INDUCTION OF RESISTANCE IN TOMATO PLANTS AGAINST *F. oxysporum* F.SP*. lycopersici* BY ARBUSCULAR MYCORRHIZAL (AM*)* FUNGI

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ABSTRACT

Systemic acquired resistance (SAR) plays an important role in the ability of plants to defend themselves against pathogens. Studied the effect of Arbuscular Mycorrhizal (AM*)* Fungi on induced resistance against Fusarium Wilt caused by *Fusarium oxysporium* f.sp. *lycopersici* have been conducted. The results showed that M+P+NPK and M+P have ability to reduce the %of disease incidence of wilt disease by 93.3%, 66.7%respectively and reduction the % of disease severity by 97.3%, 86.1% respectively While treatment with fungicide F1 and F2 gave the lowest reduction to disease incidence% by 46.6%, 66.7% respectively, disease severity% by 45.4%, 43.4% respectively. The results of the effect of AM fungi on growth parameters conducted that a significant stimulation plant growth characters that including fresh weight dry weight, root fresh weight, root dry weight, shoots length, number of leaves, and leaf area. In this connection total chlorophyll and carotenoids were significant increased in mycorrhizal tomato plants as compared to other treatments. M+P+N was the best treatment which increased total chlorophyll and carotenoids (129.2%, 107.1 %), respectively this was followed by M+P+NPK by 116.7%, 78.6 %. Studied the effect of AM fungi on total phenols and found a significant increase in total phenol contents M+P+NPK gave the highly increase 75.6 % while M+P by 47% compared to the check. While F2 and Topsin-70 were gave increase, 20.3 %, 16.3 %, respectively compared to the check. The activity of TAL, POX, PPO, ACP and ALP of mycorrhizal tomato plants were significantly increased as compared to other treatments. In the same mannes, AM fungi increasing significantly the total nitrogen, phosphorous and protein.

Keywords: *Fusarium oxysporum* f.sp. *lycopersici*- Arbuscular Mycorrhizal Fungi-Tomato plants-Induced Resistance-Fungicide-Fusarium wilt.

Abbreviations: AM: arbuscular mucorrhizae, TAL: tyrosine ammonia lyase, POX: peroxidase, PPO:poly phenol oxidase, ACP:acid phosphatase, ALK:alkaline phosphatase, M+P+NPK:AM+Fusarium+mineral fertilizer(NPK) and M+P: AM fungi+Fusarium, F1: TopsinM-70+Pathogen; F2: F2 +Pathogen, SAR: Systemic acquired resistance; JA: jasmonic acid; SA: salicylic acid.

INTRODUCTION

Tomato (*lycopersicon esculentum*, Mill) is considered as one of the most important cash crops in several production countries. World losses in tomato yield can be referred to soil-born pathogens. Fusarium wilts diseases, caused by pathogenic formae specials of the soil-inhabiting fungus *Fusarium oxysporum* (Sacc.) W.C. Snyder and H.N.Hans., cause severe losses in a wide variety of crop plants including tomato crop Interest in biological control

has increased fuelled by public concerns over the use of chemicals in the environment and the need to find alternatives to the use of chemicals for disease control. Systemic acquired resistance (SAR) plays an important role in the ability of plants to defend themselves against pathogens. SAR occurs in all or most plants in response to attack by pathogenic microorganisms, physical damage due to insects or other factors, treatment with various chemical inducers and colonization of AM fungi (Kuc, 2001; and Pozo and Azcon-Aguilar, 2007) Microbial antagonists (*Glomus mosseae, Glomus clarum*, *Glomus intraradices*, *Gigaspora margarita* and *Gigaspora gigantea*) have been used with some success for controlling wilt diseases of tomato plants (Yuen *et al*., 1994 and El- Khallal, (Samia), 2007). Root colonization by AM-fungus frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Arora *et al.,* 1992). Crop productivity in fields can increase up to 300% after the addition of AM-Fungus in experiments carried out in greenhouses (Aducci *et al.,* 1997). Shoot and root growth of tomato plants grown in soil infected with *F. oxysporum*, inhibited as compared with non-infected control. Actually, decrease in fresh weight of infected tomato shoots may be due to the toxins produced by the fungi, which affected K uptake and stomata function leading to uncontrolled transpiration and excessive loss of water leading to wilted plants (Aducci *et al.,* 1997). However, reduction in shoot dry weight might be related to increased rate of respiration, decompartementalization due to membrane degradation (Orcutt and Nilsen 2000). This represents an indirect contribution to bicontrol through the conservation of root system function, both by fungal hypha growing out into the soil and increasing the absorbing surface of the roots, and by the maintained of root cell activity through arbuscular formation (Cordier *et al.,* 1998; Whipps 2004; Morgan *et al.,* 2005). This could be mediated by effects on the level of cytokinins, which are well known factors of cell division and growth (Haberer and Kieber, 2002). The increase in shoot and root length in tomato plants especially treated with AM fungi may be related to the action of cellulose and pectinases of *Fusarium* on host cell walls which would decrease the level of lignin cell wall- bound phenolic compounds, affect mechanical properties of cell wall, result in cell wall length (Ikegawa *et al.,* 1996). The induction of defense related enzymes by VAM-fungi treatments was correlated with the percentage vascular wilt suppression in the treated plants upon challenge inoculation with the fungal pathogen. The first enzyme of tyrosine propanid metabolism pathway is ammonia tyrosine lyase (TAL), the catalyze the trans-elemination of ammonia from tyrosine to P-coumaric acid which in turn enter different biosynthetic pathways leading to lignin (Hanhlbrock and Grisebach, 1975). Increase in the TAL activity which observed in this study explains that the ISR in tomato plants treated with compost and VAM-fungi may be related to biosynthesis of lignin orginate from tyrosine, similar results were obtained by many investigators in several crops (Anderson and Guerra, 1985; Van Peer *et a*l., 1991; Podile, Lami, 1998; Meena *et al.,* 1999 and El-Zahaby, 2008). Environmental pollution problems and development of plant resistance pathogens have occurred due to the toxicity of fungicides. Instead of these chemicals, arbuscular mycorrhizal

