg / g DW for Trabzon and Rize samples, respectively, with the decrease in altitude. In general, the organic acid concentrations are in agreement with the previous values of the cherry laurel fruit collected from Turkey (Celik et al., 2011;

Yıldız et al., 2014; Ozturk et al., 2017). In general, the amounts organic acids were higher in cherry laurel fruit collected from Trabzon province than those of collected from Rize province. The results are in agreement with the previous studies which report the malic acid and ascorbic acid contents decreased with decrease in altitude (Mphahlele et al., 2014).

Sugars have a powerful impact on fruit taste and consumer acceptance. In accordance with the previous studies (Ayaz et al., 1997; Var and Ayaz, 2004), it was found that the cherry laurel fruit contains high amounts of sugar compounds, mainly glucose, fructose and sorbitol, and the results are given in Table 2. The effect of altitude on each sugar and total sugar concentrations was found to be statistically significant ($p < 0.05$). Total sugar concentrations of the cherry laurel fruit collected from low altitudes was higher than those collected at high altitude. In accordance with this finding, the amount of sugar was substantially high at low altitude in strawberry (Crespo et al., 2010), and Polygonum cognatum (Macar and Macar, 2018). Concentrations of glucose, fructose, sucrose and sorbitol in cherry laurel fruits were reported to be in the range of 0.8 - 27.62 g 100 g FW-1, 1.3 - 27.3 g 100 g FW-1, 0-0.6 g 100 g FW-1 and 0.5-14.5 g 100 g FW-1, respectively (Ayaz et al. 1997; Ayaz et al. 1998; Var and Ayaz 2004). After approximate conversion of FW to DW, the amount of sugars for both locations and altitudes are higher than these reported concentrations, while sucrose was not detected in the current study. There is contradictory information about the sucrose content of cherry laurel fruit. The absence of sucrose may be explained by factors such as its decomposition to glucose and fructose due to invertase activity, maturation level genotype (Var and Ayaz, 2004; Crespo et al., 2010). There were statistically significant differences in the total sugar content of the samples, regardless of location and altitude ($p < 0.05$). The fruit collected from Trabzon at low altitudes (TL) has the highest total sugar content (131.99 mg / g DW). In agreement with this finding, higher total sugar content was also reported in golden berry fruit grown at low altitudes (Fisher et al., 2007).

3.2.3. Phenolic compounds

Phenolic compounds determined at various wavelengths in cherry laurel fruit are shown in Table 3. Among the fourteen phenolic compounds, Procyanidin B1, Epigallocatechin, Procyanidin B2, Epicathechin, Neochlorogenic acid, Quercetin 3-β-D-glucoside and Kaempferol-3-glucoside were identified and quantitated in cherry laurel fruit for the first time. Rutin hydrate (300.12-579.80 mg / kg DW) and chlorogenic acid (282.69-944.16 mg / kg DW) were the most abundant phenolic compounds in all cherry laurel samples regardless of their location and altitude. Also, quercetin 3-β-D-glucoside was only detected in fruit collected from Trabzon province. Chlorogenic acid was found as a major phenolic compound in cherry laurel fruit by many researchers (Alasalvar et al., 2005; Karahalil and Şahin, 2011; Ozturk at al., 2017). There is no agreement on the number and amount of phenolic compounds in the previous reports. For example, in a previous study by Ayaz et al. (2004) who reported the amount of ρ-coumaric acid and ferulic acid was reported by in the range of 0.01-7.14 mg/100 mg DW and 0.14-1.0 mg 100 mg /DW, respectively, while Karahalil and Şahin (2011) found that the average amount of these compounds were 2.55 and 0.58 mg / 100 FW. In general, the amount of phenolic compounds in this study were considerably higher than those data reported for the cherry laurel fruit.

The concentrations of phenolic compounds in fruit collected at both altitudes of Trabzon province were significantly higher than those of Rize province ($p < 0.05$). As mentioned before, compared to Rize province, Trabzon province has much exposure time to sunlight. In previous reports, good correlations were observed between the concentrations of phenolic compounds and exposing time to sunlight (Mphahlele et al., 2014). In cherry laurel fruits collected from Rize province, quercetin glycoside, which is a member of flavonol that forms on the fruit peel by the effect of sunlight (Topalovic and Mikulic-Petkovsek, 2010), was not detected in Rize samples due to low exposing time to sunlight. In accordance with the observations for the total phenolic content and β-carotene content, cherry laurel fruit growing at low altitude conditions contains higher amounts of phenolic compounds than those growing at higher altitude conditions ($p < 0.05$). In agreement with current findings, amounts of phenolic compounds increased in strawberry (Crespo et al., 2010), pomegranate fruit (Mphahlele et al., 2014) and elderberry (Senica et al., 2016), when growing altitudes decreased.

