Anatolian Journal of Botany

# Anatolian Journal of Botany

**e-ISSN 2602-2818**
**Volume 7, Issue 2, Year 2023**

**Published Biannually**

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Anatolian Journal of Botany

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*Anatolian Journal of Botany*
*7(2): 101-107 (2023)*
*Research article* *doi:10.30616/ajb.1259664*

# Investigation of some quality-control parameters in Edremit yağlık olive oil

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Received : 03.03.2023
Accepted : 16.05.2023
Online : 04.07.2023

# Edremit yağlık zeytini yağında bazı kalite-kontrol parametrelerinin incelenmesi

**Abstract:** Throughout history, people have searched for the secret of long life and these researchers continue at full speed in parallel with the developments in science and technology in recent years. It is inevitable that the secret of a long and healthy life depends on nutrition and that nutrition should be with natural and high-quality nutrients. The Mediterranean diet, which is based on olive oil, has emerged as a popular diet in recent years. Due to the positive effects of olive oil on health, it is also economically valuable. For olive oil users to reach pure and high-quality olive oil, olive oils should be evaluated within the scope of some quality control parameters with the help of various analyzes. In this study, the quality parameters of free fatty acidity, peroxide value, and specific absorption($K_{232}$, $K_{270}$, and $\Delta K$) values in ultraviolet light were determined in olive oils obtained from Edremit Yağlık olives. The compliance of the values found with the Turkish Food Codex Communiqué on Olive Oil and Edible Pomace Oil (Communiqué No: 2017/26) has been evaluated. As a result of the analyzes made, the amount of free fatty acid was determined as 0.11% in %oleic acid, the average peroxide number was 0.50 meq $O_2$/kg oil, the mean$K_{232}$ values were 0.4501, the $K_{270}$values were on average 0.0446, and the mean $\Delta K$ values were -0.0248. It has been determined that all studied samples comply with the criteria for extra virgin olive oil specified in the Turkish Food Codex 2017/26 Communiqué.

**Key words:** Free fatty acidity, olive oil, peroxide number, specific absorption

**Özet:** İnsanlar tarih boyunca uzun yaşamın sırrını araştırmışlar ve bu araştırmalar son yıllarda bilim ve teknolojideki gelişmelere paralel olarak tüm hızıyla devam etmektedir. Uzun ve sağlıklı yaşamın sırrının beslenmeye bağlı olduğu, beslenmenin de doğal ve kaliteli besin maddeleri ile olması gerektiği kaçınılmaz bir gerçektir. Temelinde zeytinyağı olan Akdeniz tipi beslenme, son yıllarda popüler bir beslenme tarzı olarak karşımıza çıkmaktadır. Zeytinyağının sağlık üzerine olumlu etkileri sebebiyle ekonomik olarak da değerlidir. Zeytinyağı kullanıcılarının saf ve kaliteli zeytinyağına ulaşabilmesi için, zeytinyağlarının çeşitli tahliller yardımıyla bazı kalite kontrol parametreleri kapsamında değerlendirilmesi gerekmektedir. Bu çalışmada, Edremit Yağlık türü zeytinlerden elde edilen zeytinyağlarında kalite parametrelerinden serbest yağ asitliği, peroksit değeri ve ultraviyole ışığında özgül soğurma ($K_{232}$, $K_{270}$, and $\Delta K$) değerleri tespit edilmiştir. Bulunan değerlerin, Türk Gıda Kodeksi Zeytinyağı ve Yemeklik Pirina Yağı Tebliği'ne (Tebliğ No:2017/26) uygunluğu değerlendirilmiştir. Yapılan analizler sonucunda, serbest yağ asitliği miktarı %oleik asit cinsinden ortalama %0.11, peroksit sayısı ortalama 0.50 meq $O_2$/kg yağ, $K_{232}$ değerleri ortalama 0.4501, $K_{270}$ değerleri ortalama 0.0446 olarak, $\Delta K$ değerleri ortalama -0.0248 olarak belirlenmiştir. Çalışılan tüm örneklerin, Türk Gıda Kodeksi 2017/26 Tebliği'nde belirtilen naturel sızma zeytinyağı kriterlerine uygun olduğu belirlenmiştir.

**Anahtar Kelimeler:** Serbest yağ asitliği, zeytinyağı, peroksit sayısı, özgül soğurma

**Citation:** Kılıçel F, Kılınç S, Karapınar HS (2023). Investigation of some quality-control parameters in Edremit yağlık olive oil. Anatolian Journal of Botany 7(2): 101-107.

## 1. Introduction

The olive known since prehistoric times belongs to the *Oleaceae* (Olive Fruits) family. This family, which spreads in tropical and temperate regions, is represented by 25 genera and about 600 species in the world. The species belonging to the genus *Olea* are plants where a large part of them are trees and shrubs, and they are spread in areas with relatively difficult growing conditions. This genus includes 33 species in the world. The only species with edible fruit is *Olea europea* L., in which the cultivated olive is included. In our country, *O. europaea* species is represented by two varieties. These are *O. europaea* var. *europaea* Zhukovsky and *O. europaea* var. *sylvestris* (Miller) Lehr. The olive, which has two varieties (Delice, male olive, and wild olive), was spread in Northern, Western, and Southern Anatolia (Tokuşoğlu, 2010).

According to the data for 2021 in our country, the number of fruiting olive trees is 157.850, and from these trees, 555.833 tons of table, 1.182.147 tons of oil, a total of 1.738.680 tons, and in 2022, 2.976.000 tons of olives were produced (TUIK, 2023).

Olive oil is a natural oil with a unique taste and smell, which can vary from clear green to yellow, obtained only from the fruits of the olive tree (*Olea europaea*) by applying mechanical or physical processes (Aşık Uğurlu and Özkan, 2011). The physical processes carried out while obtaining olive oil do not bring about any change in the quality of the olive oil. Olive oil is distinguished from other seed oils by its natural state, that is, by consuming it without refining (Boskou et al., 2006).

Approximately 98% of olive oil consists of the major components triglycerides, fatty acids, and phosphatides,

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while the remaining part consists of the minor components sterols, phenolic substances, free fatty acids, hydrocarbons, aliphatic and triterpene alcohols, and volatile components (Aydın et al., 2020). Especially tocopherol and phenolic substances are among the components that are studied intensively. These components make a significant contribution to the time that fats are preserved from spoilage, especially because they have an antioxidant effect. In addition, these components have many important effects in terms of their positive contributions to human health (Covas et al., 2006; Owen et al., 2000). Apart from health, the unique aroma of olive oil is formed by minor components. It consists of aroma, taste, and smell. Taste is often associated with phenolic compounds (Visioli and Galli, 1998) and smell is associated with volatile components (Kalua et al., 2007).

The minor components of olive oil vary depending on the olive variety, climate, level of ripening, and processing conditions (Gimeno et al., 2002)

Although the consumption intensity of olive oil has been concentrated in the regions where it is produced more in the past years, it has started to be consumed in the regions where it is not produced because it is known to benefit human health today. Olive oil production is mostly concentrated in the Marmara, Aegean, Mediterranean, and Southeastern Anatolia regions (Unakıtan et al., 2012).

People's growing desire to live healthy and long has also increased their interest in olive oil. The most important feature that distinguishes olive oil from many oils is that it is a type of oil that can be used by consumers without side effects. The fact that olive oil is not subjected to heat treatment and is obtained by passing it through cold pressing is an important feature that increases its benefit to human health (Karabulut, 2013;Küçükkömürler and Uluksar, 2018). Olive oil is an important source of healthy living. It has a unique smell, aroma, and antioxidant properties. It also contains many vitamins, flavonoids, and sterols. Today, olive oil is mostly used in kitchens for cooking, salads, health care, and body care (Ünsal, 2008; Özata and Cömert, 2016; Küçükkömürler and Uluksar, 2018).

The oleic acid, hydroxytyrosol and caffeic acid found in olive oil have shown great protection factors against cancer. Olive oil consumption has benefits for the large intestine and also shows active properties in the prevention of breast cancer (Waterman and Lockwood, 2007; Kiritsakis and Shahidi, 2017).

A diet rich in extra virgin olive oil reduces the risk of cardiovascular disease (Armutcu et al., 2013). Mediterranean-type nutrition improves major risk factors for cardiovascular diseases such as lipoprotein profile, blood pressure, glucose metabolism, and antithrombotic profile (Kiritsakis and Shahidi, 2017).

In addition to being rich in monounsaturated fatty acids, olive oil contains small ingredients with antioxidant properties (Fitó et al., 2007). This is because olive trees in the Mediterranean basin have developed several antioxidant defense mechanisms to protect themselves from environmental stress (Visioli and Galli, 1998; Aparicio and García-González, 2013).

Oxidative stress is defined as an imbalance between the body's oxidant and antioxidant systems in favor of oxidants. The oxidative stress produced by free radicals has been linked to the development of various diseases, such as cancer and neurodegenerative diseases (Fitó et al., 2007). Protocatekuic and syringic acid have also been found to have antioxidant activity. Tyrosol, p-hydroxyphenylacetic acid, o-coumaric acid, p-coumaric acid, p-hydroxybenzoic acid, and vanillic acid were found to have little or no effect. Their contribution to the stability of the oil is minimal (Aparicio and García-González, 2013; Papadopoulos and Boskou, 1991).

In a study conducted to evaluate the possible differences between the antihypertensive effects of monounsaturated (MUFA) (extra virgin olive oil) and polyunsaturated fatty acids (PUFA) (sunflower oil), it was found that 8% of those who received the MUFA diet did not need drug treatment. A slight reduction in saturated fat intake combined with the use of extra virgin olive oil significantly reduces the need for a daily dose of antihypertensive drugs (Ferrara et al., 2000).

Freshly squeezed extra virgin olive oil contains oleocanthal, a compound whose pungency evokes a strong stinging sensation in the throat. This substance is one of the solutions of the anti-inflammatory drug ibuprofen. Here, the fact that oleocanthal acts as a natural anti-inflammatory compound with a profile strikingly similar to that of ibuprofen is indicative of pharmacological activity (Beauchamp et al., 2005). In this regard, it is considered anti-inflammatory and inhibitory (Kiritsakis and Shahidi, 2017).

Aging and related atherosclerosis, Morbus Parkinson's, Alzheimer's disease, and cognitive decline are major health problems in developed societies. Monounsaturated fatty acids are recognized to play a protective role against age-related cognitive decline and Alzheimer's disease (Kiritsakisand Dugan, 1985; Berr et al., 2009; Aparicio and García-González, 2013).

In this study, some quality parameters (free fatty acidity, peroxide value, and specific absorption in ultraviolet light) were analyzed in the samples of olive oils obtained from Edremit Yağlık type olives and their compliance with the Turkish Food Codex (TFC) Communiqué on Olive Oil and Edible Pomace Oil (Communiqué No: 2017/26) was evaluated.

## 2. Materials and Method

### 2.1. Material

The oils obtained from the Edremit Yağlık type olives used in the analyzes were obtained from local olive oil producers engaged in agricultural activities in the borders of Çanakkale Province-Bayramiç and Yenice District, Balıkesir Province-Edremit District during the 2020 harvest period. During the study, olive oil samples were stored in brown glass bottles andin an unlit environment.

### 2.2. Method

#### 2.2.1.Free fatty acidity determination

The free fatty acid (%FFA) content of the extra virgin olive oil sample was determined from %oleic acid using the standard AOCS Ca-5a-40 method. The determination of FFA % in oils was made by titration under non-water

conditions. 1 g of extra virgin olive oil sample was carefully weighed and shaken with the addition of 15 ml of ethylalcohol. At the end of the addition of 2-3 drops of phenolphthalein indicator, titration was performed with 0.01N NaOH solution until the onset of pink color. The consumed NaOH has been identified. Equation 1 determined the FFA results.

%FFA =( VxNxM)/(Gx10) (1)

V: Amount of NaOH spent in titration

N: NaOH normality spent in titration (0.01N)

M: Fatty acids molecular mass (282g)

G: Sample mass

**2.2.2. Determination of peroxide number**

The peroxide number (PV) of a sample of extra virgin olive oil is a measure of the amount of meq $O_2$ in kg of oil or an expression of the hydroperoxide content in the oil. Analyses were performed using the standard AOCS Cd-8b-90 method. 1 g of the olive oil sample was taken and mixed by adding 1 ml of chloroform and 1.5 ml of acetic acid. 0.1 ml of KI was dripped onto it and kept in the dark for 180 seconds. Then 25 ml of pure water and 3 drops of 1% starch solution were added to it. It is the detection of turbidity and gray color peroxide as a result of the control. Subsequently, titration was performed with 0.002 N regulated $Na_2S_2O_3$until the color of the solution was clear. The value of $Na_2S_2O_3$ spent has been determined. According to Equation 2, the peroxide number is calculated and expressed as milli equivalent gram oxygen/kg fat.

PV = (NxVx100)/G (2)

G: Sample weight

V: Amount of $Na_2S_2O_3$ consumed in titration (ml)

N: Normal of $Na_2S_2O_3$ used in titration

**2.2.3. Determination of specific absorption**

The method recommended by the standard AOCSCh 5-91 was used to determine the specific absorption values. The samples were thoroughly homogenized and filtered through filter paper before being analyzed. A 0.25 g sample was weighed in a 25 ml balloon. The balloon containing the sample was completed with hexane to 25 ml and homogenized by mixing thoroughly. The absorbance values of the samples at different wavelengths (232, 266, 270, and 274 nm) were read out in the spectrophotometer. $K_{232}$, $K_{266}$, $K_{270}$, and $K_{274}$ are calculated according to Equation 3.

$K\lambda = A\lambda/C\times$ (3)

$K_{232}$: $A_{232}/C\times L$

$K_{266}$: $A_{266}/C\times L$

$K_{270}$: $A_{270}/C\times L$

$K_{274}$: $A_{274}/C\times L$

The value of ΔK is calculated by Equation 4.

$\Delta K = K_{270}–[(K_{266}+K_{274})/2]$ (4)

$K_{232}$: Specific absorption value at 232 nm

$K_{266}$: Specific absorption value at 266 nm

$K_{270}$: Specific absorption value at 270 nm

$K_{274}$: Specific absorption value at 274 nm

$A_{232}$: Absorbance value read at 232 nm

$A_{266}$: Absorbance value read at 266 nm

$A_{270}$: Absorbance value read at 270 nm

$A_{274}$: Absorbance value read at 274 nm

C: Concentration of solution (g/100 ml)

L: Bathtub thickness (cm)

**3. Results**

**3.1. Free fatty acidity determination results**

As a result of the analysis, the amount of free fatty acidity in Edremit Yağlık type olive oil varies between 0.05% and 0.25% in terms of % oleic acid and the average 0.11% ± 0.05. The results meet the requirement of ≤0.8, which is the criterion of extra virgin olive oil determined according to the Turkish Food Codex Communiqué on Olive Oil and Pomace Oil (Communiqué No: 2017/26). It is seen that the determined results are in line with the literature. The determined analysis results are shown in Figure 1. As can be seen from Figure 1, in only two of the largest samples, our value is 0.25%, which is well below the limit value of 0.8%. According to these results, it was determined that the olive oils analyzed were of high quality in terms of free fatty acidity.

In a study conducted to determine the chemical, physical and sensory properties of olive oils produced in Çanakkale, it was reported that while the free fatty acid ratios were below the limit value specified in the TFC olive oil communiqué in all other samples except two samples, the peroxide values were generally by the standards. At the same time, it was determined that there were significant differences in terms of sensory parameters between olive oils (Öğütçü et al., 2008).

In another study conducted to reveal some characteristics of olive oils obtained from Chemlali and Chetoui variety olives taken from the northern and southern regions of Tunisia, it was determined that the free fatty acid values of Chemlali and Chetoui olive oils obtained from Southern Tunisia were between 1% and 0.9%, the free fatty acid values of olive oils obtained from Northern Tunisia were 0.1% (Chemlali) and 0.3% (Chetoui), and peroxide values were between 3.2-8.3 meq $O_2$/kg (Issaoui et al., 2010).

Various quality parameters have been examined in Queslati variety olive oils obtained from seven different regions of Tunisia. Accordingly, free fatty acid, peroxide values, UV specific absorbance values were found to be by the extra virgin olive oil standard, while palmitic acid (12.4-15.2%), oleic acid (68.1-74.7%), stearic acid (1.6-2.1%) and linoleic acid (6.7-14.9%) were the major fatty acids determined (Youssef et al., 2011).

In a study conducted to examine some properties of olive oils obtained from 11 varieties of olives belonging to the Marche region of Italy, it was revealed that 12 different free fatty acid values (0.1-0.2%) and peroxide values (3.9-8.2 meq $O_2$/kg) belonging to olive varieties were determined, at the same time eicosenoic, heptadanoic, eikosanoic, linoleic, linolenic, oleic, palmitic, stearic acid values were found and oleic acid values were highest in them (71.7-79.8%) (Cecchi and Alfei, 2013).

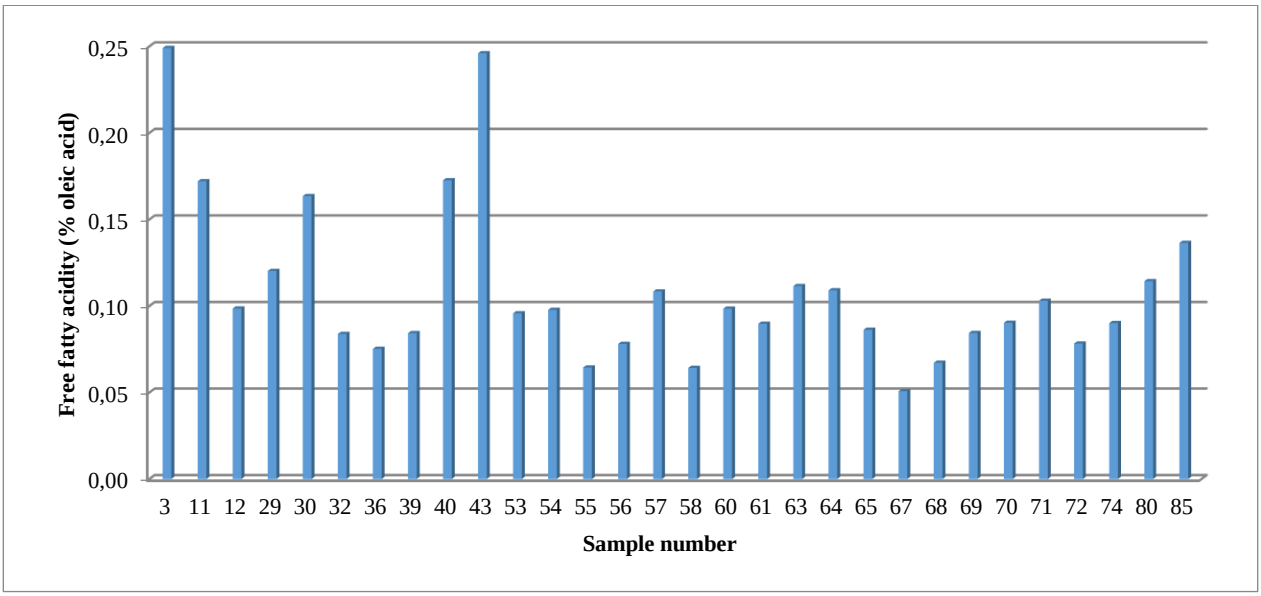


**Figure 1.** Free amounts of fatty acidity (% oleic acid)

The free fatty acidity in extra virgin olive oils of Sarıulak type olives grown in Antalya, Mersin, and Karaman was determined between 0.50% and 0.83% and peroxide values were measured in the range of 3.99-4.33 meq $O_2$/kg (Arslan et al., 2013).

### 3.2. Peroxide count determination results

As a result of the analyzes, the number of peroxides in Edremit Yağlık type olive oil ranged between 0.22 meq $O_2$/kg oil and 1.23 meq $O_2$/kg oil and was measured as 0.50 ± 0.241 meq $O_2$/kg oil. All the determined values met the requirement of ≤20, which is the criterion of extra virgin olive oil according to the Turkish Food Codex Communiqué on Olive Oil and Pomace Oil (Communiqué No: 2017/26). All samples were determined to be consistent with the literature. The results of the analysis are shown in Figure 2. In Figure 2, it is seen that the peroxide values of our samples are very, very small than the limit value of twenty. Therefore, it was determined that the samples analyzed were quality olive oils.

In a study conducted by taking samples (n=30) from 10 different olive oil varieties offered for sale in and around Nizip, some of their physical and chemical properties were examined. In the evaluations, it was determined that the acidity and peroxide values of 40% of the samples were above the values determined in the Food Codex (Türkoğlu et al., 2012).

In another study in which the peroxide values of different kinds of olive oils for the 2017 and 2018 harvest years were measured, the 2017 peroxide values of the oils of Ayvalık, Çöpaşı, Gemlik, and Yağlık varieties of olives were found between 2.00-5.00 meq $O_2$/kg, 1.99-7.43 meq $O_2$/kg, 1.49-6.47 meq $O_2$/kg and 1.99-9.90 meq $O_2$/kg, respectively, while the peroxide values of 2018 were quite low compared to the previous year and 0.88-1.63 meq $O_2$/kg respectively, It was detected between 1.00-4.00 meq $O_2$/kg, 1.00-2.75 meq $O_2$/kg and 1.00-2.50 meq $O_2$/kg (Özcan et al., 2019).

Free acid contents of the fat samples Verticillium wilt did not show a linear relationship with disease severity. The peroxide value, which gives information about the primary oxidation products in vegetable oils, was found to vary between 8.07-14.20 meq $O_2$/kg oil in oil samples obtained from diseased trees. It has not exceeded the limit of 20 meq $O_2$/kg for natural olive oil in the Turkish Food Codex Communique on Olive Oil. Peroxide values showed a direct proportion to disease severity (Yorulmaz et al., 2017).

In a study in which the aroma substances of the olive oils obtained from Gemlik and Barnea olive varieties grown in Adana province were determined, the peroxide value in olive oils was determined as 10.37 meq $O_2$/kg oil in Gemlik variety oil and 8.60 meq $O_2$/kg oil in Barnea variety oil (Kesen et al., 2014).

The peroxide value of olive oil obtained from the Gemlik variety grown in the Erzin district of Hatay province was reported as 8.85 meq $O_2$/kg (Kelebek et al., 2012).

### 3.3. Specific absorption determination results

In Edremit Yağlık type olive oils, $K_{232}$ values were determined as 0.2401-0.5922 with an average of 0.4501±0.094, and $K_{270}$ values between 0.0305-0.0628 with an average of 0.0446±0.008. ΔK values were found in the range of -0.2640-0.0037, with an average of an average of -0.0248±0.0780. According to the Turkish Food Codex Communiqué on Olive Oil and Pomace Oil (Communiqué No: 2017/26), the K value at 232 nm in extra virgin olive oil is ≤2.5; the value of K at 270 nm is ≤0.22; ΔK value should be ≤0.01. As a result of all the analyzes, it was determined that the values determined were smaller than the TFC values and were compatible with the literature. The results of the determination of specific absorption are shown in Figure 3. It was found that the $K_{232}$ values of the analyzed samples were well below 2.5, the $K_{270}$ values were well below 0.22 and the ΔK values were well below 0.01. It was determined that the samples with the analyzed olive oil consisted of quality olive oils.

In a study conducted to determine the maturity index values of the fruits of the Ayvalık olive variety harvested at 3 different maturities in 9 different regions of Çanakkale

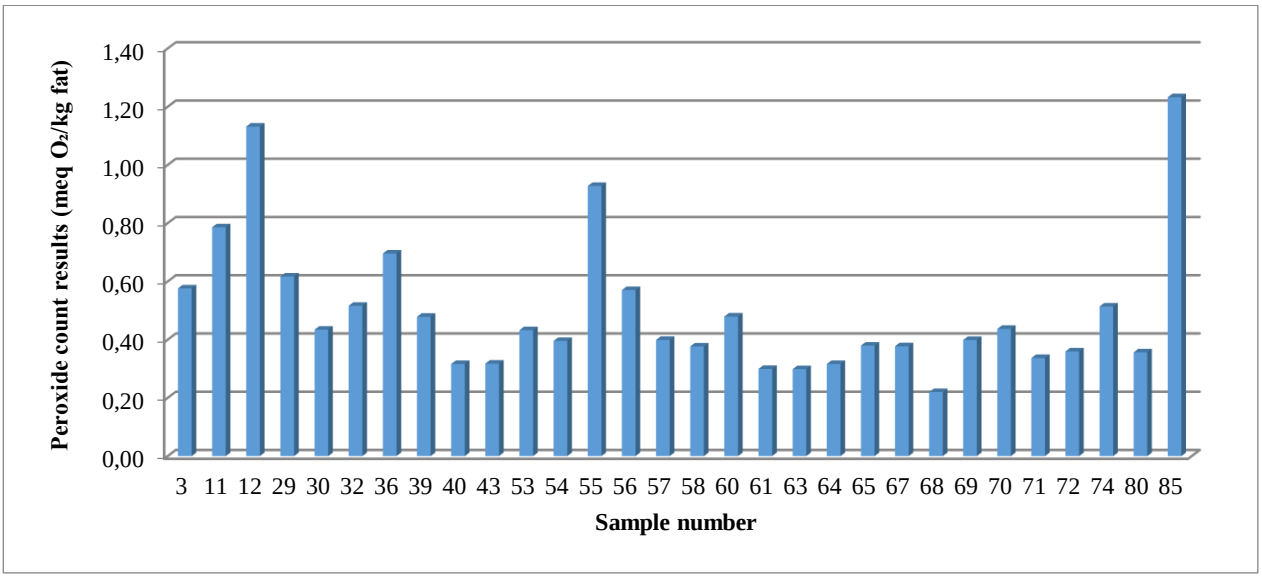


**Figure 2.** Peroxide count results (meq $O_2$/kg fat)

province Bayramiç and Edremit olive cultivation areas and the chemical properties of olive oils obtained, the $K_{232}$ value for Edremit was 1.516±0.181; $K_{270}$ value is 0.091±0.002, $K_{232}$ value for Bayramiç is 1.523±0.041; $K_{270}$ value was 0.092±0.004 (Gündoğdu and Nergis, 2020).

In another study on the quality parameters of natural olive oils produced in the Hatay region, the $K_{232}$ value was found to be the lowest at 1.67, the highest at 2.51, and the average at 2.02±0.17, the $K_{270}$ value as the lowest 0.11, the highest 0.25 and the average as 0.16±0.03, the ΔK value as the lowest 0.00, the highest 0.01 and the average 0.004±0.002 (Güler et al., 2006).

In a study conducted to determine some characteristics of olive oils obtained from G20/1 and G20/7 Clones and Gemlik variety olives, the specific absorption values in ultraviolet light were 2.57±0.08 for the G20/1 clone, the $K_{270}$ value was 0.20±0.04, the $K_{232}$ value for the G20/7 clone was 2.32±0.04, the $K_{270}$ value was 0.37±0.09 and the $K_{232}$ value for Gemlik type was 2.66±0.09,the $K_{270}$value was determined as 0.30±0.03 (Özdemir et al., 2016).

In another study conducted to determine the effect of the harvest period on the quality parameters of Beylik olives and olive oil, the $K_{232}$ value was determined as 0.67±0.03 in October, the $K_{270}$ value as 0.29±0.001, the $K_{232}$ value as 0.83±0.01 in November, the $K_{270}$ value as 0.23±0.01 and the $K_{232}$ value as 1.11±0.04 in December, the $K_{270}$ value as 0.27±0.01 (Özen and Keçeli, 2019).

In a study where the optimum harvest time of the Cornicabra olive variety was determined according to oil quality parameters at 4 different harvest times; It was found that UV-specific absorbance values and sensory quality decreased at 232 and 270 nm with peroxide as maturation increased, while free acidity amounts increased. It has been reported that the optimal maturity index for the Cornicabra olive variety should be higher than 3 and lower than 4-4.5 (Salvador et al., 2001).

In a study in which the effect of harvest time on some physical properties and pigment amounts of extra virgin olive oils obtained from Ayvalık, Domat, and Gemlik olive varieties was determined, 4 different harvest periods

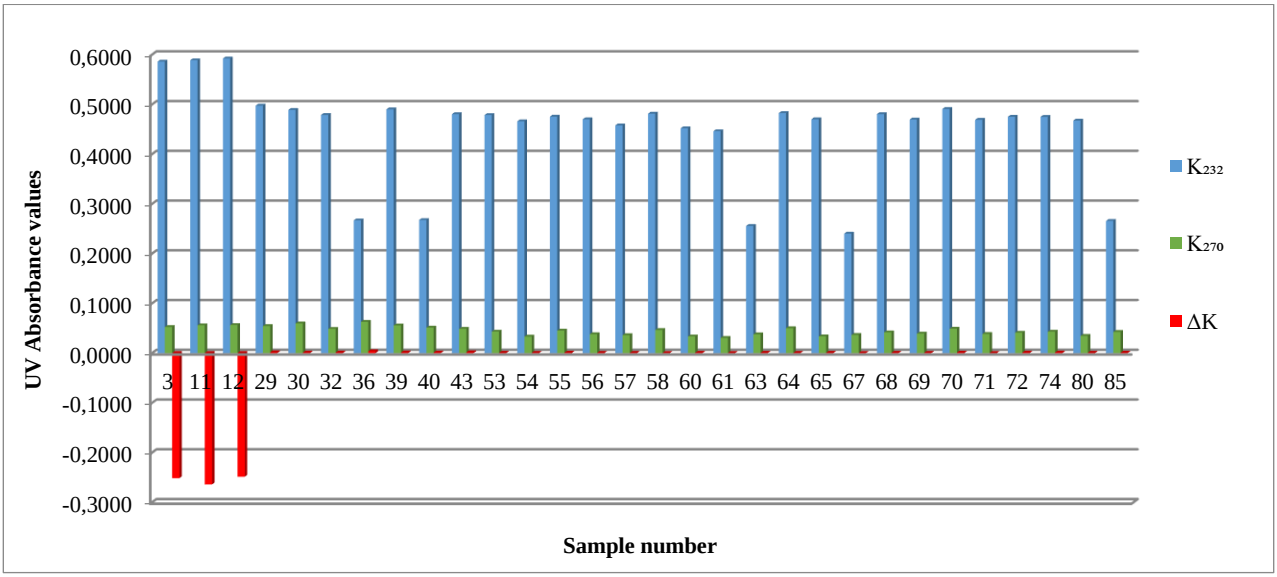


**Figure 3.** Specific absorption determination results (UV: Absorbance values $K_{232}$, $K_{270}$, ΔK)

including the first week of September, October, November, and December were selected. In extra virgin olive oils, specific absorbance ($K_{232}$, $K_{270}$) values were determined in UV light. Generally, as olive ripening increases in all varieties, a decrease in $K_{232}$ and $K_{270}$ values has been reported (Özkan et al., 2008).

Mean, standard deviations (SD), maximum and minimum values and range (i.e. maximum value – minimum value) obtained from five parameters (i.e. acidity, peroxide, $K_{232}$, $K_{270}$and ΔK) are shown in Table 1.

## 4. Discussions

In this study, it was investigated whether the olive oils obtained from Edremit Yağlık type olives harvested in 2020 complied with some quality parameters specified in the Turkish Food Codex Communiqué on Olive Oil and Pomace Oil, and the free fatty acidity, peroxide number, and specific absorption values were examined. As a result, it was determined that all of the oil samples examined remained within the natural extra virgin olive oil standard values specified in the TFC communiqué.

## Conflict of Interest

Authors have declared no conflict of interest.

## Authors' Contributions

Fevzi Kılıçel: Project administration - review and editing - original draft - data curation. Süleyman Kılınç: Data curation - writing - editing - original draft. Hacer Sibel Karapınar: Writing - review and editing - original draft - data curation.

## Acknowledgements

The authors are grateful for he financial support of the Scientific Research Project of Karamanoglu Mehmetbey University, Karaman, Turkey (Project Number 04-D-20).

**Table 1.** Data on the determination of free fatty acidity, peroxide number, and specific absorption number of Edremit Yağlık type olive oils (30 pieces)

| Saple | | Free fatty acidity (% oleic acid) | Peroxide value (meq O2/kg) | $K_{232}$ (UV) | $K_{270}$ (UV) | ΔK |
|---|---|---|---|---|---|---|
| Edremit yağlık type olive oil | Mean | 0.11 | 0.50 | 0.4501 | 0.0446 | -0.0248 |
| | St. Deviation | 0.05 | 0.24 | 0.0942 | 0.0088 | 0.0780 |
| | Minimum | 0.05 | 0.22 | 0.2401 | 0.0305 | -0.2640 |
| | Maximum | 0.25 | 1.23 | 0.5922 | 0.0628 | 0.0037 |
| | Range | 0.20 | 1.01 | 0.3521 | 0.0323 | 0.2677 |
| Turkish Food Codex Communique on Olive Oil and Pomace Oil (Communiqué No: 2017/26) | | ≤0.8 | ≤20 | ≤2.5 | ≤0.22 | ≤0.01 |

UV: Absorbance values

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*Anatolian Journal of Botany*
*7(2): 108-111 (2023)*
*Research article* *doi:10.30616/ajb.1270434*

# Titanium dioxide nano particles improving impact on sunflower seedling's emergence performance

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Received : 24.03.2023
Accepted : 19.05.2023
Online : 04.07.2023

# Titanium dioksit nano partiküllerinin ayçiçeği fide çıkışı üzerine olumlu etkileri

**Abstract:** Seed germination and seedling emergence is the main step of cultivation and improving them could yield high performance in the field. Improved seedling emergence means less sensitivity to biotic and abiotic stress factors. It is possible to enhance seedling emergence via different technologies. Nanoparticles are one of the improving technology and their impact on crop cultivation are improving day by day. The seeds of hybrid-snack type cultivar Ahmetbey and for seed treatment agent $TiO_2$ nanoparticles were used in this experiment. This study was conducted to observe the impact of seed treatment with different titanium dioxide ($TiO_2$) nanoparticles (NPs) concentrations (6, 12, and 24 mg $L^{-1}$) with dimensions of 20-50 nm during 8 hours on the emergence and seedling growth performance of snack-type sunflower cultivar Ahmetbey. Four replicates of 50 seeds in each treatment were sown in plastic trays 4 cm deep and placed in a growth chamber at 20 ± 2 °C 45 µM photons $m^{-2}$ $s^{-1}$ light for 16 h. Mean emergence time (MET), emergence percentage, seedling vigor, root-to-shoot length ratio, shoot length, and root length seedling fresh and dry weight were measured. Emergence percentage, shoot length, root length, and fresh and dry weight of seedlings increased with $TiO_2$ NPs treatments. The results revealed that 8-hour priming with water has a low impact on seeds of cv. Ahmetbey compared to any treatment of $TiO_2$ NPs. In conclusion, it is proved that the improving effects of 8 hour priming of sunflower seeds with $TiO_2$ NPs solutions on sunflower seedling emergence.

