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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): 2-Changes in the antioxidant enzymes, phenolic compounds and pathogen related- proteins.

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Abstract: Induction of plant defense against pathogen attack is regulated by a complex network of different signals. In the present study interaction between hormonal signals [jasmonic acid (JA) or salicylic acid (SA)] and bioagent [arbuscular mychorrhiza (AM) fungi] was used as new strategy to enhance tomato defense responses against wilt disease caused by *Fusarium oxysporum* (Fo). Thus changes in various physiological defenses including antioxidant enzymes, phenolic compounds and pathogenesis related (PR) proteins were investigated in leaves of tomato plants. Results appeared that production of reactive oxygen species (ROS), mainly H_2O_2 and $O_2^{\bullet-}$, and lipid peroxidation increased in tomato leaves by increasing the time of infection. Application with bioagent AM fungi and/or hormonal elicitors (JA & SA) markedly decreased these levels ,while LOX activity greatly increased as compared with infected control. SA- treated plants had the highest MDA level but JA + AM fungi treated plants recorded the highest LOX activity. Infection by *Fusarium oxysporm* significantly increased activity of antioxidant enzymes (SOD, APX and CAT) in tomato leaves at different stages of growth. The highest activity was recorded in leaves of AM fungi + JA- treated plants, while treatments with SA especially when applied alone markedly decreased H_2O_2 scavenging enzymes (APX and CAT) and greatly increased SOD activity. Thus, imbalance between H_2O_2 –generation and scavenging enzymes in leaves may reflect a defense mechanism in tomato or a pathogenicity strategy of the fungus. Levels of certain phenolic acids greatly changed in tomato leaves in response to *Fusarium oxysporum*, AM fungi and hormonal elicitors. Benzoic and Galleic acids contents markedly decreased , however, contents of coumaric, cinnamic, chlorogenic and ferulic acids increased in leaves of all treatments. Also, activity of lignification enzymes POX, PPX and PAL significantly increased in leaves of infected tomato plants. JA-treated plants caused the highest POX and PPX activities, while SA-treated plants having the highest PAL activities. High accumulation of phenolic compounds and activity POX, PPX and PAL in these plants may reflect a component of many defense signals activated by bioagent and hormonal inducers which leading to the activation of power defense system in tomato against attack. Analysis of protein electrophoresis revealed that interaction between hormone signal (JA& SA) and bioagent AM fungi mediating the expression of the majority of different PR-proteins leading to increasing defense mechanism against *Fusarium oxysporum* infection. Thus, induction of protein bands of molecular weights 35, 33, 32, 31 (PR-2, β -1, 3 glucanase), 30.5 and 27 (PR-3,-4, chitinase) in infected leaves indicated the important role which played in disease resistance. Finally, the new mechanism of the combination strategy between bioagent and hormonal signals (either synergistically or antagonistically) played important roles for increasing various defense systems and altering expression of defense genes which leading to different PR-proteins working together to increased resistance in tomato plants against wilt disease caused by *Fusarium oxysporum*. In addition, results revealed that defense mechanism in plants treated with AM fungi and JA are more effective than AM fungi plus SA- treated plants.

Key words: Biocontrol, hormonal elicitor, *Fusarium oxysporum*, tomato, antioxidant system, phenolic compounds, pathogen related proteins

INTRODUCTION

Plants responds to pathogen attack or elicitor treatments by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading (Malolepsza & Rózalska 2005).

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The defense mechanisms including the fast production of reactive oxygen species (ROS) (De Gara *et al.,* 2003); alterations in the cell wall constitution; accumulation of antimicrobial secondary metabolites known as phytoalexins (Heath 2000; Agrios 2005); activation and/or synthesis of defense peptides and proteins (Castro & Fonts 2005).

The production of reactive oxygen species (ROS) is one of the earliest cellular responses following successful pathogen recognition. The generation of ROS such as the superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are a common event associated with normal plant biochemical processes (Zhou *et al.,* 2004), and also causes oxidative damage through actions such as lipid peroxidation with membrane destruction, protein inactivation and DNA mutation (Torres *et al.,* 2006). Localized production of H_2O_2 and ($O_2^{\cdot-}$) are one of the earliest cytologically and histochemically detectable response events in plant tissue, and is probably in induction of the hypersensitive response (HR) or systemic acquired resistance (Bolwell 2004). H_2O_2 generation have direct antimicrobial activity inhibiting germination of spores of many fungal pathogens and participation in the formation of phenoxyl-radicals during phenol-polymerization within the plant cell wall (Lamb & Dixon 1997). In addition, generation of ROS induce lipid peroxidation, which lead to a loss of membrane integrity, tissue necrosis and induction of phytoalexins which synthesized by the action of lipoxygenases (LOXs). LOX metabolites may exert direct antimicrobial activity and induce or alter wound/pathogen defense gene expression (Kühn *et al.,* 2005). On the other hand, development of an antioxidant defense system in plants protect them against oxidative stress damage, by either the partial suppression of ROS production, or the scavenging of ROS which has already been produced (Torres *et al.,* 2006; Ye *et al.,* 2006; Cavalcanti *et al.,* 2007). Thus, various antioxidant enzymes such as peroxidase (POX), superoxide dismutase (SOD) catalase (CAT) and ascorbate peroxidase (APX) are participate in ROS metabolism during the pathogen attack. POX may be some of the elements of the defense systems that are stimulated in plants in response to pathogen infection like *Fusarium oxysporum* (Morkunas & Gmerek 2007). Increased in SOD activity has been implicated in pathogen-induced HR development in plants, however, reduction in catalase activity have been observed during microorganism induced HRs in plants (Delledonne *et al.,* 2002).

In compatible or incompatible interactions between plants and microorganisms, salicylic acid, Jasmonic acid and Shikimic acid-dependent pathways are involved in plant defense (Agrios 1997). Among them, shikimic acid pathway has received much more attention due to secondary metabolites such as lignin, phenolic acids and phytoalexins, which are responsible for adding mechanical rigidity and strength to cell walls and for providing barriers to infection by pathogen (Hahlbrock *et al.,* 2003; Tan *et al.,* 2004;Ončeica *et al.,* 2006). Polyphenol oxidase (PPX) is involved in the oxidation of polyphenols into quinons (antimicrobial compounds) and lignification of plant cells during microbial invation, and also may participate in the responding defense reaction and hypersensitively by inducing plant resistance against fungi (Ray *et al.,* 1998; Mohammadi & Karr 2002). Also, phenylalanine ammonia-lyase (PAL) may modulate the resistance of stress by regulating the biosynthesis of phenolic compounds (Wen *et al.,* 2005). After the contact with the pathogen a series of peptides and proteins are expressed and many of these compounds present direct antimicrobial properties (Castro & Fonts 2005). Production of chitinase (PR-3) and B-1,3- glucanase (PR-2) have been considered important in the biological control of soil borne pathogens because of their ability to degrade major component of cell walls (Chitin and B-1,3-glucan, respectively) (Kasprzewska 2003; Blade *et al.,* 2006; Cota *et al.,* 2007). Induction of disease resistance following treatment with active bioagent and/or chemical inducers has been reported to provide protection against invation of pathogen in several plant species (Pozo *et al.,* 2002; Zheng *et al.,* 2005). Bioagent arbuscular mycorrhiza (AM) is characterized by specific morphological structures of the fungus and the plant and by physiological adaptation which are mostly beneficial for both partners of the symbiosis (Franken *et al.,* 2007). The multualistic interaction between plants and arbuscular mycorrhizal (AM) fungi is believed to be regulated from the plant side among other signals by the action of phytohormones (Hause *et al.,* 2007). Evidence for phytohormones function in the establishment of AM comes mainly from application experiments or from altered endogenous levels observed during mycorrhization (Baker & Tagu 2000). Jasmonate could serve as a putative endogenous signal in mycorrhiza-induced ISR, remains to be elucidated. Moreover, colonization by an arbuscular fungus leads to elevated levels of JA in plants and induced gene expression JIP_{23}, indicating that arbuscule formation is accompanied by JA production (Liu *et al.,* 2003 ; Hause *et al.,* 2007). On the other hand, at a higher mycorrhizal colonization rate, the concentration of SA was increased in barley roots, indicating that the systemic bioprotectional effect against pathogen in mediated by SA (Khaosoad *et al.,* 2007).

The signal molecules Jasmonic acid (JA) and salicylic acid (SA) are endogenous plant growth substances that play key roles in plant growth, development and response to stresses such as pathogen attack. Jasmonic acid and its various metabolites are members of the oxylipin family. Many of them alter gene expression positively or negatively in a regulatory network with synergistic and antagonistic effects in relation to other plant hormones such as salicylate, auxin, ethylene and abscisic acid (Wasternack 2007). Signal molecules are involved in some signal transduction system, which induce particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloides and pathogenesis-related proteins (Chong *et al.,* 2005). Exogenously applied of JA and SA have been shown to move systemically through plants, resulting in the expression of a set of defense genes that are activated by pathogen injection (Lu *et al.,* 2006). The development of acquired resistance by SA may be attributed at least partly, to the SA-induced phenyl alanine-ammonia lyase (PAL) gene expression and activation (Wen *et al.,* 2005).

