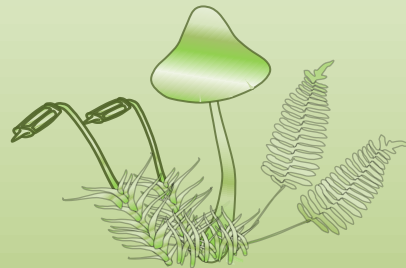


# Anatolian Journal of **Botany**



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## Erratum

### Volume 4(1)

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#### For:

<i>Adonis aestivalis</i> L. subsp. <i>parviflora</i> (Fisch. ex DC.) E.A.Busch	4423	B7 Tunceli: Pülümür, 9 km from Pülümür to Tunceli (Pülümür valley), S of Kangallı v.	1324	05.06.2014	Steppe		Unk.
<i>Adonis cyllenea</i> Boiss., Heldr. & Orph. var. <i>parviflora</i> Boiss.	Obs.	B7 Tunceli: Pülümür, between Sarıgül and Kocatepe, Buyer waterfall	2150	30.05.2017	Mt. steppe	* Rcd.	Unk.
<i>Adonis eriocalycina</i> Boiss.	3791	B7 Tunceli: Center, between Tunceli and Sütlüce, 1.8 km from Sütlüce junction	1030	23.05.2014	Steppe		Unk.

#### Read:

<i>Adonis aestivalis</i> L. subsp. <i>parviflora</i> (Fisch. ex DC.) E.A.Busch	4423	B7 Tunceli: Pülümür, 9 km from Pülümür to Tunceli (Pülümür valley), S of Kangallı v.	1324	05.06.2014	Steppe		Unk.
<i>Adonis eriocalycina</i> Boiss.	3791	B7 Tunceli: Center, between Tunceli and Sütlüce, 1.8 km from Sütlüce junction	1030	23.05.2014	Steppe		Unk.

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#### For:

<i>Consolida glandulosa</i> (Boiss. & Huet) Bornm.	3876	B7Tunceli: Pertek, 5 km from Pertek to Çemişgezek	866	24.05.2014	Steppe	*	Ir.-Tur.
<i>Consolida oliveriana</i> (DC.) Schödinger	5739	B7 Tunceli: Mazgirt, 3 km from Akdüven to Güleç	1102	05.08.2014	Steppe		Ir.-Tur.

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<i>Consolida glandulosa</i> (Boiss. & Huet) Bornm.	3876	B7Tunceli: Pertek, 5 km from Pertek to Çemişgezek	866	24.05.2014	Steppe	*	Ir.-Tur.
<i>Consolida olopetala</i> Hayek	5576	B7 Tunceli: Pülümür, 3 km from Erzincan-Erzurum-Tunceli crossroads to Pülümür	1335	22.07.2014	Steppe	* Rcd.	Unk.
<i>Consolida oliveriana</i> (DC.) Schödinger	5739	B7 Tunceli: Mazgirt, 3 km from Akdüven to Güleç	1102	05.08.2014	Steppe		Ir.-Tur.



## Flower morphology and ontogeny of endemic *Crataegus tanacetifolia* (Lam.) Pers. (*Rosaceae*) from Turkey

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### Türkiye endemiği *Crataegus tanacetifolia* (Lam.) Pers.' in (*Rosaceae*) çiçek morfolojisi ve ontogenisi

**Abstract:** In this study, flower morphology and ontogeny of Turkish endemic *Crataegus tanacetifolia* (Lam.) Pers. (*Rosaceae*) were studied using stereo, light and scanning electron microscope. *Crataegus tanacetifolia* has corymb type of inflorescence which bears 5-10 hermaphrodite flowers. The first sign in appearance of the flowers is the differentiation of apical meristem as a roundish bulge. It transforms into the floral meristem which gives rise to floral organ primordia in advanced stages. Twenty stamen primordia differentiate as roundish bulge from the sides of floral meristem. Subsequently, the stamen primordia start stretching and differentiating into filaments and anthers respectively. After a short time of stamen initiation, 5 carpel primordia differentiate from the floral meristem. In following stages, the carpel primordia elongate and form an ovary with 5 loculi. During the ovary formation, 5 styles occur on the ovary and then stigmas evolve out of styles tips.

**Key words:** apical meristem, *Crataegus tanacetifolia*, floral meristem, floral organ development, ontogeny

**Özet:** Bu çalışmada, Türkiye endemiği *Crataegus tanacetifolia* (Lam.) Pers.'in (*Rosaceae*) çiçek morfolojisi ve ontogenisi stereo, ışık ve tarayıcı elektron mikroskobu ile incelenmiştir. *Crataegus tanacetifolia* 5-10 adet hermafrodit çiçek taşıyan korimbus tipi çiçek durumuna sahiptir. Çiçeklerin ortaya çıkışındaki ilk işaret, apikal meristemin yuvarlak bir çıkıntı olarak farklılaşmasıdır. Apikal meristem ilerleyen evrelerde çiçek organlarını oluşturacak olan floral meristeme dönüşür. Floral meristemin kenarlarından 20 adet stamen taslağı yuvarlak çıkıntılar olarak farklılaşır. Ardından stamen taslakları uzamaya başlar ve sırası ile filamentler ve anterleri oluşturur. Stamen taslaklarının belirmesinden kısa bir süre sonra, floral meristemden 5 adet karpel taslağı farklılaşır. İlerleyen evrelerde, karpel taslakları uzar ve 5 lokuslu bir ovaryum oluşturur. Ovaryum oluşumu esnasında, 5 stilus ovaryumun üzerinde gelişir ve ardından stilusların üzerinde stigmalar gelişir.

**Anahtar Kelimeler:** apikal meristem, *Crataegus tanacetifolia*, floral meristem, çiçek organ gelişimi, ontogeni

**Citation:** Çetinbaş Genç A, Ünal M (2020). Flower morphology and ontogeny of endemic *Crataegus tanacetifolia* (Lam.) Pers. (*Rosaceae*) from Turkey. Anatolian Journal of Botany 4(2): 80-84.

## 1. Introduction

The genus *Crataegus* L. belongs to the subfamily *Maloideae* of *Rosaceae* and comprises approximately 200 species worldwide (Campbell et al., 2007; Benli et al., 2008). The homeland of *Crataegus* is Asian and Mediterranean Countries. According to Davis and Browicz (1972), there are 17 species, a subspecies, two varieties and dozens of hybrids, naturally grown in Turkey. *Crataegus* is characterised by a corymb which is a specialised determinate inflorescence containing a few to numerous of flowers (Dönmez, 2004). The inflorescence of *Crataegus* has one type of flower on a single corymb; hermaphrodite. Androecium consist of 5-60 stamens (Rohrer and Robertson, 1994) and gynoecium have 1-5 pistils (Dönmez, 2004). *Crataegus* species are important medicinal plants. They have been used as multi-effect traditional medicine for prevention and treatment of heart disease, insomnia and hypertension (Vitalini et al., 2009; Chang et al., 2005; Tassell et al., 2010; Dong et al., 2017). Also, they are economically important plants being used in cosmetics and food industry (Lund et al., 2017).

Sex determination in plants is among the most significant subjects that concern biologists due to the importance of reproduction and fertilization for genetical diversity. Also, flower organogenesis is a major area of plant developmental biology, because it is being one of the main basic topics and the reproductive character and the

features of reproductive organs are systematically essential (Kinney et al., 2008). The basic bisexual flower is subdivided into four whorls. Whorl 1 and whorl 2 compose the sterile parts of flower and sex determination takes place in whorls 3 and 4, which contain stamens and pistils respectively (Dellaporta and Calderon-Urrea, 1993). However, the development pattern on the whorls differs between the sexes of flowers; hermaphrodite, pistillate, staminate. The start of flower bud development comprises the conversion of the vegetative meristem into the apical meristem and the transformation of the apical meristem into the floral meristem in the upcoming stage. Floral organs; sepal, petal, stamen and carpel develop from floral meristem, but further development of stamen or pistil is selective, resulting in unisexual flowers (Çetinbaş and Ünal, 2012).

*Crataegus tanacetifolia* (Lam.) Pers. is an important species, endemic to Turkey. It is a perennial shrub or a small tree, growing up to 10 meters (Saribaş and Yaman, 2005). Most of the morphological, anatomical and ontogenic studies conducted in *Crataegus* have been concentrated on common species (Gyan and Woodell, 1987; Bura et al., 2016; Hamideh et al., 2012). To our knowledge, there are no detailed morphological and ontogenic studies that have been reported for Turkish endemic *C. tanacetifolia*. Since *C. tanacetifolia* is endemic to Turkey, there is not sufficient data regarding its



morphological and ontogenic characteristics, hence we aimed to give a detailed account of these features. Information on the development of reproductive organs will help advance our understanding of reproductive behaviour and will thus contribute to attempts to solve taxonomic problems in *Crataegus*, a rather neglected genus in this respect.

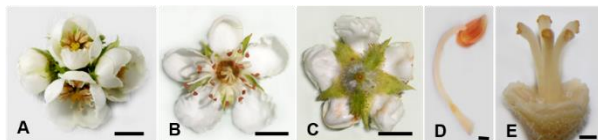
## 2. Materials and Method

The materials were collected from natural habitats within the borders of Bolu, Lake Abant Nature Park (Turkey) and morphologically analysed under stereomicroscope (Olympus 970931). At least 50 samples were studied for each phase. After fixation process by FAA (Formalin-glacial acetic acid-alcohol) solutions, they were embedded in paraffin blocks, sliced at 10 µm by Leica RM2235 rotation microtome, stained with Delafield's hematoxylin, investigated by Olympus BH-2 light microscope. For scanning electron microscope (SEM) analysis, the material was fixed in 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7.0 (Platt et al., 1983), dehydrated with an increasing ethanol gradient: from 70% up to 100% and kept in hexamethyldisilazane solution at room temperature (Topcuoglu et al., 2009). After coating with 11 nm of gold by using an automated sputter coater, they were examined with JEOL JMS-59 10LV SEM.

## 3. Results

### 3.1. Flower morphology

*Crataegus tanacetifolia* has corymb type of inflorescence and the corymb contains 5-10 actinomorph, pentamerous, hermaphrodite flowers (Fig. 1A). The flowers on the corymb are generally in different developmental stages. Gynoecium contains an ovary with 5 loculi and 5 free stigmas-styles (Fig. 1E). Twenty stamens surround the pistil (Fig. 1B,D). The flowers have 5 green, hairy sepals and 5 free, roundish, white petals. Receptacle is green and hairy (Fig. 1C). Measurements of the mature flower and flower parts of *C. tanacetifolia* were presented in Table 1.



**Figure 1.** Morphology of the corymb and the flower of *C. tanacetifolia*. A. Corymb type of inflorescence, B. Upper view of mature flower with pistils, stamens and petals, C. Bottom view of mature flower with hairy sepals, D. Mature stamen, E. Gynoecium with 5 loculi ovary and 5 free stigmas-styles. Bar: 0.5 mm (D), 1 mm (E), 1 cm (A), 0.5 cm (B, C).

**Table 1.** Measurements of mature flower and flower parts of *C. tanacetifolia*.

Flower and flower parts	Measurement (mm)
Length of the mature flower	15 ± 2
Width of the mature flower	12 ± 3
Length of the sepal	9 ± 2
Length of the petal	11 ± 3
Length of the stamen	11 ± 2
Length of the pistil	13 ± 2

### 3.2. Flower ontogeny

Development starts with the differentiation of shoot apical meristem (Fig. 2A). Tunica, located at the tip of growth cone, consists of a few layers of cells. The diameter of the apical meristem increases as a consequence of cell division. Afterwards, growth cone swells due to the cell division at corpus (Fig. 2B). With the flattening of the growth cone and then swelling up of it, the transformation from bud to flower starts (Fig. 2C). Firstly, the apical flower bud of the corymb and then the lateral flower buds differentiate (Fig. 2D). Afterwards, floral organ primordia are generated at differentiated flower buds.

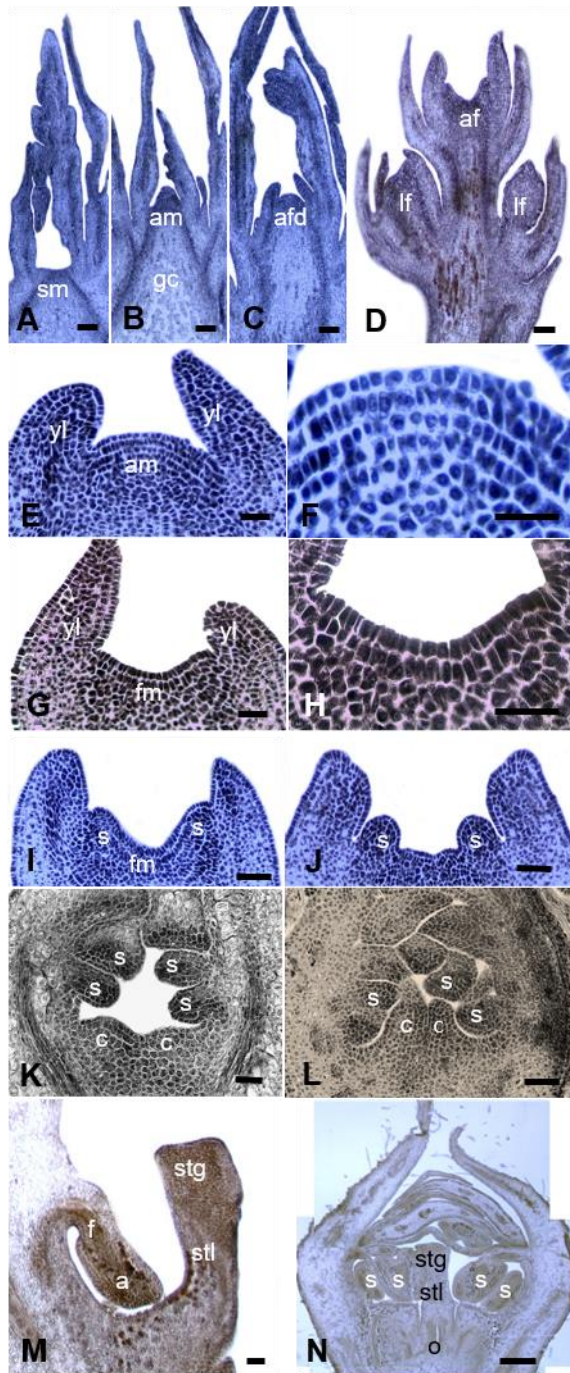
Floral organ formation starts with the formation of apical meristem. It differentiates as a roundish bulge from the vegetative meristem at growth points (Fig. 2E, Fig. 3A) and consists of well-ordered, thin walled small cells which have abundant cytoplasm and small vacuoli (Fig. 2F). Development of a flower starts with the transformation of the apical meristem to the floral meristem. During the transformation, length and width of the apical meristem increase and the apical apex becomes flattened (Fig. 2G, Fig. 3B). Therefore, the width of floral meristem (approximately 197 µm) is greater than that of apical meristem (approximately 181 µm). The floral meristem consist of well ordered, thin walled cell layers and the intercellular space of the cells are small. The cells have abundant cytoplasm, numerous vacuoli and a big nucleus which consist of a great number of nucleoli, indicates that the cells are very active in transcription (Fig. 2H).

The floral meristem gives rise to the floral organ primordia in advanced stages. Twenty stamen primordia, that are located very close to each other, differentiate as roundish bulges from the outer ring of the floral meristem (Fig. 2I,J, Fig. 3C,D). The flatness of floral meristem is still visible at this stage. Concomittant with the start of stamen primordia development, 5 roundish carpel primordia differentiate from the center of floral meristem and the flatness of the floral meristem can not be visible in this way (Fig. 2K).

While the stamens develop, the roundish shape of them begin to dissappear. Firstly anther and then filament differentiate. During this time, carpel primordia begin to develop and elongate (Fig. 2L). The extending carpel primordia create their own style, the lower part of the pistil becomes swollen and the origin of ovary and ovary loculi are formed. Stigma differentiates on the apex of the style in the advanced stage (Fig. 2M). During the initiation and development, the dimension of the flower bud and reproductive organs increase (Fig. 2N, Fig. 3E,F). Relationship between the flower bud size and flower development stages of *C. tanacetifolia* were presented in Table 2.

**Table 2.** Relationship between flower bud size and flower development stages in *C. tanacetifolia*.

Stage of flower development	Width of the flower bud (µm)	Length of the flower bud (µm)
Apical meristem	535 ± 21	436 ± 46
Floral meristem	552 ± 36	453 ± 42
Stamen primordia initiation	560 ± 14	516 ± 29
Carpel primordia initiation	573 ± 23	524 ± 32

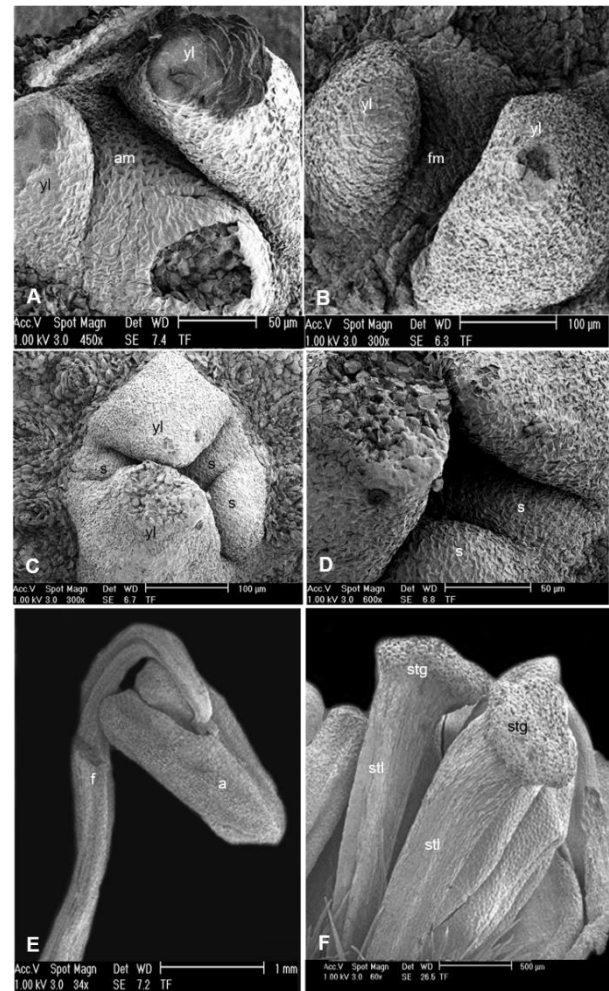


**Figure 2.** Flower ontogeny in *C. tanacetifolia*. A. Straight and flat shoot meristem, B. Shoot apical meristem and swelling of growth cone, C. Differentiation of apical flower bud, D. Lateral flower bud differentiation and becoming recognizable of corymb. E. Apical meristem, F. The cells of apical meristem, G. Floral meristem, H. The cells of floral meristem, I. Initiation of stamen primordia from floral meristem, J. Development of stamen primordia, K. Initiation of carpel primordia, L. Growing of carpel primordia, M. One of the 5 young pistil and one of the 20 young stamen, N. Mature flower. sm: Shoot meristem, am: Apical meristem, gc: Growth cone, afd: Apical flower draft, af: Apical flower, lf: Lateral flower, yl: Young leaf, fm: Floral meristem, s: Stamen primordium, a: Anther, f: Filament, c: Carpel primordium, stg: Stigma, stl: Style, o: Ovary. Bar: 50 µm (E-H, I-M), 100 µm (A-D), 200 µm (N).

**4. Discussions**

The corymbs of *C. tanacetifolia* and *C. yaltirikii* Dönmez contain 5-10 flowers, the corymb of *C. coriifolia* Sharifnia

& Zarrinkolah (Sharifnia et al., 2016) bears 15 flowers and the corymb of *C. ambigua* Mey. bears 20 flowers (Tuyakova et al., 2016). The flowers of *Crataegus* are pentamerous and petals are generally white-pink (Dönmez, 2004). Flower sizes of *Maloideae* are generally 3.4 mm and 4.1 mm. The number of organs is not related to the size of the flowers. For instance, *C. suksdorfii* Lindl. and *C. douglasii* Lindl. have larger flowers than *C. rivularis* Nutt. and *C. brockwayae* Sarg.. *Crataegus rivularis* Nutt. has more stamens than *C. douglasii* Lindl., but has less stamen than *C. brockwayae* Sarg. (Evans and Dickinson, 1996). The number of stamen differs from species to species in *Crataegus* (Evans and Dickinson, 1996). The flowers generally contain 10 stamens as in *C. transmississippiensis* Sarg. and *C. galli* L. or 20 stamens as in *C. tanacetifolia*, *C. submollis* Sarg., *C. canadensis* Sarg., *C. punctata* Jacq. (Phipps, 2012). The number of ovary loculi can also differ in *Crataegus* species. *Crataegus tanacetifolia* has 1 ovary with 5 loculi, the ovary of *C. galli* has 1-2 loculi and the ovary of *C. punctata* has 2-5 loculi. Each of the loculi of these species are linked to the style as in *C. tanacetifolia*.



**Figure 3.** SEM micrograph of the flower ontogeny in *C. Tanacetifolia*. A. Apical meristem, B. Floral meristem, C. Stamen primordia initiation, D. Stamen primordia, E. 1 of the 20 mature stamen, F. 2 of the 5 mature pistil. am: Apical meristem, fm: Floral meristem, yl: Young leaf, s: Stamen primordia, a: Anther, f: Filament, stg: Stigma, stl: Style.

Most angiosperms produce hermaphrodite flowers with functional male and female sex organs within the same flower (Aryal and Ming, 2014). During the early stages of



floral organ development, all floral organ primordia form in male and female flowers. In males, the gynoecium develops as a sterile, undifferentiated rod, while in females, anther development arrests soon after the anther primordia form, then the anthers degenerate (Juarez and Banks, 1998). Therefore, the further development of stamens or pistils is very important in determining the sex of the flower. It is thought that sex is determined by the selective inhibition of gynoecium or androecium development in hermaphrodite flower. Also, the determination process involves the differentiation of gametophyte in pistil or stamen (Dellaporta and Urrera, 1993). For instance, sex determination in pistillate flower of *Zea mays* arises as a result of programmed cell death of stamen primordia (Cheng et al., 1983). Due to the hermaphroditism, there are no atrophy or cell death on sexual organ primordia of *C. tanacetifolia*.

The developmental stages from the physiological differentiation of flower buds to the blooming of flowers have been studied by many researchers. Durner and Poling (1985) have divided the flowering into 4 stages: induction, initiation, differentiation and development. The floral induction occurs in the leaf upon exposure to a stimulus which ultimately results in the production of a flower bud at a shoot meristem (Durner, 2015). The initiation includes physiological and morphological changes occurring in the meristem upon receipt of stimuli from the leaf. Tal et al. (2017) indicated that enlargement and doming of the shoot apical meristem is a hallmark of the transition from vegetative growth to flowering. According to Evans and Dickinson (1996), the initiation of the flower buds starts with the transformation of apical meristem to the floral meristem. The morphological changes, observed during the conversion of apical meristem to floral meristem, resemble the ones in *Prunus avium* L. and *P. Persica* (L.) Batsch; apical meristem flattens and radially extends (Engin and Ünal, 2007). According to Durner and Poling (1985), differentiation is the development of specific floral organs

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on a single flower or of flowers on a single inflorescence, and development is the macroscopic production of flowers.

