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FUNGICIDE AND ARBUSCULAR MYCORRHIZA FUNGI APPLICATIONS IN TOMATO

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Abstract: Tomato is one of the important food crops of the world. It has rich essential nutrients features. However, tomato plants are sensitive to certain diseases and pests. This situation causes intense and unconscious pesticide use to avoid crop losses. It is known that mycorrhiza provide many advantages to plant. In this study, the effects of different doses of fungicide applications on some physiological parameters were examined in mycorrhiza applied and non-applied mycorrhiza tomato plants. A pesticide was applied at different doses which were, namely, recommend (R), half of recommend (R/2), and two-fold recommend (R×2). The content of proline, chlorophyll and carotenoid analysis were conducted in the plant samples. Proline values were found low in mycorrhizal than non-mycorrhizal plants in all pesticide doses ($P<0.05$). However, mycorrhiza*dose interaction was statistically significant ($P<0.01$). It was found statistically significant in chlorophyll-a ($P<0.01$), chlorophyll-b ($P<0.05$), total chlorophyll ($P<0.01$), and carotenoid ($P<0.05$) values in terms of mycorrhiza*dose interaction. We suggest that studied arbuscular mycorrhiza may increase at highly the resistance tolerance to fungicide. AMF is suitable option for low chemical input and nature conservation based sustainable agriculture.

Keywords: Tomato, Proline, Chlorophyll, Carotenoid, Mycorrhiza, Fungicide

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1. Introduction

Recent rapid population growth causes nutrition problem that is one of the biggest problems faced by humanity. To resolve this problem, studies focusing on the maximum product uptake in agriculture have been increasing. Almost all the cultivated plants in the world have been threatened by diseases, pests, and weeds (Tiryaki et al., 2010). These cause crop losses around the world (Capinera, 2005; Paini et. al., 2016). The cultural, mechanical, physical, biotechnical, and biological methods are used to solve the problems in crop production, today. Pesticide use is the most preferred chemical control method. In recent years, the pesticides are widely used in the crops are grown both in the greenhouse and in the field (Hazra and Purkait, 2019). However, while these applications cause an increase in the quality and efficiency of the products, agroecosystems and environmental protection is very important to sustain ecological balance. Unconscious and excessive applications of plant protection products in plants cause problems including phytotoxicity, residues in agricultural products with the problem in domestic and foreign markets. The need for studies to minimize the damage of these chemicals is a common problem to the world (Delen et al., 2010; Appah et al., 2020). Mycorrhizal fungi are used in the fight against plant diseases and damages in our country and world (Öztekin and Ece, 2014).

One of the most important mutualistic relationships that increase productivity and nutrient cycles between plants and microorganisms is mycorrhiza (Tilak et al., 2005). Arbuscular mycorrhizal fungi (AMF) form a mutually symbiotic with the roots of land plants and play an important role in regulating community and ecosystem functioning (Wu et al., 2021).

Mycorrhizal fungi are important in agriculture and forestry as bidirectional nutrient transfer between host and fungal endophyte (i.e., drain of host carbon and uptake of soil mineral drive many nutrient cycling processes in soil) (Xavier and Germida, 1999; Alaux et al., 2021). In community with decreased mycorrhizal fungi, weed species that are characterized by non-mycorrhizal relationships increase and the nutrient cycle can be broken.

There are separate studies showing positive or negative effects of plant-mycorrhizal relationships and fungicide use on the plant (Cordier et al., 1996; Al-Karaki, 2000; Hajiboland et al., 2010; Song et al., 2010; Abdel Latef and Chaoxing, 2011; Çekiç and Yılmaz, 2011; Öztekin and Ece, 2014; Almaca, 2014; Abdulhadi et al., 2017). Hage-Ahmed (2018) reported that there is a need to investigate the combined effects of AMF and pesticide applications on plants. Özbucak and Kabul (2019 and 2020) was determined mycorrhizal tomato had positive effect on fruit and growth parameters despite pesticide application.