Although induction of disease resistance using arbuscular mycorrhiza fungi or hormonal inducers (JA& SA) separately is well documented, there is little information about the mechanism of defense when used together. Thus, the objective of the present work was carried out to study the possible actions of the interaction between AM fungi and hormonal elicitors in the induced defense mechanisms in tomato plants against Fusarium wilt disease. Thus changes in various physiological defenses including antioxidant enzymes, phenolic compounds and pathogenesis related (PR) proteins were investigated in leaves of infected tomato plants.

MATERIALS AND METHODS

Tomato Seeds (*Lycopersecon esculentum* L.CV) after sterilization and drying were grown in large pots containing sterilized sandy loamy soil (2: 1 w/w) for 15 days. Then uniform seedlings were selected and transplanted to clay pots (5 seedling/pot) containing 10 kg of sterilized sandy loamy soil (1: 2 w/v). Pots and were divided into 7 groups (6 pots/group) and treated as the following:

- Plants of the 1st group were left without any treatments to serve as healthy control.
- Plants of the 2nd group were infected with *Fusarium oxysporum* to serve as infected control.
- Plants of the 3rd group were inoculated with AM fungi at the time of transplanting and infected with *Fusarium oxysporum* to serve as bioagent treatment.
- Plants of the 4th and 5th groups were infected with *Fusarium oxysporum* and sprayed 3 times with JA and SA, respectively, to serve as hormonal elicitor treatments.
- Plants of 6th and 7th groups were inoculated firstly with AM fungi at the time of transplanting and with JA and SA, respectively, to serve as bioagent and hormonal elicitor treatments.

AM Fungi and Fusarium Inoculation:

The arbuscular mycorrhiza species, *Glomus mosseae* (500 spore/pot) were placed 2-3 cm below the planting holes at the time of transplanting. While infection with *Fusarium oxysporum* was carried out after 2 weeks from transplanting time. Each pot was inoculated with 10ml of *Fusarium* culture suspension (10^7 cfu/ml).

Hormonal Elicitor Treatments:

At the time of *Fusarium oxysporum* infection, plants were sprayed once a week for 3 weeks with 50 µM JA or 100µM SA solution. Tween- 20 was added (0.01%) as a surfactant to hormone solution. 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 were separated from plants and used for various biochemical analysis.

Biochemical Measurements:

Assay of Reactive Oxygen Species (ROS) and Lipid Peroxidation:

The H_2O_2 content was measured according to Jana & Choudhuri (1981). Plant tissue (0.5g) was homogenized in 3ml of Na phosphate buffer (50 mM, pH 6.5) and centrifuged at 6000 x for 25 min. 3 ml of solution was mixed with 1 ml of 0.1% titanium chloride in 20% (v/v) H_2SO_4. The intensity of yellow color was measured at 410 nm. Determination of superoxide radicle (O_2^-) generated by leaves was based on the reduction of nitroblue tetrazolium (NBT) according to the method described by Doke (1983). Five leaf discs

(0.5 cm- diameter) were measured in 3 ml 0.01 M Na. phosphate buffer pH 7.8 containing 0.05% NBT and 10 mM NaN_3 for 1h. 2 ml samples of the mixture were heated at 85°C for 15 min and then cooled. The NBT reduction activity of the discs was expressed as increased in absorbance at 580 nm h^{-1} g^{-1} f. wt.

According to Heath & Packer (1968), level of lipid peroxidation was measured as the amount of malondialdhyde (MDA), which reacted with thiobarbituric acid (TBA) to form TBA-MDA complex and measured at 532 and 600 nm. Total MDA equivalents were calculated according to the following equation:

$$\text{Total MDA (nmol g}^{-1}\text{ f. wt)} = \frac{\text{Amount of Ext. buffer(ml) X amount of supernatant (ml) x [Abs532 - Abs660/155] x } 10^3}{\text{Amount of sample (g)}}$$

Enzyme Extraction and Activity Assay:

Frozen leaves were crushed to a fine powder in a mortar under liquid nitrogen. Soluble proteins of antioxidant enzymes were extracted in 50 mM of K. phosphate buffer (pH 7.0), 0.1mM EDTA, 4% polyvenylpyrrolidone (PVP) and 0.2mM ascorbic acid. After centrifugation at 10,000 x for 10 min at 4°C, the supernatant were used for assay of antioxidant enzymes. For LOX extraction, Na. phosphate buffer (0.2 M pH 6.5) containing 1% PVP, 15 mM/L mercaptoethanol and 0.25% Trition x-100 was used. While phenylalanine ammonia-lyase (PAL), B-1, 3-glucanase and chitinase were extracted using 0.1 M of Na. borate buffer (pH 7.0), sodium acetate buffer (0.5 M, pH 5.0) and 0.1 M sodium citrate buffer (pH 5.0), respectively.

Enzyme Assays:

Antioxidant Enzymes:

Guaiacol peroxidase (GPx) activity was measured by monitoring the increase in absorbance at 480nm as guaiacol reduction method according the method of Zhang (1992). The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolum(NBT) using the method of Beauchamp|& Fridovich (1971). One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction measured. CAT activity was determined by following the consumption of H_2O_2 at 240 nm for 2 min in 3 ml of a resection mixture containing 100 mM K. phosphate buffer pH 7.0 and 20 µL of 30% H_2O_2 and 30µL enzyme extract (Rao *et al.,* 1996). Ascorbate peroxidase (APX) activity was determined also according to the method described by Rao *et al.,* (1996). APX activity was recorded by following the decrease in A290 for 3 min in 1 ml of reaction mixture containing 100mM phosphate buffer pH 7.5, 0.5 mM ascorbate and 0.2 mM H_2O_2 and 30 µL enzyme extract. Following the method of Chen *et al.,* (2000) activity of polyphenol oxidase (PPO) was assayed by adding 1 ml of enzyme extract to 2 ml of catechol as a substrate and the changes in absorbance at 398 nm measured immediately.

Lipoxygerase (LOX) activity:

Activity of LOX was measured according to the method of Borthakur *et al.,* (1987). Linoleic acid was used as substrate for LOX assay which was prepared according to the method of Axelrod (1974). The reaction mixture contained 2.7 ml of 0.2 Na. phosphate buffer (pH 6.5) and 0.3 ml of substrate. The reaction was initiated by adding the enzyme extract and the change in absorbance at 234 nm was recorded for 3 min at 25°C. The specific activity was expressed as a change in the absorbance g^{-1} f wt^{-1} min^{-1}.

Assay of Phenylalanine-ammonia Lyase Activity (PAL):

Activity of PAL was measured according to the method of Assis *et al.,* (2001) with slight modifications. 1 ml of enzyme extract was incubated with 2 ml of borate buffer (50 mM, pH 8.8) and 1 ml of L-phenylalanine (20mM) for 60 min at 37°C. The reaction was stopped with 1ml of 1M HCl. The assay mixture was extracted with 3ml of toluene by vortexing for 30 sec. The absorbance of toluene phase containing trans-cininamic acid was measured at 290nm. Enzyme activity was expressed as nmol trans-cinnamic acid released $h^{-1}g^{-1}$ f.wt.

B-1, 3-glucanase and Chitinase Activities:

Total B-1, 3-glucanase activity was calorimetrically assayed by the laminarin dinitrosalicylate method (Pan *et al.,* 1991). The reaction mixture was prepared by mixing 62.5 µL of 4% laminarin and 62.5 µL of enzyme extract and incubating at 40°C for 30 min. the reaction was stopped by adding 375 µL of dinitrosalicylic acid reagent (prepared by adding 300ml of 4.5% NaOH to 880 ml containing 8.8g of

dinitrosalicylic acid and 22.5g K. Na tartarate) with subsequent heating for 5 min in a boiling water bath. The resulting colored solution was diluted with 4.5 ml of distilled water and vortexed. Products released were estimated for reducing groups at 500 nm. The enzyme activity was expressed as 1 nmol of reducing substances $min^{-1}g^{-1}$ f.wt.

Chitinase activity was assayed by increasing the release of product sugars from colloidal chitin (Tweddell *et al.,* 1994). A reaction mixture containing 1 ml of enzyme extract and 1 ml of colloidal chitin (2% w/v) in 50 mM Na. acetate buffer (pH 6.8) was incubated in a water bath for 60 min at 50°C. The released reducing sugar was measured by the somogyi-Nelson method (Somogyi 1952). Chitinase activity was expressed as 1 nmol of reducing sugars $h^{-1}g^{-1}$ f.wt.