**Key words:** $TiO_2$, seed priming, sunflower, hydropriming

**Özet:** Tohum çimlenmesi ve tarla çıkışı, ekimin ana adımıdır ve bunları geliştirmek tarla veriminde yüksek performans sağlayabilir. Geliştirilmiş fide tarla çıkışı, biyotik ve abiyotik stres faktörlerine daha az duyarlılık anlamına gelir. Farklı teknolojiler yoluyla fide çıkışını arttırmak mümkündür. Nanopartiküller gelişen teknolojilerden biridir ve bunların bitki yetiştiriciliği üzerindeki etkileri her geçen gün artmaktadır. Bu deneyde hibrid-çerezlik ayçiçeği çeşidi Ahmetbey ve tohuma ön uygulama materyali olarak titanyum dioksit ($TiO_2$) nanoparçacıkları kullanıldı. Bu çalışmada, ayçiçeği fide çıkışı ve fide büyüme performansını gözlemleyebilmek amacıyla, 8 saat boyunca 20-50 nm boyutlarında farklı titanyum dioksit ($TiO_2$) nanoparçacıkları (NPS) konsantrasyonlarında (6, 12 ve 24 mg $L^{-1}$) bekletilen tohumlar kullanılmıştır. Tohumlar her tekerrürde 50 tohum olacak şekilde (50 × 4 = 200) plastik çıkış kaplarına 4 cm derinliğinde ekilmiştir. Çıkış performansını ölçebilmek amacıyla 20 ± 2 ° C 45 µm foton $M^{-1}$ ışıkta 16 saat boyunca bir büyüme odasına yerleştirildi. Ortalama çıkış süresi (MET), çıkış yüzdesi, fide canlılığı, kök-fide boyu uzunluğu oranı, fide uzunluğu ve kök uzunluğu fide yaş ve kuru ağırlıkları ölçüldü. $TiO_2$ NP'leri tohum uygulamaları ile çıkış yüzdesi, sürgün uzunluğu, kök uzunluğu ve yaş ve kuru ağırlıkta artış gözlenmiştir. Sonuçlar, tohumların 8 saat suda bekletilmesi uygulamasının $TiO_2$ NP'leri ile yapılan uygulamalara kıyasla Ahmetbey ayçiçeği çeşidi tohumları üzerinde düşük bir etkiye sahip olduğunu ortaya koymuştur. Sonuçlar ayçiçeği tohumlarının $TiO_2$ nanopartikülleri ile 8 saat ıslatılmalarının ayçiçeği fide çıkışı için olumlu etkileri olduğunu kanıtlamıştır.

**Anahtar Kelimeler:** $TiO_2$, tohum uygulaması, ayçiçeği, hydropriming

**Citation:** Day S, Özgen Y (2023). Titanium dioxide nano particles improving impact on sunflower seedling's emergence performance. Anatolian Journal of Botany 7(2): 108-111.

## 1. Introduction

Sunflower cultivated in Türkiye is mainly oil seed type (Kaya et al., 2013) grown on around 899.254 ha (TUIK, 2022). Snack type sunflower is used in making bread, chocolate production and are consumed as a snack (Day et al., 2008). Its production is increasing with present production of around 80.435 ha (TUIK, 2022). However snack type sunflower has less yield (2490 kg $ha^{-1}$) compared to oil seed types (2610 kg $ha^{-1}$). The low yield in snack type sunflower mostly depends on farmers' habit of cultivating the seeds they obtained the year before. The seeds, in this case, have low homogeneity and their hull percentage is increased which retard the germination and seedling could stand in the field.

Seed priming could be used to increase the efficiency of seed germination and seedling fidelity under optimal and unfavourable conditions (Devika et al., 2021; Day, 2022). New approaches are developing with the increasing nanoparticle industry (Acharya et al., 2017). Several nanoparticles (NPs) such as $Al_2O_3$ NPs (Aluminium oxide), Ag NPs (Silver), $TiO_2$ NPs (Titanium dioxide), $CeO_2$ NPs (Cerium oxide), FeO NPs (Iron oxide), ZnO NPs (Zinc oxide), silicon NPs, and carbon nanotubes are used in seed germination and growth of several plant species and varieties (Haghighi et al., 2014; Prasad et al., 2017). Nanoparticles have toxic or supporting effects on plant growth depending on species and the form and concentration of nanoparticles used.

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Nanoparticles have unique physio-chemical properties, high stability, anticorrosion, and photocatalyst activity which suggests their application in many areas like cosmetics, cleaning products, transportation, energy, and agriculture (Haghighi and Teixeira da Silva, 2014). In agriculture, its usage in plant development is increasing. Particularly $TiO_2$ NPs usage is increasing due to its semiconductor properties, high visible spectrum transmittance, chemical stability, and high antimicrobial activity.

$TiO_2$ NPs can change the hormonal levels of plants during growth. Increased zeatin riboside and brassinolide in tobacco were observed after foliar application of $TiO_2$ nanoparticles (Hao et al., 2018). $TiO_2$ NPs supportive impact on plant growth, microorganism activity, and nutrient uptake was observed in barley (Marchiol et al., 2016), and wheat (Faraji and Sepehri, 2019; Zahra et al., 2019).

Seed germination stage is an important stage in sunflower cultivation. It sustain rapid germination and healthy seedlings' emergence by playing an important part in obtaining high yield for sunflower. Pre-sowing seed treatments hasten field emergence for many oil crops including canola (Day, 2022), sunflower (Bourioug et al., 2020) and soybean (Shrestha et al., 2019). $TiO_2$ biostimulation impacts has been reported successfully in many crops.

Although a limited number of studies have been carried out on the use of different nanoparticles on sunflower cultivars, to the best of our knowledge no study has been carried out to find the impact of $TiO_2$ NPs on snack-type sunflower seed germination parameters. Therefore the study aimed to compare seed germination and emergence behavior of snack-type sunflowers using hydro priming and different concentrations of $TiO_2$ NPs.

## 2. Materials and Method

The seeds of hybrid-snack type cultivar Ahmetbey were used in this experiment. $TiO_2$ nanoparticles, were purchased from a producer (NG Materials) of nanoparticles with the size of 28 nm. Distilled water and $TiO_2$ based treatments were used in priming by immersing in 200 mL water and different concentration of $TiO_2$ (6, 12 and 24 mg $L^{-1}$) for 8 hours. The immersed seeds were rinsed with distilled water after 8 h.

### 2.1 Emergence tests

Four replicates of 50 seeds in each treatment were sown in plastic trays, 4 cm deep and placed in a growth chamber (Sanyo versatile Growth chamber, Japan) at 20 ± 2 °C, 45 µM photons $m^{-2}$ $s^{-1}$ light for 16 h. The compost in trays had a pH 6.5 and electrical conductivity of 40 mS $m^{-1}$. The irrigation was done regularly two times a week using 200 ml water to adjust the water lost during evapotranspiration. The plastic trays were checked daily to count the number of emerging seedlings. The seedling emergence criteria occurred as unfolding cotyledons above the surface. The experiment ended 25 d after sowing.

The mean emergence time (MET, days) was calculated with the formula below (ISTA, 2017)

$$\mathrm{MET} = \frac{\sum n \times t}{\sum n}$$

n= number of cotyledons on the compost surface at time t

t= days from planting

Σn= final cotyledon number on the compost surface

Shoot length, root length, seedling fresh weight, and dry weight were measured for all seedlings from each replicate on the 25th day. Fresh weights of seedlings were determined soon after harvest to obtain accurate results (Day, 2016). The dry weights of the seedlings were ascertained after drying the samples in an oven at 70 °C for 48 h (Day et al., 2017). Vigor index calculation was done according to the equation given below (Raskar and Laware, 2013)

$$Vigor\ index = Germination\ percentage\ (\%) \times Seedling\ dry\ weight\ (g)$$

Root to shoot length ratio was calculated by formula described below (Khatun et al., 2013)

$$Root\ length\ (cm) \div Shoot\ length(cm)$$

### 2.2 Statistical analysis

The experimental design was randomized block design with four replicates. Germination percentage data were transformed into arcsine before analysis of variance. MSTAT-C statistical software was used for the analysis of variance and the comparisons of differences between the means were computed by Duncan's multiple range test (DMRT).

## 3. Results

MET depending on the different priming treatment did not show any differences ($F$=2.0714, $df$=12, $p$=0.1479). It ranged 8.25 to 9.28 days (Table 1).

Emergence percentages ranged 90.00 to 97.50 % with significant differences ($F$=5.2689, $df$=12, $p$=0.0110). The maximum and the minimum emergence percentage was observed using 24 mg $L^{-1}$ $TiO_2$ and distilled water-treated seeds respectively. However, no statistical differences were indicated among 6, 12 and 24 mg $L^{-1}$ $TiO_2$ treatments and they were placed in the same group (Table 1).

The seedling vigor index (Table 2) in each treatment was significantly different ($F$= 7.6766, $df$=12, $p$=0.0026). The minimum and the maximum vigor index was observed in control treatment (47.78) and 24 mg $L^{-1}$ $TiO_2$ treatment (95.25). Root-to-shoot length ratio varied between 0.75 and 0.95 ($F$=1.4364, $df$=12, $p$=0.2814).

Impact of seed treatment on the shoot length showed statistically significant differences ($F$=11.5159, $df$=12, $p$=0.0004). The result proved that 6, 12 and 24 mg $L^{-1}$ $TiO_2$ impact on the shoot and root length was similar. Control

**Table 1.** $TiO_2$ priming impacts on mean emergence time and emergence percentage

| Priming treatment | MET (day) | Emergence percentage (%) |
|---|---|---|
| Control | 9.28 ± 0.249 | 90.50 ± 1.84 b* |
| Hydro | 8.60 ± 0.300 | 90.00 ± 3.00 b |
| 6 mg $L^{-1}$ $TiO_2$ | 8.65 ± 0.059 | 97.00 ± 2.279 a |
| 12 mg $L^{-1}$ $TiO_2$ | 8.25 ± 0.341 | 97.00 ± 2.219 a |
| 24 mg $L^{-1}$ $TiO_2$ | 8.50 ± 0.067 | 97.50 ± 1.821a |

All values shown with different letters in single columns are statistically different using DMRT *: $p$<0.05; ±: Standard Error

and hydro-primed seeds had shorter shoot length compared to $TiO_2$ treated seeds (Table 3).

Seedling fresh weight ranged from 1.73 to 2.91 g plant-¹ (Table 4). Hydropriming and $TiO_2$ NPs treatments increased the fresh weight compared to the control treatment. ($F$=5.3161, $df$=12, $p$=0.0107). There were no statistical differences determined among priming treatments and took the same group statistically. Seedling dry weight showed statistical importance ($F$=6.9517, $df$=12, $p$=0.0039). The dry weight values were observed between 0.53 and 0.98 mg plant-¹.

**Table 2.** $TiO_2$ priming impacts on seedling growth parameters

| Priming treatment | Seedling vigor index | Root-to-shoot lenght ratio) |
|---|---|---|
| Control | 47.78 ± 3.563 b** | 0.75 ± 0.618 |
| Hydro | 63.78 ± 3.518 b | 0.95 ± 0.816 |
| 6 mg $L^{-1}$ $TiO_2$ | 70.28 ± 4.762 ab | 0.85 ± 0.713 |
| 12 mg $L^{-1}$ $TiO_2$ | 69.60 ± 5.184 ab | 0.88 ± 0.743 |
| 24 mg $L^{-1}$ $TiO_2$ | 95.25 ± 8.796 a | 0.82 ± 0.683 |

All values shown with different letters in single columns are statistically different using DMRT **: p<0.01; ±: Standard Error

**Table 3.** $TiO_2$ priming impacts on seedling growth parameters

| Priming treatment | Shoot lenght (cm) | Root lenght (cm) |
|---|---|---|
| Control | 11.65 ± 0.193 b** | 8.75 ± 0.485 b** |
| Hydro | 12.05 ± 1.099 b | 11.40 ± 1.175 ab |
| 6 mg $L^{-1}$ $TiO_2$ | 15.70 ± 0.311 a | 13.30 ± 0.675 a |
| 12 mg $L^{-1}$ $TiO_2$ | 15.15 ± 0.104 a | 13.30 ± 0.613 a |
| 24 mg $L^{-1}$ $TiO_2$ | 15.55 ± 0.551 a | 12.68 ± 0.634 a |

All values shown with different letters in single columns are statistically different using DMRT **: p<0.01; ±: Standard Error

**Table 4.** $TiO_2$ priming impacts on seedling growth parameters

| Priming treatment | Fresh weight g plant$^{-1}$ | Dry weight g plant-¹ |
|---|---|---|
| Control | 1.73 ± 0.137 b* | 0.53 ± 0.035 b** |
| Hydro | 2.47 ± 0.328 a | 0.71 ± 0.059 ab |
| 6 mg $L^{-1}$ $TiO_2$ | 2.46 ± 0.148 a | 0.72 ± 0.061 ab |
| 12 mg $L^{-1}$ $TiO_2$ | 2.61 ± 0.147 a | 0.72 ± 0.032 ab |
| 24 mg $L^{-1}$ $TiO_2$ | 2.91 ± 0.111 a | 0.98 ± 0.097 a |

All values shown with different letters in single columns are statistically different using DMRT *: $p$<0.05; **: $p$<0.01; ±: Standard Error

## 4. Discussions

Seed germination and early seedling growth are the critical stages for crop establishment. These stages are vulnerable to biotic and abiotic stress factors. The priming treatment could support these stages when the plantlets are under the stress from inside or outside.

$TiO_2$ priming is more effective on germination percentages, shoot length, root length, seedling fresh weight and seedling dry weight compared to hydro priming and control treatments. Germination percentage was the minimum in hydro priming treatment and it did not show diversity from control treatment statistically.

Quality of seed generally has effect on the seedling establishment and its vigor (Kandasamy et al., 2020). Seedling emergence is directly influenced by vigor because this shows ability of seeds' to emerge under optimal or adverse field conditions. Emergence delay could lead to many unwanted results like delayed harvest.

$TiO_2$ showed improving results in seedling vigor, especially in 24 mg $L^{-1}$ $TiO_2$. $TiO_2$ NPs, related to dose, particle size, and exposure time (Gohari et al., 2020), could be toxic to plant growth. But for this study beneficial impacts on seedling growth parameters were observed in line with the increased shoot length and root length seedling fresh and dry weight increased due to priming treatments. Fresh weight and shoot length increase in hemp and root length increase in flax depending on the NP's concentration were observed (Akgur and Aasim, 2022; Clément et al., 2013). Similar findings were observed for increased dry weight due to $TiO_2$ treatment in Moldavian balm (Gohari et al., 2020) and maize (Shah et al., 2021).

Considering these results 8 hour priming with water has low impact for snack type sunflower cv. Ahmetbey seeds compared to $TiO_2$ NPs. However nanoparticles have different dimension and their production process are different, this dimension range used in this research is found suitable for sunflower seed multiplication. Further studies are needed to attain standardization and achieve homogeneity, which is the main issue in using these kind of nano products in agricultural production.

## Conflict of Interest

Authors have declared no conflict of interest.

## Authors' Contributions

The authors contributed equally.

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*Anatolian Journal of Botany*
*7(2): 112-116 (2023)*
*Research article* *doi:10.30616/ajb.1286625*

# Optimizing in vitro germination of primed industrial hemp (*Cannabis sativa* L.) seeds

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Received : 23.04.2023
Accepted : 20.05.2023
Online : 04.07.2023

# Priming edilmiş endüstriyel kenevir (*Cannabis sativa* L.) tohumlarının in vitro çimlenmesinin optimize edilmesi

**Abstract:** Germination under in vitro conditions for industrial hemp (*Cannabis sativa* L., *Cannabaceae*) is highly significant for the application of biotechnological tools like genome editing. Therefore, seeds were surface sterilized followed by priming with hydrogen peroxide ($H_2O_2$) and sterile distilled water ($dH_2O$) for 24, 48, and 72 h. The primed seeds were inoculated on the Murashige and Skoog (MS) medium enriched with 1.0 mg/L benzyl amino purine (BAP) and 200 mg/L antibiotics. Exposing seeds to $H_2O_2$ was superior and 100% germination was observed. Whereas hydropriming resulted in 30-52.5% germination with a maximum of 48h priming time. The results on shoot counts revealed a maximum of 1.98 shoots from the combination of 3.0% $H_2O_2$ and 72h priming time. The results were also analyzed by constructing different statistical plots like box plots, normal plots, contour plots, and surface plots. The normal plots exhibited the significance of $H_2O_2$ concentration on both output variables. Whereas contour and surface plots classified the output data into different sub-groups and confirmed the results.

**Key words:** Germination, industrial hemp, in vitro, priming, statistical analysis

**Özet:** Endüstriyel kenevir (*Cannabis sativa* L., *Cannabaceae*) bitkisinin in vitro çimlenmesi, genom düzenleme gibi biyoteknolojik araçların uygulanması için oldukça önemlidir. Bu nedenle, tohumlar yüzey sterilize edildikten sonra 24, 48 ve 72 saat boyunca hidrojen peroksit ($H_2O_2$) ve steril distile su ($dH_2O$) ile priming uygulama yapılmıştır. Daha sonra tohumlar, 1.0 mg/L BAP ve 200 mg/L antibiyotik ile zenginleştirilmiş Murashige ve Skoog (MS) ortamı üzerinde kültüre alınmıştır. Tohumların $H_2O_2$ ile yapılan priming sonucunda daha iyi sonuç verdiğini ve %100 çimlenme olduğu gözlemlenmiştir. Buna karşı hidropriming ile yapılan çalışmada 30-52,5 çimlenme gözlenirken, en iyi sonucun 48 saatlik priming sonucu olduğu kaydedilmiştir. Sürgün sayılarına bakıldığında en fazla 1,98 sürgün, %3,0 $H_2O_2$ ve 72 saatlik priming süresi kombinasyonundan elde edilmiştir. Sonuçlar ayrıca kutu grafiği, normal grafikler, kontur grafikleri ve yüzey grafikleri gibi farklı istatistiksel grafikler oluşturularak da analiz edilmiştir. Normal grafikler, $H_2O_2$ konsantrasyonunun her iki çıktı değişkenindeki önemini açıkça göstermiştir. Kontur ve yüzey grafikleri ise çıktı verilerini farklı alt gruplara ayırmış ve sonuçları doğrulanmıştır.

**Anahtar Kelimeler:** Çimlenme, endüstriyel kenevir, in vitro, priming, istatistiksel analiz

**Citation:** Yıldırım B, Aasım M, Aytaç S (2023). Optimizing in vitro germination of primed industrial hemp (*Cannabis sativa* L.) seeds. Anatolian Journal of Botany 7(2): 112-116.

## 1. Introduction

Industrial hemp (*Cannabis sativa* L., *Cannabaceae*) is one of the most significant and commercially grown crops of the World, due to its multiple uses in different industries ranging from pharmaceutical to the fiber industry (Salentijn et al., 2014; Tremlová et al., 2021). The commercially grown area of industrial hemp is increasing significantly with an estimated compound annual growth rate (CAGR) of 16.2% up to 2028 (Aasim et al., 2022). The demand for developing new cultivars with elite characteristics (Chandran et al., 2020) enforces researchers to employ biotechnological tools like plant tissue culture. The in vitro germination followed by the seedling establishment is simultaneously a physiological process (Ventura et al., 2012), regulated by both internal and external stimuli. Exposing seeds to any external stimuli within a specific time may lead to the manipulation of a whole germination process (Paparella et al., 2015). The priming is the exogenous appliation of a physical or chemical stimulus to the seeds, and to date, various priming techniques have been developed and employed successfully for different crops under different culture conditions (Salah et al., 2015).

Germination of cannabis seeds under in vitro conditions is a major issue and needs the use of some external stimuli like water or $H_2O_2$. Hydrogen peroxide ($H_2O_2$) is an oxidant (Sevilgen and Velioğlu, 2009), that generate in response to stresses in plants (Karataş et al., 2015). The significance of $H_2O_2$ for plants is relatively high irrespective of complicated relationships, which often may lead to oxidative damage leading to cell death (Abbas et al., 2011; Hossain et al., 2015; Zhang et al., 2015). Therefore, automation of the optimum dose of $H_2O_2$ and treatment time is important. Hydropriming is another and most promising technique used in seed germination. In this study, the surface sterilized seeds of industrial hemp were treated with $H_2O_2$ and sterile distilled water ($dH_2O$) for three different treatment times to check the impact on germination and shoot counts.

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## 2. Materials and Method

The cultivated industrial hemp population "Narlısaray" was procured from Gokhoyuk Agricultural Management Directorate, Amasya, Türkiye.

The healthy seeds were selected manually and the damaged seeds were discarded. The seeds were surface sterilized following Aasim et al., (2022). The seeds were treated with ethanol (70% for 3 min), followed by treatment with $HgCl_2$ (0.10% for 10 min). Thereafter, distilled water was used for rinsing the seeds and this process was repeated three times for 5 min each for removing the traces of the disinfectant.

The surface sterilized seeds were exposed to 2.0% and 3.0% v/v $H_2O_2$ (34.5–36.5% H2O2 - Sigma-Aldrich) for 24, 48, and 72 h. The same treatment time was also used for hydropriming (Control) of industrial hemp seeds. After the respective treatment time, the seeds were transferred to the culture medium for germination.

The culture medium used in this study comprised of Murashige and Skoog (MS) medium (Murashige and Skoog 1962), 30 g/L sucrose, and 6.5% agar as a gelling agent. The pH was automated with 1.0 N HCl/NaOH to approximately 5.8 after adding 1.0 mg/L benzylamino purine (BAP). To overcome the issue of endogenic bacteria, 200 mg/L Sulcid (ampicillin and sulbactam) was also added to the culture medium after autoclaving the medium. Autoclaving of the culture medium was performed as a standard procedure employed (15 min, 121 ◦C, 1.5 atm pressure). The basal medium containing primed seeds were placed under White Light-emitting Diodes (LEDs) with temperature and light photoperiod automated at 24 ± 1 ◦C and 16 h respectively.

The experiment was performed in 4 replicates containing 10 seeds per replicate. The data regarding in vitro germination was tabulated after 21 days of culture. Whereas, shoot counts were recorded after 6 weeks of culture. The data were analyzed using two different statistical programs for a better presentation of data. The ANOVA, regression equation, and normal plots were analyzed using Minitab statistical software. The difference among the treatments was analyzed with Tukey's B. The contour and surface plots were developed with the aid of a design expert program.

## 3. Results

Application of $H_2O_2$ concentration (C) and treatment time (T) significantly affected the in vitro germination (%) and shoot counts of surface sterilized seeds of industrial hemp. The results revealed that concentration and treatment time did not affect the germination, percentage (100%). Exposing seeds to water (control treatment-hydropriming) for 24 to 72 h yielded 30.0-52.50% germination with a maximum from 48 h treatment time. The results on shoot counts ranged 1.05-1.98. The maximum shoot counts were recorded from 3.0% $H_2O_2$ for 72 h treatment time. On the contrary, minimum shoot counts were attributed to seeds used in control treatment for 72 h (Table 1). The results were further illustrated by constructing a box plot analysis of both output parameters (Fig. 1a,b).

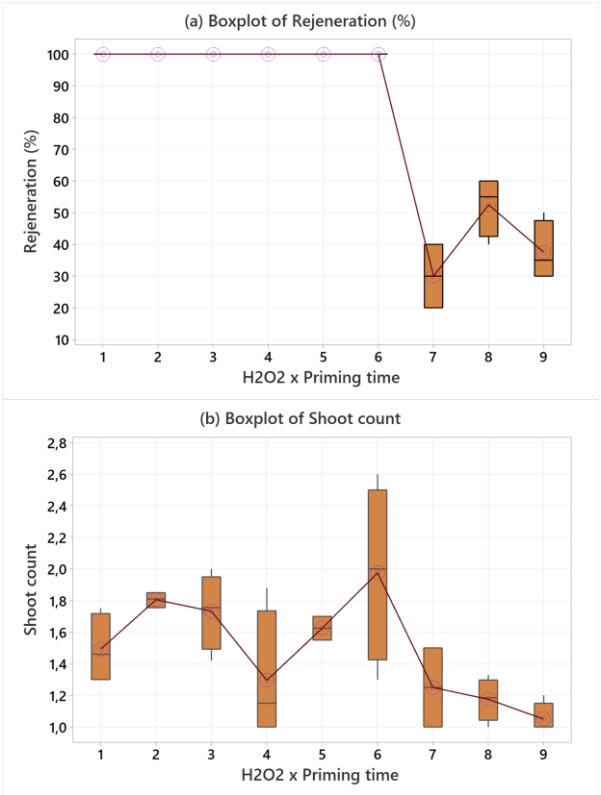


**Figure 1.** Box plot analysis of in vitro germination and shoot counts in response to priming agent concentration and treatment time.

**Table 1.** Impact of C × T of $H_2O_2$ and d$H_2O$ on the germination (in vitro) of industrial hemp (*Cannabis sativa* L.)

| $H_2O_2$ (%) | Time (h) | Germination (%) | | | Shoot counts | | |
|---|---|---|---|---|---|---|---|
| | | Mean | StDev | 95% CI | Mean | StDev | 95% CI |
| 2 | 24 | 100.0A | 0.0 | (93.9; 106.1) | 1.49ABC | 0.229 | (1.204; 1.781) |
| 2 | 48 | 100.0A | 0.0 | (93.9; 106.1) | 1.81AB | 0.0526 | (1.5161; 2.0939) |
| 2 | 72 | 100.0A | 0.0 | (93.9; 106.1) | 1.73AB | 0.241 | (1.444; 2.021) |
| 3 | 24 | 100.0A | 0.0 | (93.9; 106.1) | 1.30BC | 0.415 | (1.006; 1.584) |
| 3 | 48 | 100.0A | 0.0 | (93.9; 106.1) | 1.63ABC | 0.0866 | (1.3361; 1.9139) |
| 3 | 72 | 100.0A | 0.0 | (93.9; 106.1) | 1.98A | 0.556 | (1.686; 2.264) |
| 0 | 24 | 30.00C | 11.55 | (23.91; 36.09) | 1.25BC | 0.289 | (0.961; 1.539) |
| 0 | 48 | 52.50B | 9.57 | (46.41; 58.59) | 1.18BC | 0.1358 | (0.8861; 1.4639) |
| 0 | 72 | 37.50C | 9.57 | (31.41; 43.59) | 1.05C | 0.1000 | (0.7611; 1.3389) |

### 3.1. Normal plot analysis

Normal plot analysis was performed to check the significant level of input variables of concentration, treatment time, and interaction of C × T time. Results revealed that priming agent concentration exhibited a positive impact on in vitro germination only. The impact of treatment time and C × T remained non significant (Fig. 2a,b). Whereas, the priming agent concentration and C × T exhibited a positive impact on shoot count compared to priming time which remained non significant (Table 1).

### 3.2. Contour and surface plot analysis

The results were further analyzed with the aid of 2-D contour plots (Fig. 3a,b), and 3-D surface plots (Fig. 4a,b). The analysis of contour plots revealed the distribution of germination into 20-100% and shoot counts as 0-2.0, expressed with a different color. The results of contour plots were further confirmed with surface plots.

## 4. Discussions

Germination is a complex process and is regulated by several internal and external stimuli (Wojtyla et al., 2016). Some plant seeds are known as recalcitrant nature due to difficulties in germination under specific conditions. Plant tissue culture offers a novel technique to germinate seeds more efficiently due to controlled conditions (Karataş et al., 2016). In vitro germination of industrial hemp seeds is relatively difficult and needs some stimuli or some treatments to enhance germination.

$H_2O_2$ is the potent priming agent for enhancing in vitro seed germination of industrial hemp (Sorokin et al., 2021; Aasim et al., 2022). The results confirmed that priming seeds with

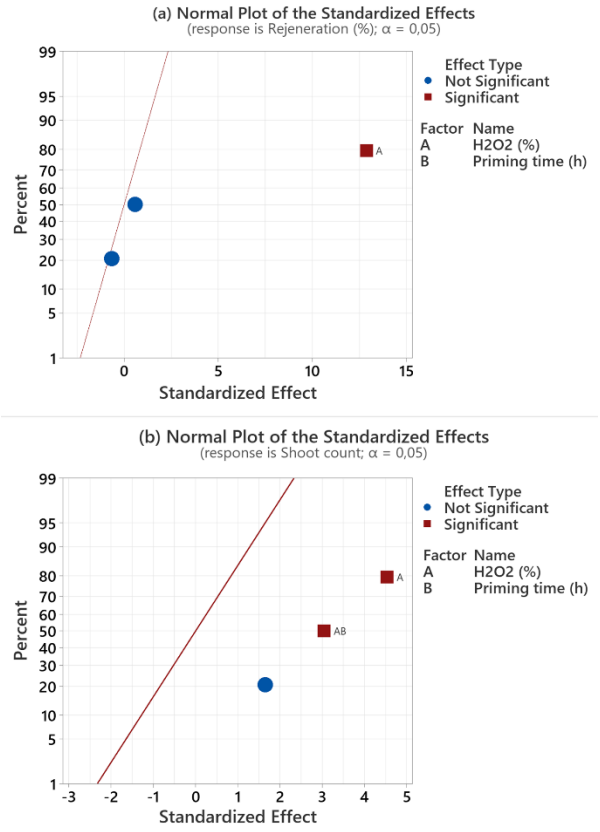


**Figure 2.** Normal plot analysis of in vitro germination and shoot counts in response to priming agent concentration and treatment time.

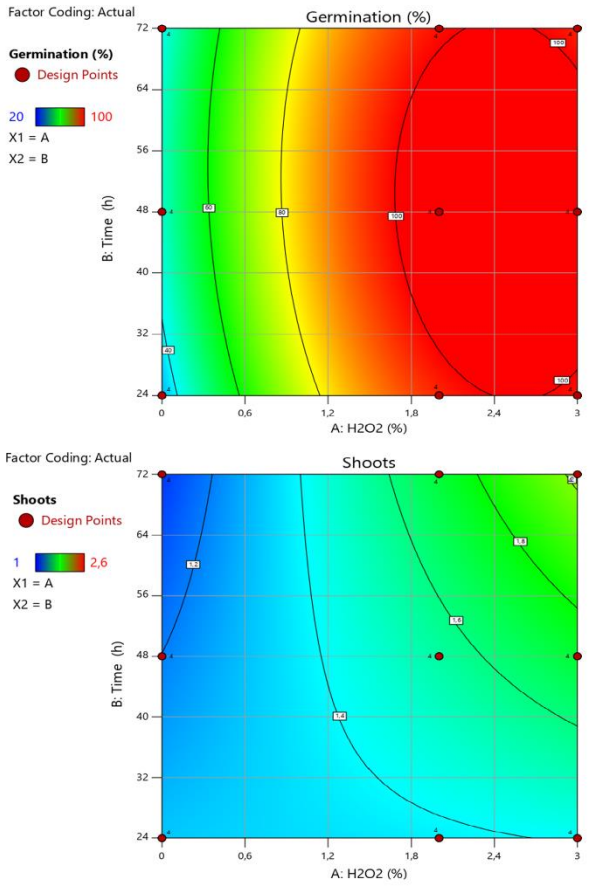


**Figure 3.** Contour plot analysis of in vitro germination and shoot counts in response to priming agent concentration and treatment time

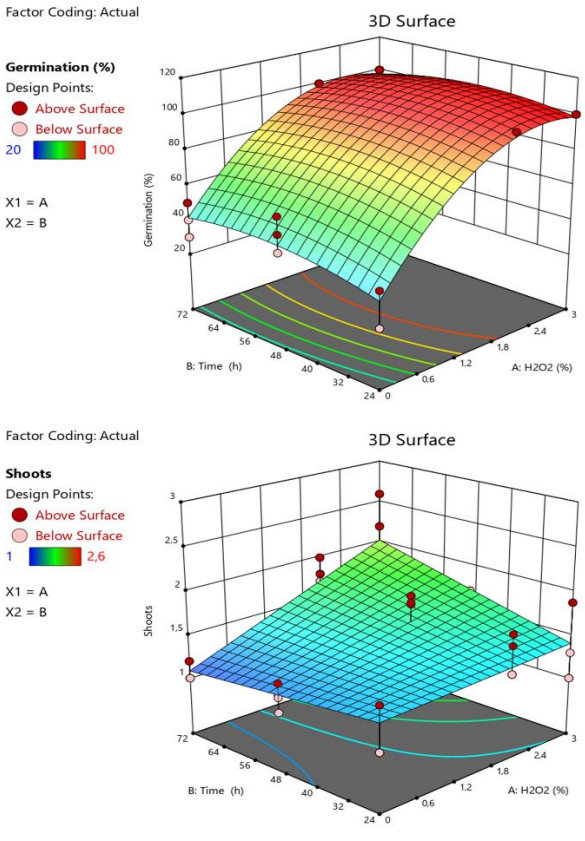


**Figure 4.** Surface plot analysis of in vitro germination and shoot counts in response to priming agent concentration and treatment time.

$H_2O_2$ yielded more germination and shoot count compared to hydropriming. The results further confirmed that treatment time also affected both output parameters but exhibited the opposite impact with the priming agent. The positive impact of $H_2O_2$ improved kinetics of water uptake ending up in higher seed germination (Anawar et al., 2011). However, the treatment time and concentration are the critical factors responsible for a positive impact on plant growth and development (Aasim et al., 2022). The results were also analyzed by box plot which exhibited great variation for germination in response to control treatment. Whereas, variable variation responses among treatments were observed for shoot counts.

