Induction and Modulation of Resistance in Tomato Plants Against *Fusarium* Wilt Disease by Bioagent Fungi (Arbuscular Mycorrhiza) And/or Hormonal Elicitors (Jasmonic Acid& Salicylic Acid): 1- Changes in Growth, Some Metabolic Activities and Endogenous Hormones Related to Defence Mechanism

Samia M. El- Khallal

Botany Department, Faculty of Girls for Arts, Sciences and Education,
Ain shams University, Cairo, Egypt.

Abstract: Biological control and hormonal inducers represents an interesting strategy to induced disease resistance against pathogen especially when applied together. In the present study, tomato plants infected by *Fusarium oxysporum* were inoculated with arbuscular mycorrhizal (AM) fungi and/ or sprayed 3 times with hormonal inducers (JA& SA). Plants were harvested at 14, 28 and 42 days after pathogen infection. Results showed that % of disease incidence in infected plants gradually increased with increasing time of infection (86% at 42 days). Treatments with AM fungi, JA and SA significantly reduced % of disease incidence. AM fungi plus JA had the highest effective (92% efficiency). Growth rate (shoot and root) and % of mycorrhiza colonization markedly inhibited in tomato plants in response to *Fusarium* wilt disease as compared with healthy control. AM fungi, JA and SA were more pronounced in increasing tomato growth especially when applied together. SA- treated plants had low effect in mycorrhization as compared with JA- treated plants. Reduction in total chlorophyll in infected leaves significantly decreased in plants treated with AM fungi and/ or SA. Also, total soluble sugars, free amino acids and total soluble proteins increased in both leaves and roots of AM and/ or JA& SA- treated plants as compared with infected control. Induction in growth rate of these treatments was associated with increased in the contents of some inorganic nutrients (N, P, K, Ca, Zn and Mn). Treatment of AM fungi plus JA had the highest N, P, Ca contents, while high levels of K, Zn and Mn were recorded in AM fungi plus SA- treated plants. Results revealed that induction in the uptake of nutrients could be responsible for increasing susceptibility of tomato plants to *Fusarium* wilt disease. On the other hand, infection with *F. oxysporum* markedly altered hormonal balance (IAA, GA$_3$, ABA, zeatin and zeatin- riboside) in leaves and roots of tomato plants. Thus, ABA was accumulated while levels of IAA, GA$_3$ and cytokinins markedly reduced in infected plants. Actually, results revealed that increase in disease incidence and decrease in growth of *Fusarium*- tomato plants could be a morphological expression of the hormonal imbalance. The reverse was true in plants treated with AM fungi plus JA or SA, where levels of IAA, GA$_3$, zeatin and zeatin- riboside significantly increased in both leaves and roots. Finally, our results suggest that reduction in disease incidence, promotion in growth and metabolic activities in tomato plants inoculated with bioagent(AM fungi) and sprayed with elicitors(JA& SA) could be related to the synergistic and cooperative effect between them; which lead to the induction and regulation of disease resistance. Thus, two signal hormones could enhance the biological activity of AM fungi in tomato , potentially through interaction signalling pathways. AM fungi plus JA more effective than AM fungi plus SA.

Keywords: Biocontrol, hormonal elicitor, *Fusarium oxysporum*, tomato, disease resistance

INTRODUCTION

Diseases of plants are a major problem for agricultural world wide. Understanding the mechanisms employed by plants to defend themselves against pathogens may lead to novel strategies to enhance disease resistance in crop plants (Pozo *et al.,* 2005). Plant defence responses are regulated by a complex net work of

Corresponding Author: Dr. Samia M. El-khallal, Dept. of Botany, Faculty of Girls for Arts, Science and Education, Ain Shams University, Cairo, Egypt.
E-mail: samia_moheb@yahoo.com

signal molecules and transcriptional regulators. Resistance genes play a role in specific recognition of a pathogen and initiate defence responses. Three key signal molecules, namely salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) mediated expression of both specific as well as basal defence responses (Jalali 2005; Stout *et al.,* 2006). Thus, elucidation of signalling pathways controlling induced disease resistance is a major objective in research on plant pathogen interactions (Buzi *et al.,* 2004; Verhagen *et al.,* 2006).

The induced resistance (IR) phenomena are often associated with an enhanced capacity to mobilize cellular defence responses against pathogen attack.The classic form of IR is systemic acquired resistance (SAR) controlled by a signalling pathway that depends on endogenous accumulation of SA (Sticher *et al.,* 1997; Durrant & Dong 2004), which is associated with the accumulation of defence compounds, such as PR proteins. In addition another kinds of JA- dependent response is the so-called induced systemic resistance (ISR), which is produced when the roots are colonized by certain non pathogenic rhizobacteria and arbuscular mycorrhizal fungus (Pieterse *et al.,* 1999). JA and SA responses show mutual antagonism where some genes are induced by both compounds, revealing complexities in the net work of defence pathways (Delaney 2004; Wasternack *et al.,* 2006).

Biological control and chemical (hormonal) inducers are two of the promising approaches to the control of plant diseases (Yu and Zheng 2006). The use of chemical inducers JA and SA represents an interesting new opportunity in controlling fungal and bacterial diseases within an environmental friendly integrated crop protection system (Ellis *et al.,* 2002). The signal molecules JA and SA are involved in some signal transduction system, which induce particular enzymes catalyzing biosynthetic reactions to form defence compounds such as polyphenols, alkaloids and pathogenesis- related (PR) proteins (Vijayan *et al.,* 1998; Métraux 2001). Such signalling molecules, when exogenously applied have been shown to move systemically through plants resulting in the expression of a set of defence genes that are activated by pathogen infection (Kozlowski *et al.,* 1999; Faheed *et al.,* 2005; Yao & Tian 2005; Conceicão *et al.,* 2006).

Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resource. Nature is bestowed with many biocontrol agents including plant growth promoting microorganisms (PGPM) could regulate plant growth by inducing defence responses in plants via an systemic resistance (ISR) and/or a systemic acquired resistance(SAR) (Akköprü & Demir 2005, Siddiqui 2006). Among the PGPM, arbuscular mycorrhizae (AM) which is involved in the most universal intimate and important symbiosis (Pozo *et al.,* 2002). Different mechanisms have been shown to play a rol in plant protection by AM fungi namely: (I) enhancement of plant nutrition, (ii) competition with the pathogen for resources and space, (iii) plant morphological changes and barrier formation, (iv) changes in biochemical compounds related with plant response, (v) alleviation of physical stresses, and (vi) changes in antagonist and/ or deleterious microbe populations in the mycorrhizosphere (Linderman 1994; Vierheilig 2004). The beneficial effects of the AM symbiosis result from a complex molecular dialogue between the two symbiotic partners(Franken *et al.,*, 2007) and Some processes occurring in this dialogue are known to be mediated by phytohormones on the plant side. Thus, phytohormones have been proposed as" suitable candidates for signalling between plants and AM fungi", and it is tempting to speculate in the autoregulation of mycorrhization (Meixner *et al.,* 2005). The elevated JA levels occurring upon mycorrhization may enhance the defence status of mycorrihizal tissues, which were shown to be less sensitive to secondary infection by pathogens (Cordier *et al.,* 1998). The role of mycorrhiza in plant defense was carried out by modulating endogenous JA levels via overexperession or suppression of JA biosynthetic genes, thus it is tempting to speculate that JA sereves as endogenous signal in mycorrhiza-induced resistance(Pozo & Azcon-guilar 2007).

On the other hand, it has been suggested that plant defence responses mediated by SA are involved in the regulation of enteric endophytic colonization (Iniguez *et al.,* 2005). Exogenous SA applicarion delays mycorrhizal colonization, it is plausible that AM fungi repress SA- dependent defence responses in the host in order to a chieve a compatible interaction(Dumas-Gaudot *et al.,* 2000). In the case of mycorrhizal plants, such attenuation could explain the delay in systemic accumulation of PR-proteins upon treatement with SA or analogs (Shaul *et al.,* 1999). Moreover, Interaction between AM fungi (*Glomus etumicatum*) and SA had the highest effect on infection of *Fusarium* wilt and disease severity in tomato was reduced by 70% (özgönen *et al.,* 2001). Although induction of disease resistance using biocontrol agent and chemical elicitors (JA& SA) separately is well documented, there is little information about the effect when used in combination. Recently, Yao & Tian (2005); Yu & Zheng (2006) reported that combination of biocontrol yeast (*Cryptococcus laurentii*) with SA and JA, respectively had a synergistic effect on the induction of fruit resistance against diseases, which might be associated with rapid increase in defence related enzyme activity than biocontrol or hormonal elicitors when used alone.

Fusarium oxysporum is a soil borne fungal pathogen that causes major economic losses by inducing necrosis and wilting symptoms in many crop plants. Thus, biological control and hormonal inducers are two of the promising approaches to the control of diseases. In this study we used hormonal inducers (JA and SA) alone or in combination with bioagent arbuscular mycorrhiza(AM) fungi to control and overcome *Fusarium* wilt disease of tomato caused by *Fusarium oxysporum* and investigated its possible modes of action through changes in growth, some metabolic activities and levels of endogenous hormones related to induced resistance in infected tomato plants.

MATERIALS AND METHODS

Plant Materials and Growth Conditions*:*
Tomato (*Lycopersicom esculantum* Mill, Castle rook cultivar) seeds were surface sterilized for 1 min in 75% ethanol, immersing for 3 min in sterile distilled water. Seeds were left to dry overnight, and then were grown in large pot containing sterilized sandy loamy soil (2:1 w/w) until transplanting and inoculation. After two weeks from sowing, the uniform seedlings were selected and transplanted to clay pots (30 cm diameter) field with 10 Kg of sterilized loamy sandy soil (2:1 w/w). Each pot was planted with 6 seedlings which were thinned to 4 after one week post planting. The pots were divided into seven groups (8 pots/ group) and treated as the follow:

- Plants of the 1st group were left without any treatments (non- infected control).
- Plants of the 2nd group were infected with *Fusarium oxysporum* (infected control).
- Plants of the 3rd group were inoculated with AM fungi and infected with *F. oxysporum* (biocontrol treatment).
- Plants of the 4th and 5th groups were infected by *F.oxysporum* and sprayed 3 times with JA and SA, respectively (hormonal inducer treatments)
- Plants of 6th and 7^{en} groups were infected with *F. oxysporum,* inoculated with AM fungi and sprayed 3 times with JA or SA, respectively (biocontrol hormonal inducer treatments). All pots were randomized in a glass house under natural conditions of day length and light intensity, and watered regulatory to near field capacity with tap water.

Pathogenic and AM fungi inoculant:
Fusarium oxyspoum f.sp. lycopersici was provided from the Regional centre for Mycology and Biotechnology, AL-Azhar University. Cairo, Egypt. It was subcultured on potato dextrose agar at 27± 1C$^{\circ}$. Each pot was inoculated with 10 ml of *Fusarium* culture suspension (10^7cfu/ ml) after two weeks from transplanting time.The AM fungal species , *Glomus mosseae* was produced by Biorize R and D. France as granular inoculums. The AM inoculate (500 spore /pot) were placed 2-3 cm below the planting hols in each at the transplanting time.

Hormonal Inducer Treatments*:*
At the time of *Fusarium* infection, plants of the 4th, 5th, 6th and 7th groups were sprayed once a week for 3 weeks with 50µM JA or 100 µM SA solutions. Tween -20 was added (0.01 %) as a surfactant to hormone solutions. All plants were sprayed until runoff.

Plant Harvest and Analysis:
Five plants from each treatment were harvested at three different stages (14, 28 and 42 days from pathogen inoculation). Leaves and roots were separated from plants and were used for different analysis. Disease incidence (%), % of mycorrhizal colonization, changes in growth parameters and some metabolic activities in both leaves and roots were evaluated at each harvest, while the content of certain elements and levels of endogenous hormone were determined only in plants of third stage.

