Effect of Composted Chicken Manure on Induction of Defense Reactions Against Tomato (*Lycopersicon esculentum* Mill.) Fusarium Wilt

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Abstract: In this investigation, responses of tomato plants growing in sandy soil with or without composted chicken manure (CM) to the phytopathogen *Fusarium oxysporum* in terms of activities of plant defense related enzymes, *i.e.* β-1,3-glucanase, peroxidase (PER), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) and levels of total phenolics were investigated. Higher levels of plant defense related enzymes in terms of β-1,3-glucanase, PER, PPO were noted between 3 and 9 days in tomato plants growing in sandy soil amended with composted CM in relative to these found in sandy soil alone upon challenge with *F. oxysporum*. Higher levels of PAL activities and total soluble phenolic content were also found in CM-treated plants and reached the maximum level 6 days after inoculation with *Fusarium*, in relative to these found in sandy soil alone. The present results suggest that the enhanced activities of defense related enzymes and elevated content of phenolics in CM-treated plants may contribute to bioprotection of tomato plants against *F. oxysporum*, the causal agent of tomato Fusarium wilt.

Keywords: composted chicken manure, induced resistance, plant defense enzymes

INTRODUCTION

Growing awareness of the environmental damage caused by the use of chemical substances for plant disease control in agriculture has raised the need to study biological alternatives, such as activating the defense response of plant crops by agents that are not toxic to the environment. Among biological alternative methods in biocontrolling plant pathogens are using compost amended substrates in cultivating plant crops. Composts have been used successfully for suppression of several plant diseases (Lewis *et al.*, 1992; Pascual *et al.*, 2002; Reuveni *et al.*, 2002; Mckellar and Nelson, 2003; Borreroa *et al.*, 2006; Youssef, 2007).

Pseudomonas spp. (Aryantha *et al.*, 2000), *Pantoea* spp. (Krause *et al.*, 2003), *Bacillus* spp. (Hardy *et al.*, 1989; Berger *et al.*, 1996), Actinomycetes (Hardy *et al.*, 1989; Aryantha *et al.*, 2000), and fungi including *Trichoderma* spp. (Smith *et al.*, 1990; Roiger and Jeffers, 1991; Sid Ahmad *et al.*, 1999) have been identified from substrates amended with composts as potential biocontrol agents against Phytophthora root and crown rot. The composition of the organic matter in the compost-amended substrate is critical to sustained biological control (Stone *et al.*, 2001). Composts may also induce systemic resistance (ISR) in plants to several plant diseases, although the results can be variable (Zhang *et al.*, 1996; Pharaud *et al.*, 2002; Krause *et al.*, 2003). Generally, systemic resistance, induced biologically or chemically in plants, is associated with an ability to resist pathogen attack by enhanced activation of cellular defense mechanisms (Sticher *et al.*, 1997; Métraux *et al.*, 2002). A mixture of sandy soil and composted CM induced protection of tomato plants against *F. oxysporum* as well as reduced severity of the visual symptoms of Fusarium wilt, as compared with tomato plants grown in sandy soil alone, i.e. without composted CMs (Youssef, 2007). The author reported that suppressiveness of the composted CM against *F. oxysporum* was due to their biotic and abiotic agents.

The purposes of this study were (1) to determine defense related enzymatic activities, i.e. ß-1,3-glucanase, peroxidase (PER), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), as well as total soluble phenolic compounds in plants upon challenge with *Fusarium oxysporum* and (2) to provide possibility of plant defense mechanism participated in the biocontrol of *F. oxysporum*.

MATERIAL AND METHODS

Compost preparation and its characteristics

Chemical properties, a number of microbial organisms and antagonistic effect against *F. oxysporum* of the composted CM3 were determined elsewhere (Youssef, 2007). CM3 was obtained from Qaloubiya goverorates.

Isolation of *F. oxysporum*

F. oxysporum was isolated by the method described by Youssef (2007) from infected tomato plants cv. GS on specific Fusarium isolating medium (peptone pentachloronitrobenzene agar (PPA) modified by Nash and Snyder (1962). A 1 cm piece of tomato stem was cut and disinfected in sodium hypochlorite (1%) for 3 min then washed several times in sterilized distilled water and dried between folds of sterilized filter paper. The pieces were put on the medium containing the following constituents in a liter of deionized water: agar, 15 g; peptone, 15 g; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g. After autoclaving at 121 °C for 20 min, the medium was amended with 0.05 g chloromphenicol, 0.75 g pentachloronitrobenzene, and 10 ml (5%) chlorotetracycline. After incubation, a hyphal tip from the margin of the developed mycelium was resubcultured on potato dextrose agar (PDA).