fungi (AM) are used as biocontrol agents for several plant pathogens, by reducing the susceptibility or increasing the tolerance of plants to plant pathogens (Rabie, 1998 and Abdalla and Abdel-Fatah, 2000). In this connection, under greenhouse experiments, our studies demonstrated that infection of tomatoes plants by *F. oxysporum* f. sp*. lycopersici*, reduced growth parameters, photosynthetic pigments, carbohydrates content, P&N, contents, total protein, reducing sugar, enzyme activities i.e. tyrosine ammonia lyase(TAL), acid and alkaline phosphatase.

MATERIALS AND METHODS

1-Preparation of the fungal pathogenic inoculum and AM Fungi:

Inoculum of the virulent isolate of *Fusarium oxysporum* f.sp*. lycopersici* was grown for 10 days at 25°C in a steam sterilized sorghum grains medium (25 g of clean sand and 75g of sorghum grains plus enough distilled water to cover the mixture (Sneh *et al.,* 1991). The inoculum growth was thoroughly mixed, added to pots of 35cm-diameter filled with clay-sandy soil (1:1w/w) at the rate 3% v/v of inoculums per pot and then spreaded uniformly onto the soil surface. The infested soils were moistened and mixed thoroughly every other day. Pots containing soil mixed with the same amount of non-infested medium were served as a check. Tomato seeds of the cultivar Super Marmande were grown in pots containing clay-sandy soil. Seedlings of 40 day-old were transplanted in soil infested with *Fusarium oxysporum* f.sp*. lycopersici* at a rate of 5 seedlings / pot. Three pots were presented one isolate. Infection percentage and disease severity were recorded 40 days after sowing on the base of the above mentioned scale. Disease incidence and disease severity were recorded according to Mihuta *et al.*, (1990).

Production of arbuscular-mycorrhizal fungi:

A mixture of arbuscular mycorrhizal fungi (AM) spores, extracted from the rhizosphere of Lycopersicum *esculantum* plants by wet sieving technique (Gerdemann and Nicolson, 1963) and multiplicated on roots of Sudan grass plants for three months. the inoculums of AM fungi added with rate 50 g/pot. This mixture contained different genera and species of VAM fungi as follows: *Glomus mosseae, Glomus clarum*, *Glomus intraradices*, *Gigaspora margarita* and *Gigaspora gigantea*. These fungi were completely identified by Prof. of Mycology, Prof Dr. Gamal M. Abdel Fattah. Department of Botany, Mansoura University, Egypt.

2-Staining of AM infected root according to (Phillips and Hayman, 1970)

3-Estimation of VAM Infection:

The rate of VAM infection was microscopically estimated according to the method of Trouvelot, *et al.,* (1986). This method calculates three parameters of infection as follows:

F: Frequency of root infection (percentage) of root segments infected.

M: Intensity of cortical infection (proportion of cortical infection in all the mycorrhizal root system).

A: Arbuscule frequency in roots (percentage) of arbuscular infection of this investigation.

4- Commercial name, recomende dose and method of application of Fungicide:

Commercial name and formulation	Recommend dose gm/L	Method of application
F2 50 % WP	3 g	Soil drenching
Topsin-M 70 % WP	1.5 g	Soil drenching

5-Analysis of growth parameter:

The growth parameters of tomato plants were measured as follow: Shoot (cm), Shoot fresh and dry weight g/ plant, Root fresh and dry weight (RFW & RDW), Number of leaves, leaf area cm^2.