Normal plot analysis is a powerful tool to express the positive or negative impact of input variables on the concerning output parameter. The results revealed that the priming agent and its concentration is more significant than other treatment time for both output parameters used in this study. Whereas, treatment time has no impact and remained non significant for both output variables. The normal plot analysis distributes the data on the graph based on significant and non significant level, expressed with different colors and shape inclined on the line (Ramazan and Uğur, 2014; Katirci, 2015). Results of normal plots exhibited the significant factors with a level of input variables. The use of normal plots has been used for investigating the impact of BAP-IBA level on in vitro regeneration of sorghum plant (Aasim et al., 2023).

## Conclusion

Germination is a complex physiological and biological process and can be enhanced by employing priming techniques. $H_2O_2$ is a potent priming agent, and priming seeds with $H_2O_2$. This proved to be more efficient for high germination and shoot count compared to hydropriming. On the other hand, treatment time had a minimum impact on germination. Results analyzed through normal, contour, and surface plots precisely confirmed the results.

## Conflict of Interest

Authors have declared no conflict of interest.

## Authors' Contributions

The authors contributed equally.

## Acknowledgements

This study was funded by the Scientific Research Council (BAP) of Sivas University of Science and Technology, Sivas, Türkiye for research project No. 2022-YLTB-TBT-0001.

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*Anatolian Journal of Botany*
*7(2): 117-121 (2023)*
*Research article* *doi:10.30616/ajb.1289077*

# Three new records of *Helotiales* for the mycobiota of Türkiye

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Received : 00.04.2023
Accepted : 23.05.2023
Online : 04.07.2023

# Türkiye mikobiyotası için üç yeni *Helotiales* kaydı

**Abstract:** *Hymenoscyphus imberbis* (Bull.) Dennis, *H. vitigenus* (De Not.) Dennis (*Helotiaceae)* and *Gemmina gemmarum* (Boud.) Raitv. (*Pezizellaceae)* species, collected from Bingöl and Van provinces, are given as new records for Türkiye. The identified samples are given with macroscopic and microscopic characters, photographs, collection localities and collector numbers.

**Key words:** *Discomycetes*, lignicolous fungi, sac fungi, Türkiye

**Özet:** Bingöl ve Van illerinden toplanan *Hymenoscyphus imberbis* (Bull.) Dennis, *H. vitigenus* (De Not.) Dennis (*Helotiaceae)* ve *Gemmina gemmarum* (Boud.) Raitv. (*Pezizellaceae)* türleri Türkiye için yeni kayıt olarak sunulmuştur. Tanımlanan örnekler makroskobik ve mikroskobik karakterleri, fotoğrafları, toplama yerleri ve toplayıcı numaraları ile birlikte verilmiştir.

**Anahtar Kelimeler:** *Discomycetes*, lignikol mantarlar, askuslu mantarlar, Türkiye

**Citation:** Akçay ME, Acar İ, Uzun Y (2023). Three new records of *Helotiales* for the mycobiota of Türkiye. Anatolian Journal of Botany 7(2): 117-121.

## 1. Introduction

The order *Helotiales*, within the class *Leotiomycetes* (*Ascomycota)* is the largest order containing the non-stromatic *Discomycetes*. It is known to include 11 families, approximately 500 genera, and almost 4000 species (Kirk et al., 2008; Baral, 2015). Members of this order usually produce brightly colored or, in some species, dark-colored apothecia, which may be stipitate or sessile. The overall shape of the apothecia is cupulate-discoid, turbinate funnel-shaped, or clavate (Korf, 1973). Most members of the *Helotiales* have minute apothecia, usually less than 2 mm in diameter. Although most of the species live as decomposers on dead plant remains, there are also a few parasitic or symbiotic species (Hosoya, 2021).

According to the latest check-lists, listing the species determined in Türkiye (Sesli et al., 2020; Solak & Türkoğlu, 2022), and the recent studies carried out on ascomycetous fungi in our country (Akçay et al., 2022; Acar & Quijada, 2022; Uzun and Kaya, 2022a; 2022b; Uzun, 2023), the order *Helotiales* is represented by the 16 families, 37 genera and 90 species in Türkiye. The most populous families are *Dermataceae* and *Helotiaceae* families (Sesli et al., 2020; Solak & Türkoğlu, 2022).

The genus *Hymenoscyphus* Gray (*Helotiaceae*), comprising more than 150 species worldwide, has attracted attention especially in Europe due to the damage they cause on various trees, especially ash, and many studies have been carried out on it (Kirk et al., 2008, Baral et al., 2014, George et al., 2022). In Türkiye, only 15 species of this genus have been reported so far without any study related to the damage they cause on trees. The genus *Gemmina* Raitv. comprises two species, and none of them has been presented from Türkiye before (Işık and Türkekul, 2018; Keleş, 2019; Sesli et al., 2020; Çetinkaya and Uzun 2021; Solak and Türkoğlu, 2021).

## 2. Materials and Method

Fungal specimens were collected from Bingöl and Van provinces of Türkiye in 2019 and 2020. Morphological and ecological characteristics of the samples were recorded during field studies, and they were photographed in their natural habitats. Then, they were taken to the laboratory and microscopic investigations were carried out on the samples. Observations of apothecia were made under a Leica EZ4 stereomicroscope with the magnifications up to 35×. Microscopic investigation of the samples was carried out under a Leica DM500 light microscope mounted with a Leica ICC50 HD camera. Drawings of some micro characters were prepared using the CorelDRAW Standard 2021 software. Reagents such as 5 % KOH and Iodine (IKI) and Melzer's reagent were used as investigation media. Identification was performed with the aid of the relevant literature (Dennis, 1956; Raitviir and Faizova, 1983; Blank, 1988). Macrofungal samples are kept in the Fungarium of the Biology Department of Van Yüzüncü Yıl University Herbarium (VANF).

## 3. Results

The determined species are presented with their macroscopic and microscopic features, habitats, substrates, collection dates and voucher numbers (eg. Acar 1155). Colour photographs of ascocarps and some microscopic features were also provided.

***Ascomycota*** Caval.-Sm.

***Leotiomycetes*** O.E. Erikss. & Winka

***Helotiaceae*** Rehm

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***Hymenoscyphus imberbis*** (Bull.) Dennis (Figs. 1-2)

**Macroscopic and microscopic features:** Apothecia 1-4 mm, cup-shaped or flat, white when fresh, drying reddish-brown or pale yellowish brown, smooth or minutely downy with a short sturdy stipe of about 1 mm, slightly tomentose at the base. Ascospores 7-11.5 × 3-4.2 µm, elliptical or slightly inequilateral, sometimes a septate, spores size based on n = 25 spores. Asci 55-85 × 6-9.4 µm, 8 spored, cylindrical-claviform, asci size based on n = 15 asci, pore blue in Melzer's reagent and croziers at the base. Paraphyses up to 2.4 µm, slightly widened apex, cylindrical, hyaline, septate in its lower part. Ectal excipulum 10-30 × 5-15 µm, polygonal, sharply defined, with a widening texture, with some VBs (persistent in dead state) in the outermost cells.

**Specimen examined:** Bingöl, Ormanardı village, on branch of *Quercus* sp., 38°49'46"N, 40°30'38"E, 1253 m, 18.04.2020, Acar 1155.

***Hymenoscyphus vitigenus*** (De Not.) Dennis (Fig. 3-4)

**Macroscopic and microscopic features:** Apothecia 0.5-3.5 mm, scattered, at first flat, then slightly convex, light yellow to yellow, ochre, exterior slightly furfuraceous. Stipe 0.2-0.5 × 1.5-3 mm, smooth, thin, usually ochraceous towards the base. Ascospores 15-20 × 4.5-6 µm, mainly cylindrical to ellipsoid, sometimes curved, large or small 3–5 or more guttulate, spores size based on n = 25 spores. Asci 100-140 × 7-12 µm, 8 spored, cylindrical-claviform, asci size based on n = 15 asci, pore blue in Melzer's reagent. Paraphyses up to 3 µm, cylindrical, clavate or lanceolate at the apex, hyaline, multiply septate and filled with many guttulate. Ectal excipulum 12-23 × 7-13 µm, hyaline thin-walled, rectangular cells, texture widening from below to upwards.

**Specimen examined:** Bingöl, Kurudere village, on branch of *Vitis vinifera*, 14.10.2020, 38°54'13"N, 40°25'15"E, 1174 m, VANF Acar 1163.

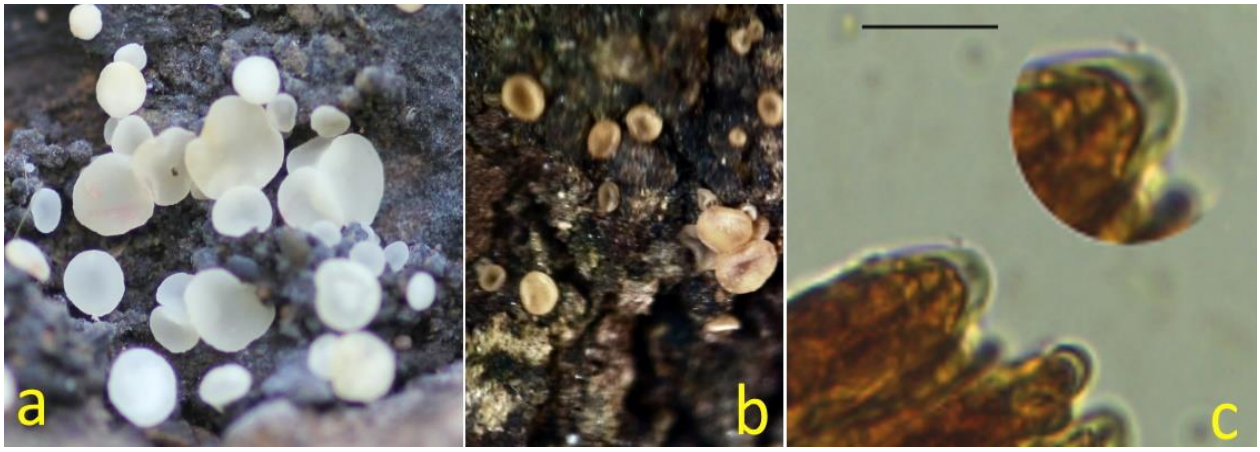


**Figure 1.** *Hymenoscyphus imberbis* a. fresh ascomata b. dried ascomata c. apex of an ascus in Melzer's reagent (Scale bar= 10 µm).

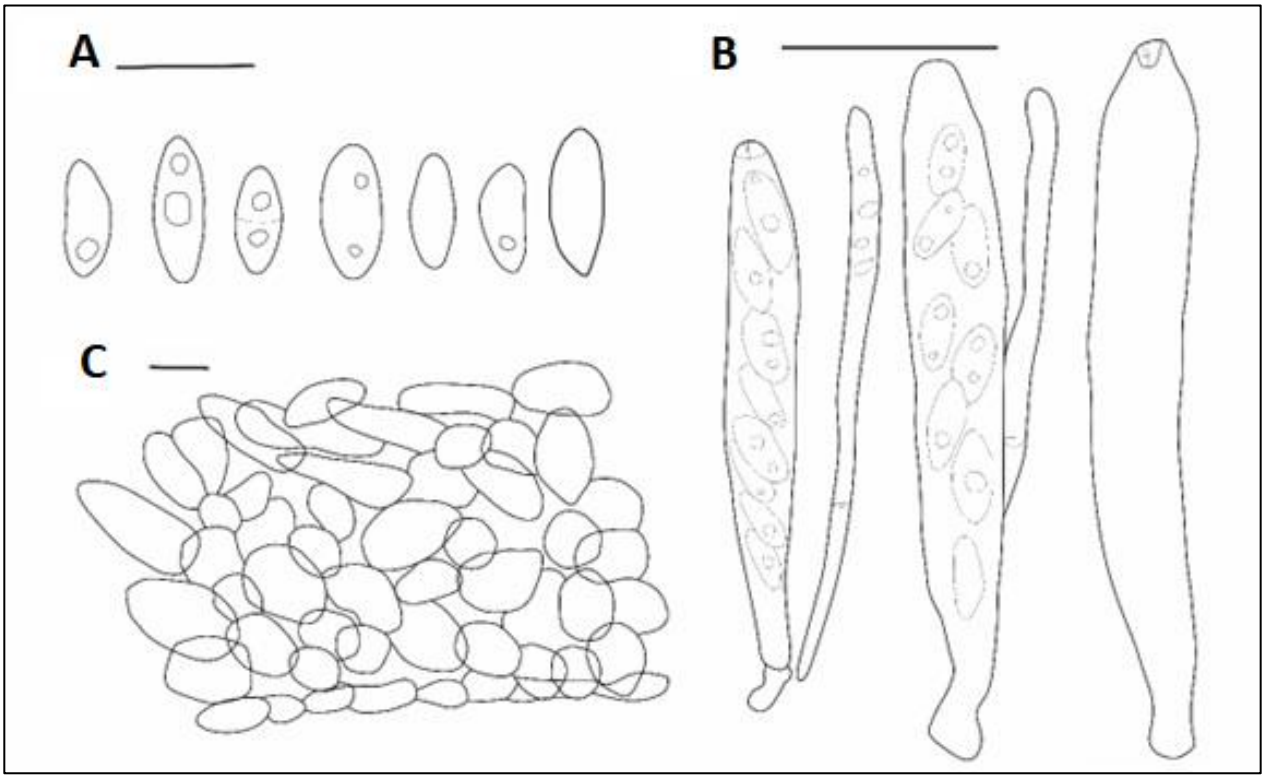


**Figure 2.** *Hymenoscyphus imberbis* A. spores B. asci and paraphysis C. ectal excipulum (Scale bars: a and c= 10 µm; b= 20 µm).

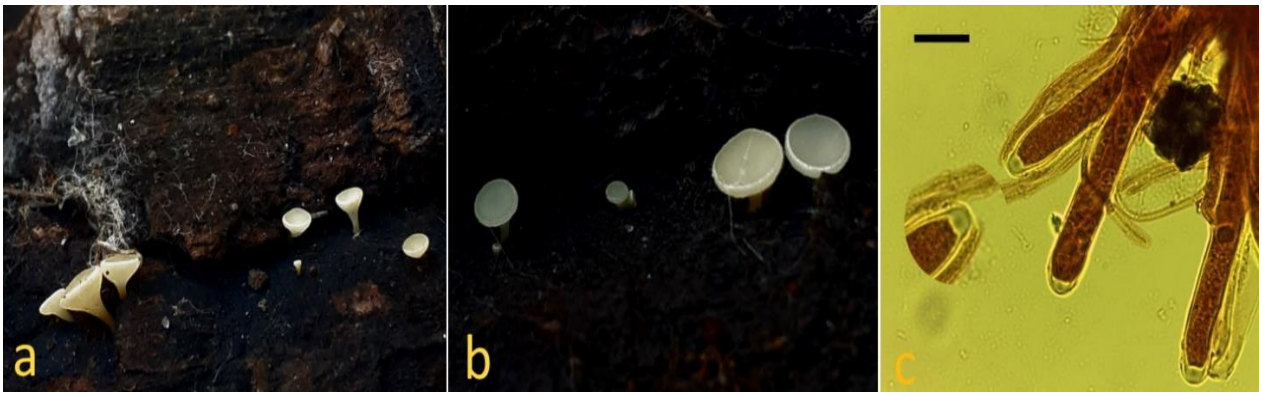


**Figure 3.** *Hymenoscyphus vitigenus* a-b. fresh ascomata c. apexes of ascus in Melzer's reagent (Scale bar= 10 µm)

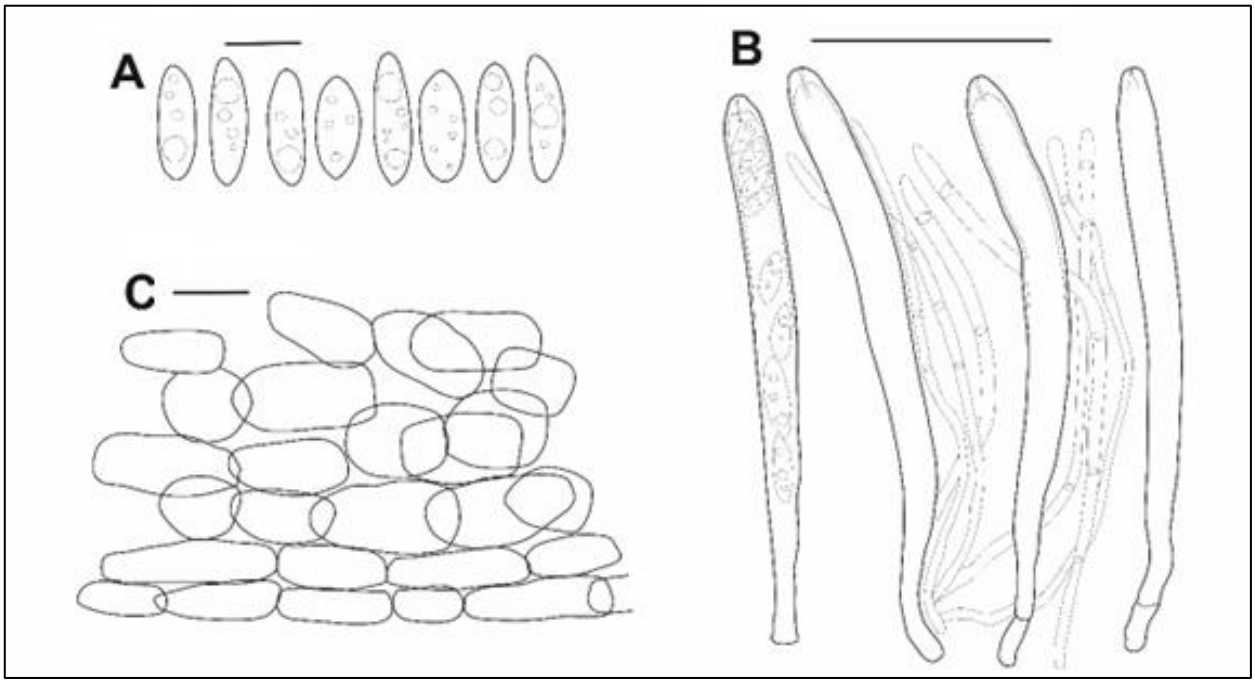


**Figure 4.** *Hymenoscyphus vitigenus* A. spores B. asci and paraphysis C. ectal excipulum (Scale bars: a and c= 10 µm; b= 50 µm)

***Pezizellaceae*** Velen.

***Gemmina gemmarum*** (Boud.) Raitv. (Fig. 5-6)

**Macroscopic and microscopic features:** Apothecia 0.3-1 mm, cup-shaped or infundibuliform when young, then broad saucer-shaped, whitish, yellowish-cream when dried. Stipe slightly longer than diameter of the disc, whitish to pale ochraceous towards to base. Ascospores 6-9.7 × 2-3 µm, elliptical, smooth, hyaline, sometimes small droplet, spores size based on n = 25 spores. Asci 35-60 × 3.3-6.5 µm, 8–spored, cylindrical, with croziers at the base, asci size based on n = 15 asci, pore blue in Melzer's reagent. Paraphyses up to 2–3.5 µm, sparse, filiform, usually even and cylindrical on all sides, sometimes clavate or tapering towards the apex, large oil droplets at the base, which oil drops make the paraphyses appear to be septate. Hair 20-50 × 3-4.6 µm curved at the apex and extended, cylindrical, 2 or 3 septate, glabrous towards the base. Ectal excipulum up to 8.5 µm, hyaline thin-walled, oblong or globose cells, texture widening from below to upwards.

**Specimen examined:** Türkiye, Van, Tuşba, MTA lodgings garden, fallen bud (poplar) scale, 04.05.2019, 38°33'31"N, 43°17'59"E, 1649 m, VANF Acar 1126.

## 4. Discussions

Three species from the order *Helotiales* were identified for the first time from Türkiye and presented as a contribution to the fungal diversity of our country. The total number of species belonging to the *Helotiales* increased from 90 to 93. *Hymenoscyphus imberbis* and *H. vitigenus* were added as the sixteenth and the seventeenth member of the genus *Hymenoscyphus* in Türkiye.

*Hymenoscyphus imberbis* is a saprotrophic species with more or less flat to slightly convex, sessile to short-stalked apothecia, like *Hymenoscyphus fagineus* (Pers.) Dennis and *Hymenoscyphus epiphyllus* (Pers.) Rehm ex Kauffman. *H. imberbis* can be distinguished from similar species by its relatively shorter and wider spores, apothecial color ranging pink to reddish-brown as it dries, and slightly downy stipe, which tapering towards the base. It can be differentiated from other *Hymenoscyphus* of the same color and habitat, such as *Hymenoscyphus vernus* (Boud.) Dennis, by the presence of croziers at the base of the asci, which do not appear in *H. vernus*, and from *Hymenoscyphus kathiae* (Korf) Baral, because it has much

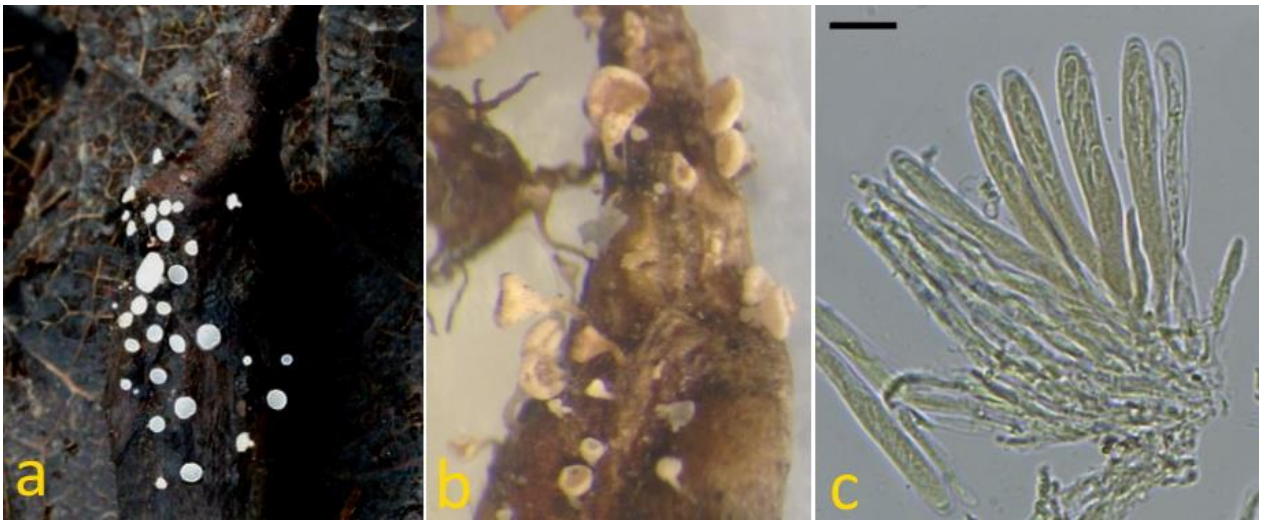


**Figure 5.** *Gemmina gemmarum* a. fresh ascomata b. dried ascomata c. asci and paraphysis in Melzer's reagent (Scale bar= 10 µm)

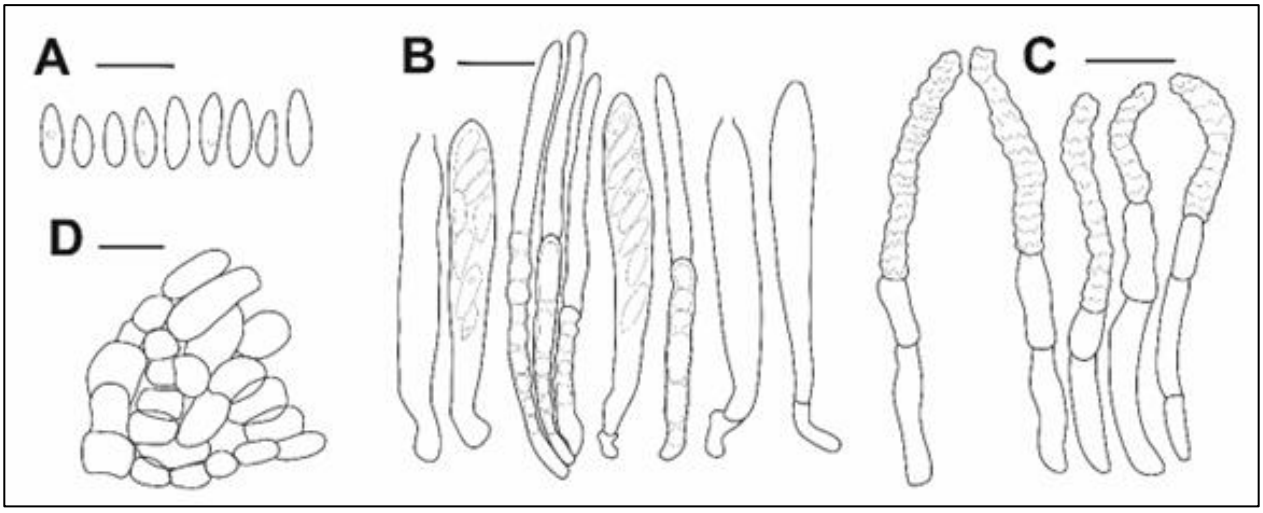


**Figure 6.** *Gemmina gemmarum* A. ascospores B. asci and paraphysis C. hairs D. ectal excipulum (Scale bars= 10 µm)

longer spores (Lizonbreve, 1992; Hengstmengel, 2009; Thompson, 2013).

*Hymenoscyphus vitigenus* grows on the rotting stems of the *Vitis vinifera* and is closely related to *Hymenoscyphus scutula* (Pers.) W. Phillips, *Hymenoscyphus caudatus* (P. Karst.) Dennis and *Helotium hyalopes* Fuckel, which grow on the bark or leave remnants of this plant (Dennis, 1964; Baral, 2015). However, *H. scutula* has much longer spores, and *H. caudatus* grows on the remains of fallen leaves of *Vitis vinifera* or different trees (Breitenbach & Kränzlin, 1984; Uzun et al., 2010). In addition, while the length of the asci of *H. vitigenus* is 100-140 µm, the ascus lengths of *Helotium hyalopes* are between 80-120 µm, and its spores have two droplets (Fuckel, 1870; Baral, 2015).

*Gemmina gemmarum* is the first member of genus *Gemmina* in Türkiye (Işık & Türkekul, 2018; Keleş, 2019; Sesli et al., 2020; Çetinkaya & Uzun 2021; Solak & Türkoğlu, 2022).

Of the two *Gemmina* species determined on the Earth, *G. gemmarum* grows on rotting residues of *Populus* sp., while *G. juniperi* grows on wood remains of *Juniperus* sp. (Raitviir, 2004; Thompson, 2013). Turkish collection of *G. gemmarum* was made on fallen bud scale of *Populus* sp.

**Conflict of Interest**

Authors have declared no conflict of interest.

**Authors' Contributions**

The authors contributed equally.

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*Anatolian Journal of Botany*
*7(2): 122-127 (2023)*
*Research article* *doi:10.30616/ajb.1287442*

# Nitrate content in roots of pepper seedlings exposed to *Phytophthora capsici*

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Received : 25.04.2023
Accepted : 24.05.2023
Online : 04.07.2023

# *Phytophthora capsici*'ye maruz bırakılan biber fidelerinin köklerinde nitrat içeriği

**Abstract:** *Phytophthora capsici* causes root rot, a deadly plant disease. Resistance to diseases is produced by the activation of many defense substances, so knowledge of this natural defense mechanism allows the development of new strategies for disease control. In this study, the response of nitrate ($NO_3^-$), which is effective in plant growth and development, in different pepper genotypes exposed to pathogen infection was investigated. For this, resistant and sensitive pepper genotypes were exposed to $10^2$, $10^3$, and $10^4$ zoospore/mL of *P. capsici-22* strain and changes in $NO_3^-$ content were determined from root samples taken on the 2nd, 4th and 6th days after infection. All zoospore concentrations resulted in an overall increase in $NO_3^-$ content in roots of CM-334 on all days. In KM-181 and SD-8 genotypes, the highest $NO_3^-$ content was determined on the 6th day of $10^3$ zoospore/mL application. In SD-8 and KM-181 genotypes, a significant decrease in the amount of $NO_3^-$ was determined on the 4th and 6th days of treatment of $10^4$ zoospore/mL. In these genotypes, a decrease in the amount of $NO_3^-$ was found with the increase in infection time at high zoospore concentration. When the three pepper genotypes were compared, the highest $NO_3^-$ content was determined in the resistant CM-334 genotype, which was exposed to $10^4$ zoospore/mL on the 6th day following the infection. In this study, changes in the amount of $NO_3^-$ in resistant and susceptible pepper genotypes indicated that $NO_3^-$ may be effective in plant defense against *P. capsici-22*.

**Key words:** Nitrate, pepper, *Phytophthora capsici*-22

**Özet:** *Phytophthora capsici* ölümcül bir bitki hastalığı olan kök çürüklüğüne neden olur. Hastalıklara karşı direnç, birçok savunma maddesinin aktivasyonu ile üretilir, dolayısıyla bu doğal savunma mekanizmasının bilinmesi, hastalık kontrolü için yeni stratejilerin geliştirilmesine olanak tanımaktadır. Bu çalışmada, bitki büyüme ve gelişmesinde etkili olan nitratın ($NO_3^-$) patojen enfeksiyonuna maruz kalan farklı biber genotiplerindeki tepkisi araştırılmıştır. Bunun için dirençli ve duyarlı biber genotipleri $10^2$, $10^3$ ve $10^4$ zoospore/mL *P. capsici*-22 izolatına maruz bırakılmış ve enfeksiyondan sonraki 2., 4. ve 6. günlerde alınan kök örneklerinden $NO_3^-$ içeriğindeki değişimler belirlenmiştir. Tüm zoospor konsantrasyonları, tüm günlerde CM-334'ün köklerindeki $NO_3^-$ içeriğinde genel olarak bir artışa neden olmuştur. KM-181 ve SD-8 genotiplerinde en yüksek $NO_3^-$ içeriği $10^3$ zoospor/mL uygulamasının 6. gününde belirlenmiştir. SD-8 ve KM-181 genotiplerinde, $10^4$ zoospor/mL uygulamasının 4. ve 6. günlerinde $NO_3^-$ miktarında önemli bir azalma saptanmıştır. Bu genotiplerde, yüksek zoospor konsantrasyonunda enfeksiyon süresinin artışı ile birlikte $NO_3^-$ miktarında azalma bulunmuştur. Üç biber genotipi karşılaştırıldığında, en yüksek $NO_3^-$ içeriği, enfeksiyondan sonraki 6. günde $10^4$ zoospor/mL'ye maruz bırakılan dirençli CM-334 genotipinde belirlenmiştir. Bu çalışmada, dirençli ve duyarlı biber genotiplerinde $NO_3^-$ miktarındaki değişimler, $NO_3^-$ 'ün *P. capsici-22* 'ye karşı bitki savunmasında etkili olabileceğini işaret etmektedir.

**Anahtar Kelimeler:** Biber, nitrat, *Phytophthora capsici*-22

**Citation:** Koç E, Karayiğit B (2023). Nitrate content in roots of pepper seedlings exposed to *Phytophthora capsici.* Anatolian Journal of Botany 7(2): 122-127.

## 1. Introduction

The soil-borne pathogen *Phytophthora capsici* Leonian, which causes root rot, causes great economic damage in pepper growing areas in many countries of the world. Root and crown rot agent *P. capsici* is effective on plant species in the *Solanaceae*, *Cucurbitaceae*, and *Leguminaceae* families (Krasnow and Hausbeck, 2015). This fungus, which was first seen on peppers in New Mexico in the world, had a wide host range in later years (Leonian, 1922). In Turkey, for the first time in 1974, the disease caused by *P. capsici* was seen in pepper cultivation areas in the Central Anatolia region and it caused sudden drying of the plants (Karahan and Maden, 1974). The disease is seen in almost all areas where pepper is grown and causes significant crop losses in years when it occurs (Leonian, 1922).The spots on the root and root collar are typical brown-colored spots and these lesions dry out after a while, causing the plant to die completely (Van Steekelenburg, 1980).

In suitable ecological conditions, zoospores released from the sporangia of the agent, which are formed abundantly in the soil irrigation water, infect the pepper seedlings from their roots, while drying from the root part of the seedling and causing its death (Leonian, 1922). These infections are more common in poorly drained areas where irrigation water or rainwater accumulates during the rainy season. The disease can be observed in different periods of the plant, depending on environmental conditions and the occurrence of infection. It causes a collapse in seedlings in the early period. It starts to appear close to fruit formation in the field and spreads in a very short time, infecting the plants and causing death (Naegele and Hausebeck, 2014). It is sometimes impossible to get products in the fields where this disease is intense. When it infects ripe fruits, it causes a large number of quality and quantity losses in the product. To date, many researchers have focused on the effects of nitrogen (N) on growth, development, and yield in plants.

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Nitrogen (N), an essential element for plant metabolism, affects a series of events involved in disease development such as inoculation, colonization, and infection as a result of plant- pathogen interaction (Zhou et al., 2017). Different N forms are effective in physiological events such as enzyme, photosynthesis rate, water balance, respiration rate, and signalling pathway. On the other hand, studies on the importance of N in plant defense against pathogens have been more limited. In this study, the changes in nitrate ($NO_3^-$) content in the roots of pepper seedlings resistant and susceptible to *P. capsici* were investigated. In the literature survey, there is no record on $NO_3^-$ content in peppers infected with *P. capsici*.

## 2. Materials and Method

### 2.1. Plant materials

In the current study, resistant Criollo de Morelos-334 (CM-334), susceptible Kahramanmaras-181 (KM-181) and Sera demre-8 (SD-8) pepper genotypes were used as plant material. Sterilized pepper seeds were placed in pots containing the same amount of sterilized soil, fertilizer and sand and left to germinate in the growing chamber. The seedlings were grown under the conditions of a photoperiod (24 ± 2 °C) of 16 hours light and 8 hours dark at two months of old.

### 2.2. *Phytophthora capsici*-22 strain and plant inoculation

*Phytophthora capsici-22* strain (Ankara University Faculty of Agriculture) to be used in the inoculation process was grown on $V_8$ agar plates (Jones et al., 1975) (Fig. 1), and zoospore were obtained from growing mycelia (Blaker and Macdonald, 1981; Ward and Stoessl, 1974; Satour and Butler, 1967; Hachler and Hohl, 1984).