In this study, we examined the tomato plants that are widely in human nutrition in Türkiye and in the world (Qasid et al., 2022), with frequently applied fungicides. Fungicide application may change proline, chlorophyll, and carotenoid metabolic activity. For this purpose, we compared the effects of different doses pesticide use on proline, chlorophyll, and carotenoid parameters in mycorrhizal and non-mycorrhizal tomato seedlings.

It is necessary to develop alternative strategies to reduce the negative effects of chemical inputs such as pesticides which are widely used in agriculture, on nature and living things. We believe that the encouraging results obtained from this study can contribute to the sustainability of the agricultural production and the promotion of the commercial use of these products.

2. Materials and Methods

2.1. Materials

In this study was used commercially purchased tomato seeds (*Solanum lycopersicum* L.) and mycorrhiza preparation (*Glomus fasciculatum, Glomus intraradices, Glomus mosseae* mixture). Antracol WP 70 (% 70 Propineb) fungicide used as pesticide.

2.2. Experimental Design

100 seeds of tomato were surface sterilized with 70% ethyl alcohol for 1 min. and 10% NaClO for 5 min., followed by rinsing 10 times with sterile-distilled water. Afterwards seeds were hold in sterile-distilled water for 20 min. and then were filtered through filter paper (Battke et al. 2003). Sterilized tomato seeds were germinated to 100 plastic cups with peat: perlite: soil mixture (2:1:1). The characteristics of the soil sandy-clay-loam (60%, 25%, 15%). 50 of plastic cups were planted with 2gr mycorrhiza and then were placed in a climate cabinet with a 14: 10 h light: dark cycle with 23.5°C-60% temperatures and humidity, respectively. and watered every other day to 60% water holding capacity (Figure 1).

Figure 1. The germination of tomato seeds in climate cabinet.

After one-month, plastic cups were removed from climate cabinet. They remained in the laboratory for 15 days. Healthy seedlings were transplanted to 20 L. pots in a greenhouse. Two seedlings were planted in each of the 24 pots. 12 pots were planted inoculum with mycorrhiza seedlings. The other 12 pots were planted with non-mycorrhiza seedlings (Figure 2, 3). Fungicide (Antracol WP 70- Propineb) application was made by spray in case of four doses, namely: (a) control, (b) recommended dose (R=0.75 g/250 ml water) (c) half of recommended dose (R/2=0.375g/250 ml water), (d) two-fold recommended dose (R*2=1.5 g/250 ml water). Pesticide sprayed to plants with days by 5 times after 24 days seedling planting. In the first flowering period was applied natural manure to plants. It was used peat, perlite, soil, fertilizer (2:1.1:1/2) for each pot. Approximately 7 days after the fifth spraying, leaf samples were taken from the different pots in each experimental group for proline, chlorophyll and carotenoid analyses.

2.3. Plant Analyses

A week after the fifth spraying treatment, leaf samples were taken from experimental groups for proline, chlorophyll, carotenoid analyses. Proline content was determined Bates et al. (1973). The leaf samples (1 g) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid solution. Supernatants were transferred to test tubes and mixed with equal volumes of glacial acetic acid and ninhydrin reagent. Test tubes were incubated in the oven for 1 h at 100 °C. The test tubes were then placed in an ice bath and thus the reaction was stopped. The samples were rigorously mixed by using a vortex after 4 mL of toluene was added to the tubes. After 50 min, toluene phases were obtained. The absorbance was measured at 520 nm on a UV-visible spectrophotometer. Photosynthetic pigment (chlorophyll a, chlorophyll b and carotenoid) contents were detected according to the method of Kaçar (1984). Fresh leaf samples (1 g) were extracted overnight with 80%

acetone at 0–4 °C. The extracts were centrifuged at 3,000 × g for 5 min. Supernatant was obtained and absorbance was read at 645 and 663 nm for chlorophyll, at 470nm for carotenoid using a spectrophotometer. The results were calculated according to Lichtentaler and Wellburn (1985) (equations 1, 2, 3 and 4).

