THE EFFECTIVENESS OF ARBUSCULAR MYCORRHIZAL FUNGI AND SALICYLIC ACID AGAINST *VERTICILLIUM DAHLIAE* INFECTING PEPPER (*CAPSICUM ANNUUM* L.)

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Abstract. This study was carried out to determine the effects of arbuscular mycorrhizal fungi (AMF), and salicylic acid (SA) on plant growth parameters and severity of wilt disease caused by *Verticillium dahliae* Kleb. in pepper. Effects of two different AMFs (Commercial AMF and *Glomus intraradices*) and two different SA doses (0.5 and 1 mM) were compared with untreated control plants after ten weeks. Both AMF species caused 62.08% and 69.07% root colonization, and the mycorrhizal dependency ratio changed from 9.08% to 59.37% in pepper plants. Commercial AMF caused a significant increase in morphological growth parameters, root colonization, mycorrhizal dependency rates and suppression of the disease severity. SA (1 mM) resulted in improved morphological growth parameters and suppression of the severity of the disease. Besides, SA and AMF applications were found to suppress the severity of Verticillium wilt disease by 21.8% and 56% in pepper plants, respectively. Thus, the applications of SA and AMF had positive contributions to the pepper plant's morphological development.
Keywords: *wilt disease, Glomus spp., plant growth regulator, disease severity, plant morphology*

Introduction

Pepper (*Capsicum annuum* L.) is an essential vegetable that accounts for 9.8% of all cultivated land and 11.1% of all vegetable production (Wang et al., 2018). Fungal diseases, however, cause 14% annual yield loss in the global vegetable production (Duran and Özkaya, 2016). Verticillium wilt, caused by the fungal pathogen *Verticillium dahliae* Kleb., is the most economically important of all the diseases that affect pepper plants (Vasileva and Todorova, 2020). The fungus can survive as microsclerotia in the soil and plant debris. Microsclerotia germinate and infect the roots of sensitive pepper seedlings (Caroll et al., 2018). *V. dahliae* colonizes the host xylem vessels, where it might block nutrient and water movements upwards (Novo et al., 2017). As a result, the pathogen causes wilting symptoms in the host plants (Carroll et al., 2018).

There have not yet, been any effective chemicals that sufficiently control the diseases (Bilgili and Güldür, 2018). Researchers have been focused on the biological management of this disease by using environmentally friendly alternatives to chemicals (Adnan et al., 2019). Enhanced nutrition, which improves host defenses, or direct restriction of pathogen development and activity, is primarily associated with biocontrol of soil-borne diseases (Rajkumar et al., 2008).

As biological control agents, arbuscular mycorrhizal fungi (AMF) infect the roots of most terrestrial plants and improve resource absorption in return for photosynthate

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(Liang et al., 2015). Different AMFs can enhance plant root tolerance or resistance at various levels under different conditions (Demir and Akköprü, 2007). Among these AMFs, *Glomus intraradices* is one of the best performing species causing improved plant development and prevented soil-borne pathogens (Aguilera-Gomez et al., 1999; Akköprü and Demir, 2005; Zheang et al., 2005; Şavur, 2015).

Amendment with certain abiotic factors (inducers) stimulates disease resistance by indirectly stimulating indigenous populations of microorganism beneficial to plant growth and antagonistic to plant pathogens (Rajkumar et al., 2008). Salicylic acid (SA), a primary regulator of plant growth, development, interactions with other species, and reactions to environmental stress, is among the inducers (Hayat et al., 2010).

The objectives of the present study were to find out the effects of AMF and SA on plant growth parameters of pepper plants infected with *V. dahliae*.

Materials and methods

Materials

A local pepper (Sera Demre 8) variety susceptible to *Verticillium dahliae* (Vd) (Coşkun et al., 2019) was used as study materials. Commercial AMF (Endo Roots Soluble - Bioglobal A.Ş.) composed of different species (*Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasillianum, Glomus etunicatum, Gigaspora margarita*) and *Glomus intraradices* (obtained from Department of Plant Protection, Faculty of Agriculture, Yüzüncü Yıl University, Turkey) which are known to have a high relative mycorrhizal dependency were employed. In the light of the findings obtained previously on SA treatments [Salicylic acid ($C_7H_6O_3$) 100%, İzmir Kimya, Turkey], the most appropriate SA doses (0.5 mM and 1 mM) were used, which would not cause adverse effects on plant growth. Vd, a highly virulent isolate obtained from pepper, was used in this study. The experiment was carried out in Turkey during 2019-2020.

