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Original article

Effects of shoot tip size on *in vitro* regeneration and virus elimination of grapevine cv. Superior Seedless

"Superior Seedless" üzüm çeşidinde sürgün ucu büyüklüğünün *in vitro* rejenerasyon ve virüs eliminasyonu üzerine etkileri

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ABSTRACT

In this study, we investigate the relation between the explant size and the success of virus elimination in 'Superior Seedless' grape variety. Plants were tested by prior to thermotherapy and *in vitro* culture by using Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (DAS-ELISA) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and verified as infected by different viruses (*Grapevine leafroll associated virus -1+3, Grapevine leafroll associated virus -4 Strains, Grapevine rupestris stem pitting associated virus*). Different size of shoot tip explants (0.1-0.5-1-5-10 mm) were excised and cultured on C_2D medium supplemented with 1 mgl^{-1} BA. All of the explants bigger than 0.1 mm 100% regenerated while 70% of the 0.1 mm explants showed complete regeneration and turned into a whole plant. Plants regenerated from 10 mm shoot tip explants have all three viruses (GLRaV-1+3, GLRaV-4 strains and GRSPaV) as founded in mother plant. Plants regenerated from 5 mm explants have two of them (GLRaV-1+3, GLRaV-4 strains) and plants regenerated from 1 mm explants have one (GLRaV-1+3). Plants regenerated from 0.1 mm explants were found completely virus-free.

INTRODUCTION

Sanitary status of the vineyard is crucial and it is important to use virus-free plant material for both satisfactory yield and quality and for conservation of local varieties, for correct amphelographic characterization and to reveal genetic potential and hence successive breeding. Virus symptoms may be different depending on the virus type and the plant species. Some may cause shape and colour disorders in shoots and leaves while the others may cause growth retention, yield loss and death. Methods (meristem and shoot tip culture, somatic embryogenesis, micro grafting, thermotherapy, cryotherapy, chemotherapy, electrotherapy etc.) for obtaining virus-free plant material are primarily based on tissue culture techniques and could be used single or combined. The most common methods are meristem or shoot tip culture and thermotherapy. Shoot tip culture was first used by Morel and Martin (1952) to clean virus infected dahlia plant (*Dahlia* sp.). Since then it is routinely being used. Meristematic zone is usually free from viruses or contain significantly lower virus concentration (Gribaudo et al. 2006). Smaller size than 0.1 mm explants referred as

meristem culture. Practically 0.1-0.2 mm size explants are used. Explant size is critically important in virus elimination. The smaller the explant the more success the elimination of pathogens. On the other hand, smaller explants have less ability to regenerate (Infante and Fione 2009, Maliogka et al. 2009, Skiada et al. 2009). Thermotherapy refers *in vivo or in vitro* heat application for several weeks in 38 °C may also be effective to keep viral proteins in infected tissue and prevent the virus to reach shoot tips by slowing down the movement (Mink et al. 1998). In our laboratory, production of virus-free base material by combining these two methods is routinely being done. In this study, we aimed to investigate the relation between the shoot tip size and the effectiveness of virus elimination in 'Superior Seedless' grape variety and to determine the optimum explant size to have virus-free and highly regenerative explants.

MATERIALS AND METHODS

The study was conducted at Manisa Viticulture Research Institution between the years 2018 and 2019. 'Superior Seedless' (*Vitis vinifera* L.) grape variety was used in this study. Mother plants were tested prior to thermotherapy and *in vitro* culture for *Grapevine rupestris stem pitting associated virus* (GRSPaV), *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll associated virus-1+3* (GLRaV-1+3), *Grapevine leaf roll associated virus-2* (GLRaV-2), *Grapevine leaf roll associated virus-4 strains* (GLRaV-4 strains), *Grapevine virus* A (GVA), *Grapevine fleck virus* (GfkV) by ELISA and/or RT-PCR methods (Table 1).

ELISA tests were conducted according to Double Antibody Sandwich Method as reported by Clark and Adams (1977). PCR was conducted with Roche LightCycler® Nano Real-time PCR system according to manufacturer's guide. For GLRaV-1 / GLRaV-3, Roche FastStart Essential DNA Green Master Mix consisting SYBR Green I and for GRSPaV, RealTime ready RNA Virus Master Probe based kits were used. PCR protocols were indicated on Table 2.

Infected plants were transferred to 10 l pots and placed in thermotherapy cabin for 12 weeks under 16 h photoperiod and 38/34 °C day/night temperatures. End of the January 2019, 1.5-2 cm apical and axillary shoot tips were excised from the plants in thermotherapy cabin. Then a surface sterilization protocol (wash 15 min under tap water with a few drops of detergent, 10 sec 70% ethanol, wash under tap water, sterilize in 5% sodium hypochlorite with 0.1% Tween for 10 min and rinse 3 times with sterile distilled water) was applied. Four different size of explants (0.1-1-5-10 mm) 22 for the 0.1 mm and 11 for each of the others were excised and cultured on petri dishes contain 20 ml C_2D medium (Chée et al. 1984), supplemented with 3% sucrose, 0.7% Difco-Bacto agar and 1 mg l^{-1} BA (N6-Benzyladanine). pH was adjusted to 5.8 prior to autoclaving at 121 °C, 105 Pa for 15 min. Petri dishes were placed in a culture room under 3000 lux, 16 h photoperiod and 25 °C temperature. 8 weeks of culture was needed for the 0.1 mm explants to regenerate while the bigger ones transferred to fresh medium after one month. Same medium formulation was used for sub-

Table 1. Virus analysis results of the mother plant prior to thermotheraphy and in vitro culture.

Sample	ArMV / FLV*	GLRaV-1+3**	GLRaV-2	GLRaV-4 rains***	GVA	GfKV	GRSPaV
Mother plant	Negative	Positive	Negative	Positive	Negative	Negative	Positive

Table 2. PCR protocols for GLRaV-1 / GLRaV-3 and GRSPaV

Name of primers	Primers (5' 3') and Probes	Size	Type of analyze	PCR cycles
GLRaV-1_9952 / F GLRaV-1_10420 / R	GAGCGACTTGCGACTT ATCGA GGTAAACGGGTGTTCT TCAATTCT (Unpublished)	469 bp	SYBR Green based RT-PCR	1 X (95°C 10 min) 45 X (95°C 20 sec/56°C 20 sec/72°C 47 sec)
GLRaV-3_ LC1 / F GLRaV-3_ LC2 / R	CGCTAGGGCTGTGGAA GTATT GTTGTCCCGGGTACCA GATAT (Osman and Rowhani, 2006)	546 bp	SYBR Green based RT PCR	1 X (95 °C 10 min) 50 X (95°C 20 sec/58°C 20 sec/72°C 55 sec)
GRSPaV/SO / F GRSPaV/SO// R	GCTTGARCCWAAGGG TGGA CCAGCMGTTCCRCCAC TAAT GCTGAAGG (Önder et al., 2016)	150 bp	One Step UPL based RT-PCR	1 X (50°C 480 sec) Reverse transcription 1 X (56°C 240 sec) Reverse transcription 1 X (95°C 30 sec) 45 X (95°C 10 sec/55°C 30 sec/72°C 10 sec)

cultures. During sub-culturing, leaf samples were taken from each plantlet (39 from 0.1 mm, 66 from 1mm, 66 from 5 mm, 96 from 10 mm) from each group and kept in 4 °C for a few days until virus analysis. According to virus analysis results only virus-free plantlets were taken to rooting step and all others were discarded. 27 virus-free plantlets (from 0.1mm explants) successfully rooted and were transferred to green house. During 2019 dormancy period, they were all tested for mentioned viruses and proved as virus-free.

RESULTS AND DISCUSSION

All of the explants bigger than 0.1 mm 100% regenerated while 70% of the 0.1 mm explants showed complete regeneration and turned into a whole plant, 30% of them still green, viable but dormant. Many researchers studied *in vitro* meristem culture of grapevines obtained similar results (Aazami 2010, Gray and Fisher 1985, Gray and Klein 1987 Salami et al. 2009). Laslo et al. (2010), reported 75% regeneration rate of shoot tip explants for 'Cabarnet Sauvignon' and 60% for 'Riesling Italian' grape varieties following 8 weeks of i*n vitro* culture. In a study on 9 different varieties belong *to Vitis rotundifolia*, best media for meristem regenerations varied depending on the varieties and different regeneration rates were obtained in the same medium (Gray and Benton 1991). Previous study on virus elimination efficiency and meristem regenerations of grapevine cv. 'Bornova Misketi' clones revealed that the regeneration rates of the clones varied from 47 to 100% (Ulas et al. 2017). As reported in previous studies, regeneration capacity depends on the plant variety and species (Peros et al. 1998). Genetic variability of *Vitis vinifera* cultivars and clones affect the regeneration capacity of the meristems and elimination efficiency of the procedure (Gray and Benton 1991, Laslo et al. 2010, Peros et al. 1998, Roubelakis-Angelakis and Zivanovitc 1991, Torregrosa and Bouquet 1996). Smerea et al. (2010) reported that the healthy status of grapevine also affected the meristem regeneration capacity. Turcsan et al. (2020) concluded that the meristem culture was a difficult technique as the regeneration capacity of the shoot tips bigger than 0.5 mm is higher but extra length also increase the virus level.

Mother plant was found positive for GLRaV-1+3, GLRaV-4 strains and GRSPaV. After 12 weeks of thermotherapy and *in vitro* culture virus tests were repeated on plants regenerated from the different size of explants. It has been found a relation between explant size and virus composition. Plants regenerated from 10 mm shoot tip explants have all three viruses (GLRaV-1+3, GLRaV-4 strains and GRSPaV) as founded in mother plant. Plants regenerated from 5 mm explants have two of them (GLRaV-1+3, GLRaV-4 strains) and plants regenerated from 1 mm explants have one (GLRaV-1+3). Plants regenerated from 0.1 mm explants were found completely virus-free (Table 3). It has been reported in some studies that elimination of GLRaV-1 was easier than that of GRSPaV-1 with both meristem-tip and shoot-tip culture (Gribaudo et al. 2006, Mannini 2003, Skiada et al. 2009). Here all explants bigger than 0.1 mm have GLRaV-1+3 may due to concentration of the virus in the mother plant or its translocation pattern as all of them are phloem-restricted viruses (Digiaro et al. 1999). As reported in a previous study where woody plant medium supplied with benzyl amino purine (BAP) (1.5 mg l^{-1}) for shoot proliferation was used, 1 mm meristems were found to be optimum to eliminate GLRaV-1 from infected grapevines cv. 'Thompson Seedless' while 3 mm meristems were revealed to carry virus particles. GFLV- and GLRaV-1-free plants (92.5 and 95%, respectively) were obtained from the optimum size (1 mm) of meristem tips (as indexed by DAS-ELISA). Of these, 85 and 87.5% plants were found negative from GFLV and GLRaV-1, respectively, as indexed by RT-PCR (Youssef et al. 2009).

Table 3. Virus presence in different size of explants after thermotheraphy and in vitro culture

Explant size	GLRaV-1+3	GLRaV-4 Strains	GRSPaV
0.1 mm	negative	negative	negative
1 mm	positive	negative	negative
5 mm	positive	positive	negative
10 mm	positive	positive	positive

Virus eradication in grapevines cv. 'Plavac mali' by using *in vitro* thermotherapy at 39 °C for 32 days and apical bud culture techniques, 26% of all regenerated plants were free from GLRaV-3. However, in different clones virus eradication success were different. Some of the clones were reported as 50% free from GLRaV-3, but in clone OB214 it was not eradicated. Similarly, GLRaV-1 was present in all regenerated plantlets of clone OB091. Virus composition and different endogenous plant regulators of different clones influences the selective virus elimination in grapevines (Hancevic et al. 2015). In another study, elimination of *Grapevine leafroll associated virus-4 Strain Pr* (GLRaV-Pr, Closteroviridae) were carried out in two grapevine cultivars, 'Mantilaria' and 'Prevezaniko', co-infected with GRSPaV (Flexiviridae). Both viruses were detected by nested RT-PCR assays. Researchers combined *in vitro* thermotherapy with meristem (≤ 0.2 mm) or shoot tip culture (≤ 0.5 cm). They found the survival and the regeneration rate of meristems was very low. On the other hand, high survival rates were observed in the cultured shoot tips accompanied with high elimination rates for both viruses. They also reported that virus elimination depends on the genotype of grapevine (Maliogka et al. 2009). Researchers found that the different source of the buds affects the *in vitro* survival rates and virus

free frequencies of grapevines. Survival rates of the shoots were higher when *in vitro* cultures were established from the middle and basal buds than from the terminal buds. Similarly, GLRaV-3- free frequencies were lower in the third axillary shoot tips than terminal, first and second axillary shoot tips (Valero et al. 2003, Wang et al. 2018).

In this study we found a positive relationship between the size of explant and *in vitro* regeneration capacity and also virus-free frequencies of the grapevine. The smaller explants (≤ 1 mm) have less or no virus, also exhibited less regeneration capacities than the bigger ones. It is known that, type of the virus and the grapevine variety also affects these phenomena. Here it may concluded that the virus concentration and the localization patterns affect the sanitary attempts. Further studies will be elucidatory to define these aspects in terms of grapevines sanitary status.

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ÖZET

Bağcılıkta, hücre içi patojenlerin neden olduğu en önemli hastalık etmeni virüslerdir. Bulaşık bitkiler zayıf gelişim, uyanmanın gecikmesi, verim ve kalitede azalma gibi belirtiler gösterebilir hatta birkaç yıl içerisinde ölebilir. Bitkileri virüslerden arındırmak için bazı yöntemler mevcuttur. Termoterapiyle birlikte uygulanan sürgün ucu kültürü en yaygın yöntemdir. Yöntemin başarısı eksplantın büyüklüğüne bağlıdır yani, eksplant ne kadar küçük olursa o kadar fazla virüsten ari bitki elde edilebilir. Öte yandan küçük eksplantların rejenerasyon kapasiteleri düşüktür. Bu çalışmada, 'Superior Seedless' üzüm çeşidinde eksplant büyüklüğü ile virüs eliminasyonu arasındaki bağlantı incelenmiştir. Bitkiler termoterapi ve *in vitro* kültür öncesinde, Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (DAS-ELISA) ve Real-time Reverse Transcription-Polymerase Chain Reaction (Real time RT-PCR) teknikleri ile test edilmiş ve farklı virüsler (Grapevine leafroll associated virus -1, -3, Grapevine leafroll associated virus -4 strains, Grapevine rupestris stem pitting associated virus) ile bulaşık oldukları doğrulanmıştır. Farklı büyüklükte (0.1-0.5-1-5-10 mm) sürgün ucu eksplantları, 1 mgl^{-1} BA içeren C_2D ortamında kültüre alınmıştır. Küçük boyutlu eksplantlarda virüs bulaşıklığı az olmuş ya da hiç saptanmamıştır.

Anahtar kelimeler: *in vitro*, eksplant büyüklüğü, virüs eliminasyonu, rejenerasyon, asma

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Original article

The effect of cover crops on composition and diversity of weeds in an apricot orchard

Kayısı bahçesindeki yabancı otların yoğunluğu ve çeşitliliği üzerine örtücü bitkilerin etkisi

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ABSTRACT

Apricot, Turkey's exports is the important role played by fruit, the weeds are the main factors that cause problems in the apricot orchards. This study was carried out in Malatya between 2014-2016 in order to determine the effects of cover crops on the control of weeds that cause problems in these areas and also on species distribution, diversity and dominance. As annual winter cover plants in the study: *Vicia villosa* Roth (hairy vetch), *Vicia pannonica* Crantz (Hungarian vetch), *V. pannonica* + Triticale (*V.pannonica* 70% + Triticale 30%) mixture and *Phacelia tanacetifolia* Bentham (lacy phacelia) and *Fagopyrum esculentum* Moench (buckwheat) was used as a summer cover plant. Weed diversity index (H) and Simpson's dominance index (Sd) values were also calculated in the study. In addition, applications were subjected to canonical discriminant analysis. The highest value of the weed diversity index (H) in the two-year period was obtained from the weed control plots. It has been determined that perennial weeds (*Convolvulus arvensis* and *Sorghum halepense*) are the dominant species in cover plants and there are also differences between practices. It has been determined that annual weeds are generally suppressed and their diversity has decreased significantly in the plots except for *F. esculentum*. The results obtained from the canonical discriminant analysis showed that the highest numbers of weed species in the 1st and 2nd years were found in the weedy control plots and that the cover crops suppressed the weeds.

INTRODUCTION

Apricot (*Prunus armeniaca* L.), which is known to have origins from Central Asia, Turkey and Western China, is one of the important crops. Apricot is produced on 580.000 hectares globally and its annual yield is 3.88 million tons (FAO 2018). It is cultivated most widely in Asia, Europe, and Africa (55.5%, 26%, and 14%, respectively). In Turkey, it is grown mainly in Malatya province. As in fruit growing, besides the total herbicides, also the tillage is widely used as a mechanical method for weed managements in apricot orchards. Instead of frequently performing the tillage, the practices that will control the weeds without damaging the balance of agro-ecosystem and/or ensure the natural

control of other damaging factors harming the fruit trees by increasing the biodiversity of the medium should be popularized (Isik et al. 2014). The use of herbicides in weed control is the most commonly preferred method. The herbicides also have many negative effects such as the problem of residues on soil, water, and foods, health risks for humans, effects on non-targeted organisms, and environmental pollution (Isik et al. 2018). In both conventional and organic farming systems, the weeds are one of the factors limiting the productivity (Stopes and Millington 1991). The most popular one among the conventional control methods is the use of chemical herbicides but there are many scientific reports emphasizing that there is no effective weed control method especially in the organic farming (Wszelaki et al. 2007). The mechanical weed control methods including handpicking and hoeing are among the most popular methods used for suppressing the weeds (Hiltbrunner et al. 2007). However, the handpicking method has also several disadvantages such as the costs and difficulty of finding workers (Kruidhof et al. 2008). The repetitive treatments applied to the soil in the hoeing method affect the stability of soil structure and increase the risk of soil erosion (Wszelaki et al. 2007). Moreover, there are few herbicides allowed to be used in organic farming and they are very expensive and non-selective chemicals; for this reason, they might damage the cultivated plant too (Knezevic 2009).