$$\text{Chlorophyll-a} = 11.75 \times A_{662} - 2{,}35 \times A_{645} \quad (1)$$

$$\text{Chlorophyll-b} = 18.61 \times A_{645} - 3.96 \times A_{662} \quad (2)$$

$$\text{Carotenoid} = 1000 \times A_{470} - 2.27 \times \text{Klorofil-a} - 81.4 \times \text{Klorofil-b} / 227 \quad (3)$$

$$\text{Total Chlorophyll} = \text{Chlorophyll-a} + \text{Chlorophyll–b} \quad (4)$$

The assumptions of data were tested with the Kolmogorov Smirnov and the Levene's tests, respectively. The variables were analyzed by two-way ANOVA/Kruskal-Walli's test. The means compared with Tukey's HSD/Dunn post-hoc test and the results were displayed by letters. The alpha level was set at 5%. All calculations were performed with Minitab 17 statistical software.

Figure 2. The planting seedlings in pots.

Figure 3. Growth of seedlings in pots.

3. Results

3.1. Proline Concentration (mM/gr/) in Leaf

The mean of proline concentration values in mycorrhizal tomato were determined lower than non-mycorrhizal tomato (P<0.05) (Table 1). Proline content of tomato leaf was found statistically significant in terms of mycorrhiza*dose interaction (P<0.001). According to Tukey test results, there were no statistical differences between control, R/2, R (P>0.05) dose in mycorrhizal plant in terms of proline concentration. However, it was statistically significant in R* dose (P<0.05). While control group has low proline content in non- mycorrhizal plant control (P<0.05), there is no significant differences between R dose (P>0.05).

3.2. Chlorophyll-a Content (mg/ml) in Leaf

The chlorophyll-a content was found statistically significant in terms of mycorrhiza*dose interaction (P<0.01) (Table 2). The mean values of chlorophyll-a content were found higher in mycorrhizal than non-mycorrhizal plant. Chlorophyll-a content was not found significant in mycorrhizal plant in all doses to Tukey (P>0.05). However, chlorophyll content in R dose was statistically significant in non-mycorrhizal plant (P<0.05). The chlorophyll-a content of R dose was found lower in non-mycorrhizal than mycorrhizal plant (P<0.05).

3.3. Chlorophyll-b Content (mg/ml) in Leaf

Chlorophyll-b content was found statistically significant in terms of mycorrhiza*dose interaction (P<0.05) (Table 3). Chlorophyll-b content was found significant in control, R/2 and R doses of mycorrhizal plant, R/2 and R doses in non-mycorrhizal plant to Tukey (P<0.05). The mean values of chlorophyll-b content were found lower in mycorrhizal than non-mycorrhizal plant. There was found statistically significant in R* dose (P<0.05).

3.4. Total Chlorophyll Content (mg/ml) in Leaf

Total chlorophyll content was found statistically significant in terms of mycorrhiza*dose interaction (P<0.01) (Table 4). In mycorrhizal and non-mycorrhizal plants were found statistically significant in R* dose than R/2 and R doses to Tukey (P<0.05). Total chlorophyll and carotenoid contents were found higher in non-mycorrhizal plant than mycorrhizal plants in R* dose (P<0.05).

3.5 Carotenoid Content (mg/ml) in Leaf

Mycorrhiza*dose interaction was found statistically significant in terms of carotenoid content (P<0.05). Carotenoid content was found higher in R dose than control and R* doses in mycorrhizal plant (P<0.05). Control and R* doses were found statistically significant from R and R/2 doses (P<0.05). The lowest content was found in R dose (P<0.05) (Table 5).