Methods

Pathogen inoculation and assessment of disease severity

Pepper seeds were surface sterilized with 2% sodium hypochlorite. Seeds were sown in plastic viols 6 cm deep and 5 cm in diameter using a 1:1 perlite and peat growth mixture. AMF inocula [2.5 g (25 spores g^{-1})] and SA (0.5 mM and 1 mM) were applied to the soil concurrently with planting.

The seedlings were placed in a growth chamber with 12 hours of fluorescent illumination at 22 ± 2°C and relative humidity of 60 - 70%. Seedlings were irrigated with distilled water and fertilized three times, with 5 mL of diluted nutrient solution (Solution A: $Ca(NO_3)_2$, KNO_3; Solution B: K_2SO_4; Solution C: KH_2PO_4; Solution D: K_2HPO_4; Solution E: $C_6H_8O_7$, $C_4H_6O_5$; Oligo-elements; Fe (Sequestrene 138), $MnSO_4$, $CuSO_4$, $ZnSO_4$, $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$) per seedling.

Vd isolate was subcultured on PDA (potato dextrose agar) medium in 9 mm diameter petri dishes for ten days at 24°C in a 12-hour dark-light cycle. Seedlings were transplanted to plastic pots (16 x 18 cm, 2-2.5 lt volume) with equal quantities of peat and perlite after six weeks. Roots of pepper seedlings were dipped in $1x10^6$ conidia/ml Vd spor suspension and let to grow for four weeks in a growth chamber. Control plants were dipped in tap water instead of Vd spor suspension.

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Four weeks after pathogen inoculation, disease symptoms were examined and evaluated (*Equation 1*). The severity of the disease was determined based on the degree of wilted leaves using a 0-5 scale (0= Healthy; 1= Less than 25% wilt in leaves; 2= 25% - 50% wilt (30% leaf loss); 3= 50% - 75% wilt (60% leaf loss); 4= 75% - 100% wilt (90% leaf loss) (Hwang et al., 1992) and vascular health 0-3 scale (0= The plants are healthy, no discoloration in the stem cross-section; 1= The plants are slightly diseased, small brown stains in the stem cross-section, 1-33% of the vascular bundles are browned; 2= The plants are moderately diseased, there are many black spots on the stem cross-section, 34-67% of the vascular bundles are browned; 3= The plants are heavily diseased, stem cross-sections are completely covered with black spots, and the plants are dried, 68-100% of the vascular bundles are browned) (Erwin et al., 1976). The following formula (*Equation 1*) was used to calculate the degree of disease severity on both scales:

$$\text{Disease Severity} = \sum \frac{(n*v)}{N*V} * 100 \qquad \text{(Eq.1)}$$

where n is the degree of disease severity on a scale, v is the number of plants in a category, N is the maximum degree of disease severity, and V is the total number of plants screened.

AMF root colonization and mycorrhizal dependency assessment

Plant roots were fixed and stained (Phillips and Hayman, 1970) and AMF root colonization was assessed under a stereoscope microscope (Leica/DFC295, Leica Microsystems Inc., Wetzlar, Germany) (4 × 10 and 10 × 10) using the grid-line intersect method according to Giovanetti and Mosse (1980). Mycorrhizal dependency assessment was determined using the method described by Declerck et al. (1995).

Plant growth parameters

A digital scale was used to determine the total fresh weight of the plants. Plants were dried at 70°C for 48 hours until they reached a consistent weight, and the dry weight was measured. The number of leaves, shoot length (cm), shoot diameter (cm), and root length (cm) of the plants were also recorded.

Statistical analysis

The study was conducted with a randomized block design with ten replications. The data were interpreted with SPSS program (v. 22.0, IBM Corp., Armonk, NY, USA). The data were analyzed using a one-way ANOVA. Duncan's multiple comparison test was used to differentiate the treatment means.

Results and discussion

Effects of two different AMF and Vd application on the plant morphology

Effects of two different AMF and Vd alone and in combination to plant growth parameters had been shown in *Table 1* and *Fig. 1*. Both AMFs caused a significant increase in plant growth parameters as compared to the untreated control plants. All plant growth parameters were declined in VD inoculated plants.