6-Estimation of photosynthetic pigments: were estimated according to Metzner *et al.,* (1965).

7-Estimation of Enzymes Activity

7-1Tyrosine ammonia lyase (TAL) according to Beaudoin-Egan and Thorpe (1985).

7-2: Polyphenoloxidase (PPO) according to Maria *et al.,* (1981).

7-3: Peroxidase (POD) according to Maxwell and Bateman (1967**).**

7-4: Phosphatase according to Abdel-Fattah (1991).

8:Estimation of total Phenol according to Maliak and Singh (1980)

9- Estimation of total nitrogen according to Hesse (1971).

10- Estimation of total phosphorus: Total phosphorus of plant was determined by the vando-molybdo-phosphoric colorimetric method in nitric acid (Jackson, 1958).

11- Estimation of Protein: The method of protein extraction followed is that described by Scarponi and Perucci (1986).

12- Statistical analysis

Data were analyzed with the statistical analysis system (SAS institute, 1988). All multiple comparisons were first subjected to analysis of variance (ANOVA) comparisons among means were made using least significant differences (LSD) at p=0.05 and Duncan's multiple range test (Duncan, 1955)

RESULTS AND DISCUSSION

Effect of AM with different levels from NPK on disease severity %, disease incidence %:

Data presented in table (1) showed that AM-fungi cause a significant reduction on these parameters. the highest reduction on the % of disease incidence and disease severity was 93.3%, 97.3%, in case of M+P+NPK, respectively this agreement with Abdalla and Aabdel-fattah (2000), Sadek, (Marwa), (2005), El- Khallal, (Samia) (2007), Metwally (2007), Mucharromah and Harahap (2007), Kapoor (2008)

Table (1): Effect of AM Fungi the percentage of disease severity, disease incidence

Treatment	Disease incidence %		Disease severity %	
	Mean	Reduction %	Mean	Reduction %

Control	١٠٠a	٠٠,٠	٨٢,٢٣a	٠,٠٠
M+P	٣٣,٣c	٦٦,٧	١١,١c	٨٦,١
M+P NPK	٦,٧d	٩٣,٣	٢,٢٣c	٩٧,٣
F1	٥٣,٣b	٤٦,٦	٤٣,٧b	٤٥,٤
F2	٣٣,٣c	٦٦,٧	٤٤,٧٣b	٤٣,٤

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test(p= 0.05)

2-Effect of the pathogen and NPK on the levels of mycorrhizal infections.

The levels of mycorrhizal colonization of tomato roots are shown in Table (2) and expressed in three parameters; frequency of mycorrhizal root segments (F %), intensity of mycorrhizal infection in the root tissue (M %), and rate of arbuscular formation in root segment (A %). The first gives an estimate of the rate of fungal colonization from the soil and of growth within the root, while the second indicates the extent of colonization by the fungus relative to root infection. The levels of mycorhhizal infection were increased significantly by increased the levels of NPK than as compared to control treatment. F% was 77.5%, 55.5%, 77.5%, 67.5%, in case M +NPK, M, M+P+ NPK and M+P , respectively, M% was 7.45%, 1.25%, 5.93%, 3.85%, in case M +NPK, M, M+P+ NPK and M+P , respectively, A% 3.15%, 0.75%, 3.15%, 1.75%, in case M+ NPK, M, M+P+ NPK and M+P,, respectively

Table (2): **levels of mycorrhizal infection in tomato roots plants infected by the pathogen and treated with NPK.**

TREATMENT	F %	M %	A %
NM	٠٠,٠	٠٠,٠	٠٠,٠
M	٥٥,٠	١,٢٥	٠,٧٥
M+ NPK	٧٧,٥	٧,٤٥	٣,١٥
M+P	٦٧,٥	٣,٨٥	١,٧٥
M+P NPK	٧٧,٥	٥,٩٣	٣,١٥

3-Effect of AM Fungi on growth parameters of plants cultivar Super Marmande grown in infested soil with *F. oxysporum* f.sp. *lycopersici*.

Data in table (3) revealed that pathogen caused reduction in growth parameters, while in case of Mycorrhizal colonization treatments give a significant stimulation in plant growth and its characters. Colonization of tomatoes plants by VA-mycorrhizal fungi often results in stimulated plant growth when compared to non-mycorrhizal plants either infected or not infected by *F. oxysporum* f.sp*. lycopersici.* In this situation, mycorrhiza are more effective for tomatoes plants in phosphate and nitrogen nutrition than those in non-mycorrhizal plants. The translocation of phosphorus through mycorrhizal hyphae to the host plant can significantly improve both plant growth under the effect of pathogen . such stimulation was related to the degree of mycorrhizal infection, **Asimi *et al*. (1980); Pacovsky, (1986); Abdel-Fattah and Shabana, (2002); Sadek, (Marwa), (2005); El-Khallal, (Samia) (2007); Metwally (2007)**; **Mucharromah and Harahap (2007) and Kapoor (2008.**

