# MEDITERRANEAN AGRICULTURAL SCIENCES

Volume: 34
Number: 3
Year: December 2021

E-ISSN: 2528-9675

e-ISSN 2528-9675

# MEDITERRANEAN AGRICULTURAL SCIENCES

*Previous Name: Akdeniz University Journal of the Faculty of Agriculture*

**The peer reviewed scientific journal of Akdeniz University Faculty of Agriculture**

**Three issues are published per year in April, August and December**

**Abbreviation of the journal: Mediterr Agric Sci**

**Owned on behalf of Akdeniz University, Faculty of Agriculture**

**Prof. Dr. Mustafa ERKAN**
**Dean**

**Publishing Manager**

**Assoc. Dr. Taki KARSLI**

**Administration Address**

Akdeniz University
Faculty of Agriculture
07058 Antalya, Turkey
Tel: +90 242 310 2412

Tel: +90 242 310 2411
Faks: +90 242 227 4564
E-Mail: ziraatdergi@akdeniz.edu.tr
Web site: www.dergipark.org.tr/en/pub/mediterranean

**Publisher**

Akdeniz University, Faculty of Agriculture
07058 Antalya, Turkey

Tel.: +90 242 310 2412
Faks: +90 242 310 2479

**Subscription**

Open access journal

**Online access free of charge**
**www.dergipark.org.tr/en/pub/mediterranean**

**Cover design**
**Assoc. Dr. Süleyman OZDERIN**

## AIM AND SCOPE

MEDITERRANEAN AGRICULTURAL SCIENCES aims to share knowledge at both national and international levels by publishing the results of research in agriculture and life sciences in both Turkish and English. Consequently this journal is a multidisciplinary platform for related scientific areas. The journal primarily publishes original research articles and accepts a limited number of invited reviews in the areas of agricultural biotechnology, agricultural economics, agricultural machinery, animal husbandry, bioenergy, biostatistics and genetics, farm structure and irrigation, field crops, food science and technology, horticulture, landscape and nature conservation, natural resources, plant protection, soil science and plant nutrition.

## ABSTRACTS AND INDEXING

MEDITERRANEAN AGRICULTURAL SCIENCES is indexed and abstracted in **CABI** data bases (**CAB Direct**), **TUBITAK-ULAKBIM** (National Data Bases-Data Base of Life Sciences), **CLARIVATE ANALYTICS, SCIENCE MASTER JOURNAL LIST** (Zoological Records) and **DRJI** (Directory of Research Journals Indexing).

e-ISSN 2528-9675

**www.dergipark.org.tr/en/pub/mediterranean**

# MEDITERRANEAN AGRICULTURAL SCIENCES

The journal was published under the name “Akdeniz University Journal of the Faculty of Agriculture” and with the number ISSN 1301-2215 until 2016.

Volume: **34** Number: **3** Year: December **2021**

e-ISSN 2528-9675

# MEDITERRANEAN AGRICULTURAL SCIENCES

Volume: **34** Number: **3** Year: December **2021**

## Contents

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 261-266
DOI: 10.29136/mediterranean.971682

# An alternative new casing material in the production of *Agaricus bisporus*

**Ersin POLAT, Omer ONEL**

Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

Corresponding author: E. Polat, e-mail: polat@akdeniz.edu.tr
Author(s) e-mail: onelomer@icloud.com

ARTICLE INFO

Received: July 16, 2021
Received in revised form: October 4, 2021
Accepted: October 6, 2021

***Keywords*:**

Patent
Mushroom
Perlite
Vermicompost
Yield

**ABSTRACT**

The study was carried out to investigate the effects of using perlite together with vermicompost on yield and quality of white button mushroom. The research was conducted in the climate-controlled mushroom growing room, located in the faculty of agriculture of Akdeniz University. Peat (Control-1) and perlite (Control-2) used as casing soil, liquid vermicompost 1 (L1) (7.6 ml $m^{-2}$), liquid vermicompost 2 (L2) (15.2 ml $m^{-2}$), liquid vermicompost 3 (L3) (22.8 ml $m^{-2}$), solid vermicompost 1 (S1) (366 g $m^{-2}$), solid vermicompost 2 (S2) (732 g $m^{-2}$), solid vermicompost 3 (S3) (1098 g $m^{-2}$), liquid+solid vermicompost 1 (LS1) (3.8 ml $m^{-2}$ + 183 g $m^{-2}$), liquid+solid vermicompost 2 (LS2) (7.6 ml $m^{-2}$ + 366 g $m^{-2}$), and liquid+solid vermicompost 3 (LS3) (11.4 ml $m^{-2}$ + 549 g $m^{-2}$) were applied into perlite as casing material. Parameters such as total mushroom yield, marketable mushroom yield, first two flushes of total yield of mushroom: 1st flush mushroom yield, 2nd flush mushroom yield, 3rd flush mushroom yield, earliness, average fruit weight, dry matter content, L, a* and b* color value were examined. As a result of the research, the highest total yield was obtained from S2 application with a value of 34.85 kg 100 $kg^{-1}$ compost compared to Control-1. It was determined that liquid, solid and liquid+solid forms of applications mixed with perlite have the most positive effects on mushroom yield, yield was increased and approved an alternative potential to traditional casing soil. Due to the positive results obtained from the research, the results of the research were recorded in a register book on 26/06/2019 and 2019/09514, through an application made to the Turkish Patent Institute.

## 1. Introduction

The production amount of cultivated mushroom is 10242541 tons across the world (FAO 2019) and the *Agaricus bisporus* constitutes a big rate of this. The production rate of this was 49364 tons in Turkey (TUIK 2019). On the other hand, white button mushroom takes the first place in the cultivation of mushroom in our country as in many countries. Casing soil necessary for stimulation of primordia and then sporophore formation of A. bisporus (Noble et al. 2003). Commercially, casing soil is an absolute and necessary substance in the farming of button mushroom. The casing soil typically consists of black peat brought to the right pH with calcium carbonate. It has been postulated that the microflora of the casing layer is necessary for fruiting body formation because it consumes a metabolite of the mushroom mycelium that is inhibitory to mushroom formation (Visscher 1988; Noble et al. 1999). For high and quality yield the preferred casing soil contains properties such as high-water holding capacity, rich in organic material, low salt level, ease of gas exchange, soil crust layer will not be formed, and having pH 7.5 (Eren and Boztok 2013).

In general, the most common casing soil used in the cultivation of mushroom is peat. However, the reduction in peat resources, low quality of the existing peat beds, high price and transport cost and the demand for increase of mushroom cultivation made it crucial to search some alternative materials to replace peat and various researches have been done on this subject (Boztok 1984; Visscher 1988; Price 1991; Erkel 1992; Nair 1997; Baysal 1999; Demirer and Özer 2000; Gülser and Pekşen 2003; Çolak 2004; Kim et al. 2018; Kerketta et al. 2019).

In addition, there are a few scientific studies suggesting that it is recommended to mix different materials, as the peat adds amendment elements to the main material, so that the new mixture can give good results (Eren and Boztok 2013; Kim et al. 2018; Kerketta et al. 2019). Although, scientific and practical studies advise the quality of this, particularly in our country no alternative has been offered to the casing material which is traditionally used in the casing soil. There are also graduate theses related to this. Casing soil is an absolutely necessary material in the cultivation of mushroom (Shandiya 1989; Sharma et al. 1996; Sharma et al. 1999; Pardo et al. 2003). The studies conducted have always been important, but the recommended procedure and mixture have not permanently solved the problems arising from the casing soil resources in our country. Perlite was previously tested as casing material. Pure and mixed perlite were used as casing soil and successful results have not been achieved (Eren 2008; Eren and Boztok 2013; Çetin and Eren 2017). The production amount of perlite was 5690027600 tons according to 2021 data. It constitutes 74% of the world perlite reservation (MAPEG 2021). Organic wastes can be broken down and fragmented rapidly by earthworms, resulting in a stable nontoxic material with good structure which has a potentially high economic value as soil conditioner for plant growth. Vermicompost is a finely divided peat like material with

excellent structure porosity, aeration, drainage and moisture holding capacity (Dominguez et al. 1997).

In this study, perlite was used as main casing material which may be an alternative to casing soil. Due to the positive results obtained from the research, the results of the research were recorded in a register book on 26/06/2019 and 2019/09514, through an application made to the Turkish Patent Institute.

## 2. Materials and methods

### 2.1. Material

The research was carried out at Akdeniz University Faculty of Agriculture (36° 53' 55.60" North latitude, 30° 38' 18.20" East longitude) in a climate-controlled mushroom production room. Perlite was used as the main material for casing soil in the study. Perlite is an acidic rock of volcanic origin which is obtained by breaking it to millimeter particles and subjecting to heat treatment between 800-1000°C. Perlite is light, sterile, aeration, high water holding capacity and high nutrient absorption capacity, inert, and neutral pH substrate. In addition to perlite, liquid and solid vermicompost as supplements were used in the study.

### 2.2. Methods

Compost was obtained from by a private compost production company in Antalya. Bags of 5 liter, having the size of 30x50 cm and about 2 kg weight, were filled. The bags were prepared based on random block design with 4 replications and each replication had 4 bags and total number was 44 bags. The growing room where research was carried out possessed moisture and heat insulation system, where the room had heating-cooling, ventilation, circulation, humidification and irrigation system infrastructure. The desired climate of the room was fully provided and was automatically controlled.

In order to complete the pre-development period of the mycelium, the temperature of the compost inside the bags was 25±1ºC and the relative humidity of the room was kept 90% approximately for 14 days. Also, the internal temperature of the compost was monitored with a soil thermometer. The mycelium pre-development compost containing bags were covered about 4 cm height with alternative casing material, which include doses and form of vermicompost. In the research, commercial peat was used as a control casing soil. Perlite was used as an alternative casing material. However, the liquid, solid and liquid+solid forms of vermicompost in different doses were treated. During the creating of doses of vermicompost, recommended application doses in vegetables were taken as a reference from square of meter. The applied doses were created with study that we have already done. Accordingly, perlite as a substrate, three different forms and three doses of vermicompost into casing material and casing material were tested. Thus, the applications made; liquid vermicompost 1 (L1) 7.6 ml $m^{-2}$, liquid vermicompost 2 (L2) 15.2 ml $m^{-2}$, liquid vermicompost 3 (L3) 22.8 ml $m^{-2}$, solid vermicompost 1 (S1) 366 g $m^{-2}$, solid vermicompost 2 (S2) 732 g $m^{-2}$, solid vermicompost 3 (S3) 1098 g $m^{-2}$, liquid + solid vermicompost 1 (LS1) 3.8 ml $m^{-2}$ + 183 g $m^{-2}$, liquid + solid vermicompost 2 (LS2) 7.6 ml $m^{-2}$ + 366 g $m^{-2}$, liquid + solid vermicompost 3 (LS3) 11.4 ml $m^{-2}$ + 549 g $m^{-2}$, traditional casing soil (Contro1-1) and pure perlite (Control-2).

Total mushroom yield, marketable mushroom yield, first two flushes of total yield of mushroom, first two flushes of marketable mushroom yield, average fruit weight parameters were determined. Yields for each flush and total yield (three flushes) for each treatment were expressed as kg 100 kg-1 compost. Additionally, the harvested mushrooms were examined for abnormalities in size and weight for marketable yield (%). Mushrooms were harvested, counted and weighed daily. At the end of each flush in the growing period, weight (g) of mushrooms with diameter of 3-5 cm per bag were determined. The yield of 2 kg of compost mushroom was determined by weighing it in "g" with a precision scale with a sensitivity of 0.01 g, and the values were calculated over 100 kg of compost. Earliness was determined by calculating the time until the first harvest in days. The dry matter content of mushrooms was taken from 2nd flush and kept in the oven at 70°C for 3 days and later the dry matter was weighted and determined by % calculation. L, a * and b * color values were measured by Minolta CR400 color chromometer.

## 3. Results

Total yield, marketable yield and two first flushes total yield is mentioned in Table 1. Also, pictures from study are given in Figures 1. Considering the total mushroom yield, the highest yield was obtained from solid vermicompost 1 (S1), solid vermicompost 2 (S2), and liquid+solid vermicompost 3 (LS3), in which the yield was 35.35 kg 100 $kg^{-1}$ compost, 35.69 kg 100 $kg^{-1}$ compost, and 34.52 kg 100 $kg^{-1}$ compost, respectively. The lowest value was recorded as 22.88 kg 100 $kg^{-1}$ compost and 22.73 kg 100 $kg^{-1}$ compost from liquid vermicompost 1 (L1) and control 2 (perlite), respectively.

**Table 1.** Effects of different casing materials on button mushroom yield

| Casing materials | Total yield (kg 100 $kg^{-1}$ compost) | Marketable yield (%) | First two flush total yield(kg 100 $kg^{-1}$ compost) |
|---|---|---|---|
| L1 | 22.88 c | 100 a | 16.98 d |
| L2 | 31.45 ab | 100 a | 25.66 c |
| L3 | 28.63 b | 100 a | 24.03 c |
| S1 | 35.35 a | 100 a | 30.32 ab |
| S2 | 35.69 a | 96 a | 31.22 a |
| S3 | 29.05 b | 99.61 a | 26.35 bc |
| LS1 | 32.93 ab | 100 a | 28.09 abc |
| LS2 | 31.18 ab | 100 a | 25.88 bc |
| LS3 | 34.52 a | 100 a | 26.72 abc |
| Control 1 | 33.16 ab | 62.92 b | 31.10 a |
| Control 2 | 22.73 c | 100 a | 16.45 d |
| $LSD_{(\%5)}$ | 5.16 | 11.46 | 4.61 |

Different letters in each column shows significantly differences at $P \leq 0.05$.

**Figure 1.** Alternative casing material: from left to right, top to bottom, Control-1; Control-2; L1; L2; L3; S1; S2; S3; LS1; LS2; LS3.

Apart from S2, S3 and control-1, the loss of marketable mushroom yield was not observed in all application, thus the data were recorded as 100%. The marketable mushroom yield was calculated for S2, S3 and control-1 as 96%, 99.61% and 62.92%, respectively. In the first two flushes of total yield, the highest values were obtained from S2 (31.22 kg 100 $kg^{-1}$ compost) and control-1 (31.10 kg 100 $kg^{-1}$ compost). However, the lowest values were obtained from L1 (16.98 kg 100 $kg^{-1}$ compost) and control-2 (16.45 kg 100 $kg^{-1}$ compost) applications. The 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ flush yield of mushroom (kg 100 $kg^{-1}$ compost) were given in Table 2. Considering the mushroom yield in the first flush, the highest values were obtained from S3 (22.03 kg 100 $kg^{-1}$ compost) and control-1 (21.66 kg 100 $kg^{-1}$ compost) applications. While the lowest values were obtained from L1 (8.35 kg 100 $kg^{-1}$ compost) and control-2 (9.55 kg 100 $kg^{-1}$ compost) applications. Considering the 2$^{nd}$ flush of mushroom yield, the highest values were achieved with L2 (12.52 kg 100 $kg^{-1}$ compost) and L3 (12.23 kg 100 $kg^{-1}$ compost) applications. While the lowest value was achieved with S3 (4.33 kg 100 $kg^{-1}$ compost) application. In the 3$^{rd}$ flush of mushroom yield, the highest value was observed in LS3 (7.80 kg 100 $kg^{-1}$ compost) application, while the lowest values were observed in S3 (2.69 kg 100 $kg^{-1}$ compost) and control-1 (2.82 kg 100 $kg^{-1}$ compost) applications.

Considering yield (g 2 $kg^{-1}$ compost), earliness (days) and average fruit weight (g $number^{-1}$) were shown in Table 3.

**Table 2.** Effects of different casing materials on yield of *A. bisporus* according to flushes

| Casing materials | 1st flush yield (kg 100 kg$^{-1}$ compost) | 2nd flush yield (kg 100 kg$^{-1}$ compost) | 3rd flush yield (kg 100 kg$^{-1}$ compost) |
|---|---|---|---|
| L1 | 8.35 e | 8.64 ab | 5.90 ab |
| L2 | 13.14 cde | 12.52 a | 5.79 ab |
| L3 | 11.8 de | 12.23 a | 4.59 ab |
| S1 | 20.15 ab | 10.17 ab | 5.04 ab |
| S2 | 20.86 ab | 10.37 ab | 4.47 ab |
| S3 | 22.03 a | 4.33 c | 2.69 b |
| LS1 | 18.83 ab | 9.26 ab | 4.85 ab |
| LS2 | 18.03 abc | 7.85 bc | 5.31 ab |
| LS3 | 16.36 bcd | 8.87 ab | 7.80 a |
| Control-1 | 21.66 a | 9.44 ab | 2.82 b |
| Control-2 | 9.55 e | 6.91 bc | 6.27 ab |
| LSD (%5) | 4.9 | 4.17 | ns |

Different letters in each column shows significantly differences at $P \leq 0.05$.

**Table 3.** Effect of different casing materials on yield, earliness and average fruit weight of *A. bisporus*

| Casing materials | Yield (g 2 kg$^{-1}$ compost) | Earliness (Days) | MushroomWeight (g fruit body$^{-1}$) |
|---|---|---|---|
| L1 | 457.62 c | 41 ab | 13.59 ab |
| L2 | 628.87 ab | 40.25 bc | 13.37 abc |
| L3 | 572.44 bc | 42 a | 14.22 ab |
| S1 | 707.03 ab | 38.5 d | 14.89 a |
| S2 | 713.71 a | 38.25 d | 14.46 ab |
| S3 | 580.96 abc | 38.75 d | 11.46 cd |
| LS1 | 658.60 ab | 39.25 cd | 13.39 abc |
| LS2 | 623.61 ab | 39.5 cd | 12.60 bc |
| LS3 | 690.37 ab | 38.75 d | 13.1 abc |
| Control-1 | 663.26 ab | 35.5 e | 10.33 d |
| Control-2 | 454.51 c | 41.75 a | 13.40 abc |
| LSD (%5) | 138.77 | 1.41 | 2 |

Different letters in each column shows significantly differences at $P \leq 0.05$.

Considering the 2 kg mushroom yield, the highest value was obtained from S2 (713.71 g 2 kg$^{-1}$ compost) application. The lowest values were obtained from L1 (457.62 g 2 kg$^{-1}$ compost) and control-2 (454.51 g 2 kg$^{-1}$ compost) applications. In earliness, the earliest harvest was obtained from control-1 (35.5 days) application, while the late harvest was achieved with L3 (42 days) and control-2 (41.75 days) applications. For the average fruit weight, the highest value was obtained with S1(14.89 g number$^{-1}$) and the lowest value was achieved with control-1 (10.33 g number$^{-1}$).

The dry matter contents were mentioned in Table 4. The highest dry matter content was achieved with S3 (7.77%), LS2 (7.76%) and control-2 (7.63%) applications. The lowest dry matter content was achieved with L3 (6.68%) application.

Parameters related to the mushroom color (L, a* and b*) are mentioned in Table 5. In terms of fruit color, the highest L values were obtained from S1 (93.5), S2 (93.24), LS3 (93.43) and control-1 (93.28) applications, while the lowest L values were obtained from L1 (91.29) and L2 (91.26) applications. The highest a* color value was observed in L2 (0.40) application and the lowest a* color value was observed in L3 (-0.10) application. Furthermore, the highest b* color value was recorded in L1 (11.58) application and the lowest value was recorded in L3 (8.38) application.

## 4. Discussion

The casing soil is one of the most important inputs affecting yield and quality in the cultivation of *A. bisporus* (Nair 1997; Pardo et al. 2003; Kerketta et al. 2019). As the conventional casing soil (peat) is not standard and based on the fact that the regions' and peat beds' different physical, chemical and biological properties directly affect the yield and quality of cultivated mushroom. In addition, casing soil (peat) obtained from the beds of lake causes the destruction and degradation of lake's beds (Price 1991; Sharma et al. 1996; Noble et al. 1999). On the other hand, our country is a great source of perlite material. It is a standard material in terms of ingredient and its effects are the same everywhere under optimum condition. To produce white button mushroom with this material, the fluctuations of the yield and quality of the mushroom will be eliminated or minimized.

Conventionally used peat (bed soil) has quite high potential to contain harmful and disease related ingredients. The primary source of *M. perniciosa* on most farms is contaminated casing soil. Generally, symptoms in the first flush indicate contamination of the casing soil. Spores of the pathogen may also survive on the surfaces of buildings, or may be carried in crop debris and, in this way, can contaminate crops. Once the pathogen is established in the crop, the main means of spread is

**Table 4.** The effects of different casing materials on mushroom dry matter content of *A. bisporus*

| Casing materials | Dry matter content (%) |
|---|---|
| L1 | 6.98 bcd |
| L2 | 6.96 bcd |
| L3 | 6.68 d |
| S1 | 6.96 bcd |
| S2 | 7.28 abcd |
| S3 | 7.77 a |
| LS1 | 7.51 ab |
| LS2 | 7.76 a |
| LS3 | 6.78 cd |
| Control-1 | 7.39 abc |
| Control-2 | 7.63 a |
| LSD $_{(\%5)}$ | 0.63 |

Different letters in each column shows significantly differences at $P \leq 0.05$.

**Table 5.** The effects of different casing materials on mushroom color

| Casing materials | Color values | | |
|---|---|---|---|
| | L | a* | b* |
| L1 | 91.29 c | 0.39 ab | 11.58 a |
| L2 | 91.26 c | 0.40 a | 10.43 ab |
| L3 | 92.98 ab | -0.10 c | 8.38 c |
| S1 | 93.5 a | 0.32 abc | 9.42 bc |
| S2 | 93.24 a | 0.015 abc | 8.86 bc |
| S3 | 91.64 bc | 0.16 abc | 9.96 abc |
| LS1 | 92.81 ab | 0.28 abc | 9.90 abc |
| LS2 | 92.21 abc | 0.34 abc | 9.47 bc |
| LS3 | 93.43 a | -0.05 bc | 9.38 bc |
| Control-1 | 93.28 a | 0.22 abc | 9.19 bc |
| Control-2 | 92.78 ab | 0.13 abc | 8.77 bc |
| LSD $_{(\%5)}$ | 1.39 | ns | 1.99 |

Different letters in each column shows significantly differences at $P \leq 0.05$.

by water splash and by excess water running off the beds and bags (Atkins 1961; Fletcher et at. 1989; Karabulut et al. 2007). In recent years, the mushroom industry in Turkey and some other countries has been suffering from an epidemic of wet bubble disease (Gea et at. 1995; Fidan et al. 1998; Ilhan and Tezcan 2000). The presence of phorids must be avoided as the adults are vectors of the dry mould *Verticillium fungicola* (Preuss) Hassebrauk, recently named *Lecanicillium fungicola* (White 1981). The infestation of mushroom flies generally occurs as the compost cools and during the introduction of spawn into the compost (Jess et al. 2007). In addition, one of the most important sources of infestation is casing material (peat and limestone mixture) which is added as a surface layer (3-4 cm deep) on the colonised compost in order to facilitate sporophore formation (Erler et al. 2009). *Trichoderma species* (green mold) is a destructive fungal disease causing epidemics in *A. bisporus* cultivation. To control green mould, it should be good hygiene in mushroom farms and compost facilities. In addition, all phases of compost preparation should be properly performed and casing soil should be disinfected (Aydoğdu et al 2020).

However, perlite with vermicompost used as substrate in new casing material is a material which is sterile, especially very clean and has no risk of disease and harmful in transportation. In addition, a lot of advantages provided by the new casing material in the form of mixture are obtained. First of all, it has a sustainable property of an alternative casing material. Taking of the certificate for organic property is extremely high. In terms of disease and harmfulness, it is a great ingredient to eradicate the use of pesticides under optimum compost and growth chamber conditions. During the harvest of mushroom, the conventional casing soil of mushroom fruit can pollute it due to the influence of mud, which may open way to the decrease of quality of visual appearance of the product. The cleaning process lead the product to its physical damage and poor quality and may lose time due to labor-force. The new alternative casing has not got such problems. There is almost no licensed pesticide in button mushroom. As an alternative, it will bring a solution to compete the pesticide, will be environmentalist and permanent. In terms of human health and food safety, there will be no risk of pesticide residues remained on the fungus and thus the consumer will safely consume it. Due to limitation of pesticides use, the consumption of mushroom will increase. The consumer will get benefit, because of both producer and healthy product food. The problems (damage and degradation of lake beds) arisen from conventionally received casing material from the lake beds, will no longer exist. Since the conventional casing soil is heavy and it increases the cost of its transportation, while the new casing soil is lighter and will reduce shipping cost.

As a result, there are many studies for alternative casing material in the cultivated mushrooms (Gülser and Pekşen 2003; Pardo et al. 2003; Eren and Boztok 2013; Kerketta et al. 2019). However, in this study it was a new approach for casing soil, in which perlite together with vermicompost application were used.

## Acknowledgement

The authors would like to thank Akdeniz University for its support.

## References

Atkins FC (1961) Mushroom Growing Today. Faber and Faber Limited; London, U.K.

Aydoğdu M, Kurbetli İ, Kitapçı A and Sülü G (2020) Aggressiveness of green mould on cultivated mushroom (*Agaricus bisporus*) in Turkey. Journal of Plant Diseases and Protection 127: 695-708.

Baysal E (1999) Utilization possibilities of waste tea leaves in the cultivation of Agaricus bisporus (Lange) Sing. Ph.D. Thesis, Karadeniz Technical University.

Boztok K (1984) Kültür mantarı (*Agaricus bisporus* L. Sing) yetiştiriciliğinde farklı kapaklık materyalin ürüne etkileri üzerinde araştırmalar. Ege Üniversitesi Ziraat Fakültesi Dergisi 21: 133-137.

Çetin M, Eren E (2017) The effect of different volimetric rates of the peat and pumice mixtures on yield and quality of mushroom (*Agaricus bisporus* (L.) Sing). Ege Üniersitesi Ziraat Fakültesi Dergisi 54(2): 207-213.

Çolak M (2004) Temperature profiles of *Agaricus bisporus* in composting stages and effects of different composts formulas and casing materials on yield. African Journal of Biotechnology 3(9): 456-462.

Demirer T, Özer İ (2000) Perlit, pomza, torf ve talaş karışımlarından oluşan örtü toprağının yemeklik mantar (*Agaricus bisporus*)'da verim ve kaliteye etkisi. Türkiye VI. Yemeklik Mantar Kongresi 2000, Bergama, İzmir, s. 265-269.

Dominguez J, Edwards CA Subler S (1997) A comparison of vermicomposting and composting. Biocycle 38(4): 57-59.

Eren E (2008) *Agaricus bisporus* üretiminde farklı örtü materyallerinin kullanılabilme olanakları. Ph.D Thesis, Department of Horticulture, Ege University.

Eren E, Boztok K (2013) Possibility of using different wastes as casing material in *Agaricus bisporus* mushroom cultivation. Iğdır University, Journal of The Institute of Science and Technology 3(1): 9-16.

Erkel İ (1992) Mantar yetiştiriciliğinde değişik örtü materyali karışımlarının kullanılma olanakları. Türkiye II. Tarımda Perlit Sempozyumu, İzmir, s. 134-139.

Erler F, Ersin Polat, Halil Demir, Huseyin Cetin and Tugba Erdemir (2009) Control of the mushroom phorid fly, Megaselia halterata (Wood), with plant extracts. Pest Management Science 65: 144-149.

FAO (2019) Food and Agriculture Organization of the United Nations Agriculture Statistics. http://www.fao.org/faostat/en/#data/QC. Accessed May 29, 2020.

Fidan O, Bora T, Özaktan H, Gümüş M (1998) Kültür mantarı üretim merkezlerinde virus ve ıslak kabarcık hastaklıkları üzerinde araştırmalar. Proceedings of the 8th Turkish Phytopathology Congress, Ankara, Turkey, pp. l31-138.

Fletcher JT, White PT, Gaz RH (1989) Mushrooms: Pest and Disease Control, 2nd edn. Intercept; Andover, U.K.

Gea FJ, Pardo A, Navano MJ, Pardo J (1995) Fungal disease of mushroom culture from Castilla-La Mancha (Spain). Incidence of Verticillium fungicola. in Science and Cultivation of Edible Fungi (G. Elliott, ed.), CRC; Rotterdam, The Netherlands, pp. 643-651.

Gülser C, Pekşen A (2003) Using tea waste as a new casing material in mushroom (*Agaricus bisporus* (L.) Sing.) cultivation. Bioresource Technology 88: 153-156.

Ilhan K, Tezcan H (2000) *Agaricus bisporus* kültür mantarındaki örümcek ağı ve ıslak kabarcık hastalıklarına karşı bazı fungisitlerin etkileti. Proceedings of the 5th Mushroom Congress, Ege University, Bergama School, Bergama, pp. 217-224.

Jess S, Murchie AK and Bingham JFW (2007) Potential sources of sciarid and phorid infestations and implications for centralised phases I and II mushroom compost production. Crop Protection 26: 455-464.

Karabulut OA, Arslan U, Kuruoglu G, Ilhan K (2007) The effect of sodium bicarbonate alone or in combination with reduced dose of prochloraz manganese on the control of wet bubble of *Agaricus bisporus.* Biological Agriculture and Horticulture 25: 55-65.

Kerketta A, Shuka CS, Singh HK (2019) Evaluation of different casing materials for growth and yield of button Mushroom (*Agaricus bisporus* (L.) Sing.). Journal of Pharmacognosy and Phytochemistry 8(4): 207-209.

Kim YG, Lee BJ, Lee SG, Lee BE, 2018. Study on new casing materials of *Agaricus bisporus*. Journal of Mushrooms 16(3): 147-154.

MAPEG (2021) Maden ve Petrol İşleri Genel Müdürlüğü. http://www.mapeg.gov.tr. Accessed May 09, 2020.

Nair NG (1997) Use of spent compost as a casing material. Mushroom News 25(9): 12-22.

Noble R, Dobrov-Pennigton A, Evered CE, Mead A (1999) Properties of peat-based casing soils and their influence on the water relation and growth of the mushroom (A*garicus bisporus*). Plant and Soil 207: 1-13.

Noble R, Fermor TR, Lincoln S, Dobrovin-Pennington A, Evered C, Mead A, Li R (2003) Primordia initiation of mushroom (Agaricus bisporus) strains on axenic casing materials. Mycologia 95(4): 620-629.

Pardo A, de Juan JA, Pardo JE (2003) Characterization of different substrates for possible use as casing in mushroom cultivation. The Journal of Food, Agriculture and Environment 1(1): 107-114.

Price S (1991) The peat alternatives manual, a guide for the professional horticulturist and landscaper, friends of the earth. London, U.K., pp. 40-51.

Shandiya TR (1989) Mushroom compost and casing research in India. Mushroom Science 12(1): 743-752.

Sharma HSS, McCall D, Lyons G (1996) Chemical changes in peat as a result of neutralising with lime during the preparation of mushroom casing. Mushroom biology and mushroom products: proceedings of the 2nd International Conference, University Park, Pennsylvania.

Sharma HSS, Furlan A, Lyons G (1999) Comparative assessment of chelated spent mushroom substrates as casing material for the production of *Agaricus bisporus*. Applied Microbiology and Biotechnology 366-367.

TUIK (2019) Turkish Statistical Institute. http://www.tuik.gov.tr. Accessed May 29, 2020.

Visscher HR (1988) Casing soil. In: Van Griensven LJLD (E.d.), The cultivation of mushrooms. Darlington Mushroom Laboratories Ltd., Rustington, Sussex, UK, pp. 73-88.

White PF (1981) Spread of the mushroom disease Verticillium fungicola by Megaselia halterata. Protection Ecology 3: 17-24.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 267-272
DOI: 10.29136/mediterranean.897989

# Effects of *Trichoderma* and PGPR applications on growth and *Verticillium* wilt of eggplant

**Melis BILGINTURAN, Gursel HATAT KARACA**

Isparta University of Applied Sciences, Faculty of Agriculture, Plant Protection Department, 32260, Isparta

Corresponding author: G. Hatat Karaca, e-mail: gurselkaraca@isparta.edu.tr
Author(s) e-mail: meliskarapire@isparta.edu.tr

ARTICLE INFO

Received: March 16, 2021
Received in revised form: October 22, 2021
Accepted: October 26, 2021

***Keywords*:**

*Solanum melongena*
*Trichoderma* spp.
PGPR
ISR
Enzyme activities

**ABSTRACT**

In this study, effects of single and combined applications of biocontrol agents; *Trichoderma* spp. (*T. atroviride*, *T. virens*) and plant growth promoting rhizobacteria (*Pseudomonas koreensis*, *Bacillus subtilis*) on growth, wilt disease severity caused by *Verticillium dahliae* and plant defence-related enzymes (peroxidase, polyphenol oxidase, phenylalanine ammonium lyase and β-1,3 glucanase) of eggplant, were investigated. It was determined that single and combined applications of biological control agents reduced the severity of wilt disease caused by the pathogen, and *T. atroviride* isolate and its combinations with bacteria were the most effective applications. Biological control agents not only increased plant growth parameters in the experimental groups they were applied, but also the activities of defence-related peroxidase, polyphenol oxidase, phenylalanine ammonium lyase and β-1,3 glucanase enzymes in the plant samples taken from these groups. Inoculations with biocontrol agents especially increased stem diameter, length, fresh and dry weights and root lengths of the eggplants, compared to the pathogen inoculated ones. Although the enzyme activities of the plants changed depending on the period after the inoculations, mostly found to be higher on the plants inoculated with the pathogen and/or biocontrol agents, compared to the non-inoculated control plants.

## 1. Introduction

Eggplant (*Solanum melongena* L.) is one of the most important vegetable crops grown all over Turkey, except some parts of the Eastern Anatolia, Central Anatolia and Black Sea Regions. It is widely cultivated after tomatoes, peppers, cucumbers, watermelons and melons since the beginning of the 17th century. According to FAO, Turkey produced a total of 822.659 tonnes of eggplant in about 23.337 hectares of land in 2019 (FAO 2021). Diseases are frequently encountered in eggplant cultivation and some important eggplant diseases caused by viruses (Sadeghi et al. 2008), bacteria (Yerchyk 2008) and fungi (Sholberg et al. 2007) were studied broadly. Common diseases caused by pathogenic fungi in eggplant cultivations are wilt, root rot, powdery mildew, white rot and gray mold, under field and greenhouse conditions (Altınok 2012). *Verticillium dahliae* Kleb. is a soil-borne fungal pathogen, which causes wilt disease and significantly decreases yield and quality in eggplant cultivation areas (Başay et al. 2011).

