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ROLE OF ARBUSCULAR MYCORRHIZA (AM) FUNGI AND LIGNIN IN BIOLOGICAL CONTROL AGAINST VASCULAR FUSARIUM WILT DISEASE

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ABSTRACT

The current study aimed to assess the biological efficacy of the triple arbuscular mycorrhiza (AM) mixture of fungi *Glomus etunicatum, G. leptotichum and Rhizophagus intraradices,* and mix it with organic matter (O) and pathogenic fungi *Fusarium oxysporum* f.sp.*lycopersici* by using voyeurism in the plastic house in the growth of the tomato plant after four and eight weeks of cultivation. The results were shown after the treatment of the tomato plant in agriculture with the mixture of mycorrhiza and the pathogenic fungi and organic matter were treated with the mixture of mycorrhiza, organic matter and pathogenic fungi together. The effect of mycorrhiza and organic matter interference on the increase in the percentage of the lignin after eight weeks was very clear. The effect of the mycorrhiza mixture, organic matter and the pathogenic fungi in the percentage of disease incidence on the leaves and roots of the tomato plant, a relative decrease in infection was observed after two and four weeks of cultivation and the percentage of the death of the plant gestures contaminated with the pathogen was low after four weeks transplantation as a result of the effect of the mycorrhiza mixture and organic matter on it.

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INTRODUCTION

Vascular wilting is a disease of the vascular tissues transmitted in plants, where the disease causes the disruption of the flow of water in the tissue of wood to the leaves, which leads to their wilting, and one of the causes of this disease is *Fusarium oxysporum* f.sp *.lycopersici* where it is common in areas with warm weather and its symptoms include drooping, yellowing, wilting and death of the lower leaves and often form on one side of the plant and may be infected in one branch or more than one plant (Nesmith *et al.*, 2014). This pathogenic fungi infects plants through the roots where innate growth within the cells of the vascular tissue transporting water block tissue cells and thus stops the movement of water within the vascular tissues transporting leading to wilting, *F. oxysporum* fungi can be transmitted through contaminated soil, remnants of old crops, contaminated seeds, water and air and can stay in the soil for several years (Nesmith *et al.*, 2014). One of the modern ways to control this disease is the compost represented by the shrub root arbuscular mycorrhiza, which has a symbiotic relationship with plant roots and varies in composition and physiological relationship by host plant type (Sun and Shahrajabian, 2023). The root arbuscular mycorrhiza frequently induces a decrease in root biomass and an increase in fungal biomass, which can spread across soil pores too narrow to permit root capillary entrance and so improve soil nutrient absorption. especially phosphorus, as well as many types of nutrients and it also notes that it has a positive role for plant growth in mineral-contaminated soils (Mohammadi *et al.*, 2011; Sharma and Mehta, 2019) as its fungal filaments can release compounds that disassemble heavy metals before being absorbed by the plant and therefore the toxic effect of minerals is not affected by the plant (Mahmood and Rizvi, 2010). Mycorrhiza is important for the growth, development and health of the plant, it improves the soil, makes minerals sustainable and improves plant growth on a regular basis because it regulates enzyme activity and increases the rate of photosynthesis, and represents vital resistance because it equips the defense mechanisms in the plant, so it is an organic fertilizer, environmentally friendly important for the plant in terms of productivity and environmental protection (Bhat *et al.*, 2017).

The substance lignin is a component of the abundant cellular wall in the plant cell and is an important secondary metabolic product for plant growth and development and is an effective defensive substance in plants where it builds physical barriers that fill the spaces outside the cell, which hinders the reproduction and movement of pathogens and acts as a major factor in vascular defense as it forms a major vascular immune mechanism in plant tissues (Lin *et al.*, 2022). There is a relationship between the presence of the shrub mycorrhiza and the amount of canine found in the cell walls where it was observed that the shrub mycorrhiza stimulates the plant to produce enzymes that contribute to increased production of lignin, including enzymes PPO, POD, PAL (Hathout *et al.*, 2010).

