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INDUCTION OF DEFENSE RESPONSES IN TOMATO PLANTS INOCULATED WITH PHYTOSTIMULATION MICROORGANISMS AGAINST *Fusarium oxysporum*

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ABSTRACT

Phytostimulation microorganisms (PSM) viz. *Trichoderma harzianum* (TH), *Bacillus subtilis* (BS) and arbuscular mycorrhizal (AM) fungi were tested individually or in mixed culture for their ability to induce pathogenesis-related (PR)-proteins (chitinase, β-1,3-glucanase, peroxidase, phenylalanine ammonia-lyase PAL) and phenolics in tomato plants grown in sterilized soilless medium artificially infested with or without the *Fusarium oxysporum f.* sp. *radicis-lycopersici* (FORT). PSM-treated plants were more developed than non-treated control or inoculation with FORT. PSM-treated plants were effective in reducing diseases produced by FORT infection. Plants application with PSM significantly increased the activity of peroxidase, chitinase, β-1, 3-glucanase and PAL and accumulated phenolics in tomato plants compared to untreated control. Among the treatments, AM fungi recorded the maximum increase in the activities of all defense–related enzymes and accumulated phenolics followed by *T. harzianum* and *B subttilis*. The maximum increase in the activities of peroxidase, β-1, 3- glucanase, PAL and accumulated phenolics were observed 6 days after application of PSM. However, the maximum increase in the activities of chitinase was observed 9 days after application of PSM. Several fold increase in the accumulation of phenolics and activities of defense enzymes was observed when the induced plants were inoculated with *Fusarium oxysporum f.* sp. *radicis-lycopersici* (FORT). These results suggest that enhanced activities of defense enzymes and elevated content of phenolics by inoculation with PSM may contribute to protection of tomato plants against *F. oxysporum*.

Key words: Arbuscular mycorrhizal (AM) fungi; *Bacillus subtilis* (BS); *Fusarium oxysporum f.* sp. *radicis-lycopersici* (FORT); Induced systemic resistance; *Trichoderma harzianum* (TH)

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INTRODUCTION

Resistance, chemical treatments, and agricultural practices such as crop rotation are the main strategies for disease management. Fungicides are used extensively to control many soil-borne diseases, but their effectiveness is variable. Furthermore, secondary infections from soil-borne inocula as well as from inocula on plant debris are difficult to control by chemical seed treatments (**Dal Bello *et al* 2002**). Public concerns with fungicide residues, as well as pathogen resistance to some pesticide, have increased the need to find alternative methods for protection against crop diseases (**Mao *et al* 1997**). In addition, there are few crop varieties that are resistant to Fusarium seedling blight. Thus biological control using antagonistic microbes alone or as supplements to minimize the use of disease management has become more important in recent years (**Daamen *et al* 1989**).

Fusarium oxysporum f. sp. *radicis-lycopersici* (FORT) is one of the most destructive pathogens of tomato (**Jones *et al* 1991; McGovern *et al* 1993**). Fusarium crown has been increasing over the last several years (**Jones *et al* 1991; McGovern *et al* 1993**) and commercial yields have been reported to be reduced by 15 percent (**Jones *et al* 1991**). Among different management practices available, the biological control methods using antagonistic microorganisms are proved a potential alternative or complementary approach to chemical fungicides to combat the disease effectively (**Attia *et al* 2004**).

Research over the past two decades has demonstrated that plants have latent defense mechanisms against pathogens, which can be systemically activated by exposure of plants to stress or infection by pathogens. Remarkably some microorganisms are also able to trigger an induced resistance that enhances the defensive capacity of the plant to a subsequent pathogen attack. This effect is not localized at the colonization site in the roots, but systemic, conferring the plant a better protection not only against a broad range of soil pathogens, but also to foliar ones. This phenomenon, called systemic acquired resistance (SQR) or induced systemic resistance (ISR), operates through the activation of defense genes and the accumulation of defense compounds at a site distant from the point of pathogen attack (**van Loon *et al* 1998 and Buell, 1999**). Interestingly, no major changes in gene expression in the plant have been related to the ISR state.