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Table 1. *Growth parameters of pepper plants treated with AMF and Vd*

Treatments	LN *	SD (cm)	RL (cm)	SL (cm)	RFW (g)	RDW (g)	SFW (g)	SDW (g)
Control	18.67 ±1.05 bc	4.17 ±0.10 c	35.48 ±0.98 ab	37.30 ±1.14 b	3.61 ±0.22 b	0.31 ±0.02 b	9.06 ±0.61 b	1.09 ±0.08 b
Vd	15.83 ±0.67 c	3.68 ±0.10 d	32.06 ±0.83 c	33.38 ±0.71 cd	3.04 ±0.19 b	0.29 ±0.01 b	5.66 ±0.35 c	0.95 ±0.05 b
AMF (Gi)	18.96 ±1.07 bc	4.66 ±0.16 b	34.31 ±0.85 bc	38.61 ±1.28 b	3.47 ±0.26 b	0.32 ±0.01 b	9.48 ±0.75 b	1.21 ±0.10 b
AMF (Gi) + Vd	16.13 ±1.05 c	3.73 ±0.11 d	33.06 ±1.07 bc	31.25 ±1.22 d	2.53 ±0.27 c	0.17 ±0.01 c	5.03 ±0.43 c	0.60 ±0.07 b
AMF (T)	26.20 ±1.45 a	5.24 ±0.11 a	37.73 ±1.12 a	42.35 ±0.59 a	5.11 ±0.29 a	0.44 ±0.03 a	14.25 ±0.80 a	1.80 ±0.11 a
AMF (T) + Vd	20.06 ±1.44 b	4.31 ±0.13 bc	34.13 ±0.94 bc	35.43 ±1.14 bc	3.68 ±0.35 b	0.28 ±0.03 b	8.22 ±0.61 b	1.11 ±0.11 b
df	5	5	5	5	5	5	5	5
F	10.64	22.99	4.10	14.03	9.93	11.98	28.45	18.39
Sig.	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000

Vd: *Verticillium dahliae*, AMF (T): Commercial AMF, AMF (Gi): AMF (*Glomus intraradices*).
* LN: leaf number; SD: shoot diameter; RL: root length; SL: Shoot length; RFW: root fresh weight; RDW: root dry weight; SFW: shoot fresh weight; SDW: shoot dry weight.
The same letters in the same column indicate the insignificant differences (P < 0.05) according to the Duncan's test findings

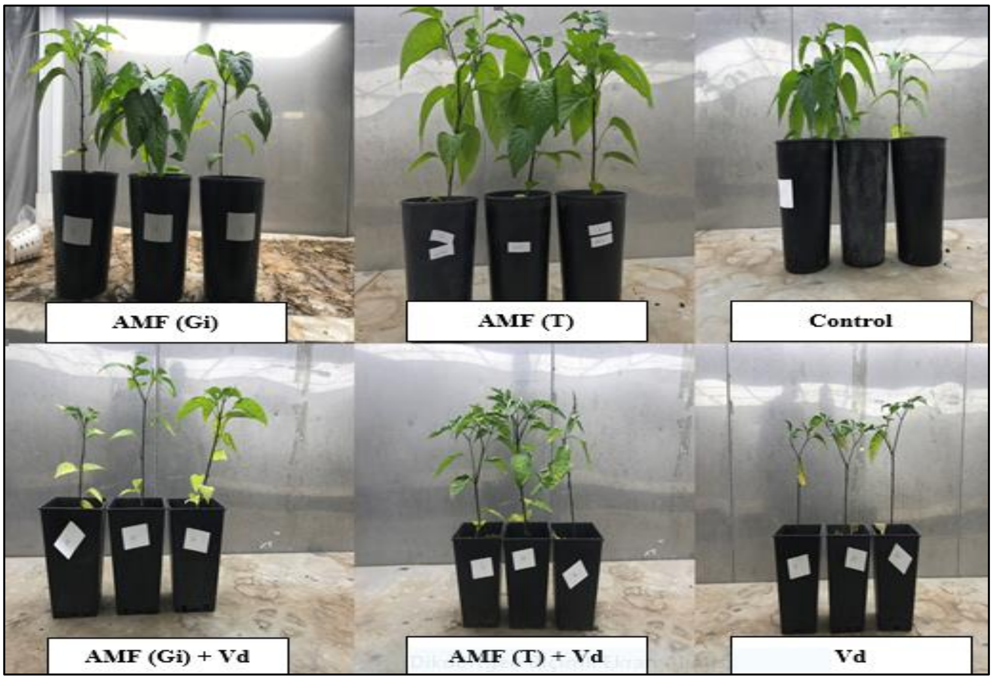

Figure 1. *The appearance of pepper plants treated with different AMFs and V. dahliae*