As in other plants (Uhl, 2011), all floral organ primordia in *C. tanacetifolia* develop as a result of periclinal division of floral meristem cells. In *C. tanacetifolia*, *C. suksdorfii* Lindl., *C. douglasii* Lindl. and *C. rivularis* Nutt., the first differentiated sex organs from floral meristem are stamens. Stamen primordia arise in roundish bulge forms as they do in *P. avium* and *P. persica* (Engin and Ünal, 2007). In *Maloideae*, 20 stamen differentiate in 1-3 rings. However, in *C. tanacetifolia*, 20 stamen primordia, that are located very close to each other, differentiate as roundish bulges from the outer 1 ring of the floral meristem and they emerge synchronized as they do in *Erigeron philadelphicus* L. (Harris et al., 1991). Stamen primordia arise out of the sides of floral meristem, as Dadpour et al. (2011) stated, and in stages where stamen primordia start emerging, the flatness of floral meristem starts to become gradually convex.

Female organ development starts with formation of carpel primordium in the center of floral meristem in *C. tanacetifolia*, as it is a usual manner in flowering plants (Gasser and Beers, 1993). But in *Maloideae* the time of differentiation of carpel primordia can differ. For instance, carpel primordia differentiate after all stamen primordia have achieved a nearly hemispherical shape in *C. suksdorfii* (Evans and Dickinson, 1996). But they differentiate shortly after the stamen initiation in *C. tanacetifolia*. The carpel primordia, which just arise in *C. tanacetifolia*, are not as roundish as stamen primordia. Uhl (2011) mentioned that various floral organs those differentiate out of floral meristem, might be morphologically different.

## Acknowledgments

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## The regulatory effects of resveratrol on the expression of renal MMP-2 and MMP-9 in the rat models of diabetes

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### Resveratrolün sıçan diyabet modellerinde renal MMP-2 ve MMP-9 ekspresyonu üzerindeki düzenleyici etkileri

**Abstract:** Hyperglycemia caused by diabetes mellitus, in sensitive tissues such as the kidney, can cause dysfunction by damaging the blood vessels. Matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, play a significant role in the degradation of proteins and angiogenesis; while epidermal growth factor receptor (EGFR) is involved in the recovery of damaged tissues. Here in, we investigated the relationship between MMP-2 and MMP-9/EGFR in the kidney tissues of streptozotocin-induced Wistar male rats. We also examined the effects of resveratrol known as a strong tissue-protective on these changes. Diabetes was induced by streptozotocin (55 mg/kg) and trans-resveratrol (20 mg/kg/day intraperitoneal) was used for treatment. Rats were divided into 4 groups as control, resveratrol (Res), diabetes (Diab) and diabetes plus resveratrol (Diab+Res). Superoxide dismutase (SOD) and catalase (CAT) levels were significantly decreased in the kidneys in the diabetic group, whereas nitrite/nitrate, urea, creatine kinase (CK) and uric acid concentrations increased. MMP-2, MMP-9, calcium-binding protein B (S100B) and platelet-derived growth factor (PDGF) concentrations of kidney were increased although reduced EGFR in the diabetes group. Resveratrol supplementation markedly restored all these structures. Diabetes activates MMP related inflammation and oxidative stress by suppressing antioxidant enzymes in the kidney tissues. Resveratrol has partly modulatory effects on diabetes-induced changes.

**Key words:** Diabetes mellitus, resveratrol, kidney, MMP-2, MMP-9

**Özet:** Diyabetes mellitusun neden olduğu hiperglisemi böbrek gibi hassas dokularda kan damarlarına zarar vererek işlev bozukluğuna neden olabilir. Matriks metaloproteinazlar (MMP'lar), özellikle MMP-2 ve MMP-9, proteinlerin bozunmasında ve anjiyogenezde önemli bir rol oynarken; epidermal büyüme faktörü reseptörü (EGFR) hasarlı dokuların iyileşmesinde rol oynar. Burada streptozotocin ile indüklenmiş Wistar erkek sıçanların böbrek dokularında MMP-2 ve MMP-9/EGFR arasındaki ilişkiyi araştırdık. Ayrıca güçlü doku koruyucu olarak bilinen resveratrolün bu değişiklikler üzerindeki etkilerini inceledik. Diyabet modeli streptozotocin (55 mg/kg) ile oluşturuldu ve tedavi için trans-resveratrol (20 mg/kg/gün intraperitoneal) kullanıldı. Sıçanlar kontrol, resveratrol (Res), diyabet (Diab) ve diyabet + resveratrol (Diab + Res) şeklinde 4 gruba ayrıldı. Diyabet grubundaki sıçanların böbreklerinde superoksit dismutaz (SOD) ve katalaz (CAT) düzeylerinin önemli ölçüde azaldığı buna karşın nitrit/nitrat, üre, kreatinin kinaz (CK) ve ürik asit konsantrasyonlarının arttığı saptanmıştır. Diyabet grubunda renal EGFR düzeyinin azalmasına rağmen MMP-2, MMP-9, kalsiyum bağlayan protein B (S100B) ve trombosit kaynaklı büyüme faktörü (PDGF) düzeyleri artmıştır. Resveratrol takviyesi tüm değişkenleri önemli ölçüde iyileştirdi. Diyabet, böbrek dokularındaki antioksidan enzimleri baskılayarak MMP ile ilişkili inflamasyonu ve oksidatif stresi aktive eder. Resveratrol, diyabetin neden olduğu bu değişiklikler üzerinde kısmen modülatör etkilere sahiptir.

**Anahtar Kelimeler:** Diyabetes mellitus, resveratrol, böbrek, MMP-2, MMP-9

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### 1. Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases involved in remodeling the extracellular matrix. Some articles have reported that MMPs could play a role in cell apoptosis, angiogenesis, tissue repair and immune response (Ning, 2017). Hyperglycemia and free oxygen reagents increase MMP-2 and MMP-9 levels by inducing MEK/ERK/NfκB pathway and often result in diabetic retinopathy (Renu, 2017; Ankita, 2019). It has been shown in many studies that diabetes-induced hyperglycemia increases the levels of end products Cystatin-C, creatine kinase (CK), urea and uric acid (Adlija, 2006; Talib, 2012; Qian, 2017) by enhancing the levels of oxidative markers such as nitrite/nitrate (Naseer, 2014). Diabetes-induced hyperglycemia also suppresses antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Xue-

Wen, 2018). Hyperglycemia is not only limited with increased glucose concentrations, but also it increased advanced glycation end-product concentrations. It increases calcium binding protein B (S100B), a protein of the S-100 protein family. S100B is glial-specific and expressed primarily by astrocytes. S100B delays the elimination of rapidly rising inflammatory markers in traumatic process and tissue damage. It is known to be related to the altered expression of S100B of Alzheimer's disease, melanoma, and type I diabetes mellitus (Guglielmo, 2013). On the other hand, when it comes to renal tissue damage, epidermal growth factor receptor (EGFR) comes into question. EGFR is a tyrosine kinase receptor expressed in the kidney and activated after renal damage (Yoshioka, 1990). This receptor plays a key role in renal electrolyte homeostasis. However, its role in renal pathology is somewhat contradictory since both beneficial and deleterious actions have been found (Melenhorst,

2008). Accumulating proof indicates a critical relevance of platelet-derived growth factor (PDGF) signaling in renal pathology (Taizo, 2012). A new PDGFR- $\beta$  aiming mouse model has ensured novel insight into the postnatal role of PDGFR- $\beta$  in aging-related glomerular remodeling after nephrectomy (Sigrun, 2004).

Resveratrol, a naturally occurring phytoalexin and polyphenolic compound, which is produced by plants against various infections. It has vasoprotective (Babacanoglu, 2013; Pektaş, 2015 and 2018), cardio-protective (Abdelgawad, 2019), anticancer (Abdelgawad, 2019), antioxidant (Akar, 2011; Pektaş 2016a, 2017), and anti-inflammatory (Sadi, 2015; Koca, 2016; Pektaş, 2016b) properties and it effects some cellular processes like angiogenesis (Pektaş, 2017), apoptosis (Pektaş, 2016a), proliferation (Pektaş, 2016a), as well as oxygen radical formation (Akar, 2011). In a recent study, resveratrol has been shown to be effective in the treatment of endometriosis by increasing MMP-2 and MMP-9 levels (Mahshad, 2019). In another study, it has been shown that decreased EGFR levels by CdCl<sub>2</sub> in the testis of mice were normalized with resveratrol supplementation (Sreyashi, 2016).

The investigation of streptozotocin (STZ)-induced diabetes on kidney tissue MMPs/EGFR and oxidative process and their modification by resveratrol will provide new insights to understand the mechanisms. Therefore, here in, we investigated the effects of STZ-induced diabetes and resveratrol supplementation on the protein levels of MMP-2/MMP-9/EGFR and oxidant/antioxidant structures in kidney tissue of rats. We hypothesized that chronic administration of supplemental resveratrol would prevent diabetic renal damage by the restoration of MMPs/EGFR and decreasing oxidative stress.

## 2. Materials and Method

### 2.1. Chemicals

Trans-resveratrol was purchased from Molekula (Gillingham, Dorset, UK) and STZ was obtained from Sigma (St. Louis, MO, USA). The purity of resveratrol was tested by HPLC followed with LC-MS and 98% of the constituent was determined as trans-resveratrol. All other chemicals used in this study were of the highest analytical grade available.

### 2.2. Animals and treatment procedure

All protocols for animal usage were approved by the Ethical Animal Research Committee of Afyon Kocatepe University (AKUHADYEK-2019/49533702-91). Experiments were performed on 8-week-old adult male Wistar rats weighing between 300-350 g. They were maintained under temperature-controlled conditions (20-22°C) with a 12-h light-dark cycle and fed with standard rodent diet: 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt mixture (chow pellet). After 1 week, the rats were randomly separated into 4 groups. The control group included 12 rats that were injected only with vehicle, 10% dimethyl sulfoxide (DMSO), for 4 weeks. The resveratrol group (12 rats) was administered a daily dose of 20 mg/kg resveratrol in vehicle intraperitoneally (i.p.) throughout the 4-week period. The diabetes group (12 rats) received a single i.p.

dose of STZ (55 mg/kg) dissolved in 0.05 M citrate buffer (pH 4.5) and daily injections of vehicle for 4 weeks. The diabetes+resveratrol group contained 9 rats that received a daily dose of 20 mg/kg resveratrol i.p. throughout the 4-week period, starting from day 2 after STZ administration. Blood glucose concentrations from the blood of the tail veins were determined weekly using Accu-Check Go (Roche, Germany) glucometer. A blood glucose concentration higher than 200 mg/dL served as the criteria for diabetes. At the end of the study period, all rats were decapitated and the kidney tissues were blotted dry, frozen in liquid nitrogen, and stored at -85 °C for further use.

### 2.3. Measurement of metabolic parameters in the renal tissue

Kidney samples were homogenized in 0.1 M phosphate buffer 1:10 (w/v), pH 7.4, and 24000 cycles/min (Ultra Turrax, IKA Works Inc., USA), and ultrasonicated at 20000 cycles/s for 1 min (Dr. Hielscher, Germany). Homogenates were centrifuged at 10000 x g and 4°C for 15 min, and the supernatants were collected. All the samples were stored at -85°C until analysis. Renal glucose, triglyceride, total cholesterol (Spinreact, Santa Coloma, Spain), CK, urea, and uric acid (Biolabo, France) levels, were determined by standard enzymatic techniques. Renal nitrite/nitrate, Cu/Zn SOD, and CAT levels were measured using assay kits (Cayman Chemical, USA).

### 2.4. Measurement of renal MMP-2, MMP-9, EGFR, S100B, PDGF, and Cystatin-C

Total protein contents were determined using the Lowry method (Lowry, 1951). The levels of renal MMP-2, MMP-9, EGFR, S100B, PDGF, and Cystatin-C concentrations were measured using commercially available rat-specific ELISA kits (eBioscience, Bender Med. Systems GmbH, Vienna, Austria) according to the manufacturer's protocols.

### 2.5. Statistical analysis

The results are given as mean  $\pm$  standard error of the mean (SEM); n is the number of rats. Statistical analyses were performed by Student's t-test for unpaired data or one-way ANOVA followed by the Bonferroni post hoc analysis where appropriate by using Prism 5.02 GraphPad software. Values were considered to be significantly different when the p-value was less than 0.05.

## 3. Results

### 3.1. Effects of resveratrol on body weights and the renal metabolic characteristics of the rats

The body weights in the diabetes group were significantly lower than those of control rats and unchanged after resveratrol supplementation (Table 1). STZ-induced diabetes increased renal glucose, triglyceride, and total cholesterol contents when compared to the control group. Resveratrol supplementation partially restored these abnormalities in rats with diabetes (Table 1). Renal levels of CK, urea, and uric acid, which are the indicators of renal damage, were increased with STZ-induced diabetes. Resveratrol administration normalized renal CK and uric acid levels but did not change urea levels (Fig. 1B, 1C, 1D). A similar marker called cystatin-C did not change in all groups (Fig. 1A).



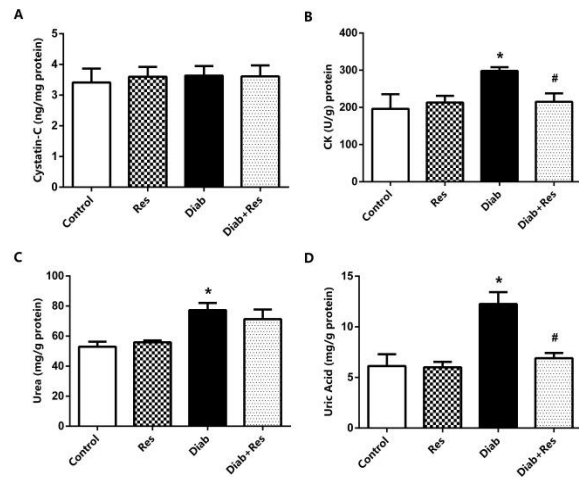
### 3.2. The effects of resveratrol on MMPs, EGFR, S100B, PDGF and the other markers in the kidney tissues

MMPs (MMP-2 and MMP-9), which are key enzymes for destructive extracellular matrix, were dramatically increased in the kidney of rats with STZ-induced diabetes. Resveratrol supplementation to rats with diabetes significantly suppressed expression levels of these enzymes (Fig. 2A, 2B). EGFR which has anti-inflammatory, anti-apoptotic, neurotrophic, and neuroprotective effects, was reduced in the diabetes group compared to control. Resveratrol showed restorative effects on the renal EGFR with STZ-induced diabetic rats (Fig. 2C). PDGF is a coagulation factor that provides proliferation and transformation of cells, regulates apoptosis and is released by many different cells such as fibroblasts, chondrocytes, and glia cells during tissue regeneration. There was a marked functional up-regulation of PDGF in the kidney of rats with diabetes. Resveratrol supplementation significantly suppressed the expression of PDGF (Fig. 2D). Expression of S100B which involves cell proliferation, protein phosphorylation, differentiation, inflammation, and apoptosis, was also apparently enhanced by diabetes. Resveratrol supplementation reduced renal S100B in the diabetic rats (Fig. 2E). Nitrite/nitrate levels were lower in the kidney samples of diabetic rats compared with rats in the control groups. Resveratrol supplementation significantly increased nitrite/nitrate levels in the kidneys of the diabetic rats from resveratrol administration during the onset of diabetes (Fig. 2F). The other results show the suppression of renal SOD and CAT activities in the STZ-induced rats (Fig. 2G, 2H). However, treatments with resveratrol did not alter CAT activities but significantly increased Cu/Zn SOD activities. As shown in figures 2G and 2H, supplementation with resveratrol to the healthy rats increased both Cu/Zn SOD and CAT activities in the kidney.

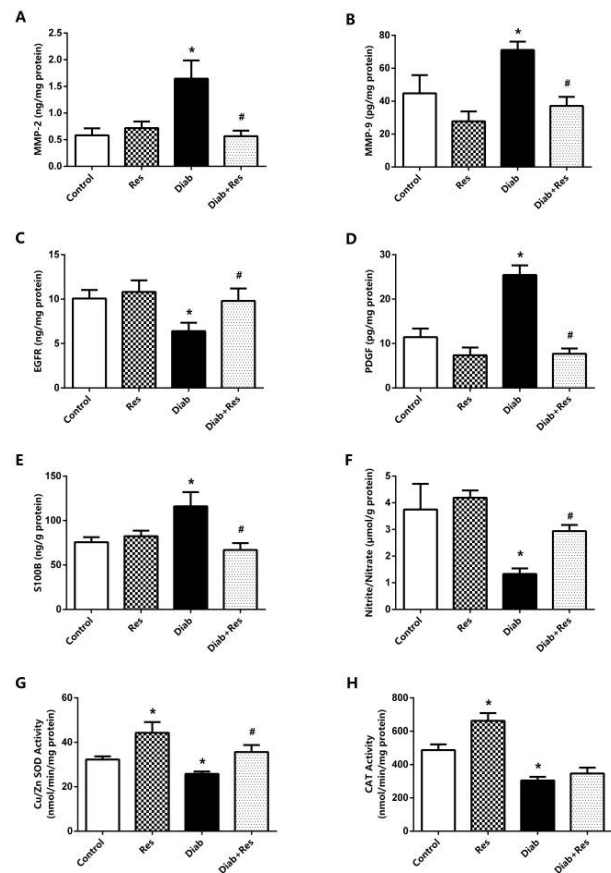
**Table 1.** Effects of diabetes and resveratrol on body weight and other metabolic parameters in the kidney tissues of STZ-induced diabetic rats.

Groups	Initial body weight (g)	Glucose (mg/g protein)	Triglyceride (mg/g protein)	Total Cholesterol (mg/g protein)
Control	439±2	91.6±7.1	265.3±2.4	11.4±2.2
Res	401±6	92.7±2.2	257.5±2.6	11.8±3.1
Diab	390±13*	134.3±12.5*	383.2±1.2*	22.1±3.1*
Diab+Res	397±5.1	95.8±9.4#	256±3.7#	11±1.2#

Values are expressed as means ± SEM, n = 6–12. Glucose, triglyceride, and total cholesterol levels in the kidney tissues of control, resveratrol (Res), diabetes (Diab), and diabetes plus resveratrol (Diab+Res) groups. Each bar represents at least six rats. \* p < 0.05, significantly different from control; # p < 0.05, significantly different from Diab.



**Figure 1.** The levels of cystatin-C (A), CK (B), Urea (C), and Uric acid (D) in the kidney tissues of control, resveratrol (Res), diabetes (Diab), and diabetes plus resveratrol (Diab+Res) groups. Values are expressed as means ± SEM, each bar represents at least six rats. \* p < 0.05, significantly different from control; # p < 0.05, significantly different from Diab.



**Figure 2.** The levels of MMP-2 (A), MMP-9 (B), EGFR (C), PDGF (D), S100B (E), Nitrite/Nitrate (F), SOD (G), and CAT (H) activity in the kidney tissues of control, resveratrol (Res), diabetes (Diab), and diabetes plus resveratrol (Diab+Res) groups. Values are expressed as means ± SEM, each bar represents at least six rats. \* p < 0.05, significantly different from control; # p < 0.05, significantly different from Diab.

### 4. Discussions

In this study, we aimed to investigate the effects of resveratrol administration on the relationship between

MMPs/EGFR and oxidative stress in kidney tissues of rats with STZ-induced diabetes. It is well known that diabetes causes insulin resistance or reduces insulin secretion and accordingly leads to hyperglycemia and hyperlipidemia (Joshua, 2014). It has also been shown several times that bodyweight decreases, especially in type I diabetes models (Akar, 2011; Sadi, 2015). Furthermore, some similar studies showed that tissue glucose, triglyceride, and cholesterol levels increased with type I STZ-induced diabetes model (Pektas, 2016b; Koca, 2016). However, resveratrol supplementations have been shown to reverse these changes (Akar, 2011; Sadi, 2015; Pektaş, 2016b; Koca, 2016). In studies performed to date, the hypoglycemic and anti-hyperlipidemic properties of resveratrol were known (Alice, 2018). This study indicated a significant reduction in body weights and also increment of renal glucose, triglyceride, total cholesterol contents with diabetes. Therewithal, resveratrol suppressed renal glucose, triglyceride, and total cholesterol contents in the STZ-induced diabetic rats. In acute or chronic kidney damage that develops with experimental diabetes models created in animals; It has been shown that plasma creatinine, CK, albumin, cystatin-C, urea and uric acid levels increase. (Yuko, 2013; Korkmaz, 2019; Safrida, 2019). In this study, a significant increase showed in the levels of renal CK, urea, and uric acid. This could be as a reflection of a diabetes-induced renal disorder. Besides, the fact that unchanged cystatin-C levels may be an indication that the severity is lower of kidney damage. On the other hand, resveratrol application significantly restored CK and uric acid levels.

It has previously been shown that resveratrol can protect against uric acid-induced damage and dysfunction by improving glycemic level (Bhatt, 2012), and it has also been shown to protect pancreatic  $\beta$  cells by lowering blood glucose levels in animals with hyperglycemia (Miki, 2011). It has been assigned that resveratrol recovered CK, and  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activities and its free form, respectively. The correction of these abnormalities by resveratrol supplementation could be valuable in the prevention of the disease. Another unfavorable alter triggered by diabetes is the increase of oxidative stress. Numerous studies demonstrated that increased oxidative end products such as malondialdehyde (MDA) in tissues of rats with diabetes (Pektas, 2016b; Koca, 2016) whereas reduced antioxidant enzyme activities such as SOD, CAT, glutathione (GSH), and glutathione peroxidase (GPx) (Sadi, 2018; Korkmaz, 2019). However, nitrite/nitrate, which is the end-products of nitric oxide known as tissue-protective, has been found to decrease with diabetes (Akar, 2011). In this study, diabetes-induced renal damage was associated with decreased nitrite/nitrate levels and activities of SOD and CAT in the kidney of rats. Prolonged hyperglycemic and hyperlipidemic medium may reflect an inadequacy in the antioxidant defense system in the diabetic condition. Moreover, resveratrol supplementation up-regulated considerably nitrite/nitrate levels and SOD activity withal there was a tendency toward augmentation of CAT activity but the differences did not achieve a significance level. Resveratrol is a potent antioxidant agent that affects endothelial nitric oxide synthase enzyme and mediates to produce nitric oxide which is in accordance with previous results found in type 2 diabetic mice or obese mice (Akar, 2011; Pektaş, 2017, 2018; Sadi, 2018). Collectively, these data suggest that

resveratrol treatment may improve renal nitric oxide bioavailability and antioxidant capacity.