The agricultural biodiversity includes the diversity and amplitude of crop-related weeds, the presence of other organisms, and ratios between different species. The diversity indexes are widely used for examining the biodiversity in agricultural products. Moreover, the diversity and dominance indexes are also used for determining the changes in the weed population on the cultivation areas (Pawlonka et al. 2014). Having an important place in agricultural biodiversity, the cover crops decrease the soil erosion, water loss, and pollution, as well as enhance the soil structure and increase the number of useful microorganisms, water infiltration, moisture ratio, and amounts of nitrogen and organic carbon (Demir and Isik 2019, Demir et al. 2019). The use of cover crops in weed control in fruit orchards gradually increases (Isik et al. 2014, Isik et al., 2018, Robacer et al. 2016). The use of cover crops in organic farming increases the organic matter content of the soil through the mixture of plant biomass and other organic agents into the soil and the protection of herbal wastes. The increase in organic matter content of the soil increases the formation of aggregate, stabilizes the soil, and decreases the erosion and flow of surface water (Demir and Isik 2019, Demir et al. 2019,). As a result of their competition with weeds for light, moisture, and nutrient, and through the allelochemicals, the cover crops prevent the germination and growth of other plants. It was reported that many plant species (*Vicia* spp., *Tripholium* spp., *Sorghum vulgare, Secale cereale*) have been successfully used as cover crops in suppressing the weeds (Mennan et al. 2009a, 2009b).

In literature, there are very few studies on the weed control in apricot growing by using cover crops. This study aims to calculate the weed diversity index (H) and Simpson dominance index (Sd) values in the apricot growing by making use of mechanical weed control, non-selective herbicide (glyphosate), and cover crops (by mowing and incorporation them into the soil). Moreover, the canonical discriminant analysis was performed and the effects of practices on the weeds were investigated.

MATERIALS AND METHODS

Experimental site and field trial

This study was carried out between 2014 and 2016 in the experimental apricot orchard of the Agricultural Faculty of Inonu University, Turkey. The study area (38.47 N - 38.34 E) has an argillaceous soil structure (52% clay, 28% silt, 20% sand, 1.7% organic matter, and pH 7.4). In the growing season 2015-2016, the mean temperature was 13.4 °C and the mean annual precipitation was 420 mm. The apricot orchard was 10-year-old and the trees were planted with 8 m (between the rows) x 8 m (between the trees in the same row).

Annual cover crops were used in the study area. *Vicia villosa* Roth (hairy vetch), *Vicia pannonica* Crantz (Hungarian vetch), *V. pannonica*, Triticale (*V. pannonica* 70% + Triticale 30%) mixture, and *Phacelia tanacetifolia* Benth (lacy phacelia) were used as winter cover crops and *Fagopyrum esculentum* Moench (buckwheat) as summer cover crop. Being a summer cover crop, *F. esculentum* was planted between 21.04.2014 and 05.05.2015, whereas other winter cover crops were between 23.10.2014 and 23.10.2015 hand spreading method. The results were recorded for summer and winter cover crops in 2015 and 2016.

The experiments were conducted in a randomized complete block design with four replications. This study also includes weedy control, herbicide, and mechanical weed control plots. The experiments were established in a 6-year-old apricot garden, and the mechanical control application was carried out at the time of sowing of the covering plants, and the herbicide application when the weeds had 2-6 leaves. Each plot was set to be 80 m^2 (4 x 20 m). The successive plots were separated as a region containing no cover crop. The seeds of cover crops were planted with plantation norm of 15 kg/ha for hairy vetch and Hungarian vetch, 30 kg/ha for lacy phacelia, and 50 kg/ha for buckwheat. The cover crops were kept at the same plots during the experiment period.

After bloom of cover crops, half of the cover crop plots was mixed down to 10 cm depth by using double-disc cultivator with two passes (incorporation process), whereas the other half was mowed (without another process) and the mowed plants were left on the soil as mulch (mowing process). In germination and active growth period, mechanical weed control by using a rotary hoe machine and glyphosate herbicide (6 l/ha) were applied.

The first weed counting was performed before incorporation the cover crops into the soil (initial counting) and the weeds were determined. In order to determine the suppressive effects of cover crops, the numbers of weeds were counted on 7th, 14th, and 28th days after the initial counting by using a 50 cm x 50 cm frame randomly thrown three times on each plot (in both incorporation process and mowing process) and the diversity and density of weeds were calculated.

Statistical analysis

At the end of the experiment, the densities of weeds observed in the study field were calculated using the formula given below.

$$\text{Density} = \frac{\text{Number of the weed species}}{\text{Number of all of the weed species}} \text{ X100}$$

Shannon-Wiener diversity index (H') was used in determining the diversity of weeds in the study field. The following formula was used in the Shannon-Wiener diversity index (H').

$H' = -\Sigma\ pi \ln(pi)$

where,

pi : ratio of i^{th} species to the others

ln: base of natural logarithm (Magurran 1988, Magurran 2004).

Simpson's dominance index (Sd) was used in determining the dominance between the weeds in the study area. The formula used in this process is given below.

$Sd = \Sigma\ ni(ni\ 1)/\ N(N\ 1)$

where,

i: number of weed species

ni: Number of individuals from the same species in the experimental practices

N: the sum of all individuals from the species in each experimental practices (Magurran 1988, Magurran 2004).

The canonical discriminant analysis was performed in order to determine the effects of cover crops on the weeds. On each sampling date, the composition of the current weed population was subjected to canonical discriminant analysis by using CANDISC practice in SAS. In order to evaluate the relationship between the presence of weed species and the cover crop practices, a vector diagram based on the total canonic coefficient of each weed species was combined in the same diagram (Isik et al. 2009a). The type and degree of the relationship between the presence of weed species and the cover crop practices were represented in a coordination dual-graph by adding a vector diagram to the distribution graph by using vectors representing the weed species (Shrestha et al. 2002). The emergence of weed species vectors and cover crop practices in the same coordination area showed the relationship between those weeds and cover crops (Legere et al. 2005).

RESULTS AND DISCUSSION

The dominant weed species in the experimental apricot orchard, in which the present study was carried out, were found to be *Amaranthus retroflexus* L., *Convolvulus arvensis* L., *Tribulus terrestris* L., *Sisymbrium officinale* (L.) Scop., *Sorghum halepense* (L.) Pers., *Lamium amplexicaule* L., *Chenopodium album* L., *Thlaspi arvense* L. and *Vaccaria pyramidata* Medik (Table 1). The densities of other weed species such as *Lactuca serriola* L., *Sinapis arvensis* L., *Glycyrrhiza glabra* L. were found to be lower than 1%. In counting practices performed in both years, the most important weed species were found to be *A. retroflexus* (22%), *C. arvensis* (16%), *T. terrestris* (16%), *S. officinalis* (14%), and *S. halepense* (7%) and the population of these weed species constituted more than 75% of the total weed population (Table 1).

Table 1. Dominant weed species in the experimental area and their relative proportion just before treatments (2015 and 2016, combined)

Weed species	Bayer code	Relative abundance (%)
Amaranthus retroflexus L.	AMARE	22
Convolvulus arvensis L.	CONAR	16
Tribulus terrestris L.	TRBTE	16
Sisymbrium officinale (L.) Scop.	SSYOF	14
Sorghum halepense (L.) Pers.	SORHA	7
Lamium amplexicaule L.	LAMAM	3
Chenopodium album L.	CHEAL	3
Thlaspi arvense L.	THLAR	3
Vaccaria pyramidata Medik.	VACPY	3
Papaver rhoeas L.	PAPRH	2
Cirsium arvense (L.) Scop.	CIRAR	2
Xanthium strumarium L.	XANST	1
Portulaca oleracea L.	POROL	1
Convolvulus galaticus Rost. ex Choisy	CONGA	1
Anthemis arvensis L.	ANTAR	1
Others		<5

Table 2. The effects of different practices on the intensity-dominance (Shannon diversity index (H´) and Simpson dominance index (Sd values) of significant weeds in cover crops (2015 and 2016, combined).

Treatments	Weeds					
	H	AMARE	CONAR	TRBTE	SSYOF	SORHA
V. villosa		Sd	Sd	Sd	Sd	Sd
FWC	1.5753	0.0000	0.2125	0.0000	0.0913	0.0000
mowing (7. days)	0.7120	0.0000	0.6046	0.0000	0.0000	0.0000
incorporation (7. days)	0.4535	0.0000	0.0551	0.0000	0.0000	0.1484
mowing (14. days)	0.8901	0.0000	0.3486	0.0000	0.0000	0.0005
incorporation (14. days)	0.6805	0.0000	0.3844	0.0000	0.0000	0.0370
mowing (28. days)	0.9193	0.0000	0.2216	0.0831	0.0000	0.0316
incorporation (28. days)	0.8876	0.0000	0.4901	0.0051	0.0000	0.0000
V. pannonica						
FWC	1.8225	0.0000	0.0211	0.0000	0.0760	0.0000
mowing (7. days)	0.7130	0.0000	0.5666	0.0000	0.0000	0.0000
incorporation (7. days)	0.6914	0.0000	0.0317	0.0000	0.0000	0.0648
mowing (14. days)	1.2755	0.0000	0.2244	0.0000	0.0000	0.0334
incorporation (14. days)	1.0021	0.0000	0.1665	0.0000	0.0000	0.0311
mowing (28. days)	1.3073	0.0000	0.1502	0.0829	0.0000	0.0095
incorporation (28. days)	0.9498	0.0000	0.4242	0.0083	0.0000	0.0010
Triticale+V. pannonica						
FWC	1.5552	0.0000	0.1275	0.0000	0.0703	0.0000
mowing (7. days)	1.2202	0.0000	0.2857	0.0000	0.0000	0.0000
incorporation (7. days)	0.5006	0.0000	0.2548	0.0000	0.0000	0.0000
mowing (14. days)	1.1298	0.0000	0.3260	0.0000	0.0000	0.0000
incorporation (14. days)	0.6630	0.0000	0.5426	0.0000	0.0000	0.0000
mowing (28. days)	1.3740	0.0000	0.3027	0.0026	0.0000	0.0176
incorporation (28. days)	1.0388	0.0000	0.3632	0.0185	0.0000	0.0013
P. tanacatifolia						
FWC	1.3022	0.0000	0.0883	0.0000	0.2582	0.0000
mowing (7. days)	0.9277	0.0000	0.5507	0.0000	0.0000	0.0010
incorporation (7. days)	0.0056	0.0000	0.5000	0.0000	0.0000	0.0000
mowing (14. days)	0.9388	0.0000	0.2886	0.0000	0.0000	0.0000
incorporation (14. days)	0.0879	0.0000	0.2442	0.0000	0.0000	0.0601
mowing (28. days)	1.1268	0.0000	0.4129	0.0076	0.0000	0.0053
incorporation (28. days)	0.0989	0.0000	0.5068	0.0020	0.0000	0.0008
F. esculentum						
FWC	1.4547	0.0349	0.0304	0.2391	0.0000	0.0015
mowing (7. days)	1.3645	0.0541	0.1913	0.0519	0.0000	0.0001
incorporation (7. days)	1.4424	0.0047	0.1831	0.0883	0.0000	0.0000
mowing (14. days)	1.4552	0.0687	0.0314	0.0810	0.0000	0.0671
incorporation (14. days)	1.5063	0.0198	0.1223	0.0519	0.0000	0.0103
mowing (28. days)	1.5681	0.0437	0.0187	0.1756	0.0000	0.0034
incorporation (28. days)	1.3274	0.0000	0.1311	0.0505	0.0000	0.0912
Mechanical weed control						
FWC	1.6488	0.0234	0.0228	0.1101	0.0786	0.0078
7. days	0.7390	0.0009	0.0373	0.0045	0.0000	0.0352
14. days	0.9107	0.0064	0.0685	0.0096	0.0000	0.0163
28. days	1.3100	0.0099	0.0951	0.0102	0.0000	0.1218
Herbicide						
FWC	1.5828	0.0121	0.0047	0.1787	0.1141	0.0022
7. days	0.6903	0.0174	0.0837	0.0381	0.0000	0.0101
14. days	0.5852	0.0218	0.0321	0.0893	0.0000	0.0062
28. days	0.5884	0.0542	0.0062	0.1443	0.0000	0.0024
Weedy control						
FWC	1.8173	0.1236	0.0056	0.0235	0.0270	0.0208
7. days	1.8057	0.1236	0.0057	0.0235	0.0273	0.0209
14. days	1.9725	0.1042	0.0065	0.0199	0.0198	0.0191
28. days	2.0711	0.0863	0.0066	0.0183	0.0160	0.0178

The Shannon diversity index (H´) and species-based Simpson dominance index (*D*) values of the top 5 weed species seen in Table 1 are given in Table 2. It was observed that, considering the average of 2 years, the highest weed diversity index (H) value was obtained in the weedy control. In the herbicide and mechanical weed control practices, the diversity of weeds was found to be higher when compared to the initial counting values. In the control plots, increases were observed in the weed diversity between the initial counting and the counting performed on the 28th day. From the aspect of weed diversity, it was found as a result of the counting practices performed before mowing and incorporation the plants that the highest weed diversity was obtained with *V. pannonica* cover crops, followed by *V. villosa*, Triticale + *V. pannonica*. In suppressing the weed species diversity by using cover crops, the highest suppression effect was obtained by incorporation *P. tanacatifolia* into the soil. When cover crops were examined in general, it was found that their incorporation into the soil gave better results compared to mowing and the effect of both processes decreased since new weeds grow as they passed time. As a result, weed diversity has increased (Table 2).

Examining the Simpson dominance index (Sd) values of top 5 most important weeds found in the study field in the 2-year period, it was determined that generally the perennial weeds [*Convolvulus arvensis* (CONAR) and *Sorghum halepense* (SORHA)] were dominant among the cover crops and there were differences between the cover crops. It was also found that the annual plants were generally suppressed and their diversity significantly decreased (except for *F. esculentum*). In the previous studies, it was reported that the cover crop practices control the weed populations but some (especially the perennial ones) species in these populations could not be suppressed. Considering the dominance values of each species reported in the previous studies, it was found that the use of the cover crops, mechanical weed control, and total herbicide suppressed the weed species. After the mowing and incorporation practices, the numbers of certain weeds decreased in the counting performed on the 7th day but their populations re-increased in 14th and 28th-day values. Kostrzewska et al. (2012) examined the effect of the summer barley and pea mixture on the weed species and they reported that, according to the Shannon-Wiener diversity and Simpson dominance values of cultivated plants grown after them, they reduced the number of weed species. In this study, it was found that the cover crops had similar effects on the weed species.

In the canonical discriminant analysis on the results obtained from the present study, it was found that 27 weed species were detected in apricot orchard at the end of 1st year and 26 species at the end of the 2nd year. In the counting performed at the end of 1st year, the highest number of weed species was observed in the weedy control plots (9.5 species), whereas the lowest number of species (4 species) was found in *P. tanacetifolia* plots. The most frequently seen weed species in *V. villosa*, *V. pannonica*, and triticale + *V. pannonica*, *P. tanacetifolia* plots was SSYOF, whereas the most frequently seen weed species in *F. esculentum* plots, mechanical weed control, and total herbicide was TRBTE. The most frequently seen weed species in the control plot was found to be AMARE. In the first counting in 2nd year, the highest number of weed species was observed again in the weedy control plot (7.5 species), whereas the lowest number (2.5 species) was obtained in the mechanical weed control plot. The most frequently seen weed species were found to be CIRAR in *V. villosa*, triticale + *V. pannonica*, and *P. tanacetifolia*, TRBTE in *F. esculentum*, *V. pyramidata* in *V. pannonica*, and SSYOF in mechanical weed control, total herbicide, and control plots. Canonical discriminant analysis was performed at the 1st counting to determine the relationship between the practices and weed species (Figure 1). The canonical discriminant analysis showed variation between the practices in terms of the weed species. It was determined that 94% of the weed correlation between the practices was explained by the 1st canonical axis and 88% was explained by the 2nd canonical axis (Table 3). Weed plot constituted a significantly separate group, whereas *F. esculentum* constituted a separate group since it couldn't establish a sufficient coverage as a cover crop and couldn't prevent the weed growth.

In the 7th day mowing in 1st year, the number of weed species was 19, whereas the number of weed species was found to be 15 in the 2nd year. In the mowing on the 7th day in the 1nd year, the highest number of weed species was found in weedy control plots (9.5 species), whereas the lowest number of weed species was found to be 1 in *V. villosa* plot.

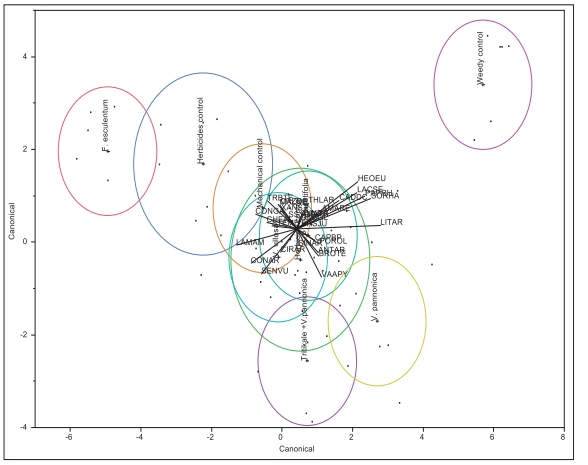

Figure 1. Diagram of 1st counting canonical discriminant analysis on weed species and practices

Table 3. The 1st and 2nd canonical axis values

Application	Counts	Cononical axis values (%)	
		1st canonical axis values	2nd canonical axis values
	1st count	94	88
Mowing	7th day	98	71
Mowing	14th day	99	81
Mowing	28th day	99	86
Incorporation	7th day	98	78
Incorporation	14th day	99	86
Incorporation	28th day	99	88

The most frequently seen weed species was found to be CIRAR in *V. villosa*, *V. pannonica*, triticale + *V. pannonica*, *P. tanacetifolia*, and TRBTE in *F. esculentum* parcel, mechanical weed control, and total herbicide. The most frequently seen weed species in control parcel was AMARE. In the mowing on the 7th day in 2nd year, the highest number of weed species was found in weedy control plots (7.5 species), whereas the lowest number was found to be 2.5 species in *V. pannonica* plot. The most frequently seen weed species was found to be SSYOF in control plot and CIRAR in other plots. The canonical discriminant analysis also showed variation between the practices in terms of weed species in the 7th day mowing practices (Figure 2). It was observed that 98% of the weed correlation between the practices was explained by the 1st canonical axis and 71% by the 2nd canonical axis (Table 3).