Several lines of experimental evidence have shown that seed treatments with bacterial or fungal antagonists were effective in protecting germinating embryos and seedlings from the damaging action of root pathogens (**Paulitz 1992**). Besides, several soil-borne rhizosphere bacteria and fungi have been shown to induce systemic resistance in plants against pathogens (**Demeyer *et al* 1998; van Loon *et al* 1998**). Use of

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naturally occurring rhizosphere microorganisms, which protect and promote plant growth by colonizing and multiplying in the rhizosphere/root cortex, could be an alternative method for plant protection.

Arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with root systems of almost all plants, also reduce root diseases caused by several soil-borne pathogens through mechanisms that are not well understood (**Linderman, 1994**). The AMF penetrates the root system improving plant nutrition and growth and altering the anatomy and architecture of the root system. These changes, together with the activation of the plant defense mechanisms, seem to be responsible for the reduction of the disease (**Azcón-Aguilar *et al* 2002; Pozo *et al* 2002a**). For example, colonization of tomato roots by *Glomus mosseae* reduce disease development in plants infected with *Phytophthora parasitica*, and the involvement of plant defense mechanisms has been pointed out (**Pozo *et al* 1996; Cordier *et al* 1998; Pozo *et al* 1998; Pozo *et al* 1999; Pozo *et al* 2002b**). Although the basic mechanisms behind pathogen inhibition are not clearly defined, the possibility that antibiosis, mycoparasitism and competition may operate synergistically has been suggested (**Paulitz, 1992**).Alterations in the isoenzymatic patterns and biochemical properties of some defense-related enzymes such as chitinases (**Pozo *et al* 1996**), chitosanases (**Pozo *et al* 1998**) and β-1,3-glucanases (**Pozo *et al* 1999**) have previously been show during mycorrhizal colonization of tomato roots, with the induction of new isoforms. These hydrolytic enzymes are believed to have a role in defense against invading fungal pathogens because of their potential to hydrolyse fungal cell wall polysaccharides (**Simmons, 1994**). Thus, the induction of these activities in mycorrhizal symbiosis may be involved in the protector effect against fungal pathogens (**Dumas-Gaudot *et al* 1996**).

The ability of *Trichoderma* strains to protect plants against root pathogens has long been attributed to an antagonistic effect against the invasive pathogen **(Dumas-Gaudot *et al* 1996)**. The *Trichoderma* isolate T39 induced plant defense against *Botrytis cinerea* in tomato, lettuce, pepper, bean and tobacco (**Demeyer *et al* 1998**). Root inoculation of *T. harzianum* induced increased peroxidase and chitinase activities in leaves of cucumber seedlings (**Yedidia *et al* 1999**). In spite of the increasing amount of research devoted to the antimicrobial activity of *Trichoderma* spp. in vitro (**Harman and Bjorkman, 1998, Inbar and Chet, 1997**), knowledge of the exact mechanisms responsible for the observed reduction of disease incidence following soil treatment with *Trichoderma* prop□agules is still incomplete. Recently, we have demonstrated that the AM fungi (*Glomus mosseae* NRC212A and *G. fasciculatum* NRC212B), *T. harzianum* (NRC2041) and *Bacillus subtilis* NRC313 suppressed *Fusarium* wilt of tomato and significantly increased the plant growth and yield in tomato (**Attia *et al* 2004**).

It's interest in this research to evaluate the effect of AM fungi (*Glomus mosseae* NRC212A and *G. fasciculatum* NRC212B), *T. harzianum* (NRC2041) and *Bacillus subtilis* NRC313 could be used to promote seedling growth of tomato during early stages when plant are most susceptible to infect by soilborne

pathogens and induction of defense mechanisms related enzymes and accumulation of phenols in tomato leaves.