MMPs family is known to cause chronic kidney disease, and kidney fibrosis present a failed wound healing in progressive chronic kidney disease (Klein, 2004). MMP-2 and MMP-9 are known to encourage cancer cell growth, including the metastasis of cancer cells (Deng, 2017) and has been shown to be profibrotic by induction of renal tubular cell epithelial-mesenchymal transition (Zhu, 2012). In the clinical studies demonstrated that serum MMP-2 and MMP-9 were higher levels in patients with type II Diabetes Mellitus (Signorelli, 2005; Derosa, 2007; Kostov, 2020). Hyperglycemia directly or indirectly (via oxidative stress or advanced glycation products) increases MMPs expression and activity (Kadoglou, 2005). The dysregulation of MMPs activity has been implicated in the pathophysiology of several diabetic co-morbidities (Thraillkill, 2009). As to our findings that MMP-2 and MMP-9 levels were significantly higher in the kidney tissues of rats with diabetes. Conveniently, we can say that results of this study are compatible compared to similar studies. Recently, a study in mice showed that resveratrol inhibits MMP3 and MMP9 expression and secretion by suppressing TLR4/NF- $\kappa$ B/STAT3 activation (Zhang, 2019). Furthermore an improved outcome was caused by resveratrol-induced reduction in plasma levels of both MMP-2 and MMP-9, as a positive correlation was observed between reductions in both MMPs and patient NIH stroke scales (Chen, 2016). In our findings demonstrated that resveratrol decreased renal MMP-2 and MMP-9 which were raised with diabetes. This could be thought to resveratrol suppress MMPs with its anti-inflammatory activity. Previously, it has been shown that down-regulated EGF via progressive renal damage in the kidney of Zucker diabetic fatty (ZDF) rats (Togashi, 2013). Similarly, Wu et al. (2019) demonstrated that EGFR levels significantly reduced in the heart tissues of STZ-induced type I diabetic mice (Wu, 2019). Here in, our findings denoted to support existing results that renal EGFR levels significantly reduced with diabetes. On the other hand, resveratrol normalized the levels of EGFR in the kidney of diabetic rats. In a similar study, resveratrol has been shown to an increase in EGFR expressions in liver tissues of diet-induced obese mice (Jin, 2019). It is possible to say that in diabetes-induced kidney damage, resveratrol shows restorative effectiveness by increasing EGFR levels. Howbeit, in our study, some of the evidence of diabetes-induced renal damage is the increase of renal S100B and PDGF levels. It can be assumed that they were secreted from infiltrating inflammatory cells and platelets for recovery of prolonged hyperglycemia medium-induced kidney damage (Abderrahmani, 2018; Katsanou, 2018; Mohammadzadeh, 2018; Shan, 2019; Yu, 2020).

In conclusion, resveratrol alleviates type 1 diabetes-induced renal damage by decreasing renal oxidative stress and stabilizing MMPs/EGFR thereby increasing the reconstructions of the kidney. Although resveratrol treatment did not shift the overall diabetic pattern toward control conditions, it significantly protected renal functions and integrity from the diabetes-induced kidney damage.

**Conflict of interest:** The authors state no conflict of interest.

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**Abbreviations:** CAT, catalase; CK, creatine kinase; Diab, diabetes; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde;

MMPs, Matrix metalloproteinases; NfκB, nuclear factor kappa B; NIH, national institutes of health. PDGF, platelet-derived growth factor; Res, resveratrol; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; STZ, streptozotocin; S100B, calcium-binding protein B; TLR4, toll like receptor 4.

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## Presence of *Cortinarius atroalbus* M.M.Moser and *C. duracinobtus* Rob. Henry (*Basidiomycota, Cortinariaceae*) in Turkey

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### *Cortinarius atroalbus* M.M.Moser ve *C. duracinobtus* Rob. Henry (*Basidiomycota, Cortinariaceae*)'nin Türkiye'deki varlığı

**Abstract:** Basidiomata were collected during the field trips in 2012 and 2015 from Trabzon, Turkey and identified based on morphological data. As a result, *Cortinarius atroalbus* M. M. Moser and *C. duracinobtus* Rob. Henry are recorded for the first time from Turkey and provided here with descriptions, figures and a short discussion. *C. atroalbus* has a conical, convex to applanate and umbonate; dark chestnut brown to blackish brown, hygrophanous pileus; adnate to nearly arcuate, pale brown to rusty brown crowded lamellae; cylindrical, slightly curved, silvery whitish to very pale brown stipe and ellipsoid, slightly verrucose basidiospores. *C. duracinobtus* has a conical to campanulate with a typically broad and umbonate, brown, dark yellow to brown pileus; sub-decurrent to adnate, sparse, dark yellow to orange brown, smooth, moderately thick and regular lamellae; cylindrical, generally curved, sometimes hollow, orange brown to dark yellowish stipe and ellipsoid, slightly verrucose and granulate basidiospores.

**Key words:** *Cortinarius*, field study, identification, new record, taxonomy

**Özet:** Bazidiyomalar 2012 ve 2015 yıllarında Trabzon, Türkiye'de yapılan arazi çalışmaları sırasında toplandı ve morfolojik yöntemlere göre teşhis edildi. Çalışmalar sonucunda *Cortinarius atroalbus* M. M. Moser ve *C. duracinobtus* Rob. Henry Türkiye'den ilk kez kaydedildi ve bu çalışmada tanımlar, şekiller ve kısa bir tartışma ile birlikte verildi. *C. atroalbus* konik, konveks veya düz, tepe çıkıntılı, koyu kestane renginden siyahımsı kahverengiye doğru değişen, higroskopik bir şapkaya; adnat veya hemen hemen arkuat, soluk kahveden paslı kahveye doğru değişen kalabalık lamellere; silindirik, hafif kıvrık, gümüş beyazından oldukça soluk kahveye değişen bir sapa ve eliptik, hafif dikenli bazidiyosporlara sahiptir. *C. duracinobtus* konikten çana doğru değişen, geniş tepe çıkıntılı, kahverengi, koyu sarıdan kahveye doğru değişen renkte bir şapkaya; dekürrent veya adnat, seyrek, koyu sarı veya portakalımsı kahverengi, düz, orta kalınlıkta, düzgün lamellere; silindirik, genellikle eğri, bazen içi boş, portakalımsı kahveden koyu sarıya doğru değişen bir sapa ve eliptik, hafif dikenli ve granüllü bazidiyosporlara sahiptir.

**Anahtar Kelimeler:** *Cortinarius*, arazi çalışması, teşhis, yeni kayıt, taksonomi

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## 1. Introduction

*Cortinarius* (Pers.) Gray is one of the largest macromycete genus and more than 130 species have been recorded in Turkey up to date. *Cortinarius atroalbus* M.M. Moser was first collected in 1991 from alpine zone of Union Peak, Windriver Mountains, Shoshone Natural Forests, Wyoming, USA; described by Moser and published in *Sydowia*. According to Moser (1993) it has relatively small, hygrophanous, dark chestnut brown pileus and ellipsoid, verrucose basidiospores. *Cortinarius duracinobtus* Rob. Henry was first described by Henry (1970) based on the samples collected from damp fir forest and characterised by small, ochre to noisette and umbonate pileus; fleshy smell and middle sized basidiospores (Henry, 1970; Tartarat, 1988).

The aim of the present study is to contribute to Turkish Mycota by introducing two *Cortinarius* species collected from Trabzon. Many *Cortinarius* species had been presented from different regions of Turkey (Türkekul, 2003; Kaya et al., 2009; Kaşık et al., 2011; Uzun et al., 2013; Akata et al., 2015; Doğan and Kurt, 2016; Sesli and Liimatainen, 2018) but *C. atroalbus* and *C. duracinobtus* haven't been reported before.

## 2. Materials and Method

About 30% of the collecting site is covered by forests and the important trees in the field are *Fagus orientalis* Lipsky, *Quercus petraea* (Mattuschka) Liebl., *Picea orientalis* (L.) Link, *Carpinus betulus* L., *C. orientalis* Mill., *Rhododendron ponticum* L., *R. luteum* Sweet, *Corylus avellana* L. and *Alnus glutinosa* (L.) Gaertn. Samples were collected from Akçaabat and Maçka districts, Trabzon, Turkey. Microscopic studies were performed according to Clémençon (2009). For these studies, a piece of basidioma is placed in 3% ammoniated water. After a while it was compressed with the help of a forceps letting the basidiospores fall on the slide. For the other structures, cross sections were obtained by a razor blade, mounted in 3% NH<sub>3</sub> solution, stained with aqueous 5% Congo red, examined under a Zeiss Axio Imager A2 trinocular microscope and the images were obtained by a Zeiss AxioCam 105 colour camera. The samples were identified with the help of the relevant literature (Henry, 1970; Moser, 1993; Breitenbach and Kränzlin, 2000; Knudsen and Vesterholt, 2008).

### 3. Results

#### *Cortinariaceae* R.Heim ex Pouzar

*Cortinarius atroalbus* M. M. Moser, Sydowia 45(2): 282 (1993) (Fig. 1)

(Syn. *Cortinarius atroalbus* var. *nigripes* M.M.Moser, Sydowia 45(2): 284 (1993))

Pileus 10-50 mm, conical, obtusely conical, convex to applanate; when young typically umbonate; dark chestnut brown to blackish brown, becoming black with 3% potassium hydroxide solution; hygrophanous; margin paler, silvery; centre dark; margin in young specimens covered by silky whitish veil remnants. Lamellae adnate to nearly arcuate, pale brown to rusty brown, moderately crowded, L= 35-50, I=1-3. Stipe 30-50(-70) × 2-5 mm, cylindrical, slightly curved, silvery whitish to very pale brownish, equal or slightly tapered towards base. Content compact, pale brownish. Odour not distinctive. Taste mild. Basidiospores [n= 48] (7-)7.5-8.5(-8.7) × (3.7-) 4-4.8 μm; on average 7.7 × 4.3 μm; Q= 1.6-2.0, Qm= 1.83, ellipsoid, slightly verrucose. Basidia 22-27(-32.5) × (6.5-)7.5-8.5(-9.6) μm; on average 25.6 × 7.9 μm, 4-spored, clavate, some with brownish contents. Cheilocystidia absent. Pileipellis consists of elongate, cellular elements. Marginal cells 15-17.7 × 4-7.4 μm. Clamp connections present at all tissues.

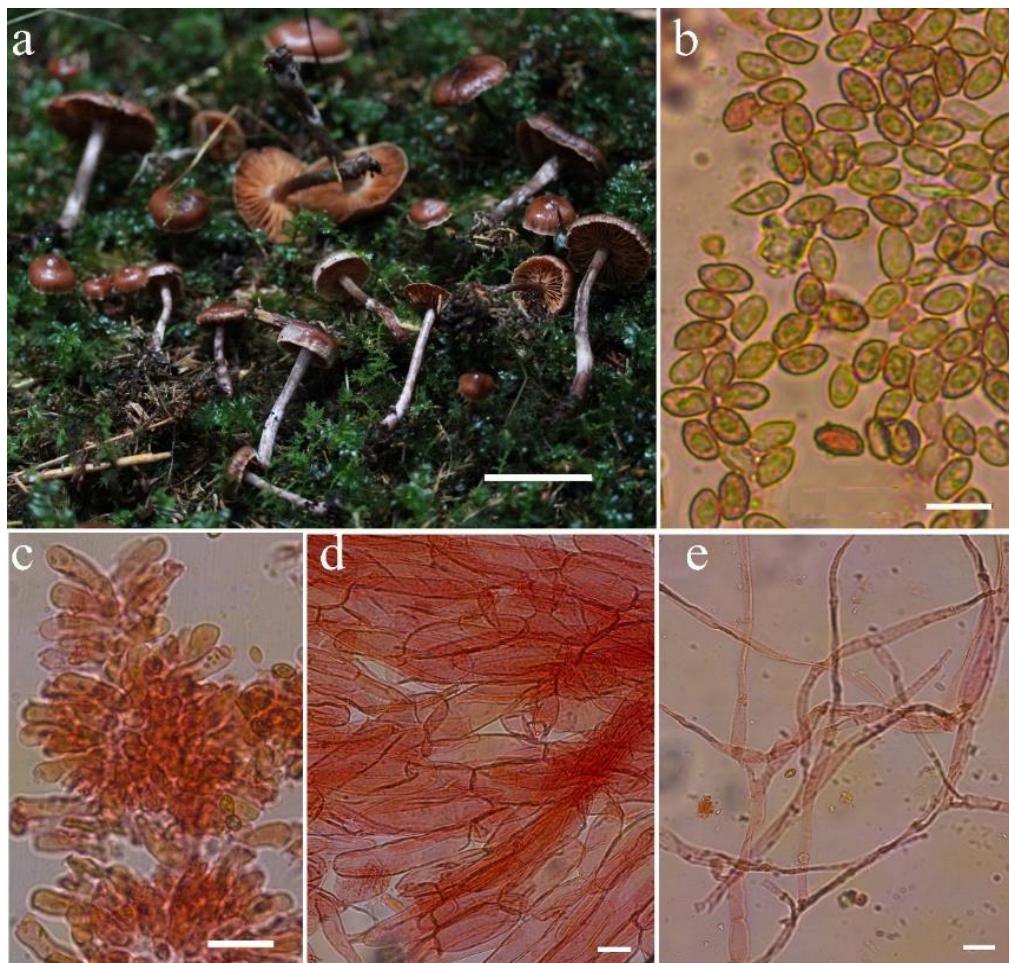
**Specimens examined:** TURKEY, Trabzon, Maçka, Sevinç neighborhood, gregarious in alpine meadow, among moss,

40°52'02.97"N, 39°36'05.50"E, 1347 m, 26 Oct. 2015, E. Sesli, KATO Fungi 3601.

*Cortinarius duracinobtusus* Rob. Henry, Bull. Trimest. Soc. Mycol. Fr. 85(4): 446 (1970) (Fig. 2)

Pileus conical to campanulate with a typically broad and large umbo, 20-30 mm, brown, orange brown, dark yellow to brown, darker at the centre, paler towards the wavy, whitish and fibrillose margin, not regular. Lamellae sub-decurrent to adnate, sparse, dark yellow to orange brown, smooth, moderately thick, margin regular, L= 15-20, I= 1-2. Content fragile, thin, pale brown or yellowish brown. Smell raphanoid, taste indistinct. Stipe cylindrical, generally curved, sometimes hollow, 40-70 × 5-8 mm, lighter than pileus, brown, orange brown with slightly whitish tint to dark yellowish, fibrillose and hard. Basidiospores [n= 45] ellipsoid, (7.3-)7.5-9(-9.6) × (4.4-)5-6(-6.5) μm; on average 8.4 × 5.2 μm, slightly verrucose and granulated. Basidia clavate, (24-)24.8-31.4(-40) × (8.2-)9.5-11.5 μm, on average 26.7 × 10.1 μm. Pileipellis consists of cylindrical and parallel hyphae. Clamp connections present at all tissues.

**Specimen examined:** TURKEY, Trabzon, Akçaabat, Hıdırnebi Plateau, gregarious to caespitose under spruce (*Picea orientalis* L.), 40°57'17.64" N, 39°25'39.75"E, 1422 m, 4 Sept. 2012, E. Sesli, KATO Fungi 3074.



**Figure 1.** *Cortinarius atroalbus*: a- basidiomata, b- basidiospores, c-basidia and basidiole, d,e-pileipellis (bars: a= 50 mm, b= 10 μm, c-e= 20).



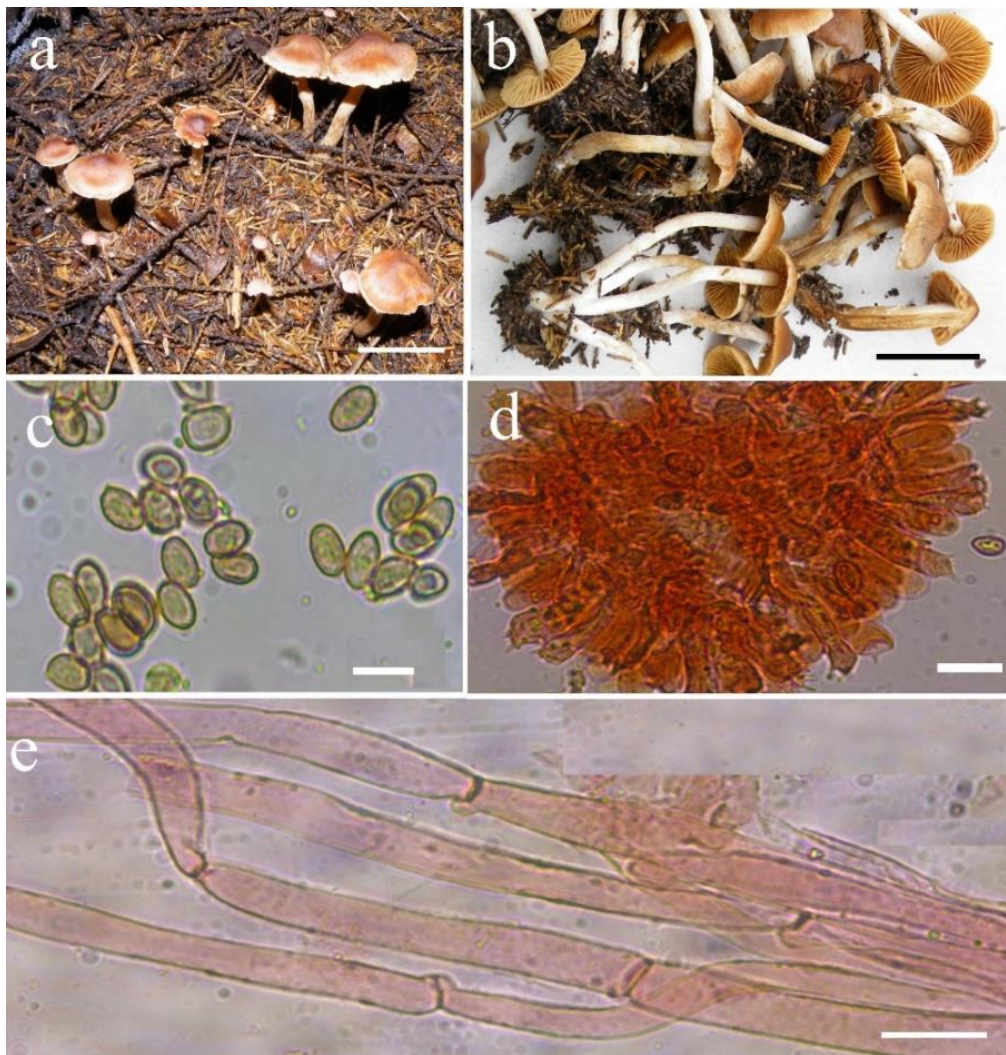
#### 4. Discussions

*Cortinarius atroalbus* and *C. duracinobtusus* are recorded for the first time from Turkey and provided here with descriptions, and photographs related to their macroscopy and microscopy. Main characteristics of the new records generally matched very well with the original descriptions. The size, shape and colour of the pileus, lamellae colour, and size and shape of basidiospores of KATO F. 3601 almost overlap with Moser (1993)'s sample. According to Mcknig and Moser (1993) *C. atroalbus* is one of the most striking species, readily identifiable in the field and known from the alpine zone under dwarf willow in Austria. Moser (1993) described this species from alpine tundra, USA. A close, but different species, *C. depressus* Fr. has  $6.5-7.5 \times$

$3.5-4 \mu\text{m}$ , narrowly ellipsoid basidiospores; 15-60 mm, dark reddish brown pileus and ochraceous to light yellowish lamellae (Knudsen and Vesterholt, 2008). *Cortinarius duracinobtusus* described by Henry (1970) shares very similar morphological characters with the one we observed in KATO Fungi 3074. A similar, but different species, *C. azureus* has an obtusely conical to plane, gray-violet or lilac-brown pileus; broadly attached lamellae and  $7-10 \times 5-8 \mu\text{m}$ , elliptical to subglobose basidiospores (Breitenbach and Kränzlin, 2000).

#### Acknowledgments

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**Figure 2.** *Cortinarius duracinobtusus*: a,b- basidiomata, c- basidiospores, d-basidia and basidiole, e-pileipellis (bars: a and b= 30 mm, c= 10  $\mu\text{m}$ , d and e= 20  $\mu\text{m}$ ).

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## Chorological contributions for some narrow-range endemic plant taxa in Turkey

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### Türkiye'deki bazı dar yayılışlı endemik bitki taksonları için korolojik katkılar

**Abstract:** The taxonomy of narrow endemic plant taxa, chorology and protective biology are an important contribution for every new population determined. Here, new population information from Turkey has been given for a total of 5 narrow-range endemic plant taxa, led by *Aethionema dumanii* (Brassicaceae), *Astragalus aytatchii* (Fabaceae), *Salvia halophila* (Lamiaceae), *Sedum hewittii* (Crassulaceae) and *Senecio olympicus* (Asteraceae). Furthermore, some features and ecological preferences, localities, distribution map and images of the species are given.

**Key words:** Endemic, chorology, new populations, Turkey

**Özet:** Tespit edilen her yeni popülasyon, dar endemik taksonların taksonomisi, korolojisi ve koruma biyolojisi için önemli bir katkıdır. Burada *Aethionema dumanii* (Brassicaceae), *Astragalus aytatchii* (Fabaceae), *Salvia halophila* (Lamiaceae), *Sedum hewittii* (Crassulaceae) ve *Senecio olympicus* (Asteraceae) olmak üzere toplam 5 dar yayılışlı endemik bitki taksonu için Türkiye'den yeni popülasyon bilgileri verilmiştir. Ayrıca türlerin bazı özellikleri ve ekolojik tercihleri, lokaliteleri, yayılış haritası ve resimleri verilmiştir.

**Anahtar Kelimeler:** Endemik, koroloji, yeni popülasyonlar, Türkiye

**Citation:** Hamzaoglu E, Koç M (2020). Chorological contributions for some narrow-range endemic plant taxa in Turkey. Anatolian Journal of Botany 4(2): 96-99.

## 1. Introduction

According to the International Union for Conservation of Nature (IUCN) criteria, the plant species, which continue their existence in nature and about which there is sufficient information, are evaluated in the threat categories, which are defined as Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT) or Least Concern (LC). Among these, the CR, EN, and VU are categories, which should be protected. It is necessary to know the extent of the area of distribution and the number of individuals for being able to decide in which category a taxon is placed (IUCN Standards and Petitions Committee, 2019). Consequently, it is an important discovery, which includes for any species the categories of threat required for protection, to find new populations, and which directly affects the threat category of a known species. According to the IUCN, species, whose lineages are under threat, are evaluated from the aspect of 5 criteria that are listed from A to E. The discovery of new populations directly affects these criteria, especially A and B. The A criterion is related to "decrease in population", whereas B is related to "extent of area of distribution" (IUCN Standards and Petitions Committee, 2019). The discovery of every new population increases the population number of the species and extends the area of distribution. Here, new distribution areas of 5 narrow-range endemic species were presented in order to contribute to the classification of the threat categories of the species.

## 2. Materials and Method

Here, the narrow endemic plant taxa given to new populations have been collected during the floristic

activities performed at different times in different provinces of Turkey. Together with the detailed address to the extent possible in the section of the distribution information for taxa, their Global Positioning System (GPS) records have also been written. Furthermore, photographs have been given, which display diagnostic characters for the taxa. The specimens collected were delivered to the GAZI and ANK herbaria to be kept. The Google Earth application was used to assess the distribution areas of the species. The width of the distribution area was calculated with the aid of a polygon drawn to include all recognized species addresses.

## 3. Results and Discussions

### Brassicaceae

*Aethionema dumanii* Vural & Adıgüzel, Turkish J. Bot. 19(4): 481 (1995), (IPNI, 2020).

**Specimens examined:** B6 Sivas: Between Şarkışla and Pınarbaşı (Malatya road), 37 S 282176-4322577, 1600 m, marl steppe, 8.8.2018, Koç 3480 & Hamzaoglu (GAZI, ANK).