Twenty weed species were found in the study field in the incorporation on 7th day in 1st year and 15 species in 2nd year. In the incorporation on the 7th day in 1st year, the highest number of weed species was found in weedy control plots (9.5 species), and the lowest number was found to be 1 in *V. villosa* parcel. The most widely seen species was found to be CIRAR in *V. villosa*, *V. pannonica*, triticale + *V. pannonica*, *P. tanacetifolia*, and *F. esculentum* and TRBTE in mechanical weed control and total herbicide parcels. The most frequently seen weed species in the control plot was found to be *A. tricolor*. In the incorporation on the 7th day in 2nd year, the highest number of weed species was found in weedy control plots (7.25 species) and no weed was observed in *V. villosa*, *V. pannonica*, triticale + *V. pannonica*, and *P. tanacetifolia* parcels. The most frequently seen weed species in the control plot was SSYOF (Figure 2). It was found that 98% of the weed correlation between the practices was explained by the 1st canonical axis and 78% by the 2nd canonical axis in the incorporation on the 7th day (Table 3).

In the first year, in the 14th day mowing practices, 17 and in the second year 18 weed species were identified. In the first year, mowing on the 14th day, the highest number of weed species was detected in weedy control plots (11.25 species) and the lowest number was determined to be 2 weed species in *V. villosa* plot. Again, the most frequently seen species were found to be CIRAR in *V. villosa*, *V. pannonica*, triticale + *V. pannonica*, and *P. tanacetifolia* plots and TRBTE weed species in mechanical weed control and total herbicide plots. The most frequently seen weed species in control parcel and *F. esculentum* was AMARE. In the mowing on the 14th day in 2nd year, the highest number of weed species was obtained in the weedy control parcel (10.25 species) and the lowest number of weed species was found to be 2.25 species in *V. villosa* plots. The most frequently seen species in control plot was found to be SSYOF (Figure 2). It was found that 99% of the weed correlation between the practices was explained by the 1st canonical axis and 81% by the 2nd canonical axis (Table 3).

In the 14th day incorporation practices, 17 weed species were observed in the 1st year and 18 species in the 2nd year. In the first year, the highest number of weed species was found in the weedy control plot (11.25 species), whereas the lowest number of weed species was found to be 1.75 species in *V. villosa* and triticale + *V. pannonica*. The most frequently seen weed species were found to be CIRAR in *V. villosa*, *V. pannonica*, triticale + *V. pannonica* plots, *P. tanacetifolia*, and *F. esculentum* and TRBTE in mechanical weed control and total herbicide parcels. The most frequently seen weed species in the control plot was AMARE. In the 14th day incorporation practices, the highest number of weed species in the 2nd year was found in the weedy control parcels (10 species), and the lowest number of weed species was found to be 1.5 species in *V. villosa*, *V. pannonica*, and triticale + *V. pannonica* vetch parcels. The most widely seen weed species was found to be TRBTE in *F. esculentum* plot, SSYOF in control plot, and CIRAR in other plots (Figure 2). It was found that 99% of the weed correlation between the practices was explained by the 1st canonical axis and 86% by the 2nd canonical axis (Table 3).

17 weed species were observed in the 1st year and 18 species in the 2nd year in the 28th day mowing practice. The highest number of weed species in the 1st year was found in the weedy control plots (12.5 species), whereas the lowest number of weed species was found to be 2.25 species in *V. villosa* plot. The most frequently seen weed species was CIRAR in *V. villosa*, *V. pannonica*, triticale + V. pannonica, *P. tanacetifolia*, and *F. esculentum* plot, TRBTE in mechanical

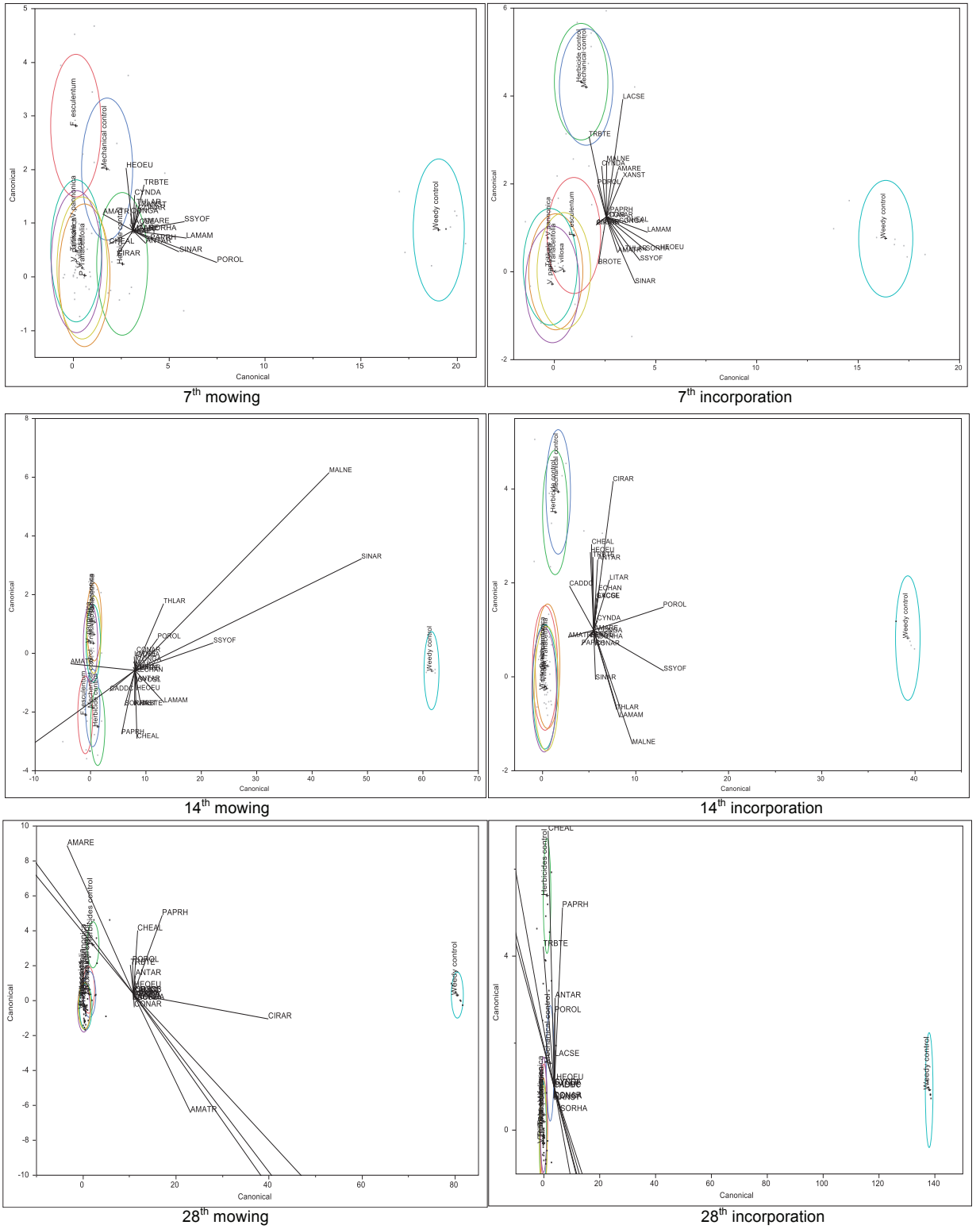

Figure 1. The diagram of 7th, 14th, and 28th day mowing and incorporation canonical discriminant analysis on weed species and cover crop practices.

weed control and total herbicide, and AMARE in control plot. In the 2nd year, the highest number of weed species in the 28th day mowing practice was found in the weedy control plots (11 species), whereas the lowest number of weed species was found to be 2 in V. pannonica and P. tanacetifolia plots. The most frequently seen species was TRBTE in *V. pannonica* and *F. esculentum*, CIRAR in *V. villosa*, triticale + *V. pannonica*, and *P. tanacetifolia*, *S. halepense* in mechanical

weed control plot, ANTAR in total herbicide plot, and SSYOF in control plot (Figure 2). It was found that 99% of the weed correlation between the practices was explained by the 1st canonical axis and 86% by the 2nd canonical axis (Table 3).

In this study, the highest number of weed species in the 28th day incorporation practices were found to be 15 in both years. In the 1st year, the highest number of weed species was found in weedy control plots (12.5 species), whereas the lowest number of species was found to be 1.5 in *V. villosa* and triticale + *V. pannonica* plot. The most frequently seen weed species were CIRAR in *V. villosa*, *V. pannonica*, triticale + *V. pannonica*, *P. tanacetifolia*, and *F. esculentum*, TRBTE in mechanical weed control and total herbicide plots, and AMATR in control plots. In the 2nd year, the highest number of weed species in the 28th day incorporation practice was found in weedy control plots (11 species), whereas the lowest number of weed species was found to be 2.25 in *V. pannonica* and *P. tanacetifolia* plot. The most frequently seen weed species was SORHA in mechanical weed control plot, ANTAR in total herbicide plot, SSYOF in control plot, and CONAR in other plots (Figure 2). It was determined that 99% of weed correlation between the practices was explained by the 1st canonical axis and 88% by the 2nd canonical axis. In all practices, the weedy control plot constituted a significantly separate group (Table 3).

As a result of this study, the cover crops yielded a significant decrease in the weed intensities and the use of cover crops is a method that can be used in suppressing the weeds. By directly competing with weeds for light and nutrients, cover crops reduce their development and seed production (Peachy et al. 1999). Fisk et al. (2001) reported that the cover crops affect the density of weeds and the weight loss in biomass by decreasing the light transmittance, changing the soil temperature, increasing the soil moisture, revealing the allelopathic chemicals, and preventing the germination of weed seedlings by acting as a physical barrier. Moreover, Akemo et al. (2000) stated that hairy vetch suppresses the weeds through its shadowing effect. Furthermore, Kitis et al. (2007) reported in their study, in which they used *Vicia sativa* L. (hairy vetch) as a cover crop of Cukurova region, suppressed the weeds by 64% in 2004 and 38% in 2005. Again, Isik et al. (2009a, 2009b) reported that hairy vetch significantly suppressed the weeds in organic paprika and lettuce growing areas. In this study, however, the most cover crop that was effective against weeds at most was found to be lacy phacelia (approx. 75%), followed by buckwheat (approx. 73%) and hairy vetch (approx. 63%). When compared to the control, the lowest effect among the cover crops was obtained from Hungarian vetch (33%), while the effect increased above 50% with the combination of Hungarian vetch and triticale. The results obtained from the cover crops are in parallel with those reported in the studies cited here. Bocianowski and Majchrzak (2019) stated that cover crop was significant effect on number of weed species and density. Also they are think that degree to cover crops affect regulation of weed infestation is diversified and depends on weed species, habitat conditions, type of catch crop as well as method of its management. Blackshaw et al. (2001) reported that the use of *Melilotus officinalis* as a live cover crop in fallow systems suppressed the weed biomass by >95%. Moreover, incorporation *M. officinalis* into the soil was found to be effective in controlling the weeds by means of this plant's residual effect. Although no such high value could be achieved in controlling the weeds in this study, it is concluded that the use of cover crops, especially in fruit orchards, can suppress the weeds. Besides suppressing the weeds, it was also determined in the present study that legume cover crops enhanced the soil in terms of nutrients. Sainju et al. (2002) reported that, by using the cover crops, the nutrient elements in the soil can be kept and it might be a necessary part of sustainable agriculture. As a result of their 3-year study on the same plots, Ngouajio et al. (2003) determined that the population in the plots fallowed in summer was larger than the population in the plots, where the cover crops were used in summer. Again, Teasdale et al. (2007) reported that the use of cover crops decreased the weed intensity and biomass weight. Moreover, in their study employing cover crops in weed control in citrus orchards in Mediterranean region, Koloren (2004) stated that there was a negative relationship between the soil coverage area of cultivated plants (cover crop) and the general weed population and the lowest weed population was found in trefoil and grass plants. In this study, in which there were gravelly plots, similar results were achieved despite different legume plants were used as cover crops.It was determined that the most important weed in the experimental area was *Amaranthus retroflexus*, followed by *Convolvulus arvensis* and *Tribulus terrestris*. The highest weed diversity index (H) value was obtained in the weedy control. When compared with the 1st count, increases in weed density were determined on the 28th day. According to the Simpson dominance index (Sd), it was determined that the cover crops generally do not suppress perennial weeds (*C. arvensis* and *S. halepense*) very well. The experiments results pointed out that annual weeds were generally suppressed in the cover crops plots other than *F. esculentum* and their diversity decreased significantly. The results obtained from the canonical discriminant analysis showed that the highest numbers of weed species in the 1st and 2nd years were found in the weedy control plots and that the cover crops suppressed the weeds. The results also provide an opportunity for effective non-chemical weed control, which is important for organic fruit production.

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ÖZET

Kayısı, Türkiye'nin ihracatında önemli rol oynayan meyvelerden olup, yabancı otlar ise kayısı bahçelerinde sorun yaratan temel faktörlerdendir. Bu çalışma, örtücü bitkilerin bu alanlarda sorun oluşturan yabancı otların kontrol altına alınmasını, ayrıca tür dağılımı, çeşitliliği ve baskınlığı üzerindeki etkilerini saptamak amacıyla 2014-2016 yılları arasında Malatya'da yürütülmüştür. Çalışmada tek yıllık kışlık örtücü bitkiler olarak: *Vicia villosa* Roth (tüylü fiğ), *Vicia pannonica* Crantz (Macar fiğ), *V. pannonica* + Triticale (*V. pannonica* %70 + Tritikale %30) karışımı ve *Phacelia tanacetifolia* Bentham (arı otu) ve yazlık örtücü bitki olarak *Fagopyrum esculentum* Moench (karabuğday) kullanılmıştır. Çalışmada yabancı ot çeşitliliği indeksi (H) ve Simpson'ın baskınlık indeksi (Sd) değerleri de hesaplanmıştır. Ayrıca, uygulamalar, kanonical discriminant analizine tabi tutulmuştur. İki yıllık dönemde yabancı ot çeşitliliği indeksinin (H) en yüksek değeri yabancı otlu kontrol parsellerinden elde edilmiştir. Çok yıllık yabancı otların (*Convolvulus arvensis* ve *Sorghum halepense*) örtücü bitkilerde baskın türler olduğu ve uygulamalar arasında da farklılıkların bulunduğu belirlenmiştir. *F. esculentum* dışındaki parsellerde tek yıllık yabancı otların genel olarak bastırıldığı ve çeşitliliğinin önemli ölçüde azaldığı tespit edilmiştir. kanonical discriminant analizinden elde edilen sonuçlar, 1. ve 2. yıllarda en çok yabancı ot türünün yabancı ot kontrol parsellerinde bulunduğunu ve kaplayıcı bitkilerinin yabancı otları bastırdığını göstermiştir.

Anahtar kelimeler: kayısı, biyoçeşitlilik, örtücü bitkiler, tür dağılımı, yabancı ot

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Original article

Determination of the control methods of *Ipomoea triloba* L. (three lobe morning glory) in cotton fields

Pamuk ekim alanlarında *Ipomoea triloba* L. (pembe çiçekli akşam sefası)'nın mücadele olanaklarının belirlenmesi

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is used as the raw material in more than fifty industries and is also the main source of raw materials used in the textile, the oil, the animal feed, and the paper industries. Additionally, it is a crop of great economic importance for its producer countries with the added value and employment opportunities. Cotton is among the crops sensitive to weed competition and its yield decreases with the weeds. For this reason, weed control treatments should be employed effectively to obtain high yields in cotton fields. In the recent years, *Ipomoea triloba* (IPOTR), which has increased in its importance in the agricultural areas of the Mediterranean Region, prevents the development of the cotton because of its invasive features and causes the harvest difficulties during by hand or machine harvest. The aim of this study is to create an effective control program against IPOTR in cotton. Field experiments were carried out in Ceyhan, Adana, Turkey in 2018 and 2019. For this purpose, impact of the treatments including Pyroxasulfone 85% (PYRS) Trifloxysulfuron sodium 75% (TRFS), Pyrithiobac-sodium 383 g/l (PYBS), Glyphosate isopropylamine salt 480 g/l (GLYI), S-metolachlor 915 g/l + Benoxacor 45 g/l + hand hoe (SMEC), inter-row rotary hoe + intra-row hand hoe (FÇEÇ) were investigated 28 day-after treatment and at the harvest during two years. It has been determined that TRFS and PYBS have an efficacy over 90%, while FÇEÇ, SMEÇ, TRFS and PYBS applications have an efficacy over 60%. Applications against IPOTR have increased the cotton lint and yield. However, crop injury was observed after GLYI application (20%).

GİRİŞ

Cotton (*Gossypium hirsutum* L.) is an industrial plant, and grown in 85 countries which have tropical and subtropical climates (Mert 2007). More than 26 million tonnes lint is obtained from 34.7 million hectares in the world (Anonymous 2020a). Leading countries of cotton production are India, China, USA, and Brazil, and Turkey ranks 7th among them (Anonymous 2020b). Although cotton production is maintained with non-GMO varieties in

Turkey, average lint yield per hectare is equal more or less to the GMO cotton varieties (Anonymous 2020c). Turkey uses traditional varieties and profitable production techniques have a significant share in the world's cotton production system. Cotton production directly or indirectly provides added value to the country's economy.