Vascular wilt diseases are among the most destructive plant pathogens and can destroy a crop in a whole cultivation area (Yadeta and Thomma 2013). Due to a large number of hosts of *V. dahliae* and the fact that it can survive in the soil for a long time, control strategies cannot be effective at the desired level. As a result of the side-effects of intensely used chemicals in agriculture on the environment and human health, biological control using beneficial microorganisms has gained importance, especially in the control of soil-borne diseases (Tjamos et al. 2004; Verma et al. 2019).

*Trichoderma* spp. and plant growth-promoting rhizobacteria (PGPR) known as biological control agents can protect plants against *V. dahliae* by colonizing the root system of plants by using mechanisms such as antibiosis against pathogens, competitive hyperparasitism-mycoparasitism and induced systemic resistance (ISR) (Tjamos et al. 2004).

In this study, the effects of single and combined treatments of *Trichoderma* species and PGPR against the severity of wilt disease caused by *V. dahliae* on eggplant, and on enzyme activities which has a role in plant defence, were investigated.

## 2. Materials and Methods

Eggplant variety 'Kemer' was raised from seed in the climate chamber for all treatments in this study. Pathogen *V. dahliae* and biocontrol agent isolates previously obtained from eggplant areas and kept in the Mycology Laboratory of the Plant Protection Department, Faculty of Agriculture, Isparta Applied Sciences University, were used in the study. Biocontrol agents were selected according to their compatible *in vitro* interactions and, *Trichoderma* isolates identified as *T. virens* and *T. atroviride*, and PGPR isolates identified as *Pseudomonas koreensis* and *Bacillus subtilis* by the MALDI-TOF method were used in this research.

To determine *in vivo* effects of biological control agents on *V. dahliae*, a modification of the method from Akhtar and Azam (2014) was used. Eggplant seedlings with 3-4 true leaves were dipped for 15 minutes into spore suspensions of *Trichoderma* isolates at $1x10^8$ spore $ml^{-1}$concentration prepared from 7 days old Potato Dextrose Agar (PDA; Merck) cultures, or suspensions with $1x10^8$-$10^{10}$ cfu $ml^{-1}$ concentration of bacteria grown in NA medium for 24-48 hours. For combined applications, root tips of the seedlings were first trimmed with a sterile scissor and then seedlings were dipped into each suspension for 15 minutes. For pathogen inoculations, seedlings were additionally kept in the *V. dahliae* conidia suspension at $10^6$ spore $ml^{-1}$ concentration for 15 minutes prepared from 7 days old PDA cultures. Positive controls were inoculated only with the pathogen and negative controls with sterile distilled water, by using the same method. Seedlings were then transferred to plastic pots with sterilized soil-peat-perlite-sand (2:1:1:1, v:v:v:v) mixture. The experiment was set up with 3 replications, with 5 plants per replication. Disease severity evaluations were made every week for 4 weeks by using a 0-4 scale of Fahima and Henis (1995) and Townsend-Heuberger formula (Townsend and Heuberger 1943) was applied to the scale values to determine the severity rates (%). Efficiencies of the treatments were also calculated by Abbott's formula (Abbott 1925). Plant growth parameters; stem diameter, stem and root lengths, fresh and dry weights were also determined.

To determine the effects of the biological control agents on the activities of enzymes related to plant defence, leaf samples of 2 grams were taken from eggplant seedlings in each application, one, seven and fourteen days after the inoculations. For use as crude enzyme extract in peroxidase, polyphenol oxidase and phenylalanine ammonium lyase analysis, 1 gram of leaf sample was homogenized with 0.1 M 2 ml phosphate buffer (pH 7.0, 4ºC). For the determination of β-1,3 glucanase activity, the remaining 1 gram sample was homogenized with 0.1 M sodium citrate buffer (pH 5.0, 4ºC). After both homogenates were centrifuged at 10000 rpm for 20 minutes, the resulting supernatants were removed (Saravanakumar et al. 2007).

Determination of peroxidase activity was carried out by the procedure using pyrogallol as a substrate. The mixture consisted of 0.32 ml of 5% pyrogallol, 0.1 ml of enzyme extract, 0.16 ml of 0.5% $H_2O_2$, 0.32 ml of 100 mM potassium phosphate buffer (pH 6.0) and 2.1 ml of purified water. The mixture was incubated for 10 minutes at 20 ± 1°C. After incubation, changes in absorbance at 420 nm wavelength has been observed for 3 minutes with 30-second intervals (Chance and Maehly 1955; Shannon et al. 1966).

Polyphenol oxidase enzyme analysis was performed using the method reported by Mayer et al. (1965). The reaction mixture contained 200 µl enzyme extract and 1.5 ml 0.1 M sodium phosphate buffer (pH: 6.5). The reaction was initiated by adding 0.5 ml of 0.01 M catechol and the absorbance values at 420 nm wavelength were measured.

Phenylalanine ammonium lyase activity was determined according to McCallum and Walker (1990). The reaction mixture consisted of the following components; 1.2 ml of 50 mM borate buffer (pH 8.8), 0.2 ml of 20 mM L-phenylalanine and 0.2 ml of enzyme extract. After mixing 1.2 ml of borate buffer and 0.2 ml of L-phenylalanine for 5 minutes at 37ºC, the reaction was stopped by adding 0.2 ml of enzyme extract and keeping it in a water bath at 37ºC for 1 hour, and then mixing with 5 N 20 µl HCl. Enzyme activity was measured at 290 nm wavelength using spectrophotometer.

β-1, 3 glucanase enzyme activity was determined using the laminarin DNSA method of Pan et al (1991). Fort he analyses, 62.5 µl of 4% laminarin and 62.5 µl of enzyme extract were taken into tubes and incubated in water bath at 40ºC for 10 minutes. The reaction was stopped by adding 375 µl of dinitrosalicylic acid and boiling it for 5 minutes. The reaction mixture was completed to 5 ml with distilled water, vortexed and absorbance was measured at 500 nm wavelength.

The data obtained in the study were subjected to analysis of variance using JMP statistical program (Ver.15.1.2) and differences among the means of the treatments were determined by the Tukey multiple comparison test ($P \leq 0.05$).

## 3. Results

### *3.1. Effects of biological control agents on wilt disease severity*

Effects of the separate or combined inoculations of eggplants with *Trichoderma* and PGPR isolates on disease severity rates caused by *V. dahliae* were shown in Table 1. No symptom was observed on eggplant seedlings in the 1st week, while wilt disease symptoms were seen in the second week and reached the maximum level in the third week. Disease severity rates decreased with the applications of biocontrol agents, especially with the application of *T. atroviride* alone or with bacteria. Although the decrease in disease severity rates was not statistically significant, the efficiency of *T. atroviride* inoculations were 75% in the second week and about 63% three and four weeks after inoculations.

### *3.2. Evaluation of plant growth parameters*

Plants inoculated with *V. dahliae* and/or biological control agents were also evaluated in terms of growth parameters (Table 2). Pathogen inoculation decreased stem diameters, stem and root lengths and fresh and dry weights, compared to non-inoculated control plants, while separate or combined inoculations of biocontrol agents increased all parameters. Inoculations of *P. koreensis*, *P. koreensis*+*T. virens* and *T. atroviride*+*B. subtilis* significantly increased stem diameters of eggplant seedlings when compared to the plants treated with pathogen solely. Separate and combined applications of *Trichoderma* and PGPR bacteria isolates significantly increased stem lengths of the seedlings and arranged in the same group with non-inoculated controls. *T. atroviride*, *B. subtilis* and combined inoculation of *T. virens* and *P. koreensis* inoculations also prevented the negative effect of the pathogen and significantly increased stem lengths of the plants inoculated with the pathogen. Although the biocontrol applications could not significantly increase stem fresh and dry weights of the plants when inoculated with the pathogen, some separate and combined inoculations of *Trichoderma* and PGPR stimulated plant growth and yielded higher values than non-inoculated control plants. All biocontrol applications except separate inoculations of *T. virens* and *P. koreensis* significantly increased root lengths of the seedlings inoculated with the pathogen. In terms of root fresh and dry weight parameters, all applications had increasing effect compared to the pathogen inoculated group, however this increase was not statistically significant.

**Table 1.** Wilt disease severity on eggplant seedlings after inoculations of *Verticillium dahliae* (Vd) with separate and combined applications of biocontrol agents and efficiencies of the treatments

| Treatments | Day 14 | | Day 21 | | Day 28 | |
|---|---|---|---|---|---|---|
| | Disease severity (%) | Efficiency (%) | Disease severity (%) | Efficiency (%) | Disease severity (%) | Efficiency (%) |
| Vd | 20.00*a** | - | 45.00 a | - | 45.00 a | - |
| Vd+*Trichoderma virens* | 8.33 ab | 58.35 | 23.33 a | 48.15 | 23.33 a | 48.15 |
| Vd+*T. atroviride* | 5.00 ab | 75.00 | 16.66 ab | 62.97 | 16.66 ab | 62.97 |
| Vd+*Pseudomonas koreensis* | 8.33 ab | 58.35 | 16.66 ab | 62.97 | 16.66 ab | 62.97 |
| Vd+*Bacillus subtilis* | 11.66 a | 41.70 | 18.33 a | 59.26 | 18.33 a | 59.26 |
| Vd+ *T. virens* + *P. koreensis* | 8.33 ab | 58.35 | 26.66 a | 40.75 | 26.66 a | 40.75 |
| Vd+ *T. virens* + *B. subtilis* | 10.00 ab | 50.00 | 25.00 a | 44.44 | 25.00 a | 44.44 |
| Vd+ *T. atroviride* + *P. koreensis* | 6.66 ab | 66.70 | 16.66 ab | 62.97 | 16.66 ab | 62.97 |
| Vd+ *T. atroviride* + *B. subtilis* | 5.00 ab | 75.00 | 16.66 ab | 62.97 | 16.66 ab | 62.97 |
| Control | 0.00 b | - | 0.00 b | - | 0.00 b | - |

*: Disease severity rates were subjected to arc sin transformation before statistical analyses, but real values were given in the table. **: Means in the same column shown by the same letter are not statistically different from each other according to the Tukey test ($P \leq 0.05$).

**Table 2.** Effects of biological control agents on growth parameters in plants inoculated with *Verticillium dahliae* (Vd)

| Treatments | SD (mm)* | SL (cm) | SFW (g) | SDW (g) | RL (cm) | RFW (g) | RDW (g) |
|---|---|---|---|---|---|---|---|
| Control | 3.268 ab** | 21.900 a | 4.836 ab | 0.666 ab | 11.393 ab* | 1.337 a | 0.176 a |
| *Trichoderma virens* | 3.309 ab | 22.820 a | 5.440 ab | 1.010 a | 11.587 ab | 1.445 a | 0.221 a |
| *T. atroviride* | 3.383 ab | 22.447 a | 5.742 ab | 0.965 a | 11.900 ab | 1.425 a | 0.203 a |
| *Pseudomonas koreensis* | 3.495 a | 23.073 a | 6.287 ab | 0.891 ab | 12.153 ab | 1.404 a | 0.226 a |
| *Bacillus subtilis* | 3.417 ab | 23.093 a | 5.905 ab | 0.878 ab | 11.520 a | 1.451 a | 0.216 a |
| *T. virens* + *P. koreensis* | 3.721 a | 24.160 a | 6.461 ab | 0.928 a | 13.700 ab | 1.618 a | 0.202 a |
| *T. virens* + *B. subtilis* | 3.447 ab | 22.907 a | 5.297 ab | 0.916 a | 11.767 ab | 1.464 a | 0.236 a |
| *T. atroviride* + *P. koreensis* | 3.467 ab | 23.320 a | 5.691 ab | 0.947 a | 15.267 a | 1.611 a | 0.235 a |
| *T. atroviride* + *B. subtilis* | 3.479 a | 22.827 a | 7.633 a | 0.867 ab | 11.580 ab | 1.518 a | 0.211 a |
| Vd | 2.443 b | 15.320 b | 3.419 b | 0.444 b | 8.340 b | 1.126 a | 0.124 a |
| Vd+ *T. virens* | 3.036 ab | 18.833 ab | 5.275 ab | 0.866 ab | 11.333 ab | 1.388 a | 0.194 a |
| Vd+ *T. atroviride* | 3.431 ab | 21.767 a | 5.487 ab | 0.865 ab | 13.600 a | 1.421 a | 0.177 a |
| Vd+ *P. koreensis* | 2.961 ab | 21.287 ab | 5.085 ab | 0.824 ab | 12.500 ab | 1.404 a | 0.187 a |
| Vd+ *B. subtilis* | 3.142 ab | 22.413 a | 5.242 ab | 0.898 ab | 12.567 a | 1.381 a | 0.202 a |
| Vd+ *T. virens* + *P. koreensis* | 3.308 ab | 22.260 a | 5.381 ab | 0.884 ab | 14.027 a | 1.383 a | 0.209 a |
| Vd+ *T. virens* + *B. subtilis* | 3.085 ab | 21.527 ab | 5.453 ab | 0.756 ab | 13.887 a | 1.430 a | 0.206 a |
| Vd+ *T. atroviride* + *P. koreensis* | 3.257 ab | 20.293 ab | 5.131 ab | 0.877 ab | 13.807 a | 1.417 a | 0.206 a |
| Vd+ *T. atroviride* + *B. subtilis* | 2.999 ab | 20.260 ab | 5.357 ab | 0.704 ab | 13.727 a | 1.411 a | 0.199 a |

*: SD: Stem diameter, SL: Stem length, SFW: Stem fresh weight, SDW: Stem dry weight, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight. **: Means in the same column shown by the same letter are not statistically different from each other according to the Tukey test ($P \leq 0.05$).

### *3.3. Enzyme activities of eggplants inoculated with V. dahliae and biocontrol agents*

Effects of the applications on defence-related enzyme activities of eggplant sedlings changed depending on the inoculated agents and time. However, almost all enzyme activities were higher in the plant samples inoculated with either pathogen and the biological agents than those in non-inoculated control plants. Peroxidase enzyme activity was found to be higher in the leaf samples taken one and seven days after inoculations of the plants with *T. atroviride* and the pathogen, while the highest enzyme activity value on the 14$^{th}$ day was obtained with *P. koreensis*+pathogen treatment (Table 3). Polyphenol oxidase enzyme activity on the first day was found to be highest in the plant samples subjected to combined inoculations with *T. atroviride* and *P. koreensis*, while enzyme activity values in plants inoculated with *T. virens* + *P. koreensis* and *T. atroviride*+pathogen were statistically in the same group. On the 7$^{th}$ day, the highest polyphenol oxidase activity was obtained by *T. virens* + *B. subtilis* combined treatment. Other *Trichoderma* and bacteria combined applications were also statistically arranged in the same group. In the last analyses made with plant samples taken on the 14$^{th}$ day after inoculations, *T. atroviride* + *B. subtilis* combined treatment yielded the highest enzyme value followed by *T. virens*+*B. subtilis* application (Table 4). The highest phenylalanine ammonium lyase activity on the 1$^{st}$ day was obtained in *T. virens* + *P. koreensis* combined treatment. Activities of this enzyme were generally lower in combined inoculations of biocontrol agents and the pathogen on the first day, while these values increased in the subsequent measurements (Table 5). As in polyphenol oxidase activity, *T. atroviride*+*P. koreensis* combined inoculations yielded the highest β-1,3 glucanase activity on the first day, followed by other *Trichoderma*+bacterium combination. Results obtained on the 7$^{th}$ day showed that single inoculation of *T. virens* resulted in the highest enzyme activity, and on the 14$^{th}$ day, all applications were statistically similar in terms of β-1,3 glucanase activity (Table 6).

**Table 3.** Effects of biological control agents on peroxidase activity (unit $ml^{-1}$) in plants inoculated with *Verticillium dahliae* (Vd)

| Treatments | Day 1 | Day 7 | Day 14 |
|---|---|---|---|
| Control | 2.672 d* | 3.729 ab | 3.463 g |
| *Trichoderma virens* | 3.458 a-d | 4.268 ab | 4.082 a-d |
| *T. atroviride* | 3.440 a-d | 3.992 ab | 3.768 c-g |
| *Pseudomonas koreensis* | 3.184 b-d | 4.081 ab | 3.778 c-g |
| *Bacillus subtilis* | 2.880 cd | 4.053 ab | 3.530 fg |
| *T. virens* + *P. koreensis* | 4.046 ab | 3.891 ab | 3.623 d-g |
| *T. virens* + *B. subtilis* | 3.514 a-d | 3.748 ab | 3.892 b-g |
| *T. atroviride* + *P. koreensis* | 3.858 a-c | 3.756 ab | 3.539 fg |
| *T. atroviride* + *B. subtilis* | 2.993 b-d | 3.704 b | 3.721 d-g |
| Vd | 3.857 a-c | 3.762 ab | 3.581 e-g |
| Vd+*T. virens* | 4.351 a | 4.212 ab | 4.255 ab |
| Vd+ *T. atroviride* | 4.405 a | 4.435 a | 4.204 a-c |
| Vd+*P. koreensis* | 3.668 a-d | 4.317 ab | 4.502 a |
| Vd+ *B. subtilis* | 3.390 a-d | 3.757 ab | 3.817 b-g |
| Vd+*T. virens*+ *P. koreensis* | 3.446 a-d | 3.912 ab | 3.980 b-f |
| Vd+*T. virens*+ *B. subtilis* | 3.845 a-c | 3.665 b | 3.931 b-g |
| Vd+ *T. atroviride* + *P. koreensis* | 3.721 a-d | 4.387 ab | 4.032 b-e |
| Vd+ *T. atroviride* + *B. subtilis* | 3.694 a-d | 4.113 ab | 4.078 a-d |

*: Means in the same column shown by the same letter are not statistically different from each other according to the Tukey test ($P \leq 0.05$).

**Table 4.** Effects of biological control agents on polyphenol oxidase activity (µg $ml^{-1}$) in plants inoculated with *Verticillium dahliae* (Vd)

| Treatments | Day 1 | Day 7 | Day 14 |
|---|---|---|---|
| Control | 36.945 e* | 39.509 e | 31.595 d |
| *Trichoderma virens* | 39.167 de | 48.415 b-e | 38.826 cd |
| *T. atroviride* | 41.458 c-e | 44.296 de | 41.868 b-d |
| *Pseudomonas koreensis* | 53.680 a-d | 45.048 c-e | 51.834 a-c |
| *Bacillus subtilis* | 46.740 a-e | 58.638 a-c | 38.774 cd |
| *T. virens*+ *P. koreensis* | 61.697 a | 58.073 a-c | 43.167 b-d |
| *T. virens* + *B. subtilis* | 54.586 a-d | 62.313 a | 57.424 ab |
| *T. atroviride* + *P. koreensis* | 62.108 a | 54.193 a-d | 54.108 a-c |
| *T. atroviride* + *B. subtilis* | 53.424 a-d | 53.834 a-d | 61.424 a |
| Vd | 44.364 b-e | 43.321 de | 38.159 cd |
| Vd+ *T. virens* | 47.185 a-e | 40.997 de | 48.005 a-c |
| Vd+ *T. atroviride* | 60.279 a | 51.236 a-e | 53.014 a-c |
| Vd+ *P. koreensis* | 50.979 a-e | 60.535 ab | 54.022 abc |
| Vd+ *B. subtilis* | 59.356 ab | 53.031 a-e | 51.526 a-c |
| Vd+ *T. virens* + *P. koreensis* | 56.296 a-c | 52.603 a-e | 52.261 a-c |
| Vd+ *T. virens* + *B. subtilis* | 57.714 ab | 48.552 b-e | 52.808 a-c |
| Vd+ *T. atroviride* + *P. koreensis* | 49.253 a-e | 58.672 a-c | 55.561 ab |
| Vd+ *T. atroviride* + *B. subtilis* | 53.304 a-d | 54.261 a-d | 52.056 a-c |

*: Means in the same column shown by the same letter are not statistically different from each other according to the Tukey test ($P \leq 0.05$).

## 4. Discussion and Conclusion

Considering the effects of *Trichoderma* and PGPR applications on the severity of wilt disease caused by *V. dahliae*, it was observed that the inoculations of biological control agents decreased wilt disease severity rates, although there was no statistically significant difference among the treatments. The lowest disease rate was obtained with the separate and combined treatments of *T. atroviride* with PGPR. In the previous studies on the effects of biological control agents against *V. dahliae*, it was also observed that single *Trichoderma* and PGPR treatments decreased disease severity rates, but the symptoms did not disappear completely and the application time of biological control agents also affected the disease severity (Amini 2017; Guenoun et al. 2018; Mokhtari et al. 2018; Zhang et al. 2018).

When the effects of the application of biological control agents together with the pathogen on plant growth parameters were examined, it was found that the combined treatments of *Trichoderma* and PGPR caused an increase in stem diameter, stem length, stem fresh and dry weights, root length, root fresh and dry weights of eggplants, compared to the treatments performed separately. It was previously reported that *Trichoderma* species (Mokhtari et al. 2018) and PGPR isolates (Guenoun et al. 2018) increased plant growth parameters in eggplant infected with *V. dahliae*.There are also reports on increased plant growth parameters as a result of the separate and combined treatments of *Trichoderma* and PGPR isolates on plants infected with different pathogens (Thilagavathi et al. 2007; Morsy et al. 2009; Chowdappa et al. 2013; Erper et al. 2013; Kumar et al. 2015).

**Table 5.** Effects of biological control agents on phenylalanine ammonia-lyase activity (µg $ml^{1}$ $h^{-1}$) in plants inoculated with *Verticillium dahliae* (Vd)

| Treatments | Day 1 | Day 7 | Day 14 |
|---|---|---|---|
| Control | 25.344 e* | 31.401 d | 26.956 d |
| *Trichoderma virens* | 53.884 a-d | 57.392 a-c | 55.845 ab |
| *T. atroviride* | 53.841 a-d | 60.617 a-c | 42.577 bc |
| *Pseudomonas koreensis* | 63.950 ab | 69.527 a | 46.913 a-c |
| *Bacillus subtilis* | 61.597 a-c | 56.477 a-c | 41.161 c |
| *T. virens* + *P. koreensis* | 70.203 a | 55.017 a-c | 47.850 a-c |
| *T. virens* + *B. subtilis* | 48.569 b-d | 59.157 a-c | 49.723 a-c |
| *T. atroviride* + *P. koreensis* | 53.492 a-d | 50.246 b-d | 38.111 cd |
| *T. atroviride* + *B. subtilis* | 53.471 a-d | 44.669 cd | 43.972 bc |
| Vd | 64.168 ab | 63.514 a-c | 58.481 a |
| Vd + *T. virens* | 52.098 a-d | 54.865 a-c | 45.257 a-c |
| Vd + *T. atroviride* | 43.274 c-e | 51.532 a-c | 45.584 a-c |
| Vd + *P. koreensis* | 46.237 b-d | 50.682 a-c | 44.277 bc |
| Vd + *B. subtilis* | 48.743 b-d | 50.094 b-d | 41.989 c |
| Vd + *T. virens* + *P. koreensis* | 42.011 c-e | 50.747 a-c | 44.647 bc |
| Vd + *T. virens* + *B. subtilis* | 43.231 c-e | 50.377 bc | 41.793 c |
| Vd + *T. atroviride* + *P. koreensis* | 47.828 b-d | 65.279 ab | 48.808 a-c |
| Vd + *T. atroviride* + *B. subtilis* | 40.246 de | 51.924 a-c | 44.495 bc |

*: Means in the same column shown by the same letter are not statistically different from each other according to the Tukey test ($P \leq 0.05$).

**Table 6.** Effects of biological control agents on β- 1,3 glucanase activity (µg $ml^{-1}$) in plants inoculated with *Verticillium dahliae* (Vd)

| Treatments | Day 1 | Day 7 | Day 14 |
|---|---|---|---|
| Control | 353.500 ı* | 336.530 h | 392.742 a |
| *Trichoderma virens* | 492.136 a-c | 553.955 a | 439.106 a |
| *T. atroviride* | 462.742 c-e | 446.227 c | 449.561 a |
| *Pseudomonas koreensis* | 433.197 e-g | 429.258 c | 417.894 a |
| *Bacillus subtilis* | 402.136 gh | 378.652 e-h | 400.167 a |
| *T. virens* + *P. koreensis* | 473.803 b-d | 420.470 c-e | 399.864 a |
| *T. virens* + *B. subtilis* | 503.955 ab | 445.167 c | 436.379 a |
| *T. atroviride* + *P. koreensis* | 520.773 a | 341.227 h | 426.379 a |
| *T. atroviride* + *B. subtilis* | 401.682 gh | 407.894 c-f | 438.500 a |
| Vd | 443.197 d-f | 501.985 b | 458.348 a |
| Vd + *T. virens* | 452.136 d-f | 424.864 cd | 407.591 a |
| Vd + *T. atroviride* | 362.742 ı | 368.197 f-h | 415.167 a |
| Vd + *P. koreensis* | 387.439 hı | 350.924 gh | 413.348 a |
| Vd + *B. subtilis* | 424.409 fg | 404.864 c-f | 412.742 a |
| Vd + *T. virens* + *P. koreensis* | 472.136 b-d | 374.561 f-h | 426.985 a |
| Vd + *T. virens* + *B. subtilis* | 373.045 hı | 385.773 d-g | 410.167 a |
| Vd + *T. atroviride* + *P. koreensis* | 505.318 ab | 422.288 cd | 413.500 a |
| Vd + *T. atroviride* + *B. subtilis* | 386.227 hı | 349.409 gh | 411.682 a |

*: Means in the same column shown by the same letter are not statistically different from each other according to the Tukey test ($P \leq 0.05$).

As a result of this study, it was found that *Trichoderma* and PGPR applications increased the activities of defence-related enzymes peroxidase, polyphenol oxidase, phenylalanine ammonium lyase and β-1,3 glucanase on eggplant seedlings inoculated with *V. dahliae.* These results showed that *Trichoderma* and PGPR isolates have the potential to stimulate enzymes involved in the defence mechanism of eggplant. Previous studies showed that *Trichoderma* spp., *B. subtilis* and *Pseudomonas* spp. treatments were responsible for the suppression of fungal diseases in plants due to the increase in peroxidase, polyphenol oxidase, phenylalanine ammonium lyase and β-1, 3 glucanase activities (Ramamoorthy et al. 2007; Thilagavathi et al. 2007; Jayalakshmi et al. 2009; Latha et al. 2009; Houssien et al. 2010; Kumar et al. 2015; Chandrasekaran et al. 2017; Li et al. 2019). However, the sampling period, the types of biological control agents and their application times, plant variety and pathogenic microorganisms can affect the changes in enzyme activities.

The results obtained in this study showed that *Trichoderma* spp. and PGPR treatments reduced the disease severity of *V. dahliae*, had positive effects on plant growth and stimulated defence responses of the plant. An increase in peroxidase, polyphenol oxidase, phenylalanine ammonium lyase and β-1,3 glucanase enzyme activities of eggplant showed that the applications of *Trichoderma* species and PGPR promoted plant defence against the pathogen. The use of resistant varieties, solarization and fertilization are the main methods used in the disease management of *V. dahliae*. In addition, sustainable agriculture intends to increase and spread the use of biological control agents in the absence of effective chemical control, to control the disease or reduce its severity.

## Acknowledgement

This study is a part of the PhD thesis project (no: OYP05268-DR-14), supported by Isparta University of Applied Sciences, OYP Institutional Coordination Unit.

## References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Akhtar MS, Azam T (2014) Effects of PGPR and antagonistic fungi on the growth, enzyme activity and Fusarium root-rot of pea. Archives of Phytopathology and Plant Protection 47: 138-148.

Altınok HH (2012) Antalya ve Mersin ili örtü altı patlıcan ekim alanlarında kurşuni küf ve beyaz çürüklük hastalıklarının yaygınlık oranlarının belirlenmesi. Bitki Koruma Büteni 52: 163-173.

Amini J (2017) Biocontrol of *Verticillium* wilt of potato caused by *Verticillium dahliae* using selected biocontrol agents. Applied Entomology and Phytopathology 84: 11-19.

Başay S, Şeniz V, Tezcan H (2011) Reactions of selected eggplant cultivars and lines to Verticillium wilt caused by *Verticillium dahliae* Kleb. African Journal of Biotechnology 10: 3571-3573.

Chance B, Maehly AC (1955) Assay of Catalases and Peroxidases. Methods in Enzymology 2: 773-775.

Chandrasekaran M, Belachew ST, Yoon E, Chun SC (2017) Expression of β-1,3-glucanase (GLU) and phenylalanine ammonia-lyase (PAL) genes and their enzymes in tomato plants induced after treatment with *Bacillus subtilis* CBR05 against *Xanthomonas campestris* pv. *vesicatoria*. Journal of General Plant Pathology 83: 7-13.

Chowdappa P, Mohan Kumar SP, Lakshmi MJ, Upreti KK (2013) Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biological Control 65: 109-117.

Erper I, Turkkan M, Atanasova L, Druzhinina IS, Karaca GH, CebI-Kilicoglu M (2013) Integrated assessment of the mycoparasitic and phytostimulating properties of *Trichoderma* strains against *Rhizoctonia solani*. Bulgarian Journal of Agricultural Science 19: 737-743.

Fahima T, Henis Y (1995) Quantitative assessment of the interaction between the antagonistic fungus *Talaromyces flavus* and the wilt pathogen *Verticillium dahliae* on eggplant roots. Plant and Soil 176: 129-137.

FAO (2021) *Eggplant production*. http://www.fao.org/faostat/en/#data/QC. Accessed 10 January 2021.

Guenoun K, Chattaoui M, Bouri M, Naghmouchi K, Raies A (2018) Biological control of growth promoting rhizobacteria against verticillium wilt of pepper plant. *Biologia*, doi: 10.2478/s11756-018-00169-9.

Houssien AA, Ahmed SM, Ismail AA (2010) Activation of tomato plant defense response against Fusarium wilt disease using Trichoderma harzianum and salicylic acid under greenhouse conditions. Research Journal of Agriculture and Biological Sciences 6: 328-338.

Jayalakshmi SK, Raju S, Usha-Rani S, Benagi VI, Sreeramulu K (2009) *Trichoderma harzianum* L1 as apotential source for lytic enzmes and elicitor of defense responses in chickpea (*Cicer arietinum* L.) against wilt diseases caused by *Fusarium oxysporum* f. sp. *ciceri*. Australian Journal of Crop Science 3: 44-52.

Kumar SPM, Chowdappa P, Krishna V, Sandhya H (2015) Induction of defense-related proteins and growth promotion in tomato by mixture of *Trichoderma harzianum* OTPB3 and *Bacillus subtilis* OTPB1 and *Pseudomonas putida* OPf1 against *Phytophthora infestans*. African Journal of Microbiology Research 9: 96-110.

Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R (2009) Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biological Control 50: 85-93.

Li M, Ma G, Lian H, Su X, Tian Y, Huang W, Mei J, Jiang X (2019) The effects of *Trichoderma* on preventing cucumber physiology. Journal of Integrative Agriculture 18: 607-617.

Mayer AM, Harel E, Shaul RB (1965) Assay of catechol oxidase, a critical comparison methods. Phytochemistry 5: 783-789.

McCallum JA, Walker JRL (1990) Phenolic biosynthesis during grain development in wheat: Changes in phenylalanine ammonia-lyase activity and soluble phenolic content. Journal of Cereal Science 11: 35-49.

Mokhtari W, Achouri M, Remah A, Boubaker H (2018) *Verticillium dahliae*-eggplant as the pathosystem model to reveal biocontrol potential of three *Trichoderma* spp. in greenhouse conditions. Atlas Journal of Biology 417-421.

Morsy EM, Abdel-Kawi KA, Khalil MNA (2009) Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents gainst *Fusarium solani* on tomato plants. Egyptian Journal of Phytopathology 37: 47-57.

Pan SQ, Ye XS, Kuc J (1991) Association of β-1, 3 glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virüs. Physiology and Moecular Plant Pathology 39: 25-39.

Ramamoorthy V, Raguchander T, Samiyappan R (2002) Induction of defence-related proteins in tomato roots treated with *Pseudomonas fluorescence* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici*. Plant and Soil 239: 55- 68.

Sadeghi MS, Behjatnia SAA, Masumi M, Izadpanah K. (2008) Characterisation of a strain of potato virus Y causing eggplant mosaic in southern Iran. Australasian Plant Pathology 37: 79-86.

Saravanakumar D, Harish S, Loganathan M, Vivekananthan R, Rajendran L, Raguchander T, Samiyappan R (2007) Rhizobacterial bioformulation for the effective management of *Macrophomina* root rot in mungbean. Archives of Phytopathology and Plant Protection 40: 323-337.

Shannon LM, Kay E, Lew JY (1966) Peroxidase Isoenzymes from Horseradish Roots. I. Isolation and physical properties. Journal of Biological Chemistry 241: 2166-2172.

Sholberg PL, Walker MC, O'Gorman DT, Jesperson GD (2007) First report of *Phytophthora capsici* on cucurbitas and peppers in British Columbia. Canadian Journal of Plant Pathology 29: 153-158.

Thilagavathi R, Saravanakumar D, Ragupathi N, Samiyappan R (2007) A combination of bio-control agents improves the management of dry root rot (*Macrophomina phaseolina*) in green gram. Phytopathologia Mediterranea 46: 157-167.

Tjamos EC, Tsitsigiannis DI, Tjamos SE, Antoniou PP, Katinakis P (2004) Selection and screening of endorhizosphere bacteria from solarized soils as biocontrol agents against *Verticillium dahliae* of solanaceous hosts. European Journal of Plant Pathology 110: 35-44.

Townsend GR, Heuberger JW (1943) Methods for Estimating Losses Caused by Diseases in Fungicide Experiments. Plant Disease Report 27: 340-343.

Verma PP, Shelake RM, Das S, Sharma P, Kim J (2019) Plant growth-promoting rhizobacteria (PGPR) and Fungi (PGPF): Potential biological control agents of diseases and pests. In: Singh DP, Gupta VK, Prabha R (Eds), Microbial Inventions in Agriculture and Environment. Springer, pp. 281-312.

Yadeta KA, Thomma BPHJ (2013) The xylem as battleground for plant hosts and vascular wilt pathogens. Frontier in Plant Science 4: 97.

Yerchyk V (2008) Virulence of strains of *Clavibacter michiganensis* subsp. *sepedonicus*. Zemdirbyste 95: 359-365.