Extraction and Analysis of Phenolic Compounds:

Frozen leaves (1g) was homogenized with 10 ml of 80 methanol and the extract left for 24 h at room temperature before centrifugation at 15.000 g for 10min. (Swain & Hillis 1959). One ml of the methanolic extract was added to 5ml of distilled water and 250 µL of Foline-ciocalteu reagent, and the solution was kept at 25°C for 3min. Then 1 ml of a saturated solution of Na_2CO_3 and 1ml of distilled water were added, and the mixture was incubated for 1h at 25°C. The absorption of the developed blue color was measured using spectrophotometer at 725nm. The total phenolic content was calculated by comparison with a standard curve obtained from a foline reaction with phenol. On the other hand, various phenolic acids in tomato leaves were determined using HPLC according to Singh *et al.,* (2002). Phenolic acids present in the samples were identified by comparing retention time (RT) of standards and by conjection. Concentrations were calculated by comparing peak areas of reference compounds with those in the samples run under the same elution conditions.

Protein Analysis:

Frozen leaves were extracted in 50 mM Tris- HCl (pH 7.0) buffer contained 10mM Na. EDTA, 5% B-mercaptoethanol and 10% glucerol. Protein fractions were characterized and identified using one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SPS-PAGE) according the method of Laemmli (1970). Proteins and molecular mass markers were visualized by staining with coomassie brilliant blue R-250 (0.5 g/L) and destained with 5% methanol/acetic acid mixture. The destained gel was photographed using a laser densitometer.

Statistical Analysis:

The recorded data were treated statistically using the one way analysis of variance as descrided by Snedecor & Cochran (1961). The means were compared by L.S.D using SPPS program version 8.

RESULTS AND DISCUSSIONS

Changes in Hydrogen Peroxide (H_2O_2) and superoxide (O^-_2):

The production of reactive oxygen species (ROS) is one of the earliest cellular responses following successful pathogen recognition (Grant *et al.,* 2000b). As shown in Table 1, infection by *Fusarium oxysporum* (Fo) significantly increased levels of H_2O_2 and O^-_2 production in tomato leaves, and its levels increased with increasing the time of infection (H_2O_2 and $O_2^{\bullet-}$ levels increased by 439.0 and 647.0% ,respectively, after 42 days from infection). Where, ROS was considered as the first defense line against pathogen and may act as direct antimicrobial agent against phyto pathogen attack (Malolepsza & Rozalska 2005; Torres *et al.,* 2006; Shetty *et al.,* 2007). Therefore, high production of H_2O_2 and $O_2^{\bullet-}$ in tomato leaves is an important element of disease resistance mechanism which are involved directly or indirectly in restricting pathogen growth and giving the time for plants to mobilize further defense reactions. Also accumulation of its levels may be related to the accelerated senescence and decreased in photosynthesis in infected tomato leaves as observed by El-Khallal (2007).

Application with bioagent AM fungi and/or hormonal elicitor JA markedly decreased levels of H_2O_2 and $O_2^{\bullet-}$ in tomato levels throughout the experiment, as compared with infected control. Unlike this, treatment with SA especially when applied alone markedly increased ROS production (Table 1). Induction or suppression of ROS generation in leaves of these treatments could be related to the activitiy of antioxidant enzymes (Table 2), which decreased levels of H_2O_2 (either by direct decomposition or oxidation) and $O_2^{\bullet-}$ (by its dismutation).Thus, SA-mediated inactivation of H_2O_2- degrading enzymes further enhance invoke hypersensitive cell death (Rao *et al.,* 1997). ROS was proposed to act synergistically in a signal amplification loop with SA to drive the HR and the establishment of systemic defence (Draper 1997). Also, JA induced H_2O_2 production in tomato (Orozcen-Cardenas *et al.,* 2001) and pepper (Ueeda *et al.,* 2006), which triggers

Table 1: Changes in the levels of hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$) in leaves of tomato plants infected with *Fusarium oxysporum* (Fo) and treated with AM fungi and hormonal elicitors (JA&SA) alone or in combination.

Treatment	H_2O_2 (µmol g^{-1} fresh wt.)			$O_2^{\cdot-}$ (OD_{560} $^{-1}$ fresh wt. h^{-1})		
	Days after pathogen inoculation					
	14	28	42	14	28	42
Control	0.87a	1.22a	1.93a	0.07a	0.12a	0.38a
Fo	3.19b	6.49b	11.02be	1.08b	1.58b	2.88b
Fo + AM fungi	2.68c	5.77c	6.62c	0.85c	1.41c	1.49c
Fo + JA	2.95d	6.03c	8.66d	0.75d	1.22d	1.40d
Fo + SA	4.89e	8.91e	11.88e	0.67e	1.43c	1.66e
Fo+ AM fungi + JA	2.66c	3.42f	4.97f	0.63e	1.20e	1.12f
Fo+ AM fungi + SA	3.88f	5.72c	10.44b	0.73f	1.19d	1.25g
LSD at 5 %	0.14	0.54	1.55	0.047	0.09	0.09

Values in columns with the same letters are not significantly at p> 0.05

expression of plant resistant and HR – related genes. On the other hand, AM fungi can elicit defense responses mediated by enhencing JA and SA signals in AM-treated plants. Thus, our results suggested that the high production of ROS (H_2O_2 and $O_2^{\cdot-}$) and the capacity of plants to controlled its concentrations might contribute to increased resistance against *Fusarium oxysporum* attack and giving the time for plants to mobilize further defense reactions. Where ROS have been involved in plant defense responses in several ways: a) reinforcing plant cell-wall through cross-linking reactions of lignin and proteins, b) acting as toxic agents against either the host plant cells, with development of HR and SAR, or against the pathogen through killing them or stopping their growth, and c) participating as a second messengers in signaling routes leading to the activation of plant defense related genes (Shinogi *et al.,* 2003 and Shimizu *et al.,*2006).

Changes in Lipid Peroxidation and Lipoxygenase Activity:

ROS (H_2O_2 and $O_2^{\cdot-}$) activity is frequently to cause membrane damage through peroxidation of fatty acids, which concided with a high activity of lipoxygenase (LOX) in tomato leaves as the results of *Fusarium oxysporum* infection (Table 2). Levels of MDA and LOX activity gradually increased in leaves of infected tomato plants with increasing the time of infection. In plants of AM fungi and hormonal elicitors (JA and SA) treatments, MDA levels markedly decreased while LOX activity greatly increased as compared with infected control. Among these treatments SA- treated plants had the highest MDA level but JA + AM fungi treated plants recorded the highest LOX activity. When plants are subjected to pathogen attack, the equilibrium between production and scavenging of ROS is broken, resulting in oxidative damage of proteins, DNA and lipids. MDA is the marker for lipid peroxidation released from cellular membranes of tissues and are formed by the reaction of ROS (H_2O_2 or/and $O_2^{\cdot-}$) with lipid molecules (Shimize *et al.,* 2006). Thus, lipid peroxidation in tomato plants has been proved to be induced by pathogens, and the subsequent products have been shown to passes antibacterial properties (Croft *et al.,* 1993) and signaling function (Melan *et al.,* 1993).

Table 2: Changes in the levels of lipid peroxidation (as indicated by malondialdehide, MDA) and lipoxygenase (LOX) activity in leaves of tomato plants infected with *Fusarium oxysporum* (Fo) and treated with AM fungi and hormonal elicitors (JA&SA) alone or in combination.

Treatment	MDA (µmol g^{-1} fresh wt.)			LOX activity (µmol products $min^{-1}g^{-1}$ fresh wt.)		
	Days after pathogen inoculation					
	14	28	42	14	28	42
Control	2.93a	3.79a	3.99a	13.05a	17.21a	21.20a
Fo	5.59b	7.44b	11.59b	18.55b	25.20b	32.15b
Fo + AM fungi	4.17c	4.72c	7.14c	21.90c	34.20c	48.90c
Fo + JA	4.67d	5.77d	8.59d	26.00d	36.75d	55.95d
Fo + SA	4.47c	5.41e	7.36e	21.35c	28.10e	42.40e
Fo+ AM fungi + JA	4.40c	5.09f	6.43f	28.81e	39.70f	63.60f
Fo+ AM fungi + SA	4.22e	5.01f	6.21g	22.00c	31.15g	48.20c
LSD at 5 %	0.38	0.19	0.20	1.86	1.98	3.60

Values in columns with the same letters are not significantly at p> 0.05

However, reduction in lipid peroxidation in infected plants treated with AM fungi plus JA might be related to the high activity of antioxidant enzymes (Fig 1), which preventing accumulation of free radicals, and consequently membrane damage. However, SA-mediated inactivation of H_2O_2 –degrading enzymes (CAT and APOX) further enhance the cellular free radical concentration that invoke hypersensitive cell death. It is appear