The main purpose of cotton production is to obtain 35-40% the average fibre yield and 60% of the un-ginned cotton obtained from the unit area is seed called cotton seed. There is 17-24% oil in the seeds from which the fibres are taken after ginning. A healthy cotton plant contains high quality fibre and high efficiency seed oil (Kolsarıcı et al. 2006), it should have ginned performance. One half of cotton production of Turkey was provided by the South-eastern Anatolia region, the other half are produced in the Aegean and Mediterranean Regions (Anonymous 2020c).

Increasing productivity and quality in agricultural production mainly depends on effective control of the weeds, which is one of the main causes of crop losses, except for environmental conditions (Güncan 2016, Tepe 2014). Cotton is one of the sensitive crops to weed competition, especially in the first 6 weeks, and the crop yield decreases with the presence of the weeds. The weeds in cotton resulted in severe yield losses, and even reach to 90% (Ahmad et al. 2003, Gönen 1999, Uludag and Uremiş 2000, Vargas et al. 1996, Zimdahl 1980).

It is reported that an effective weed control application is required in the critical period of 2-3 weeks to 7-10 weeks followed by crop emergence (Gunes et al. 2008, Kaya and Nemli 2003, Tursun et al. 2016, Uludag et al. 2004, Uludag et al. 2012, Vargas et al. 1996). Moreover, some invasive plants such as *Ipomoea triloba* L. and *Amaranthus palmeri* L., appear as weeds that cause problems in agricultural and non-agricultural fields (Üremiş et al. 2020).

Weeds prevent the development of cotton by sharing essential components such as water, nutrients, etc., which are necessary for growth of crop, and also, they reduce the amount of light that can be taken as a result of shading the plant. In addition to these damages, some weeds such as bindweed (*Convolvulus arvensis*), three lobe morning glory (*Ipomoea triloba*), rough cocklebur (*Xanthium strumarium*), black nightshade (*Solanum nigrum*), jimsonweed (*Datura stramonium*) and hooked bristlegrass (*Setaria verticillata*), especially emerge after watering, may make cotton harvest difficult and/or cause quality losses in cotton fibres (Bükün and Uygur 1997, Kadıoğlu et al. 1993, Özer et al. 1998, Özkil et al. 2019, Uludağ and Üremiş 2000, Uygur et al. 1984).

The Ipomoea genus, which includes more than five hundred species, is widely found in agriculture and non-agricultural areas in the tropical and subtropical regions of the world and continues to spread rapidly other regions of the world (Willis 1966). *Ipomoea triloba* (IPOTR) was firstly detected in cotton production areas in 1986 (Joel and Liston 1986). In Turkey, it was determined in cotton of Antalya (Özkil et al. 2019, Yazlık et al. 2014, Yazlık et al. 2018), and, in following years, it has been found in other cities of the Mediterranean region and suppress other weeds (Özkil and Üremiş 2020). It was reported that in a survey carried out in 46 different products to determine the prevalence and density of IPOTR showed that IPOTR infested cotton, soybean, corn, peanut, orange, tangerine, pomegranate gardens and eggplant fields (Özkil and Üremiş 2020). In a study conducted on glyphosate-resistant soybeans in Brazil, IPOTR was reported to cause yield loss of 70-83%. It has also been stated that IPOTR can be controlled nearly 70% with glyphosate (Ovejero et al. 2019).

IPOTR completes its vegetative growth in a short time by surrounding the cultivated plant due to its invasive nature; consequently it prevents the development of the crop. In cotton, especially in Antalya province, cotton harvest is difficult or sometimes impossible by hand or by machine (Özkil et al. 2019). The aim of this study is to create an effective weed control program in cotton against IPOTR. In cotton, there is no registered herbicide in Turkey that can be used against IPOTR. For this reason, determination of the herbicide which may control IPOTR in cotton is important for cotton producers and country's economy.

MATERIALS AND METHODS

This study was conducted to determine control strategies against IPOTR at the field conditions. The trials were carried out between 2018 and 2019 in a cotton field located in Ceyhan, Adana (37°6´8.226 E, 35°46´49.428 N). At the beginning of the trial, soil samples (0-20 cm) were taken to determine important physical and chemical features of soil (Table 1). The soil structure in the field trials has a clay structure (Table 1).

Table 1. Soil characteristics of the experimental field (0–20 cm soil depth)

Analyses	2018-2019	
	Result	Specifications
Texture (%)	84.70	Clay
pH	7.97	Mild Alkaline
Total Salt (%)	0.040	Without Salt
Organic Matter (%)	2.33	Middle
Available Potassium (K_2O) (kg/da)	186.71	High
Available Phosphorus (P_2O_5) (kg/da)	6.86	Middle
Total Lime (%)	20.60	Too Chalky

Table 2. The monthly average climate data for the period of herbicide application in IPOTR control

Applications	2018			2019		
	Avg. Temperature (°C)	Avg. Relative Humidity (%)	Avg. Wind Speed (m/s)	Avg. Temperature (°C)	Avg. Relative Humidity (%)	Avg. Wind Speed (m/s)
TRFS (One passes)	24.20	75.60	2.50	25.00	65.90	2.20
TRFS (Two passes)	24.20/29.80	75.60/49.50	2.50/3.40	25.00/29.30	65.90/75.70	2.20/2.90
PYBS(One passes)	24.20	75.60	2.50	25.00	65.90	2.20
PYBS (Two passes)	24.20/29.80	75.60/49.50	2.50/3.40	25.00/29.30	65.90/75.70	2.20/2.90
GLYI	28.60	67.60	3.50	27.70	69.10	3.00
GLYI	28.60	67.60	3.50	27.70	69.10	3.00
PYRS	17.80	64.80	3.30	23.90	38.80	2.50
PYRS	17.80	64.80	3.30	23.90	38.80	2.50
PYRS + GLYI	17.80/28.60	64.80/67.60	3.30/3.50	23.90/29.30	38.80/69.10	2.50/3.00
SMEÇ	17.80	64.80	3.30	23.90	38.80	2.50

*TRFS: 75% Trifloxysulfuron sodium, PYBS: 383 g/l Pyrithiobac-sodium, GLYI: 480 g/l Glyphosate isopropylamine salt, PYRS: 85% Pyroxasulfone, SMEÇ: 915 g/l S-metolachlor + 45 g/l Benoxacor) + hand hoe, FÇEÇ: inter-row rotary hoe + intra-row hand hoe, YOZK: weed-free control, YOKS: Full season weedy control (Ç.S.), YOKS: Full season weedy control (Ç.Ö.)

Climatic data, monthly average temperature (°C), relative humidity (%) and average wind speed (m/s) of the year during the cotton growing season were obtained using imetos 3.3 climate station in field trials (Table 2). The climatic parameters during TRFS, PYBS, and GLYI treatments were very close to each other at both years. However, these parameters at PYRS and SMEÇ in 2018 were different than in 2019.

The agronomic treatments were employed in harmony with the cotton farmer practices. Soil fertilizer (20:20-N:P) and foliar fertilizer (8 kg N, 8 kg K) were applied to support the normal development of crops. Additionally, in 2018 and 2019, the soil was surface irrigated 3 times during trials followed by application of plant growth regulator. The soil was cultivated using a rotary hoe 3 times during the cotton growing season in accordance with the cotton

Table 3. Applications carried out to control IPOTR

Applications	Active Ing.	Application Method	Application Dose	Application Frequency
Trifloxysulfuron sodium (TRFS)	75%	Foliar app.*	1.5 g/ da	1 pass
Trifloxysulfuron sodium (TRFS)	75%	Foliar app.*	1.5 g/ da	2 passes with an interval of 2 weeks
Pyrithiobac-sodium (PYBS)	383 g l^{-1}	Foliar app.*	20 ml/ da	1 pass
Pyrithiobac-sodium (PYBS)	383 g l^{-1}	Foliar app.*	20 ml/ da	2 passes with an interval of 2 weeks
Glyphosate isopropylamine salt (GLYI)	480 g l^{-1}	Foliar app.*	60 ml/ da	1 pass
Glyphosate isopropylamine salt (GLYI)	480 g l^{-1}	Foliar app.*	100 ml/ da	1 pass
Pyroxasulfone (PYRS)	85%	Soil App **	10 g/ da	1 pass
Pyroxasulfone (PYRS)	85%	Soil App **	15 g/ da	1 pass
Pyroxasulfone + Glyphosate isopropylamine salt (PYRS+ GLYI)	85% + 480 g l^{-1}	Soil. App. + Foliar App.	10 g/ da + 60 ml/ da	
(S-metolachlor + Benoxacor) + over row hoeing (SMBE)	915 g l^{-1}+ 45 g l^{-1}	Soil App * + 2 hoeing	150 ml /da	1 pass + 2 passes with an interval of 2 weeks
Inter-row rotary hoe + intra-row hand hoe (FÇEÇ)	-	-	-	3 passes with an interval of 2 weeks
Control (weed-free) (YOZK)		-	-	Throughout the season
Control (Full season weedy) (Ç.S.) (YOKS)		-	-	-
Control (Full season weedy) (Ç.Ö.) (YOKÖ)		-	-	-

* One day after the application, flood irrigation was carried out up to the field capacity.
** Soil application (pre em)
*** Soil application and foliar application (pre em and post em)
**** 1st application after planting 85% Pyroxasulfone 10 g/da;
2nd application 480 g/l Glyphosate isopropylamine salt 60 ml / da

Table 4. Application information of herbicides used

Active Substance	Application Time	Form.	Dose (g-ml/da)
75% Trifloxysulfuron sodium	Post emergence	WG	1g - 2g da^{-1}
383 g/l Pyrithiobac-sodium	Post emergence	SL	20 ml da^{-1}
480 g/l Glyphosate isopropylamine salt	Cotton 7-9. Node period	SL	60-100 ml da^{-1}
85% Pyroxasulfone	Pre-Emergence	WG	10-15 g da^{-1}
915 g/l S-metolachlor + 45 g/l Benoxacor	Pre-Emergence	EC	150 ml da^{-1}

Form. Formulation

growing technique. The pests control applications in cotton were applied with insecticides, if necessary according to the integrated control technical instructions

Field trials started on 08.04.2018 and 08.05.2019 by planting (var. Ceyhan 520). The cotton was harvested on 28.09.2018 and 03.10.2019 by hand. The trials were arranged in a randomized complete block design with 4 replicates. There was a 1.50 m allay between the blocks and 75 cm between the parcels to prevent herbicide drift. Each parcel had 4 cotton rows (75x20), and was 24m^2. In addition, two constant quadrats, each of was 1m^2, were left completely untreated as control to weed count. In the field trials, a knapsack sprayer with a boom mounted multiple flat fan nozzles was used to treat herbicides. The herbicides were applied at a pressure of 3 bar.

Some information about the applications, application dosage and application methods in the field trials are presented in Table 3. The information of herbicides is given in Table 4.

To compare efficacy of the pre or post emergence herbicides applied, the status of the weeds was visually evaluated at 7, 14, 21 and 28 days after treatment (DAT) compared to the weeds in untreated control plots. The 0-100 scale is used to assess the herbicide efficacy, where 0 equals no control and 100 equals complete died. The rates were converted according to Arc-Sin transformation before the statistical analysis, but real values were presented to clarify the interpretations.

The weeds in the quadrats in the trial plots were harvested from the soil levels on the 28 DAT and were dried at 105 °C for 24 h. (Eymirli 2011). Cotton was harvested from two rows in the middle from each plot, and the ginning efficiency (WR: %) and fibre yield (LV: kg da^{-1}) were calculated.

As some important yield component, ginning efficiency and fibre yield were calculated following formula (1 and 2).

$$GE=FWC\ (g)/((FWC\ (g)+SWC\ (g))X100 \quad (1)$$

$$LY=(LY\ (kg)xGE)/100 \quad (2)$$

Where GE means ginning efficiency, FWC means fibre weight of cotton, SWC means seed weight of cotton, LY means lint yield. The data obtained from the experiments were subjected to analysis of variance using SPSS (version 23), and the mean were compared by Duncan's multiple comparison test at 5% significance level.

RESULTS AND DISCUSSION

Crop injury symptoms caused by herbicide treatments varied heavily depending on the herbicide. TRFS resulted in temporarily growth reduction in cotton, but the symptoms disappeared in time. In parallel with our results, previous studies have also reported that TRFS in cotton may temporarily retard crop growth (O'Berry et al. 2008, Salimi et al. 2006). In the preliminary studies conducted in 2017, it was determined that some producers applied GLYI to control weed species such as IPOTR, which affect the quality of the product and make the harvest difficult, before harvest. But, it was determined that GLYI caused chlorotic and necrotic spots on the cotton leaves, and decreased the number of boll and yield of cotton.

The effects of the applications on IPOTR (%) were given in Table 5. The treatments reduced more or less dry weight of IPOTR. In both years, mechanical control treatments provided the most effective weed control compared to the herbicides. The dry matter reduction was the highest point 7 DAT with 96.5-97%, but the efficacy of treatments steadily declined due to new emergent weeds. At harvest, the impact of the treatments was higher than at 28 DAT. This case may be caused by the suppressive effect of crop on the IPOTR and other agronomic practices such as hand hoe and machine hoe. The lowest weed control provided by the GLYI and PYRD+GLYI treatments 7 DAT. Even though the impact of these treatments reached to nearly 60% 28 DAT, IPOTR recovered themselves from the adverse effects of GLYI at harvest. It was determined that the pre-emergent PYRS resulted in a moderate dry biomass reduction at 10 and 15 g da^{-1} rates.

The post-emergence herbicides, SMEÇ, FÇEÇ, PYBS and TRFS were the most effective treatments for both years. TRFS treatments reduced dry biomass accumulation at 44-46% 7 DAT, and their efficacy increased continuously until harvest. But, there were no significant differences between the treatments TRFS (1 pass) and TRFS (2 passes). Similar to TRFS, PYBS caused significant dry biomass reduction at

Table 5. The effect of the applications on IPOTR (%) at 7, 14, 21, 28 DAT and at harvest

Application	7 DAT	14 DAT		21 DAT	28 DAT	Harvest
		2018	2019			
Pre-emergence[1]						
PYRS (10 g da^{-2})	29.03±6.88 a*	36.54±3.52 a	37.64±3.75 a	39.49±2.19 a	40.92±3.59 a	59.20±7.59 a
PYRS (15 g da^{-2})	41.32±6.90 a	48.08±3.81 a	39.21±2.92 a	44.46±2.09 a	46.84±1.30 a	49.70±5.88 a
Post-emergence[1]						
FÇEÇ	97.10±2.64 a	93.10±4.51 b	86.33±3.79 a	86.52±1.87 a	80.68±1.79 b	94.69±1.16 a
PYRS+GLYI	10.62±4.28 c	37.93±3.95 d	46.04±1.55 c	59.57±2.52 c	66.10±1.48 c	22.66±6.41 c
SMEÇ	96.46±2.94 a	100.00±0.00 a	88.49±1.78 a	86.05±1.52 a	81.61±1.85 b	94.03±1.29 a
TRFS (1 pass)	46.31±3.28 b	62.07±4.39 c	73.38±1.59 b	80.88±1.73 ab	87.49±1.11 a	89.68±1.38 a
TRFS (2 pass)	44.29±3.33 b	68.97±2.19 c	69.78±2.82 b	82.74±1.59 ab	91.11±1.22 a	93.76±2.53 a
PYBS (1 pass)	50.30±4.64 b	67.24±2.66 c	74.82±2.45 b	80.49±1.87 ab	87.81±1.07 a	64.10±7.13 b
PYBS (2 pass)	40.75±5.81 b	62.07±2.65 c	64.03±2.52b	79.14±0.95 b	90.21±0.77 a	74.04±6.44 b
GLYI (60 ml da^{-2})	10.18±5.41 c	34.48±2.76 d	41.01±2.67 c	53.05±1.88 c	62.25±1.49 c	34.68±6.43 c
GLYI (100 ml da^{-2})	11.95±5.69 c	36.21±4.81 d	43.17±1.62 c	56.27±1.99 c	63.15±1.13 c	12.86±6.08 c

1 Pre-emergence and post-emergence applications have been evaluated within themselves.

* Data with the same letter in the columns are not statistically different (P≤0.05)

TRFS: 75% Trifloxysulfuron sodium, PYBS: 383 g l^{-1} Pyrithiobac-sodium, GLYI: 480 g l-1 Glyphosate isopropylamine salt, PYRS: 85% Pyroxasulfone, SMEÇ: 915 g l^{-1} S-metolachlor + 45 g l^{-1} Benoxacor) + hand hoe, FÇEÇ: inter-row rotary hoe + intra-row hand hoe

28 DAT; however, its suppressive impact on IPOTR declined at harvest. In another application, PYBS treatments, one or two pass, did not create any significant weed control efficacy on IPOTR.

Ginning efficiency as a yield component was unaffected not only by herbicides but also by hoeing and/or herbicide treatments. The results show that the impact of IPOTR on the cotton yields and LV were very similar. It was determined that TRFS (one pass) application increased yield 70% while TRFS (two passes) application increased cotton yield 11% more than TRFS (one pass) application. This increase caused by one more pass TRFS create a significant difference between these treatments. Another herbicide, PYBS (one pass) and (two passes) applications resulted in 25% and 27%

Table 6. Effects of the applications to control IPOTR in terms of the cotton yield (kg da^{-1}), the ginning efficiency (%) and the fibre yield (kg da^{-1})

Application	Yield (kg da^{-1})	ÇR (%)	LV (kg da^{-1})
Pre-emergence			
PYRS (10 g da^{-1})	208.34±34.76 b	40.03±0.76 a	83.63±14.78 a
PYRS (15 g da^{-1})	158.06±22.14 b	40.79±0.55 a	64.73±9.57 a
YOZK	537.00±32.64 a	40.56±0.46 a	218.22±14.17 b
YOKÖ	131.23±14.00 b	39.74±0.29 a	52.29±5.82 a
Post-emergence			
FÇEÇ	533.34±28.70 a	40.49±0.42 a	216.30±12.68 a
PYRS+GLYI	156.95±27.85 e	40.28±0.51 a	63.44±11.65 e
SMEÇ	523.03±31.56 a	40.61±0.44 a	212.24±12.56 a
TRFS (1 times)	374.47±31.50 c	40.36±0.41 a	151.75±13.80 c
TRFS (2 times)	479.84±34.17 ab	40.34±0.40 a	194.18±14.91 ab
PYBS (1 times)	404.44±36.86 bc	39.86±0.40 a	161.60±15.72 bc
PYBS (2 times)	392.75±23.88 bc	40.83±0.41 a	160.53±10.19 bc
GLYI (60 ml da^{-1})	246.95±35.47 d	40.66±0.57 a	100.47±14.80 d
GLYI (100 ml da^{-1})	156.94±19.08 e	40.83±0.54 a	64.17±7.99 e
YOZK	537.00±32.64 a	40.56±0.46 a	218.22±14.17 a
YOKS	129.17±19.72 e	40.63±0.62 a	52.86±8.44 e

* Data with the same letter in the columns are not statistically different. (P≤0,05)

* Each column has been evaluated in itself.

