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RESEARCH ARTICLE

Arbusclar Mycorriza Induced Antioxidant Defense Mechanism in Tomato Plant Against Fusarium Oxysporum

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A B S T R A C T

This investigation is aimed to explored the associative impact of Arbuscular Mycorrhizal fungus (AM) like *Glomus etunicatum, G. leptoticum, and Rhizophagus intraradice,* with tomato crops to induce an integrated defense against plant pathogen *Fusarium oxysporum*. The administration of AM fungi in plant can decreased a disease progression in vascular tissue. This impact can be seen on the basis of dry weight of leaf and root of plant. Increased dry weight of leafs indicates decreased *Fusarium* induced wilting due to raised chlorophyll content. This all beneficial impact of AM association is due to production of higher concentration antioxidant enzymes of tomato plants. The increased concentration of antioxidant enzymes is observed glutathione peroxidase was show 100.79U; phenylalanine ammonia lyase showed 3.09 U; Polyphenol oxidases 0.43 U respectively in presence of high organic matter. This increased antioxidant enzymes including glutathione reductase, catalase, polyphenol oxidase also helps to increase defense mechanism of plants by remove or scavenge reactive oxygen species produced during course of fungal infection. This study explores the associative role of Arbascular and mycorrhizal fungi in increased antioxidant enzyme systems in tomato plant. This induction not only helps in plants growth but also resist fungal plant pathogen like fusarium at a great extent. However, to develop this mechanism in crop development further investigations are required.

Keywords: Arbuscular mycorrhiza (AM) fungi, Antioxidant enzymes, Tomato plant, *Fusarium oxysporum*.

INTRODUCTION

The plant diseases aggressiveness is rising and causing catastrophic failure of crops. The growth of microbial toxins in finished product can endanger the health of consumers (Jackson and Taylor 1996; Khalil *et al.,* 2019; Abdelaziz *et al.,* 2022). Tomato is a perennial crop that belongs to the *Solanaceae* family and is typically planted to produce its palatable fruits (Bashir *et al.,* 2020). The financial and dietary benefits of tomatoes made this crop as one of the most valuable vegetable crop worldwide (Olatunji and Afolayan 2018). Because of its distinct flavor, abundance in nutrients, and outstanding taste this vegetable crop is utilized by the food industry in big quantity to produce various food products (Usman *et al.,* 2023). However, this industry continues to face a major danger from microbial pathogens transmitted via soil mainly fungal pathogens (Panthee and Chen, 2010; Mousa, *et al.,* 2021).

Fungal pathogens can impact on biochemical processes of cell such as photosynthesis, respiration, plasma membranes operation, and water conduction (Yaqoob *et al.,* 2024). Such microbial infection induced stress results in an excessive buildup of harmful reactive oxygen species (ROS), in plants which causes oxidative damage (Iqbal *et al.,* 2024). The

interaction of excessively generated Superoxide with biological components such lipids, proteins, and nucleic acids impairs a cell's capacity to function normally (El-Rahman *et al.,* 2012; Egamberdieva *et al.,* 2017; Hashem *et al.,* 2017). *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is one amongst the stress inducing pathogen which causes the severe reduction in yield of tomatoes through the wilt disease. (Nirmaladevi and Sirnivas 2012; Akhter *et al.,* 2015).

Pesticides, soil fumigation, choosing disease free varieties, and microbiological approaches are possible countermeasures for *Fusarium* sp. induced tomato wilt (Hanson *et al.,* 2016; Michielse and Rep, 2009). Biopesticides, are popular among other controlling strategies due to their ecological viability and eco-friendly nature, Microbiological pesticide can be incorporated in hostile environment and have a competitive nature against diseases causing microorganism (Abd El-Rahman *et al.,* 2012; Tanwar *et al.,* 2013; Wang *et al.,* 2020; Imran *et al.,* 2022).

Arbuscular mycorrhizal fungi (AMF) and plant roots can share symbiotic association to enhance the productivity of plants in ordinary and adverse environmental circumstances. Over the past ten years AMF used for adaptation of crop in responses to cold stress (Zhu *et al.,* 2010). These types of Plant growth-promoting fungi (PGPF) can also be used as efficient natural defense against various pathogenic organisms, such as *F. oxysporum* which mainly affected tomato crop. Hence, using AMF association in tomato enhances tomato crop production by improving physiological tolerance and enhancing tomato immunity against microbial pathogens (Hyakumachi, 2013). AMF association with plant can induce production of active secondary metabolism like steroids, flavonoids, terpenoids, peptides, quinones, lignans, alkaloids, phenylpropanoids, phenolics, and isocoumarins, exhibits substantial inhibitory activity against various plant pathogens (Elghaffar *et al.,* 2022).