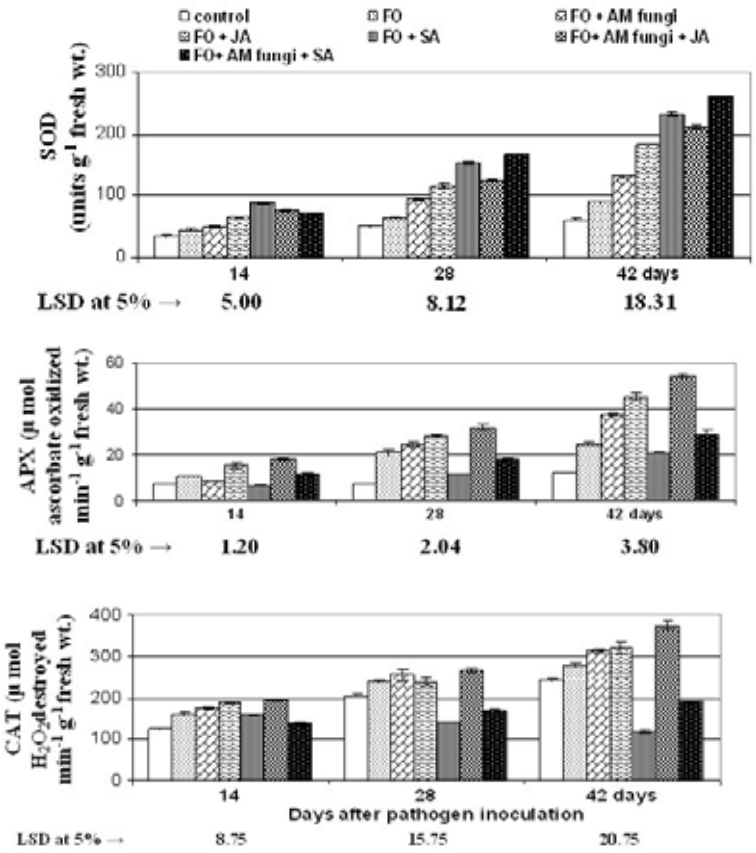

Fig. 1: Changes in the activities of SOD, APOX and CAT in leaves of tomato plants at different stages of growth, infected with *Fusarium oxysporum* (Fo) and treated with AM fungi and hormonal elicitors (JA&SA) alone or in combination.

that SA enhances H_2O_2- production to alter the cellular redox state and to provide an atmosphere that favors the generation of SA free radicals (SA^*) to initiate lipid peroxidation, hypersensitive cell death and the activation of several other defense mechanisms (Raw *et al.,* 1997). Also, Vernooig *et al.,* (1994) suggest that a specific SA-generated lipid peroxide is the translocated signals that invokes PR genes, because SA has been shown to be essential for the establishment of SAR but is not the translocated signals. In general, these results appears that AM fungi and hormonal elicitors may be requires H_2O_2 to potentate lipid peroxidation, induce PR genes and then establish SAR.

Lipoxygenase (LOX) has been implicated in the generation of ROS and play an important role in induced disease resistance and show significant antifungal activity against *Fusarium oxysporum* (Croft *et al.,* 1993, Kolomiets *et al.,* 2000; Babitha *et al.,* 2004; Huang *et al.,* 2005). It seems that the beneficial affects of either bioagent AM fungi or hormonal elicitors (JA and SA) could be partly due to changes in LOX activity in tomato leaves contributing to induced disease resistance and then antimicrobial activity. On the other hand, increased in LOX activity in mycorrhizal plants might be linked to the increased levels of JA (Hause & Fester 2005) which enhanced defensive capacity conferred by mycorrhization. Thus, various elements of induced systemic resistance have been observed in AM-treated plants, which seems-in contrast to SAR- to be dependent on the LOX products especially JA (Stumpe *et al.,* 2005).

Changes in the Activity of Antioxidant Enzymes:

Organisms protect themselves against oxidative stress by the synthesis of various antioxidant enzymes. Results in Fig.1 showed that infection by *Fusarium oxysporum* significantly increased SOD, APX and CAT activities in leaves of tomato plants at different stages of growth as compared with non-infected control.

Treatments with AM fungi and JA markedly raised these activities and the highest activity was recorded when applied together. But treatment with hormonal elicitor SA especially when applied alone markedly decreased H_2O_2 scavenging enzymes (APX and CAT) and greatly increased SOD activity.

From our result, we recorded that greater increase in the activity of antioxidant enzymes especially in AM plus JA-treated plants might be affective in scavenging mechanism to remove H_2O_2 and $O_2^{\bullet-}$ produced in leaves under *Fusarium oxysporum* infection. In banana plants, Subramaniam *et al.,* (2006) reported that levels of H_2O_2 and other enzyme activities were increased to the levels of tolerance or susceptibility to *Fusarium* wilt disease. On the other hand, accumulation of H_2O_2 (Table 1) in plants of infected control and SA-treated plants could be related to the increased in SOD activity and not coordinates with the same increase in H_2O_2- scavenging enzyme (CAT and APOX). According that increase in CAT and APX activity in pathogen infected plants did not seem to be high and/or enough to avoid the development of disease symptoms. Durner and Klessing (1996) have presented evidence suggesting that SA-mediated inhibition of CAT and APX probably results from peroxidative reactions. It appears that SA requires H_2O_2 to potentate lipid peroxidation (Table 2) as translocation signal that invokes PR genes and establish SAR (Rao *et al.,* 1997). Finally, results in Tables 1 & 2 and Fig.1 suggested that increased in levels of ROS, built up by either enhanced production and decreased scavenging potential, may contribute to the resistance reaction in tomato against *Fusarium* wilt. Finally, imbalance between H_2O_2 –generation and scavenging enzymes in leaves may reflect a defense mechanism in tomato or a pathogenicity strategy of the fungus.

Changes in Phenolic Compounds and its Related Enzymes:

Many plant phenolic compounds are known to be antimicrobial, function as precursors to structural polymers such as lignin, or serve as signal molecules (Hammerschmidt 2005). Results in Table 3 showed that total phenolic compounds markedly increased in leaves of all treated tomato plants at 42 days from pathogen inoculation. JA when applied alone or in combination with AM fungi having the highest total phenol. Increasing evidence indicates that JA induced changes in secondary metabolism constitute an ubiquitous plant defense response (Zhao *et al.,* 2005). Recently, a 6-fold increase in phenolic compounds was observed in *Hypericum perforatum* cells suspension after JA elicitation (Gadzouska *et al.,* 2007).

However, levels of certain phenolic acids greatly changed in leaves of tomato plants in response to pathogen inoculation, AM fungi and hormonal elicitors as compared with normal control. Benzoic acids and Galleic acids contents markedly decreased in all treatments under pathogen infection. Among these treatments, JA and SA-treated plants, when applied alone having high levels of Benzoic and Galleic acids, respectively. However, contents of coumaric, cinnamic, chlorogenic and ferulic acids in all treatments of *Fusarium* tomato plants increased as compared with non-infected control. JA individually or plus AM fungi highly increased these phenolic as compared with those of SA and/or AM fungi treatments. According that induction of total phenol and changes in the contents of various plenolic acids in leaves of all treatments which play an important role in plant resistance and defense against *Fusarium oxysporum* infection, which are intimately connected with ROS (Table 1). These results agree with the general speculation that when plant cells are recruited into infection, switch from normal primary metabolism to a multitude of secondary metabolism defense pathway and activation of novel defense enzymes and genes takes place (Tan *et al.,* 2004). Also, rapid esterification of phenolic compounds as cinnamic and ferulic acids into plant cell walls is a common and early response to fungal attack, resulting in cell strengthening and then enhances resistance to pathogen penetration (Stadnik & Buchenouer 2000). High accumulation of cinnamic acid in JA and SA, -AM fungi treated plants (Table3), might be related to the activation of phenyl propanoid pathway through increased in PAL activity (Fig.2) which reflect a component of a defense response of the plant against *Fusarium oxysporum* penetration. However, induction in ferulic and chlorogenic acids in infected leaves indicated that both acids are highly antifungal (Agrios 1997), supported its role in reducing disease through the formation of defense barriers and activation of defense responses. Although benzoic acid was more effective and greatly phytotoxic against soil borne root infecting fungi, its content decreased in leaves of various treatments of tomato plants and this might be because BA converted to both bound SA and gentisic acid (Schulz *et al.,* 1993; Belles *et al.,* 1999).

In parallel to the induction of various phenolic acids in tomato leaves , results in Fig.2 showed that activities of POX, PPX and PAL significantly increased in tomato plants in response to *Fusarium oxysporum*. JA-treated plants (alone or combined with AM fungi) caused the highest POX and PPX activities, while SA-treated plants having the highest PAL activities. POX and PPX are important in the defense mechanism against pathogens, through its role in the oxidation of phenolic compounds to quinines, causing increasing in antimicrobial activity. Therefore, its may be directly involved in stopping pathogen development (Shimizu 2004; Melo *et al.,* 2006); accelerating the cellular death of cells close to the infection site, preventing the advance of infection and/or by generating a toxic environment which will inhibit the growth of the pathogen inside the cells (Bi & Felton 1995).