Zhang F, Li XL, Zhu SJ, Ojaghian MR, Zang JZ (2018) Biocontrol potential of *Paenibacillus polymyxa* against *Verticillium dahliae* infecting cotton plants. Biological Control 127: 70-77.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 273-277
DOI: 10.29136/mediterranean.1013009

# A new harmful thrips species in orange in Antalya Province: *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae)

**Ekrem ATAKAN, Serkan PEHLİVAN**

Cukurova University, Agricultural Faculty, Plant Protection Department, Adana, Turkey

Corresponding author: E. Atakan, e-mail: eatakan@mail.cu.edu.tr
Author(s) e-mail: spehlivan@cu.edu.tr

ARTICLE INFO

Received: October 21, 2021
Received in revised form: November 11, 2021
Accepted: November 16, 2021

***Keywords*:**

Chilli thrips
Citrus
Damage
Turkey

**ABSTRACT**

This study discusses the damage of an invasive insect species chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) on orange trees cv. Washington in the Finike district of Antalya Province, Turkey in 2021. This harmful thrips species was recorded for the first time in a citrus grove in Turkey. Thrips cause inward curling of fresh leaves on the young shoots and also necrosis, mostly silvery-white. The biology, ecology, and control of this species are briefly given.

## 1. Introduction

According to USDA (2020) data, in 2019, a total of 92 million tons of citrus fruits were produced in the world, including 46 million tons of oranges, about 32 million tons of tangerines, 8 million tons of lemons, and 7 million tons of grapefruits. The leading countries in the world orange production in 2019 are respectively, according to their production amounts; Brazil (34%), China (16%), EU (13%), and USA (10%). Turkey ranks 7th in the world orange production with a production amount of 1.8 million tons. Although Turkey produced approximately 4.3 million tons of citrus fruit in 2019, almost all of the production is provided from the Aegean and Mediterranean Regions. 83% of Turkey's total orange production, 90% of tangerine production, 92% of lemon production, 97% of grapefruit production is provided from the Mediterranean Region of Turkey (TÜİK 2020).

There are multiple biological factors such as diseases, pests and weeds which can cause economic damage and limit the agricultural yield in citrus. Among the recorded insect species in citrus areas, thrips are recognized as a pest in general. They have been well described with their diverse life histories and habitats, in particular Thysanoptera order, which constitutes 1% of approximately 6000 thrips species, was reported as a serious pest (Morse and Hoddle 2006; Mound and Morris 2007). The feeding habits of thrips species are quite different, and they can be classified as phytophagous (plant-feeding), mycophagous (fungal-feeding), and predatory species (Morse and Hoodle 2006). Many thrips (Thysanoptera) species have been reported to feed on citrus (Rutaceae) worldwide (Blank and Gill 1997; Childers and Nakahara 2006). These species feed on the flowers, fruits, and leaves of citrus, and their typical damage is in the form of silvery scars. Scars formed on fruits negatively affect the quality of the product and thus reduce its market value (Tekşam and Tunç 2009). The most important pest thrips species attacking citrus; *Scirtothrips citri* (Moultan) (citrus thrips) and *Scirtothrips aurantii* Faure (South African citrus thrips) in South Africa (Grove et al. 2000), *Scirtothrips dorsalis* Hood (Yellow tea thrips) in East Asia (Masui 2007), and *Pezothrips kellyanus* (Bagnall) (Kelly's citrus thrips) in Australia, New Zealand (Webster et al. 2006; Froud et al. 2001) and two Mediterranean islands in Sicily (Marullo 1998), Southern Cyprus (Vassiliou 2007). Longo (1985) reported that there are more than 40 thrips species which appear in citrus areas in the world. Yet only a few species are harmful such as *Scirtothrips* spp. and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) which are considered pests as they cause scarring on young fruits.

To date, *Frankliniella occidentalis* (Pergande), *Thrips major* Uzel (Thysanoptera: Thripidae) and *T. tabaci* were reported on citrus in Turkey, however they were not taken into account as economically important pests (Nas et al. 2007; Tekşam and Tunç 2009; Ölçülü and Atakan 2013). Tekşam and Tunç (2009) reported that *T. major* was the most common species in citrus fruits in the Antalya Province, and the scarred fruit rates due to thrips was less than 2%. Although *F. occidentalis* occured intensely in the lemon flowers in the Tarsus district of Mersin Province, Turkey, no wound formation on the fruits was observed (Atakan et al. 2016). The Hawaiian flower thrips, *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) was recorded for the first time in 'Yediveren' lemons in the Eastern Mediterranean Region of Turkey (Mersin Province) (Atakan et al. 2015), this thrips causes considerable damage to lemon fruits (Atakan et al. 2021).

On September 12, 2021, in the Antalya Province, Finike district, Turkey, some damage was observed in a orange orchard with Washington variety, especially in young shoots. Collected samples were brought to the laboratory and thrips were detected on shoots and leaves. As a result of the microscopic slides, it has been determined that this species is *Scirtothrips dorsalis*, also known as chilli thrips or yellow tea thrips, which seriously damaged blueberry plants in Adana Province in 2020 and was recorded for the first time in Turkey (Atakan and Pehlivan 2021). This study provides some brief information about *S. dorsalis*, an important pest of citrus fruits, and the definition of thrips damage investigated in plant samples. It can provide basic information regarding morphological features, damage, ecology and biology of this species, and also contribute to the control studies that must be carried out before it spreads throughout larger areas.

## 2. Materials and Methods

### *2.1. Sampling*

Due to the damage observed which was similar to thrips damage especially on the leaves of the fresh shoots of the trees, in an orchard where approximately 20 years old Washington orange trees were planted in Finike, Antalya, on September 12, 2021, leaf samples were taken. The damaged leaves were randomly collected and brought to the Entomology laboratory of the Plant Protection Department of Çukurova University, Adana Province, Turkey.

### *2.2. Identification of thrips*

The thrips specimens were collected from the leaves with the help of a fine-tipped brush and placed into tubes containing 60% ethyl alcohol. Afterwards, the samples were kept in AGA (10 parts 60% ethyl alcohol, one part glycerin and one part glacial acetic acid) for two days in order to facilitate their preparation by softening their bodies and once achieved, they were reintroduced into 60% alcohol. The samples were taken separately in glass petri dishes and kept in 10% potassium hydroxide for about 1 hour at 48 degrees on the hot plate. Body contents were macerated by entering through the hind leg bases of thrips individuals with a fine-tipped needle, cleaned by passing through alcohol series and transferred to a HOYER medium to prepare their microscopic slides. Thrips preparations were kept in an oven at 47 degrees to let them dry (Mound and Kibby 1998). The morphological features of the specimens (male and female individuals) were examined under the stereoscopic microscope (40X) and identified by the first author.

## 3. Results and Discussion

### *3.1. Identification and biology*

The images obtained by making the preparations of female and male *Scirtothrips dorsalis* are shown in Figure 1a, b, and their natural appearance on the leaves is shown in Figure 2 a, b. Adult females are about 1.2 mm long, with dark wings and dark

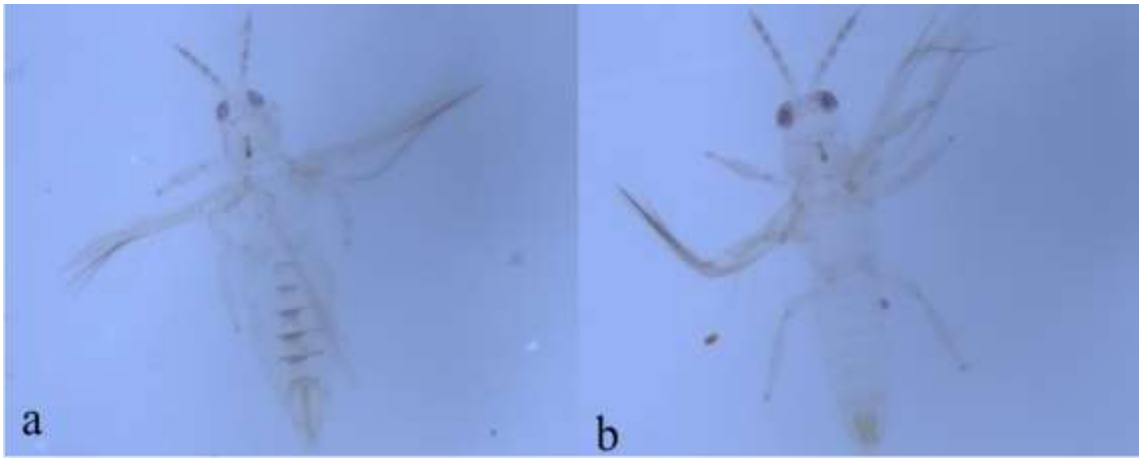


**Figure 1.** Microscopic slide images of adult female (a), and male (b) *Scirtothrips dorsalis* (Photo by E. Atakan, 2021).

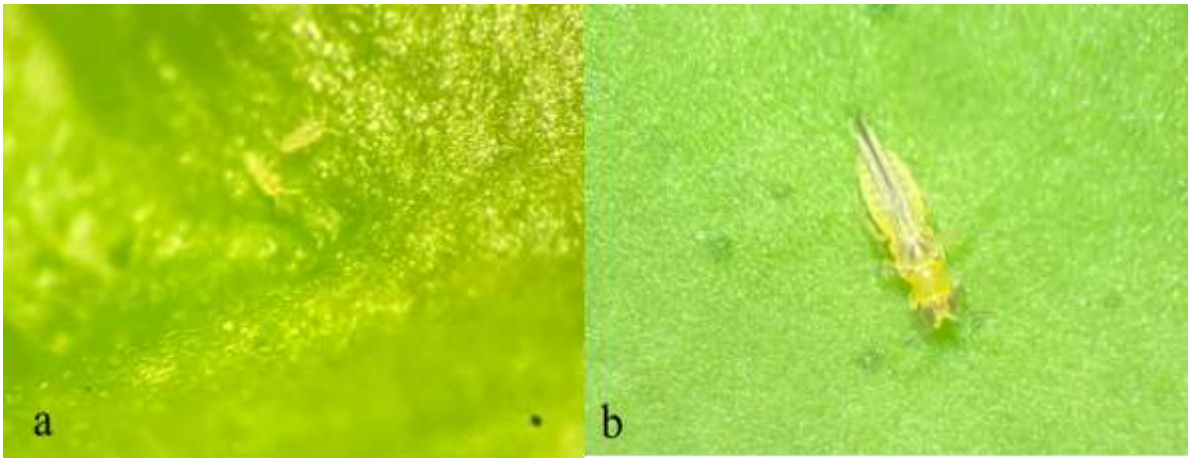


**Figure 2.** Natural appearances of the larvae (a) and adult female (b) *Scirtothrips dorsalis* (Photo by L. Bakırcıoğlu Erkılıç, 2021).

spots on the abdomen forming missing stripes visible on the back (Figure 1a). Adult males are smaller than females, have no spots on the back, and are lighter in colour (Figure 1b). Larvae are transparent or light yellowish in colour (Figure 2a). Detailed morphological diagnosis of the species has been given in a previous study (Atakan and Pehlivan 2021).

*Scirtothrips dorsalis* eggs are microscopically 0.075 mm long and 0.070 mm wide. Eggs are kidney-shaped and creamy white (Seal et al. 2009). Since thrips inserts eggs in plant tissues, it is very difficult to detect its eggs. The eggs hatch in two to seven days, depending on the temperature. Larvae and adults tend to accumulate near the midrib or borders of the host plant. The two larval stages (first and second) are completed in eight to ten days and the pupal stage lasts 2.6-3.3 days. Unlike other thrips, the pupae of this species are usually found on leaves, leaf litter, curled leaves, sometimes hidden under the sepals of flowers and fruits (Seal et al. 2009). Adults and larvae are found in the fresh leaves of the end shoots of trees.

### 3.2. Host plants

Even though *S. dorsalis* caused serious damage to the green parts of the blueberries in a greenhouse in Adana province (Atakan and Pehlivan 2021), no data are available concerning the host habitat of this species in the ecological regions where *S. dorsalis* has been detected in Turkey, up to now. To the best of our knowledge, *S. dorsalis* has been reported to infest a wide variety of host plants belonging to more than 100 plant taxa from 40 families (Mound and Palmer 1981; Venette and Davis 2004; Klassen et al. 2008; Kumar et al. 2013). Although the main hosts of *S. dorsalis* are acacia, mimosa and Saraca (Fabaceae), it has been recorded as a pest on several economically important host plants. For instance, Venette and Davis (2004) listed the potential hosts such as bananas, beans, cashews, citrus, cocoa, maize, cotton, eggplant, grapes, mangoes, melons, peanuts, peppers, strawberries, roses, sweet potatoes, tea, tobacco and tomatoes. Moreover, *S. dorsalis* is an important pest of ornamental plants in Florida (Klassen et al. 2008; Osborne 2009). Additionally, this thrips was found in 13 of 181 plant species sampled in Colombia, including citrus fruits (Ravelo et al. 2018). When this study was prepared for publication, strawberry leaves were seriously damaged due to *S. dorsalis* in autumn in Adana Province.

### 3.3. Damage

With their sucking mouthparts, adults and larvae of *S. dorsalis* absorb the cell sap of the leaves, causing the leaf to curl upwards and reduce the leaf size. Inward curls on the fresh leaves of the end shoots and also silvery-white necrosis formations are presented in Figure 3. Thrips individuals were not found in the old leaves and fruits of orange trees, and the typical signs of damage were not observed. The damage caused by *S. dorsalis* feeding may become superficially similar to mite damage (Sanap and Nawale 1987; Seal et al. 2006). In many host plants, after a dense population occurred, thrips started to feed on the upper surface of the leaves.

In this study, *S. dorsalis* individuals were detected on account of the damage to the shoots of orange trees in September. Presumably, spraying in the early period, especially in the flowering and young fruit period of citrus, may have suppressed the pest thrips. Jae-Wook et al. (2012) reported that *S. dorsalis* peaks in citrus orchards in South Korea (Jeju Island) in July or autumn, producing silver-grey or dark-coloured spots on the fruits. These injured tissues appear as a halo around the fruit stalks. According to that study, the rate of damaged fruit ranged from 0.04% to 0.09%. Moreover, *S. dorsalis* was reported in citrus fruits in Fars, Iran in 2015 (Minaei et al. 2016). According to that study, the harmful thrips species caused curling and hardening of the leaves on the fresh shoots, and silvery scars on the fruit surfaces of citrus.

### 3.4. Control

Effective control methods for *S. dorsalis* are still in the research phase. Some suggestions including crop rotation, removal of host weeds, and introducing predators or parasitoids have been made by the World Vegetable Center (AVRDC) to control this harmful thrips. Besides, it is always recommended to use insecticides from different classes in order to prevent the development of insecticide resistance. While synthetic pyrethroids can not effectively suppress *S. dorsalis* in pepper (Seal et al. 2006), soil or green parts applications of imidacloprid, one of the neonicothionide group insecticides, provides successful results in the management of *S. dorsalis* without harming beneficial insects (Seal and Kumar 2010). Foliar applications of imidacloprid from the soil much more successful

**Figure 3.** Damage symptoms on orange leaves as a result of feeding of *Scirtothrips dorsalis* (Photo by L. Bakırcıoğlu Erkılıç, 2021).

(Seal et al. 2008). However, the use of some insecticides from this group (i.e., imidacloprid) is prohibited in Turkey.

On the other hand, predatory *Orius* species (Hemiptera: Anthocoridae), known as minute pirate bugs, and entomopathogenic nematodes, *Thripinema* spp. (Tylenchida: Allantonematidae), have been reported to suppress the field populations of the pepper thrips (Kumar et al. 2017). The adults of the insidious flower bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae), effectively feed on thrips larvae and adults. Even if the thrips populations are greatly reduced, *O. insidiosus* may continue feeding on aphids, mites, moth eggs and pollens, with no significant reduction in population density. *Thripinema* species, parasitize adult female thrips, significantly reducing their egg production and thus significantly suppressing the thrips population density. Arthurs et al. (2009) evaluated two predatory mites, *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acarina: Phytoseiidae) as potential biological control agents of *S. dorsalis* and *A. swirskii* elicited the promising results in the control of *S. dorsalis* on hot pepper plants. Among the potential predators of thrips: *Chrysoperla* spp., ladybugs, predatory thrips species such as *Franklinothrips vespiformis* (Vespiform thrips), *Scolothrips sexmaculatus* (Six-pointed thrips) (Thysanoptera: Aeolothripidae), *Selenothrips rubrocinctus* (Giard) (Red banded thrips), *Leptothrips mali* (Fitch) (Black hunter thrips) (Thysanoptera: Phlaeothripidae) and predatory mites including *Amblyseius* spp., *Euseius hibisci* (Chant) and *Euseius tularensis* Congdon (Acarina: Phytoseiidae) were reported (Arthurs et al. 2009).

## 4. Conclusion

In addition to the invasive *T. hawaiiensis*, which is a problem in lemons in the Eastern Mediterranean Region of Turkey, yet another invasive species, *S. dorsalis*, was recently recorded on orange trees in a limited area in the Finike district of Antalya Province. Except for the location where the first record of this thrips species on a citrus groves was made (locations Finike and Turunçova), this thrips has not been detected yet on the citrus groves in the following locations, Sahilkent, Hasyurt and Kumluca in Antalya Province, where citrus cultivation is common. However, its distribution and economic importance in citrus orchards in the Mediterranean Region of Turkey is not known yet; these basic issues need to be investigated for control efforts. Currently, there are insecticides with temporary licenses against *T. hawaiiensis* in citrus in Turkey. Although the economic importance of newly detected *S. dorsalis* in citrus fruits in Turkey is unknown, *T. hawaiiensis* continues to be a problem in lemons. Citrus producers randomly apply different groups of insecticides against thrips, mostly in the form of mixtures. In this way, the application of pesticides may cause different ecological problems in citrus ecosystems. Solution suggestions against invasive thrips species that are harmful to citrus fruits should be sought in integrated pest control programs. In this context, there is a need for basic studies on *S. dorsalis* in citrus groves in Turkey.

## Acknowledgements

We would like to thanks to Associate Prof. Dr. Lerzan Bakırcıoğlu Erkılıç and to Halil Demirbaş (Biological Agriculture Consulting and Engineering Services, Erzin/Hatay, Turkey) for their valuable contributions to the preparation of this manuscript.

## References

Arthurs S, McKenzie CL, Chen J, Dogramaci M, Brennan M, Houben K, Osborne L (2009) Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae), on pepper. Biological Control 49: 91-96.

Atakan E, Pehlivan S (2021) First report of the chilli thrips, *Scirtothrips dorsalis* Hood, 1919 (Thysanoptera: Thripidae) in Turkey. Turkish Journal of Zoology 45(2): 156-160.

Atakan E, Pehlivan S, Achiri TD (2021) Pest status of the Hawaiian flower thrips, *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) in lemons in the Mediterranean region of Turkey. Phytoparasitica 49: 513-525.

Atakan E, Ölçülü M, Pehlivan S, Özgür O (2016) An analysis of western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in lemons: Its abundance, distribution and damage status. Journal of Entomological and Zoological Studies 4(2): 109-114.

Atakan E, Ölçülü M, Pehlivan S, Satar S (2015) Türkiye'de yeni zararlı bir thrips türü: *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae). Türkiye Entomoloji Bülteni 5: 77-84.

Blank RH, Gill GSC (1997) Thrips (Thysanoptera: Thripidae) on flowers and fruity of citrus in New Zealand. New Zealand Journal of Crop and Horticulture Science 25: 319-312.

Childers CC, Nakahara S (2006) Thysanoptera (thrips) within citrus orchards in Florida: Species distribution, relative and seasonal abundance within trees, and species on vines and ground cover plants. Journal of Insect Science 45(6): 1-19.

Froud K, Stevens PS, Steve D (2001) Survey of alternative host plants for Kelly's citrus thrips (*Pezothrips kellyanus*) in citrus growing regions. New Zealand Plant Protection 54: 15-20.

Grove T, Giliomee JH, Pringle KL (2000) Seasonal abundance of different stages of the citrus thrips, *Scirtothrips aurantii*, on two mango cultivars in South Africa. Phtyoparasitica 28(1): 43-53.

Jae-Wook H, Rok-Yeon H, Kwan-Seok L, Jeong-Heub S, Pyoung-Ho Y, Hyeog-Mo K, Dong-Hee H, Kwang-Sik K (2012) Seasonal occurrence of Yellow tea thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) in citrus orchards and its damage symptoms on citrus fruits. Korean Journal of Applied Entomology 51(1): 1-7.

Klassen W, Seal DR, Ciomperlik MA, Fieselmann DA (2008) The chilli thrips, *Scirtothrips dorsalis*: current status in the Greater Caribbean Region. Proceedings of the Caribbean Food Crops Society 44: 103-117.

Kumar V, Kakkar G, McKenzie CL, Seal DR, Osborne LS (2013) An Overview of Chilli Thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) Biology, Distribution and Management. In: Soloneski S, Larramendy M (Ed), Weed and Pest Control - Conventional and New Challenges, pp. 53-77.

Kumar V, Seal DR, Kakkar G (2017) *Scirtothrips dorsalis* Hood (Insecta: Thysanoptera: Thripidae). UF/IFAS, Entomology and Nematology. https://entnemdept.ufl.edu/creatures/orn/thrips/chilli_thrips.htm. Accessed 27 September, 2021.

Longo S (1985) Thrips on citrus groves. Proceedings of Expert Meeting Acireale. In: Cavalloro R, Dimartino E (Ed), Integrated pest control in citrus groves. Balkema, Rotterdam, Boston, pp. 209-216.

Marullo R (1998) *Pezothrips kellyanus* UN nuovo tripide parassita delle colture. Informatore Fitopatologico 48(10): 72-74.

Masui S (2007) Synchronism of immigration of adult thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) to citrus orchards with reference to their occurrence on surrounding host plants. Applied Entomology and Zoology 42(4): 517-523.

Minaei K, Bagherian SAA, Aleosfoor M (2016) *Scirtothrips dorsalis* (Thysanoptera: Thripidae) as a pest of citrus in Fars province, Iran. Iranian Journal of Plant Protection 46: 219-225.

Morse JG, Hoddle MS (2006) Invasion Biology of Thrips. Annual Review of Entomology 51: 67-89.

Mound LA, Kibby G (1998) Thysanoptera: An Identification Guide. 2nd ed. CSIRO Entomology, Canberra.

Mound LA, Morris DC (2007) The insect order Thysanoptera: classification versus systematics. Zootaxa 1668: 395-411.

Mound LA, Palmer JM (1981) Identification, distribution and host plants of the pest species of *Scirtothrips*. (Thysanoptera: Thripidae). Bulletin of Entomological Research 71: 467-479.

Nas S, Atakan E, Elekçioğlu N (2007) Doğu Akdeniz Bölgesi turunçgil alanlarında bulunan Thysanoptera türleri. Türkiye Entomoloji Dergisi 31: 307-316.

Osborne LS (2009). Chilli thrips, *Scirtothrips dorsalis* Hood. http://mrec.ifas.ufl.edu/lso/thripslinks.htm. Accessed 24 September, 2021.

Ölçülü M, Atakan E (2013) Thysanoptera species infesting the flowers of citrus in the eastern Mediterranean region of Turkey. IOBC-WPRS Bulletin 95: 63-69.

Ravelo EE, Vaca JU, Arévalo EP, Delgado L, Díaz MF, Piñeros L, Castro ÁP, Brochero H, Goldarazena A (2018) Presence and distribution of *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) in Colombia. Journal of Insect Science 18(5): 1-10.

Sanap MM, Nawale RN (1987) Chemical control of chilli thrips, *Scirtothrips dorsalis*. Vegetable Science 14: 195-199.

Seal DR, Ciomperlik M, Richards ML, Klassen W (2006). Comparative effectiveness of chemical insecticides against the chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), on pepper and their compatibility with natural enemies. Crop Protection 25: 949-955.

Seal DR, Klassen W, Kumar V (2009). Biological parameters of chilli thrips, *Scirtothrips dorsalis* Hood, on selected hosts. Environmental Entomology 39(5): 1389-1398.

Seal DR, Kumar V (2010) Biological response of chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), to various regimes of chemical and biorational insecticides. Crop Protection 29(11): 1241-1247.

Seal DR, Kumar V, Klassen W, Sabine K (2008) Response of chilli thrips, *Scirtothrips dorsalis*, and melon thrips, *Thrips palmi*, to some selected insecticides. Proceedings of the Caribbean Food Crops Society 44: 578.

Tekşam İ, Tunç İ (2009) An analysis of Thysanoptera associated with citrus flowers in Antalya, Turkey: Composition, distribution, abundance and pest status of species. Applied Entomology and Zoology 44: 455-469.

TÜİK (2020) Türkiye İstatistik Kurumu, Bitkisel Üretim İstatistikleri. http.//www.tuik.gov.tr. Accessed 4 October, 2021.

USDA (2020) Citrus: World Markets and Trade. US Department of Agriculture, Foreign Agricultural Service. https://www.fas.usda.gov/data/citrus-world-markets-and-trade. Accessed 24 September, 2021.

Vassiliou VA (2007) Chemical control of *Pezothrips kellyanus* (Thysanoptera: Thripidae) in citrus plantations in Cyprus. Crop Protection 26(10): 1579-1584.

Venette RC, Davis EE (2004) Chilli thrips/yellow thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae). Mini Pest Risk Assessment. University of Minnesota, St. Paul, MN, USA, pp. 31.

Webster KWP, Cooper P, Mound LA (2006) Studies on Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae): sex attractants, host associations and country origin. Australian Journal of Entomology 45(1): 67-74.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 279-286
DOI: 10.29136/mediterranean.1002925

# Evaluation of expert reports filed for urgent expropriation: The case of Gelemen and Tekkeköy logistics center*

**Yeşim TANRIVERMİŞ[1], Banu Sultan BAŞOĞLU[2]**

[1]Ankara University, Faculty of Applied Sciences, Department of Real Estate Development and Management, 06590, Ankara
[2]Ankara University, Graduate School of Natural and Applied Sciences, Department of Real Estate Development and Management, 06590, Ankara

Corresponding author: Y. Tanrivermiş, e-mail: aliefendioglu@ankara.edu.tr
Author(s) e-mail: banu-aksu@hotmail.com

ARTICLE INFO

Received: September 30, 2021
Received in revised form: November 11, 2021
Accepted: November 11, 2021

***Keywords*:**

Urgent expropriation
Real estate valuation
Expert witness
Quality of valuation report
Logistic center

**ABSTRACT**

In this study, the expert reports received from the court for 51 real estate parcels over the past 20 years have been examined and evaluated. The expert reports are from the urgent expropriation process as per Article 27 of the Expropriation Law No.2942, and these reports have frequently been used by public institutions. In expropriation cases, the income valuation method was used by the real estate experts. It has revealed that the zoning and usage characteristics of the real estate parcels were not taken into consideration sufficiently during valuation, the parameters used in the valuation of the real estate by the expert committee and the determination of structures, facilities and plants on the real estate parcels were incomplete. Important mistakes were made in updating the sales values of comparable real estate parcels and, in general, the quality of the valuation study remained at a low level. In order to minimize these errors, members of the expert committees should be trained and should possess sufficient experience in the field of land acquisition, expropriation and valuation. In these conditions, the valuation studies should be carried out by real estate development experts and the employment of the said experts should be made obligatory in all public institutions and companies that provide consultancy services to them. Furthermore, it is necessary and beneficial to establish a monitoring-evaluation system with the effective supervision of the expertise process.

*This article is produced from the term project titled "Expert Reports Analysis in the Process of Expropriation and Determination of Valuation within the Scope of Article 27: Example of Gelemen and Tekkekoy Logistic Center Railway Connection Line", which is conducted by Banu Sultan Başoğlu and completed under the consultancy of Assoc. Prof. Dr. Yeşim Tanrıvermiş.

## 1. Introduction

Land acquisition for investment projects cannot be achieved by means of purchasing, barter and/or transfer of the real estate in question; therefore, real estate acquisition or confiscation through different expropriation methods (i.e., purchase, full expropriation, partial expropriation, establishment of easement rights, urgent expropriation) become inevitable and valuation procedures constitute the main subject of disputes in practice in expropriation processes. Determination of real estate values is required for the realization of investments and the resolution of disputes (Açıl 1977, Tanrıvermiş et al. 2011a,b, Tanrıvermiş and Aliefendioğlu 2019a,b).

In Turkey, especially in investment projects, the value of real estate in the investment areas or routes are determined by the expert committees within the scope of Article 27, and not examining all the advantage and disadvantage aspects of the real estate negatively affects expropriation processes. The foremost of the problems encountered in the determination of the expropriation prices is whether the prices determined by the expert committees reflect the real price or not. However, not all of the positive and negative aspects affecting real estate values are taken into account by expert committees whilst evaluating real estate. Also, the real estate values are not determined in the light of laws and scientific studies have caused increases in the costs of investment projects. Since the costs determined by expert committees in urgent expropriation price determination cases affect the values in price appreciation determination and registration cases pursuant to Article 10 of the Law No. 2942, the determined costs within the scope of Article 27 are important regarding the feasibility of investment projects in Turkey. In this study, a total of 105 real estate (or parcels) is evaluated, 5 of which are in the Aşağıçınık neighborhood and 100 of them are in the Tekkeköy neighborhood. The main objective of the study was to examine the prices determined by expert committees in accordance with Article 27 of Expropriation Law No. 2942, amended by the Law No. 4650 of 2001, to reveal the effect of the values appropriated as such on the expropriation process, and to determine the effect of expert reports on price.

## 2. Materials and Methods

The material of the study consists of information obtained through literature review; data collected through surveys, interviews, counting and measurement in the study and the surrounding settlements within the boundaries of the Gelemen and Tekkeköy Logistics Center Railway Connection Line Project and data obtained from public institutions and organizations. In the study, the expropriation plan bases of the Gelemen and Tekkeköy Logistics Center Railway Connection Line Project

were obtained and the bases were superimposed with satellite images.

A parcel-based assessment form was prepared to collect data for the real estate subject to expropriation. The assessment form, which includes all factors that may affect the value of the real estate, such as the use of municipal services and the distance to the nearest province, district, village or road, was prepared at the parcel level and applied. In the study area, 51 real estate parcels with land status, for which the lawsuits under Article 27 were filed, identified at the parcel level and in line with the data obtained. The value of these properties was appraised and a questionnaire form was developed in accordance with the scope and purpose of the study.

The data on rural/urban land and lot markets has not generally been recorded and in the absence of a sound registration system, the main data collection method is surveys (Yang 1986, Ventolo and Williams 2001, Tanrıvermiş et al 2004). In the neighborhood within the scope of the study, interviews were held about the sales values and characteristics of the lands with the owners and/or operators of the lands who actually bought and sold. In the study, records and studies of real estate offices trading in the market, local governments, and other public and private institutions operating in the study area were also used.

In the real estate valuation studies, it is necessary to systematically examine the properties of real estate, to classify them, to collect and analyze data and to estimate their final values for different purposes. In the selection of employed valuation methods, the factors such as the purposes of the valuation, legal regulations, properties of the real estate and market conditions must be considered together. Market values such as comparative sales analysis (equivalent or fair value), subtraction technique (conversion value), replacement price and shadow project, income value, cost value, development analysis and mixed methods are traditionally used in valuation studies (Çağan 1977, Murray et al. 1983, Tanrıvermiş et al. 2004, Tanrıvermiş 2017). In this study, the income valuation method was used for the expropriation cases reports. In valuation based on the cost method, the cost of rebuilding the buildings on the real estate under the economic conditions on the valuation date, the age of the building, and the wear rates are taken into consideration. In the cost analysis, the cost of all materials used in construction, the net costs of special construction and systems (cost minus accumulated depreciation) are considered. In this study, the cost prices of the buildings were determined by considering the approximate cost prices of the buildings in 2018, the depreciation rates of the Ministry of Finance, and the current state of the buildings. In the examination for the determination of the expropriation value, the classes of the structures should be clearly shown and their qualities should be stated (Tutar and Pulak 2006, Tanrıvermiş et al. 2011a, b).

The conversion of a cadastral parcel, which is included in the implementation development plan, into a zoning parcel by making arrangements in accordance with the Zoning Law and related regulations is called “parceling”. A real estate that has been converted into a zoning parcel in this way is considered a land plot (Köktürk and Köktürk 2016, Tanrıvermiş 2017). There were 51 real estate in the study area and all these real estate parcels have gained land plot status.

As the real estate examined have acquired the status of land plot, it has been legally obligatory to determine the values of real estate in the form of land plot as per the comparative sale analysis method. In the valuation of the real estate that will be partially or completely expropriated, an attempt has been made to find unstructured or empty precedents as much as possible, and for this purpose, similar precedents have been identified for the valued real estate. The actual purchase and sale values of the determined comparable properties were updated by applying the Domestic Producer Prices Index (D-PPI) (2003=100) of the Turkish Statistical Institute (TURKSTAT)’ and the land plot unit values were moved to the transaction date or the valuation date (January 2019). Principally, after determining the average equivalent value with the prices for the period of January 2019, the differences, superior features and deficiencies in terms of all positive and/or negative characteristics revealed by the separate examination of the valued real estates were evaluated one by one. The effects of each of these qualities and differences on the value of the real estate have been determined, and the values of the subject real estate have been appreciated based on the comparison made in this way.

The distances of the precedent used in the appraisal and the subject real estate to each other and to the main axes as well as the distances of the precedent and the subject real estate from the district center (for example, distance to trade and business centers) were determined by using the digitized bases in the computer environment.

## 3. Results

### *3.1. Examination of the precedent parcels in the Tekkeköy neighborhood*

In the study area, 2 precedent parcels were determined for the valuation within the borders of Tekkeköy neighborhood, which are subject to expropriation. The construction conditions and zoning characteristics of these precedents differ, and accordingly, the purchase and sale values per unit of the total construction area also change. The real estate that has acquired the status of land were appraised according to the comparative sales analysis (equivalent value) method. In this method, the market price data of the real estate or its precedent are used directly in the valuation of the real estate (Denyer-Green 1998, Ventolo and Williams 2001). The surface areas of the comparable real estate are different from each other and there are differences in their quality and uncertainties and, the construction conditions are variable. For this reason, it is seen that there is a significant variation among the precedent properties in terms of the purchase and sale values per unit of the total construction area. It is observed that the average purchase and sale values of the precedent identified in the neighborhood was between 302.59 TL $m^{-2}$ and 758.72 TL $m^{-2}$ (Table 1).