Therefore, this study aims to evaluate the effect of endophytic fungi on *Fusarium oxysporum* infected tomato plants to evaluate their growth promoting activity on healthy as well as diseased tomato plants through the secure and ecofriendly method.

MATERIAL AND METHODS

A) Materials: i) Plant preparation: Seeds of Tomatoes (*Lycopersicon esculentum*) were purchased from the Department of the Agriculture's plant protection office. Prior to plantation, the seeds were surface sterilized with sodium hypochlorite (3.5%) for two to three minutes.

ii) Soil: The soil consisted loam, acquired from the Tigris bank in the Zafaraniyah neighbourhood of Baghdad. Collected soil was washed to remove its major nutrients after that it is dried and autoclaved to remove indigenous microflora. The process of autoclaving was done exclusively for 2 days (Davies and Linderman, 1997; Louis and Lime, 1988). This treated soil then subsequently supplemented with sterilized commercial peat moss at a concentration of 1.5% and Rock phosphate in appropriate concentration.

iii) AM fungi: Species of *Glomus etunicatum, G. leptoticum, and Rhizophagus intraradices* fungi were used as a consortium. For inoculation all fungal species and their associated root fragments were obtained from Department of horticulture office, Ministry of Agriculture which is propagated (Owusu-.Bennoah and Mosse, 1979) using Tomato (*Lycopersicon esculentum*) as ex plant, before infecting to plantlets were surface sterilized as stated above.

B) Experimental Set up: Fungi were grown in containers that contained the test soil (with treated tomato seeds) which is treated with a growth components and sterile distilled water is used for watering. After gap of 4 weeks, grown tomato plants were collected by cutting off the branch system, removing the soil and then root were shredded and these shards containing mycorrhizal spores were used as inoculum for later trials.

C) *Fusarium oxysporum* f. sp. *lycopersici pathogen*: A pathogenic fungus was grown on Potato Dextrose Agar medium for 5 days of incubation and after sporulation the spores were subsequently sub cultured and maintained at 25 °C under dehydrated condition. With sterilized distilled water, the suspension of spores was made. Erlenmeyer flasks containing 50 gm of sterilized seeds were filled with fungal spore's suspension. The resulting mixture was then kept at 25°C for a period of six days and then used as inoculum for tomato root infections.

D) Cultivation design: A consortium of three AM fungi is transferred as a layer of a dirt-based the inoculum 50g/pot, before being topped over an equal amount of test soil, 4.900kg/pot. Following two weeks of growth, a second dose of 25ml was introduced through irrigation of a fertilizer mixture into the pots (Yildiz, 2010). On individual fungal pad, 25gm of infecting wheat seeds containing *F. oxysporum* were put into the pots (Dewan, 1989). A control set of clean tomato seedlings were planted and grown using sterile water. These all experimental set ups were allowed to grow

in natural climatic conditions. After four weeks' interval the plantlets were harvested to separate their root and shoot systems and used for the other subsequent characteristics as follows.

a) Measurement of total root length (cm): Measurement of total root length (cm) was done as described by Ryosuke and Yoichiro, (2013). The plunked roots of AM infected plantlets and untreated plantlets were obtained after growth of 4 weeks.

b) Measurement of total dry weight (gm): Measurement of total dry weight (gm) of plantlets was done as per Shipley and Vu (2002) with some modification. The plantlets selected for the length measurement purpose were taken then weighed after their collection and this can be treated as initial weight with moisture. Plantlets then dried in oven at 60°C and again weighed (final weight) and the difference was calculated as initial and final weight.