AMF inoculation improved the growth parameters in the plants inoculated with Vd; these parameters generally remained higher than those of the control plants (*Table 1*, *Fig. 1*). AMF (T) also significantly improved the number of leaves (26.20), shoot

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diameter (5.24 cm), root length (37.73 cm), shoot length (42.35 cm), root fresh weight (5.11 g), root dry weight (0.44 g), shoot fresh weight (14.25 g) and shoot dry weight (1.80 g). Given the vast range of reactions from different plant cultivars to AMFs, the best cultivar-AMF pairings must be discovered to maximize the benefits of symbiosis (Sensoy et al., 2007). Although AM fungi positively encourage many cultivated plants against both biotic and abiotic stress conditions, recent studies have shown that the positive effect on plants may differ between varieties depending on genetic variation (Demir et al., 2010). It was reported that AMF promotes plant growth and increases plant tolerance to biotic stress factors such as Fusarium and Verticillium, and increases photosynthetic water use efficiency and yield. (Kaya et al., 2009; Karipçin and Şatır, 2016). Şavur (2015) reported that AMF species have positive contributions to the morphological development of the tomato plant. Bilgili and Güldür (2018) stated that three different Glomus species alone and in combination contributed to the morphological development, especially they caused a significant increase shoot fresh weight in pepper plants inoculated with *Fusarium oxysporum*. Güneş et al. (2019) inferred that Commercial AMF performed better than other AMF species in terms of plant development when the interactions of commercial AMF, *G. intraradices*, *Gigaspora margarita* with radish, cauliflower, spinach and nettle were examined. As a result, the effect of mycorrhizal inoculation on the growth parameters can be linked to the plant's improved nutritional condition due to AMF.

Effects of different doses of salicylic acid (SA) on plant morphology

The effects of Vd alone and in combination with two different doses of SA, and single doses of 0.5 Mm SA and 1 Mm SA on plant growth parameters in pepper plants have been shown in *Table 2* and *Fig. 2*.

Table 2. *Growth parameters of pepper plants treated with SA and Vd*

Treatments	LN *	SD (cm)	RL (cm)	SL (cm)	RFW (g)	RDW (g)	SFW (g)	SDW (g)
Control	18.67 ± 1.05 b	4.17 ± 0.10 b	35.48 ±0.98 a	37.30 ±1.14 a	3.61 ±0.22 bc	0.31 ±0.02 ab	9.06 ±0.61 b	1.09 ±0.08 b
Vd	15.83 ± 0.67 b	3.68 ± 0.10 c	32.06 ±0.83 b	33.38 ±0.71 b	3.04 ±0.19 cd	0.29 ±0.01 ab	5.66 ±0.35 d	0.95 ±0.05 b
SA (0.5 mM)	17.40 ± 0.92 b	4.32 ± 0.08 b	34.80 ±0.85 a	33.76 ±0.86 b	4.53 ±0.27 a	0.34 ±0.02 a	8.08 ±0.53 bc	1.08 ±0.07 b
SA (0.5 mM) + Vd	17.06 ± 0.70 b	3.68 ±0.10 c	35.53 ±1.02 a	32.83 ±0.87 b	2.81 ±0.19 d	0.25 ±0.01 b	6.73 ±0.36 cd	1.00 ±0.05 b
SA (1 mM)	28.80 ± 1.00 a	5.39 ±0.09 a	35.01 ±0.90 a	35.16 ±0.65 ab	4.85 ±0.22 a	0.35 ±0.01 a	14.02 ±0.45 a	1.61 ±0.04 a
SA (1 mM) + Vd	18.47 ± 1.18 b	4.32 ±0.14 b	35.59 ±1.24 a	35.04 ±0.99 ab	3.81 ±0.26 b	0.33 ±0.02 a	7.35 ±0.51 c	1.10 ±0.08 b
df	5	5	5	5	5	5	5	5
F	28.12	37.90	2.02	3.48	12.67	3.25	38.90	12.75
Sig.	0.000	0.000	0.049	0.005	0.000	0.008	0.000	0.000