When AMF was present, it was found that the quantity of Cd in pectin—a major cell wall binding site-did not rise. On the other hand, lignin content and the fraction of Cd in the root cell walls as a whole were considerably increased. Mycorrhizal colonization also had a favorable effect on lignin-related enzymes such laccase, PAL, and 4CL, whose enhanced activity is consistent with this conclusion (Lao *et al.*, 2023).

Phaeomoniella chlamydospora and *Phaeoacremonium minimum*, lignase-producing fungus linked to the fungal grapevine trunk disease Esca that affects grapevines globally, were effectively stopped from growing by lignin NCs. After a single injection into Vitis vinifera ("Portugieser") plants in a test vineyard in Germany, in planta tests demonstrated their efficacy for at least 4 years. In addition to being particularly interesting as biodegradable delivery systems to be used via trunk injection to treat the deadly fungal disease Esca, lignin NCs may also show promise in treating other fungal plant diseases (Machado *et al.*, 2020). The present study is conducted to clarify the role of Arbuscular Mycorrhiza fungi and lignin as a biological control against vascular *Fusarium* wilt disease.

MATERIAL AND METHODS

Activation of Mycorrhiza Fungi and Soil Preparation

From the Department of Research and Studies/Horticulture Department/Ministry of Agriculture/Baghdad/ Iraq, bio fertilizer was obtained for three types of shrub mycorrhiza *Glomus etunicatum, G. leptotichum,* and *Rhizophagus intraradices.* This fertilizer contains fungal hyphae, spores and infected roots in dry mixed soil previously examined, and checked to make sure there are fungal spores by wet palm and filtering according to the method of Gerdemann and Nicolson (1963).

Preparation of Soil Used in Planting

A mixed soil from the Tigris River beach was obtained from the Zaffarnia area of the surface layer deep (0-30 cm) and washed according to the method Davies and Davies Jr and Linderman (1991) for soils that lack nutrients, and sterilized according to Louis and Lim (1988) to get rid of microbiology and the types of mycorrhiza in it.

Activate Pathogenic Fungi and Test for Infection

The pathogenic fungi *F. oxysporum* f.sp. *lycopersici* was obtained from the Agricultural Research Center/ Ministry of Agriculture/Iraq, and activated the pathogenic fungi on the medium of potato dextrose agar (PDA), and the identification of the pathogenic fungi was confirmed according to the classification qualities approved by Booth (1971) and Leslie and Summerell (2008). The sensitivity of the tomato seeds (S25) to infection was tested according to the previous work of (Amran, 2005) where brown spots appear or appear snoring on the roots, indicating the incidence of pathogenic fungi and was confirmed to cut part of the infected root and re-isolate it on the PDA medium and diagnose it based on classification qualities. The strain of pathogenic fungi was preserved on the PDA in the refrigerator until laboratory studies were conducted, and the seeds of millet infected with pathogenic fungi were used in the multiplication of pathogenic fungi and used in experiments according to another groups (Dewan and Sivasithamparam, 1988).

Organic Material

The commercial bitmos type (SAB Substrate 1, Germany) was used and sterilized by the autoclave at a temperature of 121˚C and pressure of 15 pounds / inch for half an hour.

Experiment in the Plastic House

The experiment work was carried out in the plastic house by mixing sterile organic matter (bitmos) with poor sterile mixed soil (1.5%). A (50g) of the mycorrhiza mixture was added to the three fungal species (*Digitariasanguinalis*, *Solanum americanum*, and *Alternanthera caracasana*.) with 5 g phosphate rock at a concentration of 12%, then add 50 g of millet seeds contaminated with anti-fungi agent (F.O.L.). For contaminated transactions, Dewan and Sivasithamparam (1988) recommended a mixed soil (5% weight/weight) and three planting periods (0W+).Following two weeks (2W+) and four weeks (4W+) of cultivation, 50 g of sterile millet seeds were added to the mixed soil in the control container, followed by the addition of 20 *Lycopersicon esculentum* (tomato) seeds. For each pot after being sterilized with sodium hypochlorite solution and with a concentration of 3.5% so that it comes into contact with millet seeds, the mycorrhiza layer and soil and covered all the pots with 50 g of sterile mixed soil and three repeaters per treatment, the pots were watered with 750 ml of sterile water each, the plants were sown and watered whenever the soil needed to remain moist.