Determination of Certain Metabolites:
The plant photosynthetic pigments were extracted and determined by the method of Metzner *et al.,* (1965). Total soluble sugars were estimated using the phenol-sulfuric acid method according to Dubois *et al.,*(1966). Total free amino acids were estimated using ninhydrin reagent according to Moore & Stein (1954).Total soluble

proteins were determined according to the Bradford Dye-binding method (Bradford 1976) using bovin serum albumine as a standared.

For the analysis of certain elements, 1 gm of dried samples was digested with acid mixture (nitric, sulphuric, perchloric acids) as described by Champan & Pratt (1978). Total nitrogen content was determined using the micro-kjeldahl method (Jacobs 1958). Phosphorus and calcium contents were estimated following the

method of Adrian & Stevens (1977). Potassium, magnesium, zinc and manganese were determined according to the method of Allen *et al.,* (1974). The extraction and estimation of IAA, GA_3 and ABA were done according to the method reported by Guinne *et al.,* (1986). Cytokinins (zeatin and zeatin- riboside) were determined according to the method of Müller *et al.,* (1986) Determination of hormone concentrations was carried out using HPLC.

Statistical Analysis:

The recorded data were statistically analysed using the one way analysis of variance as described by Snedecor & Cochran(1969). The means were compared by L.S.D.using spps program version 8.

RESULTS AND DISCUSSIONS

Induction of Disease Resistance:

The phenomenon of systemic acquired resistance has attracted attention as a new strategy for controlling plant disease. The resistance was evident as a reduction in disease incidence compared with infected control. As shown in Table (1) % of disease incidence in tomato plants gradually increased with increasing the time of infection and reached to 83.0 % in infected control at 42 days from infection. However, % of disease incidence of non-infected plants (healthy control) was almost negligible, and this was probably due to accidental soil contamination from neighbouring inoculated soil and / or airborne spore produced of the infected plants.

Table 1: Induction of systematic resistance (as disease incidence %) in tomato plants treated with bioagent (*AM fungi*) and/or hormonal elicitors (JA&SA) against *Fusarium* wilt caused by *Fusarium oxysporum* (Fo).

Treatment	Days after pathogen inoculation					
	14		28		42	
		Efficacy (%)		Efficacy (%)		Efficacy (%)
Control	0.0[a]	00	2.0[a]	95.0	3.6[a]	95.6
Fo	16.0[b]	-	45.0[b]	-	83.0[b]	-
Fo+AM fungi	10.1[c]	36.2	18.7[c]	58.4	21.7[c]	73.8
Fo+JA	6.8d[e]	57.5	14.8[d]	67.1	19.4[c]	76.6
Fo+SA	8.1c[d]	49.3	18.0[c]	60.0	21.5[c]	74.0
Fo+AM fungi + JA	4.5[e]	71.8	7.9[e]	82.4	14.6[d]	82.4
Fo+AM fungi + SA	5.8d[e]	63.7	13.5[d]	70.0	18.3[c]	80.0
LSD at 5 %	2.11		2.45		2.26	

Values followed by the same letters are not significantly at p< 0.05.

Application with arbuscular mycorrhiza (AM) fungi and / or hormonal elicitors (JA & SA) greatly reduced % of disease incidence of tomato plants as compared with infected control (Table 1). Thus, these treatments improve plant health through reducing wilt symptoms, vascular invasion and sporulation of the pathogen. Our results are in harmony with other researchers (Buzi *et al.,* 2004; Jayaraj *et al.,* 2004; Segarra 2006). Results also showed that combination between AM fungi and JA & SA more effective in reducing disease incidence than them when applied alone. Treatment of AM fungi plus JA had a higher efficacy in inducing resistance (71.8, 77.7 and 82.4 at 14, 28 and 42 days from infection, respectively) against *Fusarium* wilt disease as compared with AM fungi plus SA treatment. This efficiency might be related to the elevated JA levels which occurring upon mycorrhization and may enhance defence status in mycorrhiza tissues (Cordier *et al.,* 1998;

Hao *et al.,* 2005). JA may modulate such a defence status in AM-plants by inducing expression of pathogenesis- and stress related genes (Wasternack & Hause 2002). Although root colonization affected negatively by SA as suggested by özgönen *et al.,* (2001), results indicated that combination between AM fungi and SA was more effective in inducing wilt disease resistance in tomato plants at different stages, as compared with AM fungi or SA when applied alone. These results indicated that two separate signal transduction pathways dependent on SA and JA are activated by diverse microorganisms and are distinct microbial pathogens (Khaosaad *et al.,* 2007).

Change in Growth Parameters:

Shoot and root growth of tomato plants grown in *F. oxysporum* pathogenized soil, markedly inhibited as compared with non-infected control. This reduction significanty increased with increasing the time of infection (Tables 1a & 1b). Application of bioagent arbuscular mycorrhiza (AM) and hormonal inducers (JA or SA) enhanced shoot and root growth of *Fusarium* infected plants, especially when applied together. 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 & Nilsen 2000). Results showed that infection by *F. oxysporum* markedly decreased fresh and dry weights in roots of tomato plants, and this may be related to the accumulation and action of phenolics produced from degradation of cell wall (lignin) mainly via depolymerization results from fungal elicitors (Steijl *et al.,* 1999).

Table 2a: Application of bioagent (AM fungi) and/or hormonal inducers (JA & SA) on the changes in shoot growth of tomato plants infected with *Fusarium oxysporum* (Fo).