Inoculation, plant growth and disease severity assessment

Tomato seeds (*Lycopersicon esculentum* Mill.) were sown in trays filled with a sandy soil. After

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germination, tomato seedlings were properly fertilized through the irrigation water with a solution of N, P and K at 50, 14 and 41 mg l^{-1}, respectively. Tomato seedlings of 30 days old were used for each inoculation trial. A pathogenic isolate of *F. oxysporum* grown on potato dextrose agar (PDA) plates for 7 days at 26 °C in the dark was used as an inoculum source (Youssef, 2007). The inoculum was prepared by washing the plates with sterile distilled water (SDW), which was then passed through sterile cheesecloth. Based on preliminary experiments, conidia were diluted to a proper concentration (5 x 10^5 conidia ml^{-1}). Healthy and uniform tomato seedlings of 30 days old, four to five, and fully expanded leaves were transplanted into pots filled with either a mixture of composted CM and sandy soil (1:4 v/v, respectively) or sandy soil alone as a control and inoculated two weeks later with 2 ml Fusarium inoculum conidial suspension or SDW to serve as control. To evaluate the role of abiotic agents, such as biochemical compounds and nutrients in composted CM3, a mixture of sandy soil and thermal sterilized CM were used. First symptoms were observed after 10-14 days from transplanting. Five weeks after inoculation, the plants were harvested and their shoot and root fresh weights were estimated. Disease severity was determined using the visual rating: (0) no symptoms, (1) slight leaf epinasty and/or apex chlorosis, (2) leaf epinasty and plant dwarfing, and (3) dead plants. Data are the mean values ± SD of three independent experiments, each with three replicates consisting of 25 plants. At various times after inoculation, leaf samples were collected and various biochemical analyses were done.

Sampling, extraction and determination of enzymatic activities

Samples of treated and untreated tomato leaves were collected at specified times after Fusarium inoculation. These were frozen immediately in liquid nitrogen and stored at -20 °C. Samples was crushed in liquid nitrogen using a prechilled mortar and pestle and then homogenized with different buffers containing 1% polyvinylpolypyrolidone (PVPP) in the proportion of 1.0 g fresh tissue to 2 ml buffer to assay different enzymes: sodium acetate buffers (50 mM, pH 5.0) for β-1,3-glucanase, and 0.2 M sodium phosphate buffer (pH 6.4) for peroxidase (PER) and polyphenol oxidase (PPO), and 0.05 M sodium borate buffer (pH 8.8, containing 5 mM β-mercaptoethanol) for phenylalanine ammonia lyase(PAL) The samples were homogenized and centrifuged at 20,000 g for 60 min at 4 °C. The supernatants were used as the crude enzyme source to assay enzymatic activities. Protein concentrations were determined by the method of Bradford (1976), using bovine serum albumin as a standard.

ß-1,3-Glucanase (EC 3.2.1.6) activity was measured by a modified method of Lima *et al.* (1997) using Azurine-crosslinked pachyman (AZCL-pachyman; Megazyme) as the substrate. The reaction mixtures contained 0.4 ml sodium acetate buffer 10 mM, pH 0.5 and 0.1 ml crude enzyme extract. After equilibrium for 5 min in a 30 °C water bath, 100 µls of AZCL-pachyman (100 mg/ 3 ml of 10 mM potassium acetate buffer, pH 5.0) were added, and the mixtures were incubated for 20 min at 30 °C with moderate shaking. The reaction was stopped by adding 700 µl of Tris (20%, w/v), incubated at room temperature for 5 min and then briefly centrifuged and the amount of blue soluble dyed fragments released from AZCL-pachyman was measured spectrophotometrically at 595 nm. Quantification of enzyme units ml^{-1} enzyme was calculated using the following equation:

$$\text{Units ml}^{-1}\text{ enzyme} = \frac{(\Delta A\ 595\ \text{test} - \Delta A\ 595\ \text{blank})(1.3)(df)}{(0.1)}$$

Where 1.3= final volume (in ml) of assay, df = dilution factor, and 0.1= volume (in ml) of enzyme used. The activities were expressed as units h^{-1} g^{-1} FW.