Table (3): Effect of AM Fungi and NPK treatments on growth parameters of plants cultivar Super Marmande grown in infested soil with *F. oxysporum* f.sp. *lycopersi*

Treatment	Shoot weight (g/ plan)		Root weight (g / plant)		Shoot height (cm)	Number of leaves	Leaf area cm^2
	Fresh	dry	Fresh	dry			
	mean	mean	mean	mean			
Control	٤٠,٣bc	7.5 bc	٣,٠cd	٠,٨ c.	49.5 c	١٥bc	٦٨٦,٦bc
P	٢٠,٥d	٤,٥d	٠,٥e	٠,١d	٣٥,٠d	8 d	٢٧٤,٢d
M	٦٥,٧ a	٩,٥a	7.7 a	٢,٣c	٣٥,٠d	١٧abc	٩٩٠,٣ ab
M+NPK	٧٠,٢ a	١٠,١a	٨,٠a	٤,٠a	49.5 c	٢١a	٩٧٦,٩ab
M+P	٥٩,٣ a	٩,٠ab	٥,٠bc	١,٩c	٤٩,٣abc	١٦abc	١١٥٣,٢a
M+P+NPK	٧٠,٠a	٩,٣ab	٦,٦ab	٣,٠b	٥٩,٠ab	١٩ab	٥٦٥,٤cd
F1	٣١,٨cd	6.5 bcd	٣,٧cd	٠,٧d	٤٥,٠bcd	17 abc	٧٤٨,١bc
F2	٢٦,٠cd	٥,٤cd	١,٥de	٠,٤d	٤٣,٠cd	١٢cd	٧٤٨,١bc

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test(p= 0.05).

4-Effect of AM Fungi and NPK treatments on total chlorophyll and carotenoids in tomato leaves of plants cultivar Super Marmande grown in infested soil with *F. oxysporum* f.sp. *lycopersici*.

Data presented in table (4) showed that Infection of tomato plants with *F .oxysporum* f.sp. *lycopersici* resulted in a decreased in pigments contents compared to control healthy. *F .oxysporum* f. sp. *lycopersici* caused highly significantly decreases in chlorophyll a, b, total and carotenoids as compared to healthy control. Increased in chlorophyll a, b, total and carotenoids of tomato plants. M+P was the best treatment which increased chlorophyll a, b, total and carotenoids (75 %, 150 %), respectively This was followed by M+P+NPK, when compared with control. F1 was gave increased in total chlorophyll and carotenoids (118.1, 171.4 %), respectively, while F2 was gave increased in total chlorophyll and carotenoids (150 %, 107.1%), respectively. Several reported confirmed decreased in photosynthetic pigment contents due to microorganism infection, **Murumkar and Chavan, (1985); Shalaby, (1988) and Mohamed, (1995)**. They stated that, chlorophyll contents of chickpea, tomato and bean significantly decreased, respectively in response to the infection with different strains of *F. oxysporum*. The reduction in chlorophyll content of infected plants may be due to destructive effect of phytopathogens or its metabolites on chloroplasts (**Hegazy, 1993 and Mohamed, 1995)** or decreased protein synthesis, **Abou-Zaid *et al.,* (1987) and Pundir *et al.,* (1991).** Such influence might subsequently lead to an inhibition in chlorophyll formation and chloroplast development, **Das, (1973)**. **Krasichkova *et al*., (1991)** detected decreases in chlorophyll and carotenoid contents and electron transport activity in cotton following infection with Fusarium wilt at early stage of development. The adverse effects of the fungus on chlorophyll pigments might come from chelate formation with iron. In fact, some authors found that the fungal toxins form iron-chelate transforming iron to become unavailable to participate in chlorophyll synthesis, **Kern, (1972) and Goodman *et al*., (1967)**. Treatments with the

bioagents favoured greater accumulation of these pigments in the infected plants. Stimulatory effect of biocontrol agents on chlorophyll contents was similar to those of *Azotobacter chroococcum* on *Zea mays*, (**El-Hoseiny and Rabie, (1979)** and *Daturia stramonium*, **Husein, (1993).**

Table (4): Effect of AM Fungi on total phenols (mg/g fresh leaves) in tomato leaves of plants cultivar Super Marmande grown in infested soil with *F. oxysporum* f.sp*. lycopersici.*