c) Glutathione peroxidase activity: Glutathione peroxidase activity was performed as described by (Rotruck *et al.,* 1973) with some modification in 0.4 mL of buffer, 0.1 mL sodium azide, 0.2 mL of reduced glutathione, 0.5 mL of enzyme extract and 0.1 mL of H_2O, was taken. The final volume adjusted to 2.0 mL with distilled water and allowed to incubate the tube at 37°C for 30 min. Then 0.5 mL of 10% trichloroacetic acid was added to stop the reaction. To determine the unused/residual glutathione content, the reaction mixture was centrifuge, and in supernatant 3.0 mL of disodium hydrogen phosphate and 1.0 mL of DTNB reagent was added. The optical density of reaction mixture was taken at 412 nm. Tubes of blank were prepared by using disodium hydrogen phosphate and 1.0 mL of DTNB reagent. The activity is expressed as µg of glutathione consumed/minute/mg protein.

d) Phenylalanine ammonia lyase (OAL) activity: Phenylalanine ammonia lyase (OAL) activity was measured as reported by (Brueske, 1980) with some modification. 0.5 mL borate buffer, 0.2 mL enzyme solution and 1.3 mL distilled water was taken in a test tube. The reaction was initiated by the addition of 1M 1 mL L-Phenylalanine solution. This reaction mixture is then incubation for 30-60 min at 32°C. The reaction was stopped by the addition of 0.5 mL of 1 M trichloroacetic acid. Control sample is monitored in same way in which phenylalanine was added after trichloroacetic acid. The optical density was measured by using spectrophotometer at 290 nm. The slope obtained on standard graph prepared by using trans-cinnamic acid.

STATISTICAL ANALYSIS

The obtained results were the mean of three determinants. Analysis of the variants were carried out on all data at $p < 0.05$ using GraphPad software (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA)

RESULTS AND DISCUSSIONS

Effect of AM fungi and organic matter on dry weight (gm) of tomato plants: The infection free conditions are essential to increase the crop of tomatoes and this can be achieved by various cultivation methods. This method includes use of chemical pesticides, irrigation techniques, use of resistant variety of tomatoes and biopesticides. Whereas not a single crop method was design to be implemented fully.

Plant root protection is a key indicator of defense mechanism of plant which can induce by different enzyme systems. For example, induction of chitinases and b-1,3-glucanase also linked with resistance against *Alternaria solani* in tomato. (Evelin *et al.,* 2009; Beltrano *et al.,* 2013) The AMF association induced plant growth is seen by increased nutritive element like phosphorous and other enzyme system such as antioxidant enzyme (Hashem *et al.,* 2015; Huang *et al.,* 2014).

The effect of all factors on the percentage of dry wt. of tomato plant (Table 1) after 4 weeks of cultivation showed the high dry wt. concentration of organic matter percentage 8.31 % in the treatment ($M^+ \times O^+ \times 2W^+$) compared with ($M^- \times O^- \times 0W^+$) which recorded a 4.03% of organic dry wt. considering the single treatments which also showed low percentages represented by the treatments (M^+) then ($4W^+$) and (O^+) reached 5.05, 7.77, and 10.10 % respectively compared to the high percentages of the treatments (M^-) than ($0W^+$) and (O-) which recorded 2.61, 1.86 and 3.83 respectively. Additionally, the dual treatments ($M^+ \times 4W^+$), ($M^+ \times O^+$) and ($O^+ \times 2W^+$) showed also low percentage 7.08, 13.92 and 5.81 % compared with the high percentage in the treatments ($M^- \times 0W^+$), ($M^- \times O^-$) and ($O^- \times 0W^+$) which showed the percentage 1.74, 1.13 and 1.23 % respectively. Despite having enough water in the soil, *F. oxysporum* infecting tomato seeds rapidly and expands via the root vascular system, interfering with water and nutrient intake and causing plant wilting (Jayamohan *et al.,* 2020). Our obtained results were showing similar type of findings where in AM infected increased root length was observed that ultimately increase the potential of vascular system of plant even in less amount of water.

Table 1. Effect of the mycorrhiza fungi, organic matter and F.o.l. on the total dry weight (gm) of tomato plants four weeks after planting.