The satellite image of the real estate in the study area and the real estate selected as appropriately comparable for valuation are shown in Figure 1. In the decisions of the Court of Cassation, the points to be considered in “selection of precedent” are stated as follows: According to subparagraph (g) of Article 11 of the Expropriation Law, the real estate to be compared must be a precedent for the real estate subject to the lawsuit in the land plots. As can be understood from the meaning of the word, the comparable real estate must be of a quality that can set a sample for the real estate subject to the lawsuit.

**Table 1.** Data on comparable properties in Tekkeköy neighborhood of Samsun Province, Tekkeköy District.

| Data and Correction Factors | 1st Sale | 2nd Sale |
|---|---|---|
| Block /Parcel # | 159/7 | 1052/6 |
| Registration at the Land Registry | Yes | Yes |
| Sales Value Declared in the Land Registry (TL) | 35000.00 | 520000.00 |
| Type on Deed | Land Plot | Masonry Structure and Land Plot |
| Neighborhood/Village | Tekkeköy | Tekkeköy |
| Plot Size ($m^2$) | 209.85 | 1216.00 |
| Actual Selling Price (TL) | 35000.00 | 520000.00 |
| Date of Sale | 05.13.2014 | 02.27.2015 |
| PPI (Valuation Date, January 2019) | 424.86 | 424.86 |
| PPI (On Sale Date) | 234.18 | 239.46 |
| Sales Value at the Valuation Date, TL (January 2019) | 63498.59 | 922605.86 |
| Unit Value on the Valuation Date (TL $m^{-2}$) | 302.59 | 758.72 |
| Description | Residential+commercial area, adjoining structures, 5 floors | Wholesale trade area height up to 10 m max |


**Figure 1.** Satellite image of comparable and real estate in the study area (TKGM 2019).

Due to the differentiation of the zoning functions and construction conditions of the examined comparable real estate, adjustments were made on the data related to the precedent parcel. In the Tekkeköy neighborhood, the zoning features of the comparable property #7 on block 159 were determined as residential and commercial area with 5 stores in an adjoining structure, while the comparable real estate # 6 on block 1052 in the Tekkeköy neighborhood was determined as a commercial area with a maximum height #10 m. As there is no precedent with the same characteristics due to the problem of buying and selling in the region, the precedent has been adjusted by considering the construction conditions and functions of the 2 real estate parcels determined as comparable. It has been decided that if the real estate #7 on block #159, which is taken as comparable to number one, was used as a residential area in its entirety, there may be a 10% drop in value from the unit price. It has been decided that if the real estate #6 on block #1052, which is taken as comparable to number two, was used as a residential area in its entirety, there may be a 35% drop in value from the unit price. The average comparable value obtained after the correction over the updated sale values of the comparable real estate was determined as 272.33 TL $m^{-2}$ for real estate #1 and 493.17 TL $m^{-2}$ for real estate #2 and the average comparable value was calculated as 382.75 TL $m^{-2}$ and the valuation study was completed based on this value.

### *3.2. Appraisal of unit values and total value of real estate*

A precedent correction was made in the Tekkeköy neighborhood, and the parameters used in the precedent correction and the possible positive and/or negative effects of these parameters on the value were determined based on the impact scores in the results of the market research conducted in the surrounding area (Table 2). A total of 51 real estate parcels within the borders of the Tekkeköy neighborhood in the study area, which will be partially and completely expropriated, have been appraised. The project line of the real estate that fall in the study area has been superimposed with the satellite images. Since the real estate examined and the comparable real estate parcels do not have similar properties in terms of zoning (cadastral parcels), it has been obligatory to make a reduction on the parcel area or value at the rate corresponding to the regulation

**Table 2.** Factors that may cause an increase or decrease in the market value of the appraised real estate in Tekkeköy neighborhood compared to comparable parcels, and their average impact scores

| Value Correction Parameters | Correction Factors (%) |
|---|---|
| Allocation of the real estate subject to expropriation as a road (decrease in value) | 35 |
| Proximity to the sea of the real estate subject to expropriation compared to comparable parcels (increase in value) | 10 |
| Allocation of the real estate subject to expropriation as a park area (decrease in value) | 30 |
| Allocation of the real estate subject to expropriation as an industrial area (increase in value) | 25 |
| Allocation of the real estate subject to expropriation as a tourism area (increase in value) | 10 |
| The real estate subject to expropriation is facing the road (increase in value) | 10 |
| Unlike the comparable land plot, no DOP is applied to the land in question (decrease in value) | 40 |
| That the real estate subject to expropriation becomes a channel (decrease in value) | 35 |
| The real estate subject to expropriation is public (Treasury) property (decrease in value) | 40 |
| The high density of construction at the location of the real estate subject to expropriation (increase in value) | 50 |
| Allocation of the real estate subject to expropriation as a park and forest area (decrease in value) | 35 |
| The real estate subject to expropriation is in a central location (transportation/socio-cultural areas) compared to comparable parcels (increase in value) | 20 |
| The real estate subject to expropriation is in a central location (socio-cultural areas) compared to comparable parcels (increase in value) | 10 |
| The real estate subject to expropriation is not in a central location (transportation, etc.) compared to comparable parcels (decrease in value) | 20 |
| The real estate subject to expropriation is not in a central location (transportation, etc.) compared to comparable parcels (decrease in value) | 25 |
| The real estate subject to expropriation is not in a central location compared to comparable parcels (decrease in value) | 20 |
| The real estate subject to expropriation is not in a central location compared to comparable parcels (decrease in value) | 30 |
| The real estate subject to expropriation is not in a central location compared to comparable parcels (decrease in value) | 10 |
| Due to the proximity of the real estate subject to expropriation to the road (increase in value) | 10 |
| The real estate subject to expropriation becomes a non-registered road (decrease in value) | 40 |
| The real estate subject to expropriation becomes a non-registered park (decrease in value) | 35 |
| The real estate subject to expropriation becomes a port back area (decrease in value) | 40 |

partnership share. Within the scope of the study, the comparable value in Tekkeköy neighborhood is 382.75 TL $m^{-2}$ and it has been determined between 160.76 TL $m^{-2}$ and 631.54 TL $m^{-2}$ considering the positive and negative characteristics of the real estate subject to expropriation.

The total 51 real estate subject to expropriation were appraised at the parcel level, and by using these values, it was decided to purchase them within the scope of Article 8 of Law No. 2942 as amended by Law No. 4650. A compromise could not be reached in the purchase transactions and the administration filed urgent expropriation cases under Article 27. The valuation reports prepared by the relevant institution in urgent expropriation cases are important as they are used as a reference point for expert committees. It was determined that 5 of the 51 real estate parcels examined in the study area had structures, 15 of them had trees and 35 real estate parcels were completely empty. A total value of 63915709.20 TL was appraised for the 51 real estate, the ground value of which was 61975669.52 TL, the structure cost was 727633.80 TL, and the tree cost was 6872.77 TL. In the study area, 145439.66 $m^2$ of the real estate have been subject to expropriation, and the average floor price has been determined as 426.13 TL $m^{-2}$, and the total average unit price, including the building and trees, as 439.47 TL $m^{-2}$.

### *3.3. Valuation process of building types*

Building structures were identified in 5 of the 51 real estate parcels in the study area, and these structures were calculated over the approximate unit costs of the building to be used in the calculation of architectural and engineering service costs. The value to be added to the ground value of the real estate, if any, of the structure on the real estate constitutes the net cost value.

The area of the reinforced concrete observation tower is 24.00 $m^2$ and it has been determined that it should be among the Class I, Group A structures in the official unit price chart. According to the results of the determination and market research, no increase or decrease in the value of the reinforced concrete observation tower on the real estate was calculated, and the total present value of the building was found to be 3672.00 TL. Since the reinforced concrete structure is 10 years old, the depreciation cost over 10% depreciation rate was taken as 367.20 TL and the net present cost value was determined as 3304.80 TL. The area of the reinforced concrete warehouse is 184.20 $m^2$ and it has been determined that it should be among the Class I, Group B structures in the official unit price chart. According to the results of the determination and market research, it has been determined that there may be a 10% decrease in the cost value due to the neglected and incomplete manufacture of the reinforced concrete warehouse on the property, and the total present value of the structure was found to be 4199.76 TL. Since the reinforced concrete structure is 10 years old, the depreciation cost over 10% depreciation rate was taken as 3779.78 TL and the net present cost value was determined as 34018.06 TL. The area of the concrete ground is 4445.00 $m^2$ and it has been determined that it should be among the class I, group A structures in the official unit price chart. According to the results of the determination and market research, no increase or decrease in the value of the concrete ground on the real estate was calculated, and the total present value of the building was found to be 680085.00 TL. Since the concrete structure is 10 years old, the

depreciation cost over 10% depreciation rate was taken as 68008.50 TL and the net present cost value was determined as 612076.50 TL.

### 3.4. Comparison of the costs in the expert reports prepared in accordance with Article 27 and the prices in the valuation reports regarding the study area and suggestions for solutions

Regarding the 51 real estate parcels among the real estate that fall into the study area, of which purchase was not successful within the scope of Article 8 of the Law No. 2942 amended by the Law No. 4650, urgent expropriation lawsuits were filed and expert reports were prepared for each real estate within the scope of Article 27 of the same law. A comparison was made of the zoning characteristics of the real estate covered by the expert reports and the valuation reports. In the expert reports, it was determined that all of the real estate have acquired the status of land plots. When 51 real estate parcels that were expropriated in the study area were examined, it was confirmed that all real estate has acquired the status of land plot. An examination of the zoning characteristics of the real estate indicated that there was no difference between the zoning properties of the real estate and the results of the study in the expert reports.

As a result of the expert committee's determination based on their own subjective opinion of whether or not the regulation partnership share was deducted from the real estate #6 on block #1873 without relying on any official documents, the board of experts made a significant mistake in calculating the price of the real estate. While there was no difference in 50 of the 51 real estate parcels in the study area, in terms of land and land plot distinction and zoning characteristics, it was ignored that the regulation partnership share was deducted in the calculation of the values of the real estate #6 on block #1873. A comparison of the properties in the expert reports and the valuation reports in terms of structures and outbuildings was made. The determination of the real estate has an important place in the evaluation of real estate that will be subject to expropriation. In the determination of real estate, it is necessary to determine the area of the real estate subject to expropriation, the trees of different ages and types in this area, and the structures in accordance with Article 11 of the Expropriation Law. The real estate in question should be evaluated in accordance with the same law.

According to the results of the study, it was determined that there were 5 buildings and 15 trees in the 51 real estate parcels in the study area. In the determinations made by the court in April 2019, it is noteworthy that no trees and structures were identified regarding the real estate subject to expropriation. While calculating the expropriation value, it is necessary to calculate not only the ground value, but also the value of the structures and trees on it. From the point of view of beneficiaries, it is a significant problem that this calculation was overlooked. While the objections to the incomplete determination of the already attached structures on the real estate have an important place in the determination of the expropriation prices in Turkey, the fact that the attached structures on the real estate were not taken into account in the relevant project makes the accuracy of the expert report questionable. According to the results of the study, the attached structures with a value of 734506.57 TL on the total real estate, including the buildings with a value of 727633.80 TL and trees with a value of 6872.77 TL, were ignored by the expert committee as part of the calculations.

In the study, the same comparable parcel was used for all of the real estate as all the 51 real estate parcels subject to expropriation are on the same route. For the valuation to be made within the scope of the study, 2 precedents, which have been the subject of real purchase and sale transactions, were found and the average comparable value was determined as 382.75 TL $m^{-2}$. The precedent value stated has been confirmed as a result of discussions with real estate offices and build-sell offices in the region. While the expert committees formed for the 51 real estate parcels in the study area are different, it is understood that the precedent for the real estate was the same. The expert committees have chosen one comparable parcel, and due to the different valuation dates, the unit comparable value has been taken as 1284.34 TL, 1287.91 TL and 1289.91 TL. Although the expert committee conducted the valuation processes on different dates, the Wholesale Price Index (WPI) on 03.03.2015, which is the date of the purchase and sale of the real estate, was taken differently.

It has been determined that the expert committees' updating the determined comparable over the Wholesale Price Index (WPI) and not using the Domestic Producer Price Index (D-PPI) in the update is contrary to judicial decisions. In the determination of the unit prices determined by the expert committees in the study area, the scoring method was used for the real estate. The unit costs were found by dividing the coefficient obtained as a result of the scoring by the comparable value. In the scoring made by the expert committee, the reason for the scores given for the case in question was not explained. In the scoring of the real estate, as depicted in Figure 2, it has been determined that different expert committees use different scoring for the real estate that are next to each other and have the same features. Different expert boards made the calculations in different ways and whilst one expert board gave 20 points to the possibility of constructing buildings according to their zoning status in the scoring, they used for real estate #1 and #2 on block #609 with the same characteristics, whereas, the other expert board did not use the mentioned parameter (Table 3, Table 4).

The fact that the parameters used in the valuation of the real estate by the expert committees do not overlap with each other reveals that the values of the real estates were determined far from reality. Even the differentiation of the main parameters to be used for two adjacent properties with the same characteristics makes the realism of the expert reports questionable.

The fact that the panel of experts determines different parameters for two real estate parcels with the same characteristics using the same comparable parcel, as can be seen in Table 3 and Table 4, and that scoring is done over these parameters clearly shows that the real market value of the real estate cannot be reflected accurately.

According to the results of the study, the total title deed area of the 51 real estate parcels is 2189926.92 $m^2$, the expropriated area is 145439.66 $m^2$, and the remaining area is 2044487.26 $m^2$ whereas the comparable value determined in the expert report ranges between 1284.34 TL $m^{-2}$ and 1289.91 TL $m^2$, and the comparable value was found to be 382.75 TL $m^{-2}$. According to the expert's report, an adjustment was made in the comparable parcels value, taking into account all the positive or negative features found for the real estate and the comparable value varies between 216.00 TL $m^{-2}$ and 650.00 TL $m^{-2}$, and the adjusted comparable value calculated according to the study results varies between 160.76 TL $m^{-2}$ and 631.54 TL $m^{-2}$. While the total floor price calculated according to the expert's report was 72447603.55 TL, the total floor price calculated based on the

**Figure 2.** Satellite image of 609 blocks and 1 and 609 blocks and 2 parcels in the study area (TKGM 2019).

**Table 3.** Parameters and scoring used for block #609 and real estate #2

| Comparison Criteria for Comparable Real Estate Subject to Litigation | Score of Comparable Parcel | 609/2 |
|---|---|---|
| Possibility of Building Based on Zoning Status | 35 | 20 |
| Utilization of Water, Electricity, Infrastructure and Energy Services | 10 | 7 |
| Area Size That Makes Sale Attractive | 3 | 5 |
| Geometric Shape of the Parcel, Construction Status | 3 | 3 |
| Distance to Public Institutions and Organizations, City Center and Social and Economic Activity Buildings | 10 | 5 |
| Availability and Ease of Access to Ring Roads | 5 | 3 |
| Preference Based on Commercial Features | 30 | 3 |
| Population Density in the Region and Distance to Schools, Religious Facilities, Settlements | 4 | 4 |
| **Total** | **100** | **50** |

**Table 4.** Parameters and scoring used for block #609 and real estate #1

| Comparison Criteria for Comparable Real Estate Subject to Litigation | Score of Comparable Parcel | 609/1 |
|---|---|---|
| Road, Water, Sewerage, Electricity, Telecom Infrastructure | 20 | 10 |
| Access to Main Roads | 15 | 7.5 |
| Distance to City Center | 25 | 10 |
| Area Size That Makes Sale Attractive | 5 | 5 |
| Parcel Geometry | 5 | 5 |
| Distance to School, Hospital, Religious Facility Areas | 30 | 12 |
| **Total** | **100** | **50** |

study results were found to be 61975669.52 TL. In the expert report, no value decrease was calculated for the remaining part, and according to the results of the study, 1205533.11 TL value decrease was appraised for the remaining part. In the expert's report, no calculations regarding the already attached structures were made. However, in the study conducted, a tree value of 6872.77 TL and a building value of 727633.80 TL were calculated. In this case, the total expropriation value calculated by the Board of Experts was found to be 72447603.55 TL and the total expropriation cost calculated according to the results of the study was 63915709.20 TL. As a result of the incomplete determination of the expert committees, a total of 8531894.35 TL was overestimated in the real estate expropriation reports. The total expropriation cost, which was assessed by the expert committees, resulted in a 13.35% higher price than it should have been (Table 5).

For the real estate subject to expropriation, the expert reports at the parcel level and the results of the study were compared in terms of price. As a result of the missing determination by the expert committee, it is seen that the real estate was valued at 11834865.96 TL higher than its real value in 21 parcels and 3302971.61 TL less than its real value in 30 parcels. The expert reports and the results of the study were compared at the parcel level, and differences were revealed in terms of price.

Expert reports and study results of 51 real estate subjected to expropriation were examined at the parcel level, and the reasons for the differences were revealed. The main reasons for the difference between the expert reports and the results of the study

**Table 5.** 609 Comparison of the expert reports and study results of the real estate in the study area in terms of comparable parcels, unit price and total price

| **Parameters** | **Expert Report** | **Study Result** |
|---|---|---|
| Number of Parcels | 51 | 51 |
| Total Area ($m^2$) | 2189926.92 | 2189926.92 |
| Expropriated Area ($m^2$) | 145439.66 | 145439.66 |
| Remaining Area ($m^2$) | 2044487.26 | 2044487.26 |
| Comparable Value (TL $m^{-2}$) | 1284.34 | 382.75 |
| | 1287.91 | |
| | 1289.91 | |
| Adjusted Comparable Value (TL $m^{-2}$) | 216.00 - 650.00 | 160.76 - 631.54 |
| Total Ground Value | 72447603.55 | 61975669.52 |
| Value of Remaining Value Decrease (TL) | - | 1205533.11 |
| Tree Value (TL) | - | 6872.77 |
| Structure Value (TL) | - | 727633.80 |
| Total Value (TL) | **72447603.55** | **63915709.20** |
| **Difference (TL)** | **8531894.35** | |
| **Increase Rate (%)** | **13.35** | |

regarding the real estate are that the zoning features of the real estate (such as no DOP deduction, DOP deduction for a 2nd time, allocation as road, park and forest in the plan) are not fully reflected in the price of the expert reports and the decrease in value due to the project in the remainder after expropriation is not taken into account by the expert committee.

In order to minimize the errors in the values determined by the expert committees, first of all, the people in the expert committees should have the necessary knowledge, experience and training. The establishment of a real estate information system at the parcel level in a way that reflects the real values of real estate throughout Turkey and making it available to the related persons, institutions and organizations will contribute to the fairness of the prices to be determined by the expert committees in determining the cost of the real estate required for land acquisition in investments made by the public and private sectors.

In the evaluation of real estate as per the Expropriation Law, it is at least necessary to take steps to ensure that the necessary information and documents can be obtained by experts and relevant institutions. For example, making a distinction between whether the real estate is land or a land plot, infrastructural studies to easily provide data such as zoning characteristics from the internet will ensure that the errors in the valuation of the real estate are minimized. In order to ensure that the deficiencies and errors in the expert reports determined within the scope of Article 27 of the Expropriation Law No. 2942, amended by the Law No. 4650 of 2001, are corrected in the lawsuits filed within the scope of Article 10 of the same law, both the relevant institution and the owner of the real estate subject to expropriation must raise objections for the deficiencies to be eliminated

## 4. Discussion and Conclusion

Within the scope of the Gelemen and Tekkeköy Logistics Center Railway Connection Line Project examined in the study, the real estate in the expropriation area were primarily classified as land and land plots, and it was determined that all of the 51 real estate subjected to expropriation were land plots. Since the real estate are land type, a precedent survey was conducted. In the study area, in Tekkeköy neighborhood, 2 comparable real estate parcels were identified, and the comparable value found according to the results of the research is 382.75 TL $m^{-2}$, and when comparable adjustment is made by considering all the positive or negative properties of the real estate, it is seen that the adjusted comparable value varies between 160.76 TL $m^{-2}$ and 631.54 TL $m^{-2}$.

It was determined by the expert committees in the study area that 51 real estate parcels qualified as land within the scope of Article 27. For the real estate in the study area, 1 precedent used in the valuation was determined and the comparable value varies between 1284.34 TL $m^{-2}$ and 1289.91 TL $m^{-2}$, and it was determined that a comparable adjustment has been made between 216.00 TL $m^{-2}$ and 650.00 TL $m^{-2}$. When the expert reports prepared for the real estate in the study area were examined, it is noteworthy that the structures and outbuildings on the 16 real estate subjected to expropriation were not taken into account in the determination of the price, and the price determination without considering the attached structures reveals the deficiency of the explorations made for the real estate. The real estate valuation starts with the identification of the real estate, and it is seen that the deficiency made in the said identification starts with shortcomings in the first stage of real estate valuation and is reflected in the price. The zoning characteristics of the real estate are essentially the most important factor in relation to the value, and the fact that this characteristic is not associated with the value prevents the real value of the real estate from being achieved. It is seen that the price differences especially for the parcels in the study area were appraised without considering the zoning characteristics of the real estate.

The fact that the expert committees used the same comparable parcel for 51 real estate in the study area and that the comparable parcel sales were updated on the Wholesale Price Index instead of the Domestic Producer Price Index (D-PPI) contradicts judicial decisions. The price index taken differently by the expert committees for the real estate chosen as a comparable parcel caused a price difference between all the valuated real estate.

In order to minimize the errors in the values determined by the expert committees, first of all, it emerges that the people in the expert committees should have the necessary knowledge, experience and training. The establishment of a real estate information system at the parcel level in a way that reflects the real values of real estate all around Turkey and making it

available to the related persons, institutions and organizations will contribute to the fairness of the prices to be determined by the expert committees in determining the cost of the real estate required for land acquisition in investments made by the public and private sectors. In these circumstances, it is considered mandatory that valuation studies should be carried out by real estate development and management experts; such experts should be employed in all public institutions and companies that will provide consultancy services to them; and expert committees to be formed by courts should primarily consist of real estate development and management experts. Lastly, the limitation of this study is that it is hard to obtainthe data, which are court reports, and the data is also very limited.

## References

Açıl AF (1977) Türkiye'de 1950-1974 Yılları Arasında Muhtelif Tarımsal Arazi Nevilerinin Kıymetleri ve Bunlardaki Değişmeler, Ankara Üniversitesi Ziraat Fakültesi Yayınları: 619, Ankara.

Çağan N (1977) Anayasa Mahkemesi ve kamulaştırmada vergi değeri. Ankara Üniversitesi Hukuk Fakültesi Dergisi 411: 247-256.

Denyer-Green B (1998) Compulsory Purchase and Compensation, Fifth Edition, Estates Gazette, London, UK.

Köktürk E, Köktürk E (2016) Taşınmaz Değerlemesi. Seçkin Yayıncılık, Ankara, s. 1374.

Murray WG, Hariss DG, Miller GA, Thompson NS (1983) Farm Appraisal and Valuation. The Iowa State University Press, Sixth Edition, 303, Iowa, USA.

Tanrıvermiş H, Gündoğmuş E, Demirci R (2004) Arazilerin kamulaştırma bedellerinin takdiri tarım arazilerinin kamulaştırma bedellerinin takdirinde kullanılabilecek kapitalizasyon faiz oranları, arazi gelirleri ve arazi birim değerleri. EDUSER Yayınları, Ankara, s. 422.

Tanrıvermiş H, Aliefendioğlu Y, Demirci R (2011a) Doğanözü Barajı göl alanı ve mücavir sahadaki arazilerin gelirleri ve kapitalizasyon oranları ile birim arazi değerlerinin araştırılması. Ankara Üniversitesi Fen Bilimleri Enstitüsü Taşınmaz Geliştirme Anabilim Dalı Yayın No: 6, Ankara.

Tanrıvermiş H, Aliefendioğlu Y, Demirci R, İşlek BG (2011b) Namazgah Barajı göl alanı ve mücavir alanda arazi gelirleri kapitalizasyon oranın birim arazi değerleri ve kamulaştırma bedellerinin analizi. Ankara Üniversitesi, Fen Bilimleri Enstitüsü Taşınmaz Geliştirme Anabilim Dalı Yayın No: 7, Ankara.

Tanrıvermiş H (2017) Gayrimenkul Değerleme Esasları, SPL Sermaye Piyasası Lisanslama Sicil ve Eğitim Kuruluşu, Lisanslama Sınavları Çalışma Kitapları Ders Kodu: 1014 (Konut Değerleme Sınavı, Gayrimenkul Değerleme Sınavı), Ankara.

Tanrıvermiş H, Aliefendioğlu Y (2019a) The Impacts of Land Acquisition and Resettlement Activities of Infrastructure Investments on Natural and Cultural Heritages and Social and Economic Life: The case of Turkey (Chapter 7), In: Handbook of Contemporary Issues in Expropriation, Eds: Pliemmer and McCluskey, Routledge Taylor & Francis Group, Oxfordshire, UK, pp:118-157.

Tanrıvermiş H, Aliefendioğlu Y (2019b) Legal and Institutional Fundaments of Expropriation and Compensation Issues in Turkey (Chapter 10), In: Handbook of Contemporary Issues in Expropriation, Eds: Pliemmer and McCluskey, Routledge Taylor & Francis Group, Oxfordshire, UK, pp: 194-228.

TKGM (2019) Tapu ve Kadastro Genel Müdürlüğü. http://parselsorgu.tkgm.gov.tr. Accessed October 11, 2019.

Tutar M, Pulak TM (2006) Açıklamalı-İçtihatlı-Notlu Karşılaştırmalı Eski-Yeni Kamulaştırma Davaları. Adalet Yayınevi, Ankara, s. 2476.

Ventolo WL, Williams MR (2001) Fundamentals of real estate appraisal. Dearborn Real Estate Education, 8th Edition, 446, Chicago, USA.

Yang WF (1986) Zirai işletmecilikte tetkik ve araştırma metodları. Ege Üniversitesi Ziraat Fakültesi Yayın No: 90, Ege Üniversitesi Matbaası: İzmir, s. 228.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 287-291
DOI: 10.29136/mediterranean.891722

# Co-digestion of spoiled maize silage with cattle manure

**Ali AYBEK[1], Mehmet SOLAK[2], Kamil EKINCI[3]**

[1]Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Biosystems Engineering Department, 46100, Kahramanmaras, Turkey
[2]Siirt University, Faculty of Agriculture, Department of Biosystems Engineering, Siirt, Turkey
[3]Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Agricultural Machinery and Technologies Engineering, 32260, Isparta, Turkey

Corresponding author: A. Aybek, e-mail: aaybek@ksu.edu.tr
Author(s) e-mail: solakmehmet@yandex.com, kamilekinci@isparta.edu.tr

ARTICLE INFO

Received: March 5, 2021
Received in revised form: October 4, 2021
Accepted: October 6, 2021

***Keywords***:

Spoiled maize silage
Cattle manure
Co-digestion
Biogas
HBT

**ABSTRACT**

In this study, spoiled maize silage (SMS) and cattle manure were co-digested at five different mixtures by the Hohenheim Batch Yield Test unit under mesophilic conditions to explore biogas production possibilities of these wastes together. The mixtures were 100% cattle manure, 100% SMS, 85% cattle manure + 15% SMS, 70% cattle manure + 30% SMS, and 55% cattle manure + 45% SMS. Chemical properties of raw materials and mixtures, including crude fat, dry matter, organic matter, and acid detergent fiber (ADF) contents were determined. As the amount of SMS in the mixtures increased, biogas and methane production increased. The highest cumulative specific biogas and methane production were determined for 100% SMS as 0.62 $Nm^3$ $kg^{-1}$ organic matter (OM) and 0.31 $Nm^3$ $kg^{-1}$ OM, respectively, where $Nm^3$ is the volume of biogas under normal conditions. Methane content of the mixture containing SMS (49.99% to 51.87%) was higher than that of cattle manure only (44.01%). Furthermore, the mixtures which had lower ADF content yielded more methane and biogas. In conclusion, the efficiency of biogas and methane production can be increased by applying the co-digestion technique.

## 1. Introduction

An increase in the world population led to increased energy demand. One of the most vital development indicators of the countries is energy consumption per capita (Ergüneş et al. 2009; Ulusoy et al. 2009; Aybek and Üçok 2017). Fossil fuels such as petroleum, natural gas, and coal are used to meet the energy demand in the World (Onurbaş Avcıoğlu et al. 2011; Yılmaz 2012; IEA 2015). Renewable energy sources are alternatives to fossil energy sources provided by nature. Renewable energy originating from rivers, wind, geothermal energy, biomass, and sun are the energy sources that do not cause environmental pollution (Temiz and Gökmen 2010). Biogas technology is at the forefront of renewable energy production. Biogas is a flammable gas originating from the anaerobic digestion of organic substances containing carbon dioxide, methane, and other small amounts of trace gasses.

Biogas technology with environmental and economic benefits is crucial (Angelidaki and Ahring 1993). Biogas production enables organic wastes to be used both as organic fertilizers and to gain an economic value by producing energy (Üçgül and Akgül 2010). Currently, many studies have been carried out determining the biogas potential of various organic wastes. Determination of biogas potentials of organic wastes is essential for the design and economics of biogas plants (Korkmaz et al. 2012). Biogas production from organic materials with high biomethane potential can be feasible (Mittweg et al. 2012a). Significant amounts of energy can be recovered from agricultural residues/wastes in Turkey for biogas production. The utilization of organic wastes in biogas production provides an economic contribution by generating energy and eliminating the adverse effects of these wastes on air, water, and soil.

Furthermore, the digestate of the anaerobic process can be applied to agricultural land as an organic soil conditioner (Yaldız 2000). Since there is a significant potential for organic wastes in Turkey, the determination of the biogas potential of these wastes and research on the possibilities of usage of biogas as renewable energy sources lead to a robust database (Aybek et al. 2015). Wastes from plant and animal production in agriculture, which have high potential in rural areas in Turkey, are the most critical materials for biogas production.

Biogas production from energy crops is widespread around the world. Of many products utilized in biogas plants, maize silage holds a key position for biogas production due to its high methane yield and chemical composition (Oslaj et al. 2010). In various regions of the world, silage maize (Zea mays L.) is commonly cultivated. The crop offers a very consistent production over a wide range of climatic and agronomic circumstances and a high energy content and strong ensiling properties. Furthermore, maize silage in dairy cows' grass or grass silage-based diets enhances feed intake, milk production, and milk protein content (Phipps et al. 1995; Khan et al. 2012). Storage of biomass is the ultimate problem; agricultural products tend to rot quickly when not properly stored. In both energy production and animal feeding, the quality of silage and the reduction of dry matter losses are vital factors to consider (Borreani and Tabacco 2010a; Borreani and Tabacco 2010b).

Microbial oxidation of silage fermentation products, such as lactic acid and residual water-soluble carbon to carbon dioxide and water is induced by oxygen penetration into the silo. There is a rise in temperature above ambient, as well as increased mass and nutritional losses (Wilkinson and Davies 2013). Aerobic deterioration - rot - is caused by failure to follow the technological discipline of silage preparation or storage or the use of inappropriate feed. With access to air, this deterioration rots silage through biological reactions. The unexpected air entry into silages creates butyric, acetic, or formic acids, rendering silage useless. Depending on the density and porosity of the plant material and the rate of silage removal, silos are opened, and the air is allowed to enter the silage. This results in the proliferation of unwanted aerobic microorganisms, such as yeasts and molds, which were previously present in the silage, as well as a rise in pH (Driehuis and Elferink 2000).

There have been many studies carried out on mono and co-digestion of maize silage. Research consisting of both laboratory and full scale investigated the possibilities of using maize silage for biogas production. It showed that maize silage is a suitable substrate for anaerobic digestion and biogas production.The observed specific methane generation for long-term maize silage processing in a mixed laboratory anaerobic reactor was 0.316 $Nm^3$ $kg^{-1}$ of VS (volatile solids) (Hutňan 2016). According to a study by Hutňan et al. (2010), specific biogas generation from maize silage reached 0.66 $m^3$ $kg^{-1}$ VS. Another study found that co-digestion of maize silage with chicken manure in 5-liter digesters led to 0.31 L $CH_4$ $g^{-1}$ VS specific methane output (Sun et al. 2016). When comparing sugar beet and maize silage, researchers discovered that maize silage had a slightly lower anaerobic conversion rate than sugar beet silage due to a large number of complex chemicals (Klang et al. 2015).

The HBT (Patent No. 10227685, 20.01.2005) is a high-efficiency discontinuous laboratory batch method (Heffrich et al. 2003) for determining the biogas and methane production potential of varying substrates according to the VDI Guideline 4630 (VDI-Richtlinie 4630 2006). An advantage of the HBT over the 2 L batch approach is the higher performance due to the significant number of digesters per batch method to determine biogas and methane production potential. Additionally, only small quantities of the substrate to be tested are needed for the analyses, making the HBT applicable for plant breeding approaches where only small amounts of the plant material are available (Mittweg et al. 2012b).

The objective of this study was to determine the biogas production efficiencies of the mixtures obtained from cattle manure and SMS by using the Hohenheim Batch Yield Test (HBT) The specific aim of this study was to determine specific biogas production as a function of mixtures of cattle manure and SMS.

## 2. Materials and Methods

### *2.1. Material preparation*

Cattle manure and SMS were obtained from livestock farms in the Eastern Mediterranean Region. The wastes were dried at room temperature for three weeks. Wastes were ground in 1 mm size according to the standards related (VDI 4630 2006) using an industrial type grinder. The main characteristics of the raw materials (cattle manure and SMS) are reported in Table 1. The moisture contents of the samples were determined as 9.26% and 6.44% (wet basis) for cattle manure and SMS, respectively. Cattle manure had an organic matter (OM) of 90.79%, while MS had an organic matter of 95.02%. Cattle manure and SMS had a crude fat content of 2.30 and 2.33%, respectively. Cattle manure was rich in Acid Detergent Fiber (ADF) of 60.19%. Values reported are on a dry weight basis except for moisture content on a wet weight basis. Compositions of feeds at the beginning of the experiment are given in Table 1. The number of replication for each mixture was 3.