After the sterilization process of the roots of the two-month-old seedlings, the seedlings were taken into the Hoagland solution and placed in the growth chamber. After 3 days, the roots of the seedlings were inoculated with $10^2$, $10^3$, and $10^4$ zoospore/mL for 1 hour. After the applications, the seedlings were placed in glass bottles containing Hoagland solution and transferred back to the growing chamber, and then random samples were taken from the roots on the 2nd, 4th, and 6th days (Koç et al., 2011; Koç and Üstün, 2012; Koç, 2022). After the samples were passed through liquid nitrogen, they were stored at -70°C until analysis.

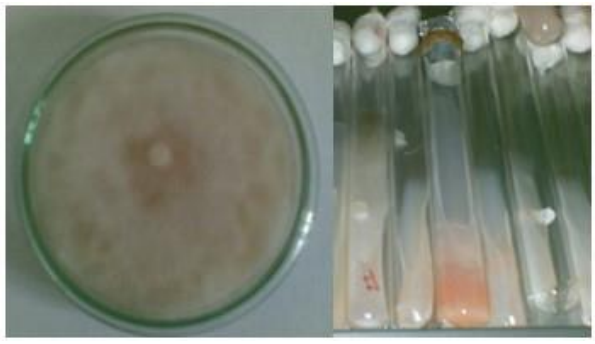

**Figure 1.** *Phytophthora capsici*- 22 strain.

### 2.3. $NO_3$ analysis

The $NO_3^-$ extraction was carried out according to Ruiz et al. (1999). The $NO_3$ amount was determined according to the salicylic acid method (Cataldo et al., 1975) and the absorbance was measured at 410 nm and calculated as µmol $L^{-1}$ $g^{-1}$ FW against the $NO_3^-$ standard.

### 2.4. Statistical analysis

The data obtained in terms of $NO_3^-$ were analyzed with repeated measures analysis of variance (ANOVA: genotype×day×treatment). Significance differences were determined by the Tukey test (5% significance level).

Capital letters indicate the difference in genotypes for the same day and treatment. Lowercase letters indicate the difference in treatments of the same genotype and the same day. Superscript lowercase letters indicate the difference in days for the same genotype and treatment.

## 3. Results

In of root all three pepper genotypes, the most disease was observed in the application of $10^4$ zoospore/mL of *P. capsici*-22 isolate on the 6th day following infection. Infection progressed more rapidly in KM-181 and SD-8 and SD-8 genotype was severely damaged, especially on the 6th day. On the 6th day of infection, severe disease symptoms such as leaf wilting, detachment of leaves, and brown lesions on stem length and roots were detected (Fig. 2).


**Figure 2.** Disease development on the 6th day in three pepper genotypes infected with $10^4$ zoospore/mL.

When the $NO_3^-$ contents of CM-334 genoype with $10^2$ and $10^4$ zoospore/mL were compared on the 2nd and 6th days, a significant increase in $NO_3^-$ content was detected in the roots of the seedlings applied with $10^4$ zoospore/mL ($p<0.05$) (Fig. 3). The difference between $NO_3^-$ contents on the 2nd and 6th days was significant in the CM-334 seedlings inoculated with $10^3$ zoospore/mL ($p<0.05$). Compared to the control, the difference between $NO_3^-$ contents measured on all days in the CM-334 genotype inoculated with $10^2$ zoospore/mL was not significant. Compared to both control and other treatments, the highest $NO_3^-$ increase was determined on the 6th day treatment of $10^4$ zoospore/mL ($p<0.05$) (Fig. 3).

An increase in the amount of $NO_3^-$ was detected on the 2nd day of infection in the roots of the KM-181 genotype exposed to $10^4$ zoospores/mL treatments. However, with the increase in the duration of infection, a significant decrease in the amount of $NO_3^-$ was determined ($p<0.05$) (Fig. 4). On the other hand, in lower zoospore/mL treatments, the increase in $NO_3^-$ content was determined on the 4th and 6th days after the infection. Increases in $NO_3^-$ contents were determined on the 4th day in the roots of the seedlings inoculated with $10^2$ zoospore/mL compared to the control, and on the 6th day in the application of $10^3$ zoospore/mL ($p<0.05$). The highest $NO_3^-$ content among all treatments was determined on the 6th day following the infection in the roots of the seedlings inoculated with $10^3$ zoospore/mL ($p<0.05$) (Fig. 4).

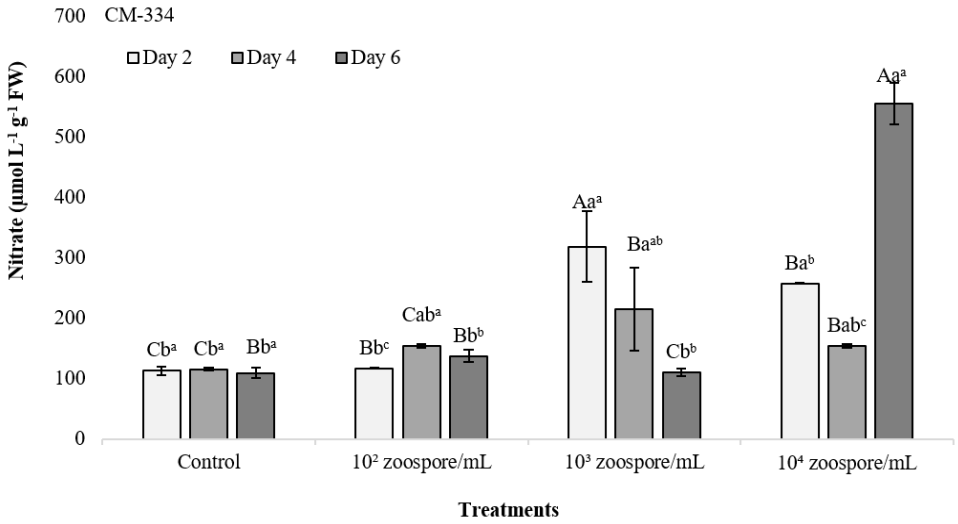


**Figure 3**. $NO_3^-$ content in roots of CM-334 genotype exposed to different zoospore concentrations (n=3)

In the roots of SD-8 seedlings, with compared to the control, a decrease in $NO_3^-$ content was determined on the 2nd and 6th days following the infection in $10^2$ zoospore/mL ($p<0.05$) (Fig. 5). Compared with the control, a decrease in $NO_3^-$ content on the 2nd and 4th days after the infection in the roots of SD-8 genotype inoculated with $10^3$ zoospore/mL and an increase in the 6th day were found, the difference was significant ($p<0.05$) (Fig. 5).

According to the control , a decrease in $NO_3^-$ content was determined on the 2nd and 6th days in $10^2$ zoospore/mL applications. The highest $NO_3^-$ increases were determined on the 4th day in the $10^2$ zoospore/mL application and on the 6th day in the $10^3$ zoospore/mL treatment, respectively ($p<0.05$). In the treatment of $10^4$ zoospores/mL, $NO_3^-$ contents decreased on all days compared to the control, and the lowest $NO_3^-$ content was determined on the 6th day ($p<0.05$) (Fig. 5).

When the control group of all three pepper genotypes were compared, the highest $NO_3^-$ amount was determined in KM-181 genotype (Fig. 4). This was followed by SD-8 and CM-334 genotypes, respectively (Fig. 3, 5). The difference between $NO_3^-$ contents in the roots of three pepper genotypes was found to be significant in $10^2$ zoospore/mL application on the 2nd day, and the highest $NO_3^-$ content was found in SD-8 genotype ($p<0.05$). When the $NO_3^-$ amounts in the roots of three pepper genotypes were compared in $10^2$ zoospore/mL application on the 4th and 6th days following the infection, the highest $NO_3^-$ was determined in KM-181 genotype ($p<0.05$) (Fig. 4). In all three pepper genotypes, the highest $NO_3^-$ contents were detected in CM-334 genotype in $10^4$ zoospore/mL treatment, and KM-181 genotype at 6th day following infection in $10^3$ zoospore/mL treatment ($p<0.05$) (Fig. 3, 4).

## 4. Discussions

Nitrogen may limit pathogen growth and affect the activation of the plant defense system. Compared with its effects on plant growth and development, the role of $NO_3^-$ in the defense of plants against pathogens is less well known (Fagard et al., 2014; Soulie et al., 2020). It has been reported that different N forms such as $NO_3^-$ are effective on plant disease resistance by using different assimilation and metabolism pathways (Mur et al., 2017). Gupta et al. (2013) found that $NO_3^-$ increased the disease resistance to *Pseudomonas syringae* pv in tobacco by stimulating the synthesis of salicylic acid and NO. Fagard et al. (2014) found that low N decreased the resistance of *Arabidopsis* to *Erwinia amylovora*. In tomato plants exposed to *Botrytis cinerea*, it was determined that low $NO_3^-$ content increased the susceptibility to the pathogen, whereas higher concentrations of $NO_3^-$ decreased the disease symptoms and

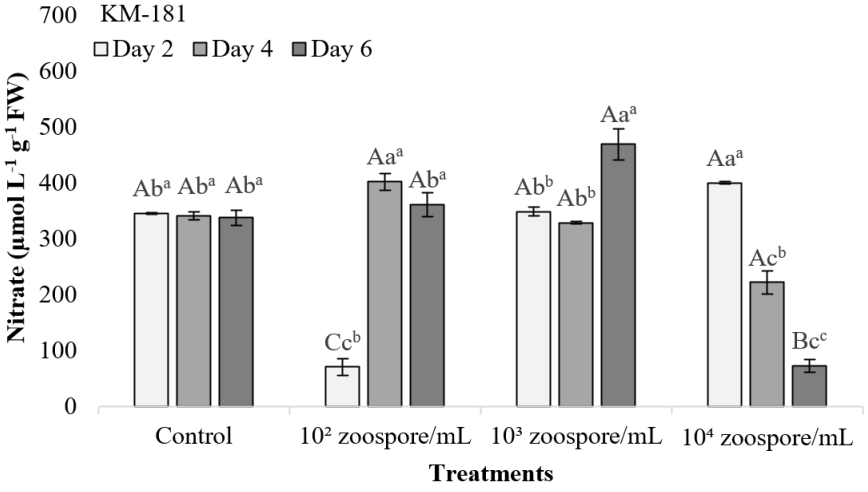


**Figure 4**. $NO_3^-$ content in roots of KM-181 genotype exposed to different zoospore concentrations (n=3)

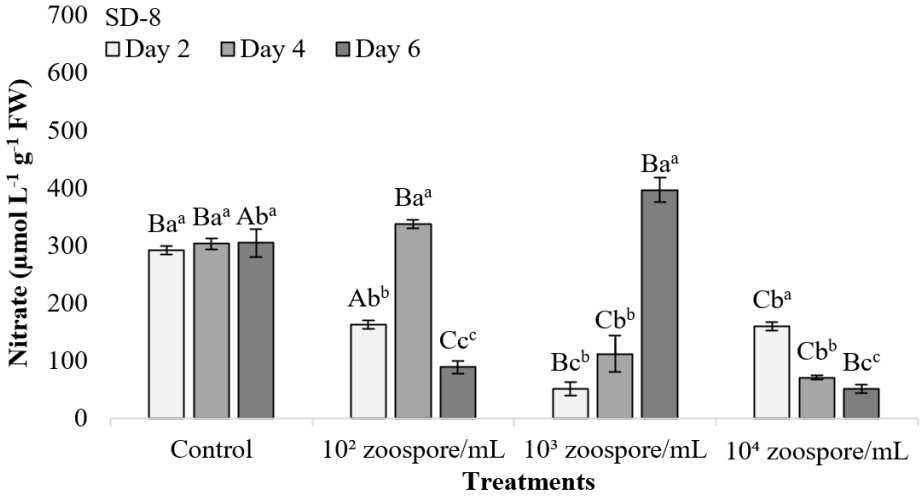


**Figure 5**. $NO_3^-$ content in roots of SD-8 genotype exposed to different zoospore concentrations (n=3)

lesion, and an increase in defense substances. Similarly, Zhou et al. (2017) and Wang et al. (2016) found that higher $NO_3^-$ in cucumber seedlings exposed to *Fusarium oxysporum* increased the tolerance to *Fusarium* wilt by inhibiting pathogen colonization due to the decrease in membrane damage and pathogen toxin production in plants. It has been reported that there is an opposite relationship between the $NO_3^-$ content in the roots and the disease index, and it has been stated that the efficacy of the disease varies depending on the $NO_3^-$ content in the root (Zhou et al., 2017). In this study, all *P. capsici* zoospore concentrations caused a general increase in $NO_3^-$ amount in the roots of CM-334 genotype on all days. A significant decrease in $NO_3^-$ content was detected on the 4th and 6th days of $10^4$ zoospore/mL application in SD-8 and KM-181 genotypes. In other words, a decrease in the amount of $NO_3^-$ was determined due to the increase in the duration of infection at high zoospore concentrations. The CM-334 genotype continued to respond to this severe stress with its high $NO_3^-$ content. This different defense response can be attributed to having different genotypes. It has been reported that the toxin produced by fungi in vitro provides N-regulation and, in general, the effects of N on disease vary depending on the host type, a pathogen type, and the duration of the infection (Bolton and Thomma, 2008). Changes in the amount of $NO_3^-$ depending on the day after the application show that it acts as a defense mechanism against the disease, and the results seem to be compatible with the data obtained from the above mentioned studies. Increases and decreases in the amount of $NO_3^-$ also indicate that there may be a connection between the occurrence of symptoms of disease (hence the severity of disease). These data also support the conclusion that CM-334 genotype have a higher resistance to *P. capsici* isolate than KM-Hot and SD-8 genotypes in our previous studies (Koç and Üstün, 2012), and have shown that $NO_3^-$ has an effect on the formation of this tolerance. The severity of the disease and the length of necrosis were determined at least in CM-334 genotype and maximum in SD-8 genotype (Koç and Üstün, 2012). It has been reported that $NO_3^-$ increases the hypersensitive response (HR)-mediated resistance to pathogens and increases the production of polyamines (Mur et al., 2017). Polyamines are also involved in systemic resistance (Mur et al., 2017). Sagor et al. (2009) and Tiburcio et al. (2014) reported that oxidative deamination of polyamines with amine oxidases reduces pathogen entry by strengthening the cell wall, while polyamine oxidase inhibitors cause weakening of the host's defense system by blocking signal transduction pathways. In a previous study, it was determined that *P. capsici* stress increased the activities of enzymes such as polyaminoxidase (PAO), diaminoxidase (DAO), which are involved in the catabolism of polyamines in CM-334 and KM-Hot pepper genotypes (Koç, 2015). In this study, in parallel with the increase in infection duration, increases in $H_2O_2$ amount and high DAO and PAO activities were detected. $H_2O_2$, which is formed as a result of catabolism of polyamines with aminoxidases such as PAO and DAO, contributes to stimulating host cell death (Yoda et al., 2003; Moschou et al., 2009). It has been stated that this DAO and PAO activities occur during hypersensitive response induction after inoculation (Cowley and Walters, 2002). Recent studies indicate that $NO_3^-$ stimulates the production of ethylene and jasmonic acid and other signaling molecules as a signal molecule, and the production of pathogenesis-related proteins (PR) and plant defensin proteins with antifungal properties (Mur et al., 2017; Soulie et al., 2020; Farjad et al., 2021). Changes such as NO, phytoalexin synthesis, SA accumulation and stimulation of defense genes encoding proteins increase resistance against pathogens (Wendehenne et al., 2014). In a previous study, PR-R and PR-S proteins in the PR-5 group and PR-2, PR-3 were found in KM-Hot pepper genotype exposed to $10^4$ zoospore/mL concentrations of *P. capsici* (Koç and Üstün, 2009). In the CM-334 genotype, it was determined that a protein with a weight of 28 kDA corresponding to Chi28 (chitinase property) of the PR-3 group was accumulated (Koç and Üstün, 2009). Therefore, changes in $NO_3^-$ content and synthesis of PR proteins after infection with *P. capsici* support studies indicating that $NO_3^-$ functions as a signaling molecule.

Developing disease-resistant genotypes and using them in cultivation is the method struggle with the highest added value in the long term in terms of health and environment. Additionally, the prevention of product loss due to disease will also increase economic gain. The development of pepper genotypes resistant to *P. capsici* is seen as the best control method against this disease. Therefore, it is important to know and use the natural defenses in the fight against pathogens and to develop new strategies for disease control.

In this study, the changes in $NO_3^-$ amounts in resistant and sensitive pepper genotypes to *P. capsici* were investigated. However, further studies are needed to better understand the molecular, biochemical and physiological changes (association with hormones such as JA, SA, ethylene that act as signal molecules in the defense system, amino acid metabolism such as glutamate and aspartate, defense-related secondary metabolites, nitrate transporters, defense-related key genes etc.) underlying the differential effect of $NO_3^-$ availability in pepper on plant susceptibility to *P. capsici.*

**Conflict of Interest**

Authors have declared no conflict of interest.

**Authors' Contributions**

EK: designing the study, conducting and supervising analyzes, manuscript writing, BK: editing of references section and nitrate analysis.

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*Anatolian Journal of Botany*
*7(2): 128-130 (2023)*
*Research article* *doi:10.30616/ajb.1285406*

# *Agaricus micromegethus,* a new record for Turkish Mycobiota

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Received : 19.04.2023
Accepted : 07.06.2023
Online : 04.07.2023

# *Agaricus micromegethus,* Türkiye Mikobiotası için yeni bir kayıt

**Abstract:** The *Agaricaceae* is a monophyletic group of saprotrophic fungi distributed worldwide. The family exhibits considerable variations such as spore color and structure of pileus covering in morphology. Agaricus is the type genus of the family containing both edible and poisonous species, with more than 500 species worldwide. This genus includes many species that are considerably important so takes attention of scientists in the fields of medicine, biochemistry and food science. This study aims to introduce a new record, *Agaricus micromegethus*, for the Turkish mycobiota based on macro- and micromorphology. An illustrated description of the record is provided.

**Key words:** *Agaricaceae, Basidiomycota,* taxonomy, Türkiye

**Özet:** *Agaricaceae*, dünya çapında yayılmış, saprotrofik mantarların monofiletik bir grubudur. Familya, morfolojik açıdan spor rengi ve pileus örtüsünün yapısı gibi önemli farklılıklar gösterir. *Agaricus* dünya genelinde 500'den fazla yenilebilir ve zehirli türleri kapsayan tip cinsidir. Bu cins oldukça önemli birçok türü içerdiğinden, tıp, biyokimya ve gıda bilimleri gibi alanlarda bilim insanlarının dikkatini çeker. Bu çalışma, makro- ve mikromorfolojiye dayanarak Türkiye'nin mikotası için yeni bir kayıt olan *Agaricus micromegethus*'u tanıtmayı amaçlamaktadır. Yeni kaydın resimli bir tanımı sunulmaktadır.

**Anahtar Kelimeler:** *Agaricaceae, Basidiomycota,* taksonomi, Türkiye

**Citation:** Acar İ, Dizkırıcı A (2023). *Agaricus micromegethus,* a new record for Turkish Mycobiota. Anatolian Journal of Botany 7(2): 128-130.

## 1. Introduction

*Agaricaceae* Chevall. is a well-known fungal family with global distribution. It is a monophyletic group of fungi that exhibits huge diversity in spore colour and structure of the pileus covering (Vellinga 2004). *Agaricus* is a large and saprotrophic genus of the family growing in forests, gardens, woods, grass-land, on roadside, rubbish dumps, fields, pasture-land, alluvial soils and manure heaps. Although members of the genus are easily recognized in the field based on the macro-morphological features alone, species delimitation is often hard due to the absence of distinguishable characters and intraspecific variability (Zhao et al., 2011). The genus is characterized by umbrella shaped carpophores with scaly to furfuraceous pileus surface. In young specimens, the lamellae are pinkish in colour, while in mature specimens they are sometimes dark brown. Lamellae are usually free-attached to stipe. *Agaricus* species have a partial veil that usually forms a ring on the stem. The spore print is dark brown and coca brown dark to purple brown-coloured spores without any germ pore (Karunarathna et al. 2016; Kuo, 2018; Saini et al. 2018).

Taxonomic monographs and other taxonomic studies of *Agaricus* species are mostly from America, Australia, Europe, India and New Zealand. However, many studies have been carried out in Türkiye for the last ten years (Güngör et al., 2015; Uzun et al., 2017; Işık et al., 2019; Keleş, 2019; Acar et al., 2020; Çağli and Öztürk, 2020; Işık, 2020; Sadullahoğlu and Uzun, 2020; Sesli et al., 2020; Uzun et al., 2020; Yeşil et al., 2020; Çevik et al., 2021; Kaşık et al., 2022; Solak and Türkoğlu, 2022). Accordingly, the total number of species in our country is presently 45. In an attempt to further knowledge of the taxonomy this genus, we describe a specimen of *Agaricus, Agaricus micromegethus* Peck., as a new record for Türkiye based on morphological characteristics. Detailed morphological descriptions and field photographs of the collected basidiomata are provided.

## 2. Materials and Method

Fresh samples of *Agaricus* were collected from Güveçli village (Bingöl) in 2019. The samples were photographed and transported to the laboratory where its fresh macroscopic details were described. Morphological descriptions of the specimens were made following Desjardin et al. (2015), Kerrigan (2016) and Siegel and Schwarz (2016). Following the methodology described by Senthilarasu and Kumaresan (2018), microscopical features (basidiospores, basidia, pileipellis and hyphae) of each dried specimen were examined. The specimens cited are deposited in the Herbarium of Van Yüzüncü Yıl University (VANF) and in the author's personal collection. Micro-morphological observations were examined from dried specimens treated with distilled water by a Leica DM500 research microscope under oil immersion. Thin sections were manually prepared from different parts of the basidiomata. At least 20 basidiospores and basidia were measured from each specimen using the Leica Application Suite (version 3.4.0) programme. The drawings used in the manuscript were made in CorelDRAW (64-bit).

## 3. Results

Short description, locality, collection date, fungarium number, and figures are given below.

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***Agaricus micromegethus*** Peck

**Pileus** 30-45 mm, at first has a slightly cylindrical or convex appearance, but later widening to plano-convex; the margins are curved inward in young specimens, then decurved; surface dry, often fimbriated with veil fragments when young; central depressed, fibrillose-scaly towards the margins, and striate in places on the margins, the ornamentation pinkish-brown, pale yellowish brown to lilac-brown over a dull background, with age the fibrils brownish, staining stronger yellow when bruised. **Lamellae** free, relatively wide and rather cramped, at first cream-buff, then pinkish-tan, finally middle -brown. **Stipe** 25-35 × 5-8 mm, usually equal, sometimes expanding towards the base, fragile, bruising yellowish to tawny; partial veil membranous-fibrillose, it is covered with appressed fibrils above and below, the annulus is superior, white, whitish to tan-colored, single, thin, and often disappears or collapses against the stipe (Fig. 1). **Spores** 4.5-5.6 × 3.2-4.3 µm, smooth, ellipsoid to ovoid, thick-walled, usually dark brown. **Pileipellis** up to 15 µm, cylindrical, sometimes sausage-shaped, yellowish brown, clamp connection not observed. **Stipitipellis** up to 10 µm, parallel, tawny, clamp connection not observed (Fig. 2).

**Specimen examined:** Türkiye, Bingöl, Güveçli village, stream edge, in meadow, 38°50′56″N, 40°31′12″E, 1080 m, 27.10.2019.

**Figure 1.** *Agaricus micromegethus* **a**. photographs of the basidiomata in the laboratory **b**. in the field

## 4. Discussions

The present study identified *A. micromegethus*, originally described by Peck (1905), based on the morphologic analyses.

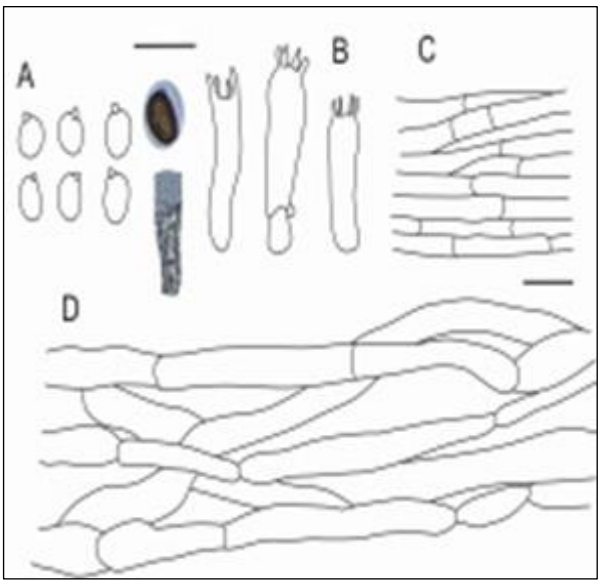


**Figure 2.** *Agaricus micromegethus* A. Basidiospores, B. Basidia, C. Stipitipellis, D. Pileipellis. Drawing by İ. Acar. **Scale Bar:** A-B= 10 µm, C-D= 20 µm

*Agaricus micromegethus* growns in grasslands and it is characterized by a small basidiocarp and pinkish to purplish fibrils on the pileus surface. As the species matures, the fibrils become brownish and turn yellowish when touched. *Agaricus comtulus* Fr. is related to *A. micromegethus* with its characteristic features that grows in meadow and slowly turn yellow when touched. However, it differs from *A. micromegethus* by cream-colored cap. *Agaricus micromegethus* is also related to *A. semotus* Fr. and *A. diminutivus* Peck, but can be distinguished by features of habitat; they are distributed in forested areas. *Agaricus micromegethus* can also be mixed with *Lepiota* species if the lamella color is not taken into account (Desjardin et al., 2015; Kerrigan, 2016; Anonymous, 2023).

*Agaricus micromegethus*, which is not very common in the world, is recorded in America, Colombia, Canada, China, and India when the literature is searched (Murrill, 1922; Sathe and Rahalkar, 1976; Liu and Bau, 1980; Devi and Thara, 2001; Kroeger and Berch, 2017). At the end of the study, thorough macro- and micro- morphological characters of the collected specimens indicated that the specimens represent *Agaricus micromegethus* for the first time from Türkiye and Europe.

## Conflict of Interest

Authors have declared no conflict of interest.

## Authors' Contributions

The authors contributed equally.

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*Anatolian Journal of Botany*
*7(2): 131-134 (2023)*
*Research article* *doi: 10.30616/ajb.1335980*

# First record of *Arpinia luteola* J.Geesink from Türkiye

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Received : 01.08.2023
Accepted : 24.08.2023
Online : 01.09.2023

# *Arpinia luteola* J.Geesink'nın Türkiye'den ilk kaydı

**Abstract:** *Arpinia luteola* J.Geesink was reported as new record for the mycobiota of Türkiye, based on the identification of the samples from Aydıntepe district of Bayburt province. This species is the first member of the genus *Arpinia* in Türkiye. A short description of the species is provided together with the photographs, related to the macro and micromorphology, and the suggested Turkish name.

**Key words:** Biodiversity, macrofungi, *Pezizales*, Türkiye

**Özet:** *Arpinia luteola* J.Geesink, Bayburt'un Aydıntepe ilçesinden toplanan örneklerin teşhisine bağlı olarak, Türkiye mikobiyotası için yeni kayıt olarak rapor edilmiştir. Bu tür *Arpinia* cinsinin Türkiye'deki ilk üyesidir. Türün kısa bir betimlemesi, makro ve mikromorfolojisine ilişkin fotoğrafları, ve önerilen Türkçe ismiyle birlikte verilmiştir.

**Anahtar Kelimeler:** Biyoçeşitlilik, makromantarlar, *Pezizales*, Türkiye

**Citation:** Uzun Y, Kaya A (2023). First record of *Arpinia luteola* J.Geesink from Türkiye. Anatolian Journal of Botany 7(2): 131-134.

## 1. Introduction

*Arpinia* Berthet is an operculate discomycete genus within *Pyronemataceae* family. It was first proposed by Berthet (1974) with the type species *A. inops* Berthet. Members of the genus are characterized by cupulate, discoid to flat, stipitate to substipitate apothecia; whitish, dull whitish, cream, pale ochraceous, dull ochraceous, yellowish ochraceous or vitelline hymenium; scurfy to irregularly crenulate margin; concolorous or paler, usually scurfy receptacle; two-layered ectal excipulum; operculate, 8 spored, pleurorhynchous asci; ellipsoid to fusiform, usually biguttulate, thick-walled, uniseriate ascospores; filiform, straight, hyaline paraphyses subclavate at the apex, and non staining nuclei with acetocarmine.

IndexFungorum (2023) lists the current names of five *Arpinia* species (*A. fusispora* Hohmeyer, *A. inops* Berthet, *A. luteola* J. Geesink, *A. microspora* (Dissing & Raitv.) Hohmeyer, *A. rahmii* Hohmeyer & Senn-Irlet). Current checklists (Sesli et al., 2020; Uzun, 2023) and the latest contributions to the pyronemataceous macrofungi of Türkiye (Keleş, 2019; Altuntaş et al., 2021; Berber et al., 2021; Kesici and Uzun, 2021; Uzun and Kaya, 2021, 2022) indicate that, any member of the genus *Arpinia* have been reported from Türkiye before. Here we present *A. luteola* as new record for the mycobiota of Türkiye.

## 2. Materials and Method

Apothecia of *A. luteola* were collected from Salmankaş village of Aydıntepe (Bayburt) district during a routine field survey in 2023. Colour photographs of the apothecia were taken at their natural habitat, and notes were taken related to their ecology, morphology and geographic position. Then some apothecia were collected and transferred to the fungarium in paper bags. Microscopic investigations were carried out under a Nikon Eclipse Ci-S trinocular microscope. Photographs related to microstructure was obtained by the aid of a DS-Fi2 digital camera. It was identified by comparing the accumulated data with Geesink (1982), Hohmeyer (1988) and Fernández Vicente and Undagoitia (2004). The samples are kept at Karamanoğlu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology.

## 3. Results

***Ascomycota*** Whittaker

***Pezizales*** J. Schröt.

***Pyronemataceae*** Corda

***Arpinia luteola*** J. Geesink

**Syn:** [*Arpinia luteola* var. *pallidorosea* Benkert, Häffner & Hohmeyer].

**Macroscopic and microscopic features:** Apothecia gregarious to somewhat caespitose, stipitate and up to 25 mm in diameter. Receptacle cup shaped at first, then discoid, and undulate at maturity. Hymenium yellowish to yellowish orange or yellowish ochraceous when young, somewhat brownish yellow towards the margin, light salmon pink to roseaeous at maturity, smooth when young, somewhat rough an furfuraceous at maturity (Fig. 1). Outer surface concolorous, smooth to furfuraceous. Outer layer of ectal excipulum with globose cells, inner layer of textura angularis. Medullary excipulum of textura intricate. Asci 120-175 × 8-10 µm, cylindrical, hyaline, octosporic, pleurorhyncal (Fig. 2a). Paraphyses 2.5-3 µm in diameter, filiform, hyaline, generally straight, some slightly enlarged towards the apex up to 4, rarely 5 µm (Fig. 2a). Ascospores

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9.5-11.5 × 5.5-6.0 µm, uniseriate, cylindrical to ellipsoid, hyaline, thick-walled, usually with two oil drops (Fig. 2b).

**Figure 1.** Ascocarps of *Arpinia luteola*

*Arpinia luteola*, also regarding varity *pallidorosea*, was reported to grow on bare ground or humus in coniferous and deciduous forests, especially of *Larix* Mill. and *Picea* A.Dietr. spp. (Geesink, 1982; Hohmeyer, 1988; Fernández Vicente and Undagoitia, 2004).

**Specimen examined:** Bayburt, Aydıntepe, Salmankaş village, poplar grove, along with decaying leaves of *Populus* sp., 40°28′N-40°00′E, 1930m, 25.06.2023, Yuzun 7335.

Suggested Turkish name for the presented species is "Sarı kepeklikadeh".

**4. Discussions**

*Arpinia luteola* was added as a new record for the mycobiota of Türkiye. This species is the first member of the genus *Arpinia* in Türkiye. General characteristics of the studied collection are in agreement with Geesink (1982), Hohmeyer (1988), Fernández Vicente and Undagoitia (2004).

Together with the synonymized var. *pallidorosea*, *Arpinia luteola* is characterized by yellowish hymenium, small and somewhat cylindrical ascospores. *Arpinia microspora* and *A. inops* differs from this species by their whitish hymenia. Larger ascospores of *Arpinia fusispora* (15-17 × 6-.6.5 µm) and *A. inops* (13-16 × 9-10 µm) also differ them from *A. luteola* (Geesink, 1982; Hohmeyer, 1988; Fernández

Vicente and Undagoitia, 2004; Rubio and Sánchez, 2005; Læssøe and Petersen, 2019)

All members of *Arpinia* seem very rare (Hohmeyer, 1988; Læssøe and Petersen, 2019) and current species generally have European distribution
have generally been reporte

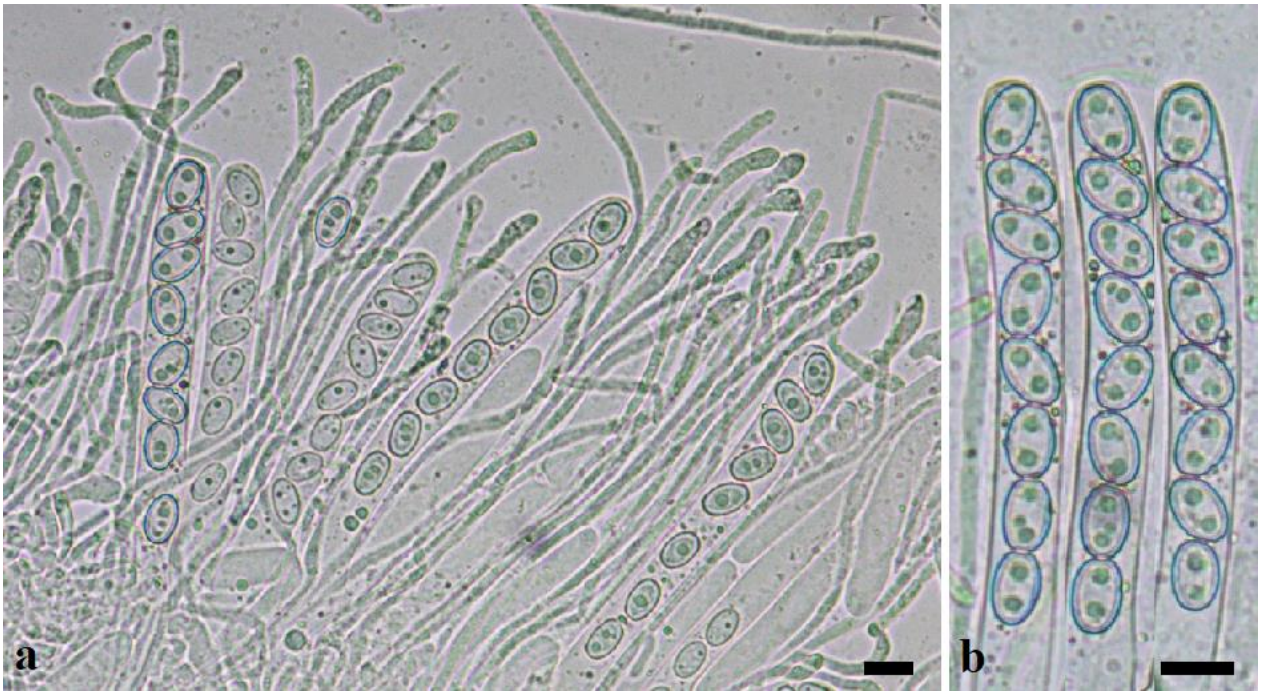


Figure 2. Asci (a,b), paraphyses (a) and ascospores (b) of *Arpinia luteola.* (in water) (bars: 10 µm)

(Berthet, 1974; Benkert, 1980; Geesink, 1982; Hohmeyer, 1988; Fernández Vicente and Undagoitia, 2004; Rubio and Sánchez, 2005; Perry et al., 2007; Carbone et al., 2021) except *A. fusispora* which was determined under beech, *Quercus* and *Fagus* spp. Though the type species of *A. luteola* was collected under *Larix* sp., our collection was made under *Populus* sp. One of the collection of synonymised *A. luteola* var. *pallidorosea* was also made from decidious forest (Hohmeyer, 1988).

