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

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ABSTRACT

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

1. Introduction

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

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

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

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

2. Materials and Methods

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

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

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

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

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

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

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

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

3. Results

3.1. Effects of biological control agents on wilt disease severity

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

3.2. Evaluation of plant growth parameters

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Discussion and Conclusion

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

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

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

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

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

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

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

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

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

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

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