MATERIAL AND METHODS

Phytostimulation microorganism(PSM)

AM fungi (*Glomus mosseae* NRC212A and *G. fasciculatum* NRC212B), *T. harzianum* (NRC2041) and *Bacillus subtilis* (NRC313), which were previously demonstrated to be effective against FORT in both greenhouse and *in vivo* were used in this study (**Attia *et al* 2004**). Mycorrhizal spores used in this study were mixtures of *Glomus* spp. (*G. mosseae* NRC212A and *G. fasciculatus* NRC212B). These spores were originally extracted by a wet sieving and decanting technique using differential centrifugation (**Allen *et al* 1979**) from multi-plated in pot cultures containing a peat: vermiculite: perlite mix 1:1:1 by volume with maize and onion grown for 4 months (**Badr El-Din *et al* 1999**) and counted under a binocular microscope using a girded filter paper.The fungal antagonist *T. harzianum* (NRC2041) was grown in yeast molasses medium for 7 days and the conidia were harvested in sterile distilled water and the final concentration was adjusted to 10^6 cfu/ml with sterile distilled water (**Kapat *et al* 1998**). *Bacillus subtilis* (NRC313) was grown at 27°C for 48 h on liquid nutrient broth media (NBM), then centrifuged at 3000 g for 15 min. and the pellet was resuspended in sterile distilled water and the final concentration was adjusted to 10^9 cfu/ml (**Thomson, 1996**).

Isolation of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORT) culture

The fungus *FORT* was isolated from wilt infected tomato plants (*Lycopersicon esculentum* L. cv. Supermarmment) using half strength potato dextrose agar (PDA) medium amended with streptomycin and single spore cultures obtained were maintained on carnation leaf agar medium (CLA) (**Burgess *et al* 1988**) for immediate use and for long term use, the culture was stored as dried filter paper cultures according to **Correll *et al* (1986)** at 4°C. The *FORT* were obtained by pouring 5 ml of sterile distilled water over a 3-days old *FORT* colony growing on sucrose nutrient agar (**Nirenberg, 1981**) and gently rotating the dish. The suspension was collected in a sterile, conical centrifuge tube, homogenized for 30s with a vortex and spore concentration determined using a hemacytometer. Spore concentrations were adjusted to 15 x 10^4 spores per ml.

Experiment design

Seeds of tomato (*Lycopersicon esculentum* L. cv. Supermarmment) were surface sterilized by immersion in 2% sodium hypochlorite, thoroughly rinsed in sterile distilled water. Peat moss, with a pH value ranging between 6.8-7.0, was autoclaved for 30 minutes at 121°C after enriched with 5g activated charcoal and 15g calcium carbonate per 100g was used as soilless medium for growth of seedlings. The amended media were dispensed into plastic pots. Tomato seeds were drenched with the cell suspension of *T. harzianum* or *Bacillus* or mycorrhizal spores suspension (250 spores/ml) as well as mixture of the bacteria and two fungi. Tomato seeds coated with Phytostimulation microorganisms either individually or in mixed culture were

grown in plastic pots in the greenhouse for five weeks. The inoculated treatments were divided in two half. One half was inoculated with FORT 3-days after treatment with PSM by five distinct 10 µl-drops of the spores suspension on the soilless medium. The other half of PSM inoculated pot was left without pathogen fungal treatment. Seedling treated with sterile distilled water served as controls and fertilized with mineral fertilizers (NPK) at a rate of 15.2 g l^{-1} applied by hand. Inoculated plants received half doses of the recommended mineral fertilizers. The moisture content of the peat moss was sustained at a proper level throughout seedling propagation. Treatments were replicated eight times in a randomized complete block design and included eight treatments: inoculated or uninoculated with FORT in combination with three PSM and control.

Leaf samples were collected at various times after FORT application (3, 6, 9, 12 and 15 days) and chemical analyses were conducted. After 35 days, data were collected on fresh of shoots and roots weight as well as total biomass of plant. Root samples were taken for biological analyses. Populations of the bacterial (cfu/g fresh root weight) and *Trichoderma* were determined using semi-selective media (**Elad *et al* 1981**). Percentage of root length colonized using the magnified intersect method described by **McGonigle *et al* (1990)**.

Protein extraction

Seedlings leaves were separated, washed under running tap water for 5 min, dried gently, weighed, and ground with a mortar and pestle. The ground matter was homogenized (2 min, 4°C) in phosphate buffer (1:2 w/v, pH 6, 0.05 M) by use of Corex tubes. The homogenate solution was centrifuged twice at 10,000*g* and 4°C, and the supernatant was collected and kept at -20°C in order to enzyme assay.