Mycorrhizal Fungi	Organic matter	Pathogenic fungi F.o.l				Mycorrhizal interaction X Organic matter O x M
		C	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	3.83	0.83	2.70	3.07	2.61 ± 0.06
	O^+	6.27	1.13	3.95	4.94	4.07 ± 0.04*
M^+	O^-	11.70	1.62	6.50	8.31	7.03 ± 0.05
	O^+	13.92	1.86	7.66	9.78	8.31 ± 0.01**
SD (0.05)	1.742 ± 0.027*					2.753 ± 0.02*
Mycorrhizal interaction × Pathogenic fungi F.o.l.xM						Mycorrhizal effect
	M^-	5.05	0.98	3.33	4.01	3.34 ± 0.034*
	M^+	12.81	1.74	7.08	9.05	7.67 ± 0.04*
LSD (0.05)						0.543 ± 0.02**
Organic interaction × pathogenic fungi F.o.l.x O						Organic effect
	O^-	7.77	1.23	4.60	5.69	4.82 ± 0.06
	O^+	10.10	1.50	5.81	7.36	6.19 ± 0.03*
(0.05) LSD	2.995 ± 0.03*					0.543± 0.03*
Pathogenic effect F.o.l		8.93	1.36	5.20	6.53	
LSD (0.05)	0.995 ± 0.05*					

Values represent the means of three replicates, Means are significantly different at P< 0.05.
M^+ = Presence of mycorrhiza, M^- = Absence of mycorrhiza, O^+ = Presence of organic matter added, O^- = Absence of organic matter, $0W^+$ =inoculation of pathogen at the same time planting, $2W^+$ = inoculation of pathogen after two weeks planting, $4W^+$ = inoculation of pathogen after four weeks planting

Effect of the mycorrhiza fungi, organic matter and F.o.l. on the total root length (cm): It was observed that after exposure of plant with AM fungi plant increased their root length. It was seen that the maximum root length increased after 4 weeks of exposure. In presence of pathogenic fungi, the maximum length was observed 15.95cm that is for (M^+ x O^+ x $4W^+$). Whereas for the (M^- x C^- x $4W^+$) 10.28 cm. the length of tomato plant root which are grown in presence of AM fungi that is in M^+ and M^- was observe to be 14.81 and 9.41 cm respectively. The mycorrhizal effect was recorded as 0.902 with 2.462 LSD 0.05. In presence of organic matter (O^+ and O^-) was observe to have13.81 and 10.71 cm respectively. The overall pathogenic effect was measured up to 1.203. Impacts of mycorrhizal bio protection were closely linked to host-AM fungus interaction. A commonly utilized critical indicator to evaluate symbiotic interactions is the mycorrhizal colonization rate (Cordier *et al.,* 1998; Slezack *et al.,* 2000). The symbiotic association of mycorrhizae will absolutely see in this study that will indicated by the increased the root length of seedlings and plantlets.

Effect of the mycorrhiza fungi, organic matter and F.o.l. on the total dry weight of root: As described in previous section the improved root length of test plant was increased and it is also replicated in terms of dry weight of root system. It was observed that after exposure of plant with AM fungi plants roots dry weight increased. It was noted that the maximum root dry weight was seen after 4 weeks of exposure. In presence of pathogenic fungi the maximum root dry weight was observed 4.24 gm that is for (M^+ x O^+ x $4W^+$). Whereas for the (M^- x C^- x $4W^+$) 1.40 gm the dry weight tomato plant root which are grown in presence of AM fungi that is in M^+ and M^- was observe to be 3.30 and 1. 40 gm, respectively. The mycorrhizal effect was recorded as 0.279 with 0.892 LSD 0.05. In terms of organic matter (O^+ and O^-) was as 2.51 and 1.83 gm respectively. The cumulative mycorrhizal effect for overall pathogenic effect was measured up to 2.003. The mycorrhiza fungi show inhibitory impact on F.o.l. and has a positive impact on tomato plant which is showed by the increased dry mass of roots of experimental plant. Somewhat different results were obtained in terms of transgenic tomato plant where the adapted verity was used in this study (Wang *et al.,* 2020).

Table 2. Effect of the mycorrhiza fungi, organic matter and F.o.l. on the total root length (cm) of tomato plants four weeks after planting

Mycorrhizal Fungi	Organic matter	Pathogenic fungi F.o.l				Mycorrhizal interaction X Organic matter O x M
		C	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	12.25	2.90	7.02	8.81	7.75 ± 0.04
	O^+	17.11	3.88	8.98	11.16	10.28 ± 0.07
M^+	O^-	23.01	5.73	11.58	14.35	13.67 ± 0.05
	O^+	27.34	6.51	13.32	16.64	15.95 ± 0.04
SD (0.05)	2.543 ± 0.04					5.007 ± 0.02**
Mycorrhizal interaction × Pathogenic fungi F.o.l.xM						Mycorrhizal effect
	M^-	14.68	3.39	8.00	9.99	9.01 ± 0.03
	M^+	25.18	6.12	12.45	15.50	14.81 ± 0.02*
LSD (0.05)						0.902 ± 0.001**
Organic interaction × pathogenic fungi F.o.l.x O						Organic effect
	O^-	17.63	4.32	9.30	11.58	10.71 ± 0.04
	O^+	22.23	5.20	11.15	13.90	13.12 ± 0.03
(0.05) LSD	3.996 ± 0.03					0.902 ± 0.02*
Pathogenic effect F.o.l		19.93	4.76	10.23	12.74	
LSD (0.05)	1.203± 0.006					