**Conflict of Interest**

Authors have declared no conflict of interest.

**Authors' Contributions**

The authors contributed equally.

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*Anatolian Journal of Botany*
*7(2): 135-139 (2023)*
*Research article* *doi:10.30616/ajb.1337945*

# Antifungal activity of *Eugenia caryophyllata, Cinnamomum* sp., *Mentha piperita,* and *Thymus vulgaris* essential oils against *Aspergillus niger*

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Received : 04.08.2023
Accepted : 31.08.2023
Online : 01.09.2023

# *Eugenia caryophyllata, Cinnamomum* sp., *Mentha piperita* ve *Thymus vulgaris* esansiyel yağların *Aspergillus niger* suşuna karşı antifungal aktivitesi

**Abstract:** *Aspergillus* species are pollutants found in both food and air. The increase in the metabolic activity of *Aspergillus* leads to spoilage of foodstuffs and large economic losses. In addition, some *Aspergillus* species have the ability to produce aflatoxins and ochratoxins, secondary metabolites called, namely, mycotoxins. Especially mycotoxins are very important in terms of food safety and human health. Since the protection of human and animal health and the prevention of economic losses is a very important issue, our study aimed to determine the antifungal activity of *Eugenia caryophyllata* Thunb., *Cinnamomum* sp., *Mentha piperita* L. and *Thymus vulgaris* L. essential oils (EO) against *Aspergillus niger* Tiegh. NRRL 321 strain. In the second step, MIC and MFC values of EOs were determined. It was determined that the MIC value of *Cinnamomum sp, M. piperita* and *T. vulgaris* EOs was 0.01 µL/mL, and *E. caryophyllata* EO was 0.5 µL/mL. It was determined that *E. caryophyllata, Mentha piperita* and *Thymus vulgaris* EOs completely inhibited radial colony growth at MIC, 2xMIC and 4xMIC values. It was determined that the inhibition of radial growth of *Cinnamomum* sp. EO varies depending on the concentration, and the inhibition rate increases as the concentration increases. As a result, evaluations should be made considering the in vivo conditions that the tested EOs showed strong antifungal activity against the *A. niger* strain.

**Key words:** Antifungal activity, cinnamon oil, clove oil, peppermint oil, thyme oil

**Özet:** *Aspergillus* türleri hem gıdalarda hem de havada bulunan kirleticilerdir. *Aspergillus* cinsinin metabolik aktivitesindeki artış, gıda maddelerinin bozulmasına ve büyük ekonomik kayıpların oluşmasına yol açmaktadır. Ayrıca bazı *Aspergillus* türleri, aflatoksinler, okratoksinler olarak adlandırılan sekonder metabolitleri yani mikotoksinleri üretme yeteneğine sahiptir. Özellikle mikotoksinler, gıda güvenliği ve insan sağlığı açısından oldukça önemlidir. İnsan ve hayvan sağlığının korunması ve ekonomik kayıpların önlenmesi çok önemli bir konu olması sebebiyle, çalışma amacımız *Aspergillus niger* Tiegh. NRRL 321 suşuna karşı *Eugenia caryophyllata Thunb., Cinnamomum* sp., *Mentha piperita* L. ve *Thymus vulgaris* L. esansiyel yağlarının (EO) antifungal aktivitesi belirlenmiştir. İkinci adımda ise EO'ların MİK ve MFK değerleri belirlendi. *Cinnamomum* sp., *M. piperita* ve *T. vulgaris* EO'ların MİK değerinin 0,01 µL/mL, *E. caryophyllata* EO'nun ise 0,5 µL/mL olduğu tespit edilmiştir. *E. caryophyllata, M. piperita* ve *T. vulgaris* EO'ların MİK, 2xMİK ve 4xMİK değerlerinde radyal koloni gelişimini tamamen inhibe ettiği belirlendi. *Cinnamomum* sp. EO'nun ise radyal büyümenin inhibisyonu konsantrasyona bağlı olarak değiştiği, konsantrasyon arttıkça inhibisyon oranın arttığı belirlenmiştir. Sonuç olarak test edilen EO'ların *A. niger* suşuna karşı güçlü antifungal aktivite gösterdiği, in vivo koşullar göz önüne alınarak değerlendirmelerin yapılması gerekmektedir.

**Anahtar Kelimeler:** Antifungal aktivite, karanfil yağı, kekik yağı, nane yağı, tarçın yağı

**Citation:** Özcan Ateş G (2023). Antifungal activity of *Eugenia caryophyllata, Cinnamomum* sp., *Mentha piperita,* and *Thymus vulgaris* essential oils against *Aspergillus niger*. Anatolian Journal of Botany 7(2): 135-139.

## 1. Introduction

*Aspergillus* species are very common both indoors and outdoors, producing numerous small, airborne spores and versatile metabolites. It has been determined that their presence in the air causes various ailments of the lungs, liver, skin and muscles (Klich et al., 2009; Kumar et al., 2017). Studies have shown that there is a relationship between upper respiratory tract, cough, wheezing and asthma symptoms in susceptible individuals, as well as severe respiratory tract infections in immunocompromised individuals and the presence of mould indoors. Studies examining indoor mould distributions have reported that the presence of *Aspergillus* species or teleomorphs are present in almost every environment and affects human health. In addition, mycotoxins (aflatoxins, fumonisins, ochratoxins, etc.) produced by the *Aspergillus* species have been found to be carcinogens, mutagens, teratogens and immunosuppressants (Klich et al, 2009; Prakash et al., 2015; Kumar et al., 2017). In addition to mycotoxin production, *Aspergillus* species also produce enzymes that can degrade many different organic substrates (Fogarty, 1994; Hua et al., 2014). Some species are xerophilic and can thrive at relatively low humidity. Indoors, they can thrive on wood, paper, paint, glue and even dirty metal doors when humidity is high. *Aspergillus* species in soil tend to occur in subtropical and temperate climates, with most of the pathogenic species in these environments (Klich, 2002; Hua et al., 2014). The most frequently reported species in soil are *A. fumigatus* Fresen, *A. niger* Tiegh., *A. flavus* Link, and *A. terreus* Thom. All four of these species are equally distributed in all biomes. It is probably not a coincidence that these are the most frequently reported species in human disease (Klich, 2002; Klich et al., 2009; Hua et al., 2014).

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Since ancient times, medicinal and aromatic plants have found wide use in many different fields. They have been widely used in various industrial areas such as traditional medicine, agriculture, food (as preservatives and sweeteners), perfumery, cosmetics and the pharmaceutical industry. Regarding the biological properties of essential oils (EOs) and their plant extracts, they have recently gained significant scientific popularity and interest. The relationships between the composition and biological properties of EOs have been investigated. Phenolic compounds in EOs have been identified and accepted in the literature as bioactive components with antimicrobial activity. (Burt, 2004; Nedorostova et al., 2009).

Therefore, to fill these gaps, the antifungal activity of commercially available EOs from *Eugenia caryophyllata, Cinnamomum* sp., *Mentha piperita* and *Thymus vulgaris* against the *A. niger* strain was evaluated. First of all, the antifungal activity was determined by the agar well diffusion method. Then, minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of EOs were determined. After the MIC values were determined, the effect of EOs at different concentrations on the radial growth of *A. niger* was investigated.

## 2. Materials and Method

### 2.1. Materials

*E. caryophyllata* and *Cinnamomum* sp. EOs Kırıntı Baharat A.Ş. (Kocaeli, Türkiye), *M. piperita* and *T. vulgaris* EOs Toroslar Group A.Ş. (Adana, Türkiye) were provided.

*A. niger* Tiegh. NRRL 321 stock culture was first inoculated on Potato Dextrose Agar (PDA) medium, and the culture was revived to check its purity.

### 2.2. Method

#### 2.2.1. Preparation of the spore suspension

*Aspergillus* strain was grown on slanted Malt Extract Agar (MEA) media at 25°C until well sporulated (7-10 days). Spores were collected by adding sterilized Tween 80 solution (0.1% v/v). Spore concentration was set at McFarland 0.5 density. The suspensions prepared to determine the MIC value were then diluted 1:10 with distilled water to obtain the final working inoculum, with a density of 2-5x$10^5$ cfu/mL.

#### 2.2.2. Determination of antifungal activity by agar well diffusion method

The antifungal activity of *E. caryophyllata, Cinnamomum* sp., *M. piperita* and *T. vulgaris* EOs was used in the method specified in NCCLS M44-A, modified by drilling a 6 mm diameter well with a cork borer set instead of a disc. For the agar well diffusion method, after planting 0.1 mL of the prepared spore solutions on the PDA medium according to the smear plate method, 20 µL of EO was added to each opened well. Zone diameters were measured after 3-5 days of incubation at 25°C (Snoussi et al., 2018; Mseddi et al., 2020). The study was carried out in 3 parallels. Amphotericin B (20 IU, 21,19 mcg, Bioanalyse, Türkiye) and fluconazole (25 mcg, Himedia, India), antifungal discs were used as positive controls.

#### 2.2.3. Determination of minimum inhibitory concentration (MIC)

MIC values were determined with minor modifications to the standard NCCLS M38-A2 method. RPMI 1640 medium (Himedia, India) containing 0.165 M MOPS with 2X essential oil and 3% DMSO was dispensed with a multichannel pipette into the wells of rows B and G microdilution plates in 100 µL volumes. Then 100 µL of spore solution was added to it. The final concentrations were 4, 2, 1, 0.5, 0.25 and 0.01 µL/mL. Row A of microplates contains RPMI 1640 broth + 3% DMSO medium containing 4 µL/mL EO and is determined as a negative control, and the last row (H) is only RPMI 1640 3% DMSO medium + culture and is defined as a positive control. Microplates were evaluated after 24-48 hours of incubation at 25°C. The first well without growth was determined as the MIC value. The MFC value was determined after the incubation at the appropriate temperature and time by planting with the drip planting method from the well defined as the MIC value and the subsequent two wells into the PDA medium (Snoussi et al. 2018; Mseddi et al., 2020). The study was carried out in three parallels.

#### 2.2.4. Effect of molds on radial growth of mycelium

Soliman and Badeaa (2002) and Gámez-Espinosa et al. (2021) by modifying the methods specified. To analyze the effect of essential oils on the radial growth of mould mycelium, the *A. niger* was grown on a PDA medium at 25°C for 7-10 days. PDA media containing 3% DMSO at 1⁄4xMIC, 1⁄2xMIC, MIC, 2xMIC, and 4xMIC concentrations of EOs whose MIC values were determined were prepared and poured into 60 mm diameter petri dishes. Single-point planting was done in the middle of the petri dish with the help of a needle loop from cultures developed in PDA medium. Petri dishes were incubated at 25°C for 7-14 days, and colony diameters were measured after incubation. As a control, inoculation was made on a PDA medium containing 3% DMSO. Petri dishes were incubated at 25°C for 7-14 days. Zone diameters were measured at the end of incubation. The study was carried out in three parallels. Inhibition of radial growth was calculated with the following equation: %I=(C-T)/C*100 (C: growth diameter (mm) in the control plate, T: growth diameter (mm) in the essential oil-containing petri dish), I: inhibition (%)).

#### 2.2.5. Statistical analysis

Results are given as mean (M) ± standard deviation (SD).

## 3. Results

The data obtained in this study were collected through the sequential application of different techniques to evaluate the antifungal activity of *E. caryophyllata, Cinnamomum* sp., *M. piperita* and *T. vulgaris* EO against *A. niger* NRRL 321. The first step was determining whether EOs have antifungal activity against the tested *A.niger* NRRL 321. For this purpose, the agar well diffusion method was used. Agar diffusion method results in mm are given in Table 1.

According to the results in Table 1, it was found that EOs of *E. caryophyllata, Cinnamomum* sp., *M. piperita*, and *T. vulgaris* against the tested *A. niger* NRRL 321 strain significantly inhibited the growth of amphotericin B antifungal agent. It was determined that *T.vulgaris* EO formed a very high inhibition zone compared to the positive

control amphotericin B antifungal. In the second step of the study, the MIC, MFC, and MFC/MIC values of the tested EOs were evaluated using the microdilution method; the results are given in Table 2.

**Table 1**. Agar disc diffusion method zone diameters in mm (M±SD)

| EO | *Aspergillus niger* NRRL 321 |
|---|---|
| *Cinnamomum sp.* | 9.54 ± 0.56 |
| *E. caryophyllata* | 12.60 ± 1.33 |
| *M. piperita* | 13.08 ± 1.85 |
| *T. vulgaris* | 36.20 ± 2.61 |
| AMP | 13.29 ± 3.48 |
| FLU | 90.00 ± 0.01 |

**Table 2.** MIC, MFC value and MFC/MIC ratio of selected EOs in µL/mL against *Aspergillus niger* NRRL 321

| EO | MIC | MFC | MFC/MIC |
|---|---|---|---|
| *Cinnamomum* sp. | 0.01 | 0.01 | 1 |
| *E. caryophyllata* | 0.5 | 4 | 8 |
| *M. piperita* | 0.01 | 0.01 | 1 |
| *T. vulgaris* | 0.01 | 0.01 | 1 |

When evaluated considering the microplate concentrations with the microdilution method, the MIC value of the first well where no growth was observed was determined. Accordingly, according to the results in Table 2, the MIC value of *Cinnamomum* sp., *M. piperita* and *T. vulgaris* EOs was determined to be 0.01 µL/mL. The MIC value of *E. caryophyllata* EO was determined to be 0.5 µL/mL. After the MIC value was determined, the wells with the MIC value on the plates and the next two wells were inoculated into the PDA medium by drip planting method. Afterwards, the PDA petri dishes were incubated at the appropriate time and temperature. The first concentration at which growth was not observed after incubation was determined as MFC. The MFC value of *Cinnamomum* sp., *M. piperita*, and *T. vulgaris* EOs was determined to be 1 µL/mL. The MFC value of *E. caryophyllata* EO was determined to be 4 µL/mL. As mentioned in Gatsing et al., 2009, Snoussi et al., 2018 and Mseddi et al. (2020), when we evaluated whether the EOs were fungicidal (MFC/MIC ratio ≤ 4) or fungistatic (MFC/MIC ratio > 4), it was determined that *Cinnamomum* sp., *M. piperita* and *T. vulgaris* EOs were fungicidal agents. *E. caryophyllata* EO was determined to be a fungistatic agent.

In the third step of our study, the effect of EOs, whose MIC value was determined, on the radial growth of *A. niger* NRRL 321 strain was also evaluated. While determining the effect on radial growth, radial growth inhibition of EOs at 1⁄4xMIC, 1⁄2xMIC, MIC, 2xMIC, and 4xMIC concentrations was investigated in line with MIC values. The results regarding radial growth inhibition are given in Table 3. It was determined that *T. vulgaris* EO completely inhibited the radial growth of the tested *A. niger* NRRL 321 strain from 1⁄2xMIC concentration. *E. caryophyllata* and *M. piperita* EO were found to inhibit radial growth at the MIC value and all subsequent concentrations. *Cinnamomum* sp. EO was found to inhibit radial growth at the MIC value and the following concentrations, with a maximum inhibition of 46.41 ± 3.20%. As clearly seen in Table 3, the inhibition rate increases with the concentration.

## 4. Discussions

The results of this study highlight the antifungal activities of herbs and essential oils, offering a promising area of research for alternative therapies and food safety.

*T. vulgaris* EO inhibited radial growth by inhibiting *A. flavus, A. parasiticus* and *A. ochraceus* isolates (Soliman and Badeaa, 2002). Kumar et al. (2008) reported that *T. vulgaris* EO at a concentration of 0.7 µL/mL completely inhibited the radial growth of *A. flavus, A. terreus* and *A. fumigatus*, while it provided 89.56% ± 3.06% inhibition on *A niger*. Al-Shahrani et al. (2017) determined the MIC and MFC values of *T. vulgaris* EO, which they obtained from Saudi Arabia, as 10 mg/mL for *A. flavus* and *A. niger*.

Mahboubi and Kazempour (2014) determined that the MIC and MFC values of *M. piperita* EO against *A. niger* were 0.5 µL/mL and 1 µL/mL, respectively, while Hu et al. (2019) found 2 mg/ml for *A. niger*. Desam et al. (2019) 1.0 µl of *M. piperita* EO gave an inhibition zone of 30.08 ± 0.08 mm against *A. fumigatus* isolate and 17.23 ± 0.23 mm for *A. variecolor*, and the MIC value of *A. fumigatus* and *A. variecolor* determined 0.50 µg/mL and 10 µg/mL respectively for isolates. The study determined that the MIC and MIC values of *M. piperita* EO were 0.01 µL/mL and completely inhibited radial growth at MIC, 2xMIC, and 4xMIC concentrations.

*Cinnamomum* sp. EO was also effective on *Aspergillus* species (Thanaboripat et al., 2007; Pekmezovic et al., 2015; Lappa et al., 2017; Moghadam et al., 2019). Hu et al. (2019) found that the MIC value of *Cinnamomum* sp. EO was 0.0625 mg/mL for *A. niger*, and Yan et al. (2021) determined it as 62.50 µL/L.The study determined that a MIC value of 0.01 µL/mL and 4xMIC concentration inhibited *A. niger* strain by 46.41% ± 3.20%. Similarly, the activity of *E. caryophyllata* EO on *Aspergillus* species was determined (Soliman and Badeaa, 2002; Vijayalakshmi et al., 2014). Hu et al. (2019) reported that the MIC value of *Eugenia caryophyllata* EO against *A. niger* isolates was 0.25 mg/mL. This study determined that the MIC value of *E. caryophyllata* EO against the tested *Aspergillus* strain

**Table 3.** Radial growth inhibition rate (in %)

| EO | 1⁄4 x MIC | 1⁄2 x MIC | MIC | 2 x MIC | 4 x MIC |
|---|---|---|---|---|---|
| *E. caryophyllata* | 59.83 ± 7.86 | 78.18 ± 4.90 | 100.00 ± 0.01 | 100.00 ± 0.01 | 100.00 ± 0.01 |
| *Cinnamomum sp.* | 0.00 ± 0.01 | 0.00 ± 0.01 | 43.18 ± 6.15 | 43.07 ± 5.42 | 46.41 ± 3.20 |
| *M. piperita* | 61.55 ± 0.27 | 81.42 ± 5.43 | 100.00 ± 0.01 | 100.00 ± 0.01 | 100.00 ± 0.01 |
| *T.vulgaris* | 100.00 ± 0.01 | 100.00 ± 0.01 | 100.00 ± 0.01 | 100.00 ± 0.01 | 100.00 ± 0.01 |

was 0.5 µL/mL, and the lowest radial growth inhibition was 50% even at 1⁄4xMIC concentration.

As a result, the antimicrobial properties of herbal sources are important when considering the problem of drug resistance. However, safety and toxicity assessments are required before using these plant oils for medicinal or food purposes. It is concluded that this study will encourage further research in the future and may assist in developing alternative therapeutic agents.

**Acknowledgements**

This study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit with project number THD-2021-3571. This study was conducted in Çanakkale Onsekiz Mart University, Faculty of Science, Biology Department, Basic and Industrial Microbiology Laboratory. For Basic and Industrial Microbiology Laboratory use, I would like to thank Dr.Tülay Bican Süerdem.

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*Anatolian Journal of Botany*
*7(2): 140-145 (2023)*
*Research article* *doi:10.30616/ajb.1343823*

# Cytotoxicity of *Usnea longissima* Ach. extracts and its secondary metabolite, usnic acid on different cells

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Received : 15.08.2023
Accepted : 03.09.2023
Online : 14.09.2023

# *Usnea longissima* Ach. ekstraktları ve onun sekonder metaboliti usnik asidin farklı hücreler üzerindeki sitotoksisitesi

**Abstract:** The biological activities of lichens, known as organisms based on a symbiotic relationship, are attracting more and more attention in traditional medicine and modern drug research. Lichens can possess various pharmacological effects such as antimicrobial, antioxidant, antitumor, anti-inflammatory, and many others due to the bioactive compounds they contain. In the present study, *Usnea longissima* Ach. and its secondary metabolite, usnic acid on human gastric adenocarcinoma cells (AGS), human colorectal adenocarcinoma cells (Caco-2), and mouse fibroblasts (NIH/3T3) were investigated. In this context, methanol and water extracts from *U. longissima* were obtained by Soxhlet extractor. The characterization of usnic acid was carried out by fourier transform infrared spectroscopy (FTIR). The cytotoxic activities of the extracts and the metabolite on cells were determined by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) analysis. Considering the median inhibitory concentration ($IC_{50}$) values, the application with the greater effect on AGS and NIH/3T3 cells was the methanol extract (373.17 µg/ml and 318.81 µg/ml, respectively). Considering the Caco-2 cells, it was determined that the water extract had the lowest $IC_{50}$ value (230.05 µg/ml). The high cytotoxic activity of usnic acid on cancer cells (AGS; $IC_{50}$: 395.03 µg/ml and Caco-2; $IC_{50}$: 462.35 µg/ml) compared to normal cell (NIH/3T3; $IC_{50}$: 472.41 µg/ml) was noted. As a result, it has been revealed that methanol and water extracts of *U. longissima*, especially usnic acid, are products that can be used within the scope of complementary therapy.

**Key words:** Anticancer, extract, lichen, secondary metabolite

**Özet:** Simbiyotik bir ilişkiye dayanan organizmalar olarak bilinen likenlerin biyolojik aktiviteleri, geleneksel tıp ve modern ilaç araştırmalarında giderek daha fazla ilgi çekmektedir. Likenler, içerdikleri biyoaktif bileşikler sayesinde antimikrobiyal, antioksidan, antitümör, antiinflamatuar ve diğer pek çok farmakolojik etkiye sahip olabilir. Mevcut çalışmada, *Usnea longissima* Ach. ve onun sekonder metaboliti usnik asidin insan gastrik adenokarsinom hücreleri (AGS), insan kolorektal adenokarsinom hücreleri (Caco-2) ve fare fibroblastları (NIH/3T3) üzerindeki sitotoksik etkileri incelenmiştir. Bu kapsamda, *U. longissima*'dan metanol ve su ekstraktları Soxhlet ekstraktörü ile elde edilmiştir. Usnik asidin karakterizasyonu fourier dönüşümlü kizilötesi spektroskopisi (FTIR) ile gerçekleştirilmiştir. Ekstraktlar ve metabolitin hücreler üzerindeki sitotoksik aktiviteleri 2,3-bis-(2-metoksi-4-nitro-5-sülfofenil)-2H-tetrazolyum-5-karboksanilid (XTT) analizi ile tespit edilmiştir. Medyan inhibitör konsantrasyonu ($IC_{50}$) değerleri dikkate alındığında, AGS ve NIH/3T3 hücreleri üzerinde daha fazla etkili olan uygulama metanol ekstraktı olmuştur (sırasıyla, 373.17 µg/ml ve 318.81 µg/ml). Caco-2 hücreleri dikkate alındığında, su ekstraktının en düşük $IC_{50}$ değerine (230.05 µg/ml) sahip olduğu belirlenmiştir. Usnik asidin normal hücreye (NIH/3T3; $IC_{50}$: 472.41 µg/ml) kıyasla kanserli hücreler (AGS; $IC_{50}$: 395.03 µg/ml ve Caco-2; $IC_{50}$: 462.35 µg/ml) üzerindeki yüksek sitotoksik etkinliği dikkat çekmiştir. Sonuç olarak, başta usnik asit olmak üzere *U. longissima*'nın metanol ve su ekstraktlarının tamamlayıcı tedavi kapsamında kullanılabilecek ürünler olduğu ortaya çıkmıştır.

**Anahtar Kelimeler:** Antikanser, ekstrakt, liken, sekonder metabolit

**Citation:** Aslan Engin T, Emsen B, Yılmaz Öztürk R, Çakır Koç R, İnan B, Özçimen D (2023). Cytotoxicity of *Usnea longissima* Ach. extracts and its secondary metabolite, usnic acid on different cells. Anatolian Journal of Botany 7(2): 140-145.

## 1. Introduction

Lichens are unique organisms formed from a symbiotic association between fungi and green algae or cyanobacteria (Ranković and Kosanić, 2019). *Usnea longissima* Ach. is a species with a long and vine-like structure that can have different structures of lichens. It is usually wrapped around tree branches or other surfaces and can sometimes be quite tall (Storaunet et al., 2014; Esseen and Ekström, 2023). This species plays an important role in ecosystems, providing shelter and a food source for organisms living in natural environments. On the other hand, it can also be used as a biological indicator for monitoring air quality (Emsen and Aslan, 2018).

The medical importance of *U. longissima* is related to its use in traditional medicine and some cultures (Rajeswari et al., 2019). In traditional medicine, certain types of lichens are associated with antibacterial properties (Somphong et al., 2023), antifungal (Rajendran et al., 2023b) and anti-inflammatory (Rajendran et al., 2023a) properties. For example, some ancient physicians suggested the use of

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lichens in the treatment of wounds and skin diseases (Guo et al., 2008). Some studies have shown that certain compounds in the content of lichens have potential biological activities. For instance, research on the antimicrobial properties of lichens has shown that they can potentially inhibit the growth of bacteria and fungi (Yang et al., 2023). In addition, many studies have been carried out on cytotoxic activities on different cells (Şahin et al., 2021; Ureña-Vacas et al., 2022).

Lichens such as *U. longissima* contain secondary metabolites with many biologically active compounds (Bharti et al., 2022). These compounds are believed to be the defense mechanisms of lichens against environmental stresses. Moreover, it is known that some of these compounds may possess antimicrobial, antiviral, antioxidant, and other pharmacological activities (Adenubi et al., 2022). Usnic acid, the most common secondary metabolite in lichens, is found in *U. longissima* (Divya Reddy et al., 2019). As a chemical structure, usnic acid, a phenolic acid, is a molecule containing a carbohydrate moiety and a phenolic ring. Usnic acid is a compound known to have antimicrobial properties. Its inhibitory effect on bacteria and fungi is considered one of the defense mechanisms of lichens against environmental stresses (Popovici et al., 2022). Therefore, usnic acid may help lichens to gain competitive advantage in their habitat. Research on the antibacterial (Bangalore et al., 2023), antiviral (Miah et al., 2023), antioxidant (Maulidiyah et al., 2023), and anticancer (Emsen et al., 2018) properties of usnic acid has drawn interest for its potential medical uses. In particular, it is thought to be used in traditional medicine to promote wound healing (Stoica Oprea et al., 2023), skin infections, and other health problems (Studzińska-Sroka et al., 2019).

In line with the above-mentioned potential of *U. longissima* and its secondary metabolite, usnic acid, we aimed to examine cytotoxic effects of different extracts of *U. longissima* and usnic acid on human gastric adenocarcinoma cells (AGS), human colorectal adenocarcinoma cells (Caco-2) and mouse fibroblasts (NIH/3T3).

## 2. Materials and Method

### 2.1. Collection and Identification of the Lichen Samples

*Usnea longissima* samples were collected from Oltu district of Erzurum province of Turkey. Samples photographed in their natural environment and were brought to the laboratory and dried. Identification was made with macroscopic and microscopic data using the literature (Purvis et al., 1992; Wirth, 1995). The species was taxonomically identified by Dr. Tubanur Aslan Engin.

### 2.2. Preparation of the Extracts and Isolation of Secondary Metabolite, Usnic Acid

Lichen specimens were air-dried at room temperature and then powdered. The extraction process involved obtaining methanol (ULME) and water (ULWE) extracts of *U. longissima* using a Soxhlet extraction apparatus with 250 mL solvent systems.

The resulting crude extract from the lichen sample was filtered using a Whatman No. 1 filter paper. The solvent was subsequently removed by evaporation through a rotary evaporator under vacuum conditions, leading to complete drying. The extract was further lyophilized to yield ultra-dry powders. The determination of functional groups within the organic compound structure and bonds present in the dried lichen powders was accomplished using a Bruker Alpha model Fourier Transform Infrared Spectroscopy (FTIR) device spectroscopy.

### 2.3. Cytotoxic Activity

The XTT method, employing 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, operates on the basis of metabolically active cells converting XTT, a tetrazolium salt, into orange formazan components. The absorbance increase, resulting from color formation, is directly proportional to the number of active cells. An advantageous aspect of the XTT method is its lack of necessity for extra agents or cell washing steps, while maintaining sensitivity even at low cell concentrations (Roehm et al., 1991). This study assessed the 24-hour cytotoxic impact by applying different extract concentrations from lichens on various cell lines, namely AGS, Caco-2, and NIH/3T3. Cells previously stored in liquid nitrogen were thawed, counted, and suspended in DMEM-F12 medium, then transferred to culture flasks and incubated under specified conditions.

For cell passage, trypsin-EDTA-treated cells were suspended, counted, and prepared for cytotoxicity tests. Seeding $10^4$ cells/well in sterile 96-well plates allowed adherence and proliferation over 24 hours before applying lichen extracts at varying concentrations for cytotoxicity assessment ($n$ = 3). Lichen extracts were sterilized through filtration before addition to cells, with dilutions prepared in DMEM-F12 medium. Following 24-hour incubation at 37°C in a 5% carbon dioxide incubator, the XTT test was conducted. Extract concentrations ranged from 50 to 1000 µg/ml, with 2% dimethyl sulphoxide (DMSO) serving as the negative control. Extract-containing media was replaced, and wells received 100 µL of 0.5 mg/mL XTT solution with phenazine. Plates were further incubated for 4 hours at 37°C, and absorbance at 450 nm was measured using a multi-plate reader to calculate cell viability as a percentage using the formula: Viability = (Extract absorbance / Control absorbance) × 100.

### 2.4. Statistical Analyses

The activities of the treatments were analysed one-way ANOVA followed by Duncan test. Probit regression analysis was used to calculate the median inhibitor concentration ($IC_{50}$) values. Heatmap analysis was utilized to investigate the similarities and dissimilarities of viabilities among the cells. All analyses were done using SPSS (version 21.0, IBM Corporation, Armonk, NY, USA).

## 3. Results and Discussion

### 3.1. Characterization of Lichen Samples

FTIR spectrums of lichen sample and usnic acid were presented in Figure 1. The absorption between the range of 3600 and 3100 $cm^{-1}$ can be assigned to the stretching vibrations of the OH groups which might be responsible for the moisture content (İnan and Özçimen, 2021). 3000-2800 corresponded to methylene C-H asymmetric stretching vibration, 1750-1500 and 1200-1000 $cm^{-1}$ which representing C=O stretching functional group and C-O functional group respectively (Bakar et al., 2014). The

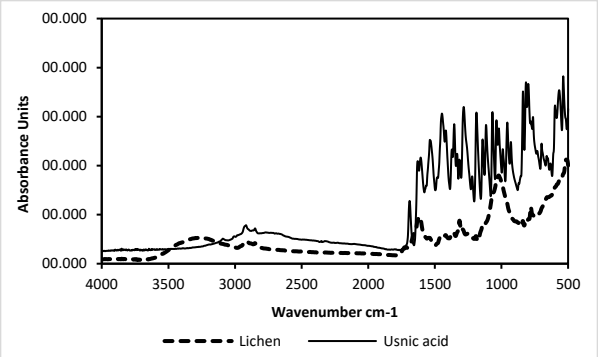


**Figure 1.** FTIR spectrums of lichen sample and usnic acid

specific bands were observed around 1690 $cm^{-1}$ and 1540 $cm^{-1}$ indicated a non-chelated aromatic ketone and conjugated chelated carbonyl (Karabacak et al., 2014).

### 3.2. XTT Viability Test

The cytotoxic effect of lichen extracts was examined on AGS, Caco-2, and NIH/3T3 cell lines. The cytotoxic effects of the lichen extracts and usnic acid on the AGS cell lines are presented in Figure 2. It was observed that the anticancer activity of the methanol and water extracts of the lichens increased with increasing concentrations. Moreover, it was found that the methanol extracts of lichen decreased the viability of the AGS cells more than the water extracts. The effect of usnic acid was almost similar to the water extracts of the lichens. The most effective concentration was found to be 1000 µg/ml of methanol extracts of lichen, with a 31% viability of AGS cells. The p-values of the concentrations of 50 and 100 µg/ml of ULME and ULWE were not significant, as can be seen in Figure 2, indicating no effect on the AGS cells. In addition to that, $IC_{50}$ values of ULME, ULWE, and usnic acid were found to be 373.17, 423.21, and 395.03 µg/ml, respectively. According to these values, the most effective application was ULME on AGS cells (Table 1).