* Ginning efficiency: ÇR%, Fibre yield: LV: kg/da

* Pre-emergence and post-emergence applications have been evaluated within themselves.

*TRFS: 75% Trifloxysulfuron sodium, PYBS: 383 g/l Pyrithiobac-sodium, GLYI: 480 g/l Glyphosate isopropylamine salt, PYRS: 85% Pyroxasulfone, SMEÇ: 915 g/l S-metolachlor + 45 g/l Benoxacor) + hand hoe, FÇEÇ: inter-row rotary hoe + intra-row hand hoe, YOZK: weed-free control, YOKS: Full season weedy control (Ç.S.), YOKS: Full season weedy control (Ç.Ö.).

yield reduction, respectively. The difference between PYBS (one pass) and (two passes) applications was not significant statistically. Therefore, PYBS (one pass) application is enough if this herbicide would be preferred by the farmer. Glyphosate application at 60 g ai ha^{-1} increased cotton yield at 46% while at 100 g ai ha^{-1} increased cotton yield at 29% compared to the weedy control. PYRS+GLYI treatment increased cotton yield as same as glyphosate application at 60 g ai ha^{-1}. Mechanical control treatments, FCEC and SMEC, provided the highest cotton yield, and they were included in the same statistical group with the un-weedy control. Pre-emergent PYRS resulted in a slight increase on cotton yield, but this difference was not significant statistically.

Buchanan and Burns (1971) reported that *I. purpurea* species caused a 21-83% yield reduction in cotton, and Crowley and Buchanan (1978) reported that *Ipomoea* genus caused a cotton yield decrease up to 88%, but their competition did not affect the ginning efficiency. In the experiment, IPOTR caused a 76% cotton yield reduction similar to Crowley and Buchanan (1978).

In brief, cultural weed control practices have an important role in cotton production fields of Turkey. Some treatments, in this context, such as preparing a good seed-bed, crop rotation, and hoeing can control the weeds effectively (Tepe 1997). However, they are not always sufficient in weed control as they are not available during weed control time. In addition, the soil tillage treatments may spread some of the weed seeds in the cotton fields or infest other cotton fields if the soil tillage machineries are not cleaned after the infested fields. Therefore, chemical weed control options are one of the most significant components of weed control management. In cotton production, agronomic practices including irrigation should be employed at the proper time when the rainfall is below than average because water deficit may create a significant crop stress and reduce the competitive ability of the crop against to the weeds. Hand hoeing, which is made to inter-row and intra-row, is an important agronomic tool to control the weeds and tillage the soil. However, high labour costs and difficulties in finding workers prevent using this tool effectively. Although IPOTR has a potency to create a strong soil bank, there is no comprehensive study to control IPOTR with soil residual herbicides in the conventional cotton (Serim et al. 2017). Further studies should conduct to create a new and cost-effective weed control program, which consist of all components related to the weed control.

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ÖZET

Pamuk (*Gossypium hirsutum* L.) elliden fazla sanayi kolunun girdisini oluşturmakta olup, özellikle tekstil, yağ, yem ve kâğıt sanayisinde kullanılan hammaddenin de ana kaynağı durumundadır. Ayrıca, yarattığı katma değer ve istihdam olanaklarıyla da üretici ülkeler açısından büyük ekonomik öneme sahip bir üründür. Pamuk özellikle çıkış sonrası ilk dönemde yabancı ot rekabetine duyarlı bitkilerden olup, verim miktarı, yabancı otların etkisiyle azalmaktadır. Bu nedenle, pamuk ekim alanlarında yüksek ve kaliteli ürün elde etmek için yabancı ot kontrol uygulamaları uygun şekilde yapılmalıdır. Son yıllarda Akdeniz Bölgesi tarım alanlarında yaygınlığı artan *Ipomoea triloba* (IPOTR) istilacı özelliğe sahip olup, pamuk bitkisinin gelişimini engellemekte, hasat döneminde ise elle veya makine ile yapılan hasadı güçleştirmektedir. Bu çalışmanın amacı, IPOTR'ye karşı pamukta etkin bir mücadele programının oluşturulmasıdır. Tarla denemeleri 2018-2019 yılları arasında Ceyhan (Adana)'da yürütülmüştür. Bu amaçla %85 Pyroxasulfone (PYRS) %75 Trifloxysulfuron sodium (TRFS), 383 g/l Pyrithiobac-sodium (PYBS), 480 g/l Glyphosate isopropylamine tuzu (GLYI) 915 g/l S-metolachlor+ 45 g/l Benoxacor) + el çapası (SMEÇ), Frezeli ara çapa makinesi + sıra üzeri el çapası (FÇEÇ) uygulamalarının etkisi 2 yıl süresince araştırılmıştır. IPOTR'ye karşı yapılan uygulamaların etkinliği kuru madde miktarı üzerinden değerlendirildiğinde; TRFS ve PYBS'nin %90'nın üzerinde, FÇEÇ, SMEÇ, TRFS ve PYBS uygulamalarının ise %60'ın üzerinde etki gösterdiği belirlenmiştir. IPOTR'ye karşı yapılan bütün uygulamalarda lif ve ürün veriminde artış sağlandığı tespit edilmiştir. Ancak, GLYI uygulamasında pamukta fitotoksisite (%20) gözlenmiştir.

Anahtar kelimeler: pamuk, *Ipomoea triloba*, mücadele, biyolojik etkinlik

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Original article

Prevalence and molecular characterization of Tomato spotted wilt virus in pepper fields in Tokat province

Tokat ilinde biber alanlarında Tomato spotted wilt virus'ın yaygınlığı ve moleküler karakterizasyonu

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ABSTRACT

The study was conducted in Tokat Center, Niksar, Erbaa and Pazar districts where peppers were grown in the summer of 2016, and leaf samples were collected from plants suspected of the virus. During the surveys, a total of 324 plant samples were collected and the infected pepper samples were subjected to DAS-ELISA test with (Tomato spotted wilt virus) TSWV-specific antiserum, and RT-PCR was performed with virus-specific primers. In DAS-ELISA studies, 324 plants were tested and 13% of the samples were found to be TSWV infected. Samples that were positive in ELISA test were subjected to RT-PCR with nucleocapsid gene specific primers in the S segment and three samples were sent for sequence analysis. According to results, Turkey TSWV isolates Np gene region have shown 98-99% nucleotide identity with the isolates from France and South Korea and grouped with them same group.

INTRODUCTION

In Turkey, pepper cultivation is widely done in greenhouse during the winter and open fields during summer months. Tokat province is one of the important vegetable production which has a microclimate in Turkey. Peppers where cultivated in open fields is more susceptible to virus infections (Bogatzevska et al. 2007). To date more than 100 viral pathogens have been detected that infect pepper crops and 43 of them have been reported to have natural infections (Edwardson and Christie 1986). *Cucumber mosaic virus* (CMV) (Chen et al. 2011, Garcia-Arenal et al. 2000, Nakazono-Nagaoka et al. 2005, Oreshkovikj et al. 2018), *Potato virus* Y (PVY) (Fereres et al. 1993, Singh et al. 2008), *Tomato spotted wilt virus* (TSWV) (Roggero et al. 2002), *Tomato mosaic virus* (ToMV) (Gilardi et al. 2004), *Tobacco mosaic virus* (TMV) (Boukema 1982), *Potato virus* X (PXV) (Palloix et al. 1994), *Beet curly top virus* (BCTV) (Chen et al. 2011), *Pepper yellow leaf curl* virus (PYLCV) (Dombrovsky et al. 2010), *Pepper mild mottle virus* (PMMoV) (Genda et al. 2007, Guldur and Caglar 2006,), and *Pepper veinal mottle virus* (PeVeMoV) (Fajinmi 2013) are the most important and common viruses infecting pepper plants.

Since TSWV first report in Australia in 1915 (Brittlebank 1919), it has been reported in over 60 countries worldwide (Karavina and Kubba 2017). In Turkey, TSWV was firstly detected in lettuce by Tekinel et al. (1969), then were determined in tobacco plants in Çanakkale region with the rate of infection

of 80-100% (Azeri 1981). Then it was also reported on tomato, pepper, eggplants, lettuce and squash from different parts of Turkey (Arli-Sokmen et al. 2005, Azeri 1994, Guldur et al. 1995, Kamberoglu and Alan 2011, Kamberoglu et al. 2009, Turhan and Korkmaz 2006, Yardimci and Kilic Culal 2009).

The TSWV is the type member of *Orthotospovirus* genus in the family Bunyaviridae (Adams et al. 2017, Milne and Francki 1984) and transmitted by thrips (Thysanoptera, Thripidae) especially *Frankliniella occidentalis* in a persistent and circulative manner (Mandal et al. 2007, Mound 2001, Todd et al. 1995). The genome of TSWV consists of three single-stranded RNAs: the large (L) negative-sense RNA and the middle (M) and small (S) ambisense RNAs (Adkins 2000, Peiro et al. 2014). The *Sw5 gene* in tomato and *Tsw* resistance in pepper provide resistance against to the TSWV. However, in different studies, researchers have reported resistance-breaking isolates in both peppers (Deligoz et al. 2014, Gabor et al. 2012, Hobbs et al. 1994, Margaria et al. 2004, Roggero et al. 2002, Sharman and Persley 2006) and tomatoes (Aramburu and Marti 2003, Debreczeni et al. 2014, Fidan 2016, Lian et al. 2013, Lopez et al. 2011, Margaria et al. 2004, Peiro et al. 2014).

In Tokat, presence of TSWV in tomato has been reported by Sin (2015), however there is no available information in pepper plants yet. In recent years, the incidence and spread of TSWV have reached high levels at pepper-producing areas in Tokat. There are many complaints about TSWV from many farmers in Tokat province and there are very few studies on the molecular studies of the virus. This study was conducted to determine occurrence and incidence of TSWV in pepper-growing areas in Tokat and to investigate whether there are isolates that breaking resistance. For this reason, this study involves field surveys combined with DAS-ELISA and RT-PCR method to determine TSWV in pepper in Tokat.

MATERIALS AND METHODS

Survey

Field surveys were carried out to detect viral agents causing disease in the pepper production fields of Tokat province. For this purpose, surveys were carried out on pepper growing fields in the Tokat Center, Turhal, Pazar, Erbaa and Niksar districts of Tokat province. A total of 324 plant samples was collected from the leaves of pepper plants showing symptoms of viruses in the survey areas.

Serological assay

Collected plant samples were subjected to DAS-ELISA test with TSWV specific antiserum.

DAS-ELISA studies were carried out according to the method reported by Clark and Adams (1977), and taking into account the rates specified by the commercial company (BIOREBA AG) from which ELISA kits were supplied.

Molecular assay

Total RNA extraction was performed from plants that gave positive reaction as a result of DAS-ELISA test and RT-PCR studies were carried out with nucleocapsid gene (Np) specific primers in the S segment of TSWV virome. RNA isolation was done according to protocol described by Astruc et al. (1996). Complementary DNA (cDNA) synthesis was performed using extracted RNA with hexamer primers. The cDNA synthesis was carried out in a total reaction mixture of 20 µl including 2.5 µl RNA, 1.0 µl hexamer primers, 0.2 µl of 25 mM dNTPs, 0.5 µl RNase inhibitor, 2.0 µl of 10x RT buffer, 1.0 µl reverse transcriptase enzyme (Thermo Scientific, USA) and sterile ultra-pure water. The reaction mixture was incubated at 25 °C for 5 min and 42 °C for 60 min, followed by incubation at 85 °C for 5 min. cDNA was used as template for reverse transcriptase-polymerase chain reaction (RT-PCR). PCR was carried out in a 25 µl mixture containing 2.5 µl of cDNA, 2 µl of 10 mM dNTPs, 2 µl of 25 mM MgCl2, 5 µL of 5X Green GoTaq Reaction buffer and 0.5 µl of 10 µM of TSWV Np specific primers (F 5' AAC CTG CAG CTG CTT TCA AGC AAG TTC 3' and R 5' ACA ACT TTT AGG ATC CTC ATG TCT AAG GTT 3') (Maiss et al. 1991), 0.25 µl of 5 units µl-1 Taq DNA polymerase (Promega, USA), and distilled water. PCRs were performed in the thermocycler (Techne Prime Thermal Cycler) using the conditions described below. The PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 sec., annealing at 52 °C for 30 sec., and elongation at 72 °C for 2 min. The final cycle was followed by extension at 72 °C for 10 min. Then, PCR products were analyzed by on 1.2% agarose gel containing ethidium bromide and visualized with a UV transilluminator.

Phylogenetic assay

For phylogenetic analysis, after RT-PCR analysis, three PCR products were sent for sequence analysis in both directions (forward and reverse) to Sanger technology. The obtained data were cleaned from beginning and end by using Chromas computer program (Chromas 2.6.6 version) and saved as a consensus file. The obtained nucleotide sequences were analyzed by Molecular Evolutionary Genetics Analysis using the Neighbour-joining tree model with 1,000 replications (software MEGAX, Kumar et al. 2018). The Bootstrap analysis was performed with 1000 replications.

RESULTS

Survey

Within the scope of the study, surveys were carried out on pepper growing areas in the districts of Tokat Central (117), Pazar (46), Erbaa (95) and Niksar (66) of Tokat province in

July-August and a total of 324 leaf samples were collected. Virus-specific symptoms like severe mosaic, chlorosis, leaf deformation, ringspots, mottling, vein clearing were observed on leaves collected during the surveys (Figure 1).

DAS-ELISA results

As a result of DAS-ELISA test, macroscopically samples showing yellow color change and samples with a value of twice the absorbance of the negative sample in the ELISA reader and more were evaluated as positive.

TSWV presence was detected in the samples tested. Out of 324 tested plants 13% of them were detected as infected with TSWV. Considering the presence of the virus by district, the infection rates were detected as 13.7% (n=16), 6.32% (n=6), 12.1% (n=8), and 26.1% (n=12), Tokat Center, Erbaa, Niksar and Pazar, respectively in peppers.

Molecular and phylogenetic results

The presence of TSWV in pepper plants was confirmed by RT-PCR using TSWV spesific primers. The expected bands (approximately 800 bp) were observed on 1.2% agarose gel (Figure 2). The sequences analysis was obtained belong to , 129 s. gene of three TSWV isolates in this study. Three Turkish TSWV isolates were sequenced and submitted to GenBank with the accession numbers: MW751975 (B1), MW751976 (B2), MW751977 (B3). Comparison of sequences of TSWV isolates showed that Turkish TSWV isolates shared 98-99% nucleotide identity with references isolates from Genbank. The nucleotide sequences of TSWV isolates were compared with reference isolates and pylogenetic analysis were performed. Based on the results, the isolates were divided into two major groups (resistance breaking (RB) and non-breaking (NRB) isolates) Turkish TSWV isolates grouped with French and South Korean NRB isolates. These isolates were not grouped with previous reported RB isolates KM379141, KM379142 and MH367502.