Treatment	Shoot length (cm)			No. of leaves/plant			No. of branches/plant			Fresh wt. (g/plant)			Dry wt. (g/plant)		
	Days after pathogen inoculation														
	14	28	42	14	28	42	14	28	42	14	28	42	14	28	42
Control	32.3[a]	40.3[ac]	65.5[a]	8.3[a]	16.8[a]	25.6a	2.0[a]	3.6a	4.3[a]	34.3[a]	56.5[a]	75.8[a]	4.7[a]	7.5[a]	11.0[a]
Fo	19.9[b]	29.8[b]	35.3[b]	5.3[b]	8.6[b]	13.6[b]	0.0[b]	0.0[b]	0.0[b]	24.6[b]	33.3[b]	41.2[b]	2.4[b]	3.5[b]	5.1[b]
Fo+AM fungi	29.3[c]	42.9[ad]	53.4[c]	6.6[c]	13.0[c]	21.0[ce]	1.0[c]	3.0[c]	4.0[a]	29.8[c]	45.7[c]	66.1[ce]	3.3[cd]	6.0[c]	8.9[ce]
Fo+JA	23.9[d]	33.9[b]	45.9[d]	6.6[c]	13.0[c]	20.0[cd]	2.0[a]	2.6[c]	3.3[ad]	29.0[c]	41.7[d]	64.2[c]	3.6[c]	5.9[c]	8.4[cd]
Fo+SA	26.2[e]	38.3[c]	48.0[e]	5.6[b]	11.7[d]	18.0[d]	1.0[c]	1.3[d]	1.6[c]	25.3[b]	37.2[e]	59.8[d]	3.0[d]	4.8[d]	7.8[d]
Fo+AM fungi+JA	28.3[f]	44.5[ad]	57.3[f]	8.0[a]	15.6[e]	23.0[e]	2.0[a]	4.3[e]	4.3[a]	32.8[a]	51.7[f]	73.0[a]	4.3[a]	7.0[a]	9.3[e]
Fo+AM fungi+SA	31.2[a]	46.4[d]	59.6[g]	6.0b[c]	14.3[f]	19.0[cd]	1.3[d]	2.0[f]	2.3[d]	29.9[c]	47.4c	67.8[e]	3.6[c]	6.1[c]	8.7[ce]
LSD at 5 %	1.45	4.35	2.11	0.95	0.52	2.00	0.04	0.50	1.65	2.40	3.81	2.90	0.45	0.80	0.75

Values followed by the same letters are not significantly at p< 0.05.

Table 2b: Application of bioagent (AM fungi) and/or hormonal inducers (JA & SA) on the changes in root growth of tomato plants infected with *Fusarium oxysporum* (Fo).

Treatment	Root length (cm)			Fresh wt (g/plant)			Dry wt. (g/plant)			% of colonization		
	Days after pathogen inoculation											
	14	28	42	14	28	42	14	28	42	14	28	42
Control	10.8[a]	18.5[a]	28.5[a]	8.5[a]	13.7[a]	22.2[a]	1.8[a]	3.6[a]	6.4[a]	-	-	-
Fo	7.1[b]	11.7[b]	16.3[b]	6.2[be]	8.2[b]	11.9[b]	1.0[b]	1.1[b]	2.3[b]	-	-	-
Fo+ AM fungi	8.9[c]	15.3[c]	24.5[c]	7.4[cd]	11.5[c]	17.8[c]	1.3[c]	2.2[cd]	4.6[c]	213[a]	56.0[a]	72.0[a]
Fo+JA	7.5[b]	13.6[d]	20.5[d]	7.1[ce]	10.8[cd]	17.2[c]	1.5[c]	2.1[c]	3.6[d]	-	-	-
Fo+SA	8.3[c]	15.0[c]	24.3[c]	6.6[e]	9.6[d]	15.5[d]	1.3[c]	1.7[d]	3.4[d]	-	-	-
Fo+AM fungi + JA	8.4[c]	16.9[e]	26.6[e]	7.7[d]	12.8[e]	19.7[e]	1.6[d]	2.34[d]	5.3[e]	28.0[b]	61.0[b]	87.0[b]
Fo+AM fungi + SA	9.6[d]	16.0[ec]	27.3[e]	7.4[cd]	10.2[d]	17.9[c]	1.3[c]	2.1[c]	4.6c	15.0[c]	40.0[c]	53.3[c]
LSD at 5 %	0.65	1.22	0.90	0.55	0.80	1.21	0.19	0.22	0.35	5.33	4.00	10.7

Values followed by the same letters are not significantly at p< 0.05.

Inoculation with arbuscular mycorrhiza (AM) fungi increased shoot and root growth of infected tomato plants as compared with plants sprayed with either JA or SA. But results showed that application with AM fungi plus JA were more pronounced in increasing shoot growth, while AM fungi plus SA had a positive effect on root growth (Tables 1a & 1b). Our results suggested that AM fungi greatly increase host tolerance against pathogen attack by compensating for the loss of root biomass or function caused by pathogen. 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). On the other hand, JA- treated plants cause the induction of several well documented defensive pathways without the removal of leaf tissue (Baldwin 1998; Redman *et al.,* 2001). In addition, jasmonates may contribute to a better growth of the plant. 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). Finally, a synergistic effect of JA and AM inoculation on shoot and root growth were found in *Allium sativum*. (Regvar *et al.,* 1996).

The increase in shoot and root length in tomato plants especially treated with AM fungi and SA 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). Similar results were obtained by özgönen *et al.,* (2001), they found that length of tomato shoot was increased by mycorrhiza plus 1mM SA, and these treatments had a potential for using in the control of *Fusarium* tomatoes. Also, SA regulated plant growth and confers plant resistance to fungal diseases with only one application (Raskin 1992). Finally, our results revealed that a synergistic action was found between AM fungi and JA & SA on the growth promotion and protection of tomato against *Fusarium*, and this might be carried out by a number of ways: including antibiotic production, induced resistance, competition between mycorrhiza and providing a physical barrier to infection (Morgan *et al.,* 2005).

Mycorrhizal Colonization:

Vescular- arbuscular mycorrhizal fungus (*Glomus moseae*) is a good colonizer of roots of many plants. Depending on this characteristic, besides the improving plant growth, it makes the root more resistant to infection of soil borne pathogen (Kasiamdari *et al.,* 2002).