Detection of chitinase

The total chitinase activity assay was based on the colorimetric determination of *p*-nitrophenyl cleaved from a chitin-analogous substrate, *p*-nitrophenyl-β-D-*N*,*N'*-diacetylchitobiose (PNP) (**Harman *et al* 1993 and Roberts & Selitrennikoff 1988**). A crude enzyme preparation and 10 µl of PNP stock solution (2 mg/ml) were added to 50 mM acetate buffer (pH 5.0) to a total volume of 0.5 ml and incubated for 2h in a water bath at 37°C. The reaction was terminated with 0.5 ml of 0.2 M Na_2CO_3. An extinction coefficient of 7 X 10^3 mM^{-1} cm^{-1} at 410 nm was used to determine *p*-nitrophenyl release from the substrate. Chitinase activity was expressed as millimoles of PNP produced per gram of fresh tissue per hour.

Detection of Peroxidase activity

Peroxidase activity was assayed spectrophotometrically at 610 nm with phenol red as a substrate. The complete reaction mixture (1 ml, 37°C) contained 10 to 20µl of a crude enzyme preparation, 50 µl of 0.2% (wt/vol) phenol red, and 50 mM sodium citrate (pH 4.2). Reactions were initiated with 10 µl of 1 mM hydrogen peroxide and stopped after 3 min with 40 µl of 2 N sodium hydroxide. The optical density was detected at 610 nm as described above. The absorbance was recorded at 610 nm and calculated

with a molar extinction coefficient of 122,000 M^{-1} cm for the oxidized product (**Ruttimann *et al* 1992**). Peroxidase activity was expressed as millimoles of phenol red oxidized per gram of fresh tissue per minute.

Detection of β–1, 3-glucanase activity

β-1, 3-glucanase activity was assayed by the laminarin-dinitrosalicylate method (**Pan *et al* 1991**). One-gram leaf samples were extracted with 5 ml of 0.05 M sodium acetate buffer (pH 5.0) by grinding at 4°C using pre-chilled pestle and mortar. The extract was then centrifuged at 10,000 g for 15 min at 4°C and the supernatant was used as enzyme source. The reaction mixture consisted of 62.5 ml of laminarin (4%) and 62.5 ml of enzyme extract. The reaction was carried out at 40°C for 10 min. The reaction was stopped by adding 375 ml of dinitrosalicylic reagent and heating for 5 min on a boiling water bath. The resulting coloured solution was diluted with 4.5 ml of distilled water, vortexed and its absorbance at 500 nm was determined. The blank consisted of crude enzyme preparation mixed with laminarin with zero time incubation. The enzyme activity was expressed as mg of glucose equivalents min^{-1} g^{-1} fresh tissue.

Detection of phenylalanine ammonialyase (PAL) activity

PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm according to **Dickerson *et al* (1984)**. Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C (**Dickerson *et al* 1984**). In reference cell, 0.4 ml of enzyme extract was taken along with 1 ml of borate buffer. Enzyme activity was expressed on a fresh weight basis (nmole of transcinnamic acid min^{-1} g^{-1}).

Detection of phenolic content

Tomato leaves (1 g) were homogenized in 10 ml of 80 percent methanol and agitated for 15 min at 70°C (**Swain and Hills, 1959**). One ml of the methanolic extract was added to 5 ml of distilled water and 250 ml of Folin Ciocalteau reagent (I M) and the solution was kept at 25°C. After 3 min 1 ml of saturated solution of Na_2CO_3 and 1 ml of distilled water were added and the reaction mixture was incubated for 1 h at 25°C. The absorption of the developed blue colour was measured using a spectrophotometer at 725 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reaction with phenol and expressed as phenol equivalents in mg g^{-1} fresh weight.

Statistics analyzes

The analyses were made with three independent samples and the experiment was repeated once with similar results. Statistical calculation was performed using IRRI STAT version 92-1.