Vd: *Verticillium dahliae*, SA (0.5mM): Salicylic acid (0.5mM), SA (1 mM): Salicylic acid (1 mM).
* LN: leaf number; SD: shoot diameter; RL: root length; SL: shoot length; RFW: root fresh weight; RDW: root dry weight; SFW: shoot fresh weight; SDW: shoot dry weight.
The same letters in the same column indicate the insignificant differences ($P < 0.05$) according to the Duncan's test findings

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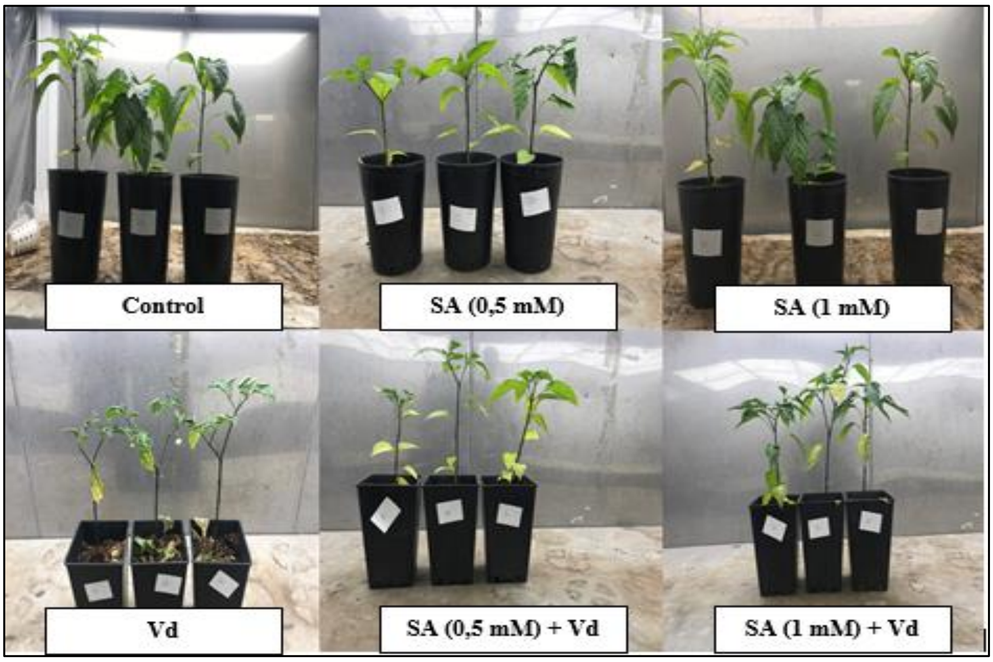

Figure 2. *The appearance of pepper plants treated with different doses of SA and Vd*

Vd caused a significant decline in shoot diameter, root length, shoot length, root fresh weight and shoot fresh weight, although it did not affect leaf number, root dry weight, and shoot dry weight. Vd combination with 0.5 Mm and 1 mM doses of SA resulted in decrease in all plant growth parameters. SA (1 mM) treatment improved in leaf number, shoot diameter, root fresh weight, root dry weight, shoot fresh weight and shoot dry weight except for root length and shoot length. SA (0.5 mM) treatment stimulated root fresh weight while it did not cause any increase in the other plant growth parameters (*Table 2*). It is shown that SA increases germination, flowering, rooting, yield and accelerates photosynthesis in plants in a number of different studies (Romanujam et al., 1998; Hayat et al., 2007; Dadaşoğlu and Ekinci, 2013; Algül et al., 2016). Elwan and El-Hamahmy (2009) examined the effect of foliar application of salicylic acid (SA 10^{-6} M and 10^{-4} M) on fruit yield and quality in pepper under salt stress conditions. They have reported that the application of SA 10^{-6} M increased the average fruit weight, fruit number and fruit yield. It is also reported that SA has a positive effect on increasing the plant height of the Kandıra pepper variety (Akpınar, 2011). Dura et al. (2016) investigated the effect of SA application against root-knot nematodes in 12 pepper cultivars of Yalova Chorba by applying different doses (3 mM/plant - 6 mM/plant -9 mM/plant) from leaves and soil. They determined that pepper plants with 9 mM SA application promoted plant growth parameters such as leaf number, plant height, root fresh and dry weight. Özdüven (2016) stated that SA applications increased yield, fruit number, leaf area, fruit firmness, total chlorophyll amount, protein content, single fruit weight and leaf K content. The study conducted by Ekbiç and Koşar (2020) studied the effects of SA doses (0, 0.5, 1 and 2 mM) on vine rootstocks and they concluded that the shoot growth was superior to the control and the degree of damage to the plants was lower with the application of 1 mM dose of SA for 1103 P rootstock. The studies conducted by other researchers earlier have been supported the current study. It has been proposed that SA's growth-promoting effects