Values representing the means of three replicates, Means are significantly different at P< 0.05
M^+ = Presence of mycorrhiza, M^- = Absence of mycorrhiza, O^+ = Presence of organic matter added, O^- = Absence of organic matter, $0W^+$ =inoculation of pathogen at the same time planting, $2W^+$ = inoculation of pathogen after two weeks planting, $4W^+$ = inoculation of pathogen after four weeks planting

Table 3. Effect of the mycorrhiza fungi and F.o.l. on the total root length (cm) of tomato plants four weeks after planting

Mycorrhizal Fungi	Organic matter	Pathogenic fungi F.o.l				Mycorrhizal interaction X Organic matter O x M
		c	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	1.03	0.14	0.70	0.82	0.67 ± 0.05
	O^+	2.25	0.28	1.35	1.72	1.40 ± 0.02*
M^+	O^-	5.10	0.60	2.70	3.51	2.98 ± 0.03
	O^+	6.20	0.72	3.30	4.24	3.62 ± 0.004
SD (0.05)	0.871 ± 0.03					1.204 ± 0.03
Mycorrhizal interaction × Pathogenic fungi F.o.l.xM						Mycorrhizal effect
	M^-	1.64	0.21	1.03	1.27	1.04 ± 0.04
	M^+	5.65	0.66	3.00	3.88	3.30 ± 0.01**
LSD (0.05)	0.892 ± 0.05					0.279 ± 0.03
Organic interaction × pathogenic fungi F.o.l.x O						Organic effect
	O^-	3.07	0.37	1.70	2.17	1.83 ± 0.03
	O^+	4.23	0.50	2.33	2.98	2.51 ± 0.02
(0.05) LSD	2.003 ± 0.002**					
Pathogenic effect F.o.l	3.65	3.65	3.65	3.65	3.65	0.279 ± 0.03
LSD (0.05)	0.447 ± 0.02					

Values representing the means of three replicates, Means are significantly different at P< 0.05
M^+ = Presence of mycorrhiza, M^- = Absence of mycorrhiza, O^+ = Presence of organic matter added, O^- = Absence of organic matter, $0W^+$ =inoculation of pathogen at the same time planting, $2W^+$ = inoculation of pathogen after two weeks planting, $4W^+$ = inoculation of pathogen after four weeks planting

Effect of the mycorrhiza fungi, organic matter and F.o.l. on the Glutathione peroxidase enzyme: Effect of the mycorrhiza fungi plus organic matter on F.o.l. and glutathione peroxidase enzyme was checked. It was observed that after exposure of plant with AM fungi plants GPOX enzyme activity was elevated. The GPOX

enzyme activity was seen after 4 weeks of exposure. In presence of pathogenic fungi, the maximum enzyme activity was observed 100.79 U that is for (M^+ x O^+ x $4W^+$) and for the (M^- x C^- x $4W^+$) 21.54 U. The GPOX enzyme activity in AM and organic matter supplemented tomato and un supplemented was observe to be 106.72 and 53.80 U respectively. The cumulative Mycorrhizal effect was recorded as 0.746 with 8.442 LSD 0.05. In case of organic matter (O^+ and O^-) was observed to be 76.16 and 84.36 U respectively. Whenever plants are affected by infectious agents, AM fungi are essential for the activation of plant defense (Bernaola *et al.,* 2018; Abo-Elyousr *et al.,* 2014). The AM fungi induce certain responses that activate the plant defense system in response to biotic stressors throughout the development of the mycorrhizal symbiosis (Pieterse *et al.,* 204). This enzyme is expressed in adverse condition and protects the enzymes.