Table 3: Changes in the contents of total phenol and some phenolic acids(µg $^{-1}$fw) in leaves of tomato plants (42 days after pathogen inoculation) treated with bioagnet AM fungi and/or hormonal elicitors (JA & SA) and grown in *Fusarium oxysporum* (Fo) pathogenized soil.

Treatment	Total phenolic acids	Benzoic acid	Coumaric acid	Galleic acid	Cinnamic acid	Chlorogenic acid	Ferulic acid
Control	330	20.5	19.2	54.1	13.2	7.5	8.5
Fo	394	11.6	35.1	31.8	25.2	10.8	11.6
Fo+AM fungi	460	12.8	42.6	12.2	36.1	12.4	13.8
Fo+JA	605	21.5	71.5	22.4	44.3	18.4	12.6
Fo+SA	527	6.55	52.8	26.0	38.5	15.6	16.4
Fo+JA+AM fungi	916	13.3	89.0	14.0	44.6	21.5	25.0
Fo+SA+AM fungi	680	8.8	73.4	17.6	39.2	17.3	22.7

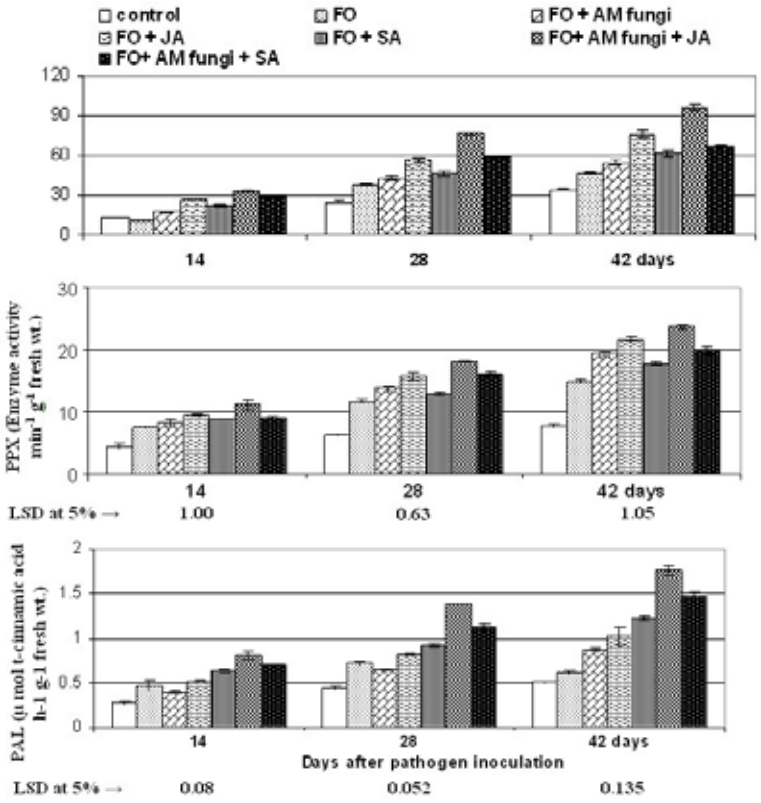

Fig. 2: Changes in the activities of GPOX, PPX and PAL in leaves of tomato plants at different stages of growth, infected with *Fusarium oxysporum* (Fo) and treated with AM fungi and hormonal elicitors (JA&SA) alone or in combination.

On the other hand, increase in PAL activity in tomato leaves is a common plant reaction to pathogen and has often been related with some forms of resistance induced by JA (Ye et al 2006), SA (Chen *et al.,* 2006) and AM fungi (Zheng *et al.,* 2005) in different plants. Also high activity of PAL in SA-treated plants (alone or plus with JA) indicated that the development of acquired resistance by SA may be attributed at least partly, to the SA induced PAL gene expression and activation. Where, SA could activate PAL by enhancing the accumulation of PAL mRNA, the synthesis of new PAL protein and its activity and consequently enhances the accumulation of phenylpropanoids such as phenolic acids (Chen *et al.,* 2006). Finally, our results suggested that phenyl propanoid compounds are more rapidly synthesized in leaves of infected tomato plants especially treated with hormonal elicitors plus bioagent. Thus accumulation of phenolic compounds and high activity of lignifications enzymes in these plants may reflect a component of many defense signals activated by bioagent and hormonal inducers which leading to the activation of power defense system in tomato against *Fusarium oxysporum* attack.

Changes in the Activities of β-1, 3-glucanase and chitinase:

β -1,3 glucanase and chitinase had been related with plant defense mechanism against pathogen infection through its ability to degrade fungal cell walls. Results in Fig.3 cleared that infection of tomato plants with

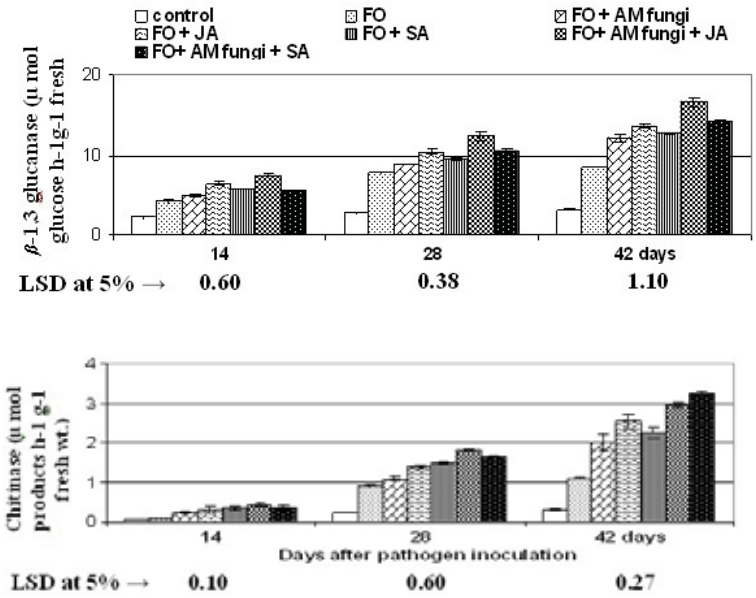

Fig. 3: Changes in the activities of ß-1,3 glucanase and chitinase in leaves of tomato plants at different stages of growth, infected with *Fusarium oxysporum* (Fo) and treated with AM fungi and hormonal elicitors (JA&SA) alone or in combination.

Fusarium oxysporum significantly increased activity of β -1, 3-glucanase and chitinase, these increments gradually increased with increasing the time of infection. In comparison between treatments, JA alone or in combination with AM fungi caused highly significant increase in β -1,3 glucanase through different stages of growth, while treatment with SA plus AM fungi at stage III (42days after pathogen inoculation) recorded the highest chitinase activity. The same results were recorded by Blade *et al.,* (2006), they found that a relationship between chitinase and melon resistance to *Fusarium oxysporum* infection, and the resistance is associated with certain isoform of these enzymes

β -1,3-glucanase and chitinase hydrolyze polymers of fungal cell walls, where fragments of cell wall were shown to induce defense reaction in plants by switching on genes responsible for the synthesis of pathogen related proteins (Shunja, *et al.,* 2007). Also, combination between JA or SA and bioagent *Cryptococcus laurentic* strongly induced chitinase and β -1,3-glucanase in sweet cherry (Yao & Tian 2005). Moreover, induction in these enzymes in tomato leaves as the result of AM colonization could play a role in bioprotection through working together against pathogen invading. This could be exerted indirectly by releasing oligosaccharides from the pathogen cell wall that could elicit host defense reactions (Pozo *et al.,* 1999; Buzi *et al.,* 2004).

Changes in Protein Profile and Induction of Pathogen-related Proteins (PR-Ps):

The SDS-PAGE electrophoretic pattern of proteins extracted from tomato leaves of various treatments are shown in photo1. The relative molecular weights and percentages of protein bands are recorded in Table 4. Results appeared that protein bands of molecular masses 65.0, 16.3 and 12.5 KDa which detected in proteins extracted from leaves of non-infected tomato plants (healthy control) were disappeared in proteins of tomato leaves in response to *Fusarium* infection, AM-fungi and both hormonal elicitors. Inoculation with AM fungi induced the appearance of especial 3 protein bands having molecular masses 64.0, 50.0 and 12.7 KDa in leaves of infected tomato plants and was not excited by JA and SA individually or in combination with AM-fungi. However, a set of seven new proteins (120, 70, 33.0, 22.0, 18.6, 4.5 and 4 KDa) were recorded in proteins extracted from leaves of plants treated with AM fungi and/or JA and SA. The highest relative concentrations of these bands were found in AM fungi plus JA-treated plants.