### *2.2. Preparation of inoculum*

Inoculum, which is a mixture of liquid + solid phase, was obtained from Gaziantep Water and Sewerage Administration (GASKI) central wastewater treatment plant. The four-layered cheesecloth was used to filter, mixed with a 1:2 ratio of buffer solution. Buffer solution; 500 mL of distilled purified water was formed from 0.1 mL of solution A, 200 mL of solution B, 200 mL of solution C, 1 mL of resazurin (0.1%, w $v^{-1}$) solution C, and 40 mL of solution E. Solution A was mixed with 13.2 g $CuCl_{22}H_2O$, 10.0 g $MnCl_{24}H_2O$, 1.0 g $CoCl_{26}H_2O$, and 8.0 g $FeCl_2H_2O$ in distilled water. Solution B was dissolved in 35 g of $NaHCO_3$ and 4 g of $NH_4HCO_3$ in pure water to complete 100 mL. Solution C was dissolved in 5.7 g of $Na_2HPO_4$, 6.2 g of $KH_2PO_4$, 0.6 g of $MgSO_47H_2O$ in pure water, and 1000 mL was completed. Solution D was dissolved in 0.5 g of resazurin pure water to complete 100 ml. Solution E consists of 95 mL of distilled water, four mL of 1 N NaOH, and 625 mg $Na_2S_9H_2O$.

### *2.3. Biogas and methane measurements*

The biogas experiment was carried out using the HBT method (Heffrich and Oechsner 2003). In this study, the prepared samples were placed into 100 mL glass syringes located in the incubator. Likewise, three inoculum syringes, each containing 30 mL of inoculum for control group samples were also placed in the sections of the incubator. The syringes were placed horizontally into the incubator at 37°C after the inoculum was placed. The methane measurement system was used to determine the methane content before the incubator was calibrated with

**Table 1.** Chemical properties of materials

| Mixes | Materials in mixtures | | Moisture content | Dry matter | Organic matter | Crude fat | ADF |
|---|---|---|---|---|---|---|---|
| | Cattle manure (%) | SMS (%) | (%) | (%) | (%) | (%) | (%) |
| Mixture-1 | 100 | - | 9.26 | 90.74 | 90.79 | 2.30 | 60.19 |
| Mixture-2 | - | 100 | 6.44 | 93.56 | 95.02 | 2.33 | 22.74 |
| Mixture-3 | 85 | 15 | 8.77 | 91.23 | 94.45 | 2.13 | 24.71 |
| Mixture-4 | 70 | 30 | 8.69 | 91.31 | 93.13 | 2.25 | 26.47 |
| Mixture-5 | 55 | 45 | 7.84 | 92.16 | 92.30 | 2.43 | 26.01 |

a calibration tube (60.5% CH4) (S-AGM plus 1010 sensor). The purpose of the calibration was to verify that the measured gas was at standard conditions (0°C and 1013 hPa). Experiments lasted for 35 days. Measurements were made every six hours for the first six days, eight and twelve hours later the subsequent days, and the methane efficiency in each sample was determined. While Equation (1) was used to calculate the normal volume of the produced gas in the glass syringes prepared for each sample of the materials studied, Equation (2) was used to determine the methane content of the formed biogas. Equation (3) was used to calculate cumulative methane over time (VDI 4630 2006).

$$V_0^n = V\left(\frac{(P-P_w)(T_0)}{(P_0)(T)}\right) \quad (1)$$

Where, $V_0^n$ is the volume of gas under normal conditions (mLN), V is the volume of gas read (mL), P is the air pressure at the time of reading (hPa), $P_w$ is the steam temperature of the water in the outside (hPa), $T_0$ is the normal temperature (273°K), $P_0$ is the normal pressure (1013 hPa), and T is the temperature of the gas which has undergone digestion in the outside (°K).

$$C_{CH_4}^n = C_{CH_4}^f\left(\frac{P}{(P-P_w)}\right) \quad (2)$$

Where $C_{CH_4}^n$ is the volumetric methane content in dry biogas (%), $C_{CH_4}^f$ is the volumetric methane content in moist biogas (%).

$$M_{CH_4}(t) = M_{CH_4}(0) + \int_{t_1}^{t_2} M_{CH_4}(t)dt \quad (3)$$

Where $M_{CH_4}(t)$ is the cumulative methane production ($Nm^3$ $CH_4$ $kg^{-1}$ (OM), $M_{CH_4}(0)$is methane production when t= 0 ($Nm^3$ $CH_4$ $kg^{-1}$ OM), and $t_2$-$t_1$ is time between two measurements (min).

### 2.4. Chemical analysis

The crude protein, crude oil, dry matter, OM, and ADF contents of the samples in the study were determined. Crude protein analysis was performed by the Kjeldahl method and crude oil analysis by TS 6317 and Foss Soxtec method. The dry matter and OM analysis were determined according to VDI 4630 (2006), standard VDI 4630 (2006), and AOAC (1990). ADF analysis were conducted by (Van Soest et al. 1991).

### 2.5. Evaluation of data

The mean and standard deviation values, statistical analyzes, and variance analyzes of the measurements made in three replicates were determined, and the obtained values were interpreted by transferring them into the figures and tables.

## 3. Results and Discussion

### 3.1. Chemical properties of materials

Results of initial chemical analysis for Mixture-1 through Mixture-5 are given in Table 1. While the highest OM content was measured for SMS (95.02%), the lowest was cattle manure at 90.79%. As the proportion of SMS in the mixture increased, the DM also increased (Table 1). The highest and the lowest ADF content were determined for cattle manure and SMS as 60.19% and 22.74%, respectively. The crude oil content of mixes, an essential parameter for biogas production, ranged from 2.13 to 2.43%.

### 3.2. Biogas and methane production values of materials

The cumulative specific methane production of mixtures and inoculum as a function of time is shown in Figure 1. The results of cumulative specific methane and biogas productions are shown in Table 2. The results of variance analysis of methane production, biogas production, and methane content are given in Table 3.

According to the test standards, cumulative specific methane production from the inoculum should be 0-0.1 $Nm^3$ $kg^{-1}$ organic matter (OM) (VDI 4630 2006). In this study, the cumulative specific methane production from the inoculum was 0.09 $Nm^3$ $kg^{-1}$ $OM^{-1}$. The cumulative methane production occurred on the first day, increased gradually, and attained the maximum value at days 30-35 (Figure 1). As the proportion of SMS in mixtures increased, cumulative specific methane production increased. The results revealed that the highest cumulative specific biogas production value was determined for Mixture-2 (SMS) as 0.62 $Nm^3$ $kg^{-1}$ OM. It was followed by Mixture-5 (0.45 $Nm^3$ $kg^{-1}$ $OM^{-1}$), Mixture-4 (0.39 $Nm^3$ $kg^{-1}$ $OM^{-1}$), Mixture-3 (0.32 $Nm^3$ $kg^{-1}$ $OM^{-1}$), and Mixture-1 (0.27 $Nm^3$ $kg^{-1}$ $OM^{-1}$) (Table 2). (Arıcı and Koçar 2015) conducted on co-digestion of a mixture of 50% cattle manure + 50% maize silage in the mesophilic conditions (37°C) showed that biogas production 0.415 $Nm^3$ $kg^{-1}$ $OM^{-1}$. A similar study on co-digestion of the mixture containing 75% cattle manure and 25% maize silage in the 37°C conditions resulted in 0.445 $Nm^3$ $kg^{-1}$ $OM^{-1}$ biogas production (Ayhan 2013). As for the cumulative methane production of the mixture, the highest cumulative methane production was measured for Mixture-2 (0.31 $Nm^3$ $kg^{-1}$ $OM^{-1}$). It is followed by Mixture-5 (0.22 $Nm^3$ $kg^{-1}$ $OM^{-1}$), Mixture-4 (0.19 $Nm^3$ $kg^{-1}$ $OM^{-1}$), Mixture-3 (0.15 $Nm^3$ $kg^{-1}$ $OM^{-1}$), and Mixture-1 (0.12 $Nm^3$ $kg^{-1}$ $OM^{-1}$) (Table 2). The results showed that the methane content in the biogas increased as the proportion of SMS in the mixture increased. Methane content from the mixture containing SMS ranged from 49.99% to 51.87%, while Mixture-1 (cattle manure) yielded a methane content of 44.01% (Table 2). The methane, biogas production, and methane content of all the mixtures were statistically significant ($P \leq 0.05$) (Table 3).

Methane and biogas production varied based on the ADF content of mixtures. Chemical analysis of the mixture showed that the lowest and the highest ADF content were determined for Mixture-2 (SMS) and Mixture-1 (cattle manure) as 22.74 and 60.19%, respectively. It was found that there was a negative correlation between the amount of ADF and methane and biogas production. Similarly, Jimenez et al. (1990) reported that Pearson's coefficient correlated the hemicellulose content in a significant and positive way for biogas production. The negative and statistically significant relationship was established for biogas production and ADF parameters.

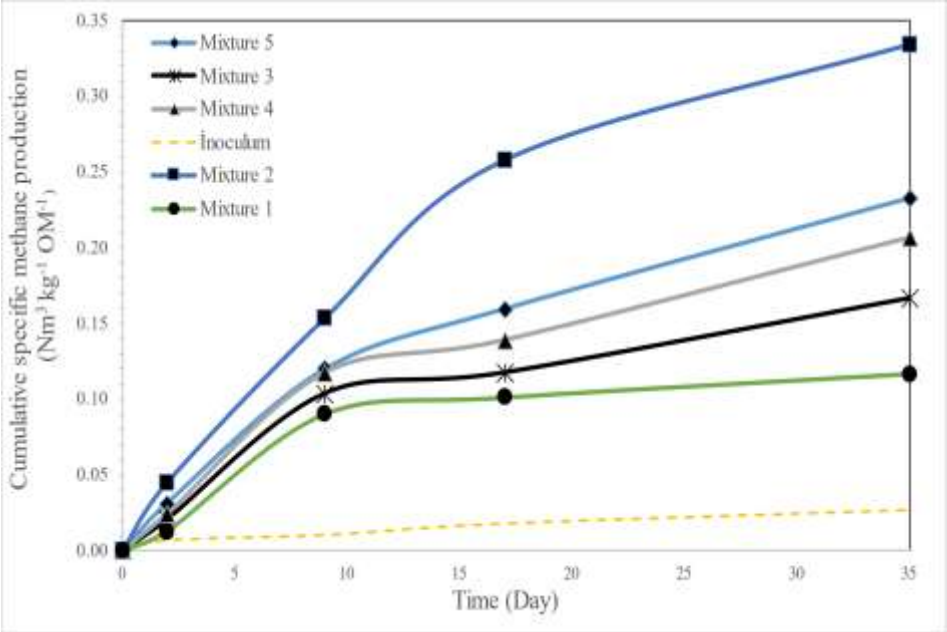


**Figure 1.** Average cumulative methane production of mixtures over time.

**Table 2.** The cumulative specific methane, biogas production and methane content in the mixes

| Mixes | Cumulative specific biogas production $Nm^3 kg^{-1} OM^{-1}$ | | | | Cumulative specific methane production $Nm^3 kg^{-1} OM^{-1}$ | | | | Methane content (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Measurements | | | | Measurements | | | | |
| | 1. | 2. | 3. | Avr. ±Stdv | 1. | 2. | 3. | Avr. ±Stdv | |
| Mixture-1 | 0.25 | 0.31 | 0.24 | 0.27±0.017 d | 0.10 | 0.14 | 0.10 | 0.12±0.009 d | 44.01 b |
| Mixture-2 | 0.69 | 0.61 | 0.56 | 0.62±0.029 a | 0.35 | 0.30 | 0.28 | 0.31±0.017 a | 49.99 a |
| Mixture-3 | 0.35 | 0.31 | 0.29 | 0.32±0.013 cd | 0.18 | 0.16 | 0.14 | 0.15±0.007 cd | 51.81 a |
| Mixture-4 | 0.39 | 0.41 | 0.38 | 0.39±0.015 bc | 0.20 | 0.21 | 0.19 | 0.19±0.005 bc | 51.87 a |
| Mixture-5 | 0.45 | 0.44 | 0.47 | 0.45±0.006 b | 0.22 | 0.23 | 0.24 | 0.22±0.003 b | 50.79 a |

$P \leq 0.05$; The differences between the cumulative specific methane, biogas productions and methane ratio averages in the biogas are indicated by different letters in the same column a, b, c, d, e.

**Table 3.** Analysis of variance of methane and biogas production in the mixes

| Biogas parameters | Source of variance | SD | SS | MS | F value | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Methane production ($Nm^3 kg^{-1} OM^{-1}$) | Between groups | 4 | 0.063 | 0.016 | 30.040 | 1.466 | |
| | Inside groups | 10 | 0.005 | 0.001 | | 1.466 | 0.000*** |
| | Total | 14 | 0.068 | | | 1.466 | |
| Biogas production ($Nm^3 kg^{-1} OM^{-1}$) | Between groups | 4 | 0.225 | 0.056 | 37.349 | 0.0250 | |
| | Inside groups | 10 | 0.015 | 0.002 | | 0.0250 | 0.000*** |
| | Total | 14 | 0.240 | | | 0.0250 | |
| Methane content (%) | Between groups | 4 | 28.497 | 32.124 | 18.996 | 0.037 | |
| | Inside groups | 10 | 16.911 | 1.691 | | 0.037 | 0.000*** |
| | Total | 14 | 145.407 | | | 0.037 | |

$P \leq 0.05$ the differences between the mean scores of methane and biogas are significant. $P \leq 0.1$ the differences between the mean scores of methane content are important (*SD*: Standard Deviation, *SS*: Some of the Squares, *MS*: Mean Square, *SEM*: Standard Error of the Mean).

## 4. Conclusions

In this study, co-digestion of SMS and cattle manure using the HBT was performed at mesophilic conditions. Preparing The mixtures tested were 85% cattle manure + 15% SMS, 70% cattle manure + 30% SMS, 55% cattle manure + 45% SMS, 100% cattle manure, 100% SMS. As the proportion of SMS in mixtures increased, cumulative specific methane and biogas production increased. The highest cumulative specific biogas production value was determined for 100% SMS as 0.62 $Nm^3 kg^{-1} OM^{-1}$. The highest cumulative methane production was measured for 100% SMS as 0.31 $Nm^3 kg^{-1} OM^{-1}$. Methane content from the mixture containing SMS ranged from 49.99% to 51.87%, while cattle manure yielded a methane content of 44.01%. Methane and biogas production changed based on the ADF content of mixtures.

In conclusion, the efficiency of biogas and methane production can be increased by applying the co-digestion technique of organic substances together. SMS and cattle manure can be used as a suitable source for biogas plants.

## Acknowledgments

We would like to thank Kahramanmaraş Sütçü İmam University Rectorate for the support under the coverage of Scientific Research Projects (Project Number: 2017/6-5) in this research.

## References

Angelidaki I, Ahring BK (1993) Thermophilic anaerobic digestion of livestock waste: The effect of ammonia. Applied Microbiology Biotechnology 38: 560-564.

AOAC (1990) Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC. The US.

Arıcı Ş, Koçar G (2015) Effect of adding maize silage as co-substrate for anaerobic animal manure digestion. International Journal of Green Energy 12(5): 453-460.

Aybek A, Üçok S, Bilgili ME, İspir MA (2015) Digital mapping and determination of biogas energy potential of usable animal manure and cereal straw wastes in turkey. Journal of Tekirdag Agricultural Faculty 12(3): 109-120.

Aybek A, Üçok S (2017) Determination and evaluation of biogas and methane productions of vegetable and fruit wastes with HBT method. International Journal of Agricultural and Biological Engineering 10(4): 207-215.

Ayhan AA (2013) Research on determination of biogas quantity from cattle manure and maize silage at different mixing ratio under mesophilic conditions. Ph.D. Thesis, Uludağ University, Bursa.

Borreani G, Tabacco E (2010a) The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science 93(6): 2620-9.

Borreani G, Tabacco E (2010b) Use of new plastic stretch films with enhanced oxygen impermeability to wrap baled alfalfa silage. Transactions of the ASABE 53(2): 635-41.

Driehuis F, Elferink SO (2000) The impact of the quality of silage on animal health and food safety: A review. Veterinary Quarterly 22(4): 212-6.

Ergüneş G, Tarhan S, Yardım MH, Kasap A, Demir F, Önal I, Uçar T, Tekelioğlu O, Çalışır S, Yumak H, Yağcıoğlu A (2009) Farm Machinery. Nobel Science and Research Center, Publish No: 49, 544, Ankara, Turkey.

Heffrich D, Oechsner H (2003) Comparison of different laboratory techniques for the digestion of biomass. Landtechnik 9: 27-30.

Hutňan M, Špalková V, Bodík I, Kolesárová N, Lazor M. (2010) Biogas production from maize grains and maize silage. Polish Journal of Environmental Studies 19(2): 323-329.

Hutňan M (2016) Maize silage as substrate for biogas production. Advances in Silage Production and Utilization 16: 173-96.

IEA (2015) Key World Energy Statistics https://www.ourenergypolicy.org/wp-content/uploads/2016/09/KeyWorld2016.pdf. Accessed 4 March 2021.

Jimenez S, Cartagena MC, Aree A (1990) Influence of lignin on the methanation of lignocellulosic waste. Biomass and Bioenergy 21: 43-54.

Khan NA, Tewoldebrhan TA, Zom RL, Cone JW, Hendriks WH (2012) Effect of corn silage harvest maturity and concentrate type on milk fatty acid composition of dairy cows. Journal of Dairy Science 95(3): 1472-83.

Klang J, Theuerl S, Szewzyk U, Huth M, Tölle R, Klocke M (2015) Dynamic variation of the microbial community structure during the long-time mono-fermentation of maize and sugar beet silage. Microbial biotechnology 8(5): 764-75.

Korkmaz Y, Aykanat S, Çil A (2012) Biogas and energy production from organic wastes. SAU Journal of Science and Letters 12: 489-497.

Mittweg G, Oechsner H, Lemmer A, Hanisch AR (2012a) Repeatability of a laboratory batch method to determine the specific biogas and methane yields. Engineering in Life Sciences 12(3): 270-278.

Mittweg G, Oechsner H, Hahn V, Lemmer A, Reinhardt-Hanisch A (2012b) Repeatability of a laboratory batch method to determine the specific biogas and methane yields. Engineering in Life Sciences 12(3): 270-278.

Oslaj M, Mursec B, Vindis P (2010) Biogas production from maize hybrids. Biomass and Bioenergy 34: 1538-1545.

Onurbaş Avcıoğlu A, Türker U, Demirel Atasoy Z, Koçtürk D (2011) Renewable energies of agricultural origin - biofuels. Nobel Academic Publishing Education Consultancy LLC, No: 72, Ankara, Turkey.

Phipps RH, Sutton JD, Jones BA (1995) Forage mixtures for dairy cows: The effect on dry-matter intake and milk production of incorporating either fermented or urea-treated whole-crop wheat, brewers' grains, fodder beet or maize silage into diets based on grass silage. Animal Science 61(3): 491-6.

Sun C, Cao W, Banks CJ, Heaven S, Liu R (2016) Biogas production from undiluted chicken manure and maize silage: a study of ammonia inhibition in high solids anaerobic digestion. Bioresource Technology 218: 1215-23.

Temiz D, Gökmen A (2010) The importance of renewable energy sources in Turkey. International Journal of Economics and Finance Studies 2(2): 23-30.

Ulusoy Y, Ulukardeşler AH, Ünal H, Alibaş K (2009) Analysis of biogas production in Turkey utilizing three different materials and two scenarios. African Journal of Agricultural Research 4(10): 996-1003.

Üçgül D, Akgül G (2010) Biomass Technology. YEKARUM Journal (1): 3-11.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74: 3583-3597.

VDI 4630 (2006) Fermentation of organic material, characterisation of substrate, collectıon of material data, fermentatıon tests, VDI Gesellschaft Energietechnik.

VDI-Richtlinie 4630 (2006) Verg¨arung organischer stoffe. VDIGesellschaft energietechnik, Dusseldorf, ICS 13.030.30; 27.190, pp. 59.

Wilkinson JM, Davies DR (2013) The aerobic stability of silage: Key findings and recent developments. Grass and Forage Science (1): 1-9.

Yaldız O (2000) Biogas Technology, Course Book. Akdeniz University Publications, Publication No: 78, pp. 181.

Yılmaz M (2012) Turkey's energy potential of renewable energy and importance of the topic from the point of electric power generation. Ankara University Journal of Environmental Sciences 4(2): 33-54.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 293-299
DOI: 10.29136/mediterranean.888603

# Evaluation of quality characteristics of three different colour tomato varieties in three ripening stages

**Selman ULUISIK**

Burdur Mehmet Akif Ersoy University, Burdur Food Agriculture and Livestock Vocational School, 15030, Burdur, Turkey

Corresponding author: S. Uluisik, e-mail: suluisik@mehmetakif.edu.tr

ARTICLE INFO

Received: March 1, 2021
Received in revised form: September 6, 2021
Accepted: October 5, 2021

***Keywords*:**

Tomato
Ripening
Cell wall
Gene expression
Pectin

**ABSTRACT**

Fruit ripening and softening indicated by firmness determines the texture transportability, and shelf life of tomato products. However, the regulatory mechanism underlying firmness formation in tomato is different in different varieties and overall softening mechanism of tomato fruit is poorly understood. Therefore, in this study, physical, biochemical, and molecular properties of three different tomato varieties; 'Sarikiz' (yellow skin colour), 'Moda' (orange skin colour) and 'Red Type Cherry' (red skin colour) at three developmental stages, mature green (MG), breaker (Br) and full ripe (R) were evaluated. For this aim, colour, texture, cell wall fractionation and pectate lyase (*PL*) gene expressions were analysed at three different ripening stages. As expected, there was a dramatic difference in colour index due to different skin colours of the varieties. For textural properties, 'Sarikiz' showed the softest while 'Moda' variety had the firmest pericarp structure. The composition of the cell wall structure at three ripening stages were also resulted with significantly different fractions. The expression of pectate lyase (PL), one of the most important cell wall modification related enzyme was also studied by semi quantitative RT-PCR. Based on biochemical and molecular studies, 'Sarikiz' showed higher pectin fraction in water and *PL* gene expression at Br and R ripening stages. Based on these results, although the tomato fruits used in this study generally show the same softening trend, they show different physiological, biochemical, and molecular changes in different softening periods.

## 1. Introduction

The cultivated tomato *Solanum lycopersicum*, is the second most widely most produced, consumed, traded and important products in the world both as raw or processed forms (Yildizhan and Taki 2018). Botanically, the tomato is described as a berry that belongs to the nightshade family Solanaceae. This family consists of 96 genera and over 3000 species in three sub-families (Waheed et al. 2020). Worldwide production of fresh market and processed tomatoes has steadily increased during the last ten years and reached annual production of about 180 million tons with a net value of over $190.4 Billion (FAO 2019). The leading countries for this production in the World are People's Republic of China (PRC) (62.8 million tons), India (19 million tons), Turkey (12.8 million tons) and the USA (10.8 million tons) and), respectively (FAO 2019).

In addition to tomato's economic importance and worldwide cultivation, it has several features that make it a convenient plant for experimental purposes, such as efficient sexual hybridization, ease of culture under a wide range of environment and high-quality sequenced genome (Van Eck et al. 2018). Like all fleshy fruits, the tomato goes through a developmental stage known as ripening, a complex process followed by softening. The softening process is the final stage that contributes to perishability of fruit, facilitating pathogen infection, postharvest decay, and reducing shelf-life and fruit quality (Wei et al. 2010). Ripening of fleshy fruits represents a highly programmed and tightly controlled developmental process leading to textural modifications, enhanced colour, as well as increased accumulation of sugars, acids and volatile compounds culminating in a diverse array of tastes and smells that vary among species (Klee and Giovannoni 2011).

There have been many studies on the physiological and biochemical changes of fruits at different stages of development (Khan et al. 2017; Trong et al. 2019). Researches on physiological and biochemical changes of tomato fruit at different stages of development have been also carried out in many different ecological regions (Raffo et al. 2002; Guil-Guerrero and Rebolloso-Fuentes 2009; Oluk et al. 2012; Gölükçü et al. 2018). However, there are currently no full reports combining physiological, and molecular changes of different tomatoes at different developmental stages in Turkey. 'Sarikiz' tomato variety is not widely consumed by the public which makes they are not always available in markets and greengrocers. This variety is not suitable for commercial production due to their relatively short storage time; however, it is consumed for its high aromatic components. 'Moda' variety is another cherry type having relatively longer shelf life and slower ripening process compared to other two varieties used in this study. 'Red type' cherry is a common variety which is largely and freshly consumed. This variety has a shelf-life longer than 'Sarikiz', but shorter than 'Moda' variety.

Consequently, it would be interesting to study ripening process of different tomato varieties to correlate various physical characteristics like colour, texture and shelf life with pectin solubilisation and a pectin degrading enzyme pectate lyase (PL) characterized by (Uluisik et al. 2016). With the help of this research, we may introduce new data and ideas how to use these different varieties for the establishment of commercial plantations for tomato consumption.

## 2. Materials and Methods

Seeds of each genotype were cultivated in Antalya, Turkey, and grown in controlled glasshouse conditions in Faculty of Agriculture, Akdeniz University. Three cherry type tomato fruits (Sarikiz, Moda and Red Cherry) were harvested from the greenhouse at three maturity stages: mature green (MG) breaker (Br) and ripe (R). The harvested fruits were immediately transported at Burdur Mehmet Akif Ersoy University to carry out all analysis.

### *2.1. Quality assessment of the fruits*

The skin colour of the tomato fruits was measured by using a Colour-Meter (PCE-CSM 1) and recorded as Hunter's L*, a*, and b* values (10 fruits from each developmental stage) and colour index (CI) was calculated according to (Nangare et al. 2016). Fruit mechanical properties were investigated by pushing a probe (6 mm) into the pericarp (PCE-PTR 200 Penetrometer) and results were expressed in Newtons (N). The assessment was performed at two opposite locations along the fruit equator after peeling around a 2-centimetre square. The average of maximum forces was recorded to represent fruit firmness at the ripening stages. Finally, total soluble solids (°Brix TSS) were determined using a digital refractometer.

### *2.2. Isolation and extraction of cell wall components*

Alcohol insoluble solids (AIS) were prepared following the procedure previously described (Lunn et al. 2013). Fractions of different cell wall pectic components were obtained by sequential chemical extraction of the cell wall material (AIS) by stirring in water (to extract water soluble pectins, WSP) for four hours at room temperature (RT). The liquid fraction was removed, and the residue treated with 50 mM of CDTA (pH 6.5) (to extract ionically bound or chelate-soluble pectins) for four hours at RT, and finally again the remaining residue were stirred with 50 mM $Na_2CO_3$+20mM $NaBH_4$ (to get covalently bound pectin fractions) overnight at 4°C. Quantification of uronic acids in fruit serum was carried out spectrophotometrically according to the method of Filisetti-Cozzi and Carpita (1991). 300 µL of each test sample and each standard was added to a test tube in triplicate along with 300 µL of boric acid solution and 5 mL of 12M sulphuric acid ($H_2SO_4$) (Fisher Scientific). The samples were then incubated at 70°C in a water bath for 40 minutes before adding 0.2 mL of the dimethyl-phenol solution. Samples were left at room temperature for 5 minutes and the colour change read at the wavelength 405 nm and 450 nm with the difference recorded. The absorbance reading at 405 nm was subtracted from that at 450 nm to correct for interference from hexoses. Averages of at three biological replicates were used for statistical analysis. Results were expressed as milligram of GA per 1 gram of AIS.

### *2.3. RNA extraction and gene expression analysis*

Total RNA was extracted from fine powdered pericarp of tomato fruits from three developmental stages of three varieties using PureLink™ Plant RNA Reagent (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. Total RNA was dissolved in 30 µL of elution buffer, and the concentration of RNA was quantified using the nanodrop (BioTek, Epoch Microplate Spectrophotometer). Total RNA was reverse transcribed into cDNA by the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The *PL* gene primers were designed using primer 3 (http://primer3.ut.ee/). For semi quantitative RT-PCR analysis, the *ELONGATION FACTOR 1-α* gene, (*LeEF-1*, GenBank accession X14449) (Pokalsky et al. 1989) was used as an internal constitutively expressed gene (house-keeping gene). Semi RT-PCR was performed in a total volume of 20 µL Thermo Scientific PCR Master Mix. Primers sequences for *PL* and *LeEF-1* for semi-RT-PCR validation were listed in Table 1.

### *2.4. Statistical Analysis*

The statistical analyses were conducted according to completely randomised design with at least ten different tomato fruits for fruit firmness and CI measurements. Three different fruits were used for cell wall analysis. All statistical analyses were performed through XLSTAT (version 2016.02.28451, Addinsoft, France). Duncan test was utilised for the comparison of means ($P \leq 0.05$).

## 3. Results

The quality analysis focused on assessment of fruit colour, firmness and TSS of the fruits. The visual appearance of three different tomato varieties at three developmental stages were shown in Figure 1. As it can be clearly seen in Figure 1, the colour changing of 'Sarikiz' has slowed down after Br stage which shows that probably it reaches maximum colour changes just after Br stage. However, when it is also looked at 'Moda' variety, there is a dramatic colour change from MG to Br and Br to R stages. This dramatic change was also supported by CI (colour index) values in which forcefully increased in three ripening stages for 'Moda' variety. 'Red type' cherry, the only red tomato in our analysis, displayed a clear change in colour from MG to Br and from Br to fully ripe tomato (Figure 1). This clear escalate was also seed in CI values which go up from 1.2 (MG) to 36.8 (R).

Colorimeters express colours in numerical terms along the L*, a* and b* axes (from white to black, green to red and blue to yellow, respectively) within the CIELAB colour sphere which are usually mathematically combined to calculate the colour indexes. The L* value was nearly similar trend for three varieties

**Table 1.** Primers used for semi quantitative RT-PCR validation

| Gene | Forward Sequence | Reverse Sequence |
|---|---|---|
| *PL* | GCGATCAGGAGTTAGAACTGG | AATCCCCTTTTGCTTTGGTT |
| *LeEF-1* | ACCTTTGCTGAATACCCTCCATTG | CACAGTTCACTTCCCCTTCTTCG |

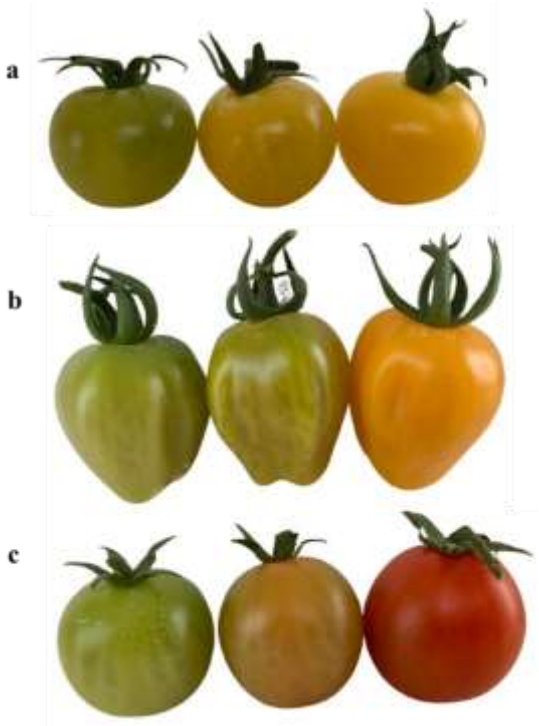


**Figure 1.** The visual differences of a) Sarikiz, b) Moda and c) Cherry at three ripening stages.

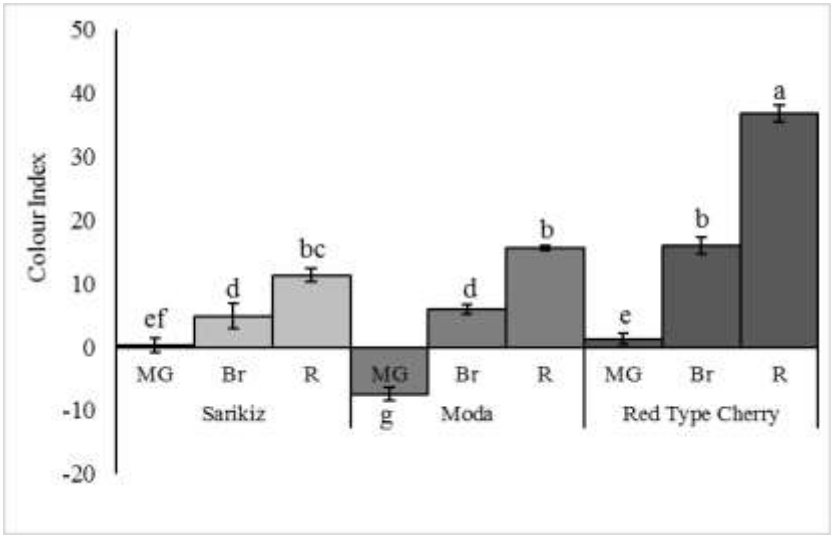


**Figure 2.** Colour index (CI) values for tomato fruits harvested at different ripening stages. Values represent means ± SE (n= 10). Different letters indicate significant differences, $P<0.05$.

at the three developmental stages (Table 2). However, the luminosity L* value decreases while other two parameters increased during ripening in tomato. This trend was seen more obvious in proceeded ripening stages of 'Red Type Cherry'. The increasing of a* value indicates the loss of green colour in which no major changes were observed in fruits in still predominantly green (MG to Br). For example, the biggest change from MG to Br was in 'Moda' variety (from -3.1 to 2.2) for a* value. However, there was more dramatic increase in a* for all varieties during ripening transition from Br to R, especially in 'Red Type Cherry' with final a* value 12.89. In overall, the total CI increased in all different colour tomatoes during three ripening stages. This is increasing values was seen much clear in 'red type' variety which are probably based on lower L* and higher a* and b* values. The degree of °Brix was higher in 'Sarikiz' in all three different ripening stags compared to other two varieties. Fully ripe 'Sarikiz' tomatoes had the highest °Brix of all tested fruits (Table 2).