RESULTS

Data in Table (1) show that the colonization of the *Trichoderma*, *Bacillus* and AM in non-inoculated control was absence (Table, 1). Populations of *Trichoderma* spp. in singly or in mixture treatments were not significantly affected by the inoculation with other microorganisms (Table, 1). Populations of bacteria (cfu/g fresh root weight) in bacterial treatment singly were greater by 17.5% in comparison with the bacterial strain in combination treatments with AM fungi + *T. harzianum* (Table, 1). The mycorrhizal colonization level reached to 69% for roots inoculated with AMF and about 72% for those inoculated with AMF and other microorganisms. No difference in percentage of mycorrhizal root colonization was found between AM or in combination with *Trichoderma*, *Bacillus* as well as pathogen.

The incidence of disease was significantly lower in PSM-treatment than in control without PSM treatment (Table, 1). Maximum levels of pathogen inside the roots were detected in non-inoculated plants with PSM, followed by those in *B. subtilis*. The minimum level of the pathogen was detected in plants colonized by *Trichoderma* or AM fungi as well as in mixed inoculation with PSM. However, plant damage was significantly reduced by pathogen inoculation in the colonized root with PSM.

Tomato plants varied in their response toward inoculation with each microorganism (Table, 1). In the absence of pathogen, colonization with any PSM was significantly affected plant development compared to non-inoculated controls. However, tomato development was inhibited by inoculation with mixture of microorganisms compared with singly inoculation (Table, 1). Exclusive inoculation with *T. harzianum, B. subtilis,* AM fungi, or in mixed culture significantly ($P<0.05$) increase of fresh weight of shoot and roots as well as total biomass of tomato seedling compared to the uninoculated plants (Table, 1). No significant differences were observed in the fresh weight of plants between inoculated plants with each microorganism. However, inoculation with mixture microorganisms resulted in depression of the plant growth, mainly at the shoot level.

In the pathogenic treatments, non-inoculated plants with PSM were considerably affected by inoculation with FORT. Losses in the weight of the plant inoculated with the pathogen alone were 46%. Growth of mycorrhizal plants was not reduced by inoculation with pathogen (Table, 1). Thus, PSM exerted a protective effect against FORT. It is remarkable that plants colonized by PSM and pathogen (whether inoculated in combination or separately) produced large roots in the noninfected plants by the pathogen than non-inoculated plants (Table, 1).

Activation of defense mechanisms

Chitinase, peroxidase, β-1,3-glucansase and PAL activities as well as the accumulation of phenolic content in leaves of tomato plants were measured at 3, 6, 9, and 15 days postinoculation. The results in Table (2) indicated that soil application with AM fungi, *T. harzianum*, and *B. subtilis* and/or inoculation with FORT, resulted in significant increase in the activities of chitinase and peroxidase in leaves of tomato plants (Table, 2). Chitinase activity peaked at 9 days in leaves of inoculated plants. While in noninoculated plants, chitinase activity increased gradually with time. At peak chitinase activity, all treatment inoculated showed a 3.2-fold increase compared with noninoculated plants. The peroxidase activity increased in leaves of tomato plants due to PSM antagonists treatments and also due to inoculation with pathogen. The increase in peroxidase activity was lasted up to 6 days after inoculation with PSM. However, noninoculated plants showed a gradual increase with time. The maximum activity of the enzyme was observed in AM fungi applied plants followed by *T. harzianum* and *B. subtilis* applied plants. When compared to control 2- to 3-fold increase in peroxidase activity was noticed due to inoculation

with PSM antagonists and/or pathogen (Table, 2). From 9 to 15 days, a two-to threefold decrease in both enzyme activities in the leaves of treated plants was observed.

Data in Table (3) show that application of PSM antagonists and inoculation with pathogen triggered the activity of β-1,3-glucansase, PAL and the accumulation of phenolic content in leaves of tomato plants. The maximum PAL activity and total phenolic content were observed in AMF inoculated plants followed by *T. harzianum* and *B. subtilis* treated plants (Table, 3). Inoculation with FORT, the activity of β-1,3-glucansase and PAL in the PSM antagonists treated plants increased several fold at 6 days after treatment and then decreased gradually. The increase PAL activity and accumulation of phenolics in the PSM inoculated tomato plants might have conferred resistance against FORT by making physical barriers stronger or chemically impervious to the hydrolytic enzymes produced by the pathogen.