Treatments with JA and SA (alone) induced the appearance of novel proteins having 30.5, 3.0 and 7KDa, but when applied with AM fungi, protein bands of molecular weights 62, 32, 9 (AM fungi + JA), 31.0 and 5.0 (AM fungi +SA) were induced in tomato leaves. In addition, a set of 13 protein bands of molecular masses

112.9, 88.6, 81.8, 53.6, 46.0, 37.0, 26.6, 21.0, 15.0, 11.5, 9.2, 7.7 and 6.0 KDa were found in proteins of all treatments and were considered as main bands (these bands were not recorded in Table 4).

Our results revealed that inhibition or induction of a number of regulatory proteins are implicated to enhance plant defense response in different ways and might be reflect strategies of bioagent and/or hormonal

Table 4: Comparative analysis of relative molecular weights and band concentrations (%) of the different types of protein bands in leaves of tomato plants (42 days after pathogen inoculation) infected with *Fusarium oxysporum* (Fo) and treated with AM fungi and/or hormonal elicitors (JA&SA).

M.wt	Control	Fo	Fo+AM Fungi	Fo+JA	Fo+SA	Fo+AM Fungi+JA	Fo+AM Fungi+SA
142.0	0.34	0.92	-	0.87	-	-	-
120.0	-	-	0.93	1.94	0.34	0.75	2.5
70.0	-	-	1.26	3.28	2.86	4.06	3.81
65.0	3.6	-	-	-	-	-	-
64..0	-	2.01					
62.0	-	-	-	-	-	2.36	-
50.0	-	1.7	4.2	-	-	-	-
33.0	-	-	2.01	2.45	1.63	2.78	2.64
32.3	-	-	-	-	-	1.34	-
31.0	-	-	-	-	-	-	2.67
30.5	-	-	-	0.92	1.86	-	-
22.0	-	-	1.32	0.96	1.41	1.85	2.31
18.0	-	-	3.8	2.81	0.33	2.44	2.7
16.3	1.97	-	-	-	-	-	-
13.0	-	-	-	0.42	0.26	-	-
12.7	-	0.17	0.42	-	-	-	-
12.5	0.32	-	-	-	-	-	-
9.0	-	-	-	-	-	1.31	-
7.0	-	-	-	3.21	4.82	-	-
5.0	-	-	-	-	-	-	1.2
4.5	-	-	1.3.4	0.84	1.1	0.36	1.5
4.0	-	-	1.95	1.85	1.3	7.33	2.7

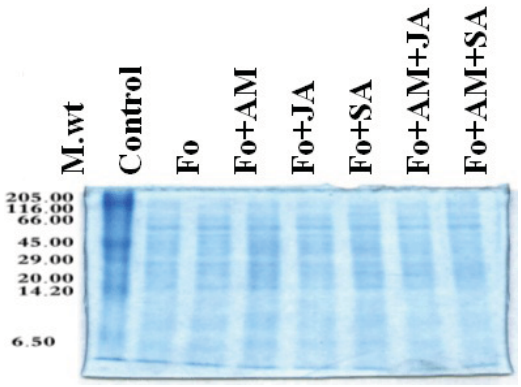

Photo1: Electrophoretic protein pattern of tomato leaves infected with *Fusarium oxysporum* (Fo) and treated with bioagent AM fungi and/or hormonal elicitors (JA&SA).

elicitors to reinforce tomato defense against *Fusarium* wilt disease. Therefore, induction of protein bands of molecular weights 35, 33, 32, 31 (PR-2, β -1, 3 glucanase), 30.5 and 27 (PR-3,-4, chitinase) (Fig. 3) and its high activities in these treatments indicated the important role which played in plant resistance. Application of JA and SA coordinary activated transcripts of different groups of defense proteins including transcript of chit, chit 3 and glu 2 and reduced common bunt infection (Lu *et al.,* 2006). B-1, 3-glucanase with molecular weights 31 and 35 KDa markedly increased in wheat leaves after treatment with JA and SA. Also, mycorrhizal fungi induced new isoform of chitinase, chitosanase and glucanase in tomato roots, and were suggested to be responsible for reduced disease symptoms upon *Phytophthora parasitica* infection (Pozo *et al.,* 1998). However, induction of low molecular weights proteins (5, 4.5 and 4 KDa) in leaves of AM fungi and/or hormonal elicitors (JA and SA) under *Fusarium oxysporum* infection might belongs to thionins family (PR-13 and 5 KDa) and presenting toxic effect against bacteria, fungi and yeast (Pennickx *et al.,* 1998). Thionin protein in plants might play a role in altering cell wall upon penetration of the epidermis by fungal hypha and act as a secondary messenger in signal transduction (Florack & Stiekema 1994). Expression of thionine gene was

triggered by JA (Anderson *et al.,* 1992). On the other hand, accumulation of 22 KDa protein (PR-5, Thaumatin-like proteins TLPs) in AM plus JA or SA-treated plants greatly exhibited antifungal activity against a variety of pathogenic fungi are postulated to play a role in enhanced plant disease resistance (Velazhahan & Muthukrishnan 2003 and 2004). Finally, appearance of 70 KDa proteins in these plants represents one of the Ca^{2+}-dependent kinase (CDPK) which acts as a signal protein in resistance mechanisms (Romeis *et al.,* 2000). However, absence of this protein in tomato plants of *Fusarium oxysporum* (infected control) may retard the triggering of resistance gene transcription which leads to pathogen-related proteins as reported by Nafie 2003.

From these results we concluded that interaction between hormones (JA& SA) and AM fungi signals mediated the expression of the majority of different PR-proteins leading to increasing defense mechanism against *Fusarium oxysporum* infection. Thus, reduction in wilt disease symptoms and enhancing in growth and metabolic activities in tomato plants treated with AM fungi and/or hormonal elicitors (El-Khallal 2007) might be related to its roles in the activation of biochemical and structural defense systems that helps word off the spread of pathogen and consequently increase tomato production. Finally, the new mechanism of the combination strategy between bioagent and hormonal signals (either synergistically or antagonistically) played important role for altering expression of defense genes leading to different PR-proteins and also working together to increased resistance in tomato plants against wilt disease caused by *F.oxysporum*. In addition, results revealed that defense mechanism in plants treated with AM fungi and JA are more effective than AM fungi plus SA- treated plants.

REFERENCES

Agrios, G.N., 1997. Plant Pathology. 4th Edition. Academic Press, San Diego, USA, pp: 93-114.

Agrios, G.N., 2005. Plant Pathology. 5th Edition. Academic Press, San Diego, USA.

Andreson, I., W. Pecker and K. schluter, J. Burges., B. Parther and K. Apel, 1992. The identification of leaf thionin as one of the main jasmonate- induced proteins of barley (*Hordeum vulgare*). Plant Mol Biol., 19: 193-204.

Axelrod, B., 1974. Lipoxygenases, ACS. Adu. Chem. Ser., 136: 324-348.

Assis, J.S., R. Maldonado, T. Munoz, M.I. Escribano and C. Merodio, 2001. Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. Postharvest Biol. Techmol., 23: 33-39.

Babitha, M.P., H.S. Prakash and H.S. Shetty, 2004. Purification and properties of lipoxygenase induced in downy mildew resistant pearl millet seedlings due to infection with *Sclerospora graminicola*. Plant Science., 166: 31-39.

Barker, S. and D. Tagu, 2000. The roles of auxin and cytokinins in mycorrhizal symbiosis. J. Plant Growth Regul., 19: 144-154.

Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: improved assays and assay applicable to acrylamide gels. Anals. Biochem, 44: 276-287.

Belles, J.M., R. Carro, J. Fayos, P. Navarro, J. Prino and V. Conejero, 1999. Gentisic acid as a pathogen-inducible signal, additional to salicylic acid for activation of plant defenses in tomato. Mol Plant Microbe Inter., 12(3): 227-235.

Bi, J.L and G.W. Felton, 1995. Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites and reactive oxygen species as components of induced resistance. J. Chem. Ecol., 21: 1511-1530.

Blade, J.A., R. Franciso, A. Queroz, P.A. Regaloda, C.P. Ricardo, and M.M. Veloso, 2006. Immunolocalization of a class III chitinase in two muskmelon cultivars reacting differently to *Fusarium oxysproum* F.S.P. melons. J. Plant Physiol., 163: 19-25.

Borthakur, A.B., B.G. Bhat and C.S. Ramadoss, 1987. The positional specification of the oxygenation of linoleic acid catalyzed by two forms of lipoxygenase isolated from Bengal gram(*Cicer arietinum*).J bioscience., 11: 257-263.

Bolwell, G.P., 2004. Role of active oxygen species and NO in plant defence responses. Current Opinion in Plant Biology., 2: 287-294.