When the flower and fruit characters and the habitat preferences stated in the original publication were taken into consideration, it was decided that the specimens collected from Sivas belong to *Aethionema dumanii*. According to the existing data, the species is an endemic plant, which prefers the marl and gypsum steppes between 840–1400 meters in Eskişehir, Ankara, and Afyonkarahisar (Vural and Adıgüzel, 1995). According to the Flora of Turkey and the East Aegean Islands, since the species is a perennial, has unilocular fruits, is shorter than 30 cm and its

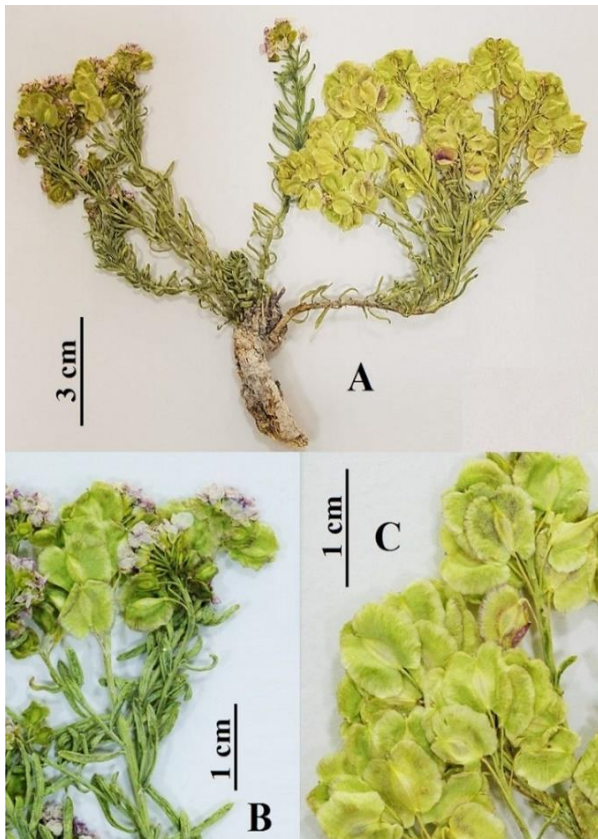
fruits have the dimensions of 6-9 × 7-9 mm, it resembles *A. enunoioides* (Boiss.) Bornm. (Hedge, 1965). However the leaves of *A. dumanii* are oblong-linear and alternately arranged (not ovate-spatulate or orbicular and the bottom leaves are not arranged opposite), the fruit edge is undulate, irregular crenate-dentate and the wings are at a width of 3-4 mm (not smooth and 1.5-2 mm) (Fig. 1). Together with the addition of the data for the new population, the upper elevation interval has reached 1600 meters, it was understood that its distribution continued towards the east and that the extent of its area of distribution became approximately 15.000 km<sup>2</sup> (Fig. 2).

#### Fabaceae

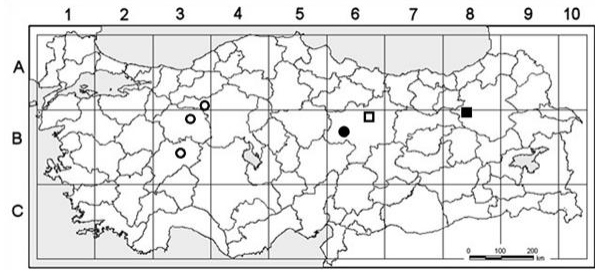
*Astragalus aytatchii* Akan & Civelek, Ann. Bot. Fenn. 38(3): 169 (2001), (IPNI, 2020).

**Specimens examined:** B8 Erzurum: Aşkale, N. of Yeşilova village, 37 S 642438-4422857, 1750 m, gypsum-bearing steppe, 29.5.2019, Hamzaoğlu 7587 & Koç (GAZI, ANK).

*Astragalus aytatchii* is a defined endemic species with specimens, which were collected from the surroundings of Hocabay Village, to the south of the Sivas Provincial Center (Fig. 3). This species, which belongs to the *Alopecuroidei* DC. (= *Alopecias* (Steven) Bunge) section, resembles *A. elatus* Boiss. & Balansa (Akan and Civelek, 2001), since its peduncle is shorter than 5 mm, its calyx is 8-10 mm long and its teeth are 3-5 mm long, the upper surface of its leaflets are without hairs and the lower surface is hairy, adpressed pilose, the stipules are 8-15 mm long, its bracts are 8-15 mm long, its standard is 18-19 mm long, and inflorescence is ovate or orbicular. However, in *A. aytatchii*, the bodies are 10-35 cm long (not 50-90 cm), the



**Figure 1.** *Aethionema dumanii* – A. Habit, B. Inflorescence and C. Fruits.



**Figure 2.** The known populations of *Aethionema dumanii* (○) and the newly determined populations (●), the known populations of *Astragalus aytatchii* (□) and the newly determined populations (■).

leaves are 6-18 cm long (not 19-36 cm), the leaflets are 9-14 pairs (not 20-24 pairs), and the calyx is 8-10 mm long (not 12-18 mm). It was stated that in the area where the species was defined on deep gypsum soil at an interval between 1500-1600 meters, approximately 100-150 mature individuals were found in the population and growing in an area of only 1000-1500 square meters. Together with the newly determined population at Aşkale, Erzurum Province, the extent of the area of distribution of the species became approximately 3000 km<sup>2</sup> (Fig. 2). When both the type and the addresses given here are taken into consideration, it is observed that the species preferred steppes with gypsum. It can be stated that the probability of finding the species at other addresses in the steppes with gypsum between Sivas and Aşkale is rather high.

#### Lamiaceae

*Salvia halophila* Hedge, Notes Roy. Bot. Gard. Edinburgh 23: 58 (1959), (IPNI, 2020).



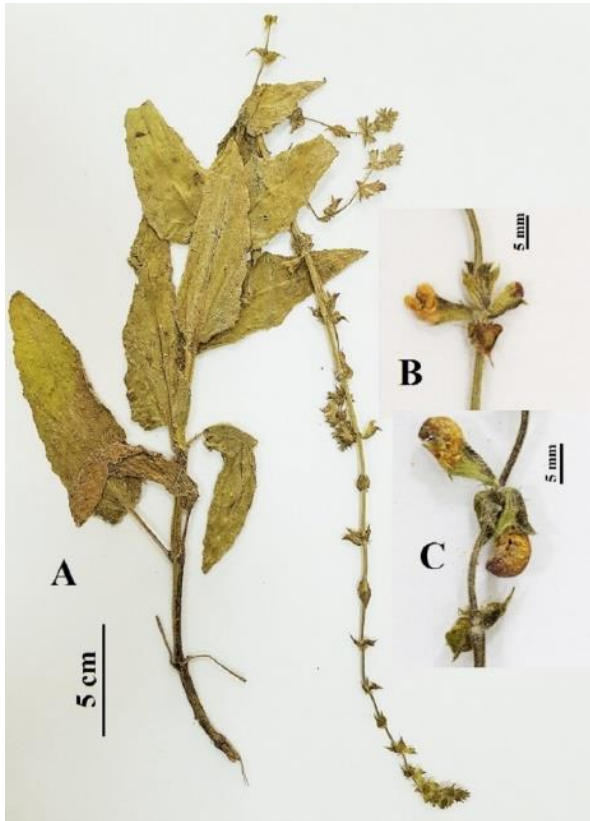
**Figure 3.** *Astragalus aytatchii* – A. Habit, B. Inflorescence.

**Specimens examined:** A3 Ankara: Beypazarı, between Kırbaşı and Uşakbükü villages, 36 T 395037-4429963, 810 m, gypsum-bearing and salty slopes, 11.6.2016, Koç 2313 & Hamzaoğlu (GAZI, ANK).

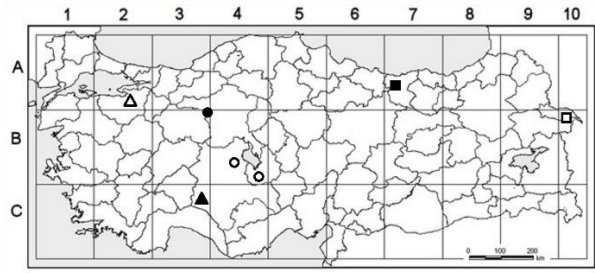
According to the distribution information given in the Flora of Turkey and the East Aegean Islands, the species is an endemic grown in the surroundings of Tuz Lake on the salty marshes (Aksaray and Konya) (Hedge, 1982). When it is compared with the depiction given in the work, it was determined that the number of flowers found in the verticillates in the Beypazarı specimens is generally 3-4 each (not 4-6) and that the length of the leaves was longer (Fig. 4). The extent of the area of distribution of taxon became approximately 4000 km<sup>2</sup> together with the Beypazarı population, which was determined at a bird's-eye view of approximately 200 km to the northwest according to Tuz Lake (Fig. 5). While the species is growing on very slightly sloped salty marshes at Tuz Lake, it was determined on the slope of a hill with gypsum, which has water seepage at Beypazarı. On the area of the species determined in the Beypazarı, species, which are frequently observed in the surroundings of Tuz Lake, were also encountered, such as *Onosma halophila* Boiss. & Heldr., *Taraxacum farinosum* Hausskn. & Bornm. ex Hand.-Mazz., and *Gypsophila oblancoolata* Barkoudah. Furthermore, a new species *Hypericum turcicum* Özbek & Hamzaoğlu from the area, was published recently (Özbek et al., 2019).

**Crassulaceae**

*Sedum hewittii* Chamberlain., Notes. Roy. Bot. Gard. Edinburgh 31(2): 325 (1972), (IPNI, 2020).



**Figure 4.** *Salvia halophila* – A. Habit, B and C. Inflorescence.



**Figure 5.** The known populations of *Salvia halophila* (○) and the newly determined populations (●), the known populations of *Sedum hewittii* (□) and the newly determined populations (■), the known populations of *Senecio olympicus* (△) and the newly determined populations (▲).

**Specimens examined:** A7 Giresun: Dereli, SW. of Aksu village, Karagöl Mountain, towards the summit of Kılınçtepe, 37 T 428850-4486785, 3000 m, rocky slopes, 9.8.2008, Hamzaoğlu 5315 & Koç (GAZI).

According to the distribution information given in the Flora of Turkey and the East Aegean Islands, the species is an endemic that is only growing at Ağrı Mountain (Chamberlain, 1972). Together with the Karagöl (Dereli, Giresun) population, which was determined approximately 530 km to the west-northwest compared to Ağrı Mountain (Ağrı-Iğdır), the extent of the area of distribution of the taxon has become approximately 6000 km<sup>2</sup> (Fig. 5 and 6). *Sedum hewittii* grows at Ağrı Mountain between 2750-3050 meters, on volcanic bedrock and moist areas. The newly determined population displays a similarity to the Ağrı Mountain population from the aspect of these characteristics.



**Figure 6.** *Sedum hewittii* – A. Habit, B. Inflorescence.



**Asteraceae**

*Senecio olympicus* Boiss., Diagn. Pl. Orient. ser 1(4): 13 (1844), (IPNI, 2020).

**Specimens examined:** C3 Konya: Between Seydişehir and Derebucak district, west of Taraşçı villag, left of Rezebeli Pass, towards the summit, 36 S 385253-4145410, 2140 m, calcareous rocks, 14.7.2011, Hamzaoğlu 6167 & Koç (GAZI).

According to the distribution information given in the Flora of Turkey and the East Aegean Islands, the species is an endemic only growing at Uludağ (Bursa) (Matthews, 1975). The population was determined at Rezebeli Pass at a bird's eye view of approximately 390 km to the south-southeast of the Uludağ population (Fig. 5 and 7). The extent of area of distribution of the taxon has become approximately 3000 km<sup>2</sup> with this new population. The edges of the lower leaves in the individuals belonging to the Rezebeli population are smooth (not remotely repandenticulate). The continuousness and the taxonomic importance of this morphological difference should be discussed by examining in detail more individuals.



**Figure 7.** *Senecio olympicus* – A. Habit, B. Capitulum.

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## Morpho-anatomical and phytochemical evaluation of *Icacina trichantha* Oliver (*ICACINACEAE*)

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### *Icacina trichantha* Oliver'in (*ICACINACEAE*) morfo-anatomik ve fitokimyasal değerlendirmesi

**Abstract:** The leaf epidermis of *Icacina trichantha*, a quintessential medicinal tropical plant was investigated with the aid of light microscopy and the chemical constituents of its leaf and root were investigated using Gas Chromatography-Mass Spectrometry (GC-MS). Hitherto, leaf epidermis data is missing and similarly, the chemical analysis of the leaf and root of the plant is undertaken in a single study for the first time. The leaf epidermis characters with which the species can be defined include paracytic and diacytic stomatal types, irregular epidermal cell shape together with angular or curved anticlinal wall patterns. However, the quantitative data appeared to overlap thus providing the range of values of both the measured and counted features. N-hexane extract of the root is rich in reducing sugars, tannins, steroids, glycosides, terpenoids and flavonoids while GC-MS analysis revealed 11 and 3 significant esterified bioactive components in the leaf and tuberous root respectively with dodecanoic acid being most abundantly present (47.85%-54.10 %) but some chemical confined to specific areas are trichothec-9-en-8-one (47.73 %) in the root and 9-octadecenoic acid (25.05 %) in the leaf. The result of this study will assist in identifying the plant even if its parts are fragmentary and also be helpful in screening the plant for drug.

**Key words:** Microscopy, taxonomy, tropical plant

**Özet:** Önemli bir tıbbi tropik bitki olan *Icacina trichantha*'nın yaprak epidermisi ışık mikroskobu yardımıyla, yaprağının ve kökünün kimyasal bileşenleri de Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) kullanılarak incelenmiştir. Halihazırda bitkinin yaprak epidermis verileri eksik durumdadır ve bitkinin yaprağı ve kökünün kimyasal analizi tek bir çalışmada ilk kez yapılmıştır. Parasitik ve diasitik stoma tipleri, düzensiz epidermal hücre şekli ve köşeli veya kavisli antiklinal duvar desenleri türün tanımlanmasında kullanılabilen yaprak epidermis karakterleri arasında yer alır. Bununla birlikte, nicel veriler, hem ölçülen hem de sayılan özelliklerin değer aralığını sağlayacak şekilde örtüşür nitelikte görülmektedir. Kökün N-heksan ekstresi indirgeyici şekerler, tanenler, steroidler, glikozitler, terpenoidler ve flavonoidler bakımından zengindir; GC-MS analizi de yaprak ve yumru kökte, en yaygın dodekanoik asit (%47.85 - %54.10) olan, sırasıyla 11 ve 3 önemli esterlenmiş biyoaktif bileşen ortaya çıkarmıştır. Buna karşın bazı kimyasalların belirli bölgelerle sınırlı (kökte trichothec-9-en-8-one, %47.73, yaprakta 9-oktadecenoik asit, %25.05) kaldığı görülmüştür. Çalışma bulguları, bitki bir bütün halinde olmasa bile bitkinin teşhisine katkı sağlayacaktır ve bitkinin drog olarak taranmasında da yardımcı olacaktır.

**Anahtar Kelimeler:** Mikroskopi, taksonomi, tropik bitki

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## 1. Introduction

*Icacina trichantha* Oliver belongs to the family *ICACINACEAE* (Hassler, 2020). It is one of the five species (*Icacina claessensii* De Wild., *I. guessfeldtii* Asch. ex Engl. *I. mannii* Oliv., *I. oliviformis* (Poir.) J.Raynal and *I. trichantha* Oliv.) of the genus and are found only in Africa. The plant is a perennial shrub up to 2 m with scandent growth above, and commonly found in crop fields, forest regrowths and waste areas in the forest and savannah. The leaves are broadly elliptic, abruptly acute at the apex and rounded at the base. Sometimes, they may be thinly pilose with simple, fascicled hairs beneath. Leaf length is about 8.0 - 10.0 cm. while the width is up to 17.0 cm. Flowers are densely crowded and subsessile with calyx nearly as long as the petals which are usually villous outside. The fruits are tomentose on the surface, ellipsoid to globose in shape and they are about 2.5 cm long (Hutchinson and Dalziel, 1958; Agyakwa and Akobundu, 1998). It is sometimes locally abundant and troublesome

as a weed in some parts of Nigeria. It is called as "Gbegbe" in Yoruba in the south west Nigeria and "Ibugo" in Igbo in the eastern Nigeria (Burkhill, 1985). The plant is extensively used in the rural areas; the leaves and tuber have folkloric uses in the treatment of malaria, constipation and food poisoning in Nigeria (Asuzu and Abubakar, 1995a; Che et al., 2016).

Phytochemicals are secondary metabolites from plants that are responsible for their medicinal properties. The presence of phytochemicals such as flavonoids, terpenoids, tannins, glycosides, reducing sugars, steroids have been reported in different extracts and parts of *I. trichantha* (Onakpa et al., 2014; Otun et al., 2015). These phytochemicals may be responsible for the antihyperglycemic, anticonvulsion, sedative, analgesic, and antimicrobial properties ascribed to the plant (Dalziel, 1937; Burkhill, 1985; Asuzu and Abubakar, 1995b; Asuzu and Aforonwa, 2008; Onakpa and Asuzu, 2013; Onakpa et al., 2014; Alawode et al., 2018).

In this investigation, the phytochemical composition of hexane extract of leaf and root using preliminary screening and GC-MS analysis was carried out in a single study for the first time, in addition to the micro-morphological evaluation of the leaf epidermis which has not been studied before. Leaf epidermal characteristics are well known to offer useful identification criteria for plants, as well as chemical characters, which also can be used to define their pharmacological usefulness. These two studied character sources of the plants (anatomy and chemistry) are important data sources for species identification and utilization of the plant as a source of drug

## 2. Materials and Method

Fresh samples of *I. trichantha* were collected from different locations across Southern Nigeria (Fig. 1), and the samples were dried and authenticated at the Lagos University Herbarium (LUH). Herbarium abbreviation follows Holmgren and Holmgren (2003). The leaves and roots were both used for the study. The former was mainly used for micro-morphological assessment, and 100 leaf samples obtained from all the individuals collected were investigated. However, the dried leaves and roots were pulverized and kept in ziplock for further investigation of chemical analysis and phytochemical screening.

For micro-morphological evaluation of the leaves, the study approach of Stace (1965), Kadiri et al. (2003), Kadiri and Olowokudejo (2016) and Ogundipe and Akinrinlade (1998) was followed while for phytochemistry, the method of Ajayi et al. (2011), Harborne (1991) and Trease and Evans (1998) was adopted.

### 2.1. Epidermal peel (leaf epidermis)

For the study, a light microscope was used. The acid soaking and counter-staining method which has proven useful for obtaining leaf epidermis from many African plants was adopted, following the approaches of Ogundipe and Akinrinlade (1998) and Kadiri and Olowokudejo (2016) with some modifications. Leaf portions of 2-3 cm<sup>2</sup> were cut from the standard median portion of the leaf lamina near the mid-rib, boiled in water for 30 minutes, and then soaked in concentrated nitric acid (HNO<sub>3</sub>) in capped specimen bottles for two to four hours to macerate the mesophyll tissue. Tissue disintegration was indicated by air bubbles, the stage at which the leaf tissues were transferred into Petri dishes containing water for separation of the epidermis using a pair of forceps and mounting needle. Tissue debris was cleared off the epidermis with an artist's fine-hair brush and washed in several changes of water. Two to three drops of sodium hypochlorite solution were dropped onto the epidermis on the slide to bleach opaque areas and allowed to soak for 30–120 seconds until a color change. The epidermis peel was mounted on the slide and then two to five drops of ethyl alcohol in a series of ascending concentrations (50%, 75%, and 100%) were added to harden the cell wall. Two to three drops of 10% aqueous Methylene Blue and one drop of 50% aqueous Safranin were later added for three to five minutes. One to two drops of glycerine were added, then the preparation was covered with a transparent coverslip and the edges were sealed with nail polish to prevent dehydration. Each slide was observed under

magnifications of ×100 and ×400 so as to capture all the features of the epidermis. These features were recorded qualitatively, and basic statistical calculations were made to show means, standard error and ranges of variations. Photomicrographs were taken using an Olympus microscope with an attached camera.

Stomata index (SI) was calculated using the formula of Stace (1965).

$$\text{Stomata index} = \frac{\text{Stomata number}}{\text{cell number per unit area} + \text{stomata number}} \times 100$$

### 2.2. Extraction of plant material

The pulverized leaf and root sample (10 g) of *Icacina trichantha* was separately extracted using Soxhlet apparatus and hexane as solvent. The hexane solvent (250 ml) was put in a round bottom flask, attached to a Soxhlet extractor and condenser on an isomantle. The pulverized sample was loaded into the Soxhlet thimble and the side arm lagged with glass wool. As the hexane was heated via the isomantle, it evaporated, condensed then dripped into the reservoir containing the thimble. The solvent flows back into the flask once its level reaches the siphon it pours back and the cycle was repeated several times for a period of 9 hr. The non-volatile extract obtained from the plant was obtained and kept in a sterile vial and stored at 4 °C for phytochemical investigations.

### 2.3. Phytochemical screening

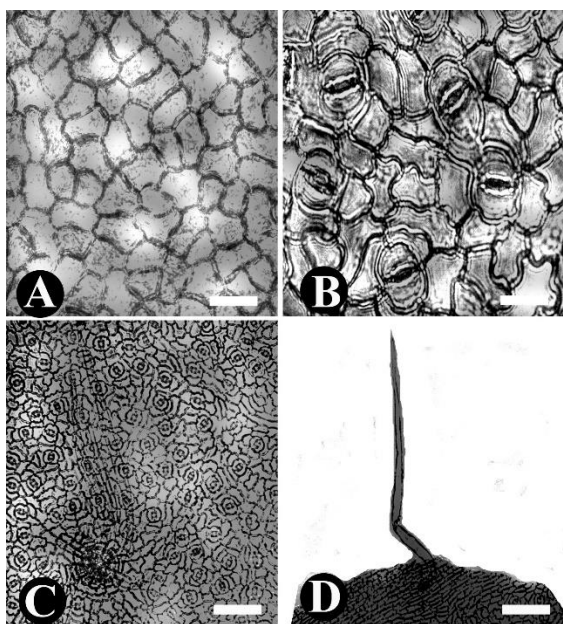
The preliminary phytochemical screening of the hexane root extract was carried out by physical observation of colour change. The following phytochemicals viz: alkaloid, saponin, flavonoid, anthraquinone, glycoside, tannins, steroid, protein and volatile were investigated using standard protocols (Trease and Evans, 1989; Sofowora, 1993; Harborne, 1999).

### 2.4. GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) of both leaves and root hexane extracts were also carried out at the University of Lagos Central Research Laboratory, Nigeria. The model of the GC-MS used for mass spectral identification of the hexane extracts of the leaves and root of *I. trichantha* was an Agilent 6890 interfaced to a 5973 mass selective detector. The capillary column (30 m x 0.25 mm x 0.25 µm film thickness) was HP-5MS. The oven temperature of 50°C for 5 minutes was firstly maintained and then set to 250 °C at 5 °C min<sup>-1</sup>. Helium (99.999%) was the carrier gas used at a flow rate of 1 ml/min, and 1 µl injection volume was employed (split ratio of 10:1). The electron-impact ionization mass spectrometry electron energy of 70 eV of was operated at an. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 40 to 450 Da. The total GC running time was 45 min. The spectra data obtained were compared with those of NIST library mass spectra.

## 3. Results

Detailed variations in the qualitative and quantitative characters of the leaf epidermis of *Icacina trichantha* are shown in Table 1. The leaf is hypostomatic and the stomatal types are paracytic and diacytic (Fig. 1B, Table 1). The epidermal cell shape is irregular on both surfaces of the leaf (Fig. 1A, B; Table 1) but the anticlinal wall pattern is either angular on the adaxial surface or curved



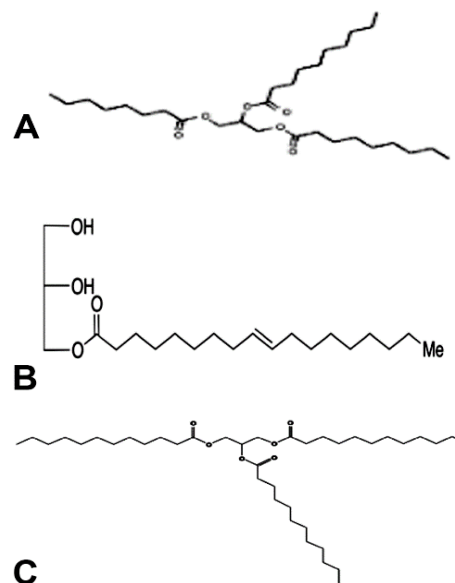
**Figure 1.** Leaf epidermis features of *Icacina trichantha*. A: Adaxial surface, B-D: Abaxial surface, D: Typical unicellular trichome found on the abaxial surface of the leaf. Scale bar = 40 $\mu$ m.

on the abaxial surface of the epidermis (Fig. 1, Table 1). Both the non-stomatal and stomatal quantitative features reasonably overlap across the one hundred leaf samples that were investigated (Table 1).