Table 4. Effect of the mycorrhiza fungi, and F.o.l. on the Glutathione peroxidase enzyme of tomato plants four weeks after planting

Mycorrhizal Fungi	Organic matter	Pathogenic fungi F.o.l				Mycorrhizal interaction X Organic matter O x M
		C	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	45.72	17.80	70.46	90.29	56.07 ± 0.06
	O^+	43.11	12.27	62.35	88.42	51.54 ± 0.03
M^+	O^-	108.35	55.33	130.33	156.63	112.66 ± 0.04
	O^+	96.43	48.16	115.15	143.41	100.79 ± 0.05
SD (0.05)	1.955 ± 0.03					26.679 ± 0.05
Mycorrhizal interaction × Pathogenic fungi F.o.l.xM						Mycorrhizal effect
	M^-	44.42	15.04	66.41	89.36	53.80 ± 0.04
	M^+	102.39	51.75	122.74	150.02	106.72 ± 0.03
LSD (0.05)	8.442 ± 0.03					0.746 ± 0.02**
Organic interaction × pathogenic fungi F.o.l.x O						Organic effect
	O^-	77.04	36.57	100.40	123.46	84.36 ± 0.05
	O^+	69.77	30.22	88.75	115.92	76.16 ± 0.02**
(0.05) LSD	35.836 ± 0.03					35.836 ± 0.04
Pathogenic effect F.o.l		73.40	33.39	94.57	119.69	84.36 ± 0.05
LSD (0.05)	1.084 ± 0.002*					

Values representing the means of three replicates, Means are significantly different at P< 0.05

M^+ = Presence of mycorrhiza, M^- = Absence of mycorrhiza, O^+ = Presence of organic matter added, O^- = Absence of organic matter, $0W^+$ =inoculation of pathogen at the same time planting, $2W^+$ = inoculation of pathogen after two weeks planting, $4W^+$ = inoculation of pathogen after four weeks planting.

Effect of the mycorrhiza fungi, organic matter and F.o.l. on the phenylalanine ammonia lyase (OAL) enzyme: Effect of the mycorrhiza fungi, organic matter and F.o.l. on the phenylalanine ammonia lyase (OAL) enzyme was checked by enzyme assay. It was observed that after exposure of plant with AM fungi plants OAL enzyme activity remain constant. The maximum OAL enzyme activity shows constant results after 4 weeks of exposure. In presence of pathogenic fungi, the maximum OAL activity observed 3.09 U that is for (M^+ x O^+ x $4W^+$). Whereas for the (M^- x C^- x $4W^+$) 1.03 U. The OAL enzyme activity in tomato which are grown in presence of AM fungi that is in M^+ and M^- was observe to be 3.36 and 1.13 U respectively. The cumulative mycorrhizal effect was recorded as 0.452 with 0.120 LSD 0.05. In presence of Organic matter (O^+ and O^-) was observe to be 2.06 and 2.44 U respectively.

Table 5. Effect of the mycorrhiza fungi, and F.o.l. on the phenylalanine ammonia lyase enzyme of tomato plants four weeks after planting

Mycorrhizal Fungi	Organic matter	Pathogenic fungi F.o.l				Mycorrhizal interaction X Organic matter O x M
		C	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	0.56	0.30	1.72	2.38	1.24 ± 0.06
	O^+	0.42	0.17	1.25	2.27	1.03 ± 0.02*
M^+	O^-	3.31	1.09	4.45	5.68	3.63 ± 0.04
	O^+	3.20	1.03	3.51	4.60	3.09 ± 0.05
SD (0.05)	0.329 ± 0.032*					1.196 ± 0.02**
Mycorrhizal interaction × Pathogenic fungi F.o.l.xM						Mycorrhizal effect
	M^-	0.49	0.24	1.49	2.33	1.13 ± 0.04
	M^+	3.26	1.06	3.98	5.14	3.36 ± 0.05
LSD (0.05)	0.452 ± 0.02*					0.120 ± 0.03
Organic interaction × pathogenic fungi F.o.l.x O						Organic effect
	O^-	1.94	0.70	3.09	4.03	2.44 ± 0.04
	O^+	1.81	0.60	2.38	3.44	2.06 ± 0.05
(0.05) LSD	1.566 ± 0.04					0.120 ± 0.02**
Pathogenic effect F.o.l		1.87	0.65	2.73	3.73	
LSD (0.05)	0.169 ± 0.02					