Measurement of the maximum load (Newton, N) on pericarp samples for the three different ripening stages of fruits are shown in Figure 3. The fruit firmness continuously decreased during the tested period in all varieties. The firmness differences between the stages for all varieties was statistically significant ($P<0.05$). Although the 'cherry' was the firmest at MG stage, the 'Moda' was slightly the firmest at the end of the ripening process but was not significantly different than the second firmest variety red type cherry'. 'Sarikiz' has a pericarp which is very juicy and

**Table 2.** L*, a* and b* mean values at three different ripening stages of tomato fruits, (n= 10).

| Samples | Ripening Stages | Colour Values | | | °Brix TSS |
|---|---|---|---|---|---|
| | | *L** | *a** | *b** | |
| Sarikiz | MG | 41.19 | 0.32 | 10.69 | 5.9 |
| | Br | 40.04 | 2.43 | 11.32 | 6.1 |
| | R | 43.02 | 3.99 | 15.89 | 6.15 |
| Moda | MG | 43.50 | -3.14 | 12.78 | 4.9 |
| | Br | 45.71 | 2.28 | 16.93 | 5.0 |
| | R | 43.66 | 8.20 | 22.56 | 5.02 |
| Red Type | MG | 41.13 | 0.29 | 10.13 | 5.05 |
| | Br | 39.65 | 4.22 | 12.36 | 5.12 |
| | R | 35.94 | 12.89 | 14.49 | 5.12 |

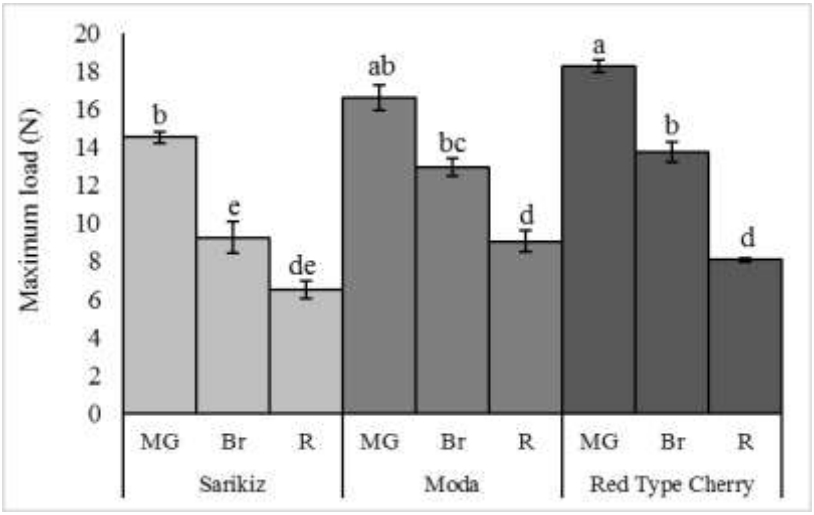


**Figure 3.** Maximum load (N) for pericarp of the tomato fruits at three different ripening stages. Values represent means ± SE (n= 10). Different letters indicate significant differences, $P<0.05$.

soft pericarp compared to other two varieties, which resulted significantly softer fruits at three developmental stages compared other two varieties. However, although the 'Sarikiz' had the softest fruits in R stage, the 'Cherry' had a total firmness loss of 55.73% from MG to R in where the 'Moda' had of 45.42%. These results suggest us that, 'Moda' variety was the firmest and probably has a longer shelf life among three varieties.

The levels of WSP (water-soluble pectin), CDTA (chelator soluble pectin), and $Na_2CO_3$ (carbonate soluble pectin) that could be extracted from the cell wall materials (CWM) of three tomato cultivars at three different ripening stages are presented (Figure 4). The fractionation of the CWM revealed a variety of differences in the levels of WSP at different ripening stages. As expected, CWM of WSP was the least at the MG stage, and there was a significant increase during shelf-life ripening in three cultivars. The cultivars 'Sarikiz' had the highest WSP with 40.76 mg UA $g^{-1}$ at the R stage, while cultivar 'Red Cherry' and 'Moda' had around 28 mg UA $g^{-1}$ of WSP at their ripe stages. The amount of CDTA pectin was found to be a higher fractionation in all cultivars at all ripening stages. The highest CDTA soluble pectin was found in R stage of all cultivars. The $Na_2CO_3$ soluble pectin of the three cultivars collected at different ripening stages decreased significantly during the storage period. Opposite to WSP, the cultivar 'Sarikiz' had the least $Na_2CO_3$ soluble pectin at R stage compared to other two cultivars. Based on cell wall fractionation analysis, the results showed that the cultivar 'Sarikiz' had the most WSP and the least $Na_2CO_3$ fraction at R stage compared to other two cultivar, which could be the reason of softer texture of 'Sarikiz'.

The *PL* gene expression was determined at a range of stages of fruit development developmental and ripening (MG, Br and R) stages of three tomato varieties. As shown in Figure 5, there was relatively strong *PL* gene expression in all varieties during all stages compared to housekeeping gene *LeEF-1*. However, it is clearly visible that, the expression of the *PL* is higher at Br stage of 'Sarikiz' compared to its MG and R stage. However, in 'Moda' e level of transcripts of PL is the highest at MG stage. The gene accumulation in 'cherry' cultivar looks similar to 'Sarikiz' in which the expression was higher in Br stage compared to other two ripening stages.

## 4. Discussion

Tomato fruits are usually harvested at MG stage either for transportation purposes or longer storage. The fruits harvested at MG stage exhibit maximum shelf life and they can ripe on their own due to its climacteric condition to the best of the ripening attributes (Sharma et al. 2020). As with all fleshy fruits, different tomato varieties show different ripening processes and mechanisms. In other words, distinct variability in postharvest ripening behavior was observed among tomato varieties when assessed in terms of colour, texture and shelf-life change based parameters. Therefore, in this study, we evaluated three different tomatoes that differ in colour, firmness and shelf-life characteristics at three different stages of ripening.

Tomato fruit changes its colour from green to red, yellow, or orange during ripening, because of chlorophyll degradation simultaneously to carotenoid biosynthesis like β-carotene (Carrillo-López and Yahia 2014). The changes in a* value was

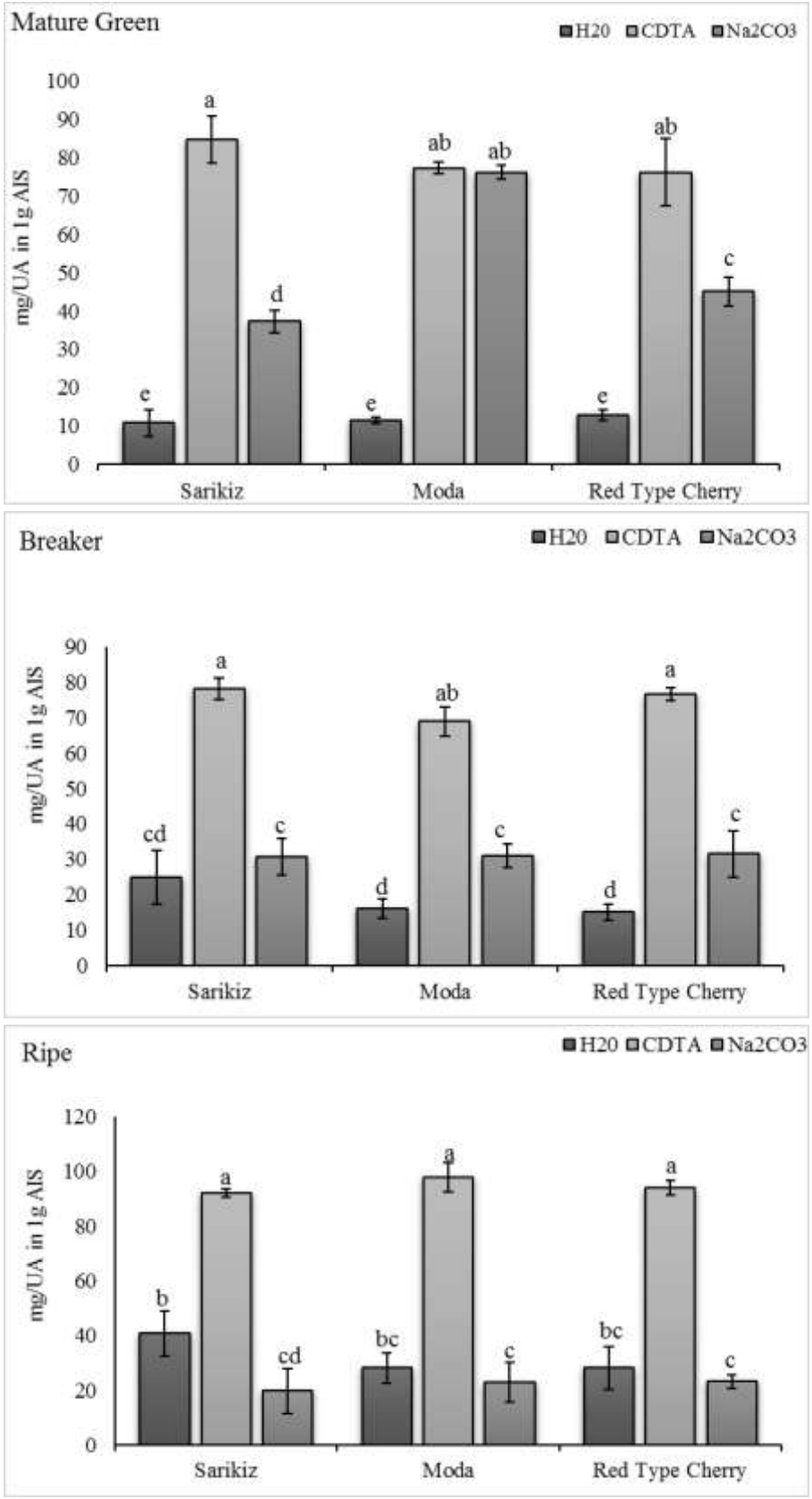


**Figure 4.** Changes in WSP, CDTA and $Na_2CO_3$ soluble pectin of Sarikiz, Moda and Red Type Cherry at MG, Br and Ripe stages. Error bars indicate the standard error for three replicates. There is a statistical difference between bars containing different letters, $P<0.05$.

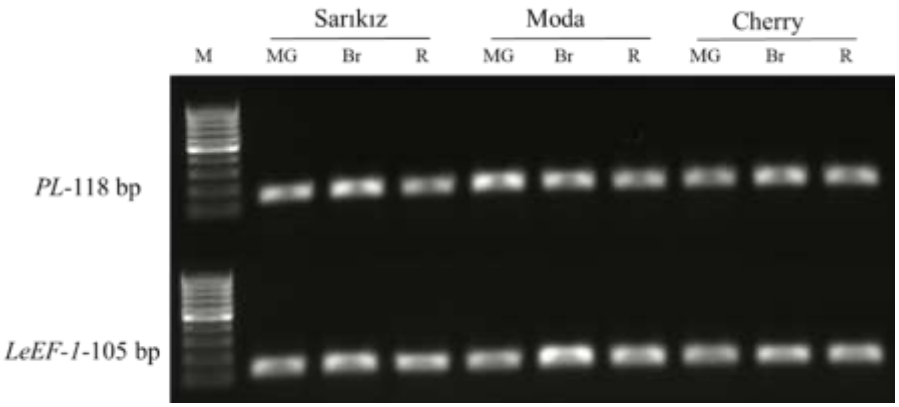


**Figure 5.** Semi quantitation RT-PCR gel electrophoresis image for the *PL* gene expression at three ripening stages of three tomato cultivars.

expected to be a good indicator for colour changes, because of a* perpendicular axes represent the green to red axis at the CIELAB colour system in which some researchers have only used a* values (Cantwell 1998). In this respect, a specific tomato ripeness stage can be determined by a* value of pericarp. However, in our case, it was impossible to evaluate the ripening stage only by a* value. Therefore, when it is also looked at b* values (blue to yellow), the gradual increase can easily be seen during ripening stages, especially in 'Sarikiz' and 'Moda' varieties. Overall, these results showed us that, the ripening process have normally gone throughout ripening process in three different colour tomatoes.

Texture is a sensory property and is recognised as a combination of different features, most likely dependent on the anatomical properties of the primary cell wall (Chylińska et al. 2017). Instrumental measurements are generally used to determine firmness related to mechanical properties of tissue. It is commonly known that tomato fruit softens quickly during development and ripening. One of the most obvious changes in the cell walls of ripening tomato fruits is the degradation of the pectic polysaccharides. This involves an increase in their solubility and a reduction in their molecular weight (Seymour et al. 1987).

Pectin content was estimated by the uronic acid concentration as galacturonic acid is the main component of pectins. Among cell-wall polysaccharides, water soluble pectin (WSP) chelator soluble pectin (CDTA) and carbonate soluble pectin ($Na_2CO_3$) are important part of cell walls and closely related to the ripening and softening of fruit (Brummell 2006).

Pectin which is loosely bound at cell wall characterized as the polysaccharides water soluble fraction (WSF). As fruit undergoes from mature to ripening transition, an increase is expected in WSF due to increase in loosely bound polyuronides, such as hydrolysis of homogalacturonan and neutral sugars present in the lateral chains of rhamnogalacturonan I (Gross and Sams 1984). In our analysis, although there was much less WSF in 'Sarikiz' compared to other cultivars, it has significantly higher amount of WSF in Br and R ripening stages. This result supports the firmness measurement results in which 'Sarikiz' was determined as the softest tomato. Moreover, it is a good hint to say that more pectin already lost its stronger bounds at Br and R ripening stages in 'Sarikiz'. Reduced amounts of WSF in 'Moda' and 'Red Cherry' at Br and R stages, suggesting that more of the pectins in fruit cell walls were covalently associated with the wall matrix. To summarize, our cell wall fractionation indicated a higher amount of WSP in 'Sarikiz' especially at Br and R ripening stages might corresponded to softer texture of this variety.

Although it would obviously be more reliable to evaluate the expression levels of genes by qPCR, due to for unforeseen reasons, we had to evaluate the expression levels of *PL* gene by semi q-RT PCR. In the present work, we evaluated the expression of *PL*, one of the main tomatoes softening related gene by semi q-RT-PCR. Silencing of a gene coding for PL enzyme improved tomato shelf-life, which is caused by alternating the levels of soluble pectin and total pectin (Uluisik et al. 2016). In our study, the expression of *PL* gene was slightly higher in 'Sarikiz' compared to other two cultivars at Br stage. The expression of the gene looks like lesser in 'Moda' especially at R stage, which could be one the reasons in lesser CWF and firmer texture of the fruit. The overall data from semi q-RT-PCR and cell wall composition led us to think that total activity of PL and other cell wall related enzymes were likely to be responsible for the wall changes that converted interconnected wall components into the WSF, mainly in 'Sarikiz'.

## 5. Conclusion

Although a great deal has been made in understanding the softening mechanism of the tomato, this complex mechanistic structure is open to new discoveries with integrating different varieties and cultivars into the research area. Therefore, in this study we evaluated quality parameters of three different colour tomatoes grown in Turkey, from physical, biochemical and molecular perspective. The softer tomato variety 'Sarikiz' at Br stage correlated with an increase in water-soluble pectin and expression level of cell wall degrading enzyme PL. Evaluation of these cultivars by different omic technologies (like transcriptomic and metabolomic) would be chance to identify different genes or compounds could be used in different breeding studies to create better quality of tomatoes.

## Conflict of Interest

The author declares that he has no conflict of interest.

## Authors Contribution

SU designed the study and carried out the experiments. He evaluated the data and wrote the manuscript.

## Acknowledgement

I extend my sincerest thanks to Hayri Üstün (Akdeniz University) for providing the fruits.

## References

Brummell DA (2006) Cell wall disassembly in ripening fruit. Functional Plant Biology 33: 103. doi: 10.1071/FP05234.

Cantwell M (1998) Optimum procedures for ripening tomatoes. In: KADER, A. (Ed.) Management of fruit ripening, Postharvest Horticulture Series. University of California, Davis, n. 9. Postharvest Outreach Program.

Carrillo-López A, Yahia EM (2014) Changes in color-related compounds in tomato fruit exocarp and mesocarp during ripening using HPLC-ApcI+-mass Spectrometry. Journal of Food Science and Technology 51(10): 2720-2726.

Chylińska M, Szymańska-Chargot M, Derylo K, Tchórzewskac D, Zdunek A (2017) Changing of biochemical parameters and cell wall polysaccharides distribution during physiological development of tomato fruit. Plant Physiology and Biochemistry 119: 327-337.

FAO (2019) Food and Agriculture Organization of the United Nations, http://www.fao.org/home/en/. Accessed 21 January, 2021.

Filisetti-Cozzi TMCC, Carpita NC (1991) Measurement of uronic acids without interference from neutral sugars. Analytical Biochemistry 197: 157-162. doi: 10.1016/0003-2697(91)90372-Z.

Gölükçü M, Kabaş A, Yeğin BA, Vuran AF, Yüksel K, Tanır A (2018) Domatesin bazı fiziksel ve kimyasal kalite özelliklerinin melezleme ile değişimi. Derim 35(2): 152-160.

Gross KC, Sams CE (1984) Changes in cell wall neutral sugar composition during fruit ripening: a species survey. Phytochemistry 23: 2457-2461. doi: 10.1016/S0031-9422(00)84075-3.

Guil-Guerrero JL, Rebolloso-Fuentes MM (2009) Nutrient composition and antioxidant activity of eight tomato (*Lycopersicon esculentum*) varieties. Journal of Food Composition and Analysis 22(2): 123-129. doi: 10.1016/j.jfca.2008.10.012.

Khan MA, Butt SJ, Khan KA, Nadeem F, Yousaf B, Javed, HU (2017) Morphological and physico-biochemical characterization of various tomatocultivars in a simplified soilless media. Annals of Agricultural Sciences 62: 139-43. doi: 10.1016/j. aoas.2017.10.001.

Klee HJ, Giovannoni JJ (2011) Genetics and Control of Fruit Ripening and Quality Attributes. Annual Review of Genetics 45: 41-59.

Lunn D, Phan TD, Tucker GA, Lycett GW (2013) Cell wall composition of tomato fruit changes during development and inhibition of vesicle trafficking is associated with reduced pectin levels and reduced softening. Plant Physiology and Biochemistry 66: 91-97.

Nangare DD, Singh Y, Kumar PS, Minhas PS (2016) Growth, fruit yield and quality of tomato (*Lycopersicon esculentum* Mill.) as affected by deficit irrigation regulated on phenological basis. Agricultural Water Management 171: 73-79. doi: 10.1016/j.agwat. 2016.03.016.

Oluk CA, Akyıldız A, Ağçam E, Keleş D, Ata A (2012) Farklı Domates Çeşitlerinin Bazı Kalite Özellikleri. Akademik Gıda 10(3): 26-31.

Pokalsky AR, Hiatt WR, Ridge N, Rasmussen R, Houck CM, Shewmaker CK (1989) Structure and expression of elongation factor 1α in tomato. Nucleic Acids Research 17: 4661-4673. doi: 10.1093/nar/17.12.4661.

Raffo A, Leonardi C, Fogliano V, Ambrosino P, Salucci M, Gennaro L, Bugianesi R, Giuffrida F, Quaglia G (2002) Nutritional value of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1) harvested at different ripening stages. Journal of Agricultural and Food Chemistry 50(22): 6550-6556. doi: 10.1021/jf020315t.

Seymour GB, Harding SE, Taylor AJ, Hobson GE, Tucker GA (1987) Polyuronide solubilization during ripening of normal and mutant tomato fruit. Phytochemistry 144: 1871-1875. doi: 10.1016/S0031-9422(00)81719-7.

Sharma L, Ramesh KV, Paul V, Pandey R (2020) Ripening index: A better parameter for colour-based assessment of ripening behaviour of tomato fruits. Plant Physiology Reports 25(1): 171-177.

Trong LV, Tuong LQ, Thinh BB, Khoi NT, Vu Thi Trong VT (2019) Physiological and biochemical changes in tomato fruit (*Solanum lycopersicum* L.) during growth and ripening cultivated in Vietnam. Bioscience Research 16(2): 1736-1744.

Uluisik S, Chapman NH, Smith R, Poole M, Adams G, Gillis RB, Besong TMD, Sheldon J, Stiegelmeyer S, Perez L, Samsulrizal N, Wang D, Fisk ID, Yang N, Baxter C, Rickett D, Fray R, Blanco-Ulate B, Powell ALT, Harding SE, Craigon J, Rose JKC, Fich EA, Sun L, Domozych DS, Fraser PD, Tucker, Grierson D, Seymour, GB (2016) Genetic improvement of tomato by targeted control of fruit softening. Nature Biotechnology 34. doi: 10.1038/nbt.3602.

Van Eck J, Keen P, Tjahjadi M (2018) Transgenic Plants, '*Agrobacterium tumefaciens*-Mediated Transformation of Tomato' Springer Protocols, Humana Press, New York, NY.

Waheed K, Nawaz H, Asif M, Rehman HR (2020) Medicinal Plants of South Asia, Tomato, Chapter 46, Elsevier, pp. 768.

Wei J, Ma F, Shi S, Qi X, Zhu X, Yuan J (2010) Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit. Postharvest Biology and Technology 56(2): 147-154. doi: 10.1016/j.postharvbio. 2009.12.003.

Yildizhan H, Taki M (2018) Assessment of tomato production process by cumulative exergy consumption approach in greenhouse and open field conditions: Case study of Turkey. Energy 156: 401-408.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 301-305
DOI: 10.29136/mediterranean.957192

# Non-destructive estimation of chlorophyll content of peanuts grown at different soil texture and salinity levels

**Cihan KARACA, Gulcin Ece ASLAN, Begum POLAT, Dursun BUYUKTAS**

Faculty of Agriculture, Department of Farm Structures and Irrigation, Akdeniz University, Antalya, Turkey

Corresponding author: C. Karaca, e-mail: cihankaraca@akdeniz.edu.tr
Author(s) e-mail: ecebacalan@akdeniz.edu.tr, btekelioglu@akdeniz.edu.tr, dbuyuktas@akdeniz.edu.tr

ARTICLE INFO

Received: June 24, 2021
Received in revised form: August 19, 2021
Accepted: August 23, 2021

***Keywords*:**

Acetone extraction procedure
CCI
Chl-a
Chl-b
Chl-a+b

**ABSTRACT**

Chlorophyll is a significant biochemical component and can be determined in the laboratory (destructive) and using various chlorophyll content measuring devices (non-destructive). In this study, destructive and non-destructive methods were used to determine chlorophyll content and compared in peanut (*Arachis hypogaea* cv. NC-7) grown under different soil texture and saline water applications. The experiment was carried out in a complete randomized block design in pots using two soil textures (clay-loam and sandy) and three irrigation water salinity (0.7, 2.1 and 3.3 dS $m^{-1}$). While the chlorophyll contents (Chl-a , Chl-b, Chl-a+b, Chl-a/b) were determined with the acetone extraction procedure, which is classified as destructive methods under laboratory conditions, the Chlorophyll Content Index (CCI) values were measured with the hand-held chlorophyll meter device (Apogee CCM-200), which is a non-destructive method. While irrigation water salinity decreased all types of chlorophyll contents (Chl-a, Chl-b, Chl-a+b) (mg $cm^{-2}$), it did not cause a statistical difference in Chl-a/b. Linear and polynomial models were fitted between the different chlorophyll contents and the CCI values under different soil textures and saline water levels. Model performances were slightly better with the polynomial model compared to the linear model in all experimental treatments. Since the difference between model performances is small, it is recommended to use the linear model due to its ease of use. In addition, the total chlorophyll content can be safely estimated under saline conditions by using portable chlorophyll meters.

## 1. Introduction

Chlorophyll (Chl) is a significant biochemical component in the molecular system responsible for the photosynthesis of peanuts (Patane and Vibhute 2014). Chlorophyll a is the most abundant form of chlorophyll within photosynthetic organisms and, for the most part, gives plants their green color. There are also other forms of chlorophyll, coded b, c, and d, enlarging the overall fluorescent signal (Patane and Vibhute 2014). The amount of chlorophyll a, b and a/b ratio in plants varies depending on species, organ (Perera and Smith 2013), agronomic practices, growth stage (Manolopoulou et al. 2016), drought stress (Khaleghi et al. 2012; Tezcan et al. 2018), salinity stress (Taibi et al. 2016), temperature stress (Martinazzo et al. 2012) and light intensity (Hazrati et al. 2016).

Various methods were developed to determine leaf chlorophyll content (Patane and Vibhute 2014). Of these methods, the spectrophotometric method is accepted as the most accurate and reliable method for determining leaf chlorophyll content (Parry et al. 2014). But the most important disadvantages of this method are time-consuming measurement process, destructive and requires well trained personnel. In recent years, nondestructive, in situ, optical techniques have become widely used to give a relative indication of leaf chlorophyll concentration (Parry et al. 2014) and provide a quick, reliable alternative to in vitro techniques (Wood et al. 1993). For this purpose, chlorophyll meters were developed which indirectly determines the amount of chlorophyll. These devices measure the transmittance of the red and infrared radiation emitted by leaves. The transmitted light is detected by the sensor as the analog signal and the inbuilt mini-processor converts this signal into SPAD or index (Chlorophyll Content Index (CCI)) by a defined arithmetic operation (Lunagaria et al. 2015). The CCM-200 uses absorbance to estimate the chlorophyll content in leaf tissue. Two wavelengths are used for absorbance determinations. One wavelength falls within the chlorophyll absorbance range while the other serves to compensate for mechanical differences such as tissue thickness. The CCM-200 measures the absorbance of both wavelengths and calculates a CCI value that is proportional to the amount of chlorophyll in the sample (Apogee 2017).

In more than 30 published papers to determine chlorophyll content for a large number of plants, the two types of chlorophyll meter developed by Minolta, (model SPAD-502) and Opti-Sciences, (model CCM-200) were commonly used (Parry et al. 2014). Chlorophyll contents were determined under water stress, salinity stress and fertilizer stress using various chlorophyll content measuring devices. Chlorophyll contents of various plants under salinity stress for beans (Beinsan et al. 2009), rice (Chandramohanan 2014), and soybean (Sabagh et al. 2015), and under water stress for olive (Khaleghi et al., 2012), cotton (Kazgöz Candemir and Ödemiş 2018), elaeagnus (Ahani

et al. 2015), canola (Meskini-Vishkaee et al. 2015), sesamum (Baştuğ et al. 2016) strawberry (Ödemiş et al. 2020), and peanut (Ngulube et al. 2018) were determined using chlorophyll meters.

On the other hand, the performance of chlorophyll content measuring devices for olives (Khaleghi et al. 2012), Asian Pear (Ghasemi et al. 2011), grape (Filimon et al. 2016), paper birch (Richardson et al. 2002), and quercus (Silla et al. 2010) under different stress conditions was evaluated and compared with spectrophotometric measurements. In this study, it was aimed to determine the chlorophyll content of peanut (*Arachis hypogaea* cv. NC-7) grown under different soil texture (clay-loam and sandy) and salinity levels (0.7, 2.1 and 3.3 dS $m^{-1}$) using the destructive method (spectrophotometer), and to compare them with the nondestructive method (CCI) for evaluating the performance of the nondestructive method.

## 2. Materials and Methods

The study was carried out at the Research and Application Farm of Agricultural Faculty at Akdeniz University between 27.05.2016 and 19.10.2016. The experimental area where the Mediterranean climate is prevailing was located at a latitude of 36°, 53', 45" N, a longitude of 30°, 38', 17" E, and an altitude of 30 m. The temperature and relative humidity ranged between 18.3-33.1°C and 21.9-82.9% at the growing season in 2016, respectively (MGM 2017).

The experiment was carried out in a complete randomized block design in pots using two soil textures, clay-loam and sandy, and began on 27.05.2016 with the planting of NC-7 (*Arachis hypogaea* cv. NC-7) peanut seeds. The crops were grown in 80 L plastic pots, 0.53 (upper diameter) × 0.41 (bottom diameter) × 0.46 (height) m deep, filled with clay-loam and sandy soils, and each pot had 4 plants. The physical characteristics of the soil were given in Table 1.

The amount of irrigation water was based on soil water deficit in four days. To prevent salt accumulation in the pots, 20% extra irrigation as leaching water was applied. Ayers and Westcot (1985) reported threshold values corresponding to 0% ($EC_i$=2.1 dS $m^{-1}$) and 50% ($EC_i$= 3.3 dS $m^{-1}$) yield reduction for peanut. Depending on the study of Ayers and Westcot (1985), three different salinity levels ($EC_i$= 0.7, 2.1, 3.3 dS $m^{-1}$) were applied including the control treatment ($EC_i$= 0.7 dS $m^{-1}$). Saline irrigation water was prepared by mixing specific ratios of three different salts (NaCI, $MgSO_4$ and $CaCl_2$) in tap water.

To determine the chlorophyll content in different salinity treatments and soil textures, a total of 54 leaves, 3 from each replication were selected and marked. The chlorophyll content index (CCI) value in the marked leaves was measured by Apogee CCM-200 portable chlorophyll meter. Similar to Pereyra et al. (2014) all measurements were carried out early in the morning to avoid variations caused by the movement of chloroplasts during the course of the day. Following CCI measurement, the marked leaves were cut and leaf chlorophyll contents (Chlorophyll-a (Chl-a) (mg $cm^{-2}$) and Chlorophyll-b (Chl-b) (mg $cm^{-2}$) were determined by UV-V (PG T60 UV VIS) spectrophotometer in the laboratory conditions, then Chlorophyll-a+b (Chl-a+b) (mg $cm^{-2}$) and Chlorophyll-a/b (Chl-a/b) were calculated. The acetone extraction method (Williams 1984) was used to directly determine leaf chlorophyll content. Since acetone extraction procedure is classified as destructive methods under laboratory conditions, for chlorophyll measurements, leaf samples were cut from the plant and analyzed only at the end of the experiment. As soon as the leaves were cut, they were transported to the laboratory in a thermal bag and chlorophyll content analysis was performed.

The experimental data were statistically analyzed by the general linear model (GLM). Means were compared using Duncan's Multiple Range Test, if necessary, to separate the means of the data at 0.05 level of significance. Regression analysis was performed between chlorophyll content (Chl-a, Chl-b Chl-a+b) obtained in vitro and CCI values measured in situ in order to determine the performance of chlorophyll meter at different salinity levels.

## 3. Results and Discussion

In order to evaluate the effect of soil textures (ST) and salinity level (SL) on Chl-a, Chl-b, Chl-a+b, Chl-a/b variance analysis results were given in Table 2.

The mean of Chl-a, Chl-b, Chl-a+b, and CCI decreased due to salinity stress. Compared to the control treatments (0.7 ds $m^{-1}$), the Chl-a content decreased by 37.9% and 62.1% in the 2.1 ds $m^{-1}$ and 3.3 ds $m^{-1}$ treatments, respectively, while the Chl-b content decreased by 32.8% and 58.0%, respectively. (Table 2). Because previous studies (Jamil et al. 2007; Sabagh et al. 2015) showed that salt stress causes an increase in the chlorophyll-degrading enzyme (chlorophyllase), thereby inducing the destruction of the chloroplast structure and instability of the pigment-protein complexes. Since salts tend to attach to clay particles in the soil, salt accumulation occurs more in clay soils than in sandy soils under the same conditions (IAARD 2008). Considering these two conditions, it would be expected that the amount of chlorophyll would be low since salt accumulation was high in clay soils. However, this research showed that the mean of Chl-a, Chl-b, Chl-a+b and CCI contents were higher in clay-loam soil than sandy soil. This phenomenon can be explained by the relationship of soil texture with plant nutrients and water. Because clay soils have finer particles that can hold soil water and nutrients better than sandy soil (Dou et al. 2016). For this reason, in this study, crop development was positively affected and increased chlorophyll content due to the fact that crops benefited more from water and nutrients in clay soil.

Different levels of irrigation water salinity and soil textures did not affect the ratio of Chl-a and Chl-b (Chl-a/b) statistically (Table 2). The Chl-a/b ranged from 1.37 to 3.48, depending on the different treatments (Data not shown). Banks and Eskins (1981) reported that the ratio of Chl-a and Chl-b was between 2.5 and 3.5 in 5 different peanut varieties. However, Monge et al. (1987) declared that this value for peanuts varied between approximately 1.2 and 2.0. The difference between Chl-a/b values in different experiments shows that this ratio is affected by many environmental factors and variety differences.

**Table 1.** Physical characteristics of soils used in the experimental pots

| No | Sand (%) | Silt (%) | Clay (%) | Texture Class | Field Capacity (%) | Permanent wilting point (%) | Bulk density (g $cm^{-3}$) | pH | EC (dS $m^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 23.7 | 27.6 | 48.7 | Clay-Loam | 27 | 13.8 | 1.20 | 6.9 | 0.989 |
| 2 | 94 | 1.5 | 4.5 | Sandy | 8.5 | 2 | 1.45 | 7.9 | 0.293 |

**Table 2.** Effect of different soil textures and salinity levels on Chl-a, Chl-b, Chl-a+b, Chl-a/b and CCI content

| | *Soil texture* | Salinity level | | | *P > F* | *Mean of ST* |
|---|---|---|---|---|---|---|
| | | **0.7 (ds m$^{-1}$)** | **2.1 (ds m$^{-1}$)** | **3.3 (ds m$^{-1}$)** | | |
| Chl-a (mg cm$^{-2}$) | **Clay-Loam** | *[£]0.38 Aa* | *0.22 b* | *0.14 c* | ** | **[†]0.25 A** |
| | **Sandy** | *0.20 B* | *0.15* | *0.08* | ns | **0.14 B** |
| | **P > F** | * | ns | ns | | |
| | **Mean of SL** | **[†]0.29 a** | **0.18 b** | **0.11 c** | | |
| | **Significance** | Soil Texture (ST): ** Salinity Level (SL): ** *ST* x *SL: ns* | | | | |
| | | **0.7 (ds m$^{-1}$)** | **2.1 (ds m$^{-1}$)** | **3.3 (ds m$^{-1}$)** | **P > F** | **Mean of ST** |
| Chl-b (mg cm$^{-2}$) | **Clay-Loam** | *[£]0.140 a* | *0.093 b* | *0.063 b* | ** | **[†]0.099 A** |
| | **Sandy** | *0.099 a* | *0.062 ab* | *0.039 b* | * | **0.067 B** |
| | **P > F** | ns | ns | ns | | |
| | **Mean of SL** | **[†]0.119 a** | **0.080 b** | **0.050 c** | | |
| | **Significance** | Soil Texture (ST): ** Salinity Level (SL): ** *ST* x *SL: ns* | | | | |
| | | **0.7 (ds m$^{-1}$)** | **2.1 (ds m$^{-1}$)** | **3.3 (ds m$^{-1}$)** | **P > F** | **Mean of ST** |
| Chl-a+b (mg cm$^{-2}$) | **Clay-Loam** | *[£]0.52 a* | *0.31 b* | *0.21 b* | ** | **[†]0.35 A** |
| | **Sandy** | *0.30* | *0.22* | *0.12* | ns | **0.21 B** |
| | **P > F** | ns | ns | ns | | |
| | **Mean of SL** | **[†]0.41 a** | **0.26 b** | **0.16 c** | | |
| | **Significance** | Soil Texture (ST): ** Salinity Level (SL): ** *ST* x *SL: ns* | | | | |
| | | **0.7 (ds m$^{-1}$)** | **2.1 (ds m$^{-1}$)** | **3.3 (ds m$^{-1}$)** | **P > F** | **Mean of ST** |
| Chl-a/b | **Clay-Loam** | *2.72* | *2.31* | *2.31* | ns | **[†]2.45** |
| | **Sandy** | *1.99* | *2.49* | *2.02* | ns | **2.16** |
| | **P > F** | ns | ns | ns | | |
| | **Mean of SL** | **2.36** | **2.40** | **2.16** | | |
| | **Significance** | Soil Texture (ST): ns Salinity Level (SL): ns *ST* x *SL: ns* | | | | |
| | | **0.7 (ds m$^{-1}$)** | **2.1 (ds m$^{-1}$)** | **3.3 (ds m$^{-1}$)** | **P > F** | **Mean of ST** |
| CCI | **Clay-Loam** | *[£]17.1 Aa* | *11.6 Ab* | *10.5 Ab* | ** | **[†]12.8 A** |
| | **Sandy** | *10.3 B* | *8.3 B* | *7.0 B* | ns | **8.6 B** |
| | **P > F** | * | * | * | | |
| | **Mean of SL** | **[†]13.4 a** | **10.0 b** | **8.8 c** | | |
| | **Significance** | Soil Texture (ST): ** Salinity Level (SL): ** *ST* x *SL:* ns | | | | |

[£]: In the italicized section, means followed by the different small letters in each row or capital letters in each column are significantly different at 5% level by Duncan test.
[†]**:** In the bold section, means followed by the different small letters in each row or capital letters in each column are significantly different at 5% level by Duncan test. *: $P<0.05$ (significant); **: $P<0.01$ (highly significant). ns: non-significant.