DISCUSSION AND CONCLUSIONS

In our present study, each microorganism was effective in reducing the number of damping off seedlings and improved the plant stand. Increased shoot, root growth and total plant biomass by PSM could be due to the growth promoting ability of PSM which was demonstrated earlier in several crops (**Attia *et al* 2004**). Disease reduction by PSM was due to the higher antagonistic potential of PSM by different means viz. antibiosis, parasitism, production of lytic enzymes, etc. Thus, the bioprotection exerted by PSM appears to be the result of a combination of local and systemic mechanisms. The same conclusion was reached by immunocytochemical studies (**Cordier *et al* 1998**). Theses studies showed that arbuscular-containing cortical cells of *G. mosseae*-mycorrhizal plants were immune to the pathogen and exhibited a localized resistance with the formation of cell wall appositions reinforced by callose adjacent to the intercellular hyphae. A compensation mechanism could also be occurring, since plants colonized by *Trichoderma* or AM fungi and pathogen showed larger roots in the non infected by the pathogen than plants colonized by AM fungi or *Trichoderma* alone. Consequently, colonized by AM fungi could respond to attack by pathogen by producing larger roots in the parts of the root system that were not infected by the pathogen. These roots would then help sustain growth by absorbing nutrients that the damaged roots could not.

Beneficial microorganisms that improved plant health through the enhancement of plant resistance/tolerance against biotic stresses include bacteria, such as *Pseudomonas* spp. or *Bacillus* spp. and fungi such as *Trichoderma* sp., *Gliocladium* sp. or mycorrhizal fungi (**Ongena *et al* 1999; Pozo *et al* 2002 a and b, Attia *et al* 2004**). Root colonization by *Trichoderma* strains frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (**Benhamou, 1989**). The same increase was observed when seeds were separated from *Trichoderma* by a cellophane membrane, which indicates that *Trichoderma* produces growth factors that increased the rate of seed germination (**Campbell and Ellis 1992**).

In the present study, PSM treatment initiated a marked increase in peroxidase activity within 6 days after inoculation. Similar findings of increase in peroxidase

proteins after application with biocontrol agents have been reported by several

workers in different crops (**Demeyer *et al* 1998; Yedidia *et al 1*999; Meena *et al* 2000; Oostendorp *et al* 2001**). Bacterization of pigeon pea seeds with *B. subtilis* increased the peroxidase activity from 1 to 7 day and reduced the Fusarium wilt incidence caused by *F. udum* (**Podile and Laxmi, 1998**). As a general rule, peroxidase activity increases earlier than chitinase activity in PSM-treated tomato plants. Peroxidase may be rapidly involved in the peroxidation of substrate molecules, leading to the accumulation of highly toxic compounds (i.e., phenolic compounds), which may contribute to resistance via their antifungal potential (**Ward, 1986**). However, these compounds may, to some extent, be toxic to the plant itself, and it seems reasonable to assume that mechanisms designed to repress peroxidase expression are activated during the resistance process in order to maintain phenolic compounds below phytotoxic levels. In that context, the decrease in peroxidase activity observed at 9 days post inoculation may reflect a process elaborated by the plant to protect itself until such activity is needed, such as upon pathogenic attack. A growing body of evidence from various studies indicates that increased resistance of arbuscular mycorrhizal roots and leaves may be associated in part with marked metabolic changes in the host, including enhanced production of peroxidases and phenolic compounds (**Spanu *et al* 1989**); accumulation of hydrolases, such as chitinases and β-1,3-glucanases, with antimicrobial potential (**Dumas-Gaudot *et al* 1996**); and deposition of structural polymers, such as lignin (**Campbell and Ellis, 1992**) and hydroxyproline-rich glycoproteins (**Benhamou, 1995**). **Yedidia *et al* (1999)** reported that inoculation of *T. harzianum* induced chitinase activities in both leaves and roots of cucumber seedlings. These proteins facilitate *Trichoderma* penetration into the host and the utilization of the host components for nutrition.