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are linked to hormonal modifications or improvements in photosynthesis, transpiration, and stomatal conductance (Rivas-San Vicente and Plasencia, 2011).

Effects of AMF and SA application on the wilt disease caused by Vd in pepper

Effects of two different AMF and two different doses of SA on the disease development in pepper are shown in *Table 3*.

Table 3. *Effects of AMF, SA and Vd application on the wilt disease parameters (0-5 and 0-3 scale) in pepper*

Treatments	0-5 scale		0-3 scale	
	Wilting (%)*	**Suppression rate (%)**	**Vascular discoloration (%)***	**Suppression rate (%)**
Vd (Control)	48.6 ±3,67 a*	-	30 ±3,69 a*	-
AMF (T) + Vd	22.6 ±3,36 c	53	13 ±3,42 b	56
AMF (Gi) + Vd	38 ±3,36 b	21.8	16 ±4,15 b	46.6
SA (0.5mM) + Vd	27.3 ±3,38 c	43.8	16 ±4,15 b	46.6
SA (1 mM) + Vd	31.4 ±3,79 bc	35.3	15 ±3,08 b	50
df	4	-	4	-
F	9.16	-	3.27	-
Sig.	0.000	-	0.013	-

Vd: *Verticillium dahliae*, AMF (T): Commercial AMF, AMF (Gi): AMF (*Glomus intraradices*), SA (0.5mM): Salicylic acid (0.5mM), SA (1 mM): Salicylic acid (1 mM).
*P < 0.05 (significant)

Vd inoculated control plants expressed the highest disease severity values for the wilted leaf index and vascular browning (48.6% and 30%, respectively). AMF (T) + Vd, AMF (Gi) + Vd, SA (0.5mM) + Vd and SA (1 mM) + Vd applications resulted in the suppression of the disease development, which was 53%, 21.8%, 43.8%, and 35.3% respectively; as well, the values of vascular health and suppression rate (56%, 46.6%, 46.6%, and 50%, respectively) should be noted. The best suppression of disease was observed in AMF (T) + Vd (53% - 56%) application according to 0-5 scale and 0-3 scales (*Table 3*).

AMF benefits plants by increasing the uptake of phosphorus and other nutrients from the soil and increasing disease tolerance in the host plant (Merina Prem Kumari and Jeberlin Prabina, 2019). Singh et al. (2010) reported that the applications of *Glomus hoi + Fusarium oxysporum, Glomus fasciculatum + Fusarium oxysporum* and *Rhizobium leguminosorum + Fusarium oxysporum* significantly reduced the severity of the disease compared to the control applications. Aljawasim et al. (2020) found that mycorrhizal plants reduced the disease severity by 46% (*Glomus clarum + Rhizoctonia solani*) and 41% (*Glomus mosseae + Rhizoctonia solani).* Ranjbar Sistani et al. (2020) examined the yield components of AMF application against *Didymella pinodes* to two different varieties of sensitive and tolerant pea plants grown in pots. They concluded that AMF

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application reduced the disease severity in both varieties. In our study, AMF (G) and AMF (T) applications significantly suppressed the severity of the disease caused by Vd in pepper plants. The above studies were in line with the results of the present study presented here.