Calculating the Percentage of the Incidence of the Disease and the Development of the Disease

The abundance of shrubs in the root system and the cutting of roots were calculated based on (Trouvelot, 1986).

Calculating the Percentage of the Lignin

The roots were dried in an electric dryer and at a temperature of 70° C for 72 hours, then grinded and sifted with a sieve with a diameter of 40 mikron, took the weight of 0.2 g of roots very precisely and put in a glass conical flask to which 2 ml of 72% of phosphoric acid was added at a rate (1 ml acid per 0.1 g roots), Put the glass flask in a water bath at a temperature of 30˚C for one hour, stirring from time to time with a glass rod, then add 56 ml of distilled water, i.e. at a rate (28 ml distilled water per 1 ml of acid), Place the flask inside the autoclave for one hour for analysis, then filter the hot solution during Sintered glass funnel on glass microfiber filters GF/C and then wash the remaining lignin accents on the filter with distilled water to remove the acid and dry the filter to a constant weight at a temperature of 105˚C and then weight and according to the accent relative to the weight taken (Jingjing, 2011) and according to the following equation:

Lignin, % = A ×100 / W

Where: A = weight of lignin, g W = oven-dry weight of sample.

The percentage of the incidence of the disease in leaves and roots has been calculated depending on (Hage-Ahmed *et al.*, 2013).

Statistical Analysis

The data were analyzed using the program SAS–Statistical Analysis System (2012) to study the effect of the factors studied according to a complete random design on the studied traits according to the different experiments applied in this study, and the significant differences between the averages were compared with the test of the least significant difference.

RESULTS AND DISCUSSION

The results of the effect of organic matter (O) and pathogen (F.O.l.), and their overlap in the abundance of shrubs in the mycorrhiza parts of the root parts (a%) on the roots of the tomato plant. After two and four weeks of cultivation showed a significant increase estimated at the following values respectively 51.98% and 8.71% in the abundance of shrubs when adding organic matter (o+) compared to the absence of organic matter (o-) where the values were 4.68%, 30.28% and as shown in tables 1 and 2.

The results of the effect of organic matter and the pathogenic fungi (F.o.l.) and their overlap in the abundance of shrubs in the root system (A%) on the roots of the tomato plant after two and four weeks of planting showed a significant increase estimated at the values of 0.331% and 8.641% in the abundance of shrubs in the root system when adding organic matter (o+) compared to the absence of organic matter (o-) where the values were 0.094 , 3.797 and as shown in the table 3 and 4. This is consistent with what Cavagnaro *et al.* (2006) and Alsheikhly and Jabbar (2013) have pointed out that the presence of organic matter in soils containing plant roots coexisting with mycorrhiza has led to the growth of mycorrhiza fungal aggregates in the root system significantly as well as to their improvement and stability within the root plots and within a short period of time and this leads to a great benefit for the plant.

Table 1. Effect of Organic Matter and Pathogen (F.o.l.)They overlap in the abundance of shrubs in the mycorrhizal parts of the (a%) root cut on the roots of the tomato plant after two weeks of planting.

Addition of the mycorrhizal mixture to the triple shrub when planting				
Organic matter	*F.o.l* pathogenic fungi			Organic matter effect
	C	0W+	2W+	
O-	5.59	2.00	6.46	4.68
O+	10.00	3.82	12.30	8.71
LSD(0.05)		1.135		0.655
Pathogenic fungi effect.	7.80	2.91	9.38	
LSD(0.05)		0.803		

Table 2. Effect of organic matter and pathogenic fungi (F.o.l.) and they overlap in the abundance of shrubs in the mycorrhizal parts of the(a%) root cut on the roots of the tomato plant after four weeks of planting.

Addition of the mycorrhizal mixture to the triple shrub when planting					
Organic matter	Pathogenic fungi (*.F.o.l)*				Organic matter effect
	C	0W+	2W+	4W+	
O-	18.83	9.57	41.20	51.50	30.28
O+	54.85	11.83	68.18	73.06	51.98
LSD(0.05)		2.006			1.021
Pathogenic fungi effect (*F.o.l.)*	36.84	10.70	54.69	62.28	
LSD(0.05)		1.431			

Table 3. Effect of Organic Matter and Pathogenic fungi (F.o.l.) and they interference in the abundance of shrubs in the root system(A%)on the roots of the tomato plant after two weeks of planting.