Linear and polynomial functions were fitted to estimate Chl-a, Chl-b, Chl-a+b content from portable chlorophyll meter (CCI) measurements in sandy and clay-loam soils. The mathematical equations of linear and polynomial functions and the determination coefficients ($R^2$) of these equations were given in Figure 1.

The relationship between CCI and chlorophyll content was described linearly in Asian pear tree by Ghasemi et al. (2011), in wheat by Lunagaria et al. (2015), in sugar maple by Van Den Berg and Perkins (2004), and was described non-lenearly by Richardson et al. (2002) in paper birch. Parry et al. (2014), on the other hand, studied 30 different studies and examined the relationship between CCI and chlorophyll content for 22 different plants and this study highlights the enormous differences in chlorophyll distribution among species and even within species.

There was a high relationship between CCI and all chlorophyll contents in both soil textures ($R^2$>0.80) (Figure 1). Depending on the increase in chloroplast uniformity, the efficiency of red light absorption increases (Parry et al. 2014). Parry et al. (2014) reported that the transmission of light through a leaf is affected by pigment concentration and pigment spatial distribution in leaves. The authors also claimed that non-uniform chlorophyll distribution caused decreases in transmission of light at lower chlorophyll concentrations. Therefore in each chlorophyll content, $R^2$ values of linear and polynomial curves of clay soils were higher compared to sandy soils.

When the different regression fitted models (linear and polynomial) were examined, the $R^2$ of the polynomial equations showed higher performances for all soil and chlorophyll content types. On the other hand, the $R^2$ of all fitted model equations was very close to each other in the same treatments. For this reason, it is recommended to use the linear equation to determine the chlorophyll contents because it is easier to use.

## 4. Conclusion

This study was conducted to determine the effects of soil texture and salinity level on the chlorophyll content in peanuts. In addition, the determination coefficients of linear and polynomial fitted curves were determined between the chlorophyll contents obtained by destructive and non-destructive methods. In this study, the chlorophyll contents (Chl-a, Chl-b, Chl-a+b and CCI) of peanuts varied statistically depending on the salinity level of the irrigation water and soil texture. The results showed that increasing salinity levels decreased the chlorophyll contents. In addition, the chlorophyll content on clay-loam soil treatments was higher compared to

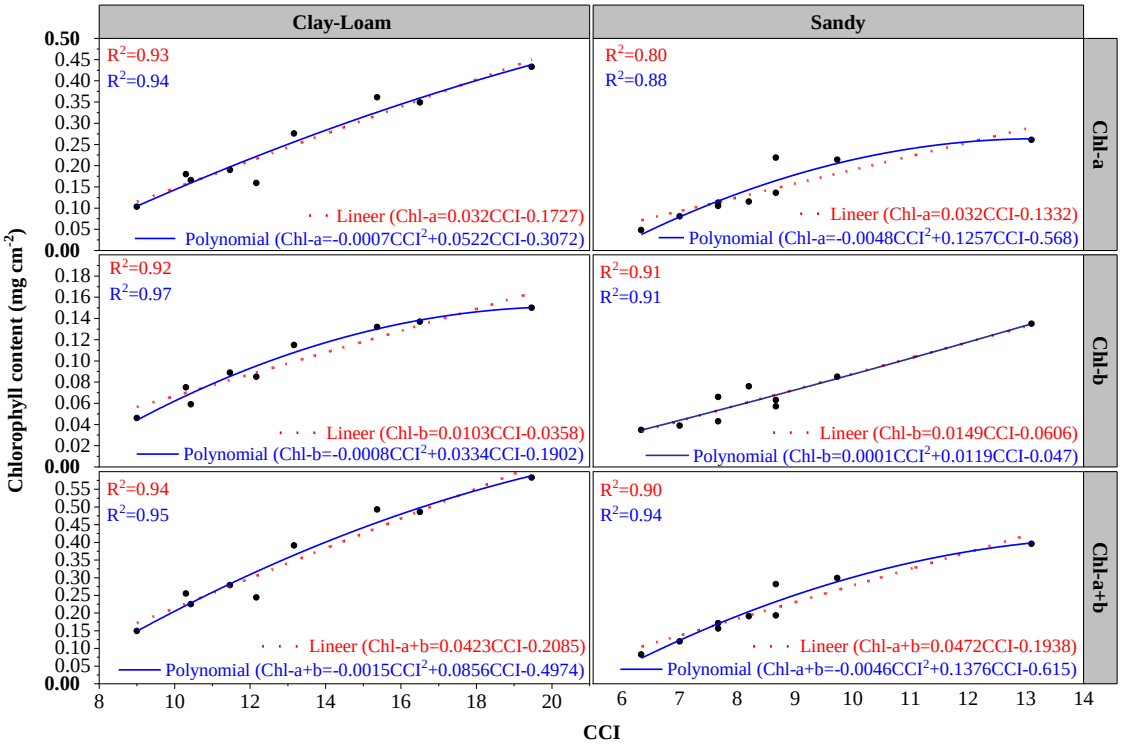


**Figure 1.** Equations based on linear and polynomial fitted curves between Chl-a, Chl-b and Chl-a+b and CCI values and the coefficients of determination of these equations.

sandy soil. The linear and polynomial models had high performance in all soil textures and chlorophyll contents. Although the polynomial model showed higher performance compared to the linear model, it is recommended to use the linear model to estimate CCI because there is no big difference in performance and it is easier to use. According to these results, the total chlorophyll content can be easily and safely estimated under the saline conditions by using the portable chlorophyll meter.

## References


Ahani H, Jalilvand H, Vaezi J, Sadati SE (2015) Effects of different water stress on photosynthesis and chlorophyll content of *Elaeagnus Rhamnoides*. Iranian Journal of Plant Physiology 5: 1403-1410.

Apogee (2017) CCM-200 Chlorophyll Content Meter Web Manual. Apogee Instrument.

Ayers AS, Westcot DW (1985) Water Quality for Agriculture. FAO Irrigation and Drainage Paper 20, Rome.

Banks DJ, Eskins K (1981) Analysis of normal and mutant peanut chloroplast pigments by liquid chromatography. Peanut Science 1: 40-42.

Baştuğ R, Karaca C, Büyüktaş D, Aydınşakir K, Dinç N (2016) The effects of deficit irrigation on water use, yield and quality properties of sesame (*Sesamum indicum* L.) grown in lysimeters. In 13. Ulusal Kültürteknik Kongresi. Antalya, Turkey, pp. 256-266. (In Turkish)

Beinsan C, Camen D, Sumalan R, Babau M (2009) Study concerning salt stress effect on leaf area dynamics and chlorophyll content in four bean local landraces from Banat area. In 44th Croatian & 4th International Symposium on Agriculture. Opatija, Croatia, pp. 416-419.

Van Den Berg AK, Perkins TD (2004) Evaluation of a portable chlorophyll meter to estimate chlorophyll and nitrogen contents in sugar maple (*Acer saccharum* Marsh.) leaves. Forest Ecology and Management 200: 113-117.

Chandramohanan KT (2014) A study on the effect of salinity stress on the chlorophyll content of certain rice cultivars of Kerala State of India. Agriculture, Forestry and Fisheries 3: 67.

Dou F, Soriano J, Tabien RE, Chen K (2016) Soil texture and cultivar effects on rice (*Oryza sativa*, L.) grain yield, yield components and water productivity in three water regimes. PLoS ONE 11: 1-12.

Filimon RV, Rotaru L, Filimon RM (2016) Quantitative investigation of leaf photosynthetic pigments during annual biological cycle of *Vitis vinifera* L. table grape cultivars. South African Journal for Enology and Viticulture 37: 1-14.

Ghasemi M, Arzani K, Yadollahi A, Ghasemi S, Sarikhani Khorrami S (2011) Estimate of leaf chlorophyll and nitrogen content in Asian Pear (*Pyrus serotina* Rehd.) by CCM-200. Notulae Scientia Biologicae 3: 91-94.

Hazrati S, Tahmasebi-Sarvestani Z, Modarres-Sanavy SAM, Mokhtassi-Bidgoli A, Nicola S (2016) Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L.. Plant Physiology and Biochemistry 106: 141-148.

IAARD (2008) A Practical Guide To Restoring Agriculture After A Tsunami. In Indonesian Agency for Agricultural Research and Development, Indonesia and NSW Department of Primary Industries.

Jamil M, Rehman SU, Kui JL, Jeong MK, Kim HS, Eui SR (2007) Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. Scientia Agricola 64: 111-118.

Kazgöz Candemir D, Ödemiş B (2018) Effects of foliar sulfur applications in reducing water stress applied to the cotton plant (*Gossypium hirsutum* L.) during different development (In Turkish) periods. Derim 35: 161-172.

Khaleghi E, Arzani K, Moallemi N, Barzegar M (2012) Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content

index under water stress in *Olea europaea* cv. Dezful. International Scholarly and Scientific Research and Innovation 6: 2108-2111.

Lunagaria MM, Patel HR, Pandey V (2015) Evaluation and calibration of noninvasive leaf chlorophyll meters for wheat. Journal of Agrometeorology 17: 51-54.

Manolopoulou E, Varzakas T, Petsalaki A (2016) Chlorophyll determination in green pepper using two different extraction methods. Current Research in Nutrition and Food Science Journal 4: 52-60.

Martinazzo EG, Ramm A, Bacarin MA (2012) The chlorophyll a fluorescence as an indicator of the temperature stress in the leaves of *Prunus persica*. Brazilian Journal of Plant Physiology 24: 237-246.

Meskini-Vishkaee F, Mohammadi MH, Neyshabouri MR, Shekari F (2015) Evaluation of canola chlorophyll index and leaf nitrogen under wide range of soil moisture. International Agrophysics 29: 83-90.

MGM (2021) General Meteorological Statistics of Provinces. Turkish State Meteorological Service. https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m =ANTALYA. Accessed 7 June, 2021.

Monge E, Val J, Heras L, Abadia J (1987) Photosynthetic Pigment Composition of Higher Plants Grown under Iron Stress. In: Biggins J (Ed.), Progress in Photosynthesis Research: Proceedings of the VIIth International Congress on Photosynthesis Providence, Rhode Island, USA, Springer Netherlands, Dordrecht, pp. 201-204.

Ngulube M, Mweetwa AM, Phiri E, Muriu SC, Chalwe H, Shitumbanuma V, Brandenburg RL (2018) Effects of biochar and gypsum soil amendments on groundnut (*Arachis hypogaea* L.) dry matter yield and selected soil properties under water stress. African Journal of Agricultural Research 13: 1080-1090.

Ödemiş B, Kazgöz Candemir D, Evrendilek F (2020) Responses to drought stress levels of strawberry grown in greenhouse condiations. Hortic. Studies 37: 113-122.

Parry C, Blonquist JM, Bugbee B (2014) In situ measurement of leaf chlorophyll concentration: Analysis of the optical/absolute relationship. Plant, Cell and Environment 37: 2508-2520.

Patane P, Vibhute A (2014) Chlorophyll and nitrogen estimation techniques: A Review. International Journal of Engineering Research and Reviews 2: 2348-697.

Perera CO, Smith B (2013) Technology of Processing of Horticultural Crops. In: Handbook of Farm, Dairy and Food Machinery Engineering. London, pp. 259-315.

Pereyra MS, Davidenco V, Núñez S, Argüello JA (2014) Chlorophyll content estimation in oregano leaves using a portable chlorophyll meter : relationship with mesophyll thickness and leaf age. Agronomía and Ambiente 34: 77-84.

Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153: 185-194.

Sabagh AEL, Omar AE, Saneoka H, Barutçular C (2015) Comparative physiological study of soybean (*Glycine Max* L.) cultivars under salt stress. Yuzuncu Yil University Journal of Agricultural Sciences 25: 269-284.

Silla F, González-Gil A, González-Molina ME, Mediavilla S, Escudero A (2010) Estimation of chlorophyll in quercus leaves using a portable chlorophyll meter: effects of species and leaf age. Annals of Forest Science 67: 108-108.

Taibi K, Taïbi F, Ait Abderrahim L, Ennajah A, Belkhodja M, Mulet JM (2016) Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L.. South African Journal of Botany 105: 306-312.

Tezcan A, Aslan GE, Kaman H, Can M, Sayici A, Gökçen U, Ekizoğlu H (2018) Assessing different irrigation regimes regarding chlorophyll content of the sweet bell pepper. Journal of Agriculture and Ecology Research International 15: 1-7.

Williams S (1984) Official Methods of Analysis of the Association of Official Analytical Chemists (14th ed.). In Association of Analytical Communities, USA.

Wood CW, Reeves DW, Himelrick DG (1993) Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status, and crop yield: A review. Proceedings of the Agronomy Society of New Zealand 23: 1-9.

Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES
(2021) 34(3): 307-313
DOI: 10.29136/mediterranean.972937

# Actinorhizal plants of Turkey: formation of nodules, cluster roots and ectomycorrhizal roots

**Ian T. RILEY, Eniola A. OLOWU, Kaddijatou JAWNEH, Müge ATLI**

Department of Plant Production and Technologies, Faculty of Agricultural Science and Technologies, Nigde Omer Halisdemir University, Nigde, 51240, Turkey

Corresponding author: I. T. Riley, e-mail: ian@riley.asia
Author(s) e-mail: eniolowu@gmail.com, kjawneh@gmail.com, mugeatli51@gmail.com

ARTICLE INFO

Received: July 20, 2021
Received in revised form: July 27, 2021
Accepted: November 12, 2021

***Keywords***:

Actinorhizae
Ectomycorrhizae
Nodulation
Proteiod roots

**ABSTRACT**

None of the known native actinorhizal species in Turkey, *Alnus glutinosa* and *A. orientalis* (Betulaceae), *Datisca cannabina* (Datiscaceae), *Elaeagnus angustifolia* and *Hippophae rhamnoides* (*Elaeagnaceae*), and the widely-planted exotic *Casuarina cunninghamiana* (Casuarinaceae), have confirmed records of actinorhizae in Turkey. This study determined the capacity of representative actinorhizal plants in Turkey to form nodules, cluster roots and ectomycorrhizal roots in a typical central Anatolian soil with and without amendment of soil and nodule extracts, as well as in soil from Adana and Izmir. Nodulation was confirmed experimentally for *E. angustifolia* and *C. cunninghamiana* in Niğde soil (the latter only with addition Adana or Izmir soil), but only observationally for *A. glutinosa* during sample collection in Rize. Cluster roots developed strongly in *C. cunninghamiana*, and likewise ectomycorrhizal roots in *Allocasuarina verticillata* (included as a reference species) but only to a lesser extent in *C. cunninghamiana*. The nodulation status of the natives, *D. cannabina* and *H. rhamnoides*, remains to be investigated.

## 1. Introduction

Actinorhizal plants with nitrogen-fixing nodules induced by species of the Actinobacteria, *Frankia*, are important endemics and economic exotics in many climatic and floristic regions worldwide. Actinorhizal species are found in 24 genera in eight families (Benson and Silvester 1993), however, when these species are reported in specific locations there is often an untested assumption that they are actinorhizal and will be nodulated and fixing nitrogen. However, this can be an invalid assumption, for example, Paschke (1997) makes the point that for *Dryas* (Rosaceae), although widespread in Europe and the USA, there were no confirmed reports of nodulation throughout that range at the time of writing. Also, his review of 80 actinorhizal species in the western USA did not cite evidence for nodulation of any of these species. Markham (2009) addressed this issue experimentally and was unable to confirm that *Dryas integrifolia* can nodulate in response to inoculation with several *Frankia* strains. Markham attributed this apparent inconsistency to there being non-nodulating species in otherwise actinorhizal genera, but lack of nodulation in other contexts could also be a result of non-conducive conditions or absence of compatible *Frankia*.

Turkey is a regarded as having high floristic richness (Kier et al. 2005) with nearly two thirds of the country in Mediterranean or Mediterranean-influenced continental climate zones with these areas possessing rich floras. However, unlike, for example, Australia with >60 putative actinorhizal species in the Casuarinaceae (Riley 2021) and 80 species in varies families in the western USA (Paschke 1997), the number of actinorhizal species in Turkey is low and their nodulation status undetermined. There are five known native actinorhizal species in Turkey, *Alnus glutinosa* and *A. orientalis* (Betulaceae), *Datisca cannabina* (Datiscaceae), *Elaeagnus angustifolia* and *Hippophae rhamnoides* (*Elaeagnaceae*). *Casuarina cunninghamiana* (*Casuarinaceae*) is the only introduced actinorhizal species and is widely used as an ornamental, landscape and windbreak tree in provinces along the Mediterranean and Aegean coasts (Riley and Korkmaz 2019). Although, *E. angustifolia* and *H. rhamnoides* are widely distributed species that produce edible fruit, their current horticultural use in Turkey is minimal.

Although there is no direct evidence of nodulation of actinorhizal plants in Turkey, the authors of a molecular study of *Frankia* report collecting nodules from *A. orientalis* at two sites in Turkey (Ormancık, Adana Province and Demirtaş, Antalya Province) (supplementary data in Põlme et al. 2014). There is no similar incidental information available for other native species, and given that nodulation of *Casuarina* grown outside its natural range does not occur unless compatible *Frankia* are also introduced (Simonet et al. 1999), it should not be assumed that *C. cunninghamiana* is nodulated in Turkey.

With actinorhizal species having great potential for agroecosystem restoration and improvement in contexts with infertile or degraded soil (e.g. Roy et al. 2011), and as pioneer species in assisted secessional restoration (e.g. Parrotta 1999), it is important that their nodulation status/potential is determined in target contexts. Therefore, the aim of this study was to determine

the capacity of representative actinorhizal plants in Turkey to nodulate in a typical central Anatolian soil with and without amendment of soil and nodule extracts (that could supply compatible *Frankia*). In addition, root responses such as formation of cluster roots and ectomycorrhizal associations were assessed. The data obtained provides the first evidence of nodulation of the test species (*A. glutinosa*, *C. cunninghamiana* and *E. angustifolia*) both experimentally and observationally (during sampling for materials used as experimental amendments) in Turkey.

## 2. Materials and Methods

Seeds were sourced from Australia and Turkey as detailed in Table 1. Seeds of *A. glutinosa* and depulped *E. angustifolia* were stratified in moist sand at 5°C for at least 6 weeks to promote germination. Soil (<200 mm deep) and nodules were variously obtained from the root zones of actinorhizal species as detailed in Table 1. Basic analysis of the soil was performed by the Niğde government soil laboratory (TC Niğde İl Özel İdaresi Toprak ve Su Analiz Laboratuvarı, Niğde, Turkey) as shown in Table 2. In addition, during the collection of soil samples, exposed roots were examined for the presence of nodules, and the roots systems of mechanically uprooted mature *E. angustifolia* trees in Niğde were likewise examined for nodules.

Plants were grown in mixtures of soil and peat (Emin Torf, Yeniçağa, Bolu Province, Turkey) in plastic pots in a greenhouse as detailed below for all experiments. The addition of field soil (either directly or as an aqueous suspension) was used as a potential source of *Frankia* in the experiments. In the final two experiments, suspensions of nodules of *A. verticillata* (Table 1) were also included as an inoculum.

The amendments of soil and nodule suspensions were prepared as follows. Air-dried soil (100 mL) from Adana, Niğde and Rize were separately suspended in 100 mL tap water, vigorously shaken for 5-10 min and allowed to settle. The supernatants were decanted and applied as an inoculum in the first and second experiments. Air-dried nodules (0.5 g air dried) from *A. verticillata* were crushed in 50 mL of skimmed milk and applied (1 mL per plant) as an inoculum in the final two experiments.

**Table 1.** Seed, soil and nodule sources used in greenhouse experiments to assess the **f**ormation of nodules, cluster roots and mycorrhizal roots in actinorhizal plants in Turkey.

| Material | Actinorhizal species or associated species | Collection details | Expt |
|---|---|---|---|
| Seed | *Allocasuarina verticillata* | 26.I.2019, Linden Park, SA, Australia, 34°56'23" S, 138°38'34" E, planted, public garden, collected by IT Riley | 1,2,3,4,5 |
| | *Casuarina cunninghamiana* | 27.X.2018, Antalya, Antalya, Turkey, 36°53'06" N, 30°40'54" E, planted, public garden, collected by IT Riley | 1,2,3,4,5 |
| | *Elaeagnus angustifolia* | 2.X.2018, Niğde Ömer Halisdemir University, Niğde, Turkey, 37°56'17.5" N, 34°37'34.4" E, planted/volunteer, collected by M Altı and IT Riley, stratified before planting | 1,2 |
| | *Alnus glutinosa* | X.2018, Ordu, Turkey, 40°58'34" N, 37°58'03" E, natural stand (exact location unknown), collected by E Ekbiç | 1,2 |
| Soil | *C. cunninghamiana* | 17.X.2018 (Expts 1 and 2) and 23.X.2019 (Expt 4), Çukurova University, Sarıçam, Adana, Turkey, 37°03'29" N, 35°21'24.7" E, adjacent planted *C. cunninghamiana*, collected by IT Riley and EA Olowu | 1,2,4 |
| | | 17.I.2020, Ege University, Erzene, Bornova, Izmir, Turkey, 38°27'19" N, 27°13'34" E, adjacent planted *C. cunninghamiana*, collected by IT Riley | 5 |
| | *E. angustifolia* | Various dates, Niğde Ömer Halisdemir University Campus, Niğde, Turkey, in fields, 37°56'17.5" N, 34°37'34.4" E, with *E. angustifolia* within 100 m radius, collected by the authors | 1,2,3 |
| | *A. glutinosa* | 22.VI.2018, Yaylaköy, Çamlıhemşin, Turkey, 40°51'36" N, 41°00'03" E, collected by IT Riley | 1,2 |
| Nodules | *A. verticillata* | 25.VII.2019, Nepean Bay, Kangaroo Island, SA, Australia, 35°44'14" S 137°35'57" E, trees uprooted by roadworks, collect by IT Riley, MH Ryder and JR Rathjen. | 4,5 |

**Table 2.** Analysis of soils from three locations used in the growth medium to assess the **f**ormation of nodules, cluster roots and mycorrhizal roots in actinorhizal trees in Turkey.

| Location | Site | Associated actinorhizal species[1] | Collection | Saturation (%) | pH | Salt (%) | $CaCO_3$ (%) | Olsens P (ppm)[2] | K (ppm)[2] |
|---|---|---|---|---|---|---|---|---|---|
| Adana | Garden | *Casuarina cunninghamiana* | 23.X.2019 | 84 | 7.7 | 0.05 | 31 | 6.8 | 137 |
| Izmir | Garden | *C. cunninghamiana* | 18.I.2020 | 57 | 7.9 | 0.04 | 17 | 5.8 | 127 |
| Niğde | Field | *Elaeagnus angustifolia* | 11.IX.2020 | 45 | 7.5 | 0.03 | 26 | 4.4 | 192 |

[1]Soil collected adjacent to the listed tree, except in Niğde, where specimens of the tree occurred within a 100-m radius of the soil collection site. [2]Elemental not oxide.

Five experiments were conducted in growth medium consisting of various combinations of soil and peat, with and without amendment, to assess root responses including nodulation, cluster root and ectomycorrhizal root development. The greenhouse (37°56'38" N, 34°37'51" E) was located on the campus of Niğde Ömer Halisdemir University, Turkey, with automatic ventilation, evaporative coolers and shade-cloth for cooling in summer and central heating for winter, however, the temperature was not rigorously controlled.

Experiment 1: *C. cunninghamiana* was grown in a full factorial experiment with four inoculation treatments, and sterilised or unsterilised growth medium (75% Niğde soil plus 25% peat) in a randomised complete block design (4 replicates). The inoculation treatments (1 mL per plant) were the three soil suspensions as described above plus an uninoculated control. The sterilisation treatment was three cycles of autoclaving at 121°C for 60 min with at least 48 h between cycles. *C. cunninghamiana* seeds were germinated in peat and transplanted to the treatment pots (round plastic pots, 125 × 95 mm) on 5 November 2018. Inoculation treatments were applied 7 days after transplanting. The plants were watered as needed and in a manner to minimise the risk of cross contamination. By early February, 10 weeks after planting, many plants were chlorotic, so a low rate of complete soluble fertiliser (5 mL of 8 g 4.5 $L^{-1}$, Thrive Soluble Fertiliser, Yates, Clayton, Vic., Australia) was applied weekly (but with minimal visual response). Plants were harvested for assessment after 20 weeks.

Experiment 2: *A. glutinosa*, *A. verticillata*, *C. cunninghamiana* and *E. angustifolia* were grown in an unsterilised growth medium (25% Niğde soil plus 75% peat) inoculated with a combined suspension of Adana and Rize soil (prepared as described above). The plants were germinated in peat and single plants transplanted to each pot (round plastic pots, 165 × 140 mm) on 10 March 2019. Other details were the same as experiment 1, although no fertilizer was applied. Plants were harvested for assessment after 52 weeks.

Experiment 3: *A. glutinosa*, *A. verticillata*, *C. cunninghamiana* and *E. angustifolia* were grown in the same growth medium as in experiment 2 but in larger pots (square plastic pots, 115 × 183 mm) with no inoculum. One to two plants per pot were transplanted to 20 replicate pots on 17 April 2019. Other details were the same as experiment 2. Plants were harvested for assessment after 47 weeks.

Experiment 4: *A. verticillata* and *C. cunninghamiana* were grown in a full factorial experiment with and without inoculation in unsterilised growth medium (25% Adana soil plus 75% peat) in a randomised complete block design with five replicates. The inoculum (1 mL $plant^{-1}$) was *A. verticillata* nodule suspension as described above. Seeds of the plants were germinated directly in the growth medium (in the same pots as experiment 1) in a growth chamber (~25°C), sown on 20 December 2019 and transferred to the greenhouse on 22 January 2020. Inoculation was applied 35 days after germination. The plants were watered as needed and from below to minimise the risk of cross contamination. Plants were harvested for assessment after 31 weeks.

Experiment 5: *A. verticillata* and *C. cunninghamiana* were grown in full factorial experiment using Izmir soil (25%) with four replicates but otherwise as in experiment 4. The seeds were sown into pots in the growth chamber on 24 January 2020 and transferred to the greenhouse 28 February 2020. Inoculation was applied 35 days after germination. Plants were harvested for assessment after 26 weeks.

At harvest, plant height and visual health were recorded. Shoot colour was scored on a four-point scale with zero being uniformly chlorotic (yellow) through to three for fully green. The plants were removed from pots and gently washed. Roots were assessed for their length, visual health, and the presence of nodules, cluster roots and mycorrhizal roots. Root health was scored on a three-point scale with one being poorly developed through to three for healthy and well developed. Roots were examined under a stereomicroscope to count nodules and cluster roots, and to observe any other root responses. Ectomycorrhizal roots were scored as absent (-), present (+) or present at moderate to high density (++).

Data processing, analysis and visualisation was performed with R (www.R-project.org). Analysis of variance was conducted on complete data sets, e.g. plant height, however, differences between plants species were not statistically meaningful and the analysis could not done in data sets with many zero values. Given that the key responses needed to meet the aims of the study were categorical in nature, exploratory statistics were (Tukey 1997) used, where appropriate.

## 3. Results

Across the five experiments, nodules, cluster roots and ectomycorrhizal roots were variously observed depending on the plant species, soil and amendments. The key results are presented below for each plant species.

### 3.1. *Alnus glutinosa*

In the experiments that include *A. glutinosa* (experiments 2 and 3), although it germinated readily, its growth was poor with leaves chlorotic turning necrotic, and plants dying before harvest. Although no experimental data was obtained on the nodulation of *A. glutinosa* in Turkish soils, in the field where the soil was collected, nodules were observed (Figure 1a) with many occurring on roots within the litter layer.

### 3.2. *Allocasuarina verticillata*

*Allocasuarina verticillata*, included in this study as a reference species not currently grown in Turkey, was included in all but the first experiment with no nodulation observed despite being inoculated with a presumptively compatible nodule suspension in experiments 4 and 5. Nevertheless, the plants grew well in all cases, growing as well as, or better than, *C. cunninghamiana* (Table 3 and Figure 1g, h), with shoot scores of 3 in Adana and Izmir soil, but exhibited mild chlorosis in Niğde soil with shoot scores of 2.4. Their root systems appeared healthy and well developed (scored as 3 in all experiments), but were not highly branched. Cluster roots were uncommon within only a single cluster root found on four plants (two plants in experiment 3 and one each in experiments 4 and 5; Table 2). In contrast, ectomycorrhizal roots were present in abundance in all cases. These were short, thick, mostly unbranched roots, growing perpendicular to the main root often with some terminal swelling (Figure 1i) consistent with range in ectomycorrhizal root morphology described by Brundrett and Tedersoo (2020). This not only contrasted with their lack of nodules and rare cluster roots in this species, it also contrasted with the low numbers of ectomycorrhizal roots found on *C. cunninghamiana*, the only other test species to have evident ectomycorrhizal roots.

Although no nodules were found in these experiments, they were obtained from uprooted trees in a natural stand in Australia (Figure 2), where they occurred at low density on relatively thick roots near the crown or the plants. Efforts to find them on smaller, surface roots surrounding undisturbed trees were unsuccessful.

### 3.3. *Casuarina cunninghamiana*

*Casuarina cunninghamiana* was included in all experiments with nodules, cluster roots and ectomycorrhizal roots developing variously across the experiments. In experiment 1, the plants grew slowly with a moderate proportion of chlorotic


**Figure 1.** A. *Frankia* nodule (coralloid) on *Alnus glutinosa* growing naturally in Rize Province, Turkey; B, C, *Frankia* nodules, young unlobed and older lobed (coralloid) nodules, on *Elaeagnus angustifolia* container grown in 25% Niğde soil; D, cluster root in *Casuarina cunninghamiana* container grown in 25% Niğde soil; E, F, *Frankia* nodules with prolific development of nodules roots on *C. cunninghamiana* container grown in 75% Niğde soil for 1 year (Expt 2) and in 25% Adana soil for 7 months (Expt 4); G, H, growth of *Allocasuarina verticillata* and *C. cunninghamiana* in 25% Adana soil after 7 months (Expt 4) and 25% Izmir soil after 6 months (Expt 5); and I, mycorrhizal roots in *A. verticillata* that are short lateral roots that grow perpendicular to the main root often thicker with a terminal lobe (see in plants grown in 25% Niğde, Adana and Izmir soil, Epxts 3, 4 and 5, respectively).

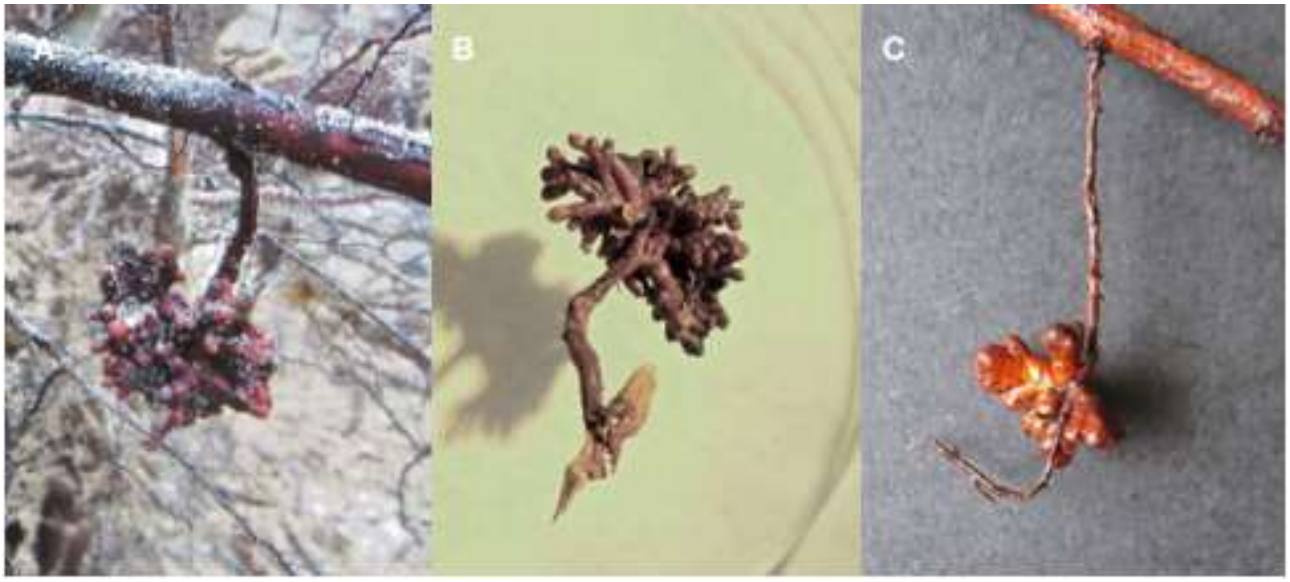


**Figure 2.** *Frankia* nodules (coralloid) on *Allocasuarina verticillata* from a natural stand at Nepean Bay, Kangaroo Island, SA, Australia. The tree was uprooted by roadworks, and nodules were found on short roots descending from larger lateral roots and less than 1 m from the crown of the tree. A and B are the same nodule, in situ in a light sandy soil and washed in the laboratory (about 50 mm across) and B is a younger, lobed but less branched nodule (about 20 mm across).