As for the Caco-2 cells, cytotoxic activity of the lichen extracts was determined at a concentration of 50 µg/ml (Fig. 3). Similar to the anticancer activity results of the AGS cells, viability of the Caco-2 cells decreased with increasing concentrations. However, the lichen extracts and usnic acid were more effective on Caco-2 cells in comparison with the AGS cells. Cell viability rates at concentrations of 500-1000 µg/ml of ULWE and ULME were not significantly different ($p > 0.05$) from each other. The IC50 values for ULME, ULWE, and usnic acid were found to be 368.02, 230.05, and 462.35 µg/ml, respectively. According to these values, the most effective application was ULWE on Caco-2 cells (Table 1).

In Figure 4, the cytotoxic effect of ULME, ULWE, and usnic acid on NIH/3T3 cells was shown. It was observed that lichen extracts showed a cytotoxic effect on NIH/3T3 cells starting at a concentration of 50 µg/ml. In this analysis, ULME ($IC_{50}$: 318.81 µg/ml) was found to be the most effective on fibroblast cells in comparison with ULWE ($IC_{50}$: 367.28 µg/ml) and usnic acid ($IC_{50}$: 472.41 µg/ml). Between the concentrations of 50-1000 µg/ml, viability of NIH/3T3 cells decreased from 81% to 34% during the experiments. Statistically, the p-values of ULWE and usnic acid at a concentration of 50 µg/ml were not statistically

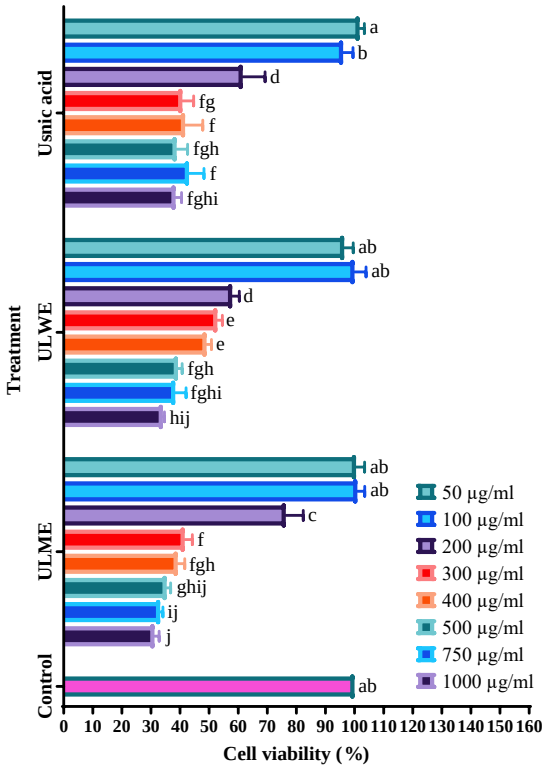


**Figure 2.** Viability rates obtained by XTT analysis in AGS cells treated with *U. longissima* extracts and its secondary metabolite (Mean ± Standard Deviation, $n = 3$) (Values indicated by different letters differ from each other at the level of $p < 0.05$). ULME: Methanol extract of *U. longissima*; ULWE: Water extract of *U. longissima*.

**Table 1.** $IC_{50}$ values (µg/ml) resulting from cytotoxic activities of *U. longissima* extracts and its secondary metabolite on different cells

| Cell | Treatment | IC50 (Limits) | Slope ± Standard error of the mean (Limits) |
|---|---|---|---|
| AGS | ULME | 373.17 (349.35–397.75) | 1.18 ± 0.09 (1.16–2.03) |
| | ULWE | 423.21 (392.79–457.03) | 1.66 ± 0.07 (1.51–1.81) |
| | Usnic acid | 395.03 (364.87–428.01) | 1.49 ± 0.08 (1.32–1.66) |
| Caco-2 | ULME | 368.02 (347.41–390.01) | 1.96 ± 0.07 (1.82–2.10) |
| | ULWE | 230.05 (207.35–254.06) | 1.18 ± 0.06 (1.05–1.30) |
| | Usnic acid | 462.35 (402.44–539.25) | 0.82 ± 0.08 (0.66–0.99) |
| NIH/3T3 | ULME | 318.81 (286.90–354.88) | 0.97 ± 0.05 (0.86–1.09) |
| | ULWE | 367.28 (335.22–404.13) | 1.29 ± 0.06 (1.16–1.42) |
| | Usnic acid | 472.41 (412.94–549.49) | 0.83 ± 0.06 (0.71–0.95) |

ULME: Methanol extract of *U. longissima;* ULWE: Water extract of *U. longissima*

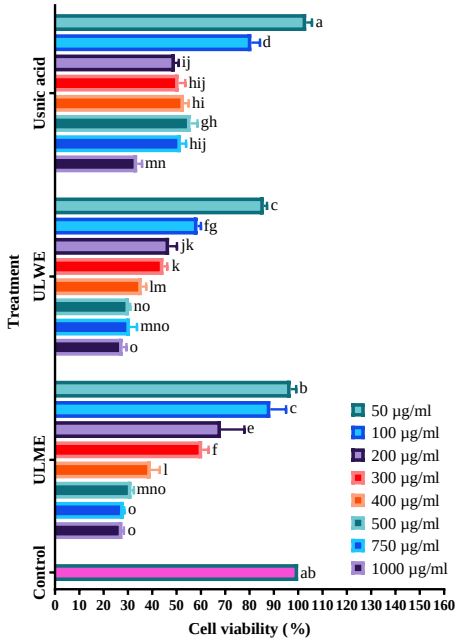


**Figure 3.** Viability rates obtained by XTT analysis in Caco-2 cells treated with *U. longissima* extracts and its secondary metabolite (Mean ± Standard Deviation, $n = 3$) (Values indicated by different letters differ from each other at the level of $p < 0.05$). ULME: Methanol extract of *U. longissima*; ULWE: Water extract of *U. longissima*.

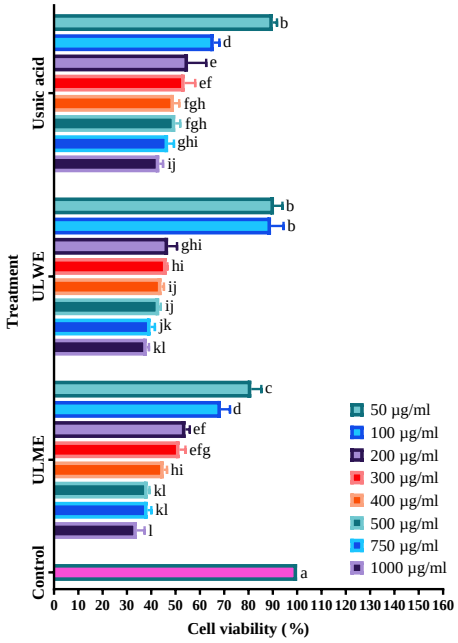


**Figure 4.** Viability rates obtained by XTT analysis in NIH/3T3 cells treated with *U. longissima* extracts and its secondary metabolite (Mean ± Standard Deviation, $n = 3$) (Values indicated by different letters differ from each other at the level of $p < 0.05$). ULME: Methanol extract of *U. longissima*; ULWE: Water extract of *U. longissima*.

significant. Similarities and differences of different concentrations of ULME, ULWE, and usnic acid on the cells were measured by heatmap analysis. Accordingly, the 50 µg/ml applications of all treatments showed a green gradient on all cells, indicating low cytotoxicity. In addition, the experiments at 500-1000 µg/ml showed high cytotoxicity and were close to each other with the red gradient (Figure 5).

ULME and ULWE exhibited distinct effects on cancer cell lines, with variations likely attributed to factors such as extract concentration, the specific type of cancer cell being tested, and the duration of exposure during cytotoxicity analysis. For example, a study was conducted focusing on eight different lichens and their impact on various cancer cell lines, including L1210 (lymphocytic leukemia), 3LL (Lewis lung carcinoma), K562 (chronic myelogenous leukemia), U251 (glioblastoma), DU145 (prostate carcinoma), and MCF7 (breast adenocarcinoma). The study also included non-cancerous cells represented by the Vero cell line (African green monkey kidney cell line). The results indicated that certain lichens demonstrated heightened activity in terms of cytotoxic effects. Notably, the diethyl ether fractions of *Cladonia convoluta* and *C. rangiformis*, along with the n-hexane fraction of *P. caperata*, exhibited particularly noteworthy anticancer potential (Bézivin et al., 2003).

The researchers conducted a study exploring the impact of methanol extracts from lichens, namely *Parmelia sulcata*, *Flavoparmelia caperata*, Evernia prunastri, *Hypogymnia physodes*, and *Cladonia foliacea*, on colon cancer cells (HCT-116). These extracts were tested across concentrations ranging from 50 to 1000 µg/ml for durations of 24 and 72 hours. The most significant inhibition of cell growth was observed at the highest concentration (1000 µg/mL) after both exposure periods. Notably, extracts from

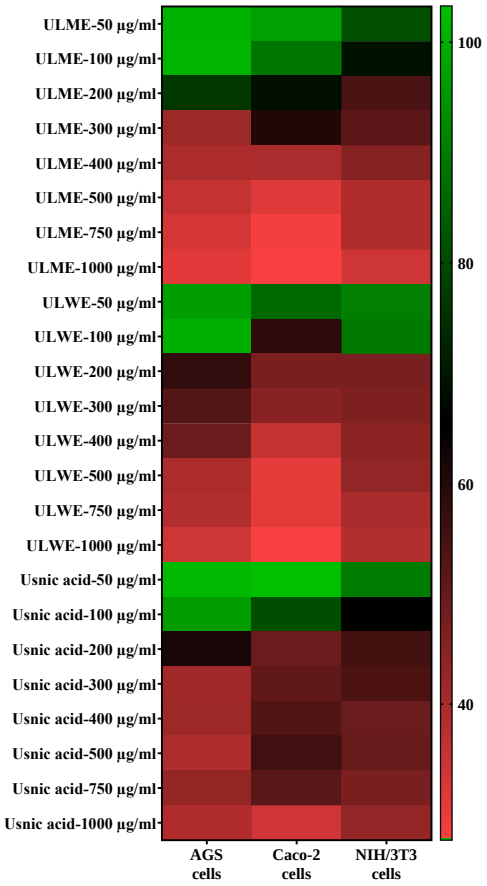


**Figure 5.** Heatmap of viability rates obtained in the cells treated with different concentrations of *U. longissima* extracts and its secondary metabolite. (High and low activities were represented by green and red colour, respectively). ULME: Methanol extract of *U. longissima*; ULWE: Water extract of *U. longissima*.

*Flavoparmelia caperata*, *Hypogymnia physodes*, and *Cladonia foliacea* exhibited considerable dose- and time-dependent suppression of cell growth (Mitrović et al., 2011). In another study, cytotoxic properties of lichens gathered from Vietnam National Park were investigated. One extract obtained from *Usnea fammea* Stirt., derived using methanol, demonstrated substantial cytotoxic effects against MCF-7 cells. However, these tested lichen extracts only displayed slight cytotoxicity on fibroblasts at a screening concentration of 100 µg/ml. The lichen extracts exhibited diverse cytotoxic activities against MO-91 cells, but the effective concentrations were over 50 mg/mL (Nguyen et al., 2019). In a separate study, the chemical composition of extracts from *Parmelia conspersa* and *Parmelia perlata* lichens was explored, assessing their antimicrobial, antioxidant, and anticancer attributes. The extracts' cytotoxic effects on RD, Hep2c, and L2OB cells ranged from 76.33 to 163.39 µg/mL (Manojlović et al., 2021). Lichen compounds induce cell death through various molecular mechanisms, including cell cycle arrest, apoptosis, necrosis, and inhibition of angiogenesis. Brisdelli et al. highlighted these mechanisms as the primary pathways through which lichen compounds exert their lethal effects (Brisdelli et al., 2013). The researchers emphasized that lichen compounds have been scientifically proven to possess anticancer properties, reinforcing their potential in combatting cancer cells (Manojlović et al., 2021).

**Conflict of Interest**

There is no conflict of interest in any form between the authors.

**Authors' Contributions**

The authors contributed equally.

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*Anatolian Journal of Botany*
*7(2): 146-153 (2023)*
*Research article* *doi: 10.30616/ajb.1342763*

# Macromycetes determined in Çamlı (Of-Trabzon) village and its environs

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Received : 14.08.2023
Accepted : 10.09.2023
Online : 14.09.2023

# Çamlı (Of-Trabzon) mahallesi ve çevresinde belirlenen makromantarlar

**Abstract:** The study was carried out on the macrofungi samples collected from Çamlı (Of-Trabzon) district and its close environs. As a result, 118 macrofungi species within 63 genera, 38 families, 8 orders and 3 classes belonging to *Ascomycota* and *Basidiomycota* were determined. Ten of the determined species belong to *Ascomycota* while 108 belong to *Basidiomycota*. In the region, the most crowded order is *Agaricales*, the most crowded families are *Inocybaceae* and *Psathyrellaceae*, and the most crowded genus is *Inocybe* in terms of taxa number. The determined species are listed together with their localities, habitats/substrates, geographical position, collection date and voucher numbers.

**Key words:** Biodiversity, macrofungi, Trabzon, Türkiye

**Özet:** Çalışma, Çamlı mahallesi (Of-Trabzon) ve yakın çevresinden toplanan makromantar örnekleri üzerinde gerçekleştirilmiştir. Sonuçta *Ascomycota* ve *Basidiomycota* bölümlerine ait 3 sınıf, 8 takım, 38 familya ve 63 cins içinde dağılım gösteren 118 makromantar türü belirlenmiştir. Belirlenen türlerden 10 tanesi *Ascomycota*, 108 tanesi ise *Basidiomycota* bölümüne aittir. Bölgede tür çeşitliliği bağlamında en kalabalık takım *Agaricales*, en kalabalık familyalar *Inocybaceae* ve *Psathyrellaceae*, en kalabalık cins ise *Inocybe*'dir. Yörede belirlenen türler, lokalitesi, habitatı/substratı, toplandığı coğrafi koordinat, toplanma tarihi ve toplayıcı numaraları ile birlikte listelenmiştir.

**Anahtar Kelimeler:** Biyoçeşitlilik, makromantarlar, Trabzon, Türkiye

**Citation:** Keleş A, Kaya A (2023). Macromycetes determined in Çamlı (Of-Trabzon) village and its environs. Anatolian Journal of Botany 7(2): 146-153.

## 1. Introduction

A study on macrofungal biodiversity of Çamlı and the surrounding villages (Dumlusu, İrfanlı, Kiraz, Yemişalan, Çamlıtepe) within Of district of Trabzon province was performed between 2018 and 2021. The area is situated between 40°52′-40°57′ northern latitudes and 40°14′-40°17′ eastern longitudes, and covers about %20 of the surface area of district Of (Fig. 1). Phytogeographically the research area falls in Colchis sector of the Euro-Siberian floristic area within Holarctic flora kingdom (Davis, 1965). According to meteorologic data of Trabzon meteorological station, the region has an annual average temperature of 14.8°C, and a rainfall of 828 mm. Members of *Alnus* Mill., *Carpinus* L., *Castanea* Mill., *Fagus* L., *Fraxinus* L., *Buxus* L., *Larix* Mill., *Pinus* L., *Rhododendron* L. and *Tilia* L. are the main components of forest vegetation of the region.

Some local lists (Sesli 1993, Keleş et al., 2014; Oruç et al., 2021; Uzun and Kaya, 2022) were presented within the boundaries of Rize and Trabzon provinces, and some new records were also presented from the region (Keleş, 2019a) and the neighboring regions (Keleş, 2019b, 2020; Sesli 2021, 2022, 2023; Keleş et al., 2022; Yeşilyurt et al., 2023). However, the current checklists (Sesli et al., 2020; Uzun, 2023) and the contributory studies, presented after checklists, indicate that the area is among the unstudied regions of Türkiye in terms of macrofungal biodiversity. The study aims to determine the macrofungal composition of the region, and to make a contribution to the mycobiota of Türkiye.

## 2. Materials and Method

Macromycete samples were collected from Çamlı and neighboring villages (Dumlusu, Yemişalan, Kiraz, Çamlıtepe, İrfanlı), including Of central district, during periodic field trips between 2018 and 2021. During field studies, available habitats were traced and upon finding the fruit bodies, necessary data related to macromorphology and ecology were noted and they were photographed at their natural habitats. Then some of them were collected and transferred to the fungarium in paper boxes. After drying, the samples were put in zip lock polyethylene bags as fungarium materials. Micromorphological data was obtained from the dry samples upon the investigations carried out under a light microscope. Twenty to 25 measurements were performed to obtain an average size of micromorphologic structures. Then the samples were identified by comparing the accumulated data with the relevant literature (Phillips, 1981; Breitenbach and Krânzlin, 1984-2000; Bresinsky and Besl, 1990; Hansen and Knudsen, 1992, 2000; Jordan, 1995; Bessette et al., 1997, 2007; Krânzlin, 2005; Bessette and Bessette, 2006; Antonin and Noordeloos, 2010; Beug et al., 2014; Siegel and Schwarz, 2016). The specimens are kept at Yüzüncü Yıl University.

## 3. Results

The determined taxa are listed in alphabethical order. Current names of the species are in accordance with IndexFungorum (2023).

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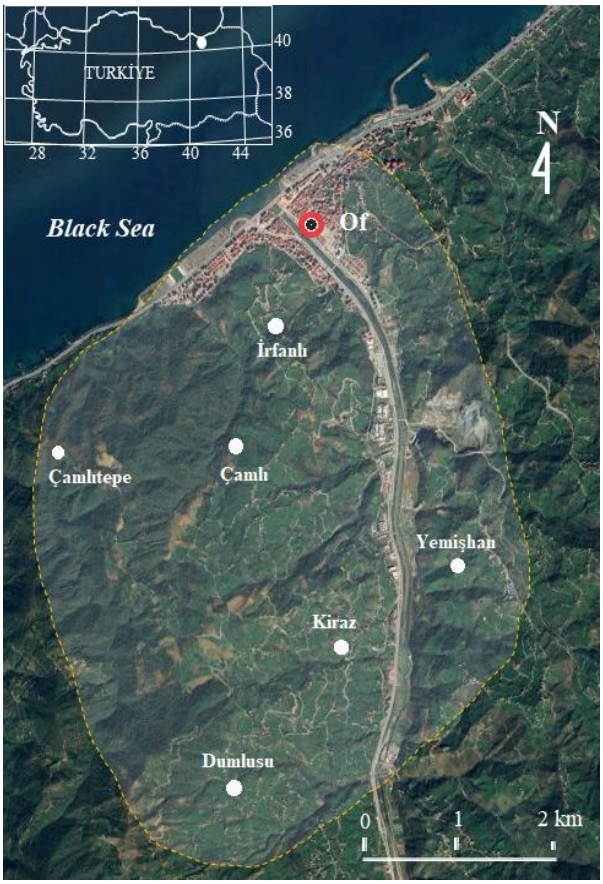


**Figure 1.** Macrofungi collection sites.

***Ascomycota*** Whittaker

***Pezizales*** J. Schröt.

***Helvellaceae*** Fr.

1. ***Dissingia leucomelaena*** (Pers.) K. Hansen & X.H. Wang

Çamlı village, aronud highways nursing station, among needle litter under *Pinus* sp., 40°55′N-40°16'E, 20 m, 12.10.2021, AK 5802.

2. ***Helvella compressa*** (Snyder) N.S. Weber

Yemişalan village, cemetary, under decidious wood, 40°54′N-40°17′E, 174 m, 23.10.2021, AK 5828.

3. ***Helvella crispa*** (Scop.) Fr.

İrfanlı village, around Ulusoy residences, under *Larix* sp., 40°56′N-40°15′E, 80 m, 12.10.2019, AK 5592.

**4.** ***Helvella elastica*** Bull.

İrfanlı village, around Ulusoy residences, under *Actinidia* Lindl. sp., 40°56′N-40°15′E, 80 m.,16.11.2019, AK 5517; aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16'E, 20 m, 16.09.2019, AK 5799; 23.10.2021, AK. 5843; Kiraz village, under *Pinus* sp., 40°54′N-40°15′E, 245 m., 16.11.2020, AK 5816; Çamlı village, Of Çay factory garden, under *Larix* sp., 40°55′N-40°16'E, 18 m, 07.12.2021, AK 5875.

**5.** ***Helvella latispora*** Boud.

İrfanlı village, around Ulusoy residences, under *Populus* L. sp., 40°56′N-40°15′E, 80 m, 28.10.2018, AK 5504.

**6.** ***Helvella macropus*** (Pers.) P. Karst.

Çamlı village, Of Çay factory garden, under *Larix* sp., 40°55′N-40°16'E, 18 m, 07.11.2019, AK 5661.

***Pezizaceae*** Dumort.

**7.** ***Peziza repanda*** Wahlenb. Ex Fr.

İrfanlı village, Ulusoy Multi-Program Anatolian Vocational High School garden, on decaying hardboards, 40°56′N-40°16′E, 8 m, 13.05.2021, AK 5751.

***Pyronemataceae*** Corda

**8.** ***Aleuria aurantia*** (Pers.) Fuckel

Dumlusu village, roadside, on soil, 40°53′N-40°16′E, 203 m, 24.10.2021, AK. 5860.

***Sarcoscyphaceae*** Le Gal ex Eckblad

**9.** ***Sarcoscypha coccinea*** (Jacq.) Lambotte

İrfanlı village, Ulusoy Multi-Program Anatolian Vocational High School garden, on soil, 40°56′N-40°16′E, 8 m, 13.05.2021, AK 5756.

***Sordariomycetes*** O.E. Erikss. & Winka

***Xylariales*** Nannf.

***Xylariaceae*** Tul. & C. Tul.

10. ***Xylaria polymorpha*** (Pers.) Grev.

Çamlı village, *Corylus* L. sp. garden, on decaying stump, 40°55′N-40°16′E, 148 m, 23.10.2021, AK 5661.

***Basidiomycota*** R.T. Moore

***Agaricales*** Underw.

***Agaricaceae*** Chevall.

11. ***Agaricus moelleri*** Wasser

İrfanlı village, Ulusoy Multi-Program Anatolian Vocational High School garden, under *Larix* sp., 40°56′N-40°16′E, 8 m, 07.11.2021, AK 5681;

12. ***Lepiota cristata*** (Bolton) P. Kumm.

Kiraz village, among grass in *Corylus* sp. garden, 40°54′N-40°15′E, 245 m, 07.07.2018, AK 5424.

13. ***Leucocoprinus cepistipes*** (Sowerby) Pat.

Dumlusu village, on processed dry tea remains, 40°53′N-40°16′E, 50 m, 12.10.2019, AK. 5574.

***Amanitaceae*** E.-J. Gilbert

14. ***Amanita caesarea*** (Scop.) Pers.

İrfanlı village, around Ulusoy residences, under Larix sp., 40°56′N-40°15′E, 80 m, 28.10.2018, AK 5490.

15. ***Amanita citrina*** Pers.

İrfanlı village, around Ulusoy residences, meadow 40°56′N-40°15′E, 80 m., 12.10.2021, AK 5798; İrfanlı village, around İrfaniye mosque, under decidious forest, 40°56′N-40°15′E, 140 m, 28.10.2018, AK 5509.

16. ***Amanita mairei*** Foley

Çamlı village, among grass in *Corylus* sp. garden, 40°55′N-40°16′E, 148 m., 12.10.2021, AK 5810.

17. ***Amanita muscaria*** (L.) Lam.

Yemişalan village, around flour mill, under Carpinus sp., 40°55′N-40°17′E, 169 m, 18.08.2020, AK 5714.

18. ***Amanita rubescens*** Pers.

Çamlı village, cemetary, on soil under Pinus sp., 40°55′N-40°15′E, 217 m, 07.07.2018, AK 5453.

19. ***Amanita vaginata*** (Bull.) Lam.

Çamlı village, among grass in Corylus sp. garden, 40°55′N-40°16′E, 148 m, 12.10.2021, AK 251, 5816.

***Bolbitiaceae*** Singer

20. ***Conocybe tenera*** (Schaeff.) Kühner

Dumlusu village, among grass in *Corylus* sp. garden, 40°52′N-40°16′E, 323 m, 16.10.2020, AK. 5718.

21. ***Conoybe rickenii*** (Jul. Schaff.) Kühner

Çamlı village, among grass under *Pinus* sp., 40°55′N-40°16'E, 20 m., 06.04.2019, AK 5536.

***Cortinariaceae*** Singer

22. ***Cortinarius cinnamoviolaceus*** M.M. Moser

Çamlı village, Of Çay factory garden, on soil among needle litter, 40°55′N-40°16'E, 18 m, 07.11.2019, AK 5663.

23. ***Galerina heterocystis*** (G.F. Atk.) A.H. Sm. & Singer

Çamlı village, aronud highways nursing station, among grass under *Pinus* sp., 40°55′N-40°16'E, 20 m, 01.01.2021, AK 5743.

***Entolomataceae*** Kotl. & Pouzar

24. ***Entoloma ameides*** (Berk. & Broome) Sacc.

Yemişalan village, under decidious forest, 40°54′N-40°17′E, 174 m, 18.10.2021, AK 5777.

25. ***Entoloma conferendum*** (Britzelm.) Noordel.

Çamlı village, Engineering Faculty garden, meadow, 40°55′N-40°16'E, 15 m, 21.07.2019, AK 5571.

26. ***Entoloma rhodopolium*** (Fr.) P. Kumm.

Dumlusu village, among grass in *Corylus* sp. garden, 40°53′N-40°16′E, 90 m, 24.10.2021, AK 5869.

27. ***Entoloma subradiatum*** (Kühner & Romagn.) M.M. Moser

Yemişalan village, among grass in *Corylus* sp. garden, 40°54′N-40°16′E, 149 m, 23.10.2021, AK 5820.

***Hydnangiaceae*** Gäum. & C.W. Dodge

28. ***Laccaria laccata*** (Scop.) Cooke

Yemişalan village, cemetary, under *Larix* sp., çayırlık, 40°54′N-40°17′E, 180 m, 23.10.2021, AK 5830; Çamlı village, under *Pinus* sp., 40°55′N-40°16'E, 20 m, 23.10.2021, AK: 5841; 07.12.2021, AK. 5874.

***Hygrophoraceae*** Lotsy

29. ***Hygrocybe citrina*** (Rea) J.E. Lange

Yemişalan village, around Mimar Sinan mosque, under decidicous forest, 40°54′N-40°17′E, 174 m, 18.09.2021, AK 5766.

30. ***Hygrocybe conica*** (Schaeff.) P. Kumm.

İrfanlı village, around İrfaniye mosque, under decidicous forest, 40°56′N-40°15′E, 140 m, 16.11.2018, AK 5524.

31. ***Hygrocybe punicea*** (Fr.) P. Kumm.

Çamlı village, Engineering Faculty garden, meadow, 40°55′N-40°16'E, 15 m, 21.07.2019, AK, 5572.

***Incertae sedis***

32. ***Clitocybe fragrans*** (With.) P. Kumm.

İrfanlı village, cemetary, among needle litter under *Pinus* sp., 40°56′N-40°15′E, 71 m, 07.07.2018, AK. 5428.

33. ***Clitocybe phyllophila*** (Pers.) P. Kumm.

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16'E, 20 m, 16.11.2018, AK 5515.

34. ***Crucibulum laeve*** (Huds.) Kambly

İrfanlı village, cemetary, on decaying herb remains, 40°56′N-40°15′E, 71 m, 07.07.2018, AK 5439; around Ulusoy residences, on decaying twigs, 40°56′N-40°15′E, 80 m, 28.10.2018, AK 5798.

35. ***Cyathus olla*** (Batsch) Pers.

Çamlı village, on woody remains in meadow, 40°55′N-40°16E, 148 m, 21.07.2019, AK, 5568.

***Inocybaceae*** Jülich

36. ***Inocybe bresadolae*** Massee

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, under decidious forest, 40°56′N-40°16′E, 8 m, 17.07.2019, AK 5551.

37. ***Inocybe dulcamara*** (Pers.) P. Kumm.

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16'E, 20 m, 16.11.2018, AK 5513; 06.04.2019, AK 5537; İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, decidious forest, 40°56′N-40°16′E, 8 m, 11.10.2019, AK 5794.

38. ***Inocybe geophylla*** P. Kumm.

Çamlı village, cemetary, under *Pinus* sp., 40°55′N-40°15′E, 217 m, 06.04.2019, AK 5543.

39. ***Inocybe hirtella*** Bres.

İrfanlı village, around Ulusoy residences, under *Populus* sp., 40°56′N-40°15′E, 80 m, 28.10.2018, AK 5496.

40. ***Inocybe hystrix*** (Fr.) P. Karst.

Yemişalan village, around group houses, among grass in *Corylus* sp. garden, 40°54′N-40°17′E, 172 m, 16.11.2020, AK 5827.

41. ***Inocybe praetervisa*** Quél.

Yemişalan village, around group houses, under *Pinus* sp., 40°54′N-40°17′E, 172 m, 11.10.2021, AK 5783.

42. ***Inocybe queletii*** Konrad

Çamlı mah. aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16′E, 20 m, 16.11.2018, AK 5512.

43. ***Inocybe subcarpta*** Kühner & Boursier

Dumlusu village, among grass in *Corylus* sp. garden, 40°53′N-40°16′E, 203 m, 05.11.2019 AK 5623.

44. ***Inocybe taxocystis*** (J. Favre & E. Horak) Senn-Irlet

Çamlı village, cemetary, under *Pinus* sp., 40°55′N-40°15E, 217 m, 06.04.2018, AK 5542.

45. ***Inocybe tenebrosa*** Quél.

İrfanlı village, around İrfaniye mosque, under *Pinus* sp., 40°56′N-40°15′E, 140 m, 19.11.2018, AK 5532.

46. ***Pseudosperma rimosum*** (Bull.) Matheny & Esteve-Rav.

İrfanlı village, among grass in Corlylus sp. garden, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5412; Çamlı village, 40°56′N-40°15′E, 24 m, 23.09.2018, AK,5471.

***Lycoperdaceae*** Chevall.

47. ***Lycoperdon molle*** Pers.

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, under *Larix* sp., 40°56′N-40°16′E, 8 m, 17.07.2019, AK 5557.

48. ***Lycoperdon pratense*** Pers.

Yemişalan village, around flour mill, meadow, 40°55′N-40°17′E, 191 m, 18.08.2020, AK 5714.

***Macrocystidiaceae*** Kühner

49. ***Macrocystidia cucumis*** (Pers.) Joss.

Çamlı village, among grass in *Corylus* sp. garden, 40°55′N-40°16′E, 148 m, 07.07.2018, AK, 5454.

***Marasmiaceae*** Roze ex Kühner

50. ***Gerronema nemorale*** Har. Takah.

Kiraz village, on decaying twigs in *Corylus* sp. garden, 40°54′N-40°15′E, 245 m, 07.07.2018, AK 5424.

51. ***Marasmius bulliardii*** Quél.

İrfanlı village, on decaying twigs, 40°56′N-40°15′E, 24 m, 28.10.2018, AK 5489.

52. ***Marasmius capillaris*** Morgan

İrfanlı village, on *Corylus* sp. remains, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5410.

53. ***Marasmius cohaerens*** (Pers.) Cooke & Quél.

Kiraz village, on *Alnus* sp. stump, 40°54′N-40°15′E, 245 m, 07.07.2018, AK. 5421.

54. ***Marasmius siccus*** (Schwein.) Fr.

İrfanlı village, on *Corylus* sp. remains, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5409.

***Mycenaceae*** Overeem

55. ***Atheniella delectabilis*** (Peck) Lüderitz & H. Lehmann

İrfanlı village, in *Corylus* sp. garden, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5411.

56. ***Atheniella flavoalba*** (Fr.) Redhead, Moncalvo, Vilgalys, Desjardin & BA Perry

İrfanlı village, cemetary, on herb remains, 40°56′N-40°15′E, 71 m, 07.07.2021, AK 5433.

57. ***Mycena abramsii*** (Murrill) Murrill

İrfanlı village, around İrfaniye mosque, under *Pinus* sp., 40°56′N-40°15′E, 140 m, 12.10.2021, AK 5806.

58. ***Mycena flavescens*** Velen.

Çamlı village, on decaying Corylus sp. twigs, 40°56′N-40°16′E, 148 m, 12.10.2021, AK 5814.

59. ***Mycena galericulata*** (Scop.) Gray

Yemişalan village, on *Alnus* sp. stump, 40°54′N-40°16′E, 149 m, 23.10.2021, AK 5821.

***Omphalotaceae*** Bresinsk

60. ***Collybiopsis ramealis*** (Bull.) Millsp.

İrfanlı village, cemetary, under *Pinus* sp., 40°56′N-40°15′E, 71 m, 07.07.2018, AK 5436.

61. ***Collybiopsis vaillantii*** (Pers.) R.H. Petersen

İrfanlı village, cemetary, on decaying *Pinus* sp. branch, 40°56′N-40°15′E, 71 m, 07.07.2018, AK 5432.

62. ***Gymnopus dryophilus*** (Bull.) Murrill

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, under Larix sp., 40°56′N-40°16′E, 8 m, 17.07.2019, AK 5678.

63. ***Gymnopus erythropus*** (Pers.) Antonín, Halling & Noordel.

İrfanlı village, among grass in *Corylus* sp. garden, 40°56′N-40°15′E, 24 m, 23.09.2018, AK 5459.

***Physalacriaceae*** Corner

64. ***Armillaria cepistipes*** Velen

Dumlusu village, among grass in *Corylus* sp. garden, 40°53′N-40°16′E, 50 m, 12.10.2019, AK 5854.

65. ***Armillaria mellea*** (Vahl) P. Kumm.

Yemişalan village, on *Corylus* sp. stump, 40°54′N-40°16′E, 149 m, 23.10.2021, AK 367; Dumlusu village, 40°53′N-40°16′E, 203 m, 24.10.20121 AK 5605.

66. ***Strobilurus trullisatus*** (Murrill) Lennox

İrfanlı village, cemetary, among needle litter under *Pinus* sp., 40°56′N-40°15′E, 71 m, 07.07.2018, AK 5417.

***Pluteaceae*** Kotl. & Pouzar

67. ***Pluteus plautus*** (Weinm.) Gillet

Kiraz village, on decaying *Corylus* sp. stump, 40°54′N-40°15′E, 245 m, 07.07.2018, AK 5423.

***Psathyrellaceae*** Vilgalys, Moncalvo & Redhea

68. ***Britzelmayria multipedata*** (Peck) D. Wächt. & A. Melzer

İrfanlı village, on decaying *Corylus* sp. twigs, 40°56′N-40°15′E, 24m., 07.07.2018, AK 5406.