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll		Carotenoides	
			Mean	Increase %	mean	Increase %
Control	٠,٦bc	٤,٦b	b٥,٢	١١٨,١	٣,٨a	١٧١,٤
P	٠,٧b	٥,٢a	٦,٠a	١٥٠,٠	٢,٩ab	١٠٧,١
M	١,٣a	٤,٢c	٥,٥ab	١٢٩,٢	2.9 ab	١٠٧,١
M+NPK	٠,٩b	4.3 bc	٥,٢ab	١١٦,٧	2.5 b	٧٨,٦
M+P	١,٤a	4.9 b	6.3 a	١٦٢,٥	٣,٥a	١٥٠,٠
M+P+NPK	١,١a	4.4 bc	5.5 ab	١٢٩,٥	2.9 ab	١٠٧,١
F1	١,٣a	4.7 b	6.0 a	١٥٠	٣,٨a	١٧١,٤
F2	٠,٥bc	1.8 d	٢,٤c	٠٠,٠	١,٤c	٠٠,٠

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test(p= 0.05).

5-Effect of AM Fungi on total phenols.

Data presented in table (5) showed a significant increase in phenol contents in all treated. M+P+NPK gave the highly increase 75.6 % compared to the check. While F2 and Topsin-70 were gave increase, 20.3 %, 16.3 %, respectively compared to the check . AM colonization led to a significant increase in the phenolic content of tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici*, suggesting that these parameters are implicated in disease resistance and although they are found in healthy as well as diseased plants, their synthesis or accumulation seems to be accelerated after AM colonization. These results are in agreement with that of, **El-Khallal, (2007)** who recorded an increase in various physiological defenses including antioxidant enzymes, phenolic compounds and pathogenesis related (PR) proteins in tomato plants colonized with AM fungi and infected with *Fusarium oxysporum*. Many plant phenolic compounds are known to be antimicrobial, function as precursors to structural polymers such as lignin, or serve as signal molecules and constitute an active defense response, **Hammerschmidt, (2005)**. These defense responses may include the elaboration of cell wall thickenings usually accompanied by the synthesis and deposition of lignin, a polymer of aromatic phenolics. These thickening limited the infection process and played an important role as a physical barrier to stop the pathogen invasion. These thickening were confirmed by our TEM investigations. Results from many studies suggest that esterification of phenols to cell wall materials and the accumulation and deposition of phenols in and on cell walls is usually considered as an increase in resistance to fungal hydrolytic enzymes as well as a physical barrier against fungal penetration**, Saldajeno *et al.*, (2008).**

Table (5): Effect AM Fungi on total phenols (mg/g fresh leaves) in tomato leaves of plants cultivar Super Marmande grown in infested soil with *F. oxysporum* f.sp*. lycopersici*.

Treatment	Total Phenols mg/100 g fresh weight	
	Mean	Increase %
P	٢٠٨,٨٤b	٠٠,٠
M+P	٣٠٧a	٤٧
M+P+NPK	٣٦٦,٨a	٧٥,٦
F1	٢٤٢,٨٠b	١٦,٣
F2	٢٥١,٩٣b	٢٠,٦

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test(p= 0.05),

6-Effect AM Fungi and fungicides application on activity of TAL, POX, PPO, ACP and ALP Enzymes of tomato plants of cultivar Super Marmande grown in soil infested with *F. oxysporum* f.sp*. lycopersici*.

The effects of AM Fungi and fungicide application on the activity of TAL, POX, PPO, ACP and ALP **enzymes** of tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici* are presented in table (6). The data revealed that, significantly increased the activity of TAL, POX, PPO, ACP and ALP **enzymes** of tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici*, in the root of tomato plants colonized by AM fungi. AM as compared to control a highly significantly decreased was observed in the activity of TAL, POX, PPO, ACP and ALP **enzymes** of tomato plants infested with *Fusarium oxysporum* f.sp. *lycopersici* with fungicide or without application fungicide, however the rate of reduction in, the activity of TAL, POX, PPO, ACP and ALP **enzymes** was remarked in non-mycorrhizal plants that infected with *Fusarium oxysporum* f.sp. *lycopersici* alone particularly. The present study revealed increase in the POX (Preoxidase) activity due to AM-fungi. POX is involved in lignification leading to disease resistance, **Lagrimini *et al.*, (1987)** for lignification, specific cell wall peroxidase are thought to be required to generate H_2O_2, **Van Huysatee, (1987),** this is agreement with, **Nandakumar *et al.*, (2001)**, who published that ISR in rice has been correlated with an increase in activity of pathogensis related peroxidase in PGPR treated plants inoculated with the rice sheath pathogens, *Rhizoctonia solani.* Extracellularly secreted plant peroxidases (POXs) are considered to catalyze the generation of reactive oxygen species (ROS) coupled to oxidation of plant hormone indole-3-acetic acid (IAA) and defense-related compounds salicylic acid (SA), aromatic monoamines (AMAs) and chitooligosaccharides (COSs), describe H_2O_2-dependent and H_2O_2-independent mechanisms for ROS generation, respectively, plant POXs oxidize SA, AMAs and COSs in the presence of H_2O_2 via a conventional POX cycle, yielding the corresponding radical species, such as SA free radicals. These radical species may react with oxygen, and superoxide (O_2) is produced. Through the series of reactions 2 moles of O_2 can be formed from 1 moles of H_2O_2, thus leading to oxidative burst. It has been revealed that the ROS induced by SA, AMAs and COSs triggers the increase in cytosolic Ca^{2+} concentration, Actually POXs transduce the extracellular signals into the