Regarding the effect of phenolic compounds on antioxidant activity determined by DPPH and ABTS assays were evaluated by taking into consideration the results from both of the location and altitude. All the phenolic compounds were well correlated with DPPH and ABTS values. Positive correlations were observed between the DPPH scavenging activity and phenolic compounds which ranged from $r= 0.636$ (neochlorogenic acid) to $r= 0.976$ (rutin hydrate). There

were also good correlations between the ABTS results and phenolic compounds which ranged from r= 0.506 (gallic acid) to r= 0.942 (epigallocatechine).

Table 3. Individual phenolic compounds (mg / kg DW) at 280 nm, 320 nm and 360 nm in cherry laurel samples grown in different locations and altitudes
Çizelge 3*. Farklı bölge ve yüksekliklerde yetiştirilen karayemiş örneklerinin 280, 320 ve 360 nm de bireysel fenolik bileşenleri (mg / kg KM)*

Wavelength	Phenolic Compounds (mg / kg DW)	TH	TL	RH	RL
280 nm	Gallic acid	83.51± 0.24b	111.43±0.40d	63.67± 1.63a	99.29± 1.70c
	Procyanidin B1	1.80± 0.33b	2.40± 0.63c	0.07± 0.00a	1.38± 0.25b
	Epigallocatechin	153.29±6.05b	217.49± 2.53c	52.04± 2.75a	51.49± 0.02a
	Catechin	19.70±0.93b	35.21± 1.38c	11.24± 1.27a	17.06± 1.11b
	Procyanidin B2	55.42± 1.02c	56.99± 0.27c	10.43± 1.62a	34.10± 1.28b
	Epicathechin	61.48± 0.22b	123.60± 0.44d	41.23± 0.72a	86.40± 0.14c
320 nm	Neochlorogenic acid	0.21± 0.04a	2.09± 0.09c	0.14± 0.02a	1.64± 0.02b
	Chlorogenic acid	447.67± 1.30b	944.16± 4.01d	282.69± 0.02a	509.70± 3.45c
	Caffeic acid	8.13± 0.21c	12.54± 0.00d	3.99± 0.01a	4.47± 0.23b
	ρ-Coumaric acid	2.76± 0.00b	5.88± 0.07d	0.46± 0.19a	4.94± 0.16c
	Ferulic acid	0.58± 0.01a	16.69± 0.46c	0.10± 0.02a	12.68± 1.05b
360 nm	Rutin hydrate	517.94± 3.52d	579.80± 0.07c	300.12± 2.86a	434.39± 0.82b
	Quercetin 3-β-D-glucoside	2.91± 0.14a	5.13±0.09b	0	0
	Keampherol 3-gliKaempferol-3-glucoside	5.06± 0.00c	5.31± 0.00d	4.86± 0.03a	4.96± 0.07b

Means with different letters in the line for each position and cherry laurel samples are significantly different ($p < 0.05$), Trabzon High (TH), Trabzon Low (TL), Rize High (RH), Rize Low (RL), Dry Weight (DW)

4. Conclusion

The present study exhibited that the location and altitude had a great effect on the compositional and antioxidant properties of cherry laurel fruit. The some bioactive components of cherry laurel fruit (malic acid from 117.01 to 124.72 mg/g DW, ascorbic acid from 0.43 to 0.46 mg/g DW) increased with increasing altitude while some bioactive contents of fruit (Total phenolic content from 23.32 to 21.90 mg GAE/100g DW, antioxidant activity from 24.60 to 23.09 µmol trolox / 100 g DW, β-carotene from 6.75 to 5.19 mg/kg DW) decreased with increasing altitude . The fruit from Trabzon province had superior features in terms of some quality properties (total phenolic content, organic acid, sugar content and all the individual phenolic compounds examined) and antioxidant characteristics. This may be due to differences in exposing time of sunlight and the amount of rainfall. These results are considered to be of practical importance in the nutritive value and bioactive properties of cherry laurel fruit.

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