Salicylic acid, as well as serving as a growth regulator, acts as a signal in the plant's defense mechanism against pests and diseases, promoting plant's resistance positively (Raskin, 1992; Arıcı and Yardımcı, 2001). Various studies have shown that SA increases the resistance to pathogens in different plants and decreases the disease severity. In this regard, Şahbaz and Akgül (2016) found that the application of SA at a dose of 50 g/100 L water three times, both *Fusarium oxysporum* f.sp. *vasinfectum* and *V. dahliae* have been the most successful application in reducing disease severity in cotton plants. Tutar and Erkılıç (2016) stated that the application of 500 and 1000-ppm concentrations of SA and BABA (DL-β-aminone-butyric acid) in the eggplant plant inhibited *V. dahliae* by 27.8% and 33.3%, respectively. Erkılıç et al. (2018) stated that three soil applications (SA, BABA and ASM (Acibenzolor S-Methyl)) in olive prevented the suppression of the *V. dahliae* in the vascular discoloration up to 90%. ASM also was seen as the most effective application, it was determined that different concentrations of SA prevented the development of disease at a rate of 52% to 76%.

Effects of different AMFs and Vd application on root colonization and mycorrhizal dependency

Effects of two different AMF and Vd application on root colonization and mycorrhizal dependency in pepper are shown in *Table 4*.

Table 4. *Effects of different AMFs and Vd application on root colonization and mycorrhizal dependency*

Treatments	Root colonization (%)	Mycorrhizal dependency (%)
AMF (Gi)	62.08 ±4.84 ab	9.08
AMF (Gi) + Vd	54.79 ±3.16 b	-
AMF (T)	69.07 ±2.19 a*	59.37
AMF (T) + Vd	54.95 ±4.87 b	-
df	3	-
F	2.98	-
Sig.	0.043	-

Vd: *Verticillium dahliae,* AMF (T): Commercial AMF, AMF (Gi): AMF (*Glomus intraradices*)
*P < 0.05 (significant)

The rates of colonization in pepper plants with different AMFs and Vd application varied between 54.95% and 69.07%. On the other hand, mycorrhizal dependence rates vary between AMF (Gi) 9.08% and AMF (T) 59.37%, but mycorrhizal dependence did not occur in applications with Vd. AMF root colonization and mycorrhizal dependency in the plant root were adversely affected by treatments that included the Vd pathogen (*Table 4*).

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AMF is under the influence of biotic and abiotic conditions; positively or negatively, it influences the colonization rates in the root zone (Akköprü and Demir, 2005). Çetinkaya and Dura (2009) determined that the fungal colonization rate was between 26% - 30% with Endo-Roots applications in corn plant under field conditions. Yıldız (2010) stated that the root colonization rate of *Glomus sp.* was recorded as 61% in pepper, 71% in cucumber, and 72% in tomato. Aslanpay (2011) reported that AMF colonization rates of pepper varieties varied between 3.05% - 38.66%, and mycorrhizal dependence rates varied between 2.52% and 20.64%. Different pathogens can reduce AMF colonization on a variety of hosts. Aysan (2008) investigated the effects of *G. mosseae, G. fasciculatum* and *Sclerotinia sclerotiorum* applications on root colonization in bean plants. It was determined that the lowest colonization rate was in *G. mosseae* + *Sclerotinia sclerotiorum* and *G. fasciculatum* + *Sclerotinia sclerotiorum* treatments. Demir et al. (2015) stated that different AMF applications, including Vd pathogen applications, had a negative effect on root colonization. Our study showed similar results with the above studies.

Conclusion

The use of beneficial biological agents or growth regulators in the crop production should be among the important objectives in ensuring sustainable, safe and healthy production and their contribution to plant development and yield. This study aimed to determine the effects of AMF and SA on plant growth parameters and disease severity against wilt disease caused by *V. dahliae* in pepper. It was determined that SA and AMF applications generally had positive contributions to the morphological development of pepper plants and disease severity. Commercial AMF resulted in the best in morphological growth parameters, root colonization, mycorrhizal dependency rates and suppressing severity of the disease. SA (1 mM) resulted in improved morphological growth parameters and suppression of the severity of the disease. After considering the beneficial effects of these agents on the plant's growth, their practical effect cannot be ignored. Besides, the obtained encouraging results against *V. dahliae* could help plant protection efforts. We believe that the results of our study can contribute to more detailed research in this direction.

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