Addition of the mycorrhizal mixture to the triple arbuscular when planting				
Organic matter	Pathogenic fungi (*.F.o.l)*			Organic matter effect
	C	0W+	2W+	
O-	0.110	0.027	0.146	0.094
O+	0.372	0.064	0.558	0.331
LSD(0.05)		0.053		0.031
Organic matter effect	0.241	0.046	0.352	

Table 4. Effect of Organic Matter and Pathogenic fungi (F.o.l.)and they overlap in the abundance of shrubs in the root system (A%) on the roots of the tomato plant after four weeks of plant.

Addition of the mycorrhizal mixture to the triple arbuscular when planting					
Organic matter	Pathogenic fungi (*.F.o.l)*				Organic matter effect
	C	0W+	2W+	4W+	
O-	1.707	0.322	5.658	7.502	3.797
O+	8.812	0.540	11.523	13.687	8.641
LSD(0.05)		0.662			0.338
Pathogenic fungi effect	5.260	0.431	8.591	10.595	
LSD(0.05)		0.441			

The results of the effect of mycorrhizal interference and organic matter and the pathogenic fungi (F.o.l.) in the percentage of lignin in the roots of the tomato plant after four weeks at the mycorrhizal interference in (2W+) showed an urgent increase in the percentage of lignin to 52.59%, which is more than the intervention of the pathogenic fungi only in (2W+) where it reached 37.61%,There is also an increase in the percentage of lignin at the interference of mycorrhiza and organic matter (O × M) to 36.42%, and this result is more than the interference of mycorrhiza(M+) alone and the interference of organic matter (O+) alone, where the results were respectively 33.60%, 27.73% and as shown in table 5. The presence of mycorrhiza increases nutrient absorption and promotes plant growth, including the phosphorus element found in organic matter (Plassard *et al.*, 2019), which activates enzymes responsible for the formation of lignin such as PAL and POD (Lin *et al.*, 2021). It also increases phenolic compounds in plant cell walls, which gives the plant a susceptibility to disease resistance as lignin prevents the loss of nutrients as well as prevents pathogenic fungus from penetrating plant cells (Lin *et al.*, 2022).

Table 5. Effect of mycorrhiza interference, organic matter and the pathogenic fungi in Percentage of Lignin in the roots of the tomato plant after four weeks of planting.

Mycorrhiza fungi	Organic matter	Pathogenic fungi (*F.o.l*)				Interference of mycorrhiza & organic matter (O × M)
		0^-	$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	21.11	4.25	11.65	22.35	14.84
	O^+	25.78	7.55	15.10	27.75	19.05
M^+	O^-	43.52	10.40	21.45	47.74	30.78
	O^+	48.28	17.40	27.41	52.59	36.42
LSD (0.05)			2.358			10.621
Interference of mycorrhiza & pathogenic fungi (*F.o.l* x M)						Mycorrhiza effect
	M^-	23.45	5.90	13.38	25.05	16.94
	M^+	45.90	13.90	24.43	50.17	33.60
LSD (0.05)			3.185			0.864
Interference of mycorrhiza & organic matter (O × M)						Organic matter effect
	O^-	32.32	7.33	16.55	35.05	22.81
	O^+	37.03	12.48	21.26	40.17	27.73
LSD (0.05)		10.962				0.864
Pathogenic fungi effect	34.67	9.90	18.90	37.61		
LSD (0.05)		1.196				

Mycorrhiza interference and organic matter also had a clear effect on the results that showed that the percentage of seedling death after two weeks of cultivation decreased significantly to 14.17% at triple dendritic mycorrhiza interference and organic matter. As for the mycorrhiza interference (M+ only), the percentage was 15.42% and when organic interference (O+) only, the result was 19.58% and as shown in table 6, as the presence of mycorrhiza increases the absorption of nutrients such as phosphorus, potassium and sodium, so phenols increase and the effectiveness of the peroxide enzyme increases (Liu *et al.*, 2020) which leads to an increase in the resistance of the plant to diseases as a result of good nutrition of the plant and reduces the death of seedling (Abohatem *et al.*, 2011).