The result of the phytochemical analysis from this study is as presented (Fig. 2, Table 2). The hexane extract of the root of *I. trichantha* is rich in reducing sugars, tannins, steroids, glycoside, terpenoids and flavonoids, while saponins, alkaloid, anthraquinone and the volatile oil is absent. GC-MS analysis of n-hexane extracts of both the leaf and the root of *I. trichantha* were reported (Table 3). Eleven significant bioactive components were found in the leaf while the root revealed 3 bioactive components in high percentage concentration. Dodecanoic acid was revealed to be the major component in both extracts with the leaf (54.10 %) having a higher concentration than the

**Table 1.** Qualitative and quantitative characteristics of the leaf epidermis of *Icacina trichantha*

Features	Surfaces	
	Adaxial surface	Abaxial surface
Cell shape	Irregular	Irregular
Wall pattern	Angular	Curved
E. cell No./ field	56	68
E. cell length ( $\mu$ m)	28.0(42.0 $\pm$ 2.0)58.5	30.0(48.0 $\pm$ 2.0)65.2
E. cell width ( $\mu$ m)	12.0(15.0 $\pm$ 2.0)25.3	10.5(12.0 $\pm$ 2.0)16.0
Stomata	Absent	Present
Stomata No./ field	Absent	42
Stomatal type	Absent	Paracytic, diacytic
Stomatal length ( $\mu$ m)	Absent	22.0(27.0 $\pm$ 2.0)30.0
Stomatal width ( $\mu$ m)	Absent	8.5(10.0 $\pm$ 2.0)12.0
Stomatal index (%)	Absent	38.2
Trichome	Absent	Long unicellular trichomes present



**Figure 2.** Structures of some of the chemicals found in *I. trichantha* A: 9-Octadecadienoic, B: Methoxyacetic acid, C: Dodecanoic acid and B were found in the leaf while C occurred in the root.

root (47.85 %). The root in addition revealed the presence of Trichothec-9-en-8-one (47.73 %). The leaf extract also revealed 9-Octadecenoic acid (25.05 %). Other prominent components in the leaf extract were acetamide (6.21 %) and methoxyacetic acid (5.58 %) (Table 3).

#### 4. Discussions

A combined evaluation of the leaf epidermis and chemical features of *I. trichantha* carried out in the study has shown that the documented characters are useful for identification. They are also potentially suitable for differentiating the species from any other related species in the family Icacinaceae. Those features that seem to be good for defining the species include hypostomatic leaves, paracytic and diacytic stomatal types, and the presence of simple unicellular trichomes which are usually restricted to the abaxial surface of the leaf. However, the taxonomic relevance of epidermal features has been expounded by several workers, as being good for identification, delineation, classification and in resolving taxonomic intricacies (Davis and Heywood, 1963; Stace, 1965;

**Table 2.** Phytochemical analysis of the tuberous root of *Icacina trichantha*

Phytochemical	Result
Reducing sugars	+
Tannins	+
Saponin	-
Alkaloids	-
Steroids	+
Glycoside	+
Flavonoids	+
Anthraquinone	-
Terpenoids	+
Volatile oils	-
Proteins	-

**Key:** + = present, - = absent



**Table 3.** Chemical composition of leaf and root of *Icacina trichantha* as revealed by Gas Chromatography–Mass Spectrometry (GC-MS)

S/N	Chemicals	Concentration in leaf (%)	Chemicals	Concentration in root (%)
1	Carbonic acid, octadecyl vinyl ester, Carbonic acid, octadecyl prop-1-en -2-yl ester	0.362%-0.433%	Dodecanoic acid, 1,2,3-propanetriyl ester, Trichothec-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-ester, monoacetate, (3.alpha.,7.alpha.)-ester, Dodecanoic acid, 1-(hydroxymethyl) -1,2-ethanediy ester	28.211%-83.84%
2	Sulfurous acid, 2-propyl tetradecyl ester	0.366%-0.70%	Dodecanoic acid, 1,2,3-propanetriyl ester, Cyclododecanol, 1-aminomethyl-ester	19.642%-58.37%
3	Octacosane, Octadecane, 3-Eicosene esters	0.616%-1.19%	Trichothec-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-, monoacetate, (3.alpha.,7.alpha.)-ester	33.650%-100.00%
4	Heptacosane, 1-chloro-, Tritetracontane ester	0.756%-1.46%	Trichothec-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-, monoacetate, (3.alpha.,7.alpha.)-ester, Trimyrustin	14.086%-41.86%
5	Hexadecane, 1-iodo-, Docosane, 9-octyl- ester	0.835%-1.61%	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one ester	4.411%-13.11%
6	Tritetracontane ester	0.698%-1.35%	-	-
7	Oleic Acid, Dodecanoic acid, 2,3-dihydroxyprop yl ester, Methyl nonyl ether	2.197%-4.23%	-	-
8	Heptadecane, Docosane, 1,22-dibromo- ester	0.698%-1.35%	-	-
9	Methoxyacetic acid, tridecyl ester, Diethylene glycol monododecyl ether, Cyclohexane, 1R-acetamido-2,3-cis- epoxy-4-cis-formyloxy-ester	1.577%-3.04%	-	-
10	Cyclohexane, 1,1'-(2-propyl-1,3-propanediyl)bis-ester, Cyclohexanol, 2-(2-propynyloxy)-trans-ester, Cyclohexanol, 2-(2-propynyloxy)- 2-Dodecanol ester	1.140%-2.20%	-	-
11	9-Octadecenoic acid, Hexadecanoic acid, 2-hydroxy-, methyl ester, 9-Octadecenoic acid (Z)-, octadecyl ester	5.038%-9.70%	-	-
12	9-Octadecenoic acid (Z)-, 2-hydroxy-1-hydroxymethyl)ethyl ester, 2-Butoxyethyl oleate, Oleic acid, 3-hydroxypropyl ester	1.077%-2.07%	-	-
13	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	3.089%-5.95%	-	-
14	Cyclohexane, 1R-acetamido-2,3-cis-epoxy-4-cis-formyloxy-ester, Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl-ester, Cyclohexanone, 2-(2-propenyl)-ester	0.292%-0.56%	-	-
15	2-Methyltriacontane ester, Methoxyacetic acid, decyl ester	1.280%-2.47%	-	-
16	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Oleoyl chloride	15.838%-30.51%	-	-
17	Acetamide, 2-chloro-N-(2-cyanoethyl)-ester, 2-Dodecanol, 3-Heptafluorobutyroxydodecane	6.214%-11.97%	-	-
18	1-Methoxy-3-hydroxymethylheptane, Cyclobutanone, oxime, Methoxyacetic acid, 2-tridecyl ester	5.577%-10.74%	-	-
19	Dodecanoic acid, 1,2,3-propanetriyl ester, Dodecanoic acid, 1,2,3-propanetriyl ester, Piperidine, 3-(bromomethyl)-ester	51.916%-100.00%	-	-

Okundipe and Akinrinlade, 1998; Kadiri, 2003; Kadiri and Olowokudejo, 2016). Characteristically, the quantitative data overlap significantly among the one hundred individuals investigated thus providing the value range of the data upon which the species can be defined or differentiated from another related species. Environmental factors have been implicated in influencing the expression of morphological characters both quantitatively and qualitatively (Stace, 1965).

*Icacina trichantha* incorporates interesting character constituents in line with Otun et al. (2015) who reported

the presence of tannin, flavonoid, glycoside, terpenoid and steroid in hexane extract and the absence of saponin. These chemicals underlie their medicinal value. Phytochemicals are responsible for the biological activities of plants. Flavonoids are hydroxylated phenolic compounds known to be synthesized as a defense against microbial infection (Kumar and Pandey, 2013); consequently they exhibit pharmacological potentials such as: antimicrobial, cytotoxicity, anti-inflammatory and antitumor properties. Tannins have the ability to bind protein and metal ions from solution, hence, its benefit in the prevention of cancer activity and treatment of

inflammatory conditions (Olajide et al., 2004, Okuda and Ito, 2011; Otun et al., 2015).

The GC-MS results of hexane leaf extracts of *I. trichantha* revealed the abundant presence of an ester 9-Octadecadienoic acid which have been reported to exhibit antiinflammatory and antiarthritic property (Lalitha Rani et al., 2009). It may also be useful as anti-cancer, hepatoprotective, nematocide, insectifuge, anti-histaminic, anticoronary, anti-eczemic, anti-acne, 5-alpha reductase inhibitor and anti-androgenic agent (Vohra and Kaur, 2011). In addition, dodecanoic acid was also detected in both leaf and root hexane extracts and its antifungal activity has been reported which may attribute its use as an antimicrobial agent (Jagtap et al., 2009). Therefore, the chemical diversity in *I. trichantha* alludes to its

quintessential medicinal uses in the folkloric health care system.

However, the information provided from the morphological and chemical assessment of the plant appears to be helpful in identifying and characterizing the species, and they would assist in crude drug research of the plant.

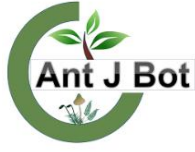
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## The checklist of the macromycetes determined in Gaziantep province

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### Gaziantep yöresinde belirlenen makromantarların kontrol listesi

**Abstract:** This study was based on the findings of macrofungal studies performed within the boundaries of Gaziantep Province between 2009 and 2019. Tracing the researches carried out in the region, a list of 404 taxa belonging to 203 genera, 80 families, 23 order and 8 classes within Ascomycota and Basidiomycota have been compiled.

**Key words:** Biodiversity, macrofungi, Turkey

**Özet:** Bu çalışma 2009 ve 2019 yılları arasında Gaziantep il sınırları içinde makromantarlar üzerinde gerçekleştirilmiş çalışmaların bulgularına dayanmaktadır. Bölgede gerçekleştirilen çalışmalar taranarak Ascomycota ve Basidiomycota bölümleri içinde yer alan 8 sınıf, 23 takım, 80 familya ve 203 cinsine ait 404 taksonu içeren bir liste oluşturulmuştur.

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

**Citation:** Uzun Y, Kaya A (2020). The checklist of the macromycetes determined in Gaziantep province. *Anatolian Journal of Botany* 4(2): 106-115.

#### 1. Introduction

There has been an increase in the number of taxonomic studies on Turkish macromycota, especially in the last three to four decades. The latest checklists on the macrofungi of Turkey (Sesli and Denchev, 2014; Solak et al., 2015) contain about 2400 macromycete taxa, three of which were presented as new species (Intini et al., 2003; Işiloğlu et al., 2009, 2010; Watling et al., 2010). Following the checklists, some local lists (Demirel and Koçak, 2016; Güngör et al., 2016; Demirel et al., 2017; Kaşık et al., 2017; Türkekul and Işık, 2017, 2019; Uzun et al., 2017; Demirel and Allı, 2019; Yıldız et al., 2019; Sadullahoğlu and Uzun, 2020; Uzun et al., 2020), some new species (Sesli et al., 2015, 2017, 2018; Vizzini et al., 2015, 2016; Taşkın et al., 2016; Sesli and Vizzini, 2017; Doğan et al., 2018; Kaygusuz et al., 2020) and many new records (Acar and Uzun, 2017; Çolak et al., 2017; Topçu Sesli and Sesli, 2017; Akata et al., 2018; Doğan et al., 2018; Işık and Türkekul, 2018; Sesli et al., 2018; Akçay, 2019, 2020; Keleş, 2019; Şen and Allı, 2019; Işık, 2020) were also presented.

Gaziantep is a province of Turkey within Southeastern Anatolian region and is among the studied regions of Turkey. In Gaziantep, the first mycological study related to macrofungi was carried out by Kaya (2009) in a restricted area (Huzurlu High Plateau). Starting from 2012, three local lists from Araban (Kaya et al. 2012), Şehitkamil and Yavuzeli (Kaya et al. 2014), and İslahiye (Uzun et al. 2015a) districts were presented. Following these studies 83 macromycete taxa have been reported as new records for the macromycota of Turkey from the region (Karacan et al., 2015, Kaya and Uzun, 2015, 2018; Kaya et al., 2015, 2016, 2018; Uzun et al., 2015b; Uzun et al., 2016; Uzun et al., 2017a,b,c; Uzun et al., 2018a,b,c). Lastly a contributory list was also presented about the macromycete taxa of the region by Kaya et al. (2019).

The current study was based on the findings of the studies that have so far been carried out within the boundaries of

Gaziantep province. The aim of the study is to present the macromycetes of Gaziantep province as a complete list and to contribute to the knowledge of the mycobiota of Turkey.

#### 2. Materials and Method

The researches published on macromycetes of Gaziantep province were traced and a list of the macromycete taxa was prepared together with the references they were presented in. During preparation of the list, only the taxa presented in a peer reviewed article were considered, and those presented in conference papers, graduate theses or project reports were not included. The authors names of fungal taxa are abbreviated according to Kirk & Ansell (1992) and Kirk et al. (2004). The systematic of the taxa follows Cannon and Kirk (2007), Kirk et al. (2008), and Index fungorum (accessed 20 December 2019).

#### 3. Results

The list of the taxa, reported from the region within the boundaries of Gaziantep province are given in alphabetical order together with the references they were presented in.

*Fungi* R.T. Moore

*Ascomycota* Caval.-Sm.

*Dothideomycetes* O.E. Erikss. & Winka

*Patellariales* D. Hawksw. & O.E. Erikss.

*Patellariaceae* Corda

1. *Patellaria atrata* (Hedw.) Fr.: (Kaya et al., 2019).

*Leotiomycetes* O.E. Erikss. & Winka

*Helotiales* Nannf.

*Dermateaceae* Fr.

2. *Mollisia cinerea* (Batsch) P. Karst.: (Hedw.) Fr.: (Kaya et al., 2015).

3. *Mollisia hydrophila* (P. Karst.) Sacc.: (Hedw.) Fr.: (Kaya et al., 2019).

4. *Mollisia ligni* (Desm.) P. Karst.: (Hedw.) Fr.: (Kaya et al., 2019).



5. *Mollisia melaleuca* (Fr.) Sacc.: (Hedw.) Fr.: (Kaya et al., 2019).
6. *Tapesia fusca* (Pers.) Fuckel: (Uzun et al., 2015a).
7. *Tapesia strobilicola* (Rehm) Sacc.: (Uzun et al., 2015a).
8. *Trichobelonium kneiffii* (Wallr.) J. Schröt.: (Hedw.) Fr.: (Kaya et al., 2015).

**Helotiaceae** Rehm

9. *Ascocoryne cylichnium* (Tul.) Korf: (Kaya et al., 2019).
10. *Bisporella citrina* (Batsch) Korf & S.E. Carp.: (Uzun et al., 2015a).
11. *Bisporella sulfurina* (Quél.) S.E. Carp.: (Kaya et al., 2018).
12. *Cenangium ferruginosum* Fr.: (Kaya et al., 2019).
13. *Hymenoscyphus calyculus* (Fr.) W. Phillips: (Kaya et al., 2019).
14. *Hymenoscyphus fructigenus* (Bull.) Gray: (Kaya et al., 2019).
15. *Hymenoscyphus herbarum* (Pers.) Dennis: (Kaya et al., 2019).
16. *Hymenoscyphus janthinum* (Fr.) Lambotte: (Uzun et al., 2015a).
17. *Hymenoscyphus scutula* (Pers.) W. Phillips: (Uzun et al., 2015a).
18. *Hymenoscyphus serotinus* (Pers.) W. Phillips: (Kaya et al., 2019).
19. *Phaeohelotium umbilicatum* (Le Gal) Dennis: (Kaya, 2009).

**Hyaloscyphaceae** Nannf.

20. *Calycina conorum* (Rehm) Baral: (Uzun et al., 2017d).
21. *Dasyscyphella nivea* (R. Hedw.) Raitv.: (Uzun et al., 2015a).
22. *Discocistella grevillei* (Berk.) Svrček: (Uzun et al., 2017d).
23. *Hyalopeziza millepunctata* (Lib.) Raitv.: (Uzun et al., 2017d).
24. *Lachnellula subtilissima* (Cooke) Dennis: (Uzun et al., 2015a).
25. *Lasiobelonium horridulum var. capitatum* Dougloud: (Kaya et al., 2015).
26. *Lasiobelonium variegatum* (Fuckel) Raitv.: (Uzun et al., 2017c).
27. *Perrotia flammea* (Alb. & Schwein.) Boud.: (Uzun et al., 2015a).
28. *Rodwayella citrinula* (P. Karst.) Spooner: (Uzun et al., 2017d).

**Lachnaceae** Raitv.

29. *Lachnum fuscescens* (Pers.) P. Karst.: (Kaya et al., 2018).
30. *Lachnum virgineum* (Batsch) P. Karst.: (Uzun et al., 2015a).
31. *Neobulgaria pura* (Pers.) Petr.: (Uzun et al., 2015b).
32. *Trichopeziza subsulphurea* (Svrček) Baral: (Uzun et al., 2015b).

**Marthamycetaceae** H.O. Baral, G. Lantz, Hustad & Minter

33. *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter: (Kaya et al., 2014).
34. *Cyclaneusma niveum* (Pers.) DiCosmo, Peredo & Minter: (Kaya et al. 2018).
35. *Naemacyclus fimbriatus* (Schwein.) DiCosmo, Peredo & Minter: (Kaya et al., 2019).

**Rutstroemiaceae** Holst-Jensen

36. *Rutstroemia firma* (Pers.) P. Karst.: (Kaya et al., 2019).

**Sclerotiniaceae** Whetzel

37. *Ciboria rufofusca* (O. Weberb.) Sacc.: (Kaya et al., 2019).
38. *Sclerotinia sclerotiorum* (Lib.) de Bary: (Kaya et al., 2019).
39. *Sclerotinia trifoliorum* Erikss.: (Kaya and Uzun, 2018).

**Rhytismatales** M.E. Barr ex Minter

**Rhytismataceae** Chevall.

40. *Coccomyces delta* (Kunze ex Fr.) Sacc.: (Kaya and Uzun, 2018).
41. *Coccomyces dentatus* (J.C. Schmidt) Sacc.: (Kaya and Uzun, 2018).
42. *Lophodermium arundinaceum* (Schrad.) Chevall.: (Kaya et al., 2019).
43. *Propolis farinosa* (Pers.) Fr.: (Kaya et al., 2019).

**Orbiliomycetes** O.E. Erikss. & Baral

**Orbiliales** Baral, O.E. Erikss.

**Orbiliaceae** Nannf.

44. *Hyalorbilia inflatula* (P. Karst.) Baral & G. Marson: (Kaya et al. 2018).
45. *Orbilia aristata* (Velen.) Velen.: (Kaya et al., 2018).

**Pezizomycetes** O.E. Erikss. & Winka

**Pezizales** J. Schröt.

**Ascobolaceae** Boud. ex Sacc.

46. *Ascobolus carbonarius* P. Karst.: (Uzun et al., 2018b).
47. *Ascobolus crenulatus* P. Karst.: (Uzun et al., 2018b).
48. *Ascobolus foliicola* Berk. & Broome: (Uzun et al., 2018b).
49. *Ascobolus immersus* Pers.: (Uzun et al. 2018b).
50. *Ascobolus stercorarius* (Bull.) J. Schröt.: (Kaya et al., 2014).
51. *Saccobolus glaber* (Pers.) Lambotte: (Uzun et al., 2018b).
52. *Thecotheus holmskioldii* (E.C. Hansen) Eckblad: (Uzun et al., 2018b).
53. *Thecotheus pelletieri* (P. Crouan & H. Crouan) Boud.: (Kaya and Uzun, 2015).

**Ascodesmidaceae** J. Schröt.

54. *Lasiobolus cuniculi* Velen.: (Uzun et al., 2018b).

**Caloscyphaceae** Harmaja

55. *Caloscypha fulgens* (Pers.) Boud.: (Kaya et al., 2019).

**Helvellaceae** Fr.

56. *Barssia hellenica* Kaounas, Agnello, P. Alvarado & Slavova: (Uzun et al., 2018b).
57. *Helvella acetabulum* (L.) QuéL.: (Kaya et al., 2019).
58. *Helvella compressa* (Snyder) N.S. Weber: (Uzun et al., 2015a).
59. *Helvella costifera* Nannf.: (Uzun et al., 2015a).
60. *Helvella dissingii* Korf: (Kaya et al., 2019).
61. *Helvella lacunosa* Afzel.: (Kaya et al., 2019).
62. *Helvella leucomelaena* (Pers.) Nannf.: (Kaya et al., 2019).
63. *Helvella leucopus* Pers.: (Kaya et al., 2019).
64. *Helvella macropus* (Pers.) P. Karst.: (Kaya et al., 2019).
65. *Helvella monachella* (Scop.) Fr.: (Kaya et al., 2019).
66. *Paxina queletii* (Bresadola) Stangl: (Kaya et al., 2019).

**Morchellaceae** Rehb.

67. *Mitrophora semilibera* (DC.) Lév.: (Kaya et al., 2019).  
 68. *Morchella deliciosa* Fr.: (Kaya et al., 2019).  
 69. *Morchella elata* Fr.: (Kaya et al., 2019).  
 70. *Morchella esculenta* (L.) Pers.: (Kaya et al., 2019).  
 71. *Verpa conica* (O.F. Müll.) Sw.: (Kaya et al., 2019).

**Pezizaceae** Dumort.

72. *Iodophanus carneus* (Pers.) Korf: (Kaya et al., 2019).  
 73. *Marcellina atroviolacea* Brumm.: (Uzun et al., 2018b).  
 74. *Marcellina rickii* (Rehm) Graddon: (Uzun et al., 2018b).  
 75. *Peziza badia* Pers.: (Kaya et al., 2019).  
 76. *Peziza cerea* Sowerby ex Fr.: (Kaya et al., 2019).  
 77. *Peziza fimeti* (Fuckel) E.C. Hansen: (Kaya et al., 2019).  
 78. *Peziza pseudoviolacea* Donadini: (Kaya et al., 2019).  
 79. *Peziza vesiculosa* Bull.: (Kaya et al., 2019).  
 80. *Peziza violacea* Pers.: (Kaya et al., 2019).  
 81. *Sarcosphaera coronaria* (Jacq.) J. Schröt.: (Kaya et al., 2019).  
 82. *Terfezia boudieri* Chatin: (Kaya et al., 2019).  
 83. *Terfezia olbiensis* Tul. & C. Tul.: (Uzun et al., 2015a).