redox signals that eventually stimulate the intracellular Ca^{2+} signaling required for induction of defense responses, On the other hand, IAA can react with oxygen and plant POXs in the absence of H_2O_2, by forming the ternary complex enzyme-IAA-O_2, which readily dissociates into enzyme, IAA radicals and O_2, extracellularly produced hydroxy radicals derived from O_2 mediate the IAA-induced cell elongation. Here a novel model for IAA signaling pathway mediated by extracellular ROS produced by cell-wall POXs is proposed. In addition, possible controls of the IAA-POX reactions by a fungal alkaloid **(Kawano, 2004).** AM symbioses have a significant impact on plant interactions with other organisms. Increased resistance to soil-borne pathogens has been widely described in mycorrhizal plants. Among the potential mechanisms involved in the resistance of mycorrhizal systems, the induction of plant defenses is the most controversial **(Pozo *et al.*, 2002).** During mycorrhizal colonization, modulation of plant defense responses occurs, potentially through jasmonic acid (JA) dependent signaling pathway. Where, JA-responsive genes and genes involved in JA biosynthesis are expressed in arbuscule containing cells, and mycorrhizal roots are associated with increased levels of endogenous JA. This increase occurs after the onset of mycorrhization, likely associated with fully established mycorrhizas **(Hause *et al.,* 2002)**. Elevated levels of basal JA production could be related to the increased resistance of mycorrhizal plants to pathogens **(Hause *et al*., 2007).** JA signaling pathway is characterized by the production of a cascade of PR proteins. Where a number of biochemical and physiological changes has been associated with mycorrhizal colonization. These include; the production of antifungal chitinases, glucanases, and oxidative enzymes **(Pozo *et al,*. 2002)** such as peroxidases, polyphenoloxidases and lipoxygenases (**Pozo and Azcon-Aguilar, 2007**); Low molecular weight compounds with antimicrobial properties (phytoalexins) can also accumulate **(Yao et al., 2003)**; cell death and deposition of callose and lignin **(Saldajeno et al., 2008)** and activation of genes involved in plant defense responses such as those coding for PR proteins and defenses **(Liu et al., 2007).** Our results indicated that, AM colonization led to a significant increase in the phenolic content and the activities of the investigated defense enzymes TAL and PPO of tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici*, suggesting that these parameters are implicated in disease resistance and although they are found in healthy as well as diseased plants, their synthesis or accumulation seems to be accelerated after AM colonization. These results are in agreement with that of **El-Khallal, (2007)** who recorded an increase in various physiological defenses including antioxidant enzymes, phenolic compounds and pathogenesis related (PR) proteins in tomato plants colonized with AM fungi and infected with Fusarium oxysporum. Many plant phenolic compounds are known to be antimicrobial, function as precursors to structural polymers such as lignin, or serve as signal molecules and constitute an active defense response (**Hammerschmidt, 2005).** These defense responses may include the elaboration of cell wall thickenings usually accompanied by the synthesis and deposition of lignin, a polymer of aromatic phenolics. These thickening limited the infection process and played an important role as a physical

barrier to stop the pathogen invasion. These thickening were confirmed by our TEM investigations. Results from many studies suggest that esterification of phenols to cell wall materials and the accumulation and deposition of phenols in and on cell walls is usually considered as an increase in resistance to fungal hydrolytic enzymes as well as a physical barrier against fungal penetration **(Saldajeno *et al.,* 2008).** In addition to cell wall thickening, phenolics seem to inhibit disease development through different mechanisms involving the inhibition of extra cellular fungal enzymes (cellulases, pectinases, lactase, xylanase), inhibition of fungal oxidative phosphorylation, nutrient deprivation (metal complexation, protein insolubilisation), and antioxidant activity in plant tissues, **Hammerschmidt, (2005)**. We suggest that the increase in phenolic content in tomato plant inhibited the *Fusarium oxysporum* f.sp. *lycopersici* growth .

Table (6): Effect of AM Fungi on activity of TAL, POX, PPO, ACP and ALP .