Table1. Proline concentration (mM/gr) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	0.063^{Bb}	0.006	0.010	0.055	0.075	0.108^{Ba}	0.003	0.004	0.105	0.113	0.086	0.010	0.026	0.055	0.113
R/2	0.060^{Bb}	0.005	0.009	0.054	0.071	0.143^{Aa}	0.003	0.006	0.137	0.148	0.101	0.019	0.046	0.054	0.148
R	0.074^{Bb}	0.013	0.023	0.060	0.101	0.137^{ABa}	0.002	0.003	0.135	0.141	0.106	0.015	0.037	0.060	0.141
R*	0.143^{Aa}	0.005	0.009	0.133	0.149	0.160^{Aa}	0.003	0.005	0.156	0.165	0.151	0.005	0.011	0.133	0.165
G	0.085	0.011	0.037	0.054	0.149	0.137	0.006	0.020	0.105	0.165					
P-Value	Mycorrhiza: 0.000; Dose: 0.000; Mycorrhiza×Dose:0.000***														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
*statistically significant (p<0,05); ***statistically significant (p<0.001)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 2. Chlorophyll-a content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	31.824^{Aa}	0.391	0.678	31.067	32.374	32.631^{Aa}	0.117	0.203	32.454	32.853	32.227	0.257	0.629	31.067	32.853
R/2	32.701^{Aa}	0.265	0.459	32.188	33.072	32.518^{Aa}	0.071	0.123	32.376	32.590	32.610	0.129	0.317	32.188	33.072
R	32.242^{Aa}	1.299	2.251	29.704	33.995	27.586^{Bb}	0.612	1.060	26.503	28.621	29.914	1.223	2.996	26.503	33.995
R*	31.709^{Aa}	0.394	0.682	31.206	32.486	31.707^{Aa}	0.264	0.457	31.314	32.209	31.708	0.212	0.520	31.206	32.486
G	32.119	0.328	1.137	29.704	33.995	31.110	0.640	2.215	26.503	32.853					
P-Value	Mycorrhiza:0.022; Dose:0.001; Mycorrhiza×Dose:0.001**														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
**statistically significant (p<0.01)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 3. Chlorophyll-b content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	24.608^{Aa}	1.839	3.185	22.226	28.226	26.296^{Aa}	0.532	0.922	25.455	27.282	25.452	0.936	2.292	22.226	28.226
R/2	15.760^{Ba}	1.379	2.388	14.304	18.516	18.696^{Ba}	0.952	1.648	16.956	20.234	17.228	0.996	2.440	14.304	20.234
R	14.762^{Ba}	0.295	0.511	14.400	15.347	15.494^{Ba}	0.234	0.405	15.228	15.960	15.128	0.235	0.575	14.400	15.960
R*	19.693^{ABb}	0.078	0.136	19.538	19.791	28.918^{Aa}	2.485	4.304	24.674	33.279	24.305	2.343	5.740	19.538	33.279
G	18.706	1.268	4.394	14.304	28.226	22.351	1.744	6.043	15.228	33.279					
P-Value	Mycorrhiza:0.001; Dose:0.000; Mycorrhiza×Dose:0.017*														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
*statistically significant (p<0.05)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 4. Total Chlorophyll content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	56.432^{Aa}	2.039	3.532	53.293	60.257	58.927^{Aa}	0.423	0.733	58.308	59.736	57.679	1.086	2.660	53.293	60.257
R/2	48.461^{Ba}	1.127	1.952	47.148	50.704	51.214^{Ba}	0.897	1.553	49.543	52.613	49.838	0.891	2.182	47.148	52.613
R	47.004^{Ba}	1.451	2.513	44.104	48.534	43.080^{Ca}	0.407	0.705	42.463	43.849	45.042	1.106	2.710	42.463	48.534
R*	51.402^{ABb}	0.316	0.547	50.997	52.024	60.625^{Aa}	2.229	3.860	56.883	64.593	56.013	2.295	5.621	50.997	64.593
G	50.825	1.236	4.282	44.104	60.257	53.461	2.165	7.500	42.463	64.593					
P-Value	Mycorrhiza:0.012; Dose:0.000; Mycorrhiza×Dose:0.001**														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
*statistically significant (p<0.05)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 5. Carotenoid content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	8.226^{BCa}	0.527	0.912	7.179	8.847	7.866^{Ba}	0.190	0.329	7.514	8.165	8.046	0.263	0.644	7.179	8.847
R/2	10.938^{ABa}	0.386	0.668	10.281	11.617	10.319^{Aa}	0.143	0.248	10.041	10.519	10.629	0.230	0.564	10.041	11.617
R	12.005^{Aa}	0.112	0.194	11.788	12.160	10.681^{Aa}	0.244	0.422	10.206	11.015	11.343	0.320	0.783	10.206	12.160
R*	9.830B^{Ca}	0.428	0.742	8.974	10.290	6.895^{Bb}	0.855	1.480	5.375	8.332	8.362	0.783	1.918	5.375	10.290
G	10.250	0.454	1.573	7.179	12.160	8.940	0.521	1.806	5.375	11.015					
P-Value	Mycorrhiza:0.012; Dose:0.000; Mycorrhiza×Dose:0.001**														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
*statistically significant (p<0.05)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