The results of the percentage incidence of the disease on the leaves of the tomato plant after four and four weeks of cultivation were at the interference of triple arbuscular mycorrhiza and organic matter 14.99%, 19.26% and in the case of mycorrhiza interference (M+) only, the results were 16.10%, % 12.55%, and when the interference of organic matter (O+) only the results were 19.69%, 26.47% as shown in table 7 and 8.

The results of the percentage of the disease occurred on the roots of the tomato plant after two and four weeks of cultivation when the interference between triple mycorrhiza and organic substance %25.05, 31.93 %, and when the mycorrhiza interference, the results were 26.30 %, 35.17 %. When the organic matter interference, the results were 29.95 %, 39.19 %, and as shown in the table 9 and 10.

The mycorrhiza fungus has a clear effectiveness in reducing the severity of vascular wilting disease in the tomato plant because the mycorrhiza fungus improves the nutritional status of the plant, causes a morphological change of roots and induces plant resistance (El-Batanony *et al.*, 2007), if mycorrhiza stimulates the enzymes peroxidase, chitinase and polyphenol-oxidase that have a significant role in increasing plant resistance of diseases (Hemissi *et al.*, 2011). The results showed that the effect of mycorrhiza interference and organic matter has reduced the incidence of root and leaf injury this may be attributed to mycorrhiza helped the plant to grow well because it improved the ability of the plant to absorb water and nutrients, as it helped the plant to grow properly led to the resistance of the plant to the disease (Castañeda-Gómez *et al.*, 2022).

Table 6. Effect of the triple arbuscular mycorrhiza mixture, organic matter and the pathogenic fungi and their overlaps in Percentage of death of seedling contaminated with the pathogen on the tomato plant after two weeks of cultivation.

Triple mycorrhiza mixture	Organic matter	Pathogenic fungi (*F.o.l*)		Interference of mycorrhiza and organic matter (O × M)
		0W+	2W+	
M-	O-	30.00	26.67	28.34
	O+	28.33	21.67	25.00
M+	O-	21.67	11.67	16.67
	O+	20.00	8.33	14.17
LSD(0.05)		2.188		6.501
Interference of mycorrhiza and pathogenic fungi *F.o.l* x M				Mycorrhiza effect
	M-	29.17	24.17	26.67
	M+	20.84	10.00	15.42
LSD(0.05)		2.498		1.415
Interference of organic matter and pathogenic fungi				Organic matter effect
	O-	25.84	19.17	22.50
	O+	24.17	15.00	19.58
LSD(0.05)		6.217		1.415
Pathogenic fungi effect (*F.o.l*)		25.00	17.09	
LSD(0.05)		1.415		

Table 7. Effect of the triple arbuscular mycorrhiza mixture, organic matter and the pathogenic fungi and their overlaps in disease incidence on the leaves of the tomato plant after two weeks of cultivation.

Triple arbuscular mycorrhiza mixture	Organic matter	Pathogenic fungi		Interference mycorrhiza and organic matter (M x O)
		0W+	2W+	
M-	O-	31.13	23.30	27.22
	O+	28.87	19.90	24.39
	O-	22.20	12.23	17.22
	O+	20.00	9.97	14.99
	LSD(0.05)	2.159		6.637
		Interference mycorrhiza and pathogenic fungi		Mycorrhiza effect
	M-	30.00	21.60	25.80
M+	M+	21.10	11.10	16.10
	LSD(0.05)	2.584		1.529
		Interference organic matter and pathogenic fungi		Organic matter effect
	O-	26.67	17.77	22.22
	O+	24.44	14.94	19.69
	LSD(0.05)	6.326		1.529
Pathogenic effect		25.55	16.35	
LSD(0.05)		1.529		

Table 8. Effect of triple shrubby mycorrhiza mixture, organic matter and the pathogenic fungi and their interference in the percentage incidence of disease incidence on the leaves of the tomato plant after four weeks of transplantation.