Buzi, A., C. Chilosi, D. De Sillo and P. Magro, 2004. Induction of resistance in Melon to *Didymella bryoniae* and *Sclerotinia Sclerotiorum* by seed treatments with acibenzolor-S-Metlyl and methyl jasmonate but not with salicylic acid. J. Phytopathology., 152: 34-42.

Castro, M.S. and W. Fontes, 2005. Plant defense and antimicrobial peptides. Protein and Peptide Letters., 12: 11-16.

Cavalcanti, F.R., M.L. Resende, S.P. Lima, J.A. Silveira and J.T. Oliveira, 2007. Activities of antioxidant enzymes and photosynthetic responses in tomato pre-treated by plant activators and inoculated by *Xanthomonas vesicatoria*. Physiol Mol Plant Pathol., 68(4-6): 198-208.

Chen, C., R. Belanger, N. Benhamou and T.C. Paulitz, 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythiuem aphamidermatum*. Physiol Mol Plant Pathol., 56: 13-23.

Chen, H., A.D. Jones and G.A. Howe, 2006. Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. FEBS letters., 580: 2540-2546.

Chong, T.M., M.A. Abdullah, N.M. Fadzellah, O.M. Lai and N.H. lajis, 2005. Jasmonic acid elicitation of anthraquinones with some associated enzymic and non-enzymic antioxidant responses in *Mornida elliptica*. Enzyme and Microb Technology., 36: 469-477.

Conćeicao, L.F., F. Ferreres, R.M. Tavores and A.C. Dios, 2006. Induction of phenolic compounds in *Hypericum pertoralum* L. cells by *Colletotricohum gloeosprioides* elicitation. Phytochemistry., 67: 149-155.

Cota, I.E., R. Troncaso-Rojas. R.Sotelo-Mundo, A. Sanchez-Estrada and M.E. Tiznado-Hernandez, 2007. Chitinase and ß-1, 3-glucanase enzymatic activities in response to infection by *Alternaria alternate* evaluated in two stages of development in different tomato fruit varieties. Scientica Horticulturae., 112: 42-50.

Croft, K.P.F. Juttner and A.J. Slusarenko, 1993. Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* PV. Phaseolicola. Plant Physiol., 101: 13-24.

De Gara, L., M. de Pinto and F. Tommasi, 2003. The antioxidant systems via-á-via reactive oxygen species during plant-pathogen interaction. Plant Physiol Biochem., 41: 863-870.

Delledonne, M., I. Murgia, D. Ederle, P.F. Sbicego, A. Biondian, A. Polverari and C. Lamb, 2002. Reactive oxygen intermediates modulates nitric oxide signaling in the plant hypersensitive disease-resistance response. Plant Physiol Biochem., 40: 605-610.

Doke, N., 1983. Involvement of superoxide generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestance* and to hyphal wall components. Physiol Plant Pathol., 23: 345-357

Draper, J., 1997. Salicylate, superoxide synthesis and cell suicide in plant defense. Trends Plant Sci., 2: 162-165.

Durner, J. and D.F. Klessig, 1996. Salicylic acid is a modulator of tobacco and mammalian catalase. J. Biol. Chem., 271: 28492-28501.

El-Khallal, S.M. and A.A. Yussry, 2000. Role of salicylic acid in growth of tomato seedlings infected by *Fusarium Solani.* J. Union Arab Biol., Cairo., (9B): 25-36.

El-Khallal. S.M., 2007. Induction and Modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (arbuscular mycorrhiza) and hormonal elicitors (jasmonic acid and salicylic acid): 1 Changes in growth, some metabolic activities and endogenous hormones related to defense mechanism (in press).

Florack, D.E. and W.J. Stiekema, 1994. Thionins: Propertie, possible biological roles and mechanisms of action. Plant Molecular., 26: 25-37.

Franken, P., K. Donges, V. Grunwold, K. Gerhard, K. Rexer, M. Tamasloukht, A. Waschke and D. Zeuske, 2007. Gene expression analysis of arbuscule development and functioning., 68: 68-74.

Gadzouska, S., A. Delaunnay; M. Spasenoski, S. Maury; C. Joseph and D. Hağege, 2007. Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphtodianthrones. Plant Cell Tissue and Organ Culture., 89(1): 1-13.

Grant, M.I., S. Brown, M. Adams, A. Kinght, A. Ainslie and J. Mansfield, 2000b. The RPMI plant disease resistance increase in cytosolic calcium that in necessary for the oxidative burst and hypersensitive cell death. Plant J., 23: 441-450.

Hahlbrock, K., P. Bednarek., I. Ciolkowski, B. Hamberger, A. Heise, H. Liedgns, E. Logemann, T. Nurnberger, I. Somssich and J. Tan, 2003. Non-self recognition, transcriptional reprogramming, and secondary metabolite accumulation during plant/pathogen interactions. Proc. Natl. Acad. Sci. USA., 25 (100 suppl. 2): 14569-14576.

Hammerschmidt, R., 2005. Phenols and plant-pathogen interactions: The saga continues. Physiol Mol Plant Pathol., 66: 77-78.

Heath, M., 2000. Hypersensitive response related death. Plant Mol Biol., 44: 321-334.

Heath, R.I. and L. Packer, 1968. Photoxidation in isolated chloroplasts: 1-Kinetics and stoichiommetry of fatty acid peroxidation. Archives of Biochemistery and Biophysics., 125: 1189.

Hause, B. and T. Fester, 2005 Molecular and cell biology of arbuscular mycorrhizal symbiosis. Planta., 221: 184-196.

Hause, B., C. Mrosk, S. Isayenkov and D. Strack, 2007. Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry., 68: 101-110.

Hause, B., W. Maier, O. Miersch, R. Kramell and D. Strack, 2002. Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiol., 130: 1213-1220.

Iniguez, A.L., Y. Dong, H.D. Carter, B.M. Ahmer, J.M. Stone and E.W. Triplett, 2005., Regulation of enteric endophytic bacterial colonization by plant defense. Mol Plant- Microb Interact., 18: 169-178.

Jana, S. and M.A. Choudhuri, 1981. Glycolate metabolism of the three submerged aquatic angiosperm during aging. Aquat Bot., 12: 345-352.

Kasprzewska, A., 2003. Plant chitinases - regulation and function. Cell Mol Biol lett., 8: 809-824.

Khaosaad, T., J.M. Garcia-Garrido, S. Steinkellner and H. Vierheilig, 2007. Talk-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol and Biochem., 39: 727-734.

Kolomiets, M.V., H. Chen, R.J. Gladon, E.J. Braun and D.J. Hannapel, 2000. A leaf lipoxygenase of potato induced specifically by pathogen infection. Plant Physiol., 24: 1121-1130.

Künke, B.N. and D.M. Brooks, 2002. Cross talk between signaling pathway in pathogen defense., Curr. Opin. Plant Biol., 5: 325-331.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature., 227: 680-685.

Lamb, C. and R.A Dixon, 1997. The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol., 48: 251-257.

Liu, J.V., L.A. Blaylock, G. Endre, J. Cho, C.D. Town, K.A. Vanden Bochi and M.J. Harrison, 2003. Transcript profiling coupled with spatial expression analysis reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. Plant Cell., 15: 2106-2123.

Lu, Z.X., D. Gaudet, B. Puchalski, T. Despins, M. Frick and A. Laroche, 2006. Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defense-gene expression. Physoil Mol Plant Pathol., 67: 138-148.

Malolepsza, U. and S. RÓzalaska, 2005. Nitric oxide and hydrogen peroxide in tomato resistance. Nitric oxide modulates hydrogen peroxide level in O.hydroxyethylorutin-induced resistance to *Botrytis cinerea* in tomato. Plant Physiol Biochem., 43: 623-635.

Melan, M.A., X. Dong, M.E. Endara, K.R. Ausubel, F.M. and T.K. Peterman, 1993. An *Arbidopsis thaliana* lipoxygenase gene can be induced by pathogen, abscisic acid, and methyl jasmonate. Plant Physiol., 101: 441-450.

Melo, G.A., M.M. Shimizu and P. Mazzafera, 2006. Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. Phytochemistry., 67: 277-285.

Michē, L., F. Battistoni., S. Gemmer., M. Belghazi and B. Reinhold-Hurek, 2006. Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the *Endophyte Azoareus* SP. Mol Plant. Microb Interact., 19(5): 502-511.

Mohammadi, M. and A. Karr, 2002. β-1, 3-glucanase and chitinase activities in soybean root nodules. J. Plant Physiol., 159: 245-256.

Morkunas, I. and J. Gemerek, 2007. The possible involvement of peroxidase in defense of yellow lupin embryo axes against *Fusarium oxysporum*. J. Plant Physiol., 164: 497-506.

Nafie, E.M., 2003. The possible induction of resistance in *Lupinus termis* L. Against *Fusarium oxysporum* by *Streptomyces chibaensis* and its mode of action. II: Alleviating oxidative stress associated with infection. International J. Agric Biol., 5(4): 473-480.