Results in Table (1b) Showed that application with JA greatly induced mycorrhization in roots of infected tomato plants and reached to maximum AM colonization 87% at the third stage. While SA inducer slightly increased AM colonization in tomato roots as compared with infected *Fusarium* plants. The first contact of mycorrhizal of hypha with roots was significantly accelerated upon treatment with JA (Regvar *et al.,* 1996). The increased JA levels of mycorrhizal roots might be linked to the enhanced defensive capacity conferred by mycorrhization (Hause & Fester 2005). However, Medina *et al.,*(2003) reported that SA may delay root colonization, but did not affect the maximal degree of root colonization Actually, during mycorrhiza formation, modulation of plant defense responses occurs, potentially through cross-talk between SA and JA dependent signalling pathways. This modulation may impact plant responses to potential enemies by priming the tissues for a more efficient activation of defence mechanisms(Pozo & Azcón-Aguilar 2007). On the other hand, results appeared that In infection with *F. oxysporum* markedly affected AM colonization in tomato plants, and this because fusarium hyphae might due to mycorrhizal disorganization, probably induced by a strong reaction of the host cell characterized by the moasive accumulation of phenolic- like compounds and the production of hydrolytic enzymes such as chitinase (Azcon- Aguilar & Bara 1996; Hao *et al.,* 2005). Thus, induction in resistance in tomato plants treated with AM fungi against *Fusarium* wilt disease could be related to the activation of plant defence responses by mycorrhiza formation provides a certain protection against pathogen.

Changes in Some Metabolic Activities and Hormonal Levels:

One of the most important indicators of physiological activity is the rate of photosynthesis, which is related to the chlorophyll content of plants. Table 3 should that total chlorophyll gradually decreased in leaves of infected tomato plants with increasing the time of infection. Reduction in total chlorophyll in tomato leaves as a result of *Fusarium* wilt disease may be a consequence of the fungal effect on the release of transported toxins which leads to the liberation of reactive oxygen species. Achore *et al.,* (1993) found that toxins which produced by *Fusarium* were induced inhibition of chlorophyll biosynthesis. Also, decreased in biomass and chlorophyll content in tomato plants might results from high level of lipid peroxidation (El- Khallal 2007, in press) mediating cell damage in tomato tissues.

Table 3: Changes in total chlorophyll and total soluble sugars (mg/g fresh wt.) in leaves and roots of tomato plants inoculated with bioagent AM fungi and/or sprayed with hormonal inducers (JA & SA) and grown in *Fusarium oxysporum* (Fo)pathogenized soil.

Treatment		Total chlorophyll			Total soluble sugars		
		Days after pathogen inoculation					
		14	28	42	14	28	42
Leaves	control	0.120	0.199[a]	0.448[a]	5.18[a]	8.89[a]	17.11[a]
	Fo	0.035	0.084[b]	0.124[b]	3.94[b]	9.40[b]	19.16[b]
	Fo + AM fungi	0.084	0.147[c]	0.310[c]	4.72[c]	10.65[c]	21.04[b]
	Fo + JA	0.345	0.107[d]	0.222[d]	4.92[d]	9.94[d]	19.66[b]
	Fo + SA	0.253	0.134[e]	0.289[e]	4.87[d]	10.87[c]	20.57[bd]
	Fo + AM fungi + JA	0.113	0.160[f]	0.362[f]	5.12a	12.79[e]	25.41[c]
	Fo +AM fungi + SA	0.127	0.175[g]	0.391[g]	4.95[d]	13.38[f]	22.25[d]
LSD at 5 %		Non	0.012	0.021	0.115	0.36	1.97
Roots	control				2.15[a]	3.24[a]	3.07[a]
	Fo				1.73[b]	2.02[b]	4.74[ab]
	Fo + AM fungi				2.05[c]	3.86[c]	7.12[bc]
	Fo + JA				1.82[d]	3.53[d]	6.37[bc]
	Fo + SA				1.88[d]	3.69[d]	6.69[bc]
	Fo+ AM fungi + JA				2.06[c]	5.10[e]	8.639[c]
	Fo+ AM fungi + SA				1.95[e]	4.37[f]	7.23b[c]
LSD at 5 %					0.078	0.165	2.895

Values followed by the same letters are not significantly at p< 0.05.

As shown in Table (3) content of total chlorophyll significantly increased in leaves of tomato plants treated with AM fungi and/or JA& SA as compared with infected- control. Among all treatments, AM fungi plus SA had the highest chlorophyll content. These results indicated that treatments with bioagent and/or hormonal elicitors markedly affected the efficiency of photosynthetic apparatus in infected tomato leaves with a better potential for resistance. According that, the decrease in photophosphorylation rate which usually occurring after an infection (Hutcheson & Buchanan 1983) can be compensated by an increase in efficiency of the photosynthetic apparatus. Results showed that AM fungi- treated plants had the highest chlorophyll content, while JA-treated plants recorded the lowest chlorophyll content. Hi chlorophyll content in AM- treated plants might be ascribed to the influence of mycorrhizal fungi on the development processes leading synthesis of chloroplasts enzymes (Abo-Ghalia & El- Khallal 2005). Also, high chlorophyll content in SA-treated plants could be attributed to its stimulatory effect on rubiso a ctivity (Khodary 2004). However, JA may affect photosynthesis indirectly either as a stress modulating substance, or the alteration in gene expression (Metadiev *et al.,* 1996).