4. Discussion

In this study, proline concentration, chlorophyll a, b, total chlorophyll, and carotenoid quantity values were found statistically significant. It has been found that proline concentration of plant leaf statistically significant in terms of mycorrhiza*dose interaction (P<0.01) (Table 1). However, the mean values of proline concentration were found lower in mycorrhizal than non-mycorrhizal plant in all pesticide doses. Most plant synthesizes proline amino acid from glutamine when exposed to stress. (Tort et al., 2004). It is organic compound that is synthesized and accumulated in plant's stress condition. Claussen (2005) was reported that proline is a reliable indicator of the environmental stress in tomato. It has been reported that short-term AZX exposure to the aquatic macrophyte *Myriophyllum quitense* Kunth. occurred oxidative stress and DNA damage occurred (Garanzini and Menone, 2015).

Proline might play a critical role in protecting plants under stress (Velázquez, et al., 2010). Matysik et al. (2002) reported that proline is organic indicator substance which increases the resistance of plants to stress conditions. Many studies have shown a positive correlation between stress tolerance to synthesis of proline (Asraf and Foolad, 2007; Topaloğlu 2010; Özdener and Kutbay, 2011; Yıldıztekin and Tuna, 2015). Ghosh et al. (2022) reported that proline is an antioxidative defense molecule that scavenges reactive oxygen species (ROS) with its metal chelator properties. In our study, proline concentration was found to be higher than the control at all doses applied to fungicide in non-mycorrhizal plant. This increase may be evidence that the stress of tomato from fungicide applications. It has been suggested that the toxic substances which were produced using fungicides inhibits protein synthesis induces change in the enzymatic system and disturbs nitrogen metabolism. Fungicide treatments in cotton (*Gossypium hirsutum* L.) caused accumulation of reactive oxygen species (ROS) (Mohamed et. al. 2018). However, mycorrhizal plants have low proline concentration in all doses. This show that mycorrhizal plants are less affected by fungicide application.

Many studies on abiotic stresses have shown that human activities such as excessive use of pesticides and fertilizers, deforestation and irrigation negatively affect plant growth, development, and yield. However, it has been reported that several studies have confirmed that plants infected with AMF is more resistant to abiotic stress such as drought, salinity, fungicide, and heavy metal contamination (Claussen, 2005; Çetinkaya and Dura, 2010; Erzurumlu and Kara, 2014; Ganugi et al., 2019). Diagne et al. (2020) reported that AMF improved plant growth parameters some species such as *Solanum lycopersicum* L. (Bona et al., 2016), *Cucurbita maxima* Duchesne (Al-Hmoud et. al., 2017), *Piper longum* L. (Gogoi, 2011), *Phaseolus vulgaris* L. (Ibijbijen et al., 1996) in stressed conditions. *Glomus* genera have different reproductive organs which are compatible to unstable environment conditions (Azimi et al., 2018). Gonzalez-Chavez et al. (2002) reported *Glomus intraradices* and

Glomus mosseae vesicular arbuscular mycorrhiza could be suitable for the reconstruction and rehabilitation of plant communities in harsh environmental conditions (Gonzalez-Chavez et al., 2002). Zhu et al. (2009) was reported that the *Zea mays* leaf proline content in was lower in mycorrhizal plant with AM fungus than nonmycorrhizal plants under temperature stress.