Treatment	TAL Activity $\Delta A_{233}.g^{-1}.min^{-1}$	POX Activity ΔA_{470} min^{-1} g^{-1}	PPO Activity ΔA_{420} min^{-1} g^{-1}	ACP Activity $\Delta A_{410}.g^{-1}.min^{-1}$	ALP Activity $\Delta A_{410}.g^{-1}.min^{-1}.$
Control	٨,٣b	٣,٣b	٠,٩c	7.2 ab	1.4 d
P	٦,٢c	٥,١b	١,٠c	4.5 c	0.7 e
M	8.6 b	5.8 b	1.1 bc	8.0 a	2.1 c
M+NPK	8.8 ab	6.0 b	1.2 bc	8.2 a	2.1 c
M+P	8.8 ab	8.2 ab	1.7 b	8.1 a	2.9 b
M+P+NPK	٩,٤a	13.4 a	2.9 a	8.2 a	6.0 a
F1	٨,٣b	٧,٢b	٠,٩c	٧,٢ab	1.7 c
F2	٨,١b	٥,٥b	١,٠c	٦,٨b	1.9 c

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test(p= 0.05),

7-Effect of AM Fungi on total nitrogen, phosphorus and protein of tomato plants cultivar Super Marmande grown in infested soil with *F. oxysporum* f.sp*. lycopersici*.

Our results showed that AM Fungi increased total nitrogen, phosphorus and protein in tomato plants infected with or without pathogen . Colonization of tomato plants by AM fungi often results in stimulated plant growth when compared to non-mycorrhizal plants infected or not infected by *F. oxysporum* f.sp*. lycopersici.* In this situation, mycorrhizae are more effective for tomato plants in phosphate and nitrogen nutrition than those in non-mycorrhizal plants. The translocation of phosphorus through mycorrhizal hyphae to the host plant can significantly improve both plant growth, **(Pacovsky, 1986)** and levels of nodulation, **Asimi *et al*., (1980) and Abdel-Fattah and Shabana, (2002).** Such stimulation was related to the degree of mycorrhizal infection The highly significant shoot biomass production by mycorrhizal plants, could be attributed to enhanced inorganic nutrition absorption and greater rates of photosynthesis, **Cooper, (1984).** AM Fungi have been said to affect both uptake and accumulation of nutrient. Moreover, our results showed that,

inoculating tomato plants with arbuscular mycorrhizal fungi significantly increased the root biomass production, where mycorrhizal roots have been known to absorb phosphorus faster than non-mycorrhizal plants, **Jakobsen *et al.,* (1992)** and enhance the absorption of nutrient from the soil, this could have resulted in a higher root biomass in mycorrhizal plants. These results were in good agreement with the findings of **El-Ghandour *et al.,* (1995).**there are significant differences were observed with regard to leaf area. Total phosphorus, nitrogen, protein and total phenol contents in mycorrhizal tomatoes plants were significantly greater than those of non-mycorrhizal plants infected or not infected with *F. oxysporum* f. sp*. lycopersici*. These observations are in good agreement with other studies of **Ames *et al.,* (1983); Gianinazzi-Pearson *et al*., (1981) and Abdel-Fattah, (1991). Sanders and Sheikh (1983),** they reported that the inflows of phosphorus to mycorrhizal roots can be greater than inflows to comparable non-mycorrhizal roots by 2.5 times, also it is evident from the present study that mycorrhizal association was one way of guaranteeing phosphorus absorption from reserves in the soil. . Results revealed that infection with *Fusarium oxysporum* markedly decreased N and P contents shoot system of tomato plants as compared with non- infected control. Reduction in N and P uptake in tomato tissues could be correlated with pathogenesis when root tissues already attacked, affecting its ability to take up water and nutrients from soil or they may be leached out from macerated tissues**, Nafie (2003).** Bioagents application AM Fun influenced the N and P contents in the direction of enhancing growth and reducing the disease symptoms. Treatment with AM fungi had a higher N and P contents than those of VAM fungi. Increase in P supply as a direct consequence of VAM fungi colonization had positive effect on N accumulation, leaf area and biomass production in *Vicia faba,* **Jia *et al.,*(2004); Bucher (2007). However, Kasiamdari *et al.,* (2002), Fritz *et al.,* (2006)** reported that improved in P nutrition with or without mycorrhizal colonization appeared only in the form of stimulated plant growth and had little effect in reducing the disease rating , suggesting other disease suppression mechanisms may be involved. One possibility is that defense gene expression is mediated by a signaling mechanism that senses the level of P in the root, resulting in an up regulation of defence genes (catalase, chitinase and glucanase genes). Finally, results revealed that application with VAM fungi could be effective in enhancing uptake of some inorganic nutrients (N, P, K, Ca, Zn and Mn) which play a role in a decrease in the incidence of wilt *Fusarium* disease. Thus improvement in plant nutrition can enhance plant

Table (7) : Effect of AM on total nitrogen, phosphorus and protein of dry weight of tomato plants .