Soluble sugars are involved in the responses to a number of stresses, and act as nutrient and metabolite signalling molecules that activate specific or hormonal- crosstalk transduction pathways, resulting in important modifications of gene expression (Couée *et al.,* 2006). Total soluble sugars in both leaves and roots of infected tomato plants treated or non- treated with bioagent and/ or hormonal elicitors decreased at 14 days from pathogen inoculation, and then significantly increased to reached maximum value at 42 days from time infection as compared with non- infected control (Table 3). Treatment of AM fungi plus JA had the highest soluble sugars in roots and this might be related to increased JA levels in mycorrhiza roots which could enhance the sink-strength of mycorrhizal roots and thereby stimulate carbohydrate biosynthesis in the shoots and their transport into the roots (Hause *et al.,* 2002). While increased of soluble sugars in leaves of AM- SA treated plants could be related the high chlorophyll content and then increased photosynthetic rate. These results revealed that accumulation of soluble sugars in infected tomato plants- especially treated with bioagent and

hormonal elicitors indicated the relationship between sugar regulation and activation of the systemic resistance. Blee & Anderson (2000) recorded that high levels of total soluble sugars in AM- treated plants might be due to the enhanced transcription of genes involved in the catabolism of sucrose which was correlated to the enhanced transcription of defence genes. Therefore, a sensor system based on the flux of carbohydrates regulates defence gene expression in plant cell containing arbuscules. On the other hand increase in soluble sugar in plants grown in pathogenized soil may be due to retarded rate of translation under the influence of cell wall degrading enzymes or toxins produced of transfer via phloem elements (Heiser *et al.,* 1998).

As shown in Table (4) levels of free amino acids and total soluble protein significantly decreased in leaves and roots of tomato plants infected with *Fusarium   oxysporum*, and this reduction was increased with increasing the time of pathogen infection. *Fusarium oxysporum* may accelerate the decline in soluble protein if plants are undergoing natural senescence when attack by the pathogen occurs. The significant decrease in the protein content in tomato tissues as a result of pathogen infection may be due to its compartmention in light harvesting complex protein or in some other activities related to a hypersensitive response (Chandra & Bhatt 1998). Retardation in the synthesis of the main nitrogenous compounds in leaves might be related to a depletion of energy demand. Thus, it is hard to predict how the presence of *F. oxysporum* and/ or its metabolites can influence the capacity of tissues in energy conservation(Nafie 2003). It may be speculated that the toxins produced by *F. oxysporum* might act as uncoplulers and inhibit ATP synthesis (Obwald & Diewirt 1995).

Table 4: Changes in the contents of free amino acids and total soluble proteins (mg/g fresh wt.) in leaves and roots of tomato plants inoculated with bioagent AM fungi and/or sprayed with hormonal inducers (JA&SA) and grown in *Fusarium oxysporum*(Fo) pathogenized soil.

Treatment		Free amino acids			Total soluble protein		
		Days after pathogen inoculation					
		14	28	42	14	28	42
Leaves	Control	0.32[a]	0.86a	1.71[a]	4.06[a]	9.54[a]	14.05[a]
	Fo	0.18[b]	0.47[b]	1.06[b]	2.66[b]	6.94[b]	8.91[b]
	Fo+AM Fungi	0.24[c]	0.66[c]	1.65[c]	3.47[c]	8.24[c]	11.41[cd]
	Fo+JA	0.41[d]	0.79[d]	1.72[a]	3.61[c]	8.61[c]	12.15[c]
	Fo+SA	0.37[e]	0.70[e]	1.68a	3.42[c]	8.23[c]	12.65[d]
	Fo+JA+AM Fungi	0.51[f]	1.04[f]	2.91[d]	3.84[c]	9.39[c]	12.65[d]
	Fo+SA+AMFungi	0.47[g]	0.91[a]	2.85[e]	3.71[c]	8.94[c]	7.22[e]
LSD at 5 %		0.032	0.060	0.035	0.520	0.915	5.987
Roots	Control	0.43[a]	0.48[ae]	1.20[a]	2.07[a]	3.09[a]	7.28[a]
	Fo	0.12[b]	0.23[b]	0.63[b]	1.57[b]	2.08[b]	3.20[b]
	Fo +AM Fungi	0.19[b]	0.39[c]	1.07[c]	1.73[c]	2.88[a]	6.06[c]
	Fo+JA	0.16[b]	0.45[a]	0.98[d]	1.87[d]	3.01[a]	5.83[d]
	Fo+SA	0.19[b]	0.38[c]	0.87[e]	1.83[d]	2.66[c]	4.81[e]
	Fo+JA+AM Fungi	0.13[b]	0.52[e]	1.49[f]	2.04[e]	3.18[a]	7.09[f]
	Fo+SA+AMFungi	0.20[b]	0.46[a]	1.33[g]	1.91d[e]	3.00[a]	6.28[g]
LSD at 5 %		0.152	0.050	0.094	0.090	0.145	0.165

Values followed by the same letters are not significantly at p< 0.05.

Results indicated that treatment with JA elicitor alone or in combination with bioagent AM fungi had more effective in increasing free amino acids and total soluble proteins in infected tomato plants, as compared with all treatments. Jasmonates could act directly (transcriptional regulation) or indirectly (via the changes of N partitioning) on N storages as soluble proteins and particular VSPs (Meuriot *et al.,* 2004). In lupine seedlings, El- Bahay & Moursy (2003) reported that close correlation between the levels of nucleic acids and total soluble

proteins in response to SA pointed to exert their action mechanism upon DNA- RNA synthesizing protein machinery at transcriptional and/ or translocational levels with magnitudes. Results showed that infection with *F.oxysporum* primarily alters growth of tomato plants with concomitant reduction of N acquisition due to negative feedback regulation mechanisms. Changes in the levels of total soluble proteins in pathogenized tomato plants may be due to a promotion in the conversion of other amino acids seems to be on the expense of soluble proteins. Finally, interaction between bioagent (AM fungi) and hormonal elicitors(JA& SA) had effective to overcome the wilt disease symptoms mediated by restoring the metabolic alterations imposed by infection.

Some inorganic nutrients including N, P, K, Ca, Zn and Mn play an important roles in disease development. Microbial interactions with plant roots are known to profoundly affect plant nutrient status. Thus data in Table (5) revealed that infection with *Fusarium oxysporum* markedly decreased N and P contents in both leaves and roots of tomato plants (at 42 days from pathogen inoculation) 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).