DISCUSSION AND CONCLUSION

Pepper ranks second after tomato in terms of vegetable cultivation and vegetable production is an important source of income for farmers. Previously, pepper leaf samples were collected from pepper growing areas Tokat Central, Turhal, Pazar, Erbaa and Niksar districts of Tokat in July-August 2016. During the surveys, typical TSWV symptoms

Figure 1. Symptoms on infected plants collected during surveys

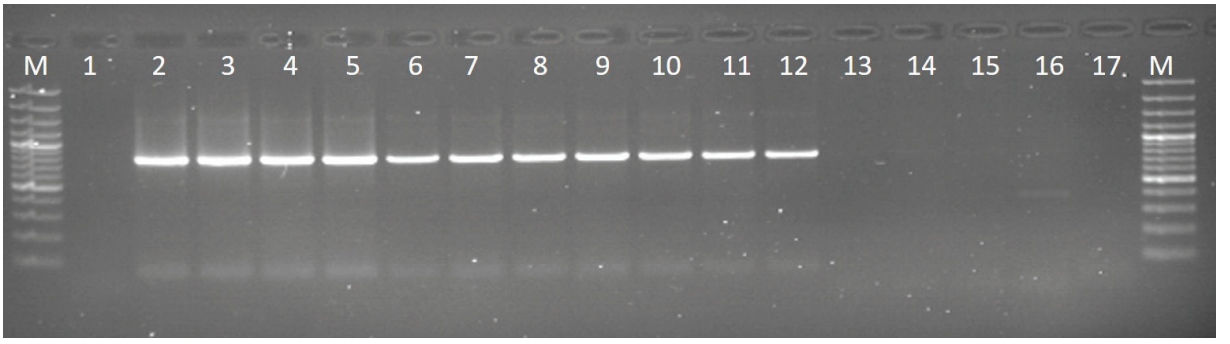

Figure 1. Agarose gel images of RT-PCR results. M: 100 bp Ladder (Fermantas), 1: Negative control, 2-12: TSWV positive samples, 13-17: TSWV negative samples

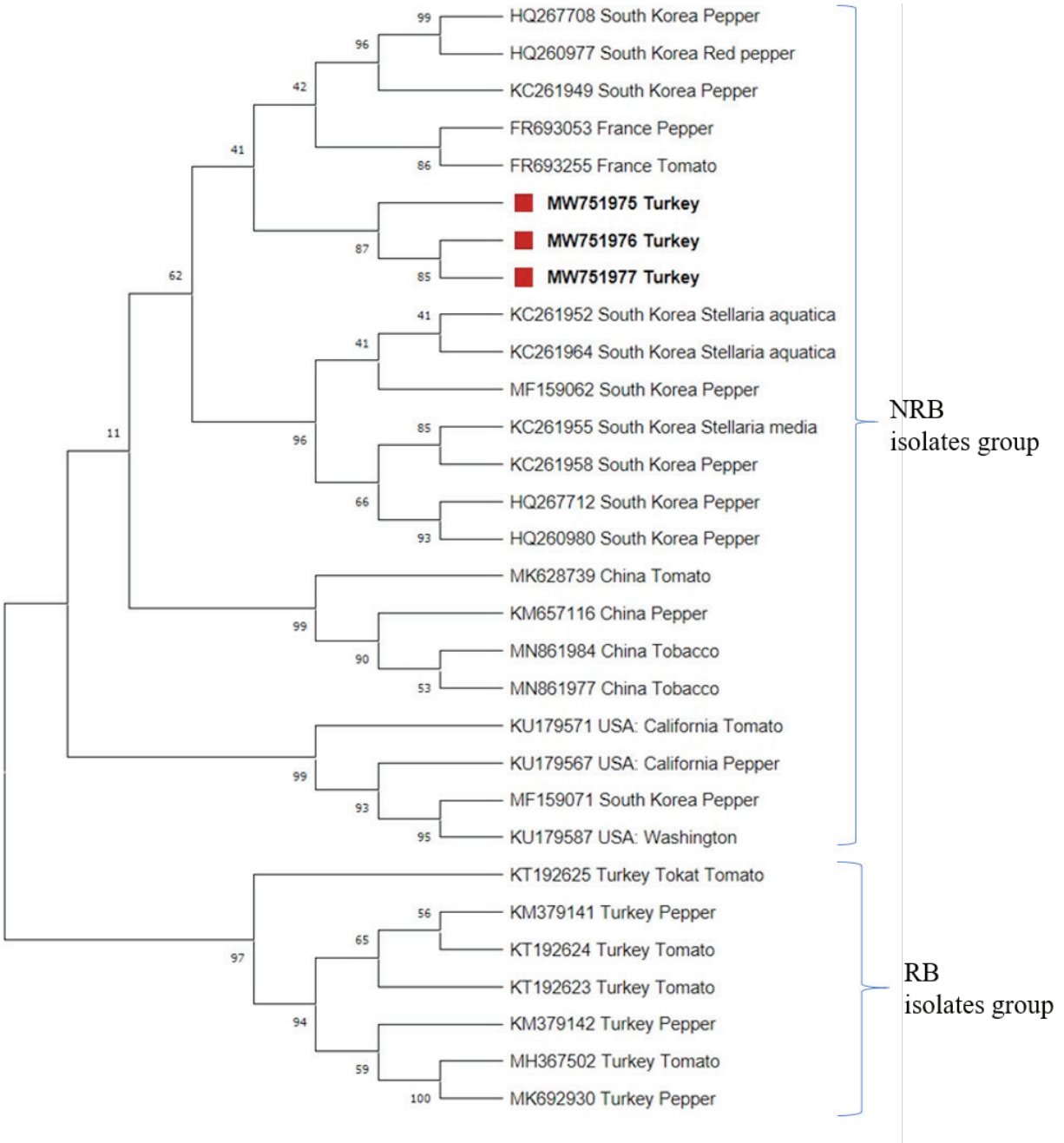

Figure 3. Pylogenetic tree of three Turkish isolates and references isolates. Sequences obtained in this study are shown in bold colour and red mark.

such deformations on the leaves of the plants, yellowing, discoloration of the veins, mosaic, excessive whitening, deformations in the fruits, and ring spot symptoms were observed surveyed area. The results of survey observations were similar to the reported studies of Deligoz et al. (2014), Buzkan et al. (2013), and Bozdogan and Kamberoglu (2015).

The presence of TSWV and other important viruses such as CMV, PVY, PMMoV, and AMV were detected by DAS-ELISA in the samples collected during the surveys. According to DAS-ELISA test results, the rates of viruses were 43%, 25%, 13%, 15.3%, 11.9%, 9.4% CMV, TSWV, AMV, PMMoV, PVY respectively (not published). Similarly, the presence of these viruses in peppers has been reported by different researches (Arli-Sokmen et al. 2005, Buzkan et al. 2013, Ozdag and Sertkaya 2017). Ozdag and Sertkaya (2017) reported that PVY, CMV, *Beet western yellows virus* (BWYV), *Potato leaf roll virus* (PLRV), *Potato X virus* (PVX), *Tomato mosaic virus* (ToMV) and TSWV were identified in pepper growing areas of Iskenderun and Samandağ districts in Hatay. TSWV also infect other vegetables. Bozdogan

and Kamberoglu (2017) reported that TSWV was detected in 526 samples (88%) from 156 tomato (81%), 316 pepper (92%), 54 lettuce (93%) plants, which are collected from greenhouses in Antalya province with DAS-ELISA tests.

TSWV was firstly observed in tomatoes in the region, and typical symptoms were also observed in peppers over time. The presence of the TSWV was serologically and molecularly determined in this study. The TSWV isolates did not group based on geographical origin, as previously reported by Zindovic et al. (2014) and Karavina and Gubba (2017), but was grouped as resistance breaking (RB) and non-breaking isolates (RB). In Turkey, RB isolates reported in Samsun and Antalya provinces in pepper plants. Molecular analysis of the TSWV nucleocapsid gene showed that Turkish TSWV isolates from this study were very similar (98-99%, 99.02%) to each other that non-breaking isolates at nucleotide and amino acid sequence levels. These isolates were showed relativity with the previously reported RB isolates KM379141, KM379142 and MK692930. In pylogenetic tree, the isolates were divided into two major groups as RB and NRB isolates. RB isolates reported by Deligoz et al. (2014) from Samsun province (KM379141, KM379142) and Fidan and Sarı (2019) (MK692930, MH367502) from Antalya province showed different clustering in the pylogenetic tree. Three Turkish TSWV isolates grouped with NRB isolates.

Pepper production constitutes an important source of income for Tokat province. In recent years, farmer have given up pepper farming due to yield losses caused by diseases and pests especially viral diseases that do not have chemical control. Due to the easy transmission of viruses [mechanically and by insects (*Frankliniella occidentalis*)] and open field cultivation, yield losses cannot be avoided. While cultural precautions are the most important method to be taken for the management of viral diseases, it is necessary to know their molecular structures well for the correct diagnosis and detection of them. By the improvements on molecular techniques, the diagnosis of viral agents can be done in a shorter time and more accurately in all production areas. In this study, the important viruses were determined both serologically and molecularly in Tokat. Previously, TSWV infections in tomato plants were reported by Sin (2015). This is the first molecular assay that had been employed in characterizing TSWV in pepper in Tokat province. Only partial nucleocapsid gene of S segment of TSWV were studied in this study. It is reported that the TSWV RB isolates related to NSm protein, which has a point mutation on the cell to cell movement gene, caused resistance breaking on TSWV resistant tomato (Fidan and Sarı 2019, Lopez et al. 2011). However, this study also showed that Np gene region of TSWV isolate can be us for disciriminate of TSWV isolate as RB and NRB. And, more isolates and further studies are needed for evolutionary analysis of TSWV isolates. This information will shed light on future studies.

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ÖZET

Çalışma kapsamında 2016 yılı yaz döneminde biber yetiştirilen Tokat ili Merkez, Niksar, Erbaa ve Pazar ilçelerine sürveyler düzenlenmiş ve virüs şüphesi gösteren biber bitkilerinden yaprak örneği toplanmıştır. Sürvey çalışmaları sonucunda toplanan 324 adet bitki yaprak örneği *Tomato spotted wilt virus* (TSWV)'e spesifik antiserum ile DAS-ELISA testine tabii tutulduktan sonra virüse spesifik primerlerle RT-PCR işlemi gerçekleştirilmiştir. Testlenen örneklerin %13'nün TSWV ile enfekteli olduğu tespit edilmiştir. ELISA testinde pozitif çıkan örnekler S segmentinde yer alan Nucleocapsid proteine (Np) spesifik primerlerle RT-PCR işlemine tabi tutulmuş ve pozitif bant elde edilen örneklerden 3'ü sekans analizine gönderilmiştir. Analiz sonuçlarına göre Türkiye TSWV izolatlarının Np gen bölgesi Fransa ve Güney Kore izolatları ile %98-99 nükleotid benzerliği göstermiştir ve filogenetik ağaçta aynı grupta kümelenmiştir.Anahtar kelimeler: biber, RT-PCR, Tomato spotted wilt virus, Tokat

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Original article

The determination of developmental time, parasitism rate at different temperatures and parasitism behavior at constant temperature of *Aenasius arizonensis* Girault 1915 (Hymenoptera: Encyrtidae)

Aenasius arizonensis Girault 1915 (Hymenoptera: Encyrtidae)'in farklı sıcaklıklarda gelişme süresi, parazitleme oranı ve sabit sıcaklıkta parazitleme davranışının belirlenmesi

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ABSTRACT

Aenasius arizonensis Girault, 1915 (Hymenoptera: Encyrtidae) is a primary parasitoid species of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Some biological characteristics of *A. arizonensis* at different temperatures were studied to evaluate its potential role against *P. solenopsis* in biological control. Six temperatures (15, 20, 25, 30, 35±2, and 25/35±2 °C) were studied to determine the most suitable temperature for parasitism. The results showed that the highest parasitism rate (79%) and the optimum developmental time from parasitism to adult emergence (16-17 days) were recorded at 25 °C. Although developmental periods were shorter at 30 and 35 °C, parasitism rate was lower at higher temperatures. The longest developmental period and lowest parasitism rate were recorded at 20 °C.The host preference of *A. arizonensis* was studied on different mealybug species and this parasitoid parasitized only *P. solenopsis*. The parasitism behavior of *A. arizonensis* based on the increasing the number of the host was carried out at 25 °C, where the best results were obtained in terms of parasitism rate during this study. The regression curve showed that *A.arizonensis* can be classified as Holling Type II. In conclusion, this study revealed that *A. arizonensis* can be an effective biological control agent to use against *P. solenopsis* and 25 °C is an optimum temperature for the mass-rearing of this parasitoid.

INTRODUCTION

Mealybugs are known as one of the most important pests in terms of agriculture. They can damage all parts of plants causing economic losses to the crops (Nagrare et al. 2009). Due to climate changes, invasive mealybug species became more important pests throughout the world (Mani and Shivaraju 2016). Generally, the *Phenacoccus* genus has been recorded in the Mediterranean region for 10-15 years (Kaydan et al. 2013, Mendel et al. 2016). This genus includes

approximately 180 species. Among them, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is one of the most dangerous invasive mealybug species, recorded in Turkey in 2012 (Kaydan et al. 2013). *P. solenopsis* has a wide-range of host plant spectrum (202 host plants from 55 families) (Garcia et al. 2016). The species has been reported on 72 host plants from 55 families in Turkey (Çalışkan and Ulusoy 2018).

Biological control of Cotton mealybug can be potential prevent the spread of this pest. There are many studies have been conducted to determine parasitoids and predators of *P. solenopsis* (Ben-Dov et al. 2012, Çalışkan et al. 2016, Çalışkan-Keçe et al. 2018, Hayat 2009). *Aenasius arizonensis* Girault (Hymenoptera: Encyrtidae) is one of the most effective solitary endoparasitoids and reported in Turkey in 2018 (Çalışkan-Keçe et al. 2018, Hayat 2009). This parasitoid is specialized in *P. solenopsis* and does not parasitize other mealybug species (Shera et al. 2017).

Aenasius arizonensis represented 95% of the parasitoids under field conditions (Solangi and Mahmood 2011). The efficacy of *A. arizonensis* has been studied in both laboratory and field conditions. Ram and Saini (2009) reported that field parasitism with this parasitoid species ranged between 37.6-72.3%. Aga et al. (2016) and Kahya et al. (2019) found that third nymphal instar and adult stage of *P. solenopsis* were preferred by *A. arizonensis*. Moreover, many studies were conducted about *A. arizonensis* and its parasitism behavior against *P. solenopsis* (Bodlah et al. 2010, Prasad et al. 2011, Spargo et al. 2013). The effect of different temperatures on *A. arizonensis* parasitism has been studied and minimum-maximum thresholds were determined as 11.5 and 11.2 °C by He et al. (2017). Joodaki et al. (2018) studied the functional response of *A. arizonensis* at different temperatures and the maximum parasitism rate was found at 25, 30, and 35 °C during their study. Moreover, effects of different temperatures on *A. bambawalei* Hayat (=*Aenasius arizonensis* Girault) (Hymenoptera: Encyrtidae) were studied by Zhang et al. (2016) and found that higher temperatures have negative effects on the reproductive capacity of *A. bambawalei*.

This study carried out to determine developmental period (from parasitism to adult emergence) and parasitism rate at 15, 20, 25, 30, 35, and 25/35 °C. In addition, parasitism behavior of *A. arizonensis* based on different hosts species and the number of the increasing host were studied within this study. The main objective is to detect the most suitable temperature and the host consumption capacity of *A. arizonensis* for mass-rearing under controlled conditions.

MATERIALS AND METHODS

The study was carried out at Nedim Uygun Biological Control Laboratory (Çukurova University Agriculture Faculty Plant Protection Department/Adana/Turkey) and Biological Control Research Institute in Adana/Turkey. Six different temperatures (15, 20, 25, 30, 35±2 °C and 25/35±2 °C) were tested in climate cabinets for finding out the most suitable temperature for parasitism. In addition, parasitism behavior of *A.arizonensis* depending on the number of the host was estimated at 25 °C, 65% ± 10 RH, and 16:8 (L:D). Moreover, parasitism behavior of *A. arizonensis* on the different host was found during this study. Stock culture of *A. arizonensis* and *P. solenopsis* were mass-reared at Biological Control Research Institute and individuals, which were used in these experiments, were obtained from the stock cultures.

Developmental time and parasitism rate of Aenasius arizonensis at different temperatures

Experiments were conducted using adults of *P. solenopsis* and *A.arizonensis*. Cotton leaves were placed in Petri dishes (9 mm) including 10 *P. solenopsis* adult females. Moreover, one female and 2 males of *A. arizonensis* were released into each Petri dish for 24 hours then removed. Experiments were done at 15, 20, 25, 30, 35±2 °C and 25/35±2 °C and 10 replicates were used for each temperature. Experiments were examined daily. Mean total developmental period (from parasitism to adult emergence) and parasitism rate at each temperature were calculated and recorded.

Parasitism behavior of Aenasius arizonensis on different hosts

Parasitism behavior of *A. arizonensis* on different hosts was studied. *Planococcus citri* Risso (Hemiptera: Pseudoccocidae) and *Phenacoccus madeirensis* Green (Hemiptera: Pseudoccocidae) were used as a host during this experiment. These mealybug species were cultured in climate rooms in Nedim Uygun Biological Control Laboratory (Cukurova University Agriculture Faculty Plant Protection Department/Adana/Turkey) and Biological Control Research Institute in Adana/Turkey. Totally, 100 mixed individuals of *P. citri* and *P. madeirensis* were placed into different plastic boxes with sprout potatoes. After that, 10 females and 20 males of *A. arizonensis* were released into these boxes for 24 hours then removed. Experiments were conducted at 25 °C, 65% ±10 RH and 16:8 (L:D) in climate cabinets and controlled daily.

Parasitism behavior of Aenasius arizonensis with the increasing number of host (Phenacoccus solenopsis)

According to results of parasitism rate results at different temperatures, 25 °C was found the most suitable temperature for parasitism and these experiments were conducted at

25 °C. *P. solenopsis* individuals adapted to cotton leaves were placed into Petri dishes where 1 female, 2 males of *A. arizonensis* were released into the Petri dishes for 24 hours then removed. Each Petri dish included 5, 10, 20, 40 and 80 individuals (the number of hosts were determined according to the geometric increase) of *P. solenopsis*. This experiment was carried out through 10 replicates at 25 °C, 65%±10 RH and 16:8 (L:D). Results were controlled and recorded daily.

Statistical analysis

Analysis were done with SPSS 23 statistic program. One-way ANOVA and Duncan multiple comparison tests ($p<0.05$) were used for the determination statistical differences between developmental time and parasitism rate of *A. arizonensis* at different temperatures. The logistic regression test was applied to detect the relationship between parasitoid and increasing the number of host. R, R2, and p values were calculated by SPSS 23 statistic program. According to Holling (1959), the type of the parasitoid was determined through the above tests.

RESULTS AND DISCUSSION

Developmental time of Aenasius arizonensis at different temperatures

The longest mean total developmental period of *Aenasius arizonensis* from parasitism to adults emergence was obtained at 20 °C (43.9±0.8 days) for female, (42.8±1.4 days) for male, while the shortest one was found at 30 °C (13.6±0.4 days) for female and (14.4±0.8 days) for male. Optimum values were found at 25 °C for both developmental periods (16.7±0.3 and 17.0±0.5 days) for females and males, respectively (Table 1). The developmental periods of *A. arizonensis* at different temperatures were estimated as a whole (from parasitism to adults emergence), as the immature stages of such endo-parasitoid are completely internal of the host (*P. solenopsis*). This parasitoid lays their eggs into the mealybugs and eggs, larval and pupal stages are developed inside host (*P. solenopsis*). According to visual observation under the stereomicroscope, mealybugs were paralyzed after parasitism in 24 hours. It was estimated that egg hatching occurred within 72 hours inside a host, and also 5-7 days later, the colour of parasitized mealybug individuals started to change (mostly brownish black colour) and powdery was vanished (Figure 1). Consequently, *P. solenopsis* are paralyzed after parasitism and parasitized mealybug individuals are enlarged and turn into brownish black in 5- 7 days. Finally, *A. arizonensis* individuals became adults (from parasitism to adults emergence) within 15-17 days at 25 °C, 65% ± 10 RH and 16:8 (L:D). Although the shorter developmental periods from parasitism to adults' emergence were detected at 30 and 35 °C, the parasitism rate was reduced from 79% to 50% above 25 °C. The changeable temperature 25/35±2 °C was not suitable for *A. arizonensis* because the developmental period increased to 18.0±0.8 days (female), and 17.1±0.7 days (male). Significant differences were found between temperatures and the developmental periods from parasitism to emergence of males and females ($p<0.05$). Ram (2016) studied developmental period of *A. arizonensis* at different temperatures (20, 25, 30, 35±2 °C) and found that the adult emergence of *A. arizonensis* from parasitisation was completed in 15.8 days for male and 17.8 days for female at 25 °C. In addition, Vijaya (2011) studied some biological characteristics of *A. bambawalei* at different temperatures and stated that the parasitoid completed its development between 20 and 35 °C and maximum fecundity was obtained at 23-33 °C. Abdin et al. (2012) reported that adult emergence from pupa occurred after 12-17 days at 28 °C, 70% RH, 18:6 h (L:D). He et al. (2017) found that 19-37 °C was the suitable temperature for the development of *A. arizonensis*.