Proline results were found like chlorophyll-a, b, total chlorophyll, and carotenoid quantity values in present study. Abiotic stresses factors such as heavy metals, nutrient deficiency and pesticides have negative effect on chlorophyll biosynthesis (Sharma et al., 2020). It was known that the use of pesticide to reduce the amount of chlorophyll and negative effect on the CO2 fixation, Hill reaction and electron transport system (Hopkins, 1995; Sharma et al., 2016). It has been reported plants infected mycorrhiza have higher chlorophyll content (Akay and Kararslan, 2012). Chlorophyll and carotenoid contents were decreased in line with dose increase. On the other hand, it was reported that Antracol WP 70 (Propineb) fungicide cause a reduction in the chlorophyll content (Özörgücü et al., 1990). Sharma et al. (2020) was reported that fungicide reduce photosynthesis by reducing amount photosynthetic pigments. Also, similar results have been reported by Tort et al. (2004). However, all chlorophyll and carotenoid values were found higher in infected mycorrhizal plants in all pesticide doses in our study. AMF watermelon plants higher photosynthetic rate, chlorophyll contents, and biomass accumulation showed to non-AMF watermelon plants, and they are enhancing resistance to soil borne fungal diseases (Wu et al., 2021). It shows that, chlorophyll-a, and carotenoid quantity values such as proline of mycorrhizal plants not affected by pesticide application.

In the present study was investigated fungicide resistance of mycorrhiza in tomato plant in terms of some physiological parameters. In the comparison of these parameters, positive results were determined on resistance of mycorrhiza against pesticide. It is well-known that AM fungi not only stimulate the growth of plants but also contribute to enhancing plant tolerance to abiotic stresses factors (Charest et al., 1993; Augé, 2001). Mycorrhiza is considered as a stimulant for superoxide dismutase, catalase, and peroxidase in leaves. AMF symbiosis can alter plant physiology in a way to cope with stresses under stressful conditions (Miransari et al., 2008). It has reported that knowledge on the mechanisms of dealing with pesticides is limited (Hage-Ahmed et al., 2018). Murrel et al. (2020) reported that AMF colonization can also increase secondary metabolite and defense gene regulation in crop plants. AMF have different strategies as morphological adaptation, protective molecules, and changes in gene expression to deal with organic pollutants (Lenoir et al., 2016; Diagne et al., 2020). It has been documented that some herbicide applications in some crop plants affect AMF root colonization within a few days, reaches balance within a few weeks (Santos et al., 2006).

Today, the damages to the environmental health of fungicide widely used in the agriculture has been scientifically proven. The biggest problem related to pesticides used in the prevention of bacterial and fungal diseases of the damage is irrational and uncontrolled use. The unconscious use of pesticides leads to the accumulation of this in the nature that are not tolerated its damages. Therefore, we must develop alternative applications or methods that will reduce the damages that may occur due to the use of fungicide. Recent mycorrhiza studies indicate that AMF applications that reduce the effects of abiotic stress can be an alternative for sustainable agriculture. The use of arbuscular mycorrhizal fungi (AMF) may be an alternative to improve the defense mechanisms of plants. It has been reported that arbuscular mycorrhizal fungi (AMF) effectively induce phenolic profiles and antioxidant activities in leaves of Potato (*Solanum tuberosum* L.) (Fritz et. al., 2022). Kaymak (2022) stated that alternative environment-friendly methods should be applied in agriculture.

5. Conclusion

In this study, a potential fungicide resistance was tested in mycorrhizal applications. Arbuscular mycorrhiza fungus affected plant growth-promoting traits despite fungicide application. Studied arbuscular mycorrhiza may increase at highly the resistance tolerance to fungicide. Therefore, AMF is suitable option for low chemical input and nature conservation based sustainable agriculture. Thus, it is necessary to conduct further studies on the mechanism of AM fungi in terms of enzymatic.

Author Contributions

T.Ö. (100%) supervised the research, suggested the research methods, structured the paper and edited the manuscript. D.K. (100%) initiated the research idea, developed, organized, analyzed, and interpreted the data and wrote the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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