**Pyronemataceae** Corda

84. *Aleuria exigua* Rifai: (Kaya et al. 2016).  
 85. *Cheilymenia catenipila* J. Moravec: (Kaya et al., 2016).  
 86. *Cheilymenia fimicola* (Bagl.) Dennis: (Kaya et al., 2019).  
 87. *Cheilymenia pulcherrima* (P. Crouan & H. Crouan) Boud.: (Uzun et al., 2018b).  
 88. *Cheilymenia theleboloides* (Alb. & Schwein.) Boud.: (Kaya et al., 2019).  
 89. *Cheilymenia vitellina* (Pers.) Dennis: (Kaya et al., 2016).  
 90. *Geopora arenicola* (Lév.) Kers: (Kaya et al., 2019).  
 91. *Geopora arenosa* (Fuckel) S. Ahmad: (Kaya et al., 2019).  
 92. *Geopora sumneriana* (Cooke) M. Torre: (Kaya et al., 2019).  
 93. *Geopyxis majalis* (Fr.) Sacc.: (Kaya et al., 2016).  
 94. *Geopyxis vulcanalis* (Peck) Sacc.: (Kaya et al., 2016).  
 95. *Humaria aurantia* (Clem.) Häffner, Benkert & Krisai: (Kaya et al., 2016).  
 96. *Humaria hemisphaerica* (F.H. Wigg.) Fuckel: (Kaya et al., 2019).  
 97. *Hypotarsetta insignis* (Berthet & Rioussset) Donadini: (Kaya and Uzun, 2015; Kaya et al., 2019).  
 98. *Inermisia gyalectoides* (Svrček & Kubička) Dennis & Itzerott: (Uzun et al., 2018a).  
 99. *Kotlabaea deformis* (P. Karst.) Svrček: (Kaya et al., 2016).  
 100. *Lamprospora carbonicola* Boud.: (Uzun et al., 2018c).  
 101. *Lamprospora miniata* De Not.: (Uzun et al., 2018c).  
 102. *Lamprospora dictydiola* Boud.: (Uzun et al., 2018c).  
 103. *Octospora areolata* (Seaver) Caillet & Moyne: (Uzun et al., 2018c).  
 104. *Octospora axillaris* (Nees) M.M. Moser: (Uzun et al., 2018c).  
 105. *Octospora coccinea* (P. Crouan & H. Crouan) Brumm.: (Uzun et al., 2018c).  
 106. *Octospora excipulata* (Clem.) Benkert: (Uzun et al., 2018c).

107. *Octospora gemmicola* Benkert: (Uzun et al., 2018c).  
 108. *Octospora grimmiae* Dennis & Itzerott: (Kaya et al., 2019).  
 109. *Octospora itzerottii* Benkert: (Uzun and Kaya, 2017a).  
 110. *Octospora leucoloma* Hedw.: (Uzun et al., 2018c).  
 111. *Octospora musci-muralis* Graddon: (Uzun et al., 2018c).  
 112. *Octospora orthotrichi* (Cooke & Ellis) K.B. Khare & V.P. Tewari: (Uzun et al., 2018c).  
 113. *Octospora polytrichi* (Schumach.) Caillet & Moyne: (Uzun et al., 2018c).  
 114. *Octospora rustica* (Velen.) J. Moravec: (Uzun et al., 2018c).  
 115. *Pseudombrophila merdaria* (Fr.) Brumm.: (Kaya and Uzun, 2015).  
 116. *Pulvinula archeri* (Berk.) Rifai: (Karacan et al., 2015).  
 117. *Pulvinula carbonaria* (Fuckel) Boud.: (Karacan et al., 2015).  
 118. *Pulvinula johannis* Lantieri: (Kaya et al., 2016).  
 119. *Pulvinula laeterubra* (Rehm) Pfister: (Karacan et al., 2015).  
 120. *Pustularia patavina* (Cooke & Sacc.) Boud.: (Kaya and Uzun, 2015).  
 121. *Pyronema domesticum* (Sowerby) Sacc.: (Kaya et al., 2016).  
 122. *Pyronema omphalodes* (Bull.) Fuckel: (Kaya and Uzun, 2015).  
 123. *Scutellinia trechispora* (Berk. & Broome) Lambotte: (Kaya et al., 2016).  
 124. *Scutellinia umbrorum* (Fr.) Lambotte: (Kaya, 2009).  
 125. *Sepultariella semi-immersa* (P.Karst.) Van Vooren, U.Lindem. & Healy: (Uzun et al., 2018b).  
 126. *Smardaea planchonis* (Dunal ex Boud.) Korf & W.Y. Zhuang: (Kaya et al., 2016).  
 127. *Tarsetta catinus* (Holmsk.) Korf & J.K. Rogers: (Kaya et al., 2019).  
 128. *Tarsetta cupularis* (L.) Svrček: (Kaya et al., 2019).  
 129. *Tricharina gilva* (Boud. ex Cooke) Eckblad: (Kaya and Uzun, 2015).  
 130. *Tricharina ochroleuca* (Bres.) Eckblad: (Kaya et al., 2016).  
 131. *Tricharina praecox* (P. Karst.) Dennis: (Kaya et al., 2016).  
 132. *Trichophaeopsis bicuspis* (Boud.) Korf & Erb: (Kaya et al., 2016).  
**Sarcoscyphaceae** Le Gal ex Eckblad  
 133. *Komposcypha chudei* (Pat. ex Le Gal) Pfister: (Kaya and Uzun, 2018).  
 134. *Pithya cupressina* (Batsch) Fuckel: (Kaya and Uzun, 2018).  
 135. *Pseudopithyella minuscula* (Boud. & Torrend) Seaver: (Kaya and Uzun, 2018).  
**Sarcosomataceae** Kobayasi  
 136. *Strobiloscypha cupressina* B. Perić & Pfister: (Kaya and Uzun, 2018).  
**Tuberaceae** Dumort.  
 137. *Tuber borchii* Vittad.: (Kaya et al., 2019).  
**Sordariomycetes** O.E. Erikss. & Winka  
**Diaporthales** Nannf.  
**Valsaceae** Tul. & C. Tul.  
 138. *Valsa sordida* Nitschke: (Kaya et al., 2019).

**Hypocreales** Lindau

**Nectriaceae** Tul. & C. Tul.

139. *Dialonectria episphaeria* (Tode) Cooke: (Kaya et al., 2019).

**Sordariales** Chadef. ex D. Hawksw. & O.E. Erikss.

**Lasiosphaeriaceae** Nannf.

140. *Lasiosphaeris hirsuta* (Fr.) A.N. Mill. & Huhndorf: (Kaya and Uzun, 2018).

**Xylariales** Nannf.

**Diatrypaceae** Nitschke

141. *Diatrype bullata* (Hoffm.) Fr.: (Kaya et al., 2019).  
 142. *Diatrype disciformis* (Hoffm.) Fr.: (Kaya et al., 2019).  
 143. *Diatrype stigma* (Hoffm.) Fr.: (Kaya et al., 2019).

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

144. *Hypoxylon rubiginosum* (Pers.) Fr.: (Uzun et al., 2015a).  
 145. *Xylaria hypoxylon* (L.) Grev.: (Uzun et al., 2015a).

**Basidiomycota** R.T. Moore

**Agaricomycetes** Doweld

**Agaricales** Underw.

**Agaricaceae** Chevall.

146. *Agaricus arvensis* Schaeff.: (Kaya, 2009).  
 147. *Agaricus bitorquis* (Quél.) Sacc.: (Kaya et al., 2019).  
 148. *Agaricus campestris* L.: (Kaya et al., 2019).  
 149. *Agaricus moelleri* Wasser: (Kaya et al., 2019).  
 150. *Agaricus pseudopratensis* (Bohus) Wasser: (Kaya 2009).  
 151. *Agaricus sylvaticus* Schaeff.: (Kaya et al., 2019).  
 152. *Agaricus xanthodermus* Genev.: (Kaya et al., 2019).  
 153. *Battarrea phalloides* (Dicks.) Pers.: (Kaya et al., 2019).  
 154. *Bovista aestivalis* (Bonord.) Demoulin: (Kaya et al., 2019).  
 155. *Bovista nigrescens* Pers.: (Kaya et al., 2019).  
 156. *Bovista plumbea* Pers.: (Kaya et al., 2019).  
 157. *Coprinus comatus* (O.F. Müll.) Pers.: (Kaya et al., 2019).  
 158. *Crucibulum laeve* (Huds.) Kambly: (Kaya et al., 2019).  
 159. *Cyathus olla* (Batsch) Pers.: (Kaya et al., 2019).  
 160. *Cyathus stercoreus* (Schwein.) De Toni: (Kaya et al., 2019).  
 161. *Cystoderma amianthinum* (Scop.) Fayod: (Kaya et al., 2019).  
 162. *Cystodermella cinnabarina* (Alb. & Schwein.) Harmaja: (Kaya et al., 2019).  
 163. *Echinoderma jacobi* (Vellinga & Knudsen) Gminder: (Kaya 2009).  
 164. *Lepiota cristata* (Bolton) P. Kumm.: (Uzun et al., 2015a).  
 165. *Leucoagaricus erioderma* (Malençon) Bon: (Kaya, 2009).  
 166. *Leucoagaricus leucothites* (Vittad.) Wasser: (Kaya et al., 2019).  
 167. *Leucoagaricus serenus* (Fr.) Bon & Boiffard: (Kaya, 2009).  
 168. *Leucocoprinus badhamii* (Berk. & Broome) Locq.: (Kaya 2009).  
 169. *Lycoperdon excipuliforme* (Scop.) Pers.: (Kaya et al., 2019).  
 170. *Lycoperdon molle* Pers.: (Kaya et al., 2019).  
 171. *Lycoperdon nigrescens* Pers.: (Kaya, 2009).

172. *Lycoperdon perlatum* Pers.: (Kaya et al., 2019).

173. *Lycoperdon pratense* Pers.: (Kaya et al., 2019).

174. *Lycoperdon utriforme* Bull.: (Uzun et al., 2015a).

175. *Macrolepiota excoriata* (Schaeff.) Wasser: (Kaya et al., 2019).

176. *Macrolepiota mastoidea* (Fr.) Singer: (Kaya et al., 2019).

177. *Macrolepiota procera* (Scop.) Singer: (Kaya et al., 2019).

178. *Tulostoma brumale* Pers.: (Kaya et al., 2019).

179. *Tulostoma fimbriatum* Fr.: (Kaya et al., 2019).

180. *Tulostoma melanocyclum* Bres.: (Kaya et al., 2019).

**Amanitaceae** R. Heim ex Pouzar

181. *Amanita excelsa* (Fr.) Bertill.: (Kaya et al., 2019).

182. *Amanita gemmata* (Fr.) Bertill.: (Uzun et al., 2015a).

183. *Amanita muscaria* (L.) Lam.: (Uzun et al., 2015a).

184. *Amanita pantherina* (DC.) Krombh.: (Uzun et al., 2015a).

185. *Amanita rubescens* Pers.: (Uzun et al., 2015a).

186. *Amanita vaginata* (Bull.) Lam.: (Kaya, 2009).

**Bolbitiaceae** Singer

187. *Bolbitius titubans* (Bull.) Fr.: (Kaya et al., 2019).

188. *Conocybe apala* (Fr.) Arnolds: (Kaya et al., 2019).

189. *Conocybe deliquescens* Huskn. & Krisai: (Kaya et al., 2014).

190. *Conocybe filaris* (Fr.) Kühner: (Kaya et al., 2019).

191. *Conocybe rickenii* (Jul. Schäff.) Kühner: (Kaya et al., 2019).

192. *Galeropsis desertorum* Velen. & Dvořák: (Kaya et al., 2019).

**Chromocyphellaceae** Knudsen

193. *Chromocyphella muscicola* (Fr.) Donk: (Uzun et al., 2017c).

**Cortinariaceae** R. Heim ex Pouzar

194. *Cortinarius trivialis* J.E. Lange: (Uzun et al., 2015a).

195. *Cortinarius turgidus* Fr.: (Kaya 2009).

196. *Hebeloma crustuliniforme* (Bull.) Quél.: (Uzun et al., 2015a).

197. *Hebeloma sinapizans* (Paulet) Gillet: (Kaya, 2009).

**Cyphellaceae** Lotsy

198. *Chondrostereum purpureum* (Pers.) Pouzar: (Kaya et al., 2019).

**Entolomataceae** Kotl. & Pouzar

199. *Entoloma rusticoides* (Gillet) Noordel.: (Kaya et al., 2019).

200. *Entoloma sinuatum* (Bull.) P. Kumm.: (Uzun et al., 2015a).

**Hydnangiaceae** Gäum. & C.W. Dodge

201. *Laccaria laccata* (Scop.) Cooke: (Kaya et al., 2019).

**Hygrophoraceae** Lotsy

202. *Ampulloclitocybe clavipes* (Pers.) Redhead, Lutzoni, Moncalvo & Vilgalys: (Kaya et al., 2019).

203. *Hygrocybe cantharellus* (Fr.) Murrill: (Kaya, 2009).

204. *Hygrocybe conica* (Schaeff.) P. Kumm.: (Kaya et al., 2019).

205. *Hygrophorus agathosmus* (Fr.) Fr.: (Kaya, 2009).

206. *Hygrophorus camarophyllus* (Alb. & Schwein.) Dumée, Grandjean & Maire: (Kaya, 2009).

207. *Hygrophorus chrysodon* (Batsch) Fr.: (Uzun et al., 2015a).

208. *Hygrophorus discoxanthus* (Fr.) Rea: (Kaya, 2009).

209. *Hygrophorus eburneus* (Bull.) Fr.: (Uzun et al., 2015a).
210. *Hygrophorus purpurascens* (Alb. & Schwein.) Fr.: (Uzun et al., 2015a).
- Hymenogastraceae** Vittad.
211. *Galerina graminea* (Velen.) Kühner: (Kaya et al., 2019).
212. *Galerina marginata* (Batsch) Kühner: (Kaya et al., 2019).
- Incertae Sedis**
213. *Panaeolina foeniseccii* (Pers.) Maire: (Kaya et al., 2019).
214. *Panaeolus ater* (J.E. Lange) Kühner & Romagn. ex Bon: (Kaya et al., 2012).
215. *Panaeolus fimicola* (Pers.) Gillet: (Kaya et al., 2019).
216. *Panaeolus olivaceus* F.H. Møller: (Kaya et al., 2019).
217. *Panaeolus papilionaceus* (Bull.) Quél.: (Kaya et al., 2014).
- Inocybaceae** Jülich
218. *Crepidotus pallidus* (Berk. & Broome) Knudsen: (Uzun et al. 2017b).
219. *Crepidotus variabilis* (Pers.) P. Kumm.: (Uzun et al., 2015a).
220. *Inocybe amblyospora* Kühner: (Kaya et al., 2019).
221. *Inocybe bongardii* (Weinm.) Quél.: (Kaya et al., 2019).
222. *Inocybe cincinnata* (Fr.) Quél.: (Kaya et al., 2019).
223. *Inocybe geophylla* (Bull.) P. Kumm.: (Kaya et al., 2019).
224. *Inocybe perbrevis* (Weinm.) Gillet: (Kaya 2009).
225. *Inocybe pusio* P. Karst.: (Kaya 2009).
226. *Inocybe rimosa* (Bull.) P. Kumm.: (Kaya et al., 2019).
227. *Phaeomarasmium erinaceus* (Fr.) Scherff. ex Romagn.: (Kaya et al., 2019).
- Lyophyllaceae** Jülich
228. *Lyophyllum decastes* (Fr.) Singer: (Uzun et al., 2015a).
- Marasmiaceae** Roze ex Kühner
229. *Calyprella capula* (Holmsk.) Quél.: (Kaya et al., 2019).
230. *Henningsomyces candidus* (Pers.) Kuntze: (Kaya et al., 2019).
231. *Macrocystidia cucumis* (Pers.) Joss.: (Kaya et al., 2019).
232. *Marasmius anomalus* Lasch ex Rabenh.: (Kaya et al., 2019).
233. *Marasmius chordalis* Fr.: (Kaya, 2009).
234. *Marasmius epodioides* Bres.: (Kaya et al., 2019).
235. *Marasmius oreades* (Bolton) Fr.: (Kaya et al., 2019).
236. *Marasmius wynneae* Berk. & Broome: (Kaya et al., 2019).
- Mycenaceae** Roze
237. *Hemimycena lactea* (Pers.) Singer: (Kaya et al., 2019).
238. *Mycena crocata* (Schrad.) P. Kumm.: (Kaya et al., 2019).
239. *Mycena haematopus* (Pers.) P. Kumm.: (Uzun et al., 2015a).
240. *Mycena inclinata* (Fr.) Quél.: (Kaya 2009).
241. *Mycena meliigena* (Berk. & Cooke) Sacc.: (Uzun et al., 2017b).
242. *Mycena pura* (Pers.) P. Kumm.: (Kaya et al., 2019).
243. *Mycena rosea* Gramberg: (Kaya et al., 2019).
244. *Mycena seynii* Quél.: (Kaya et al., 2019).
245. *Scytinotus violaceofulvus* (Batsch) Courtec.: (Kaya et al., 2019).
246. *Xeromphalina campanella* (Batsch) Kühner & Maire: (Kaya et al., 2019).
247. *Xeromphalina caudicinalis* (With.) Kühner & Maire: (Kaya et al., 2019).
- Niaceae** Jülich
248. *Cyphellopsis anomala* (Pers.) Donk: (Kaya et al., 2014).
249. *Flagelloscypha minutissima* (Burt) Donk: (Kaya et al., 2014).
250. *Lachnella alboviolascens* (Alb. & Schwein.) Fr.: (Kaya et al., 2019).
251. *Lachnella villosa* (Pers.) Donk: (Uzun et al., 2017b).
- Omphalotaceae** Bresinsky
252. *Gymnopus dryophilus* (Bull.) Murrill: (Kaya et al., 2019).
253. *Gymnopus erythropus* (Pers.) Antonín, Halling & Noordel.: (Kaya et al., 2019).
254. *Gymnopus ocior* (Pers.) Antonín & Noordel.: (Kaya et al., 2019).
255. *Gymnopus quercophilus* (Pouzar) Antonín & Noordel.: (Kaya et al., 2019).
256. *Omphalotus olearius* (DC.) Singer: (Kaya et al., 2019).
257. *Omphalotus olivascens* H.E. Bigelow, O.K. Mill. & Thiers: (Kaya et al., 2014).
- Physalacriaceae** Corner
258. *Armillaria borealis* Marxm. & Korhonen: (Kaya et al., 2019).
259. *Armillaria mellea* (Vahl) P. Kumm.: (Kaya et al., 2019).
260. *Armillaria ostoyae* (Romagn.) Herink: (Kaya 2009).
261. *Cryptomarasmium corbariensis* (Roum.) T.S. Jenkinson & Desjardin: (Uzun et al., 2017b).
262. *Hymenopellis radicata* (Relhan) R.H. Petersen: (Uzun et al., 2015a).
263. *Strobilurus stephanocystis* (Kühner & Romagn. ex Hora) Singer: (Kaya et al., 2019).
264. *Strobilurus tenacellus* (Pers.) Singer: (Kaya et al., 2019).
265. *Xerula pudens* (Pers.) Singer: (Uzun et al., 2015a).
- Pleurotaceae** Kühner
266. *Hohenbuehelia petaloides* (Bull.) Schulzer: (Kaya et al., 2019).
267. *Pleurotus dryinus* (Pers.) P. Kumm.: (Kaya et al., 2019).
268. *Pleurotus eryngii* (DC.) Quél.: (Kaya, 2009).
269. *Pleurotus ostreatus* (Jacq.) P. Kumm.: (Kaya et al., 2019).
- Pluteaceae** Kotl. & Pouzar
270. *Pluteus nanus* (Pers.) P. Kumm.: (Kaya 2009).
271. *Pluteus romellii* (Britzelm.) Sacc.: (Kaya et al., 2019).
272. *Volvopluteus gloiocephalus* (DC.) Justo: (Kaya et al., 2019).
- Psathyrellaceae** Vilgalys, Moncalvo & Redhead
273. *Coprinellus disseminatus* (Pers.) J.E. Lange: (Kaya et al., 2019).
274. *Coprinellus ephemerus* (Bull.) Redhead, Vilgalys &

Moncalvo: (Kaya et al., 2019).

275. *Coprinellus impatiens* (Fr.) J.E. Lange: (Kaya, 2009).  
 276. *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson: (Kaya et al., 2019).  
 277. *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo: (Kaya et al., 2019).  
 278. *Coprinopsis lagopides* (P. Karst.) Redhead, Vilgalys & Moncalvo: (Kaya et al., 2019).  
 279. *Coprinopsis nivea* (Pers.) Redhead, Vilgalys & Moncalvo: (Kaya et al., 2019).  
 280. *Lacrymaria lacrymabunda* (Bull.) Pat.: (Kaya, 2009).  
 281. *Parasola auricomma* (Pat.) Redhead, Vilgalys & Hopple: (Kaya et al., 2014).  
 282. *Parasola plicatilis* (Curtis) Redhead, Vilgalys & Hopple: (Kaya et al., 2019).  
 283. *Psathyrella bipellis* (Qué.) A.H. Sm.: (Kaya et al., 2019).  
 284. *Psathyrella candolleana* (Fr.) Maire: (Kaya et al., 2019).  
 285. *Psathyrella lutensis* (Romagn.) Bon: (Kaya et al., 2012).

**Pterulaceae** Corner

286. *Pterula multifida* (Chevall.) Fr.: (Kaya et al., 2019).

**Schizophyllaceae** Qué.

287. *Schizophyllum amplum* (Lév.) Nakasone: (Kaya et al., 2019).  
 288. *Schizophyllum commune* Fr.: (Kaya et al., 2019).

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

289. *Agrocybe molesta* (Lasch) Singer: (Kaya et al., 2019).  
 290. *Agrocybe pediades* (Fr.) Fayod: (Kaya et al., 2019).  
 291. *Agrocybe praecox* (Pers.) Fayod: (Kaya et al., 2014).  
 292. *Agrocybe vervacti* (Fr.) Singer: (Kaya et al., 2019).  
 293. *Cyclocybe cylindracea* (DC.) Vizzini & Angelini: (Kaya et al., 2019).  
 294. *Hypholoma fasciculare* (Huds.) P. Kumm.: (Kaya et al., 2019).  
 295. *Protostropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys: (Kaya et al., 2019).  
 296. *Psilocybe coprophila* (Bull. : Fr.) P. Kumm: (Kaya et al., 2019).  
 297. *Stropharia aeruginosa* (Curtis) Qué.: (Kaya et al., 2019).  
 298. *Stropharia coronilla* (Bull.) Qué.: (Kaya et al., 2019).

**Tricholomataceae** R. Heim ex Pouzar

299. *Arrhenia lilacinicolor* (Bon) P.-A. Moreau & Courtec.: (Uzun et al., 2018a).  
 300. *Arrhenia retiruga* (Bull.) Redhead: (Kaya et al., 2019).  
 301. *Arrhenia rickenii* (Hora) Watling: (Kaya et al., 2019).  
 302. *Arrhenia spathulata* (Fr.) Redhead: (Kaya et al., 2019).  
 303. *Cellypha goldbachii* (Weinm.) Donk: (Uzun et al., 2018a).  
 304. *Clitopaxillus alexandri* (Gillet) G. Moreno, Vizzini, Consiglio & P. Alvarado: (Kaya et al., 2019).  
 305. *Clitocybe gibba* (Pers.) P. Kumm.: (Kaya et al., 2019).  
 306. *Clitocybe nebularis* (Batsch) P. Kumm.: (Uzun et al., 2015a).