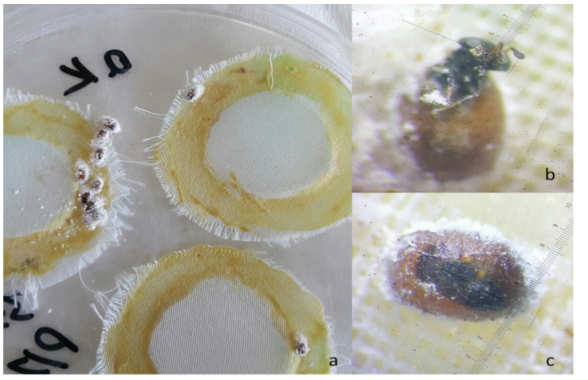

Figure 1. a) parasitized mealybug adults b) the hatching of Aenasius arizonensis, c) before hatching of Aenasius arizonensis

Table 1. Developmental period and parasitism rate of Aenasius arizonensis at different temperatures

Temperature (°C)	Parasitism rate (%mean±SE)	Developmental period of female (pupa to adult) (mean/day±SE)	Developmental period of male (pupa to adult) (mean/day±SE)
15	0	0	0
20	24.0±0.5*c	43.9±0.8c	42.8±1.4c
25	79.0±0.3a	16.7±0.3b	17.0±0.5b
30	61.0±0.5ab	13.6±0.4a	14.4±0.8a
35	54.0±0.7b	15.1±0.9a	14.3±0.9a
25/35	46.0±0.8b	18.0±0.8b	17.1±0.7b

*Each column showed that same letters are not statistically significant (Duncan test, $p<0.05$)

As shown in Table 1, the parasitism rate was determined at the 6 different temperatures. Maximum parasitism rate was obtained at 25 °C as it increased to 79.0%, while it ranged between 54.0-61.0% at 30 and 35 °C. Lower parasitism rate was also obtained at the changeable temperature (25/35±2 °C). The lowest parasitism rate was found as 24.0% at 20 °C. At 15 °C, no parasitization was determined. Significant differences were determined among temperatures in terms of parasitism rate for male and female ($p<0.05$). He et al. (2017) found that parasitism rate tendency increased from 19 to 31 °C. However, high temperatures (higher than 31 °C) had negative effects on parasitism rate of *A. arizonensis*. Moreover, Zhang et al. (2016) studied the parasitism rate of *A. bambawalei* at different temperatures, and demonstrated that when the temperature rose to 36 °C, the parasitism rate decreased to 52.0%. Thimmegowda (2017) recorded temperature's tolerance of *A. arizonensis* at 27, 32, 35 and 38 °C, and the optimum developmental duration from oviposition to pupal stage and fecundity values were obtained at 27 °C. Many researchers have also studied the effects of temperature on the parasitism rate. For example, Daane et al. (2004) found that 24.7 °C was the optimum temperature of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) for parasitism on *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae). As shown in the present study, parasitism rate affected easily by higher temperature.

Parasitism behavior of Aenasius arizonensis on different hosts

Parasitism behavior of *Aenasius arizonensis* on different host were determined during this study. According to results of this experiment, *A. arizonensis* did not lay eggs on *P. citri* and *P. madirensis*. Shera et al. (2017) used 6 different mealybug species for parasitism situation of this parasitoid and *P. solenoopsis* were found only host of *A. arizonesis*.

Parasitism behavior of Aenasius arizonensis with increasing the number of host (Phenacoccus solenopsis)

Parasitism behavior of *Aenasius arizonensis* was determined at 25 °C. The results showed that there was a strong correlation between *P. solenopsis* and *A. arizonensis* (R=0.83, R^2=0.97). Besides, the parasitism increased as the number of host until 40 individuals. This situation suggested that parasitism rate increased with host density directly proportional. However, the breaking point was 40 prey/individuals for *A. arizonensis* (Table 2 and Figure 2).

Table 2. Functional and numerical response of *Aenasius arizonensis*

Number of hosts Phenacoccus solenopsis (Mean)	Number of parasitized mealybug individuals (Mean±SE)
5	3.0±0.1
10	7.0±0.2
20	9.0±0.2
40	16.0±0.2
80	15.0±0.2

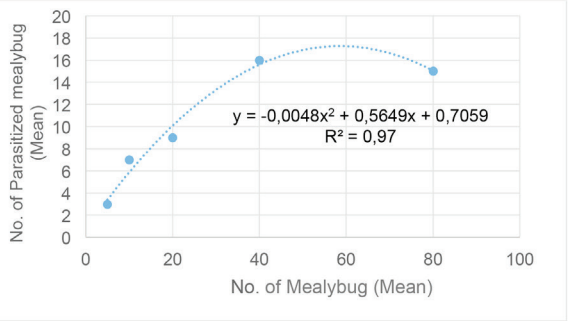

Figure 1. Quadratic regression curve of *Aenasius arizonensis*

Accordingly, *Aenasius arizonensis* can be classified as Type II model (Holling 1959).The R and R^2 values were found as 0.83 and 0.97, respectively (Figure 2). This solitary parasitoid parasitized an adult female of *P. solenopsis* with higher parasitism rates at 25 °C than 1st , 2nd and 3rd nymphal stages of this mealybug. Although Type II model seems less suitable than Type III model in terms of functional response, Type II functional response is generally fitted for parasitoid species (Fernandez-Archex and Corely 2003, Holling 1965). Joodaki et al. (2018) studied the numerical and functional response of *A. bambawalei* and classified it as Type II model Holling (1959). In addition, Chong and Oetting (2006) studied Madeira mealybug parasitoid, *Anagyrus* sp. nov.nr. *Sinope* and classified the parasitoid as type II functional response when opposed to the different numbers of *P. madeirensis*.

Phenacoccus solenopsis, Cotton mealybug, has been cause economically important damages on crops in Turkey since 2012. *Aenasius arizonensis* is one of the most effective parasitoids of this pest and has been detected in Turkey since 2018. This study aimed to find out some biological characteristic of this parasitoid in laboratory conditions and to enhance the mass-rearing opportunities of *A. arizonensis*. According to results of this study, *A. arizonensis* can be mass-produced at 25 °C successfully and can be used as an effective biological control agent properly against *P. solenopsis* in biological control studies.

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ÖZET

Aenasius arizonensis Girault, 1915 (Hymenoptera: Encyrtidae) *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)'in önemli parazitoitlerinden biri olarak bilinmektedir. Yapılan bu çalışmada *A. arizonensis*'in farklı sıcaklıklarda bazı biyolojik özellikleri belirlenerek *P. solenopsis*'e karşı biyolojik mücadelede kullanım potansiyeli değerlendirilmiştir. Bu parazitoit için en uygun parazitleme sıcaklığını belirlemek için 6 farklı sıcaklık (15, 20, 25, 30, 35±2 ve 25/35±2 °C) çalışılmıştır. Elde edilen bulgulara göre parazitleme ile ergin çıkışı arasında en iyi gelişme süresi (16-17 gün) ve en yüksek parazitleme oranı (79%) 25 °C'de elde edilmiştir. 30 ve 35 °C'de daha kısa gelişme süresi bulunmasına rağmen parazitleme oranının yüksek sıcaklıklarda düştüğü tespit edilmiştir. En uzun gelişme süresi ve en düşük parazitleme oranı 20 °C'de bulunmuştur. *A. arizonensis*'in parazitlemede farklı konukçuları tercih çalışmaları yapılmış ve sadece *P. solenopsis*'i parazitlediği belirlenmiştir. En iyi parazitlemenin elde edildiği 25 °C'de A. arizonensis'in artan konukçu yoğunluğuna bağlı parazitleme davranışı çalışılmıştır. Elde edilen regresyon eğrisine göre bu parazitoit Holling Tip II olarak sınıflandırılmıştır. Sonuç olarak, *A. arizonensis*, *P. solenopsis* ile mücadelede etkili bir parazitoit olarak kullanılabileceği ve bu parazitoitin kitle üretiminin 25 °C'de başarılı bir şekilde gerçekleştirilebileceği sonucuna ulaşılmıştır.

Anahtar kelimeler: *Aenasius arizonensis*, farklı sıcaklık, parazitleme davranışı, *Phenacoccus solenopsis*

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Original article

Determination of grapevine stem fungal diseases in Malatya province

Malatya ilinde asma gövde fungal hastalıklarının belirlenmesi

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ABSTRACT

Grapevine (*Vitis vinifera* L.) is one of the most prevalent and long-standing cultivated crops in the world due to its non-selective climate and soil demands, types of usage and having a wide range of varieties. Turkey has a rich grapevine gene pool because of its favorable climate zone. Viticulture, which has an important role in agriculture, faces many problems in the process of production to marketing. Fungal diseases take an important place by limiting the production wherever grapevine is cultivated. It is aimed to determine the stem fungal diseases that cause drying in viticulture areas of Malatya province with this research. Arapgir, Yesilyurt, Battalgazi and Darende districts of Malatya province, where grapevine is intensely cultivated, were surveyed in two different vegetation periods, and samples were taken from symptomatic plants. Identification of these isolates were performed based on morphological and molecular examinations. As a result of this study, *Botryosphaeria* spp., *Phaeomoniella chlamydospora*, *Fomitiporia mediterranea*, *Cytospora viticola*, *Neoscytalidium dimidiatum*, *Dothiorella* spp., *Lasiodiplodia* spp. were identified as causal agents for the diseases observed in Malatya province.

INTRODUCTION

Turkey is a country located on the most ideal climate zone for vineyard cultivation in the world. It has a viticulture culture dating back to the past and is gene source of the vine (*Vitis vinifera* L.). Anatolia is a very rich gene center not only with its vineyards and grape production but also with its culture vine and wild vine species.

When the evaluation possibilities of the grape, which is also called the "Fruit of Heaven" among the people, are examined, it is one of the rare plants with many alternatives in the world and in our country. The main evaluation methods of grape can be listed as follows; besides its fresh consumption, it is used in various ways such as vinegar, wine, molasses and dried nuts. Pickled vine leaves are also a product whose economic value has increased in recent years (Cabaroğlu 2015).

The most important grape-producing countries are Spain, France, Italy, USA, Turkey and China. According to 2019 data from FAOStat, Turkey ranks 5th (400.997 ha) in terms of vineyard area and 6th (4.208.908 tons) in terms of production in the world (FAOStat 2019). Of the 4.208.908 tons of grapes produced, approximately 38.3% are table grapes, 11.6% seedless table grapes, 26.1% seedless raisins,

INTRODUCTION

14% raisin with seed and 10% wine grape. Turkey exports 40-45% of seedless raisins, nearly half of the world production, hereby been a significant income for the country's economy.

After the Aegean, Mediterranean, Central Anatolia and Western Anatolia regions, Eastern Anatolia ranks fifth in terms of grape production and vineyard area as it has 4.4% of the vineyards. When evaluated provinces in the region, Malatya is the second after Elazig in terms of both cultivation area and production (Table 1) (Anonymous 2020).

Table 1. The production areas (da) and production amounts (tons) of the provinces located in Eastern Anatolia

Province	Production area (da)	Production amount (tons)
Elazığ	108.568	94.463
Malatya	38.920	22.812
Bingöl	1.600	640
Tunceli	2.781	2.869
Van	410	232
Muş	3.848	2.114
Bitlis	5.315	3.139
Hakkari	6.485	5.131
Eastern Anatolian	167.927	131.400
Turkey	4.009.970	4.208.908

Grape is an important agricultural product whose production faces many problems from growing to storage and from processing to marketing. The most important of these problems are fungal diseases, which are increasing in importance day by day in all countries where vineyards are grown, reaching economic dimensions and limiting grape production due to the damage, they cause (Göktaş 2008).

Among the fungal diseases that cause significant losses, powdery mildew (*Uncinula necator* Burr.), grape downy mildew (*Plasmopara viticola* Berl. & De Toni), anthracnose (*Elsinoe ampelina* Shear.) and gray mold (*Botrytis cinerea* Pers.) are of great importance due to direct damage to the product. Apart from these, the dead arm (*Phomopsis viticola* Sacc.), which causes significant damage to the shoots, the esca (kav) disease (Anonymous 2008) determined to be caused by a group of factors (*Stereum hirsutum*, *Phellinus igniarius*, *Phaeoacremonium* spp., *Phaemoniella chlamydospora*), petri disease caused by *Phaeoacremonium oleophilum*, *Phaemoniella chlamydospora* (Crous and Gams 2000, Crous et al. 1996), retrograde death disease by *Eutypa lata* which causes branch and trunk drying (Rappaz 1984), cancer in the ligaments and black dead arm disease (*Botryosphaeria* spp.) are the diseases frequently encountered in the cultivation areas (Urbez-Torres 2011). *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Macrophomina phaseolina*, *Verticillium dahliae*, *Armillaria mellea*, *Rhizoctonia solani* and *Rosellinia necatrix* cause root rot and plant death (Gubler et al. 2004, Petit and Gubler 2005, van Coller et al. 2005).

Although the vineyard area is spread over a large area in Malatya province, the average yield per unit area is low. Diseases and pests are among the main reasons for the low yield. Fungal diseases observed in different stages of production cause significant product losses. Considering the share of agriculture in the increasing population and total income, it is important for our country to ensure high efficiency and quality in agriculture and to fight fungal diseases in this respect (Kiracı et al. 2015).

There are many wood tissue diseases that cause economic losses in vineyard cultivation. The observation of intense drying in the vineyards of Malatya province in recent years and the fact that research conducted upon the complaints from the producers did not reveal the cause of the fungal stem diseases in the vineyard areas. Thus, this study aimed to determine the fungal agents in the wood tissue in the vineyards of Malatya province.

MATERIALS AND METHODS

Survey

The villages of Arapgir, Yesilyurt, Battalgazi and Darende districts of Malatya province where viniculture is concentrated were determined as survey areas to determine fungal stem diseases (Figure 1). Sampling was carried out in two different periods considering the vegetation period in the vineyard areas selected to represent the region. The first sampling was performed in June-July when fungal ligament

Figure 1. The surveyed area in the province of Malatya

diseases were widespread, and the second sampling was performed in September and October just before harvesting in the region. The guided sampling method reported by Bora and Karaca (1970) was used.

Isolation and morphologic identification

Transverse sections were taken from the part/tissue of plants including trunk and 2-year-old shoots of vine stocks. About 3-4 mm pieces containing both diseased and healthy tissue were cut out with the help of sterile scalpel from parts where symptoms observed, kept in 1% sodium hypochlorite for 2-3 minutes, and dried between sterile blotting papers (Whatman Filter Papers 110 mm, Germany) for 15 minutes. The excised sections were placed in Petri dishes (90 mm diam.) containing potato dextrose agar (PDA; Merck, Germany) and Malt Extract Agar (MEA; Merck, Germany) supplemented with streptomycin (Sigma Aldrich, USA) (100 mg/l) and incubated at 22 ± 2 °C and 12 hours light photoperiod in an incubator (ME-352 PE, Panasonic, Japan). After about 7-10 days of incubation, hyphal tips of each fungal colonies were transferred into another plate containing fresh PDA to obtain pure cultures.

Morphological features of fungal agents (colony color, micelle growth, pycnidia formation, and conidial shape) was evaluated in PDA medium. The conidia of the fungal isolates were measured and photographed in a trinocular research microscope (Nikon Eclipse E200, Japan). Diagnostics were done according to Fischer (2006), Fischer et al. (2016), Larignon and Dubos (1997), Lawrence et al. (2017), Urbez-Torres et al. (2010).

Pathogenicity studies

Pathogenicity was conducted with three inoculated plants per selected isolates on a 2-years-old potting Köhnü grape variety in a completely randomized experimental design. Plants were left in the glasshouse (23–26 °C) for 3 weeks. Dense suspensions of conidia (~1.107 conidia/ml) were prepared by flooding the surface of two-week-old PDA cultures of selected isolates with sterile water and gently rubbing the surface with a sterile rod. Grapevine stems were injured with a sterile scalpel, 200 µl of the spore suspension were poured directly on a wound. Control plants were inoculated with sterile distilled water. Three weeks after inoculation, vascular lesions were recorded, by removing the bark from the stem and measuring the necrotic lesions downwards and upwards at the inoculation site. The presence of necrosis was used as an indicator of pathogenicity.

Molecular studies

Pathogenicity tests of morphologically pre-diagnosed isolates were conducted and isolation of total genomic DNA from mycelia (50 mg) taken from single spore cultures grown on PDA medium of the isolate isolated from disease symptoms was carried out using DNeasy Blood and Tissue kit (Qiagen, Germany) and the protocols suggested by the manufacturer.