The increased accumulation of chitinase and β-1,3-glucanase due to the application of biocontrol agents, in addition to hydrolysing chitin and β-1,3 glucan respectively which are the major components of the fungal cell walls, might have also released elicitors from the walls of fungi which in turn might have triggered various defense related activities in tomato (**Ren and West, 1992**). Plants treated with *Pseudomonas* strains 69-28 and 13 initially had higher levels of PAL, but when these plants were inoculated with the pathogens, the levels were reduced compared with other treatments or control (**Chen *et al* 2000**). The authors concluded that early induction of PAL by PGPR might have resulted in the activation of defenses, but subsequent pathogen challenge did not induce higher PAL levels (**Chen *et al* 2000**).

It can be concluded that colonization by PSM confers a significant reduction in disease development. The results also indicated that the applications of PSM resulted in increased plant growth compared with plant where PSM was not applied. Total plant biomass values were greatest in PSM plant compared with control plants. However, total plant biomass in the inoculated plants with each a PSM were equivalent to total plant biomass in the plant inoculated with mixed PSM. This suggest that PSM treatment stimulated plant growth, possible *via* growth promotion of plant,

direct negative effects on the soilborne pathogens, indirect effects on the pathogen by induction of disease resistance or a combination.

Soil application of PSM increased the activities of defense enzymes and accumulation of phenols in tomato. In addition, challenge inoculation with *FORT* also resulted in further increase of these enzymes and phenols in tomato leaves. This study indicated the usefulness of PSM in protecting tomato plants against Fusarium wilt by induction of systemic resistance.

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تحفيز الوسائل الدفاعية في نباتات الطماطم المعاملة بالميكروبات المحفزة للنمو النباتي ضد فطر الفيوزاريوم أوكسيسبوريم

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اختبار الميكروبات المحفزة لنمو النبات (تريكوديرما هارزيانم والباسيلس سبتلس وفطريات الميكوريزا) منفردة أو خليط لمقدرتها على إنتاج الإنزيمات المضادة لنشاط الممرض (الكيتينيز وبيتا 1-3 جلوكانيز والبيروكسيديز والفينيل ألانين أمونيا ليز (PAL) والفينولات بنباتات الطماطم النامية في تربة معقمة وغير معقمة ملقحة بفطر الفيوزاريوم أوكسيسبوريم.

وقد أظهرت النتائج أن النباتات الملقحة بالميكروبات المحفزة للنمو منفردة أو على من غير الملقحة المصابة بفطريات الفيوزاريوم أظهرت كفاءة عالية فى مقاومة الإصابة بالفطر الممرض. ولقد أدى تلقيح النباتات بالميكروبات المحفزة للنمو إلى حدوث زيادة معنوية في نشاط الإنزيمات المضادة للفطر الممرض (الكيتينيز وبيتا 1-3 جلوكانيز والبيروكسيديز والفينيل ألانين أمونيا ليز (PAL) وتراكم الفينولات بنباتات الطماطم بالمقارنة بالنباتات غير الملقحة وأظهرت فطريات الميكوريزا أعلى زيادة في معدل نشاط الإنزيمات الدفاعية بالإضافة إلى زيادة تراكم الفينولات الكلية في أنسجة نباتات الطماطم يليه التريكوديرما ثم الباسيلس سبتلس. وجد أن الحد الأقصى لزيادة نشاط إنزيم البيروكسيديز وبيتا 1-3 جلوكانيز و PAL وكذا تراكم الفينولات بلغ 6 أيام من التلقيح بالميكروبات المحفزة للنمو بينما كان الحد الأقصى لإنزيم الكيتينيز بعد 9 أيام من التلقيح. ولوحظ عدم إصابة النباتات بالفيوزاريوم في نشاط هذه الإنزيمات وكذلك تراكم الفينولات [illegible] أضعاف.

من النتائج السابقة نستنتج أن تحفيز إنتاج الإنزيمات المضادة للفطر الممرض وتراكم الفينولات داخل أنسجة النبات عن طريق التلقيح بالميكروبات المحفزة للنمو

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النباتقدتساهمفحيامةينباتاتلطامطممن الإصابةبفطريالتلفيوزاريم.

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