**Table 3.** Formation of nodules, cluster roots and ectomycorrhizal roots in actinorhizal plants, *Allocasuarina verticillata* (Ave), *Casuarina cunninghamiana* (Ccu) and *Elaeagnus angustifolia* (Ean), in five greenhouse experiments using various growth medium and amendments. Growth medium included topsoils from Adana and Izmir, Turkey, adjacent to *C. cunninghamiana*, and Niğde, Turkey, adjacent to *E. angustifolia*, in 25 or 75% mixtures with peat (A25, I25 and N25/75, respectively) to ensure drainage in container grown plants. Amendments included supernatants of soil suspensions from soil collected adjacent to the three actinorhizal plants species, *Alnus glutinosa* (Agl-S, Rize, Turkey), *C. cunninghamiana* (Ccu-S, Adana) and *E. angustifolia* (Ean-S, Niğde), and a suspension *A. verticillata* nodules (Kangaroo Island, Australia) prepared by homogenising air-dried nodules in skimmed milk

| Expt | Species | Medium | Amendment | Reps | Growth (weeks) | Plant height (mm) | Shoot/root score | Nodules[2] | Cluster roots[2] | Ectomycorrhizal roots[3] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ccu | N75 | None | 10[4] | 20 | 82 | 1.3/1.7 | 0 | 9 (9) | nd |
| | Ccu | N75 | Agl-S | 10 | 20 | 73 | 1.8/2.0 | 0 | 11 (10) | nd |
| | Ccu | N75 | Ccu-S | 10 | 20 | 70 | 1.3/2.0 | 0 | 8 (8) | nd |
| | Ccu | N75 | Ean-S | 10 | 20 | 72 | 1.2/2.3 | 0 | 15 (8) | nd |
| 2 | Ave | N25 | Agl/Ccu-S | 5 | 52 | 499 | 2.4/3.0 | 0 | 1 (2) | ++ |
| | Ccu | N25 | Agl/Ccu-S | 5 | 52 | 554 | 2.4/3.0 | 2 (4) | 1 (2) | + |
| | Ean | N25 | Agl/Ccu-S | 5 | 52 | 901 | nd/2.8 | 348 (3) | 1 (2) | - |
| 3 | Ave | N25 | None | 5 | 47 | 589 | 2.4/3.0 | 0 | 1 (1) | ++ |
| | Ccu | N25 | None | 5 | 47 | 160 | 1.0/2.6 | 0 | 57 (5) | + |
| | Ean | N25 | None | 5 | 47 | 611 | nd/2.6 | 56 (5) | 0 | - |
| 4 | Ave | A25 | None | 5 | 31 | 423 | 3.0/3.0 | 0 | 0 | ++ |
| | Ave | A25 | Ave-N | 5 | 31 | 471 | 3.0/3.0 | 0 | 0 | ++ |
| | Ccu | A25 | None | 5 | 31 | 229 | 3.0/3.0 | 1 (2) | 14 (5) | + |
| | Ccu | A25 | Ave-N | 5 | 31 | 289 | 3.0/3.0 | 6 (2) | 8 (4) | + |
| 5 | Ave | I25 | None | 4 | 26 | 240 | 3.0/3.0 | 0 | 1 (1) | ++ |
| | Ave | I25 | Ave-N | 4 | 26 | 334 | 3.0/3.0 | 0 | 0 | ++ |
| | Ccu | I25 | None | 4 | 26 | 188 | 3.0/3.0 | 0 | 7 (4) | + |
| | Ccu | I25 | Ave-N | 4 | 26 | 304 | 3.0/3.0 | 8 (2) | 2 (2) | - |

[1]Shoot/root scores are means on a four-point scale from zero for poor to three for healthy (see the text for details); *E. angustifolia* (Ean) shoots were not scored as the plants were dormant at that time. [2]Nodule and cluster root numbers are means for positive pots only. Positive pot numbers are given in parentheses. [2]Ectomycorrhizzal roots were scored as -, not present, +, present and ++, present at moderate to high density, with occurrence was consistent across replicates. [3]Replicates in Expt 1 include 5 replicates each for sterile and non-sterile soil treatments that were combined given these treatments had no observable effect. nd, not determined.

plants (overall shoot score of 1.5). This poor growth (Table 3) was attributed to the high proportion of Niğde soil which made for a heavy, hard-setting growth medium and the plants being grown over winter months with shorter day lengths even though the greenhouse was heated, consequently a greater proportion of peat was included in the subsequent experiments. (However, this was ineffective in Niğde soil, but growth and shoot scores were much better in experiments with Adana and Izmir soil). Although there was no nodulation in experiment 1, the roots were generally well developed (overall score of 2) and much longer than the shoots (mean root-shoot ratio of 1.8). Cluster roots developed in 35 of the 40 pots with means per treatment ranging from 8 to 15 cluster roots per pot, with no statistically significant response to inoculation treatment. Ectomycorrhizal roots were not scored in experiment 1.

Experiment 2, conducted in response to the lack of nodulation in experiment 1, included a combined inoculum and unsterilised Niğde soil with more peat and growing period extending over a full year to maximise the probability of nodulation. In total, nine nodules (with air-dry weight ranging from 0.03 to 1.67 g and 6 to 25 mm long; a representative sample is shown in Figure 1e) developed with some in all *C. cunninghamiana* replicates (2 nodules $pot^{-1}$). The range from small to large with a highly branched, lobed structure and prolific nodule roots (Figure 1e) as described by Torrey (1976) and illustrated by Batista-Santos et al. (2015; their Figure 1 with zero salt treatment). However, cluster roots and ectomycorrhizal roots only occurred in small numbers (Table 3).

In the remaining experiments, nodulation of *C. cunninghamiana* nodulation occurred in plants grown in Adana and Izmir soil with and without inoculation (experiments 4 and 5), but not in Niğde soil (experiment 3). The mean nodule number ranged from one to eight per pot (Table 3) with air-dry weight ranging from 0.01 to 1.36 g and 4 to 15 mm long (a representative range of nodules are shown in Figure 1f) and, being younger nodules than in experiment 2, these were lighter in colour. Again, the nodules had abundant nodules roots. Although nodules occurred in inoculated treatments, the source of the *Frankia* is mostly likely to have been the field soil because inoculation failed to lead to nodulation of the homologous control (*A. verticillata*). No nodules were found on shallow roots of *C. cunninghamiana* exposed when sampling soils for these experiments.

Cluster roots also formed in *C. cunninghamiana* root systems in these three experiments (Table 3) with mean numbers ranging from 2 to 57 clusters per pot in 20 of the 23 pots. The largest numbers of cluster roots were in the Niğde soil (all pots with mean of 57 per pot; a representative cluster root is shown in Figure 1d). Although, ectomycorrhizal roots developed on plants in the three soils, the numbers were again relatively low compared to *A. verticillata*.

### 3.4. *Elaeagnus angustifolia*

*Elaeagnus angustifolia*, the only actinorhizal species tested that is grown in Niğde, grew well and freely nodulated in Niğde soil (experiments 2 and 3) with 348 and 56 nodules per pot, respectively. The nodules range from small single-lobed nodules to multi-lobed nodules as shown in Figure 1b, c. There were two non-nodulated plants in experiment 2. Under these conditions, *E. angustifolia* grew taller than the sheoaks but only as single stemmed plants with no development of side branches. Development of cluster roots was limited with only two examples seen in experiment 2. No ectomycorrhizal roots were found. The roots of *E. angustifolia* were well developed and highly branched with nodules on the main and lateral roots, but the roots were easily broken, with some browning and splitting of the cortex, so these were not scored as being as healthy as the sheoak roots in the same experiments (Table 3). In the field, *E. angustifolia* nodules could not be found on the shallow roots of young to mature trees, but large nodules were observed on larger roots of uprooted trees close to the main trunk (a similar situation to that described above for *A. verticillata*).

## 4. Discussion

A key finding is confirmation that *A. glutinosa*, *E. angustifolia* and *C. cunninghamiana* nodulate in Turkish soils, at least in the contexts in which the observational and experimental data were obtained. *A. glutinosa* and *E. angustifolia* nodulation was confirmed by field observation and *E. angustifolia* and *C. cunninghamiana* in the experiments. With *C. cunninghamiana* it appears that compatible *Frankia* might only occur in areas where it is currently grown, as nodulation did not occur in treatments without amendment with soil from such locations. The reference plant, *A. verticillata,* did not nodulate in any experiment, even with the suspension of conspecific nodules, which is not unexpected as *Allocasuarina* spp. are generally less readily nodulated than *Casuarina* spp. and compatible *Frankia* strains are less adaptable to establishment beyond their native range (Simonet et al. 1999).

The prolific nodulation of *E. angustifolia* seedlings grown in pots with a proportion Niğde soil contrasts starkly with the difficulty in finding nodulated plants in the field. In the uprooted mature trees examined only a few large nodules were found, so it appears that the reliance *E. angustifolia* has on biologically-fixed nitrogen might decline as the plant matures. Confidently quantifying the relative proportion of nitrogen acquired as biologically-fixed nitrogen in mature trees is technically challenging and requires considerable data collection over several years (Boddey et al. 2000). Nodules can be readily obtained from some mature actinorhizal trees and have been used as a source of inoculum, for example *A. glutinosa* (Markham and Chanway 1999) and *Casuarina equisetifolia* (Karthikeyan et al. 2013), for both research and production purpose. However, for the present study, efforts to find nodules on shallow roots of both *A. verticillata* in native stands and *C. cunninghamiana* in Turkey were unsuccessful. Nodulation in mature legumes is considered to be less common as they recycle much of their nitrogen (Sprent 2005). Bauters et al. (2016) report no detectable nodules on mature leguminous trees in late successional forest plus their seedlings and herbaceous legumes in the understory were not nodulated. Therefore, in low-productive, semiarid contexts, such as Central Anatolia, with other soil nutrient limitations, decline in nodulation as trees mature seems likely.

Cluster roots, which form in the Casuarinaceae in response to P or Fe deficiency (Arahou and Diem 1997; Reddell et al. 1997), occurred in moderate to large numbers in *C. cunninghamiana,* particularly in Niğde soil, a soil which causes significant Fe chlorosis (experiments 1 and 3) in this species. A low instance of structures scored as cluster roots were found in *A. verticillata* and *E. angustifolia* but given the low incidence and numbers, it is not possible to be confident that these were formed or functioning in response to a particular nutrient deficiency. Cluster roots are known to occur inconsistently in *Allocasuarina* (Diem et al. 2000) but have not been reported in *A. verticillata* or *E. angustifolia*, so the possibility that they can develop in these taxa deserves further investigation.

In contrast, ectomycorrhizal roots developed in high numbers in *A. verticillata* from the resident mycorrhizal fungi in Turkish soil, and to a lesser extent in *C. cunninghamiana*. *E. angustifolia* did not appear to have ectomycorrhizal roots, despite being grown in soil where this species is common under conditions favourable for ectomycorrhizal root development in *C. cunninghamiana*. The Elaeagnaceae is variously reported to form both vesicular arbuscular mycorrhizal and ectomycorrhizal associations (Gardner 1986; Harley and Harley 1987; Nedelin 2014), but primary reports on ectomycorrhiza in *E. angustifolia* are not easily found.

In conclusion, it is likely and, as expected, that the native actinorhizal plants of Turkey will be nodulated where they occur naturally, and this has been confirmed for *A. glutinosa* in Rize and *E. angustifolia* in Niğde. The exotic *C. cunninghamiana* is likely to be nodulated where it is currently established in Turkey, but its use in new areas or the introduction of other Casuarinaceae, as has been proposed (Riley 2021), would best be accompanied with compatible *Frankia* inoculum. The establishment of other root structures/associations in both *Allocasuarina* and *Casuarina* adaptive for infertile soil are likely to require specific intervention, but this remains to be confirmed for vesicular arbuscular mycorrhizal associations. Also, the nodulation of status of the natives, *D. cannabina* and *H. rhamnoides*, remains to be investigated.

## Acknowledgements

E Ekbiç (Ordu University), F Ersin (Ege University), S Serçe (Niğde Ömer Halisdemir University), JR Rathjen and MH Ryder (University of Adelaide) are gratefully acknowledged for their help in obtaining materials used in this study. Special thanks also to NF Tutar for her technical assistance with experiment 3. Authors, MA, KJ and EO, are grateful for scholarships provided by Ayhan Şahenk Foundation and KJ for the support provided by the Turkish Council of Higher Education.

## References

Arahou M, Diem HG (1997) Iron deficiency induces cluster (proteoid) root formation in *Casuarina glauca*. Plant Soil 196: 71-79.

Batista-Santos P, Duro N, Rodrigues AP, Semedo JN, Alves P, da Costa M, Graça I, Pais IP, Scotti-Campos P, Lidon FC, Leitão AE, Pawlowski K, Ribeiro-Barros AI, Ramalho JC (2015) Is salt stress tolerance in *Casuarina glauca* Sieb. ex Spreng. associated with its nitrogen-fixing root-nodule symbiosis? An analysis at the photosynthetic level. Plant Physiology and Biochemistry 96: 97-109.

Bauters M, Mapenzi N, Kearsley E, Vanlauwe B, Boeckx P (2016) Facultative nitrogen fixation by legumes in the central Congo basin is downregulated during late successional stages. Biotropica 48: 281-284.

Benson DR, Silvester WB (1993) Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. Microbiological Reviews 57: 293-319.

Boddey RM, Peoples MB, Palmer B, Dart PJ (2000) Use of the $^{15}N$ natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutrient Cycling in Agroecosystems 57: 235-270.

Brundrett MC, Tedersoo L (2020) Resolving the mycorrhizal status of important northern hemisphere trees. Plant Soil 454: 3-34.

Diem HG, Duhoux E, Zaid H, Arahou M (2000) Cluster roots in Casuarinaceae: role and relationship to soil nutrient factors. Annals of Botany 85: 929-936.

Gardner IC (1986) Mycorrhizae of actinorhizal plants. MIRCEN Journal of Applied Microbiology and Biotechnology 2: 147-60.

Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British flora. New Phytologist 105: 1-102.

Karthikeyan A, Chandrasekaran K, Geetha M, Kalaiselvi R (2013) Growth response of *Casuarina equisetifolia* Forst. rooted stem cuttings to *Frankia* in nursery and field conditions. Journal of Biosciences 38: 741-747.

Kier G, Mutke J, Dinerstein E, Ricketts TH, Küper W, Kreft H, Barthlott W (2005) Global patterns of plant diversity and floristic knowledge: global plant diversity. Journal of Biogeography 32: 1107-1116.

Markham JH, Chanway CP (1999) Does past contact reduce the degree of mutualism in the *Alnus rubra* - *Frankia* symbiosis? Canadian Journal of Botany 77: 434-441.

Markham JH (2009) Does *Dryas integrifolia* fix nitrogen? Botany 87: 1106-1109.

Nedelin T (2014) Ectomycorrhiza – nature and significance for functioning of forest ecosystems. Forestry Ideas 20: 3-29.

Parrotta JA (1999) Productivity, nutrient cycling, and succession in single- and mixed-species plantations of *Casuarina equisetifolia*, *Eucalyptus robusta*, and *Leucaena leucocephala* in Puerto Rico. Forest Ecology and Management 124: 45-77.

Paschke MW (1997) Actinorhizal plants in rangelands of the western United States. Journal of Range Management 50: 62-72.

Põlme S, Bahram M, Kõljalg U, Tedersoo L (2014) Global biogeography of *Alnus*-associated *Frankia* actinobacteria. New Phytologist 204: 979-988.

Reddell P, Yun Y, Shipton WA (1997) Cluster roots and mycorrhizae in *Casuarina cunninghamiana*: their occurrence and formation in relation to phosphorus supply. Australian Journal of Botany 45: 41-45.

Riley IT (2021) A case for assessing *Allocasuarina* and *Casuarina* spp. for use in agroecosystem improvement in semi-arid areas with a focus on Central Anatolia, Turkey. Frontiers of Agricultural Science and Engineering 8: 568-582.

Riley IT, Korkmaz LN (2019) Identity of the *Casuarina* sp. in Turkey. Turkish Journal of Weed Science 20: 159-168.

Roy S, Khasa D, Greer C (2011) Combining alders, frankiae, and mycorrhizae for the revegetation and remediation of contaminated ecosystems. Canadian Journal of Botany 85: 237-251.

Simonet P, Navarro E, Rouvier C, Reddell P, Zimpfer J, Dommergues Y, Bardin R, Combarro P, Hamelin J, Domenach A-M, Gourbière F, Prin Y, Dawson JO, Normand P (1999) Co-evolution between *Frankia* populations and host plants in the family Casuarinaceae and consequent patterns of global dispersal. Environmental Microbiology 1: 525-533.

Sprent J (2005) West African legumes: the role of nodulation and nitrogen fixation. New Phytologist 167: 326-330.

Torrey JG (1976) Initiation and development of root nodules of *Casuarina* (Casuarinaceae). American Journal of Botany 63: 335-344.

Tukey JW (1997) Exploratory Data Analysis. Addison-Wesley, Reading, MA, USA.

# Acknowledgement of reviewers

MEDITERRANEAN AGRICULTURAL SCIENCES thanks to reviewers listed below for their enormous contribution to the articles published in Volume 34.

**Akbudak, M. Aydin**
**Akman, Haşmet Emre**
**Aktaş, Türkan**
**Aliefendioğlu, Yeşim**
**Alkan, Sezai**
**Aslan, Baran**
**Aslan, Gülçin Ece**
**Atasever, Savaş**
**Aydinoğlu, Bilal**

**Başayiğit, Levent**
**Bayındır Erol, Alime**
**Boyacı, Sedat**
**Bozbuğa, Refik**
**Bozoğlu, Mehmet**
**Budak, Mesut**
**Büyüktaş, Kenan**

**Canpolat, Sirel**
**Cemek, Bilal**
**Coşge Şenkal, Belgin**

**Çaliş, Özer**
**Çanci, Hüseyin**
**Çeliktopuz, Eser**
**Çetin, Öner**
**Çikili, Yakup**

**Dağlı, Fatih**
**Demir, Okan**
**Duru, Serdar**

**Ekinci, Kamil**
**Elekcioğlu, Naime Zülal**
**Elmasulu, Safinaz**
**Erdoğan, Metin**
**Erem, Fundagül**
**Eren, Erkan**
**Erper, İsmail**
**Erper, İsmail**
**Ertürk, Umran**
**Ertürk, Yakup Erdal**
**Esetlili, Mustafa Tolga**
**Esetlili, Mustafa Tolga**

**Genç, Serdar**
**Goncu, Serap**
**Gözükara, Gafur**
**Gülyüz, Fetih**
**Güney, Osman İnanç**
**Gürdil, Gürkan**
**Gürdil, Gürkan**

**Irmak Yilmaz, Funda**

**İrvem, Ahmet**

**Kaçar, Gülay**
**Karabağ, Kemal**
**Karaca, İsmail**
**Karakayacı, Zuhal**
**Karakuş, Ferda**
**Karaş, Ertuğrul**
**Karsli, Taki**
**Kayalak, Selma**
**Kayişoğlu, Birol**
**Kenanoğlu Bektaş, Zerrin**
**Keskin, Nurhan**
**Kibar, Beyhan**
**Kocabaş Oğuz, Işın**
**Koçak, Burak**
**Konyalı, Aynur**
**Korkmaz Turgud, Firdevs**
**Kul, Ertuğrul**
**Kutlar, İlkay**
**Küçükbasmacı Sabır, Ferhan**
**Küçükyumuk, Cenk**
**Küden, Ayzin**

**Mencet Yelboga, Makbule Nisa**

**Ödemiş, Berkant**
**Ödemiş, Berkant**
**Özdemir, Zahide**
**Özgönen Özkaya, Hülya**

**Polat, Süleyman**

**Sabir, Ali**
**Sözer, Salih**

**Şahin, Özge**

**Tan, Sibel**
**Taşcıoğlu, Yavuz**
**Taşkın, Turgay**
**Tavali, İsmail Emrah**
**Topaloğlu, Fatih**

**Uygur, Veli**
**Uzun, Bülent**

**Ünal, Dilek**
**Ünal, Halil**

**Yakupoğlu, Gökçen**
**Yaşar, Baran**
**Yavuzaslanoğlu, Elif**
**Yazar, Atilla**

# Volume content, Volume 34

***Number*: 1 (April 2021)**

***Number*: 2 (August 2021)**

**Number: 3 (*December* 2021)**

# Author index

# INSTRUCTIONS FOR AUTHORS

**Scope**

MEDITERRANEAN AGRICULTURAL SCIENCES is a multidisciplinary platform for the related scientific areas of agriculture and life sciences. Therefore, the journal primarily publishes original research articles and accepts a limited number of invited reviews in agricultural biotechnology, agricultural economics, agricultural machinery, animal husbandry, bioenergy, biostatistics and genetics, farm structure and irrigation, field crops, food science and technology, horticulture, landscape and nature conservation, natural resources, plant protection, soil science and plant nutrition.

**General rules**

Manuscripts within the scope of MEDITERRANEAN AGRICULTURAL SCIENCES can be submitted. The submitted manuscript must be unpublished, must not be simultaneously submitted for publication elsewhere, nor can the copyright be transferred somewhere else. Responsibility for the work published in this journal remains with the author(s). Moreover, the author(s) must comply with the ethical rules of science and scientific publications (International Committee of Medical Journal Editors and Committee on Publication Ethics). The journal is not responsible for these issues.

**Manuscript submission**

The manuscripts should be submitted to the journal by using online system: www.dergipark.org.tr/en/pub/mediterranean. A copy of the "Copyright Transfer Agreement" signed by all authors who contributed to the manuscript should be submitted by the corresponding author. Those manuscripts requiring an Ethics Committee Report should be supplied a copy of the report by the Ethics Committee. Ethics Committee Report which is the name of the board, date and number of approval, etc. information should be specified in the manuscript (on the Manuscript Cover Page and Material-Method).

**Review process, proof and publishing**

The manuscript submitted to the journal is subject to preliminary assessment by the Editorial Board. The Board has the right to decline the manuscript without initiating the peer review process in the event the manuscript does not meet the journal's criteria.

Manuscripts that meet the basic requirements of the journal are sent to three referees for review by experts in the particular field of science.

If all or a majority of the reviewers do not find the manuscript suitable for publication, the author is informed and documents are not returned.

Should the manuscript as is be found suitable for publication by reviewers; the author is informed of the final decision.

Should the manuscript is found publishable but requires revision as suggested by the review team; the areas where revisions are required are sent to the author with the referee's suggestions. The author is expected to return the corrected manuscript, or a letter of rebuttal within thirty days, including the last revised version of the manuscript, correction list and "Copyright Transfer Agreement" sent to Editor. Should the author(s) do not accept the reasons for the revision, they are required to present scientific evidence and record the sources giving reason for this rejection in the letter of rebuttal. The Editorial Board takes the final decision by taking the referee reports into account and the compliance with the requirements for correction and the authors are notified of the final decision for publication.

Before publishing, the proof of the accepted manuscript is sent to the corresponding author for a final check. The corresponding author is expected to return the corrected final proof within 10 days. All authors can access their article on the web page of the journal (www.dergipark.org.tr/en/pub/mediterranean).

***MEDITERRANEAN AGRICULTURAL SCIENCES*is free of charge.**

**Manuscript preparation guidelines**

Manuscript submitted to the journal should consist of main two parts: the first page and the manuscript.

**1.The coverpage:** Should contain the title, names of the author(s) and addresses including the corresponding author's name and full contact details in addition the author(s) must contain the ORCID number(s).

**2. Manuscript:** Manuscripts should be prepared on A4-size paper in 12 point, Times New Roman font, double line spaced, leaving 3 cm blank spaces on all four margins of each page. Each page of the manuscript and each line on page should be numbered.

The manuscript should not be longer than **16** pages, double line spaced, including the "References" section (excluding any figures and tables). The information (name and title) of the author(s) should not be given in the article. A total of Tables or Figures should not be more than 8 in the manuscript, and must have the following sections:

**Title:** Must be short and inclusive, not to exceed fifteen words, and the first letter of the first word to be written in uppercase and rest in lowercase letters, in bold.

**Abstract:** The abstract should not exceed 250 words, and it should summarize the objective of the study, the methods employed and the results.

**Keywords:** A maximum of **five keywords**, directly related to the subject matter and not employed in the title, should be recorded directly below the abstract.

**Introduction:** In this section, the subject of the study should be summarized, previous studies directly related to the study should be evaluated with the current knowledge of the subject, and the issues associated with production of the information needed are highlighted. Finally, the objective of the study should be clearly and explicitly stated. *Section titles within the manuscript:* except for the "References" all the main and sub-titles should be numbered. The first letters of the first words in the titles should be written in capital letters. Main titles should be written in bold and the sub-titles in italics.

**Material and methods:** In this section, all the materials employed in the study, the methods used, criteria evaluated, sampling methods applied, experimental design with statistical analysis and the confidence limits should be clearly explained. In addition, information regarding the permit (name of the board, date and number no of approval) of the studies requiring Ethics Committee permit should be provided in this section (Method).

**Results:** In this section the findings of the study should be presented clearly and explicitly with the help of figures, tables, and statistical analysis. Duplication of data presented in the Figures and Tables should be avoided, and the most appropriate tool should be employed.

**Discussion and Conclusion:** The findings of the study should be discussed with the results of previous studies, in terms of their similarity and contrast, and information gap filled by the study should be emphasized. Finally, conclusions and recommendations should be given. The manuscript

layout of this section can be entitled "Results and Discussion" and / or "Conclusions" depending on author(s) preference.

For the reviews, the author(s) can make appropriate title arrangements.

**Acknowledgement:** People who contribute to the manuscript and/or the study and the funding agency (project numbers, if any) must be specified.

**References:** In the text, "the author's surname and the year" method should be used for identification of references. A reference identified by means of an author's surname should be followed by the date of the reference in parentheses. For identification of references provided by two authors, "and" should be used between the surnames of authors. When there are more than two authors, only the first author's surname should be mentioned, followed by 'et al.'. In the event that an author cited has had two or more works published in the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish between the works. When more than one reference is given at the end of a sentence, the references should be chronologically ordered, those of same date in alphabetical order.

Examples:

Burton (1947), Sayan and Karaguzel (2010), Keeve et al. (2000), (van Harten 2002), (Karaguzel and Altan 1995), (Burton 1947; Keeve et al. 2000; Yilmaz 2004a, b; Karaguzel 2005, 2006; Gulsen et al. 2010; Sayan ve Karaguzel 2010).

References should be listed at the end of the manuscript in alphabetical order in the References section. The original language of reference should be employed and journal's name should not be abbreviated. Authors are fully responsible for the accuracy of the references they provide.

Examples:

**Journal:**

Karagüzel O (2003) Farklı tuz kaynak ve konsantrasyonlarının Güney Anadolu doğal *Lupinusvarius*'larının çimlenme özelliklerine etkisi. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 16: 211-220.

Keeve R, Loupser HL, Kruger GHJ (2000) Effect of temperature and photoperiod on days to flowering, yield and yield components of *Lupinusalbus* (L.) under field conditions. Journal of Agronomy and Crop Science 184: 187-196.

**Book:**

Taiz L, Zeiger E (2002) Plant Physiology. 3rd Edition, Sinauer Associates, Massachusetts.

**Book chapter:**

Van HartenAM (2002) Mutation breeding of vegetatively propagated ornamentals. In: Vainstein A (Ed), Breeding for ornamentals: Classical and Molecular Approaches. Kluwer Academic Publishers, Dordrecht, pp. 105-127.

**Institution publications with unknown author name(s):**

TSI (2005) Agricultural Structure.T.C. Prime Ministry State Institute of Statistics, Publication No. 1579, Ankara.

**DOI and received information from the internet**:

Gulsen O, Kaymak S, Ozongun S, Uzun A (2010) Genetic analysis of Turkish apple germplasm using peroxidase gene-based markers. doi:10.1016/j.scienta.2010.04.023.

FAO (2010) Statistical database.http://faostat.fao.org/site/339/default.aspx. Accessed 27 July, 2010.

**Theses:**

Sever Mutlu S (2009) Warm-season turfgrass species: Adaptation, drought resistance and response to trinexapac-ethyl application. PhD Thesis, The University of Nebraska, Nebraska.

Girmen B (2004) Gazipaşa yöresinde doğal yayılış gösteren hayıtların (*Vitexagnus-castus* L.) seleksiyonu ve çoğaltılabilme olanakları. Yüksek Lisans Tezi, Akdeniz Üniversitesi Fen Bilimleri Enstitüsü, Antalya.

**Full-text congress/symposium book:**

Hawkes JG (1998) Current status of genetic diversity in the world. In: Zencirci N, Kaya Z, Anikster Y, Adams WT (Eds), The Proceedings of International Symposium on *In Stu* Conservation of Plant Genetic Diversity. CRIFC, Ankara, Turkey, pp. 1-4.

Kesik T (2000) Weed infestation and yield of onion and carrot under no-tillage cultivation using four crops. In: 11th International Conference on Weed Biology. Dijon, France, pp. 437–444.

**Figures and tables:** In submitted manuscripts all photographs, graphics, figures, diagrams and the like must be named as "Figure", and lists of numerical values as "Table". All figures and tables should be numbered and placed at the end of the manuscript. The font of the letters within Figures and Tables used should be no larger than 8 points. Figure and table widths should be 8 cm or 17 cm and, if necessary, dimensions of up to 17x23 cm. The images should be in JPG format with 600 dpi resolution and should be informative in explaining the results. The authors must check the printing quality of the figures and should use high quality figures suitable for printing. Use of vertical lines in the tables is unacceptable, statistical significance should be stated using *P* values as much as possible, or using the "*" symbols for which description should be given. Small case lettering should be used for statistical groupings, and the statistical comparison method and significance level specified. Table and figure captions and descriptions should be short, concise, and descriptive. Abbreviations should be explained immediately if used within the Figures and tables. Those images composed of pieces should be grouped and converted into high-quality TIF format.

**Units:** For manuscripts SI (System International d'Units) unit system is used. In units, "/" should not be used and there should be a space between the units (for example: 5.6 kg $ha^{-1}$, instead of 5.6 kg/ha; 18.9 g $cm^{-3}$, instead of 18.9 g/$cm^3$; 1.8 µmol $s^{-1}$ $m^{-2}$, instead of 1.8 µmol/s/$m^2$).

**Abbreviations and symbols:** Abbreviations should not be used in the manuscript title or in the subtitles. The necessary abbreviations at their first mention should be given in parentheses. Universal rules must be followed in the use of abbreviations and symbols.

**Latin names and chemicals:** The authority should not be used in the manuscript title when Latin names are used. The authority should be given when the Latin names are first used in the abstract and the text. For example: "*Lupinusvarius* (L.) is ....", "*L. varius* ... grown in the.. " Latin names should be written in italics. The trade mark of chemicals used in the studies should not be given unless it is absolutely necessary to do so.

**Formulas:** In manuscripts, formulas should be called "Equation", numbered as necessary, the numbers next to the formulas leaning right shown in brackets and the equations should be fitted in a single line (double-column, 8 cm), if possible.

The author (s) is encouraged to visit the web site (www.dergipark.org.tr/en/pub/mediterranean) to see the latest issue of the journal.

**MEDITERRANEAN AGRICULTURAL SCIENCES**

e-ISSN 2528-9675

Journal web page: www.dergipark.org.tr/en/pub/mediterranean

**Address:**

Faculty of Agriculture
Akdeniz University
07058 Antalya, TURKEY

Phone: +90 242 310 2412
Fax: +90 242 310 2479
E-mail: ziraatdergi@akdeniz.edu.tr

## COPYRIGHT TRANSFER AGREEMENT

Please note that publication of this article **can not** proceed until this signed form is submitted.

| | |
|---|---|
| Author(s) | |
| Article title | |

Corresponding Author's Contact Information

| | | | |
|---|---|---|---|
| Name | | Address | |
| E-mail | | | |
| Phone | | Fax | |

As the author (s) of the article submitted, we hereby accept and agree to the following terms and conditions.

a) I/We acknowledge that the Faculty of Agriculture at Akdeniz University does not carry any responsibility until the article arrives at the Bureau of Editor in Chief of the MEDITERRANEAN AGRICULTURAL SCIENCES.

b) I/We confirm that this article is in compliance with ethical rules, carries the ethical permission documents for the conditions required and will not cause any damage or injury when the materials and methods described herein are used.

c) The author(s) here take the full responsibility for the contents of the article.

d) The article has not been previously published and has not been submitted for publication elsewhere.

e) All the authors have seen, read and approved the article.

f) We accept that by disclaiming the copyright of the article, we transfer this right to the Faculty of Agriculture at Akdeniz University and authorize the Faculty of Agriculture at Akdeniz University in respect to publication of the article.

Except for the above issues, the author (s) reserve (s) the following rights

a) The author(s) retain (s) all proprietary rights, other than copyright, such as patent rights.

b) The author(s) can use the whole article in their books, teachings, oral presentations and conferences.

c) The author (s) has/have the right to reprint/reproduce the article for noncommercial personal use and other activities.

Whether accepted for publication or not, articles submitted to the journal are not returned and all the materials (photographs, original figures and tables, and others) is withheld for two years and is destroyed at the end of this period of time.

This document must be signed by all of the authors. If the authors are from different institutions, the signatures can be submitted on separate forms. Nevertheless, all the signatures must be wet signatures.

| ***Author(s) Name(s)** | **Address** | **Date** | **Signature** |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

*: The number of colon must be equal to the number of authors. If insufficient, it must be increased.

**If the submitted article is not accepted for publication, this document is null and void.**

**PLEASE SIGN THE FORM AND UPLOAD ONLINE SYSTEM.**