PCR studies were performed using ITS4 and ITS5 primer pair for the internal transcribed spacers (ITS) rDNA site (White et al. 1990), Bt2a and Bt2b primer pair for the β-tubulin (TUB2) gene region (Glass and Donaldson 1995), LROR and

Table 2. Primers and PCR conditions used in molecular studies

Locus	Primers	PCR condition	
ITS	ITS4 (5'-TCC TCC GCT TAT GC-3') ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3')	95 °C 3 min 35 cycle 95 °C 1 min 54 °C 45 sec 72 °C 45 sec 72 °C 10 min	White et al. (1990)
β-Tubulin	BT2a (5'GGT AAC CAA ATC GGT GCT GCT TTC-3') BT2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3')	95 °C 3 min 35 cycle 95 °C 1 min 58 °C 45 sec 72 °C 45 sec 72 °C 10 min	Glass and Donaldson (1995)
LSU	LROR (5'-ACC CGC TGA ACT TAA GC-3') LR5 (5'-TCC TGA GGG AAA CTT CG-3')	95 °C 3 min 35 cycle 95 °C 1 min 58 °C 45 sec 72 °C 45 sec 72 °C 10 min	Woudenberg et al. (2009)
TEF1- α	EF1-986R 5'-TA CTT GAA GGA ACC CTT ACC-3' EF1-728F 5'-CA TCG AGA AGT TCG AGA AGG-3'	95 °C 3 min 35 cycle 95 °C 1 min 58 °C 45 sec 72 °C 45 sec 72 °C 10 min	Carbone and Kohn (1999)

LR5 primer pair for large subunit (LSU) (Woudenberg et al. 2009), EF1-728F and EF1-986R primer pair for Translation elongation factor 1-alpha (TEF1-α) (Carbone and Kohn 1999) (Table 2). In DNA amplification for each reaction, 5 µl of 10 × enzyme buffer, 0.2 mM of dNTPs, 2 mM MgCl2, 0.5 µl of each primer, 1-unit Taq DNA polymerase and 2 µl of DNA were added to a total of 50 µl of PCR mixture. PCR amplification conditions were 3 min initial denaturation at 95 °C, 1 min at 95 °C, 54 °C for ITS and 58 °C for TUB2, LSU and TEF 1-α, 72 °C for 45 seconds (35 cycles) and 10 min at 72 °C as a final step. Depending on the quality of the DNA bands obtained, PCR products were selected for sequencing. DNA nucleotide sequences of PCR products were matched with existing fungal species listed in the NCBI (National Center for Biotechnology Information) library using the BLASTn algorithm (Boratyn et al. 2013). Access numbers of some isolates diagnosed according to these results were deposited in the library of NCBI GenBank. The dendrogram showing the genetic kinship between the isolates was created using the maximum likelihood method and the obtained phylogenetic tree was confirmed with 1,000 repetitions (Bootstrap, p-distance, pairwise deletion) (Kumar et al. 2016).

RESULTS

Survey studies were carried out in the districts of Arapgir, Yesilyurt, Darende and Battalgazi (Table 3) and their villages, which are the most important vineyard areas of Malatya, in June-July and September-October, and the disease symptoms (Figure 2) were detected in 2018, considering the phenology of the vine.

A total of 133 vineyard samples were collected; 47 from Arapgir, 30 from Yesilyurt, 27 from Battalgazi, and 29 from Darende. The collected samples were transferred to the laboratory and the fungi were isolated and purified. Morphological diagnoses of the purified isolates were made by considering criteria such as colony shape, culture color, micelle structure, and asexual reproduction structures (Figure 3). The number of isolates obtained from each district as a result of morphological diagnosis are presented in Table 4.

Figure 2. Symptoms of apoplexy in grapevine (a), mottling on grains (b), white rot in vascular tissue (c), color change in the form of the letter 'V' (d) and bending and discoloration in the plant stem (e)

Pathogenicity test of isolates diagnosed at genus or species level as a result of diagnoses made by considering morphological criteria were tested against 2-years-old Köhnü grape variety. Brown-black discoloration of the peeled wood in the cut part and the bark tissue of the plant were evaluated as pathogen at the end of the 21 days (Figure 4). At the same time, the plant was cut horizontally from the part where the cut was, and a brown-black color change was observed. As a result of pathogenicity, *Botryosphaeria* spp., *Fomitiporia mediterranea*, *Phaeomoniella chlamidospora*, *Cytospora viticola*, *Neoscytalidium dimidiatum*, *Dothiorella*

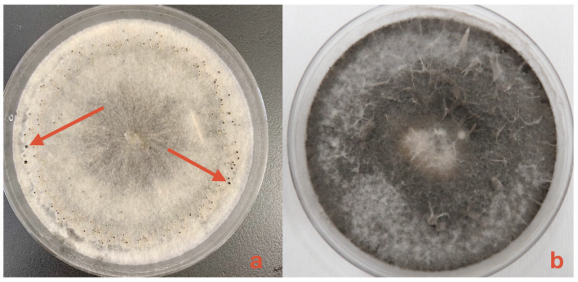

Figure 3. *Cytospora viticola* colony growth and pycnidia on PDA (a) and colonies of *Neoscytalidium dimidiatum* on PDA (b)

Table 3. The number of samples taken in from each district in Malatya province

Province	District	Number of samples		Total
		1st Period	2nd Period	
Malatya	Arapgir	25	22	47
	Yesilyurt	18	12	30
	Battalgazi	15	12	27
	Darende	14	15	29
Total	72	61	133	

Table 4. Number of isolates obtained from each district

Species	Number of isolates				
	Arapgir	Yeşilyurt	Battalgazi	Darende	Total
Botryosphaeria spp.	33	10	13	7	63
Fomitiporia mediterranea	18	7	5	5	35
Phaeomoniella chlamidospora	12	3	3	3	21
Cytospora viticola	4	2	1	1	8
Dothiorella spp.	3	2	2	7	14
Neoscytalidium dimidiatum	6	4	3	3	16
Phaeoacremonium spp.	7	4	3	2	16
Lasiodiplodia spp.	2	1	-	-	3
Alternaria spp.	12	4	2	2	20
Fusarium spp.	8	4	3	2	17
Epicoccum nigrum	5	3	1	1	10
Cladosporium spp.	7	5	3	2	17
Aspergillus spp.	5	7	5	4	21
Torula herbarum	3	3	1	2	9
Curvularia spp.	8	4	5	2	19
Beauveria bassiana	6	4	2	2	1
Total	139	67	52	45	290

spp., *Phaeoacremonium* spp. and *Lasiodiplodia* spp. were noted to cause necrosis in the stem of Köhnü grape variety; however, *Alternaria* spp., *Fusarium* spp., *Epicoccum nigrum*, *Cladosporium* spp., *Aspergillus* spp., *Torula herbarum*, *Curvularia* spp. and *Beauveria bassiana* did not cause any symptoms. Pathogens were re-isolated from symptomatic tissues of inoculated plants to fulfill Koch postulates and stored in oblique agar and sterile filter papers.

The sequences of randomly selected fungal isolates (*Phaeomoniella chlamydospora*, *Dothiorella viticola*, *Botryosphaeria dothidea*, *Fomitiporia mediterranea*, *Lasiodiplodia viticola* and *Phaeoacremonium viticola*) amplified with ITS primers were deposited in GenBank with the accession nos. MW692120, MW692121, MW692122, MW692123, MW692124 and MW692125, respectively.

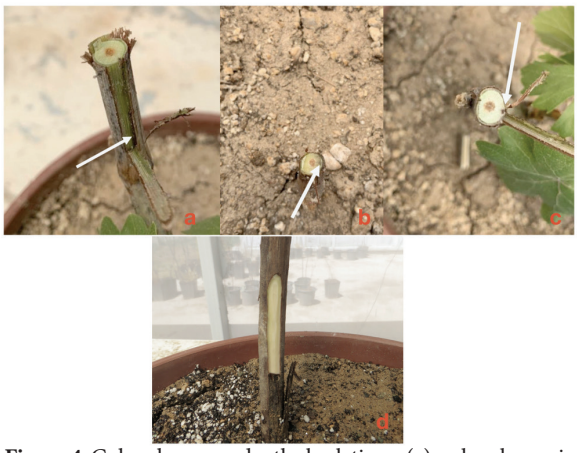

Figure 4. Color change under the bark tissue (a), color change in vascular tissue (b, c) and control (d)

Among the isolates identified as causing stem diseases in vineyards using morphological diagnosis and pathogenicity test, a detailed molecular diagnosis of *Neoscytalidium dimidiatum* and *Cytospora viticola* isolates which were detected for the first time in Turkey, was done using ITS, BTU, LSU and Tef 1- α primers for PCR analysis (Figure

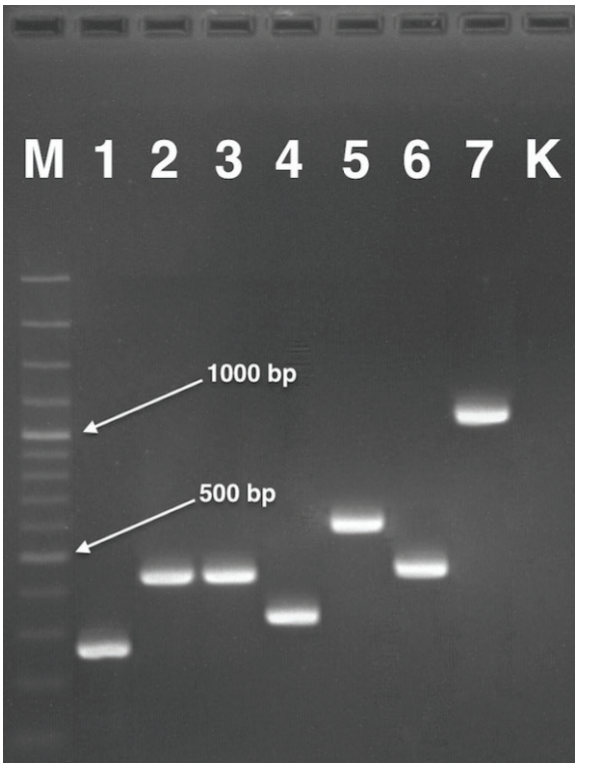

Figure 5. Agarose gel with DNA marker (M, Termoscientific, USA), TEF 1-α (1), ITS (2), β-Tubulin (3) of *Cytospora viticola* isolate and TEF 1-α (4), ITS (5), b-Tubulin (6) LSU primers (7) of *Neoscytalidium dimidiatum* and water control (K)

5) and similarity ratio was assessed by molecular analysis of sequences obtained with species existing in the GenBank database by using the BLAST analysis program and Mega 7 software on NCBI's web page.

Cytospora viticola isolate formed bands of different sizes depending on the primer used. In PCR processes using TEF 1-α primers 200-300 bp bands, in PCR processes using ITS primers a 400-450 bp and in PCR processes using β-Tubulin primers bands between 400-450 bp were obtained. Accession numbers (MK706295, MK715441 and MK715442) of the *C. viticola* isolate were obtained from NCBI, and the species verification was made by comparing the ITS, BTU, TEF1-alpha sequences of the isolate with the sequences of the same species.

Neoscytalidium dimidiatum isolate formed bands of different sizes depending on the primer used. In PCR processes using TEF 1-α primers, band formation between 250-350 bp occurred, band formation between 550-600 bp in PCR processes using ITS primers, band formation between 450-500 bp in PCR processes using b-Tubulin primers band formation between 1100-1200 bp occurred in PCR processes using LSU primers. Accession numbers (MK816354, MK816355, MK813853, and MK813852) of *N. dimidiatum* isolate were obtained from NCBI, and species verification was made by comparing the ITS, LSU, BTU, TEF1-alpha sequences of the isolate with the sequences of the same species.

DISCUSSION AND CONCLUSION

Within the scope of the study, surveys were carried out taking into account vine phenology in the villages of Arapgir, Yesilyurt, Darende and Battalgazi districts of Malatya in June-July and September-October when fungal disease symptoms began to be observed in 2018. A total of 133 plant samples and a total of 2.400 decares were surveyed and samples were taken from the main stem and shoots of plants showing signs of diseases. Of the diseased plant samples collected, 290 fungus isolates were isolated and 176 of these were determined as pathogens.

Moreno-Sanz et al. (2013), carried out a study in Northern Spain after considering that the low yield in the vineyards in the last 10 years was related to fungal stem diseases. Non-pathogenic fungi detected (55%) were *Cladosporium* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Gliocladium* spp., *Epicoccum* spp., *Aspergillus* spp., *Ulocladium* spp., *Phialophora* spp., *Sporothrix* spp., *Nigrosporra* spp. and pathogenic fungi detected (31%) were *Cylindrocarpon* spp., *Botryosphaeria* spp., *Phaeoacremonium* spp. In our study, sampling was done on plants showing similar symptoms such as reverse death, wilt, dead arms and color change in the leaves in the vineyards. Among the samples collected 60.6% were identified as pathogenic and 39.4% were non-pathogenic. When other studies were examined in terms of pathogen and saprophyte factors, it was seen that the distribution of pathogen and saprophyte fungal agents that cause drying in the vineyards was almost the same.

Results of surveys and diagnostic studies we conducted in the study showed that the most common pathogen detected at a rate 21.7% in Malatya vineyards was *Botryosphaeria* spp. Siebert (2001) stated that *Botryosphaeria* spp. fungus has become the most common in vineyard areas in the world in recent years, has a wide host range and is widespread in worldwide. It has been reported that this disease causes high amounts of economic losses in viticulture.

Lawrence et al. (2017) reported that because *Cytospora* canker has some similar general symptoms as esca, it is considered a part of connective body diseases. In our study, eight *Cytospora viticola* isolates were detected. The symptoms seen in diseased plants are not caused by a single pathogen but by the pathogen complex. For this reason, it is not possible to diagnose the agent by just observing the symptoms.

Similar to our work, as a result of isolations made from plants showing symptoms such as wilting and necrosis in leaves, drying and shrinkage in fruits, and in some cases collapse (apoplexy) of the whole vine in the middle of the growing season in vineyards areas in California in 2012, *Rolshausen* et al. (2013) detected the presence of *Neoscytalidium dimidiatum* based on morphological characters. For the first time Akgül (2019) reported two different isolates, *Lasiodiplodia exigua* and *Neoscytalidium novaehollandiae*, after fungal isolation from symptoms of xylem necrosis and wedge-shaped dark brown spots in the wood tissue in the study in the bond areas. In our study, 16 isolates of *N. dimidiatum* were determined to cause trunk diseases in Malatya vineyards. *Neoscytalidium* agent has been reported to cause disease in different hosts in our country in recent years (Dervis et al. 2019, Dervis et al. 2020, Kurt et al. 2019, Oksal and Özer 2021, Oksal et al. 2020a, 2020b, Ören et al. 2020)

Fungal diseases of the stem are thought to enter from the wound formed in the plant during pruning and training (Eskalen and Gubler 2001). It has been stated that the greatest factor in the spread of stem fungal pathogens in vineyards is pruning tools (Mugnai 1999).

The fight against fungal diseases that cause yield and quality losses in the vineyards in the world and in our country should be done on time and following the conditions. One of the ways to increase yield and quality during production

of cultivated plants is to protect the plant from diseases and pests. The agents that we obtained from vineyards in Malatya, many of which are wound and vulnerability pathogens, enter the plants during cultural processes or through natural openings and continue their lives in the plant body for a period of 2-3 years without any symptoms. When the plants reach the harvest period, symptoms occur depending on climatic conditions. It has been determined that none of the fungal agents we have identified in the vineyard areas of Malatya province are found in a single plant, but generally in a complex. This situation makes control difficult and in some cases impossible. The reason for this is that the periods and forms of transmission of each pathogen are different and that a single cultural measure or a single chemical is not sufficient to combat these diseases.

Producers beginning to abandon cultural measures, the lack of quarantine and hygiene measures, and climate change have caused major problems in the vineyards, especially in recent years. In control of fungal stem diseases, many of which are wound pathogens, it is of great importance to remove or destroy pruning residues from the field, avoid pruning on rainy and cool days, and apply a protective fungicide within 24 hours after pruning.

This study aimed to determine the fungal pathogens in the vineyard areas that have an important place in plant production and to perform morphological and molecular diagnoses. *Botryosphaeria* spp., *Phaeomoniella chlamydospora*, *Fomitiporia mediterranea*, *Cytospora viticola*, *Neoscytalidium dimidiatum*, *Dothiorella* spp. and *Lasiodiplodia* spp. were found to be the most common fungal agents in the vineyards of Malatya province at the end of the study.

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ÖZET

İklim ve toprak istekleri yönünden çok seçici olmayışı, değerlendirme şekilleri ve çeşit açısından zengin olması nedeniyle asma (*Vitis vinifera* L.) dünyada en yaygın ve en eski kültür bitkilerinden biridir. Bağcılık için dünyanın en elverişli üretim alanlarından biri olan ülkemiz zengin asma gen potansiyeline sahiptir. Bitkisel üretimde önemli bir yere sahip olan bağcılık, günümüzde üretimden pazarlamaya kadar geçen süreç içerisinde bir çok sorunla karşı karşıyadır. Bu sorunlar içerisinde üretimi sınırlandıran fungal hastalıklar önemli yer tutmaktadır. Çalışmada Malatya ili bağ alanlarında kurumalara sebep olan gövde fungal etmenlerinin saptanması amaçlanmıştır. Bu amaçla Malatya ilinde bağcılığın yoğun olduğu Arapgir, Yeşilyurt, Battalgazi ve Darende ilçelerinde vejatasyon peryodu dikkate alınarak iki farklı dönemde örnekleme yapılmış ve hastalık belirtisi görülen bitkilerden numuneler alınmıştır. Hastalıklı bitki numunelerinden 290 adet fungal izolat elde edilmiştir. Fungal izolatların tanısı morfolojik özelliklerine ve moleküler yöntemlere göre yapılmıştır. Tanılama çalışmaları sonucunda *Botryosphaeria* spp., *Phaeomoniella chlamydospora*, *Fomitiporia mediterranea*, *Cytospora viticola*, *Neoscytalidium dimidiatum*, *Dothiorella* spp., *Lasiodiplodia* spp. hastalık etmenleri saptanmıştır.

Anahtar kelimeler: *Vitis vinifera*, fungal hastalıklar, Botryosphaeriaceae, Malatya

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