69. ***Candolleomyces candolleanus*** (Fr.) D. Wächt. & A. Melzer

İrfanlı village, cemetary, on decaying twigs, 40°56′N-40°15′E, 71 m, 07.07.2018, AK 5430; Çamlı village, cemetary, among herb remains, 40°55′N-40°15′E, 217 m, 07.07.2018, AK 5452; İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, around decaying stump, 40°56′N-40°16′E, 8 m, 21.07.2019, AK 5579.

70. ***Coprinellus disseminatus*** (Pers.) J.E. Lange

İrfanlı village, around Ulusoy residences, on damp soil under decidious forest, 40°56′N-40°15′E, 80 m, 12.10.2021, AK 5802; Çamlı village, Engiinering Faculty garden, 40°55′N-40°16’E, 20 m, kütük üzeri, 11.10.2021, AK, 5796.

71. ***Coprinellus domesticus*** (Bolton) Vilgalys, Hopple & Jacq. Johnson

Yemişalan village, around flour mill, roadside, among grass under decidious trees, 40°55′N-40°17′E, 160 m, 07.11.2019, AK 5672.

72. ***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, around *Platanus* sp. stump, 40°56′N-40°16′E, 8 m, 07.11.2021, AK 5682.

73. ***Coprinopsis echinospora*** (Buller) Redhead, Vilgalys & Moncalvo

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, kütük üzeri, 40°56′N-40°16′E, 8 m, 09.09.2021, AK 5782.

74. ***Coprinopsis urticicola*** (Berk. & Broome) Redhead, Vilgalys & Moncalvo

İrfanlı village, cemetary, on decaying Urtica sp. remains, 40°56′N-40°15′E, 71 m, 07.07.2021, AK 5458.

75. ***Lacrymaria lacrymabunda*** (Bull.) Pat.

Yemişalan village, around Mimar Sinan mosque, on soil with woody remains, 40°54′N-40°17′E, 174 m, 18.09.2021, AK 5762.

76. ***Psathyrella piluliformis*** (Bull.) P.D. Orton

Dumlusu village, mountain road, on *Alnus* sp. stump, 40°52′N-40°16′E, 335 m, 05.11.2019, AK 5629.

77. ***Psathyrella pygmaea*** (Bull.) Singer

İrfanlı village, on decaying *Corylus* sp. twigs, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5405.

78. ***Psathyrella spadiceogrisea*** (Schaeff.) Maire

İrfanlı village, cemetary, among grass under *Pinus* sp., 40°56′N-40°15′E, 71 m, 07.07.2018, AK 5430.

***Schizophyllaceae*** Quél.

79. ***Schizophyllum commune*** Fr.

Yemişalan village, around flour mill, on decaying stump, 40°55′N-40°17′E, 160 m, 07.11.2019, AK 5670.

***Strophariaceae*** Singer & A.H. Sm.

80. ***Agrocybe dura*** (Bolton) Singer

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, among grass under Platanus sp., 40°56′N-40°16′E, 8 m, 20.04.2021, AK 5751.

81. ***Hypholoma fasciculare*** (Huds.) P. Kumm.,

Dumlusu village, mountain road, around *Alnus* sp. stump, 40°52′N-40°16′E, 335 m, 16.11.2020, AK 5715.

***Tricholomataceae*** R. Heim ex Pouzar

82. ***Tricholoma terreum*** (Schaeff.) P. Kumm.

Çamlı village, aronud highways nursing station, among needle litter under Pinus sp., 40°55′N-40°16’E, 20 m, 07.12.2021, AK 5876.

***Tubariaceae*** Vizzini

83. ***Cyclocybe cylindracea*** (DC.) Vizzini & Angelini

Çamlı village, around *Colylus* sp. stump, 40°55′N-40°16′E, 148 m, 23.09.2018, AK 5475; İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, 40°56′N-40°16′E, 8 m, 17.07.2019, AK 5446.

84. ***Tubaria conspersa*** (Pers.) Fayod

Kiraz village, among woody debris, 40°54′N-40°15′E, 245 m, 07.07.2018, AK 5434.

***Boletales*** E.-J. Gilbert

***Boletaceae*** Chevall

85. ***Leccinum holopus*** (Rostk.) Watling

Çamlı village, among grass in *Corylus* sp. garden, 40°55′N-40°16E, 148 m, 23.09.2018, AK, 5482.

86. ***Xerocomellus chrysenteron*** (Bull.) Šutara

İrfanlı village, around Ulusoy Multi-Program Anatolian Vocational High School, under *Larix* sp., 40°56′N-40°16′E, 8 m, 17.07.2019, AK 5550.

87. ***Xerocomus subtomentosus*** (L.) Quél.

Çamlı village, among grass in *Corylus* sp. garden, 40°55′N-40°16E, 148 m, 23.09.2018, AK, 5469.

***Diplocystidiaceae*** Kreisel

88. ***Astraeus hygrometricus*** (Pers.) Morgan

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16’E, 20 m, 23.10.2021, AK 5840.

***Paxillaceae*** Lotsy

89. ***Gyrodon lividus*** (Bull.) Sacc.

Yemişalan village, under decidious trees, 40°54′N-40°17′E, 174 m, 18.09.2021, AK 5781.

90. ***Paxillus involutus*** (Batsch) Fr.

Yemişalan village, among grass under *Alnus* sp., 40°54′N-40°17′E, 180 m, 18.09.2021, AK 5771.

91. ***Paxillus rubicundulus*** P.D. Orton

Yemişalan village, around Mimar Sinan mosque, under Larix sp., 40°54′N-40°17′E, 174 m, 23.10.2021, AK 5829.

***Sclerodermataceae*** Corda

92. ***Scleroderma citrinum*** Pers.

Dumlusu village, roadside, on soil, 40°53′N-40°16′E, 203 m, 24.10.2021 AK. 5859.

***Suillaceae*** Besl & Bresinsky

93. ***Suillus americanus*** (Peck) Snell

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16’E, 20 m, 23.10.2021, AK 5514.

94. ***Suillus flavidus*** (Fr.) J. Presl

Dumlusu village, around mountain road, under *Pinus* sp., 40°52′N-40°16′E, 349 m, 16.10.2020, AK. 5808.

***Cantharellales*** Gäum.

***Cantharellaceae*** J. Schröt.

95. ***Cantharellus cibarius*** Fr.

Yemişalan village, under decidious trees, 40°54′N-40°17′E, 174 m, 18.09.2021, AK 5768.

96. ***Clavulina cinerea*** (Bull.) J. Schröt.

Dumlusu village, Kıbrıs place, among woody debris, 40°53′N-40°16′E, 90 m, 24.10.2020, AK 5851.

97. ***Craterellus tubaeformis*** (Fr.) Quél.

Dumlusu village, Kıbrıs place, among grass in *Corylus* sp. garden, 40°53′N-40°16′E, 90 m, 24.10.2020, AK 5852.

***Hydnaceae*** Chevall.

98. ***Clavulina coralloides*** (L.) J. Schröt.

İrfanlı village, around İrfaniye mosque, on decaying *Pinus* sp. twigs, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5415; Yemişalan village, under decidious trees, 40°54′N-40°17′E, 174 m, 23.10.2021, AK 8334.

***Geastrales*** K. Hosaka & Castellano

***Geastraceae*** Corda

99. ***Geastrum fimbriatum*** Fr.

Çamlı village, around decaying stump under decidious trees, 40°55′N-40°17E, 197 m, 23.10.2021, AK, 5590.

100. ***Geastrum rufescens*** Pers.

Yemişalan village, cemetary, under decidious trees, 40°54′N-40°17′E, 175 m, 23.10.2021, AK 382; Yemişalan village, around Mimar Sinan mosque, 40°54′N-40°17′E, 174 m, 23.10.2021, AK 5763.

***Polyporales*** Gäum.

***Laetiporaceae*** Jülich

101. ***Laetiporus sulphureus*** (Bull.) Murrill

Çamlıtepe village, on *Salix* sp. stump, 40°54′N-40°15′E, 245 m, 16.11.2020, AK 5726.

***Polyporaceae*** Fr. ex Corda

102. ***Cerioporus squamosus*** (Huds.) Quél.

Yemişalan village, around Mimar Sinan mosque, on Morus sp. stump, 40°54′N-40°17′E, 174 m, 20.04.2020, AK 5713.

103. ***Ganoderma adspersum*** (Schulzer) Donk

Kiraz village, on *Corylus* sp. stump, 40°54′N-40°15′E, 245 m, 16.11.2020, AK 5814.

104. ***Trametes hirsuta*** (Wulfen) Lloyd

Dumlusu village, mountain road, on decaying Carpinus sp. twigs, 40°52′N-40°16′E, 335 m, 16.1012020, AK 5723.

105. ***Trametes ochracea*** (Pers.) Gilb. & Ryvarden

İrfanlı village, on decaying *Corylus* sp. twigs, 40°56′N-40°15′E, 24 m, 07.07.2018, AK 5402.

106. ***Trametes pubescens*** (Schumach.) Pilát

Dumlusu village, on decaying *Alnus* sp. branch, 40°52′N-40°16′E, 349 m, 05.11.2019, AK 5637.

107. ***Trametes versicolor*** (L) Lioyd

Çamlı village, on decaying *Alnus* sp. branch 40°55′N-40°16E, 148 m, 23.09.2018, AK 5478.

***Russulales*** Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

***Auriscalpiaceae*** Maas Geest.

108. ***Auriscalpium vulgare*** Gray

İrfanlı village, around İrfaniye mosque, on decaying pine cone, 40°56′N-40°15′E, 174 m, 19.11.2019, AK 5526.

***Russulaceae*** Lotsy

109. ***Lactarius chrysorrheus*** Fr.

Dumlusu village, among grass in *Corylus* sp. garden, 40°53′N-40°16′E, 50 m, 12.10.2019, AK 5604.

110. ***Lactarius flavidus*** Boud.

İrfanlı village, around İrfaniye mosque, among needle litter under *Pinus* sp., 40°56′N-40°15′E, 174 m, 19.11.2019, AK 5518.

111. ***Lactarius glyciosmus*** (Fr.) Fr.

Dumlusu village, Kıbrıs place, among grass in *Corylus* sp. garden, 40°53′N-40°16′E, 90 m, 11.10.2021, AK. 5788.

112. ***Lactarius salmonicolor*** R. Heim & Leclair

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16’E, 20 m, 16.10.2020, AK 5733.

113. ***Lactarius semisanguifluus*** R. Heim & Leclair

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16′E, 217 m, 16.11.2020, AK 5736.

114. ***Russula cavipes*** Britzelm.

İrfanlı village, around İrfaniye mosque, under *Pinus* sp., 40°56′N-40°15′E, 174 m, 16.11.2018, AK 5520, 5521.

115. ***Russula cyanoxantha*** (Schaeff.) Fr.

Kiraz village, around *Corylus* sp. stump, 40°54′N-40°15′E, 245 m, 07.07.2018, AK 5435.

116. ***Russula medullata*** Romagn.

Çamlı village, cemetary, among grass, 40°55′N-40°15E, 217 m, 07.07.2018, AK 5427.

117. ***Russula melzeri*** Zvára

İrfanlı village, under decidious trees, 40°56′N-40°15′E, 149 m, 07.07.2018, AK 5440.

118. ***Russula paludosa*** Britzelm.

Çamlı village, aronud highways nursing station, under *Pinus* sp., 40°55′N-40°16’E, 20 m, 07.07.2018, AK 5511.

## 4. Discussions

One hundred and eighteen macromycete species were determined from the research area. Ten of the determined taxa (%8.5) belong to *Ascomycota* and 108 (%91.5) to *Basidiomycota*. The taxa are distributed in 3 classes (*Agaricomycetes* 108, *Pezizomycetes* 9, *Sordariomycetes* 1) and 8 orders. The order-wise distribution of the determined taxa is presented in Figure 2.

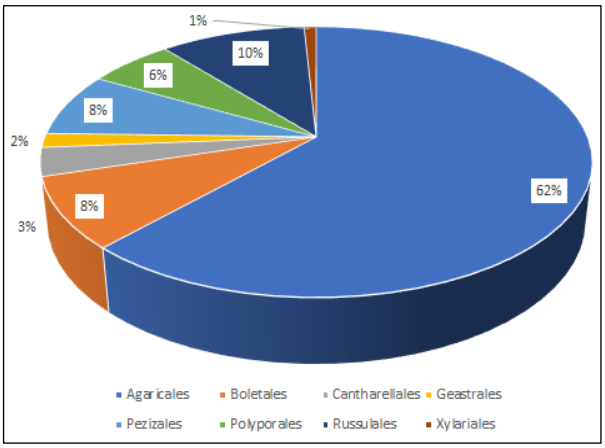


**Figure 2.** Orderwise distribution of the determined taxa.

The taxa are distributed in 38 families and 63 genera. Four of the determined 118 species are currently in Incertae sedis position. *Inocybaceae* and *Psathyrellaceae* are the most crowded families, each with 11 taxa while *Russulaceae* is the second with 10 taxa. *Amanitaceae, Helvellaceae* and *Polyporaceae* follow it, each with 6 taxa. *Marasmiaceae* and *Mycenaceae* comprise 5 taxa while *Entolomataceae* and *Omphalotaceae* comprise 4 taxa. In the region six families (*Agaricaceae, Boletaceae, Cantharellaceae, Hygrophoraceae, Paxillaceae, Physalacriaceae*) are represented with three taxa, seven families (*Bolbitiaceae, Cortinariaceae, Geastraceae, Lycoperdaceae, Strophariaceae, Suillaceae, Tubariaceae*) are represented with two taxa, while the rest of 14 families are represented with only one taxon.

The genus *Inocybe* was found to be the most crowded genus with 10 taxa. It is followed by *Amanita* with 6 taxa. *Helvella, Lactarius* and *Russula* are represented with 5

taxa, while *Entoloma*, *Marasmius* and *Trametes* are represented with 4 taxa. Four of the genera (*Coprinellus*, *Hygrocybe*, *Mycena*, *Psathyrella*) are represented with 3 taxa, and 12 of them (*Armillaria*, *Atheniella*, *Clavulina*, *Clitocybe*, *Collybiopsis*, *Conocybe*, *Coprinopsis*, *Geastrum*, *Gymnopus*, *Lycoperdon*, *Paxillus*) are represented with 2 taxa while the other 39 genera are represented in the region with only one taxon.

**Table 1.** Similarity percentages of Çamlı village with the studies performed in neighboring regions

| Neighboring study | # of Identical taxa | Total taxa | Similarity (%) |
|---|---|---|---|
| Sesli (1993) | 11 | 64 | 17.19 |
| Demirel et al. (2010) | 17 | 126 | 13.49 |
| Akata et al. (2014) | 28 | 216 | 12.96 |
| Keleş et al. (2014) | 23 | 126 | 18.25 |
| Akata and Uzun (2017) | 42 | 212 | 19.81 |
| Oruç et al. (2021) | 43 | 109 | 39.45 |

Except *Mycena ustalis* (Keleş, 2019a), all of the determined species are new for the research area.

Thirty four of the taxa are edible, 72 are inedible while 12 are more or less poisonous. Local people generally have a suspicious attitude towards naturally growing mushrooms. Tough %28.33 of the determined taxa are edible, none of them are collected and consumed by the locals. The first author's expressions are also in the same way, as being a resident of the region.

The determined taxa were compared with the studies carried out in neighboring regions and some similarities were observed (Table 1). The reason for this similarity and dissimilarity may be the common climate, vegetation and sampling period.

**Conflict of Interest**

Authors have declared no conflict of interest.

**Authors' Contributions**

The authors contributed equally.

**Acknowledgements**

The authors would like to thank Yüzüncü Yıl University Research Fund for its financial support (FYL-2019-8024).

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*Anatolian Journal of Botany*
*7(2): 154-160 (2023)*
*Research article* *doi: 10.30616/ajb.1342073*

# Evaluation of the bioactivities of turmeric spices of different origins

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Received : 13.08.2023
Accepted : 10.09.2023
Online : 27.09.2023

# Farklı kökenli zerdeçal baharatlarının biyoaktivitelerinin değerlendirilmesi

**Abstract:** Our study aimed to compare the in vitro bioactivities of turmeric spice samples obtained from three different sources (India, Pakistan, and Indonesia). Our study involved the determination of total phenolic and flavonoid content, in vitro antioxidant activities, tyrosinase enzyme activity, and antimicrobial activity (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans*) analyses in three different spice samples. Each spice sample was dissolved in 250 mL of ethanol and stirred on a magnetic stirrer for 36 hours. After filtering out the solid parts, the residues were dissolved again in 250 mL of ethanol separately. This process was repeated three times. After the filtration steps, all filtrates were combined. The remaining solvents in the filtrate were evaporated using an evaporator. The residue of the extracts was placed in Eppendorf tubes and stored in a freezer until use. It has been determined that turmeric of Indian origin, with its high phenolic and flavonoid content, exhibits more potent antioxidant and antityrosinase effects than those from Indonesia and Pakistan. However, antimicrobial activity could not be detected within the studied concentration range of 10 µg/mL to 400 mg/mL.

**Key words:** Antimicrobial, antioxidant, antityrosinase, turmeric

**Özet:** Çalışmamız, üç farklı kaynaktan elde edilen zerdeçal baharat örneklerinin in vitro biyoaktivitelerini (Hindistan, Pakistan ve Endonezya) karşılaştırmayı amaçlamıştır. Çalışmamızda, toplam fenolik ve flavonoid içeriğin belirlenmesini, in vitro antioksidan aktivitelerini, tirozinaz enzim aktivitesini ve antimikrobiyal aktiviteyi (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* ve *Candida albicans*) üç farklı baharat örneğinde analiz edildi. Her bir baharat örneği 250 mL etanol içinde çözüldü ve 36 saat boyunca manyetik bir karıştırıcıda karıştırıldı. Katı parçalar süzüldükten sonra artık kalıntılar ayrı ayrı 250 mL etanol içinde tekrar çözündü. Bu işlem üç kez tekrarlandı. Süzme adımlarının ardından, tüm süzme sıvıları birleştirildi. Süzme sıvısındaki artan çözücüler buharlaştırıcı kullanılarak uzaklaştırıldı. Özütlerin kalıntısı Eppendorf tüplerine konuldu ve kullanıma kadar dondurucuda saklandı. Hindistan kökenli zerdeçalın yüksek fenolik ve flavonoid içeriği ile Endonezya ve Pakistan'dan gelenlere göre daha güçlü antioksidan ve antitirosinaz etkileri sergilediği belirlendi. Bununla birlikte, 10 µg/mL ile 400 mg/mL arasındaki incelenen konsantrasyon aralığında antimikrobiyal aktivite tespit edilemedi.

**Anahtar Kelimeler:** Antimikrobiyal, antioksidan, antitirozinaz, zerdeçal

**Citation:** Canbolat F, Özcan Ateş G (2023). Evaluation of the bioactivities of turmeric spices of different origins. Anatolian Journal of Botany 7(2): 154-160.

## 1. Introduction

Medicinal herbs have been used as a source of medicine from ancient times to the present day. Among these, turmeric is a spice derived from the rhizomes of *Curcuma longa (C. longa)*, a flowering plant in the ginger family. *C. longa* is commonly cultivated in China, India, and other Asian countries, and it is a perennial herbaceous plant (Trigo-Gutierrez et al., 2021; Artar and Öztürk, 2022). Turmeric is a polyphenolic plant, and like many other plant materials, variations in the polyphenolic content of *C. longa* from different geographical regions have been observed (Zorofchian Moghadamtousi et al., 2014; Karaman and Köseler, 2017). More than 100 components have been isolated from turmeric. Turmeric comprises approximately 60-70% carbohydrates, 8.6% protein, 5-10% fat, 2-7% dietary fiber, 3-5% curcuminoids (with 50-70% curcumin content), and 5% volatile oil and resin, as indicated by Trujillo et al. (2013). It also contains noteworthy quantities of vitamins and minerals, including vitamin C (ascorbic acid), vitamin E (α-tocopherol), vitamin B3 (niacin), potassium (K), magnesium (Mg), phosphorus (P), and calcium (Ca), as outlined by Karaman and Köseler (2017).

The main components of the plant are volatile oil called turmerone and coloring agents called curcuminoids (Noorafshan and Ashkani-Esfahani, 2013; Artar and Öztürk, 2022). Curcumin ($C_{21}H_{20}O_6$), one of the three curcuminoids found in turmeric, is considered as the most important polyphenol associated with health. It has been reported that curcumin positively reduces oxidative stress, and its activities extend to various chronic diseases. It reduces oxidative stress and increases the activities of antioxidant enzymes, thus reducing the risk of various chronic diseases (Artar and Öztürk, 2022). While curcumin is soluble in acetone and ethanol, it is insoluble in water. Curcumin is a hydrophobic polyphenolic flavonoid (Akbay and Pekcan, 2016). The methoxy, hydroxyl, and carbonyl groups in curcumin, which have a polyphenolic structure, confer antioxidant and radical scavenging properties on turmeric (Wright, 2002; Boroumand et al., 2018).

Additionally, some curcumin analogs have recently exhibited inhibitory properties against the enzyme tyrosinase (Du et al., 2011). Tyrosinase is a multifunctional enzyme containing copper. Tyrosinase catalyzes the oxidation of phenolic compounds to quinone derivatives. Tyrosinase is an enzyme involved in melanin formation.

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Melanin is the black pigment in hair and skin, and it is necessary to protect the skin against ultraviolet rays. This pigment is produced by melanocyte cells. However, abnormal melanin production leads to dermatological disorders (Akter et al., 2021). The increase in tyrosinase activity in some cancer cells has drawn attention to the importance of tyrosinase in treating these cancer types. Lee et al. (2009) reported that some curcumin analogs exhibited inhibitory activity against tyrosinase (Lee et al., 2009). This situation has attracted our interest in evaluating turmeric's antityrosinase and antioxidant activities.

Furthermore, the antimicrobial properties of turmeric have been included in the literature. Empirical evidence substantiates that the antimicrobial effect is linked to the methoxy and hydroxyl groups present in the chemical configuration of curcumin within turmeric. Molecular docking studies have suggested that functional groups in curcumin form hydrogen bonds and nonspecific hydrophobic interactions with the catalytic region of the FtsZ protein, which is involved in bacterial cell division, thereby inhibiting bacterial cell division (da Silva et al., 2018).

Curcuminoids, with their polyphenolic structure, are present in turmeric at a rate of 3-5%. An average teaspoon of turmeric contains approximately 30-90 mg of curcumin. Studies in humans have shown that curcumin consumption of up to 12000 mg/day is well-tolerated. For the most effective therapeutic effect, it is recommended to consume 4000-8000 mg/day of curcumin based on safety and toxicity profiles (Çöteli and Karataş, 2017). However, the phenolic composition within turmeric, specifically the curcuminoid content, may vary, ranging from 2% to 9%, depending on geographical conditions (Trujillo et al., 2013).

Therefore, comparing the bioactivities of turmeric with varying phenolic contents based on geographical conditions can provide valuable insights into its consumption, which has a positive impact on public health and is a part of our daily diet. Our study aimed to compare the in vitro bioactivities of turmeric spice samples obtained from three different sources (India, Pakistan, and Indonesia). Our study involved the determination of phenolic and flavonoid content, antioxidant activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ABTS cation radical scavenging activity, and cupric ion-reducing (CUPRAC) activity. Additionally, we analyzed tyrosinase enzyme activity and antimicrobial activity in three different spice samples (India, Pakistan, and Indonesia).

## 2. Materials and Method

### 2.1. Reagent and standards

The chemicals utilized for investigating bioactivity were procured from Sigma-Aldrich (Schnelldorf, Germany). The microorganisms used for assessing antimicrobial properties were obtained from the American Type Culture Collection (ATCC). The spice samples from India, Pakistan, and Indonesia were obtained from international online sales platforms.

### 2.2. Preparation of extracts

Samples of turmeric spice from three different origins, namely India, Pakistan, and Indonesia, were individually weighed (20 grams each). Each weighed spice sample was dissolved in 250 mL of ethanol and stirred on a magnetic stirrer for 36 hours. After filtering out the solid particles, the residues were dissolved again in 250 mL of ethanol separately. This process was repeated three times. Following the filtration steps, all the filtrates were combined. The remaining solvents in the filtrate were evaporated using an evaporator at 40°C. The residue from the extracts was placed in Eppendorf tubes and stored in a freezer until use. Sample preparations were conducted across a wide concentration range (10 µg/mL - 400 mg/mL), and subsequent analyses were carried out.

### 2.3. The total phenolic and total flavonoid contents

The quantification of total phenolic content (TPC) was conducted using the Folin–Ciocalteu method with slight modifications, following the approach described in earlier studies (Sarikurkcu et al., 2018; Yırtıcı et al., 2022). A 96-well microplate was employed, where 12.5 µL of Folin–Ciocalteu reagent (diluted 1:9) was added to 25 µL of the extract and 187.5 µL of ultrapure water in each well. Subsequently, a mixture of 25 µL of 20% (w/v) sodium carbonate was introduced. The absorbance was gauged at 760 nm through a microplate reader. The outcomes of TPC were presented in milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract).

For evaluating total flavonoid content (TFC), a modified aluminum chloride colorimetric technique was used (Sarikurkcu et al., 2018; Yırtıcı et al., 2022). In this procedure, the extract was combined with methanol (25 µL), 5% sodium nitrite (10 µL), and ultrapure water (100 µL) within each well of a 96-well microplate. Then, 10% aluminum chloride (15 µL) was added to the mixture. After an incubation period of five minutes, 100 µL of 1 M sodium hydroxide and 50 µL of ultrapure water were incorporated, and the absorbance was assessed at 510 nm. A standard curve was devised using rutin, and the findings were expressed as milligrams of rutin equivalent (RE) per gram of extract (mg RE/g extract).

### 2.4. Bioactivities

#### 2.4.1. Antioxidant tests

The assessment of DPPH radical scavenging activity was conducted with specific adaptations based on prior research (Sarikurkcu et al., 2018; Yırtıcı et al., 2022). Different concentrations (10 µg/mL - 400 mg/mL) of the extracts (10 µL) were blended with 190 µL of a DPPH solution (0.004%). Following a 30-minute incubation period at room temperature, the absorbance was gauged at 517 nm. The proportion of DPPH radical scavenging manifested by the spice extracts was calculated using Equation 1. The same procedures were replicated utilizing Trolox as a standard, and the DPPH radical scavenging activities of the spice extracts were conveyed in terms of Trolox equivalent (mgTE/g extract).

Inhibition (%) = [(Acontrol–Asample)/Acontrol] x 100 (1)

$A_{control}$; control absorbance and $A_{sample}$; sample absorbance

ABTS cation radical scavenging activity was assessed with adjustments derived from the methodology presented by Sarikurkcu et al. (2018). The $ABTS^{\cdot+}$ was generated by reacting 7 mmol/L of 2,20-azinobis(3-ethylbenzothiazoline -6-sulfonic acid) diammonium salt with 2.45 mmol/L of potassium persulfate over 16 hours at room temperature.

Varied concentrations (10 µg/mL - 400 mg/mL) of the extracts (10 µL) were combined with 190 µL of the prepared ABTS solution and incubated for six minutes at room temperature. Subsequently, the absorbance was measured at 734 nm. The proportion of ABTS cation radical scavenging demonstrated by the spice extracts was determined using the supplied equation. The activities of the spice extracts were conveyed in terms of Trolox equivalent (mgTE/g extract).

CUPRAC reagent was freshly prepared by combining 10 mM copper(II) chloride, 7.5 mM neocuproine, and 1 M ammonium acetate (pH 7) at a ratio of 10:1:1 (v/v/v). Various extracts (10 µg/mL - 400 mg/mL) concentrations (25 µL) were mixed with 175 µL of the CUPRAC reagent and incubated for 30 minutes at room temperature. Subsequently, the absorbance was measured at 450 nm. The antioxidant potential of the extracts was quantified in terms of milligrams of standard equivalent per gram of extract and compared against the positive controls represented by Trolox.

### 2.4.2. Enzyme inhibition test

The tyrosinase inhibitory potential of the extracts was evaluated utilizing the dopachrome method outlined by Sarıkurkcu et al. (2018). In alignment with this procedure, 25 µL of each extract (10 µg/mL - 400 mg/mL), 40 µL of tyrosinase solution, and 100 µL of sodium phosphate buffer (pH 6.8) were combined and thoroughly mixed. The resultant mixture was subjected to an incubation period at 25°C for 15 minutes. After adding 40 µL of l-3,4 dihydroxyphenylalanine (L-DOPA), the mixture was again incubated at 25°C for 10 minutes. Subsequently, the absorbance was gauged at 492 nm. To determine the inhibitory effect of the scutellarin compound, employed as the positive control, against the tyrosinase enzyme, a well-defined procedure was followed. Initially, 1 mg of scutellarin compound was dissolved in dimethyl sulfoxide (DMSO) and then subjected to a tenfold dilution with distilled water. The enzyme activity was assessed across five distinct concentrations of the scutellarin compound.

### 2.4.3. Antimicrobial test

The antimicrobial activity of ethanol extracts of the spices was determined using a combination of the microdilution method, the NCCLS M07-A9 method, and the method of Özcan Ateş and Kanbur (2023) (NCCLS, 2012; Özcan Ateş and Kanbur, 2023). Initially, cultures of *Escherichia coli (E. coli) ATCC 25922*, *Klebsiella pneumoniae (K. pneumoniae)* ATCC 700603, *Staphylococcus aureus (S. aureus)* ATCC 25923, and *Candida albicans (C. albicans)* ATCC 10231 were revived on Tryptic Soy Agar (Merck, Germany) Petri dishes at 37°C for 24 hours. Then, ethanol extracts of the samples were prepared in the range of 10 µg/mL - 400 mg/mL using Muller Hinton Broth (MHB) (BD, USA) medium. From the prepared concentrations, 100 µL was added to the wells of U-bottomed microplates. In row, A of the plates, only MHB (4 mg/mL) medium was added as a sterility control. Samples were distributed in rows B and G. In row H, MHB + inoculum suspension (100 µL) was added to control microbial growth. After incubating at 37°C for 24 hours, 20 µL of sterile 1% 2,3,5 triphenyl tetrazolium chloride (Merck, Germany) solution was added to the wells and further incubated for 30 minutes. The lowest concentration without a visible red-pink coloration was identified as the minimum inhibitory concentration (MIC). The study was conducted in triplicate.

### 2.5 Statistical evaluation

Statistical analysis of the substance content and biological activities of spices from different sources was conducted using SPSS version 23. The results are expressed as the mean ± standard error of the mean (SEM) based on three separate experiments performed in triplicate. A one-way analysis of variance (ANOVA) was employed, followed by the Tukey post hoc statistical test, as the groups showed a normal distribution according to Normality Analysis. Differences were considered statistically significant when the p-value (P) was less than 0.05 at a 95% confidence interval.

## 3. Results

The quantities of extracts obtained from 20 grams of turmeric spice samples originating from India, Pakistan, and Indonesia were determined to be 1.30 g, 1.15 g, and 1.86 g, respectively. The spice content and in vitro bioactivity analysis results obtained from the extracts are presented in Table 1, based on three repeated analyses.

**Table 1.** Substance content and bioactivity analysis results of three different spice samples

| Analyze | Sample | Mean ± std.dev | One way ANOVA (P) |
|---|---|---|---|
| Fenolic (mgGAE/g extract) | India | 75.46 ± 1.82 | P < 0.05 |
| | Pakistan | 24.74 ± 2.57 | |
| | Indonesia | 68.03 ± 1.30 | |
| Flavanoid (mgRE/g extract) | India | 42.11 ± 1.77 | P < 0.05 |
| | Pakistan | 13.54 ± 2.14 | |
| | Indonesia | 60.56 ± 1.80 | |
| ABTS (mgTE/g extract) | India | 132.23 ± 4.86 | P < 0.05 |
| | Pakistan | 61.58 ± 3.60 | |
| | Indonesia | 129.32 ± 4.96 | |
| DPPH (mgTE/g extract) | India | 44.58 ± 3.86 | P < 0.05 |
| | Pakistan | 34.09 ± 3.86 | |
| | Indonesia | 48.39 ± 3.83 | |
| CUPRAC (mgTE/g extract) | India | 86.42 ± 5.63 | P < 0.05 |
| | Pakistan | 46.15 ± 1.74 | |
| | Indonesia | 71.08 ± 2.79 | |
| Tyrosinase Enzyme inhibition ( $IC_{50}$ µg/mL) | India | 38.50 ± 2.28 | P < 0.05 |
| | Pakistan | 301.36 ± 4.21 | |
| | Indonesia | 46.29 ± 1.80 | |
| *E. coli, K. pneumoniae, S. aureus, C. albicans* (MIC; mg/mL) | India | >400 | |
| | Pakistan | >400 | |
| | Indonesia | >400 | |

The outcomes are presented as the mean ± standard deviation (std.dev). Values denoted by distinct superscripts within the same row differ as determined by Tukey's honestly significant difference post hoc test at a 5% significance level. $IC_{50}$ (mg/mL) signifies the concentration at which 50% inhibition is achieved for all samples. MIC (mg/mL) stands for Minimum Inhibitory Concentration.

When Table 1 is examined, TPC and TFC in each spice sample were statistically different (P < 0.05). Regarding phenolic content, the Indian extract exhibited the highest level, whereas the Pakistani extract showed the lowest (Figure 1). On the other hand, the Indonesian extract displayed the greatest quantity of flavonoid content, whereas the Pakistani extract demonstrated the lowest (Fig. 1).

The antioxidant activity assessments of the spices were reported as mgTEs/g using ABTS, DPPH, and CUPRAC

analyses (Table 1). Upon statistical evaluation of the ABTS and DPPH analysis results, it was found that the antioxidant activity level of the Pakistan extract was significantly lower compared to the Indian and Indonesian extracts ($P < 0.05$) (Fig. 1). When comparing the CUPRAC analysis activities of the spices, it was observed that the activity levels of each spice were significantly different ($P < 0.05$). The extract with the highest activity was the Indian extract (Fig. 1).

When the Tyrosinase Enzyme inhibition ($IC_{50}$ µg/mL, 50% inhibition concentration) of the spice extracts is examined in Table 1, the $IC_{50}$ level of each spice sample was found to be significantly different from each other ($P < 0.05$) (Figure 1). The most effective spice extract in enzyme inhibition was the Indian extract (Figure 1). Tyrosinase Enzyme activity levels of each spice are given in Figure 2.