307. *Clitocybe odora* (Bull.) P. Kumm.: (Uzun et al., 2015a).  
 308. *Clitocybe phyllophila* (Pers.) P. Kumm.: (Kaya, 2009).  
 309. *Cotylidia diaphana* (Cooke) Lentz: (Kaya, 2009).  
 310. *Infundibulicybe geotropa* (Bull.) Harmaja: (Kaya et al., 2019).  
 311. *Lepista nuda* (Bull.) Cooke: (Kaya et al., 2019).  
 312. *Lepista sordida* (Schumach.) Singer: (Uzun et al., 2015a).  
 313. *Leucopaxillus gentianeus* (Qué.) Kotl.: (Kaya et al., 2019).  
 314. *Melanoleuca cognata* (Fr.) Konrad & Maubl.: (Kaya et al., 2019).  
 315. *Melanoleuca excissa* (Fr.) Singer: (Kaya et al., 2019).  
 316. *Melanoleuca polioleuca* (Fr.) Kühner & Maire: (Kaya et al., 2019).  
 317. *Melanoleuca stridula* (Fr.) Singer: (Kaya et al., 2019).  
 318. *Myxomphalia maura* (Fr.) H.E. Bigelow: (Kaya et al., 2019).  
 319. *Pseudoclitocybe cyathiformis* (Bull.: Fr.) Singer: (Kaya et al., 2019).  
 320. *Resupinatus taxi* (Lév.) Thorn, Moncalvo & Redhead: (Uzun et al., 2018a).  
 321. *Resupinatus trichotis* (Pers.) Singer: (Kaya et al., 2019).  
 322. *Rimbachia neckerae* (Fr.) Redhead: (Uzun et al., 2018a).  
 323. *Tricholoma anatolicum* H.H. Doğan & Intini: (Uzun et al., 2015a).  
 324. *Tricholoma batschii* Gulden: (Kaya, 2009; Uzun et al., 2015a).  
 325. *Tricholoma equestre* (L.) P. Kumm.: (Uzun et al., 2015a).  
 326. *Tricholoma terreum* (Schaeff.) P. Kumm.: (Kaya et al., 2019).  
 327. *Tricholoma virgatum* (Fr.) P. Kumm.: (Kaya, 2009).
- Tubariaceae** Vizzini
328. *Tubaria conspersa* (Pers.) Fayod: (Kaya et al., 2019).
- Typhulaceae** Jülich
329. *Typhula fistulosa* (Holmsk.) Olariaga: (Kaya et al., 2019).  
 330. *Typhula setipes* (Grev.) Berthier: (Uzun et al., 2017b).
- Auriculariales** J. Schröt.
- Auriculariaceae** Fr.
331. *Exidia glandulosa* (Bull.) Fr.: (Kaya et al., 2019).  
 332. *Exidia nigricans* (With.) P. Roberts: (Kaya et al., 2019).
- Boletales** E.-J. Gilbert
- Boletaceae** Chevall.
333. *Boletus edulis* Bull.: (Kaya et al., 2019).  
 334. *Boletus erythropus* Pers.: (Kaya et al., 2019).  
 335. *Boletus reticulatus* Schaeff.: (Kaya, 2009).  
 336. *Xerocomellus chrysenteron* (Bull.) Šutara: (Kaya et al., 2019).  
 337. *Xerocomellus porosporus* (Imler ex Watling) Šutara: (Kaya, 2009).
- Diplocystidiaceae** Kreisel
338. *Astraeus hygrometricus* (Pers.) Morgan: (Kaya et al., 2019).



**Gomphidiaceae** Maire ex Jülich

339. *Chroogomphus rutilus* (Schaeff.) O.K. Mill.: (Kaya et al., 2019).

**Paxillaceae** Lotsy

340. *Melanogaster ambiguus* (Vittad.) Tul. & C. Tul.: (Uzun et al., 2015a).

341. *Melanogaster broomeanus* Berk.: (Uzun et al., 2015a).

**Rhizopogonaceae** Gäum. & C.W. Dodge

342. *Rhizopogon luteolus* Fr.: (Kaya et al., 2019).

343. *Rhizopogon roseolus* (Corda) Th. Fr.: (Kaya et al., 2019).

**Sclerodermataceae** Corda

344. *Pisolithus arhizus* (Scop.) Rauschert: (Kaya et al., 2019).

345. *Scleroderma cepa* Pers.: (Kaya et al., 2019).

346. *Scleroderma polyrhizum* (J.F. Gmel.) Pers.: (Uzun et al., 2015a).

347. *Scleroderma verrucosum* (Bull.) Pers.: (Kaya et al., 2019).

**Suillaceae** Besl & Bresinsky

348. *Suillus collinitus* (Fr.) Kuntze: (Kaya et al., 2019).

349. *Suillus granulatus* (L.) Roussel: (Kaya et al., 2019).

350. *Suillus luteus* (L.) Roussel: (Kaya et al., 2019).

**Tapinellaceae** C. Hahn

351. *Tapinella panuoides* (Fr.) E.-J. Gilbert: (Kaya et al., 2019).

**Cantharellales** Gäum**Clavulinaceae** Donk

352. *Clavulina cinerea* (Bull.) J. Schröt.: (Kaya et al., 2019).

353. *Clavulina coralloides* (L.) J. Schröt.: (Uzun et al., 2015a).

**Gastrales** K. Hosaka & Castellano**Gastraceae** Corda

354. *Gastrum minimum* Schwein.: (Kaya et al., 2019).

355. *Gastrum pectinatum* Pers.: (Kaya et al., 2019).

356. *Gastrum rufescens* Pers.: (Uzun et al., 2015a).

357. *Gastrum triplex* Jungh.: (Uzun et al., 2015a).

358. *Sphaerobolus stellatus* Tode: (Kaya et al., 2019).

**Gomphales** Jülich**Gomphaceae** Donk

359. *Gautieria monticola* Harkn.: (Uzun et al., 2015a).

360. *Gautieria trabutii* (Chatin) Pat.: (Kaya et al., 2019).

361. *Gomphus clavatus* (Pers.) Gray: (Uzun et al., 2015a).

362. *Ramaria flava* (Schaeff.) Quél.: (Uzun et al., 2015a).

363. *Ramaria stricta* (Pers.) Quél.: (Kaya, 2009).

**Hymenochaetales** Oberw.**Hymenochaetaceae** Imazeki & Toki

364. *Phellinus hartigii* (Allesch. & Schnabl) Pat.: (Kaya et al., 2019).

365. *Phellinus igniarius* (L.) Quél.: (Kaya et al., 2014).

366. *Phellinus pomaceus* (Pers.) Maire: (Kaya et al., 2019).

**Hysterangiales** K. Hosaka & Castellano**Hysterangiaceae** E. Fisch.

367. *Hysterangium clathroides* Vittad.: (Uzun et al., 2015a).

**Phallales** E. Fisch.**Phallaceae** Corda

368. *Phallus impudicus* L.: (Kaya et al., 2019).

**Polyporales** Gäum.**Ganodermataceae** Donk

369. *Ganoderma adpersum* (Schulzer) Donk: (Kaya et al., 2019).

370. *Ganoderma applanatum* (Pers.) Pat.: (Kaya et al., 2014).

371. *Ganoderma lucidum* (Curtis) P. Karst.: (Kaya et al., 2019).

**Meruliaceae** Rea

372. *Abortiporus biennis* (Bull.) Singer: (Kaya et al., 2014).

373. *Bjerkandera adusta* (Willd.) P. Karst.: (Kaya et al., 2019).

**Phanerochaetaceae** Jülich

374. *Terana coerulea* (Lam.) Kuntze: (Kaya et al., 2019).

**Polyporaceae** Fr. ex Corda

375. *Cerioporus varius* (Pers.) Zmitr. & Kovalenko: (Kaya, 2009).

376. *Fomes fomentarius* (L.) Fr.: (Kaya et al., 2019).

377. *Lentinus arcularius* (Batsch) Zmitr.: (Kaya et al., 2019).

378. *Lentinus brumalis* (Pers.) Zmitr.: (Kaya et al., 2019).

379. *Lentinus tigrinus* (Bull.) Fr.: (Kaya et al., 2019).

380. *Lenzites betulina* (L.) Fr.: (Kaya et al., 2019).

381. *Royoporus badius* (Pers.) A.B. De: (Kaya et al., 2019).

382. *Trametes hirsuta* (Wulfen) Lloyd: (Kaya et al., 2019).

383. *Trametes trogii* Berk.: (Kaya et al., 2014).

384. *Trametes versicolor* (L.) Lloyd: (Kaya et al., 2019).

385. *Trichaptum abietinum* (Dicks.) Ryvarden: (Uzun et al., 2015a).

386. *Trichaptum fuscoviolaceum* (Ehrenb.) Ryvarden: (Uzun et al., 2015a).

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

387. *Lentinellus cochleatus* (Pers.) P. Karst.: (Uzun et al., 2015a).

388. *Lentinellus micheneri* (Berk. & M.A. Curtis) Pegler: (Kaya et al., 2019).

**Hericiaceae** Donk

389. *Hericium abietis* (Weir ex Hubert) K.A. Harrison: (Kaya, 2009).

**Russulaceae** Lotsy

390. *Lactarius deliciosus* (L.) Gray: (Kaya et al., 2019).

391. *Lactarius piperatus* (L.) Pers.: (Kaya, 2009).

392. *Lactarius torminosus* (Schaeff.) Gray: (Kaya et al., 2019).

393. *Russula albonigra* (Krombh.) Fr.: (Kaya, 2009).

394. *Russula brevipes* Peck: (Uzun et al., 2015a).

395. *Russula delica* Fr.: (Kaya, 2009).

396. *Russula virescens* (Schaeff.) Fr.: (Kaya et al., 2019).

**Stereaceae** Pilát

397. *Stereum hirsutum* (Willd.) Pers.: (Kaya et al., 2019).

**Bankeraceae** Donk

398. *Sarcodon imbricatus* (L.) P. Karst.: (Kaya, 2009).

**Thelephorales** Corner ex Oberw.**Bankeraceae** Donk

399. *Hydnellum caeruleum* (Hornem.) P. Karst.: (Kaya et al., 2019).

**Thelephoraceae** Chevall.

400. *Thelephora terrestris* Ehrh.: (Kaya et al., 2019).

**Dacrymycetes** Doweld

**Dacrymycetales** Henn.

**Dacrymycetaceae** J. Schröt.

401. *Calocera cornea* (Batsch) Fr.: (Kaya et al., 2019).

402. *Dacrymyces capitatus* Schwein.: (Kaya et al., 2019).

403. *Dacrymyces stillatus* Nees: (Kaya et al., 2019).

**Tremellomycetes** Doweld

**Tremellales** Fr.

**Hyaloriaceae** Lindau

404. *Myxarium nucleatum* Wallr.: (Uzun et al., 2016).

**4. Discussions**

Compiling the overall macrofungal taxa that had been presented so far, Gaziantep was determined to hosts a total of 404 taxa within *Ascomycota* and *Basidiomycota*. One hundred and forty five (%35.89) of them belong to *Ascomycota* while 259 (%64.11) belong to *Basidiomycota*.

The taxa determined in the region were found to distribute in eight classes (Table 1) and 23 orders (Table 2). The most diverse class and the order were found to be *Agaricomycetes* and *Agaricales* respectively.

Eighty macromycete families were represented in Gaziantep. *Pyronemataceae*, *Agaricaceae*, *Tricholomataceae*, *Psathyrellaceae*, and *Pezizaceae* are the first 5 most crowded families in the region. The most crowded genus is *Octospora* with 12 taxa. It was followed by *Helvella*, *Agaricus*, *Inocybe* and *Mycena* respectively.

Eighty eight of the 404 taxa are edible, but only six taxa, *Agaricus campestris*, *Coprinus comatus*, *Pleurotus ostreatus*, *Terfezia boudieri*, *Tricholoma anatolicum*, and *Volvopluteus gloiocephalus*, are collected and consumed in the region by local people. *Terfezia boudieri* have local economic importance while *Tricholoma anatolicum* have international economic importance (Kaya et al., 2019). Two hundred and eighty three of the determined taxa were regarded as inedible while 33 are more or less poisonous.

Among the determined taxa, 103 are lignicolous, 18 are coprophilous, 7 are pyrophilous, 24 are bryophilous and the rest of the taxa are terricolous. Twenty two of them were also determined as hygrogeous.

The presented articles indicate that Gaziantep hosts the first locality in Turkey for 95 of the determined species. These species are *Aleuria exigua*, *Arrhenia lilacinicolor*, *Ascobolus carbonarius*, *A. crenulatus*, *A. foliicola*, *A. immersus*, *Barssia hellenica* *Bisporella sulfurina*,

**Table 1.** Distribution of the determined taxa in classes.

Division	Class	# of taxa
Ascomycota	<i>Pezizomycetes</i>	92
	<i>Leotiomycetes</i>	42
	<i>Sordariomycetes</i>	8
	<i>Orbiliomycetes</i>	2
	<i>Dothideomycetes</i>	1
Basidiomycota	<i>Agaricomycetes</i>	255
	<i>Dacrymycetes</i>	3
	<i>Tremellomycetes</i>	1

**Table 2.** Distribution of the determined taxa in orders.

Division	Order	# of taxa
Ascomycota	<i>Pezizales</i>	92
	<i>Helotiales</i>	38
	<i>Xylariales</i>	5
	<i>Rhytismatales</i>	4
	<i>Orbiliiales</i>	2
	<i>Diaporthales</i>	1
	<i>Hypocreales</i>	1
	<i>Patellariales</i>	1
	<i>Sordariales</i>	1
	<i>Agaricales</i>	185
	<i>Boletales</i>	19
	<i>Polyporales</i>	18
	<i>Russulales</i>	11
	<i>Geastrales</i>	5
<i>Gomphales</i>	5	
Basidiomycota	<i>Dacrymycetales</i>	3
	<i>Hymenochaetales</i>	3
	<i>Thelephorales</i>	3
	<i>Auriculariales</i>	2
	<i>Cantharellales</i>	2
	<i>Hysterangiales</i>	1
	<i>Phallales</i>	1
	<i>Tremellales</i>	1

*Calycina conorum*, *Cellypha goldbachii*, *Cheilymenia catenipila*, *Cheilymenia pulcherrima*, *C. vitellina*, *Chromocyphella muscicola*, *Coccomyces delta*, *C. dentatus*, *Cortinarius turgidus*, *Cotylidia diaphana*, *Crepidotus pallidus*, *Cryptomarasmius corbariensis*, *Cyclaneusma minus*, *C. niveum*, *Discocistella grevillei*, *Geopyxis majalis*, *Geopyxis vulcanalis*, *Helvella compressa*, *Humaria aurantia*, *Hyalopeziza millepunctata*, *Hyalorbilia inflatula*, *Hymenoscyphus janthinum*, *Hypotarzettia insignis*, *Hypoxyton rubiginosum*, *Inermisia gyalectoides*, *Komposocypha chudei*, *Kotlabaea deformis*, *Lachnella villosa*, *Lachnum fuscescens*, *Lamprospora carbonicola*, *L. dictydiola*, *L. miniata*, *Lasiobolium horridulum* var. *capitatum*, *L. variegatum*, *Lasiobolus cuniculi*, *Lasiosphaeris hirsuta*, *Lepiota jacobi*, *Leucoagaricus erioderma*, *L. serenus*, *Marcelleina atroviolacea*, *M. rickii*, *Mollisia hydrophila*, *Mycena meliigena*, *Myxarium nucleatum*, *Neobulgaria pura*, *Octospora areolata*, *O. axillaris*, *O. coccinea*, *O. excipulata*, *O. gemmicola*, *O. itzerottii*, *O. musci-muralis*, *O. orthotrichi*, *O. polytrichi*, *O. rustica*, *Orbilium aristata*, *Perrotia flammea*, *Phaeohelotium umbilicatum*, *Pithya cupressina*, *Pseudombrophila merdaria*, *Pseudopithyella minuscula*, *Pulvinula archeri*, *P. carbonaria*, *P. johannis*, *P. laeterubra*, *Pustularia patavina*, *Pyronema domesticum*, *P. omphalodes*, *Resupinatus taxi*, *Rimbachia neckerae*, *Rodwayella citrinula*, *Saccobolus glaber*, *Sclerotinia trifoliorum*, *Scutellinia trechispora*, *Sepultariella semi-immersa*, *Smardaea planchonis*, *Strobiloscypha cupressina*, *Tapesia strobilicola*, *Thecotheus holmskioldii*, *T. pelletieri*, *Tricharina gilva*, *T. ochroleuca*, *T. praecox*, *Trichobolium kneiffii*, *Trichopeziza subsulphurea*, *Trichophaeopsis bicuspidis* and *Typthula setipes*.

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## Biofilm formation mechanism in fungi

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## Mantarlarda biyofilm oluşum mekanizması

**Abstract:** The biofilm problem is a problem that is commonly encountered in many areas of industry and causes serious economic losses. It is stated in the literature that biofilms can be removed from surfaces by appropriate cleaning methods. However, biofilm formation gains continuity over time. Biofilm studies are generally on bacteria. Microorganisms that cause infection in humans include bacteria, viruses, and fungi. In biofilm-borne infections, there may be only one or a combination of various microorganisms. Nowadays, new searches are in progress due to the ineffectiveness of synthetic drugs against fungal diseases, their side effects, and the increase of the number of pathogenic microorganisms that are rapidly resistant to existing antifungals. Therefore, the prevention of biofilm formation is now one of the most important studies worldwide.

**Key words:** Antifungal, biofilm, fungal resistance

**Özet:** Biyofilm sorunu endüstrinin birçok alanında yaygın olarak rastlanılan bir sorundur ve ciddi anlamda ekonomik kayıplara neden olmaktadır. Literatürde uygun temizleme yöntemleri ile biyofilmlerin yüzeylerden uzaklaştırılabileceği belirtilmektedir. Ancak zamanla biyofilm oluşumu süreklilik kazanır. Biyofilm çalışmalarının geneli bakteriler üzerinedir. İnsanlarda enfeksiyona neden olan mikroorganizmalar arasında bakteriler, virüsler ve funguslar bulunmaktadır. Biyofilm kaynaklı enfeksiyonlarda etken tek olabileceği gibi bazı mikroorganizmaların karışımında olabilir. Günümüzde artan fungal hastalıklara karşı sentetik yapılı ilaçların yetersiz kalması, yan etkileri ve mevcut antifungallere hızla direnç kazanan patojen mikroorganizmaların sayısının artması ile yeni arayışlar devam etmektedir. Bütün bunlara bağlı olarak da biyofilm oluşumunun önlenmesi artık tüm dünyada önem arz eden çalışmaların başında gelmektedir.

**Anahtar Kelimeler:** Antifungal, biyofilm, fungal direnç

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### 1. Introduction

Biofilm is a matrix created by microorganisms, which consists of extracellular polymeric material, allows them to adhere to any surface, interface, or to each other, and in which microorganisms showing and creating different phenotypes depending on growth rates and gene transcription are embedded (Szafranski et al., 2017).

There are a limited number of studies on fungal biofilms in the literature. Fungal biofilm-forming species are limited to a few species that are differentiated as endogenous and exogenous in medical use and easy to isolate rather than in industrial use. The main ones are; *Candida* Berkhout spp. (*C. albicans* (C.P.Robin) Berkhout), *Cryptococcus neoformans* (San Felice) Vuill., *Blastoschizomyces* Salkin, M.A. Gordon, Sams. & Rieder spp., *Malassezia* Baill. spp., *Trichosporon* Behrend spp., *Pneumocystis* P.Delanoë & Delanoë spp., *Saccharomyces* Meyen spp., *Aspergillus* P.Micheli ex Haller spp. (*A. fumigatus* Fresen.) and *Coccidioides immitis* C.W.Stiles (Andersen et al., 2014). The fungal cells in the biofilm matrix differ from those that develop planktonically. Biofilms protect cells against adverse environmental conditions such as UV, metal toxicity, pH, osmotic changes, dehydration, antimicrobial agents, and disinfectants (Dimakopoulou-Papazoglou et al., 2016).

This research aims to support future studies for the ease of treatment and the elimination of high costs by examining the fungal biofilm mechanism in the prevention of biofilms caused by fungi in the industrial and medical sectors.

### 1.1. Fungal biofilms

#### 1.1.1. Biofilms in industrial, environmental, and agricultural fields

It is stated that biofilms cause economic losses due to their negative effects in industrial, environmental, and agricultural areas (Van and Michiels, 2010). The damage on surfaces (on instruments), product contaminations, energy losses and disease-causing infections due to biofilms cause losses worth millions of dollars in the United States. Biofilms are responsible for product and capital equipment damage (pipe clogging, rusting, and water pollution) every year in the industry (Güvenç and Ekmekcioğlu, 2016).

#### 1.1.2. Biofilms in the food industry

Biofilms, one of the most important sources of foodborne infections, cause serious problems in the food industry. Among the sectors affected by fungal biofilms is a wide range of products such as seafood, dairy products, poultry, and meat enterprises (Akan and Kınık, 2014). Due to biofilm formation, many problems such as film accumulation on the food surface, microbial colonization in milk storage tanks, contamination in heat exchangers, and sports adhesion on the surface of the packaging material arise (Brooks and Flint, 2008). In the milk industry, the biofilm matrix predominantly includes milk residues, proteins, and minerals like calcium phosphate, etc, and biofilm is the first important reason that comes to mind when any contamination occurs in dairy products (Simões et al., 2010).



### 1.1.3. Biofilms in medicine

In biofilm-borne infections, the factor can be a single bacterium or a mixture of bacteria and fungal species. Infections caused by fungi negatively affect human health. Many medically important fungi produce biofilms, including *Candida*, *Aspergillus*, *Cryptococcus* Vuill., *Trichosporon*, *Coccidioides* C.W.Stiles, and *Pneumocystis*. The use of broad-spectrum antibiotics, neutropenia, parenteral nutrition, permanent catheters, immunosuppression, and disruption of mucosal barriers secondary to surgery, chemotherapy, and radiotherapy are important factors for these infections (Allison et al., 2000).

The mechanisms used by biofilm-related organisms that cause infections in humans have not yet been fully understood. Among the proposed mechanisms is the emergence of infection in the blood or urinary system due to cells or cell groups separated from the biofilm on implants, endotoxin production, continuation of existence against the host immune system by gaining antimicrobial agent resistance through genetic substance transfer through showing structural resistance (Dimakopoulou-Papazoglou et al., 2016). In models of *Candida* biofilms, yeast cells adhere to a live or inert surface and initially maintain a yeast-like morphological form. *Candida albicans* biofilms are comprised primarily of yeast-form and hyphal cells, both of which are required for biofilm formation. Formation is a sequential process involving adherence to a substrate (either abiotic or mucosal surface), proliferation of yeast cells over the surface, and induction of hyphal formation. As the fungal biofilm matures, yeast-like growth is repressed and hyphal growth expands. As the hyphae spread across the surface, an extracellular matrix is secreted and surrounds the fungal biofilm, thereby gluing the hyphae together. The expanded hyphal growth and surrounding matrix can be considered characteristic features of fungal biofilms. *Aspergillus* biofilms can form both on abiotic and biotic surfaces. The initial colonizing cells that adhere to the substrate are conidia. Mycelia (the hyphal form) develop as the biofilm matures. Although *Cr. neoformans* forms hyphae in the course of mating, no hyphae have been observed in *Cr. neoformans* biofilms to date. Similarly, *Pneumocystis* species do not produce hyphal structures as part of their biofilms. Thus, hyphal formation is not a uniform feature of fungal biofilms (Foreman et al., 2009; Fanning and Mitchell, 2012).

Biofilms formed by microorganisms are responsible for approximately 65% of nosocomial infections. Diseases such as inflammation of the internal wall of the heart, periodontitis, cystic fibrosis are some of the diseases caused by these microorganisms. The biofilm matrix reduces the penetration of antimicrobial agents (Szafranski et al., 2017). Besides, 2% of the plastic surgery operations performed in the USA require replacement of the prosthesis due to biofilm infections. In addition, these microbial communities are naturally more resistant to antibiotics and other forms of antimicrobial therapy. This causes recurrent infections in clinical terms. Orthopedic prostheses, catheters, contact lenses, intrauterine devices, ophthalmic implants, vascular stents, and mechanical heart valves can be listed among the examples of medical materials that cause biofilm infections (Costerton et al., 1999). Fungal biofilms are an important health problem

that causes about 80% of hospital infections however, infections caused by them are complicated.