Values representing the means of three replicates, Means are significantly different at P<0.05
M^+ = Presence of mycorrhiza, M^- = Absence of mycorrhiza, O^+ = Presence of organic matter added, O^- = Absence of organic matter, $0W^+$ =inoculation of pathogen at the same time planting, $2W^+$ = inoculation of pathogen after two weeks planting, $4W^+$ = inoculation of pathogen after four weeks planting

Table 6. Effect of the mycorrhiza fungi, and F.o.l. on the Polyphenol oxidases enzyme enzyme of tomato plants four weeks after planting

Mycorrhizal Fungi	Organic matter	Pathogenic fungi F.o.l				Mycorrhizal interaction X Organic matter O x M
		C	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	0.11	0.05	0.21	0.31	0.17 ± 0.03
	O^+	0.09	0.03	0.17	0.24	0.13 ± 0.005**
M^+	O^-	0.44	0.15	0.57	0.72	0.47 ± 0.04
	O^+	0.35	0.12	0.47	0.61	0.39 ± 0.02*
SD (0.05)	0.034 ± 0.005**					0.138 ± 0.025**
Mycorrhizal interaction × Pathogenic fungi F.o.l.xM						Mycorrhizal effect
	M^-	0.10	0.04	0.19	0.28	0.15 ± 0.04
	M^+	0.40	0.14	0.52	0.67	0.43 ± 0.02
LSD (0.05)	0.047 ± 0.006*					0.012 ± 0.007*
Organic interaction × pathogenic fungi F.o.l.x O						Organic effect
	O^-	0.28	0.10	0.39	0.52	0.32 ± 0.03
	O^+	0.22	0.08	0.32	0.43	0.26 ± 0.004**
(0.05) LSD	0.203 ± 0.05*					0.012 ± 0.005*
Pathogenic effect F.o.l		0.25	0.09	0.36	0.47	
LSD (0.05)	0.017 ± 0.01*					

Values representing the means of three replicates, Means are significantly different at P< 0.05
M^+ = Presence of mycorrhiza, M^- = Absence of mycorrhiza, O^+ = Presence of organic matter added, O^- = Absence of organic matter, $0W^+$ =inoculation of pathogen at the same time planting, $2W^+$ = inoculation of pathogen after two weeks planting, $4W^+$ = inoculation of pathogen after four weeks planting

Effect of the mycorrhiza fungi, and F.o.l. on the Polyphenol oxidases enzyme µmol ketone/min of tomato plants four weeks after planting: Effect of the mycorrhiza fungi, organic matter and F.o.l. on the Polyphenol oxidases enzyme µmol was checked by enzyme assay. It was observed that after exposure of plant with AM fungi plants PPO enzyme activity was somewhat remain constant at different experimental set up. It was observed that the maximum PPO enzyme seen somewhat constant after 4 weeks of exposure. In presence of pathogenic fungi, the maximum PPO activity observed 0.39 µmol ketone/min that is for (M^+ x O^+ x $4W^+$). Whereas for the (M^- x C^- x $4W^+$) 0.13 µmol ketone/min. the PPO enzyme activity in tomato which are grown in presence of AM fungi that is in M^+ and M^- was observe to be 0.43 and 0.15 U respectively. The Mycorrhizal effect was recorded as 0.012 with 0.047 LSD 0.05. In effect of organic matter (O^+ and O^-) was observe to be 0.26 and 0.32 µmol ketone/min respectively. While PAL is engaged in phenylpropanoid metabolism, which is directly related to the production and accumulation of phenols, lignin, and antitoxin, PPO is the main enzyme involved in the process of oxidation of polyphenols to quinons, which are antimicrobial chemicals (Constabel *et al.,* 1995).

CONCLUSION

After obtaining above results it was concluded that the entophytic AM fungal association shows positive impact when implemented in agriculture field with the organic matter. It was determined that this fungus have positive effect on antioxidant enzyme system of tomatoes. The elevated concentration of this enzyme activity after AM association helps plants in their patho-protecting activity. The phosphatase and antioxidant enzymes improvise photosynthesis and ultimately helped to boost defense against FOL wilting disease in tomato. The plant shows good impact on their growth which will determined in terms of leaves, height and other parameters of the tomato plant. This study proposed that the AM fungi can work as a potential biological disease controlling agent in controlling of *Fusarium* wilt disease as well as in stimulated phytohormones production and organics matter degradation. However, the more efforts should be required to exploit the AM fungi as a commercial biofrtilizer.

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