Table 5: Changes in the content of certain elements (%) in leaves and roots of tomato plants (42 days after pathogen inoculation) treated with bioagent AM fungi and/or hormonal elicitors (JA & SA), and grown in *Fusarium oxysporum* (Fo) pathogenized soil.

	Treatment	N	P	K	Ca	Zn	Mn
Leaves	Control	3.05	0.81	4.68	4.16	71.5	53.5
	Fo	1.73	0.46	2.71	1.85	50.3	36.2
	Fo+ AM Fugi	2.41	0.95	3.35	3.20	73.8	56.2
	Fo+JA	2.38	0.71	3.19	3.45	64.5	50.8
	Fo+SA	2.08	0.68	3.46	3.06	68.3	47.2
	Fo+AM Fugi + JA	3.16	1.23	3.85	4.08	76.7	58.5
	Fo+AM Fugi + SA	2.88	0.94	4.21	3.65	73.1	54.6
Roots	Control	2.41	0.56	6.21	3.88	92.3	68.7
	Fo	1.26	0.27	4.88	2.05	64.3	37.8
	Fo+ AM Fugi	2.24	0.61	6.81	2.81	96.3	71.3
	Fo+JA	2.16	0.52	5.08	2.36	74.6	62.0
	Fo+SA	1.94	0.47	5.71	2.18	80.2	60.8
	Fo+AM Fugi + JA	2.83	0.68	7.13	3.41	106.5	76.4
	Fo+ AM Fugi +SA	2.56	0.60	7.82	3.16	92.8	70.3

Applying with bioagent (AM) and / or elicitors (JA &SA) influenced the N and P contents in the direction of enhancing growth and reducing the disease symptoms. Treatment with AM fungi plus JA had a higher N and P contents than those of AM fungi plus SA treatment (Table 5). Increase in P supply as a direct consequence of AM 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 suppersion mechanisms may be involved. One possibility is that defence gene expression is mediated by a signalling mechanism that senses the level of P in the root, resulting in an upregulation of defence genes (catalase, chitinase and glucanase genes). Goulas *et al.,* (2003). Meuriot *et al.,*(2004) reported that jasmonate have been implicated in the control of accumulation and mobilization of N reserves in plant roots. Finally, induction in biomass and photosynthetic rate could related to high N and P contents, which could be responsible for increasing susceptibility of tomato plants to wilt disease induced by *F. Oxysporum*

Contents of K and Ca^{2+} in both leaves and roots of *F. oxysporum* infected tomato plants markedly decreased as compared with these contents in non-infected control(Table 5). Reduction in K and Ca^{2+} contents in infected leaves higher than that of the corresponding roots. In comparison between JA and SA inducers, results showed that JA- treated plants had a high calcium content, while a higher potassium level was found in SA-treated plants. Interaction between AM fungi and JA or SA increased K and Ca contents in infected tomato plants as compared with other treatments.

Reduction in K and Ca contents in infected tomato tissues might be related to toxins produced by the fungi which affected K^+ uptake and stomata function leading to uncontrolled transpiration, excessive loss of water, and finally wilted plants (Aducci *et al.,* 1997). While AM fungi and /or hormonal elicitors increased K^+ contents in infected plants could be via increasing the level of K^+ impede the flow of nutrients from host to pathogen and retard the spread of *F. oxysporum* and its toxins (Nafie 2003). Also an adequate K^+ supply dose promote cell wall thickening that helps plant to resist disease (Shuman 1994). However, Low level of Ca^{2+} content in all infected plants as compared with non-infected plants (healthy control) might be responsible for decreasing the integrity of cell membranes and hence increase their permeability and induced leakage from tissues involved by *Fusarium oxysporum* . Orcutt & Nilsen (2000) reported that calcium under *Fusarium oxysporum* pathogensis may interfere with the uptake of toxin ions from the soil.

As shown in Table (6) contents of both Zn and Mn greatly decreased in leaves and roots of tomato plants infected with *F. oxysporum* as compared with healthy control. Foliar spraying (3 times) with either JA or SA significantly increased Zn and Mn contents , and treatment with AM fungi plus JA had the highest value in both leaves and roots. The essential micronutrients Zn is reported among other micronutrients which can increase resistance in plants to pathogen (Hardham 1992; Shirasu *et al.,* 1999). Zinc was shown to reduce the incidence of root disease in rice and wilt disease in cotton when applied as a root dip prior to transplant or as a soil amendment, respectively (Agrios 1988). Mn may prove to be the most important in the development of resistance of plants to both root and foliar diseases of fungal origin (Altomare *et al.,* 1999). In addition,Mn is required for several physiological functions of plants such as photosynthesis , synthesis for some amino acids , hormones, phenols and lignin which it plays a major role in the growth and disease resistance of plants.

Finally, results revealed that application with AM fungi and / or hormonal inducers JA & SA 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 development and also might make the plant more resistant to, or compensate for, the effect of disease.

Changes in Endogenous Hormone Levels:

Plant growth regulators are implicated in several plant/pathogen interactions. Infection with *Fusarium oxysporum* significantly affected the hormonal balance in the leaves and roots of tomato plants at 42 days from pathogen inoculation (Fig.1). As compared with non- infected control (healthy plants), *Fusarium oxysporum* markedly reduced levels of IAA, GA_3, cytokinin (zeatin and zeatin - riboside) and increase ABA level in tissues of tomato plants . Results indicated that reduction in growth rate of infected tomato plants could be directly related to the hormonal imbalance as the result of pathogen infection, which reduced cell division and /or cell elongation. Plants inoculated with AM fungi had a positive effect on the phytohormone levels in tissues Absisic acid is known to be involved in plant interactions with pathogenic fungi, and its increased plant susceptibility to phytopathogenic microorganisms (Audenaert *et al.,* 2002). Infection of tomato plants by pathogenic *Fusarium oxysporum*, produced a drastic increased in ABA level in both leaves and roots as compared with non-infected control (Fig. 1C). An increase in the level of ABA causes an increased resistance against pathogen can explained by the induction of stomatal closure or by the inhibition of cellose degradation (Adie *et al.,* 2007). Also, ABA markedly increased in infected tomato plants and inoculated with AM fungi and this may caused by at least four processes: (1) stimulation of ABA biosynthesis by the host, (2) release of ABA or its precursor by the fungus, stimulation of biosynthesis of plant ABA and/ or inhibition of its metabolism by the fungus (Bothe *et al.,* 1994; Kettner & Dörffling1995). On the other hand, AM fungi induced endogenous ABA production in plants to suppress SA- dependent defence mechanisms(Mohr&Cahill 2007).Thus, negative modulation of SA- dependent signalling is probably one of the mechanisms by which ABA determines susceptibility of AM fungi- treated tomato plants to *Fusarium oxysporum*.

Results in Figure 1 appeared that application with JA and SA alone or in combination with AM fungi greatly decreased ABA in both roots and leaves of tomato plants, as compared with infected control. SA treatments had the lowest ABA value. These results revealed that defence response against *Fusarium* wilt
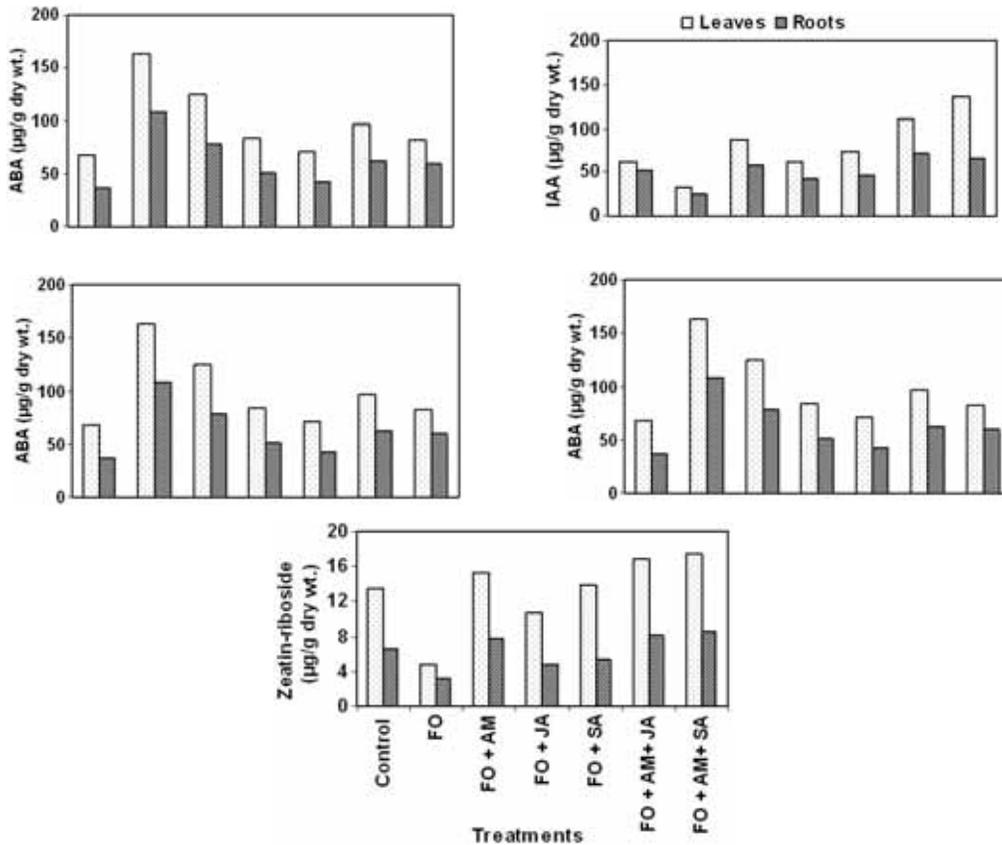
Fig. 1: Changes in endogenous growth hormones (µg⁻¹ dry wt.) in leaves and roots of tomato plants (42 days after pathogen inoculation) treated with bioagent AM fungi and/or hormonal elicitors (JA & SA), and grown in Fusarium oxysporum (Fo) pathogenized soil.

disease in these treatments was concomitant with a depression in ABA level and consequence activation of gene action responsive to both elicitor signals and allied transduction pathways. The inverse relationship between the level of ABA and the induction of certain disease-resistance components led to the assumption that ABA has an antagonistic interaction with the JA/ET defense pathway that is necessary for resistance to the pathogen *Fusarium oxysporums*(Anderson *et al.,* 2004). However, ABA also seems to interfere with signalling involving SA, leading to reduced plant resistance against pathogen (Adie *et al.,* 2007).

Changes in the ABA level in tomato plants of various treatments showed that ABA, JA and SA might control the expression of different but overlapping sets of genes to influence the outcome of plant – pathogen interactions and induced defences. On the other hand, phytohormone balance or imbalance in tomato plants infected with *F. oxyoporum* and treated with bioagents and / or elicitors can be regulated by its role on synthesis, degradation / inactivation transport and compartmentation. Finally , our results indicate that arbuscular mycorrhiza affect the hormone levels in infected tomato plants by intervening in each of these processes and / or by sending hormone messages (Torelli *et al.,* 2000).

Beside the roles of AM fungi, JA and SA in disease resistance when applied individually, our results indicated that interaction between bioagent and hormonal elicitors (JA &SA) had the beneficial effect and more pronounced on the induction of resistance in tomato plants against *Fusarium* wilt disease as compared when applied alone. Therefore, reduction in disease incidence, promotion in growth, metabolic activities, uptake of nutrients and hormonal balance in these treatments could be related to the synergistic and cooperative effect between bioagent and hormonal elicitors especially JA, on the induction and regulation of disease resistance.

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