The MIC (mg/mL) values obtained from the antimicrobial analysis of the spice samples extracted in ethanol are provided in Table 1. At the end of the analysis, it was determined that the MIC values of the spice samples were >400 mg/mL.

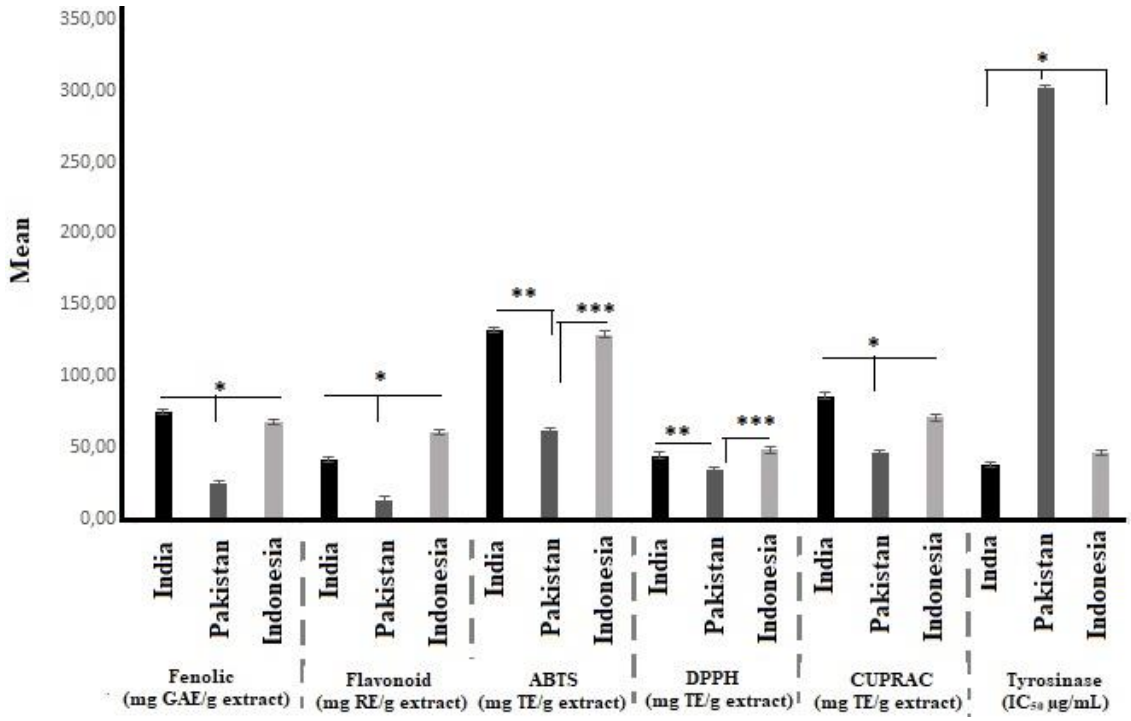


**Figure 1.** The substance content and bioactivity analysis outcomes for three spice samples were subjected to a one-way analysis of variance (ANOVA) along with the Tukey post hoc statistical test. Differences were deemed significant when the p-value (P) was less than 0.05 within a 95% confidence interval. *,**,***; Signs of statistical significance.

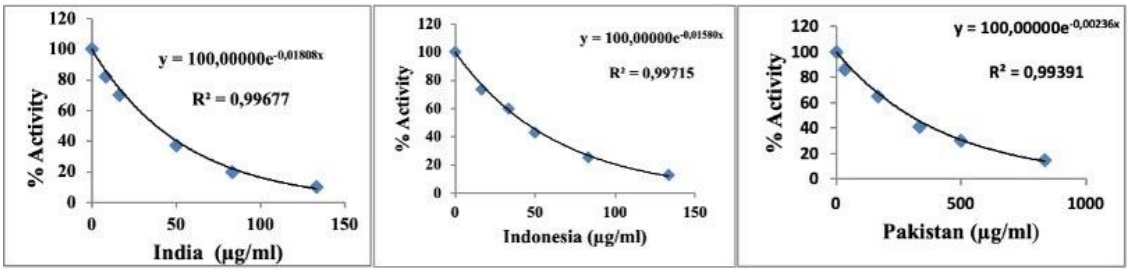


**Figure 2.** Tyrosinase Enzyme Activity results of spices

## 4. Discussions

This study has highlighted significant variations in bioactivity, TPC, and TFC among different types of turmeric. Among the three analyzed turmeric types, the extracts from Indian and Indonesian origins exhibited considerably stronger antioxidant activity. Additionally, these extracts contained higher levels of TPC and TFC than the extract from Pakistan. An appropriate solvent is crucial for extracting active compounds from plants and spice species containing bioactive substances. In the study by Akter et al. (2019), methanol was used as the extraction solvent to determine turmeric's antioxidant and flavonoid content (Akter et al., 2019). In the research conducted by Ivanović et al. (2021), turmeric samples were extracted using different solvents, including 80% methanol, 80% ethanol, and ultrapure water. The study indicated the highest phenolic content in the ethanol-extracted sample (Ivanović et al., 2021). Considering the findings from the literature, ethanol solvent was chosen in our study to effectively extract phenolic and flavonoid compounds from turmeric, to achieve high extraction yields. The analysis results revealed the following TPC (mg GAE/g extract) and TFC (mg RE/g extract) values: for the Hindustani extract, 75.46 ± 1.82 and 42.11 ± 1.77; for the Pakistani extract, 24.74 ± 2.57 and 13.54 ± 2.14, and for the Indonesian extract, 68.03 ± 1.30 and 60.56 ± 1.80 (Table 1, Figure 1). In general, our extraction results align with the findings of

Sahin (2018), who investigated the effects of turmeric extraction (Sahin, 2018). In the study conducted by Sahin in 2018, using various extraction methods, TPC in turmeric was found to be approximately 46.50 (mg GAE/g extract) (Sahin, 2018). The ethanol extraction method employed in our study has facilitated the extraction of high levels of phenolic and flavonoid compounds from turmeric samples. Existing literature studies have identified that the antioxidant property of turmeric originates from its contained phenolic and flavonoid compounds (Çöteli and Karatas, 2017). In the study conducted by Eleazu et al. in 2015, the polyphenolic content and in vitro antioxidant capacity of six new turmeric accessions were investigated. At the end of the study, the antioxidant capacity of two specific accessions was found to be different from the others. It was reported that the ratio of phytochemical compounds in turmeric directly influenced its antioxidant capacity (Eleazu et al., 2015). Our study observed that the antioxidant activity analysis of the Pakistan extract, which contains lower levels of phenolic and flavonoid compounds compared to the extracts from India and Indonesia, was significantly lower when compared to the other spices (Table 1, Figure 1). Our study is consistent with existing literature data. Upon assessing the $DPPH^{\cdot}$ and $ABTS^{\bullet+}$ scavenging activity of distinct turmeric varieties in our study, as detailed in Table 1 and Figure 1, it becomes evident that the extracts originating from India and Indonesia, characterized by elevated phenolic content, demonstrate correspondingly heightened $DPPH^{\cdot}$ and $ABTS^{\bullet+}$ scavenging activity. In the study conducted by Du et al. in 2011, the antioxidant activities of polyphenolic curcumin analogs were investigated using DPPH analysis.

Based on the analysis results, the study concluded that the samples exhibited higher antioxidant activity than vitamin C (Du et al., 2011). In the study conducted by Ak and Gulcin in 2008, the antioxidant and radical scavenging properties of curcumin were investigated. The study concluded that curcumin could be an antioxidant in both pharmacology and the food industry (Ak and Gulcin, 2008). In the study conducted by Tanvir et al. (2017), the antioxidant capacity of various turmeric species grown in Bangladesh was investigated. Turmeric samples from the Khulna and Chittagong regions in Bangladesh were collected for the study. The antioxidant capacities of the extracts obtained using different solvents were also examined. At the end of the study, the ethanolic extract of turmeric grown in the Khulna region was found to have the highest antioxidant capacity. Similarly, in our study, the antioxidant capacity of turmeric plants varied depending on the region and extraction solvent. Our data and literature indicate that turmeric's levels of bioactive compounds and antioxidant capacity can vary according to climatic and geographical conditions (Tanvir et al., 2017).

Antioxidants are recognized as reducers. Their capacity to donate electrons enables them to counteract the effects of free radicals and reactive oxygen species, reducing these elements from higher-valence states to lower-valence states. The redox potential, or the reducing ability of antioxidants, holds substantial importance as an indicator of their effectiveness in combating oxidative stress. In our study, when evaluating the reducing power of turmeric extracts on copper ions, it was found that the one with the highest effect was turmeric from India (Table 1, Figure 1). Although all the turmeric species used in the study exhibited high antioxidant activity, the turmeric variety originating from India was identified to have the highest activity among the three spice types. Our findings are consistent with the literature regarding the antioxidant activity levels of turmeric, as supported by previous studies (Noori et al., 2022).

All of the turmeric extracts studied for tyrosinase inhibition exhibit effective inhibitory capabilities. Regarding tyrosinase inhibition, the most effective extract is from India, followed by extracts from Indonesia and Pakistan, respectively (Table 1, Figure 1, Figure 2). Previous studies have reported that phenolic compounds possess inhibitory abilities through competitive inhibition or by exhibiting chelating properties with the copper atom at the center of the tyrosinase enzyme (Kim and Uyama, 2005). In this context, the high tyrosinase inhibition ability of the Indian extract among the investigated turmeric extracts can be attributed to its elevated phenolic content. Various studies have also observed similar approaches (Oskoueian et al., 2012). The study by Du et al. (2011) investigated the antioxidant and antityrosinase activities of curcumin analogs. The research revealed that curcumin analogs exhibited potent activity (Du et al., 2011). Akter et al. (2021) reported that active molecules extracted from turmeric exhibited antityrosinase activity (Akter et al., 2021). The turmeric extracts used in our study also showed high antityrosinase activity (Table 1, Figure 1, Figure 2). Our study results are in accordance with the existing literature.

Our study determined that the MIC values of spice samples from India, Pakistan, and Indonesia, extracted in ethanol, were >400 mg/mL (Table 1). Niamsa and Sittiwet (2009) determined the MIC values of *C. longa* rhizome water extract as follows: 400 mg/mL for *E. coli* ATCC 25922, 600 mg/mL for *S. aureus* ATCC 25923, and 1600 mg/mL for *K. pneumoniae* ATCC 10031 (Niamsa and Sittiwet, 2009). In their study, Gupta et al. (2015) determined that petroleum ether, benzene, chloroform, methanol, and aqueous solutions of turmeric extracts at concentrations of 50 and 100 mg/mL exhibited inhibition zones similar to the gentamicin antibiotic disk against *S. aureus* ATCC 6571 and two clinical *S. aureus* isolates (Gupta et al., 2015). In the study by Kasta et al. (2020), it was observed that the ethanol extract of *C. longa* exhibited inhibition zones similar to antibiotic disks at a concentration of 500 mg/mL against *E. coli*, *S. aureus*, and *C. albicans* cultures. In our study, despite conducting bioactivity analyses over a wide concentration range (10 µg/mL - 400 mg/mL), the MIC values could not be determined for concentrations lower than 400 mg/mL, as the microdilution method was employed for evaluation. In our study, the samples exhibited no antimicrobial activity within the 10 µg/mL concentration range to 400 mg/mL. Additionally, concentrations exceeding 400 mg/mL were not investigated in our study. As a result, no antimicrobial effects of the samples against the tested microorganisms were observed within the specified concentration range. Based on our study's data and existing literature, it was observed that antimicrobial activity exhibited a dose-dependent pattern. Our findings indicated that the samples displayed robust antioxidant and antityrosinase activity within the range of 10 µg/mL to 400 mg/mL, while no antimicrobial activity was evident within this concentration range. Consequently, the principal bioactive attributes of the samples were identified as antioxidant and antityrosinase activity.

## 5. Conclusion

Our findings highlight that the phenolic and flavonoid content variations of turmeric plants grown in different geographical regions also influence their bioactivities. Moreover, we have determined that turmeric originating from India, with its high phenolic and flavonoid content, exhibits more potent antioxidant and antityrosinase effects than those from Indonesia and Pakistan. However, antimicrobial activity could not be detected within the studied concentration range. These results suggest that turmeric spice samples extracted using ethanol as a solvent may possess antimicrobial activity at higher concentrations. These insights underscore the potential of turmeric, particularly in terms of antioxidant activity, in the context of health benefits.

## Conflict of Interest

Authors have declared no conflict of interest.

## Authors' Contributions

F.C. is the first author of this study. F.C. contributed to the study's design, conceptualization, methodology, TPC, TFC, antioxidant and antityrosinase activity analysis, and manuscript writing. G.O.A. contributed to the methodology, antimicrobial analysis, and manuscript writing.

## Acknowledgements

We thank Dr. Nükhet Nilüfer ZORBA for providing the standard microbial cultures used in this study.

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*Anatolian Journal of Botany*
*7(2): 161-165 (2023)*
*Research article* *doi:10.30616/ajb.1344402*

# Comparative leaf and stem anatomy of *Tamarix tetrandra* (*Tamaricaceae*) species from different habitats

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Received : 16.08.2023
Accepted : 20.10.2023
Online : 24.10.2023

# Farklı habitatlarda yayılış gösteren *Tamarix tetrandra* (*Tamaricaceae*) türünün karşılaştırmalı yaprak ve gövde anatomisi

**Abstract:** In this study, the leaf and stem structure of *Tamarix tetrandra* Pallas ex. Bieb. species, which are distributed in riparian and salt marshes, were examined in terms of anatomical and micromorphological aspects. Specimens of the species have been preserved in 70% alcohol for anatomical study. Herbarium specimens were used for micromorphological studies. The studies showed that there were differences in anatomy and micromorphology. It was found that the stomata were embedded in the epidermis in the samples distributed in the salt marsh. In addition, stem epidermal cell length, sclerenchyma cell diameter, sieve tube cell diameter, and pith cell diameter were found to be greater in riparian species. In the correlation analysis, a positive correlation was observed between leaf lower surface stomata width and upper epidermis cell width, and between stem sclerenchymatic cell diameter and upper epidermis cell width in species distributed in salt marshes. The studies did not find intensive salt accumulation in the stem and leaf structures of the species that spread in the salt marsh. Salt uptake is thought to be inhibited in these species.

**Key words:** Anatomy, micromorphology, riparian, salt marsh, *Tamarix tetrandra*

**Özet:** Bu çalışmada nehir kenarında ve tuzlu bataklıklarda yayılış gösteren *Tamarix tetrandra* Pallas ex. Bieb. türünün yaprak ve gövde yapısı anatomik ve mikromorfolojik yönden incelenmiştir. Türe ait örnekler anatomik incelemeler için %70'lik alkol içerisinde stok örnek haline getirilmiştir. Mikromorfolojik incelemelerde herbaryum örnekleri kullanılmıştır. Yapılan incelemelerde anatomik ve mikromorfolojik yönden farklılıklar olduğu belirlenmiştir. Tuzlu bataklıkta yayılış gösteren örneklerde stomaların epidermise gömülü olduğu tespit edilmiştir. Ayrıca nehir kıyısında yayılış gösteren türlerde ise gövde epidermis hücre boyu, sklerenkima hücre çapları, kalburlu boru hücre çapları ve öz hücre çaplarının daha büyük olduğu saptanmıştır. Yapılan korelasyon analizinde tuzlu bataklıkta yayılış gösteren türlerde yaprak alt yüzey stoma en ile üst epidermis hücre en arasında ve gövde sklerenkima hücre çap ile üst epidermis hücre en arasında pozitif korelasyon gözlemlenmiştir. Yapılan incelemelerde tuzlu bataklıkta yayılış gösteren türlerin gövde ve yaprak yapılarında yoğun bir tuz birikimine rastlanmamıştır. Bu türlerde tuz alınımının engellendiği düşünülmektedir.

**Anahtar Kelimeler:** Anatomi, mikromorfoloji, nehir kıyısı, *Tamarix tetrandra,* tuzlu bataklık

**Citation:** Akbulut MK (2023). Comparative leaf and stem anatomy of *Tamarix tetrandra* (*Tamaricaceae*) species from different habitats. Anatolian Journal of Botany 7(2): 161-165.

## 1. Introduction

*Tamarix* L. (*Tamaricaceae*) is naturally distributed in Asia, Europe, and northeastern and southwestern Africa (Sheidai et al., 2019). It is mostly found in temperate and subtropical regions. It is represented by approximately 70-75 species worldwide (Villar et al., 2019). There are 8 species of this family in Türkiye (Güner et al., 2012), one of which is endemic (*Tamarix duezenlii* Çakan & Ziel.). *Tamarix tetrandra* Pall. ex M.Bieb., one of these species, has a very wide distribution.

Plants are capable of adapting to various environmental conditions. Salty soils create different ecological living conditions for most plants (Dölarslan and Gül, 2012). While many plant species cannot adapt to very low levels of salinity (glycophytes), some plant species can adapt to high levels of salinity (halophytes) (Doğru and Canavar, 2020). This situation can be morphological as well as anatomical. Most plants cannot tolerate high levels of salt in the soil (Glenn and Brown, 1999). Plants (halophytes) adapted to these environments have developed adaptation mechanisms at the cell, tissue and whole plant level (Aslamsup et al., 2011). These mechanisms can be classified as osmotic tolerance, ion excretion, and tissue tolerance (Tiryaki, 2018).

The salt tolerance of plant species can vary depending on the environment. Even plants of the same species can have different levels of salt tolerance (Doğru and Canavar, 2020). The osmotic potential of water in saline soils can decrease and become unavailable to plants (physiological drought) (Marschner, 2011). Halophytes use the salts that accumulate in their leaves to compensate for the low osmotic potential of the soil (Lauchli and Epstein, 1984). Leaf cells store salts in vacuoles and maintain a level that does not affect enzyme activity and metabolic activities in organelles and cytoplasm (Lauchli and Epstein 1984). This is very important for plants adapted to high salinity soils. Several studies have been carried out to determine the anatomical characteristics of *Tamarix*. Some of these have focused on the leaf structures (Abbruzzese et al., 2013; Alaimo et al., 2013) of the species and others on the stem (Waly, 1999; Oladi et al., 2017) structures.

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The aim of this study was to determine the anatomical and micromorphological characteristics of the *Tamarix tetrandra* Pall. ex M.Bieb. species found in different habitats. In this context, we compared the leaf and stem structures of *Tamarix tetrandra* found in riparian and salt marshes from an anatomical and micromorphological point of view.

## 2. Materials and Method

Samples of *Tamarix tetrandra* Pallas ex. Bieb. were collected in April in the Çarşamba (Aşıklı) district of Samsun. Some of the collected plants were herbarium samples, and some of them were made into stock samples in 70% alcohol (MKA 201, MKA 202, OMUB, 8222). Stem and leaf sections of the species were examined and measured using the Zeiss AxioLab A1 microscope and the Zeiss Axiocam 105 imaging system. After taking the anatomical sections, an average of 30 measurements were taken for each character (Table 1) using the same microscope. Glycerine (50%) was used for section examinations. In addition, the leaf surfaces of the study species were also examined from a micromorphological point of view.

Micromorphological studies were carried out to determine the general appearance of the epidermis and stomata on the leaf surfaces (measurements of stomatal and epidermal cell sizes were taken from superficial leaf sections using the Zeiss Axiocam 105 camera imaging system). Scanning electron microscopy (SEM) was used to determine surface characteristics. For scanning electron microscopy (SEM), the samples were attached to the stubs with double-sided carbon tape and coated with 12.5-15 nm gold-palladium (SEM coating system, SC7620). Examinations and photographs were made in a JEOL JMS-7001F scanning electron microscope (SEM) with a voltage of 5-15 KV. Statistical analyses were also carried out on the anatomical characteristics of the taxa. PAST 4.04 packages were used for the analysis (averages and correlation).

## 3. Results

The leaves of both habitat samples have a scale-like shape. In addition, there is a vascular bundle in the centre of the leaf structure in both habitat samples. *Tamarix*, which spreads along the riparian, has a single layer of epidermis, the cortex layer has 5-7 rows. Just below the cortex there is a sclerenchymatic sheath structure. The stem centre consists of parenchymatic cells. In salt marsh samples, a single layer of epidermis was identified in the stem structure. In addition, the cortex layer consists of 4-8 rows of elongated and cylindrical cells. In addition, the centre of the stem is made up of parenchymatic (cylindrical) cells. There is a sclerenchymatic sheath layer beneath the cortex.

The anatomical studies of the leaves compared the salt marsh and riparian species. It was found that the length and width of the lower epidermal cells and the width of the stomata on the lower surface of the leaves were greater in the *Tamarix tetrandra* species that spread in the salt marsh (Fig1, Fig. 3). It was observed that the leaf upper epidermis cell length and width and leaf lower surface stomata length were larger in the species distributed along the riparian. The leaves of species found in both habitats have a single layer of epidermis (Fig. 1). In addition, the mesophyll has a unifacial leaf type formed by parenchymatic cells. In the anatomical examinations made on the stem, it was determined that the stem epidermis cell width and trachea cell diameters were larger in species distributed in salt marshes. It was found that stem epidermis cell length, sclerenchyma cell diameter, sieve tube cell diameter, and pith cell diameter were greater in the riparian species (Fig. 2).

The micromorphological studies revealed conical papillae structures on the upper leaf surfaces of the species found in both habitats. The swelling was observed on the tops of the papillae structures on the upper surface of the leaves of the species that spread along the riparian. In addition, reticulated ornaments were identified on the surface structures of the papillae (Fig. 3). The upper parts of the papillae structures of the salt marsh species have a pointed end. Flat wavy ornamentation was found in the surface structures of the papillae. In the examinations made on the lower surface of the leaves, it was determined that there were reticulate ornaments on the surface structures of the species that spread along the riparian. In addition, epidermis cell structures and stomata were clearly observed on the lower surface. In salt marsh species, the stomata are prominently embedded in the epidermal layer. Moreover, the stomata are closed in both samples examined (Fig. 3). Crystal-like structures and amorphous structures were observed on the stomata margins and epidermis surfaces. Wavy ornaments were also found on the lower leaf surfaces of the samples examined (Fig. 3).

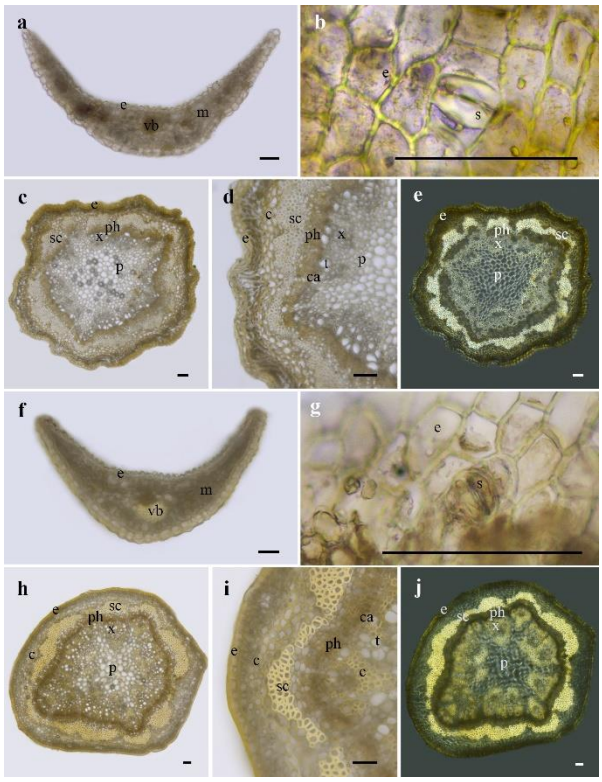


**Figure 1.** Stem and leaf anatomy of riparian and salt marsh specimens of *Tamarix tetrandra.* a-e: Riparian specimens (a-b leaf, c-e stem), f-j: Salt marsh specimens (f-g: leaf, h-j: stem). e: epidermis, vb: vascular bundle, m: mesophyll, sc: sclerenchyma, c: cortex, ph: phloem, x: xylem, ca: cambium, t: trachea, p: pith, s: stomata, scale bar: 100µm

**Table 1.** Measurements and characters of *Tamarix* from different habitats

| Character/Habitat | Riparian samples | | Salt marsh samples | |
|---|---|---|---|---|
| | Mean (µm) | Std. error | Mean (µm) | Std. error |
| Leaf lower epidermis cell length | 32,030 | 1,011 | 28,397 | 1,616 |
| Leaf lower epidermis cell width | 24,518 | 1,249 | 20,502 | 0,504 |
| Leaf upper epidermis cell length | 26,771 | 1,358 | 33,621 | 1,477 |
| Leaf upper epidermis cell width | 15,612 | 0,397 | 28,358 | 0,861 |
| Stomata length | 21,983 | 0,623 | 27,419 | 1,040 |
| Stomata width | 16,645 | 0,580 | 16,462 | 0,878 |
| Stem epidermis cell length | 16,096 | 0,664 | 16,225 | 1,017 |
| Stem epidermis cell width | 13,558 | 0,468 | 12,957 | 0,504 |
| Stem sclerenchyma cell diameter | 8,486 | 0,462 | 9,531 | 0,648 |
| Stem trachea cell diameter | 11,977 | 0,754 | 10,323 | 0,641 |
| Stem sieve tube diameter | 4,494 | 0,168 | 5,326 | 0,200 |
| Stem pith cell diameter | 24,729 | 0,847 | 34,027 | 1,205 |

Correlation analysis was performed on anatomical data for samples from both habitats. In the correlation analysis, positive correlations were observed between leaf lower epidermis cell length and upper epidermal cell width, leaf lower epidermis cell length, and stem sclerenchyma cell diameter in riparian species. A negative correlation was determined between the stomata length and stem sieve tube diameter (Fig. 4). On the other hand, a positive correlation was observed between leaf upper epidermis cell width and stomata width and leaf upper epidermis cell width and stem sclerenchyma cell diameter in salt marsh species. There was also a negative correlation between leaf lower epidermis cell width and leaf upper epidermis cell width, stem epidermis cell length, and stem pith cell diameter (Fig. 4).

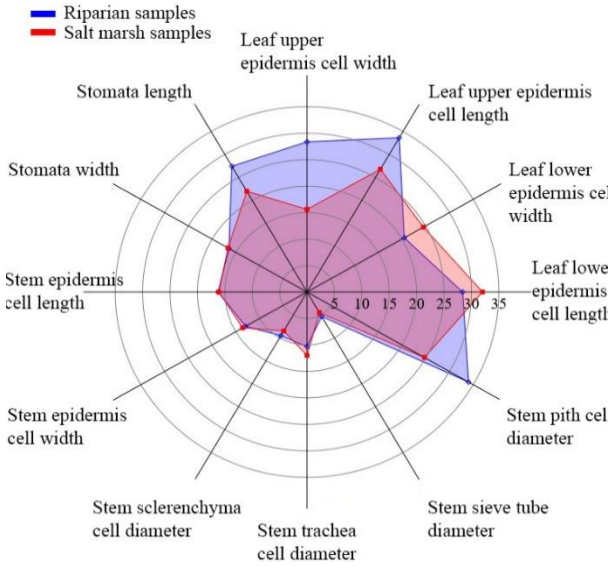


**Figure 2.** Mean of anatomical values of salt marsh and riparian samples.

## 4. Discussions

In this study, the leaf and stem structure of *Tamarix tetrandra*, which is distributed in the riverside and salt marsh area, were examined in terms of anatomy and micromorphology. Areas with abundant water but high soil salinity are similar to areas with low water availability (Munns and Tester, 2008). Although the salt marshes in our study area have high water availability, they have low water potential for *Tamarix* (and other species) that occur in this habitat.

Plants are able to adapt to salt marshes (areas) by performing certain functions. The first is the inhibition of salt uptake or storage in the protoplast (Dölarslan and Gül, 2012). Intense salt accumulation was not observed in the stem and leaf structures of salt marsh species. Salt intake is

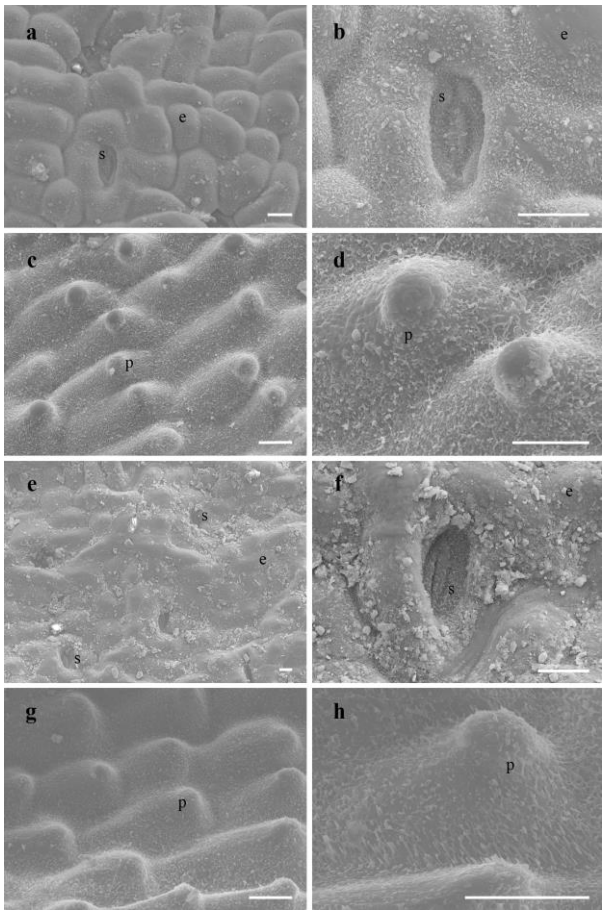


**Figure 3.** SEM image of salt marsh and riparian samples for micromorphological study. a-d: Riparian specimens (a-b: lower epidermis, c-d: upper epidermis), e-h: Salt marsh specimens (e-f: lower epidermis, g-h: upper epidermis). e: epidermis, s: stomata, p: papillae, scale bar: 10 µm

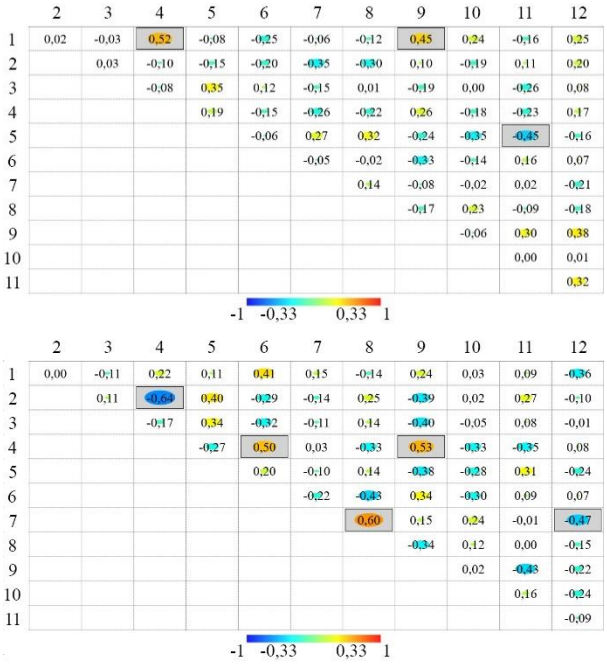


**Figure 4.** Correlation analysis for anatomical characters (The first analysis is for riparian samples and the second analysis is for salt marsh samples). 1: Leaf lower epidermis cell length, 2: Leaf lower epidermis cell width, 3: Leaf upper epidermis cell length, 4: Leaf upper epidermis cell width, 5: Stomata length, 6: Stomata width, 7: Stem epidermis cell length, 8: Stem epidermis cell width, 9: Stem sclerenchyma cell diameter, 10: Stem trachea cell diameter, 11: Stem sieve tube diameter, 12: Stem pith cell diameter.

thought to be inhibited in these species. It is noted that some characters are lost during the adaptation phase of plants to saline environments (Grigore and Toma, 2017, 2021). In the examined specimens of *T. tetrandra*, no loss of anatomical characteristics was observed in the leaf and stem structure. According to Grigore and Toma (2021), salt-secreting structures are rarely observed in *Tamarix* species growing in saline soils. In our study, no salt glands were found, especially in the samples distributed in salt marshes.

Micromorphological studies showed that the stomata were embedded in the epidermis. This condition is known as enhanced xeromorphic properties to reduce sweating. For species that spread in saline environments, this mechanism is also very important. The first of the changes that can occur in a short time when plants are under salt stress is the closing of stomata and the slowing down of carbon assimilation (Doğru and Canavar, 2020). In addition, stomata closure prevents transpiration and causes a decrease in stomatal conductance (Munns and Tester, 2008). It has been suggested that papillae structures are concentrated around stomata, reducing airflow (Maricle et al., 2009). Both of our habitat samples are located on the papillae leaf upper surface. However, no stoma was detected on the upper surface of the examined species. In a micromorphological study of *Tamarix* species, Abbruzzese et al., (2013) observed salt glands on the leaf surfaces of the species. Salt glands were not found in our micromorphological studies. This may be due to differences in species or environmental conditions.

Salinity causes differentiation in the transmission system (Flowers and Colmer, 2015). In our study, we found that the stem tracheal cells of salt marsh species were larger in diameter, while the sieve tube cells were smaller. *Tamarix* can show differences in development and distribution even over short distances in response to changes in soil salinity (Mumcu and Korkmaz, 2021). Salty environments have a negative effect on stem development in plants, but slow down vascular development (Zafar et al., 2015). It was determined that trachea diameters were larger and sieve tube diameters were smaller in salt marsh samples compared to riparian samples. There are smaller xylem diameters in *Tamarix*, especially in fast-drying areas (Long et al., 2021).

*Tamarix* has the ability to adapt to its environment. Therefore, it can have an active distribution in different habitats. It reveals anatomical differences in adaptation to the environment. In particular, the change in stomatal sizes, the stomatal structures embedded in the epidermis, and the differences in tracheal cell diameters clearly reveal this situation. Anatomical and micromorphological studies of the small leaf structures have shown that the anatomical structures can vary between species occurring in different habitats.

**Acknowledgements**

I would like to thank Dr. Gülsüm Mumcu and Prof. Dr. Hasan Korkmaz for their help in collecting plant samples.

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