Fungi are increasingly recognized as able to adopt a biofilm phenotype both on live and abiotic surfaces. Much of the work in fungal biofilm research has focused on *Candida* species involved in indwelling medical device infection. Although vast ranges of fungal species are isolated from CRS patients, *Candida* species are rarely seen. *Aspergillus fumigatus*, however, is a frequent sinonasal pathogen and is known to form biofilms on bronchial epithelium (Fanning and Mitchell, 2012).

### 1.2. Fungal biofilm formation

It has been determined that the structure of the biofilm is specific to the species in pure cultures and to the substrate in multiple cultures. It is known that the structure of heterogeneous biofilms is mostly irregular. Microorganisms in biofilms have been proven to be resistant to environmental conditions such as pH, temperature, pollutants, hydraulic shock, antibiotics and toxic substances (Dimakopoulou-Papazoglou et al., 2016).

Biofilm formation has been identified in prokaryotic microorganisms such as Gram-negative *Pseudomonas aeruginosa* Schröter and eukaryotic fungi such as *S. cerevisiae* (Desm.) Meyen, *C. albicans*, and *C. glabrata* (H.W. Anderson) S.A. Mey. & Yarrow. There are serious differences between their fungal biofilm-forming forms and free-living forms, and different opinions are announced about the situations that require bacteria to form biofilms. When we compile these studies, we can list the causes of biofilm formation as follows. These are defense, adhesion, and colonization, the formation of a viable environmental and community building. Facilitating the storage of foods and removal of waste are among other advantages of biofilm formation. Because the yeasts are found in clusters and in the extracellular matrix, they become difficult to phagocyte and the humoral immune system components are prevented from reaching the yeasts. The distinctive feature of *C. albicans* biofilms is that different morphological forms coexist. *C. albicans* biofilm formation occurs in 3 stages: Early phase (0-11 hours), intermediate phase (12-30 hours), and mature phase (38-72 hours) (Fleming and Rumbaugh, 2017; Kumamoto, 2005).

### 1.3. The biofilm formation mechanism

It is very common to find microbial biofilms in the environment and even on fomite surfaces with other commensal species. Bacteria and fungi can both form biofilms on surfaces, but some factors that arise during their formation are different (D'Acunto et al., 2015). A better understanding of the structure and mechanisms of biofilms will reveal potential treatment goals. Biofilm formation can be defined as a 4-step process:

**Attachment:** It is the first phase in which planktonic-structure microorganism is reversibly attached to the surface. The bacteria attach to the surface in a short time as a result of the flagella movement and the electrostatic and physical interactions (Brownian motion) it has with the surface it is attached to. In yeast, attachment is directly related to the surface. Attachment molecules that are different from bacteria are divided into two groups as nonspecific and specific. Their molecules are the surface

hydrophobic molecules and the receptor-ligand level including Als 1, Als 3, Ace 2, Bcr 1, Hwp 1, Sun 41, Eap 1, Mnt 1, Mnt 2, Och 1, Pmr 1, respectively. The hydrophobicity of the surface also positively affects biofilm formation. Yeast cells line up in the basal layer and hyphae elements in the ESM (exopolysaccharide matrix) line up on this layer in a biphasic structure on the hydrophobic surfaces of silicone elastomer or PVC discs. However, on irregular or rough 'polymethylmethacrylate' surfaces, it has been observed that mature biofilm consists only of yeast cells and ESM (Chandra et al., 2001).

**Irreversible Attachment:** The irreversible attachment in mushrooms is mostly explained by studies in medicine. The first thing to do after the implantation of medical devices is the formation of a "preparatory film" by various macromolecules in different body fluids surrounding the medical device such as saliva, mucus, serum, or blood through accumulation on the surface. Microorganisms usually adhere to this film layer, not to the surface of the bare device. The first adhesion is in a reversible loose attachment style. This can turn into a tight adhesion with exopolymer production (Pascual, 2002).

**Colonization and Microcolony Formation:** Bacteria cells attached to the surface divide and multiply to form microcolonies. Quorum sensing (QS) is a mechanism of microbial communication dependent on cell density that can regulate several behaviors in bacteria such as secretion of virulence factors, biofilm formation, competence, and bioluminescence (Albuquerque and Casadevall, 2012). The bacterial population grows gradually with the newly formed microcolonies. The Quorum Sensing (QS) signal system is activated, which allows the bacteria to detect the population density around it. This stage is also the stage in which the biofilm phenotype begins to emerge, which may result in the microorganism gaining a resistant structure (D'Acunto et al., 2015). The existence of fungal QS systems was revealed ten years ago after the discovery that farnesol controls filamentation in the pathogenic polymorphic fungus *C. albicans*. In the past decade, farnesol has been shown to play multiple roles in *C. albicans* physiology as a signaling molecule and inducing detrimental effects on host cells and other microbes. In *S. cerevisiae*, two other aromatic alcohols, phenylethanol and tryptophol were found to be QSMs regulating morphogenesis during nitrogen starvation conditions (Albuquerque and Casadevall, 2012).

In fungi, exopolymers form the layer called glycocalyx (slime) by wrapping the film layer formed by macromolecules. Microorganisms multiply in this slime layer, resulting in a thick film layer. It is an important condition for nosocomial fungal infections that *Candida* species both play a role as a continuous focus of infection and get rid of the body's defense mechanisms and the effect of antifungal therapy as a result of adherence to medical devices through slime factor (Pittet et al., 1997).

**Rupture:** After biofilm formation and maturation, flagella or hyphae are synthesized as a result of genetic arrangements in bacteria or fungus cells in the biofilm. In this way, the activated microorganism cells are ruptured from the top layer of the biofilm. The ruptured planktonic cells migrate to new foci. This can lead to the spread of the biofilm and sometimes the transition of local infections to the systemic state (Kumamoto, 2011).

### 1.3.1. Fungal quorum sensing

The QS mechanism in eukaryotic microorganisms was not known until the discovery of the chemical called farnesol. The QS studies in fungi are much newer than bacteria. The fact that the cell morphology of *C. albicans*, a human pathogen, was controlled by "farnesol" was identified in 2001 (Hornby et al., 2004). The molecule called farnesol has been shown to prevent germ tube formation in the presence of three different triggers (L-proline, N-acetylglucosamine, swine, and bovine serums). It has been determined that this extracellular molecule is highly thermostable, produced in amounts proportional to the number of yeast cells in the range of 23-43 °C, is effective on different *C. albicans* origins, and is not affected by the composition and structure of the medium. In a study in which more than forty farnesol analogs were tested, it was shown that the QS activity was highly related to the structure of the molecule, and this finding was due to a specific communication with a farnesol receptor (Shchepin et al., 2003). The same researchers demonstrated that the QS molecules were taken into the cell as a result of the examinations with labeled probes displaying QS activity (Shchepin et al., 2005).

The biofilm structure of *C. albicans* consists of basal yeast layer, a dense hyphae structure, and an extracellular matrix. Considering the concentration of farnesol, it is thought that farnesol can prevent biofilm formation. When the farnesol concentration reaches 300µM/l, biofilm formation is completely prevented (Shchepin et al., 2005). Since the discovery of farnesol, different QS molecules have been identified in different fungal species. Examples of other QS molecules are peptide-1 in *Cr. neoformans*, oxilipines found in *A. nidulans*, and tyrosol inducing filamentation in *C. albicans*. In *S. cerevisiae*, phenylethanol and triptofol are aromatic alcohols that stimulate pseudo-hyphal growth at low concentrations. These compounds are also produced by *C. albicans* (Shchepin et al., 2003).

Farnesol does not affect germ tube formation when added to *A. nidulans* (Eidam) G.Winter cultures; however, it triggers apoptosis in these species. Farnesol-induced apoptosis is associated with mitochondrial function. When *A. nidulans* and *C. albicans* were cultured together on the same medium, *A. nidulans* growth was prevented (Ramage et al., 2011). As a result, it suggests that farnesol may reduce competition with other microorganisms.

Unlike *A. nidulans*, farnesol does not affect the growth of *A. niger* Tiegh., but it causes significant changes in its morphology. At farnesol concentrations higher than 10 mM, the conidiation is completely suppressed and a 10-fold reduction takes place in the intracellular level of cAMP (Semighini et al., 2006).

It is imperative that *C. albicans*, which is in yeast form in the gastrointestinal lumen, form a germ tube to show pathogenicity through invasion. Farnesol's ability to prevent this transformation suggests that it can be used as a new class antifungal, especially in immunosuppressed individuals. However, contrary to this *in vitro* effect, it is also stated that accumulation of lipophilic farnesol under *in vivo* conditions changes the intestinal membrane permeability and decreases protein kinase C activity by reducing the production of diacylglycerol, and paves the

way for the entry of yeast cells, which have accumulated in a small area in large numbers, to the systemic circulation, leading to apoptosis (Hornby et al., 2004).

*Candida albicans* is also protected from oxidative stresses such as hydrogen peroxide and superoxide anion produced by some bacteria such as *Enterococcus faecalis* and phagocytes of the host, where it is cultured together. When *C. albicans* culture supernatants in the stagnation phase were added to *C. albicans* cultures in the logarithmic reproduction period, it was shown that they continued living under oxidative stress, under which they normally died, and this resistance occurred due to antioxidant molecules resulting from increased in CAT1, SOD1, SOD2 and SOD4 gene expression. In the same study, it was found that alphatocopherol, another molecule of QS, did not produce such a protective effect and the effect was due to farnesol (Westwater et al., 2005).

Farnesol was tested on the crepe, crater, concentric, and flat colony variants of *C. parapsilosis*, and it was observed that those except the flat type colony variant prevent biofilm formation. Besides, farnesol has been shown to reduce the reproduction and development and trigger apoptosis of *A. nidulans*, which does not produce a measurable amount of QS molecules, although it does not affect its morphogenesis (Laffey and Butler, 2005). From this point of view, it can be said that *C. albicans* uses QS molecules to gain an advantage against other microorganisms that it coexists with. A similar inhibitory effect was observed on *A. fumigatus* and *Fusarium graminearum* Schwabe (Semighini et al., 2006). It was shown that another dimorphic fungus, *Ceratocystis ulmi* (Buisman) C. Moreau, also had a QS molecule with similar effects, which was not effective on *C. albicans* and that this species was not affected by farnesol (Hornby et al., 2004).

Tyrosol is the second molecule of QS discovered after farnesol. Tyrosol also regulates morphogenesis depending on concentration density like farnesol (Semighini et al., 2006). Studies on *C. albicans* have shown that germ tube formation accelerates in cultures less diluted in terms of tyrosol. Based on this result, *C. albicans* morphogenesis is thought to be controlled depending on environmental conditions. Tyrosol induces cell growth and germ tube formation at low cell density. It accelerates the conversion from yeast form to filamentous form. In the presence and absence of tyrosol, the formation of germ tubes in *C. albicans* cells was examined, and in the presence of tyrosol, 55% of the cells at 1 hour and 80% at 2 hours were observed to form germ tubes (Chen et al., 2004).

## 2. Discussion and conclusion

The biofilm formed in the medical tools and materials used can be formed by bacteria and yeast. The presence of fungal biofilms rather than bacterial biofilms is increasingly investigated in clinical studies. The source for these microorganisms can be the patient's own flora, the hands of healthcare professionals, tap water, or environmental surfaces (Douglas, 2002).

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Traditional treatments used in medicine today are not considered clinically sufficient due to the presence of bacteria that are resistant to treatment and the emergence of new resistant/highly resistant strains (biofilm-forming bacteria, yeasts, and mycobacteria). While determining future diagnosis-treatment-follow-up methods, the processes and conditions before the biofilm is formed should be taken into consideration with a proactive approach. As more detailed and specific information about the morphology and physiology of biofilms formed by fungi, which is a factor of chronic infections, is obtained; the results obtained from antifungal developments will show a noticeable improvement.

As an alternative to existing antibiomics in the treatment of microbial diseases, the discovery of inhibitory agents against the QS mechanism in microorganisms is very important. This will once again emphasize the importance of elucidating the QS mechanism. Since antifungal resistance shows species-specific features in fungi, diagnosis at the species level is of great importance. In addition to the presence of resistance at the species level, isolate-specific resistance development can also be observed, so species-level identification studies with yeasts are as important as the prevention of fungal biofilms (Al-Hatmi et al., 2016).

Another important point in fungi is rapid spread. *C. auris* Satoh & Makimura is a new yeast strain that causes invasive infections and is resistant to the existing antifungals. *C. auris* causes serious outbreaks in patients hospitalized in many countries and progresses with high mortality. Its rapid spread is another matter as important as its resistance to antifungals (Baillie and Douglas, 2000). Therefore, it is very important to correctly identify the organism and to treat it properly with the new antifungal approaches to control this pathogen. Because the problems originating from this fungal pathogen cannot be overcome with the existing methods.

Biofilm studies have been continuing with an increasing speed especially in the last three years. While the microorganism alone is not completely defeated in the face of man, the new target is in a different appearance and a stronger community. Combating infections caused by biofilm appears to be very difficult, as it is directly related to tissue damage during application. Reliable sampling and measurement techniques must be developed to control biofilm formation.

As a result, after the literature review; we think that increasing the number of studies on biofilm is important, especially in vivo studies should become widespread, analysis of genes and gene products associated with biofilms, i.e. proteomics studies, should continue increasing, and that a “problem-target-solution” approach should be adopted in overcoming the problems caused by fungal biofilms by researching new molecules that prevent biofilm formation.

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**Alkayış, M. Fatih (2019). Türkiye Türkçesinde Bitki Adları  
Hiperyayın, ISBN 978-605-281-343-0, 432 pages**

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**Abstract:** Plants have been used by mankind in many areas from past to present. In this respect, plants are indispensable for human beings, named by each nation in different ways and these nomenclatures have often been the subject of examination for linguists. The book “Türkiye Türkçesinde Bitki Adları” prepared by Alkayış is one of those investigations. In this study, the book is introduced and some evaluations are made on the book.

**Key words:** Plant names in Turkish, Turkish plant names, vernacular plant names

**Özet:** Bitkiler, geçmişten günümüze insanoğlu tarafından birçok alanda kullanılmıştır. Bu bakımdan insanoğlu için vazgeçilmez bir öneme sahip olan bitkiler, her millet tarafından farklı şekillerde adlandırılmış ve bu adlandırılmalar, dilciler için çoğu zaman bir inceleme konusu olmuştur. Alkayış tarafından hazırlanan “Türkiye Türkçesinde Bitki Adları” isimli kitap da bu incelemelerden biridir. Bu çalışmada söz konusu kitap tanıtılmış ve kitap üzerine bazı değerlendirmelerde bulunulmuştur.

**Anahtar Kelimeler:** Türkçede bitki adları, Türkçe bitki adları, yerel bitki adları

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## 1. Introduction

Plants have been used by mankind in many areas from past to present. They are an indispensable asset for human beings in fields such as medicine, anthropology, pharmacy, literature, art, biology, botany and gastronomy. Sait Faik once said “if one does not know the names of flowers and fishes, he can’t write stories”. In fact, he mentions about the importance of plant and animal names in literature. Likewise, plant names have an important place in classical Turkish literature. Hayali, in the following couplet says, likens figured saplings in the garden to light-faced houris that have come down from the sky thanks to newly opened flowers and flowers shining with dew (Kaya, 2015).

Cemende her nihâli gökden inmiş hûra benzettüm

Çiçekler gördüm anların yüzünde nûra benzettüm Hayâlî  
(Tarlan, 1992).

Various ways of plants naming that have such an importance in society have been followed. Plants have been named in different ways according to the cultural background of the societies as well as their appearance, usage or their habitats. Turkish language has many plant names produced in this way. For example, *Antirrhinum* plant whose flowers resemble the mouth of a lion is called as snapdragon. Similarly, plants such as mountain poplar, garden terraces, island bulbs, Cretan tulips are named in this way due to the geography where they grow. Naming plants in Turkish is very important for the vocabulary of Turkish Language. To find Turkish equivalents or reproduce new names to plant taxa which are of Latin or latinized origin and use these plant names in Turkish forms enriches the vocabulary of the Turkish language. Therefore, different studies have been conducted regarding plant names. “Türkiye Türkçesinde Bitki Adları” (2019) published by Alkayış, is one of the most important studies on plant names

in Turkish language. The author states the aim of his work as the determination of the modes of plant naming and to reveal the richness of Turkish in terms of plants by a structural and conceptual investigation of the plant names in Turkish.

In this study, the book titled “Türkiye Türkçesinde Bitki Adları” prepared by Alkayış is introduced and some evaluations are made on the book.

## 2. The book

Türkiye Türkçesinde Bitki Adları (Plant Names in Turkish)

The book was published by Hyper Publications in İstanbul in 2019. The study, except for the Kısaltmalar (abbreviations), Simgeler (symbols) and Önsöz (preface), consists of six parts: Giriş (Introduction), İnceleme (Review), Bitki Adlarında Geçen Yapım Ekleri (derivational suffixes in plant names), Türkiye Türkçesi Bitki Adları Sözlüğü (dictionary of Turkish plant names), Sonuç (conclusion) and Bibliyografya (bibliography).

The abbreviations (Kısaltmalar) section includes the abbreviations of publication names, language codes, and some other abbreviations.

In the preface, the author first emphasizes the importance of plants, then gives some information about his work. In addition, the author reserves a paragraph of appreciation to the people who helped when creating his work.

Introduction is the first part of the study and it is a transition to the review section. In this chapter the author, referring to the importance of plants in human life in the first paragraph, says, “nature takes its naturalness, beauty and vitality from plants. It is actually plants that bring nature into poetry, painting and various art branches”; in this way he emphasizes the importance of plants in nature. From



sixteenth page, the author provides information on ways to name plants, and he supports this with information giving examples in Turkish, German, English etc. The author later mentions that plant names are important not only for linguists, but also for anthropologists, pharmacists, ecologists and ethnobotanists. This, of course, increases the value of the study even more. Lastly the author gives information about the methods he used while preparing the study.

The review part is the second part of the study and it is one of the main parts. This section consists of three titles: “Basit Yapılı Bitki Adları (the simple structured plant names)”, “Türemiş Bitki Adları (derivative plant names)” and “Birleşik Bitki Adları (compound plant names)”.

Basit Yapılı Bitki Adları section is the first title of the review section. This section was handled under two subtitles as “Türkçe Kökenli Bitki Adları (Turkish originated plant names)” and “Alıntı Kökenli Bitki Adları” (derivative plant names). Alkayış states that Turkish originated plant names do not occupy much place in the study, only 46 samples such as “ağu”, “çöpür”, “erik”, “enek”, in this structure, could be detected. The author moves to the derivative plant names section, he states that excerpts of plant names used in Turkish came from the Arabic Persian, Armenian, Latin, Greek, French, English, Italian, Spanish, Bulgarian, Chinese, Georgian, Serbian, Slavic, Mongolian, Russian, Portuguese and Polish languages and then he gives the examples of plant names derived from these languages under the same title. Some examples are as follow:

**bezir** (<Ar. *bezr*)  
**gül** (<Far. *gul*)  
**kiraz** (<Yun. *Kerasi*)  
**feliks** (Lat. *felix*)  
**funda** (İt. *fonda*)

“Türemiş Bitki Adları” section is the second title of the review section. Under this heading, Alkayış identifies the origins of derivative words and gives examples. For the origins of the words that could not be determined in the examples he uses “?” sign. In addition, Alkayış separates the existing words one by one in this section. Some examples are as follow:

**alça** (< T. *al+ça*)  
**şarlık** (< Ar. *şa'r + T. lik*)  
**tüllü** (Fr. *tulle + T. lü*)  
**söbelek** (? *söbe + T. lek*)

“Birleşik Bitki Adları” section is the third and last title of the review section. This section, occupies the widest place in the review section, and is handled under two subtitles as “Yapı Bakımından Birleşik Bitki Adlarının Kuruluşu (structural formation of compound plant names)” and “Kavram Bakımından Birleşik Bitki Adlarının Kuruluşu (conceptual formation of compound plant names)”. Under the title of “Yapı Bakımından Birleşik Bitki Adlarının Kuruluşu”, Alkayış classifies what kind of structures the plant names consist of. These structures are handled in six different ways as noun phrase, adjective phrase, adjective-verb group form, repeat group form, adjective group form and sentence form. Alkayış has subclassified these six forms among themselves too. Conceptually, “Kavram Bakımından Birleşik Bitki Adlarının Kuruluşu” section is handled in seven different ways, as plant names taken from human characteristics, animals, organs, goods and objects,

analogies, species, and geographical places. Alkayış later subclassifies these forms among themselves too.

“Bitki Adlarında Geçen Yapım Ekleri (derivation suffixes in plant names)” section constitutes the third part of the study. In this section, derivational suffixes are examined under four subtitles; noun noun, verb noun, verb verb and verb noun derivational suffixes. In this section, besides one Mongolian, one Persian, one Arabic and 31 Turkish plant names, there are derivation suffixes of 34 nouns from nouns, three verbs from nouns, seven verbs from verbs and 37 nouns from verbs.

“Türkiye Türkçesinde Bitki Adları Sözlüğü (dictionary of plant names in Turkish)” section constitutes the fourth part of the study. This part, which constitutes the most comprehensive part of the study, includes the pages from 147 to 425. In this section the author lists the plant names used in Turkish and their dialects within the framework of lexicography rules and creates a dictionary. The meaning of each word given as per item is given here. However, for words that are differently expressed in different sources, meaning is not given separately, only one meaning that is considered to be common is given. The origin of each headword, which structure the words consist of, from which sources the words are taken is indicated in parentheses. Below are some examples from the “Türkiye Türkçesinde Bitki Adları Sözlüğü” section.

**ak arpa:** (< T. *ak + arpa*) Bir çeşit beyaz arpa. (DS, I, 140; DS, XII, 4410)

**akbacak:** (T. *ak + OFar. pāçak*) Çiğ olarak yenilen bir ot. (DS, I, 141)

**bostan:** (< Far. *būstān*) Hıyar, salatalık, karpuz (*bostan borusu*) (DS, II, 742; DS, XII, 4463; AAT, 207; Erz. İ.A., III, 48)

**eşek papatyası:** (T. *eşek + Yun. papadia + T. sı*) bk. Beyaz papatya. (TSBAS, 103)

**kabağaç** (T. *kaba + ağaç*) (And. Ağz.: *kabaaç, kabaç, kabağaç*) Kalın gövdeli meşe ağacı. (DS, VIII, 2577)

**mekrikarmudu:** (? *mekrik + Far. emrūd*) Sarı renkli, küçük yaz armudu. (DS, IX, 3153)

**saat çiçeği:** (Ar. *sā'at + T. çiçeği*) Bir tür çiçek. (TS, 2, 1874)

**şifi:** (?) Fidan. (DS, X, 3776; DS, XII, 4726)

**zülbeya** (Ar. *zūlbeyā* “*beyā* dolmuş, dolu”), (And. Ağz.: *zülee*) Geç olgunlaşan kara ve küçük bir erik çeşidi. (DS, XI, 4401, 4402)

“Sonuç” (conclusion) section is the fifth part of the study. In this part, Alkayış discussed the plant names in Turkish in terms of structure and concept, and showed that Turkish has a rich plant culture.

The bibliography section is the sixth and final part of the study. In this section, the author lines up the sources he refers to in alphabetical order while creating his work.

This book titled “Türkiye Türkçesinde Bitki Adları” is a valuable study and was prepared to reveal the richness of vocabulary of Turkish language on plant names. It is obvious that not only linguists, but also pharmacists,

ecologists, ethnobotanists and many other scientists will benefit from this study. For such a valuable study, I would

like to thank Assoc. Prof. Dr. Alkayış and wish him success in his future studies.

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