Treatment	Total nitrogen mg.g^{-1}		Total phosphorus mg.g^{-1}		Total protein mg.g^{-1}	
	Mean	Increase%	Mean	Increase%	Mean	Increase%
Control	٢,٣c	٢٨٣,٣	٣١,٦bc	٧٩,٢	١٤,٤c	٢٨٣,٣
P	٠,٦d	٠٠,٠	١٧,٦c	٠٠,٠	٣,٨e	٠٠,٠
M	٥,٢ a	766.7	٥٩,٦ a	238.6	32.5 a	755.2
M+NPK	٥,٠ab	٧٣٣,٣	٣٨,٩b	١٢٠,٣	٣١,٣ab	٧٣٣,٣
M+P	٤,٨ab	٧٠٠,٠	٤٤,٥b	١٥٢,٢	٣٠,٠ab	٧٠٠,٠
M+P+NPK	٤,٦ab	٦٦٦,٧	٤١,٥b	١٣٥,٣	٢٦,٨ab	٦٦٦,٧
F1	٤,٠ab	٥٦٦,٧	٣٢,٨b	٨٥,٧	٢٥,٠b	٥٦٦,٧
F2	٣,٤bc	٤٦٦,٧	٣١,٧bc	٧٨,٩	٣٨,٨a	٤٦٦,٧

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test(p= 0.05).

Finally, the results obtained here concluded that the application of bioagent (AM fungi) could be used to overcome the negative effect of Fusaruium wilt disease in tomato plants. In this connection, these bioagents treatments are best beneficial effects as compared to chemical agents (Topsin M-70 and Rhizolex-T).

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استحثاث المقاومة ضد مرض الذبول الفيوزارمى في الطماطم *Fusarium oxysporium* f.sp. *lycopersici* باستخدام الفطريات الجذرية الشجيرية.

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تم دراسة تأثير الفطريات الجذرية الشجيرية (AM) على استحثاث المقاومة وتثبيط فطر *Fusarium oxysporium* f.sp. *lycopersici* وجد أن فطريات الميكروهيزا باضافة التسميد المعدني وبدون إضافة تحدث تثبيط لنسبة الإصابة بنسبة 93.3%و 66.7% على التوالي وتحدث تثبيط لشدة الإصابة بنسبة 97.3 %و 86.1% على التوالي بينما تعطى المبيدات الفطرية (توبسين ام-٧٠ والريزولكس- تى) تثبيط لنسبة الإصابة بنسبة ٤٦,٦% و ٦٦,٧% وشدة الإصابة بنسبة ٤٥,٤% و٤٣,٤ % على التوالي .إضافة فطريات الميكروهيزا باضافة التسميد المعدني وبدون إضافة أدى إلى تحسين صفات النمو وتقليل تأثير المرض على صفات النمو المختلفة((الوزن الطازج والجاف للمجموع الخضري والمجموع الجذري – طول المجموع الخضري والجذري- عدد الأفرع/نبات- عدد الأوراق المركبة – مساحة الورقة/ بالسم٢).

دراسة التأثير على الكلوروفيل الكلى وصبغة الكاروتين فوجد أنه في حالة التسميد المعدني مع الميكروهيزا كان الأفضل حيث أعطى نسبة زيادة ١٢٩,٢ % و١٠٧,١% على التوالي بينما أعطت في حالة عدم الإضافة زيادة ١١٦,٧ % و ٧٨,٦ % بينما أعطت النباتات المعاملة بالمبيدات الفطرية توبسين-م-٧٠ زيادة ١١٨,١ % في الكلوروفيل الكلى ١٧١,٤ % في صبغة الكاروتين وريزولكس-تى زيادة الكلوروفيل الكلى ١٥٠ % في صبغة الكاروتين ١٠٧,١ % على التوالي.

تم دراسة التأثير على الفينولات الكلية فوجد أنه في حالة إضافة التسميد المعدني مع الميكروهيزا كان الأفضل حيث أعطى نسبة زيادة ٧٥,٦ % بينما أعطت زيادة في حالة عدم الإضافة ٤٧% بينما أعطت النباتات المعاملة بالمبيدات الفطرية توبسين-م- ٧٠ وريزولكس-تى زيادة في الفينولات الكلية بنسبة١٦,٣ % و ٢٠,٦ % على التوالي

وجد أن فطريات الميكروهيزا تحدث زيادة في نشاط إنزيمات Tyrosine Ammonia Lyase Peroxidase, Poly Phenol Oxidase, Alkaline Phosphatase, Acid Phospatase التي تستحث المقاومة لمرض الذبول بالمقارنة بالنباتات الغير ملقحة بهذه الفطريات.

وجد أن فطريات الميكروهيزا تحدث زيادة معنوية في المحتوى الكلى للنيتروجين والفوسفور و البروتين.