Triple mycorrhiza mixture	Organic matter	Pathogenic fungi			Interference of mycorrhiza and organic matter
		0W+	2W+	4W+	
M-	O-	51.13	33.33	28.87	37.78
	O+	47.67	28.87	24.47	33.67
M+	O-	38.20	17.80	15.53	23.84
	O+	34.33	12.33	11.13	19.26
LSD(0.05)		2.509			11.216
Interference mycorrhiza and pathogenic fungi					Mycorrhiza effect
	M-	49.40	31.10	26.67	35.72
	M+	36.27	15.07	13.33	21.55
LSD(0.05)		3.558			1.033
Interference organic matter and pathogenic fungi					Organic matter effect
	O-	44.67	25.57	22.20	30.81
	O+	41.00	20.60	17.80	26.47
LSD(0.05)		9.118			1.033
Pathogenic fungi effect		42.83	23.08	20.00	
LSD(0.05)		1.278			

Table 9. Effect of the triple mycorrhiza shrubby mixture, organic matter and the pathogen and their overlaps in the percentage incidence of the disease incidence on the roots of the tomato plant after two weeks planting.

Triple mycorrhiza mixture	Organic matter	Pathogenic fungi		Interference of mycorrhiza and organic matter
		0W+	2W+	
M-	O-	43.30	33.33	38.32
	O+	40.00	29.70	34.85
M+	O-	33.00	22.10	27.55
	O+	31.30	18.80	25.05
	LSD(0.05)	3.008		4.923
		Interference mycorrhiza and pathogenic fungi		Mycorrhiza effect
	M-	41.65	31.52	36.58
	M+	32.15	20.45	26.30
	LSD(0.05)	3.219		2.033
		Interference organic matter and pathogenic fungi		Organicmatter effect
	O-	38.15	27.72	32.93
	O+	35.65	24.25	29.95
	LSD(0.05)	4.715		2.033
Pathogenic fungi effect		36.90	25.98	
LSD(0.05)		2.033		

Mycorrhiza interference compensates for the mass of the roots affected by the disease by means of extended and scattered hyphae between soil minutes, increasing the surface area of absorption, which reflects positively on the plant and increases its ability to resist diseases (Morgan *et al.*, 2005; Maghribi *et al.*, 2018). Wang *et al.* (2022) have pointed out that mycorrhiza fungi work to stimulate genes in the plant when it is infected with vascular wilt disease caused by *F. oxysporum* fungus, which leads to stimulating the plant to resist the disease, and this is considered a genetic quality trait that makes mycorrhiza fungi act as biological control agents to control vascular wilting disease, which is caused by *F. oxysporum* fungus.

Table 10. Effect of the triple mycorrhiza mixture, organic matter and pathogen and their interference in Percentage of the disease incidence on the roots of the tomato plant after four weeks of planting.

Triple mycorrhiza mixture	Organic matter	Pathogenic fungi			Interference mycorrhiza and organic matter
		$0W^+$	$2W^+$	$4W^+$	
M^-	O^-	66.67	52.60	40.00	53.09
	O^+	60.00	46.00	35.50	47.17
M^+	O^-	52.20	35.30	27.70	38.40
	O^+	47.30	27.00	21.50	31.93
LSD(0.05)		4.106			9.526
Interference mycorrhiza and pathogenic fungi					Mycorrhiza effect
	M^-	63.34	49.30	37.75	50.13
	M^+	49.75	31.15	24.60	35.17
LSD(0.05)		4.336			2.319
Interference organic matter and pathogenic fungi					Organic matter effect
	O^-	59.44	43.95	33.85	45.75
	O^+	53.65	36.50	28.50	39.55
LSD(0.05)		9.136			2.319
Pathogenic fungi effect		56.54	40.23	31.18	

CONCLUSIONS

The shrubby mycorrhiza fungus defined as a fungus that naturally coexists with the roots of plants, it is represented with organic matter in addition to being an environmentally friendly organic fertilizer that is considered an anti-vascular wilting disease in the tomato plant, which is caused by the pathogenic fungus *Fusarium oxysporum f. sp. lycopersici* and is free of harmful chemical compounds, so it is safe and environmentally friendly.

AUTHOR CONTRIBUTIONS

The authors alone are responsible for the content and writing of the paper.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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