Orozcon-Cardenas, M.L., J. Narvaez-Vasquez and C.A. Ryan, 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin and methyl jasmonate. Plant Cell., 13: 179-191.

Pan, S.Q., X.S. Ye and J. Kuc, 1991. Association of ß-1, 3-glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with pernospara tabacive or leaf inoculation with tobacco mosaic virus. Physiol. Mol. Plant Pathol., 39: 25-39.

Penninckx, I., B. Thomma, A. Buchala, J.P. Metraux and W.F.Broekaert, 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defens in gene in arabidopsis. Plant Cell, 10: 2103-2113.

Pozo, M.J., C. Azcon-Aguilar, E. Dumas, Gaudot., S. and J.M. Barea, 1998. Chitosanase and chitinase activities in tomato roots during interactions with arbuscular mycorrhiza fungi or *Phytophthora parasitica*. J. Exp Bot., 49: 1729-1739.

Pozo, M.J., C. Azcon-Aguilar, E. Dumas-Gaudot and J.M. Barea, 1999. B-1, 3-glucanase activities in tomato roots inoculated with arbuscular mycorrhiza fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. Plant Science., 141: 149-157.

Pozo, M.J., C. Cordier, E.Dumas-Gaudot, S. Gianinazzi, J.M. Barea and C. Azcon-Aguilar, 2002. Localized versus systemic effect of arbuscular mycorrhiza fungi on defence response to *phytophtora* infection in tomato plants. J. Exp Bot., 53: 525-534.

Rao, M.V., G. Paliyath and D.P. Ormrod, 1996. Ultraviolet- B-radiation and ozone-induced biochemical changes in the antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol., 110: 125-136.

Rao, M.V., P. Gopinadhan, D.P. Ormrod, D.P. Murr and C.B. Watkins, 1997. Influence of salicylic acid on H_2O_2 production, oxidative stress, and H_2O_2 metabolizing enzymes. Plant Physiol., 115: 137-149.

Romeis, T., P. Piedras and J.D. Jones, 2000. Resistance-gene-dependent activation of a calcium-dependent protein kinase (CDPK) in the plant defense response. Plant cell, 5: 321-386.

Ray, H., D.S. Douches and R. Hammerschmiat, 1998. Transformation of potato with cucumber peroxidase. Expression and disease response. Physiol Mol Plant Pathol., 53: 93-103.

Schoutten, A., K.B. Tenberge, J. Vermeer, J. Stewart, L. Wagemakers and B. Williamson., 2002. Functional analysis of an extracellular catalase of *Botrytis cinerea*. Mol Plant Pathol., 3: 327-338.

Schulz, M., H. Schnable., B. Manthe, B. Schweihofen and I. Casser, 1993. Uptake and detoxification of salicylic acid by *Vicia faba* and *Fagoprum esculentum*. Phytochemistry., 33: 291-294.

Shetty, N.P., R. Mehrabi, H., Lutken, A. Haldrup, G.H. Kema, D.P. Collenge and H.J. Jorgenson, 2007. Role of hydrogen peroxide during the interaction between the hemibiotrophic fungal pathogen *Septoria tritici* and wheat. New phytologist., 174(3): 637-637.

Shimzu, N., N. Hosogi, G.S. Hyon, S. Jiang, K. Inoue and P. Park, 2006. Reactive oxygen species (ROS) generation and ROS induced lipid peroxidation are associated with plant membrane modifications in host cells in response to AK-toxin from *Alternaria alternate* Japanese pear pathotype. J. Gen Plant Pathol., 72: 6-15.

Shinogi, T., T. Suzuki, T. Kurihara, Y., Narusaka and P. Park, 2003. Microscopic detection of reactive oxygen species generation in the compatible and incompatible interaction of *Alternasia alternata* Japanese pear pathotype and host plant. J Gen Plant Pathol., 69: 7-16.

Shunja, T., K. Hanai, I. Galis, K. Suzuki, K. Matsuoka, H, Matsuoka and M. Soite, 2007. Characterization of Nt chit IV, a class IV chitinase induced by β-1, 3-, 1-6-glucan elicitor from *Alternaria alternate* 102: Antagonistic effect of salicylic acid methyl jasmonate on the induction of Nt chit IV. Biochemical Biophysical Research Communication., 353: 311-317.

Singh, U.P., B.K. Sarma, D.P. Singh and A. Bahadur, 2002. Plant growth-promoting rhizobacteria – mediated induction of phenolics in pea *Pisum sativum* following infection with *Erysiphe pisi*., Curr. Microbiol.

Snedecor, G.M and W.C. Cochran., 1969. Statistical Method. 6th Ed. Lowa Univ. Press, Ames, Iowa, USA.

Somogyi, M., 1952. Notes on sugar determination. The Journal of Biol Chem., 195: 19-23.

Stadnik, M.J. and H. Buchenauer, 2000. Inhibition of phenylalanine ammonia-lyase suppresses the resistance induced by benzothiadiazole in wheat to *Blumeria graminis* F.SP. tritici. Physiol Mol Plant Pathol., 57: 25-34.

Stumpe, M., J.G. Carsjens, I. Stenzel, G. Göbel, I. Lang, B. Hause and I. Feussner, 2005. Lipid metabolism in arbuscular mycorrhizal roots of *Medicage truncatula*. Phytochemistry., 66: 781-791.

Subramaniam, S., M. Moziah, M. Sariah, M.P. Puad and R. Xavier, 2006. Bioassay method for testing Fusarium wilt disease tolerance in transgenic banana. Scientia Horticulturae., 108: 378-389.

Swain, T. and W.E. Hillis, 1959. The phenolic constituents of *Prumus domestica* 1. the quantitative analysis of phenolic constituents. J. Sci. Food Agric., 10: 63-68.

Tan, J.B. Schneider, A. Svatos, P. Bendnarek, J. Liu and K. Hahlbrock, 2004. Universally occurring phenylpropanoid and specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves. Phytochemistry., 65: 691-699.

Torres, M.A., D.G. Jonathan and J.L. Dangl, 2006. Reactive oxygen species signaling in response to pathogen. Plant physiol., 141: 373-378.

Tweddell, R.J., S.H. Jabaji-Hare and P.M. Charest, 1994. Production of chitinase and β-1, 3-glucanase by *Stachybotrys elegans*, a mycoparasite of *Rhizoctonia solani*. Appl Environ. Microbiol., 60: 489-495.

Ueeda, M., K. Masaharu and K. Nishi, 2006. Contribuation of jasmonic acid to resistance against phytophthora blight in *Capsicum ammum* CV. SCM 334. Physiol Mol. Plant Pathol.

Velazhahan, R. and S. Muthukrishnan, 2003/4. Transgenic tobacco plants constitutively overexpressing a rice thaumotin-like protein (PR-5) show enhanced resistance to *Alternaria alternata*. Biol Plant., 47: 347-354.

Vernooij, B., L. Friedrich, A. Morse, R. Reist, R. Kolditz-Jawhar, E. Ward, S. Uknes, H. Kessmann and J. Ryals, 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant Cell., 6: 959- 965.

Wen, P.F, J.Y. Chen, W.F. Kong, Q.H. Pan, S.B., Wan and W.D. Huang, 2005. Salicylic acid induced the expression of phenylalanine ammonia- lyase gene in grape berry. Plant Science., 169: 928-934..

Wasternack, C., 2007. Jasmonates : an update on biosynthesis, signal transduction and action inplant stress response, growth and development. Annals of Botany, 100: 681-697.

Yao, H.J and S.P. Tian, 2005. Effects of pre-and post harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Posthorwest Biol. Technol., 35: 253-262.

Yao, H.J. and S.P. Tian, 2005. Effects of a biocontrol agent and methyl jasmonate on postharvest disease of peach fruit and the possible mechanisms involved. J. Applied Micro., 98: 941-950.

Ye, S.F., H.Y. Zhou, Y. Sun, L.Y. Zou and J.Q. Yu, 2006. Cinnamic acid causes oxidative stress in cucumber roots and promotes incidence of *Fusarium* wilt. Enviro and Exp. Bot., 56: 255-262.

Zhang, X.Z., 1992. "Methodology of Crop Physiology" Agricultural Press, Beijing, pp: 142-212.

Zhao, J., L.C. Daves and R. Verpoorte, 2005. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol. Adv., 23: 283-333.

Zheng, H.Z., C.I. Cui, Y.T. Zharg, D. wary, Yu. Jing and Y.K. Kum, 2005. Active changes of lignifications-related enzymes in pepper response to *Glomus intraradices* and/or *Phytophthora capsici*. J. Zhejiang Uni Sci., 6B(8): 778-786.

Zhu, H.H. and Q. Yao, 2004. Localized and systemic increase of phenols in tomato roots induced by Glomus versiforme Inhibit *Ralstonia Solanacearum*. J. Phytopathology., 152: 537-546.