PER activity was assayed according to a modified method of Hammerschimidt *et al.* (1982). Briefly, the assay mixture in a total volume of 3 ml contained 10 mM potassium phosphate buffer, pH 7.5 at 25 °C, 2 mM H_2O_2 and 9 mM guaiacol as the substrate. After dilution of 5 µl of crude enzyme extract, the increase in absorbance was measured spectrophotometrically at 470 nm at intervals of 30 seconds up to 2 min. Activities of PER were expressed as enzyme units min^{-1} mg^{-1} protein

PPO activity was determined according to a modified method of Canal *et al.* (1988). The reaction mixtures in a total volume of 3 ml contained 0.5 ml of crude enzyme extract and 2.5 ml of 500 mM pyrogallol as substrate in 0.02 M potassium phosphate buffer (pH 0.7). The increase in absorbance was measured spectrophotometrically at 420 nm over 2 min at the steepest increase at 25 °C. One unit of enzymatic activities is defined as the amount of enzyme giving, under the assay conditions, a change in absorbance of 0.001 per min. PPO activities were expressed as enzyme units min^{-1} g^{-1} FW.

PAL activity was assayed by measuring the amount of trans-cinnamic acid formed at 290 nm as described by Beaudoin-Eagan and Thorpe (1985). The reaction mixtures consisted of 100 µl of plant extract and 900 µl of 6 µM L-phenylalanine in 500 mM Tris-HCl buffer, pH 8.5. After 60 min at 37 °C, the reaction mixture was stopped by the addition of 0.05 ml N HCl and measured spectrophotometrically at 290 nm. PAL activities were expressed as µmoles min^{-1} mg^{-1} protein

Extraction and determination of phenolic content

Total phenolic compounds were determined using the method described by Singleton and Rossi (1965). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10, 000 g for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5 ml of distilled water. One-hundred µl of this extract was diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of sodium carbonate (20%) were added and the contents were mixed thoroughly. The color was developed and absorbance was measured spectrophotometrically at 650 nm after 60 min. The

standard curve was prepared using catechol. The absorbance was converted to the phenolic content in terms of catechol equivalent. The results were expressed as µg of phenolics g^{-1} FW.

RESULTS

Assessment of disease severity and plant growth

Data presented in Table (1) show that disease severity on tomato plants infected by *F. oxysporum* was significantly decreased in soil amended with unsterilized CM relative to that found in sandy soil alone. It was evident that the disease index reached a value of 2.2 and zero for plants grown in sandy soil inoculated and non-inoculated with *F. oxysporum*, respectively. Data also show that addition of non-sterilized CM3 to soil resulted in stimulation of vegetative growth in infected and non-infected tomato plants. The fresh weight of plants grown in the soil-CM mixture was significantly higher than those grown in soil alone. In addition, Data in Table (1) also show that sterilized CM exhibited a potential role in reducing the disease severity and increasing fresh weight, suggesting that manure's abiotic agents having a capability to protect tomato plants from *F. oxysporum*.

Table (1): Effect of composted CM on infection by *F. oxysporum* and shoot fresh weight (FW) of tomato plants grown in thermal sterilized sandy soil alone or combined with non-sterilized and sterilized CM.

Treatments	Inoculated plants		Non-inoculated plants	
	Disease Severity	Fresh weight/plant	Disease Severity	Fresh weight/plant
Soil only (control)	2.21 (± 0.12)	15.1 (± 1.9)	0.0	23.4 (± 1.3)
Soil + CM3	1.07 (± 0.07)	21.3 (± 1.3)	0.0	31.6 (± 2.1)
Soil + CM3*	1.81 (±0.08)	18.7 (± 1.2)	0.0	29.3 (± 1.3)

Control: soil without composted CM . CM3* represents sterilized CM at 120 °C for 40 min.

Induction of defense enzymatic activities

Sandy soil amended with CM increased β-1,3-glucanase activities in tomato plants following infection with *F. oxysporum*, compared to these found in tomato plants grown in soil alone (Fig. 1). The concentration of β-1,3-glucanase was increased gradually and reached maximum levels between 6-9 days, then declined. Similar trends were also found in terms of PER and PPO activities, as shown in Fig. (1). Significant increases in PAL activities were noted in tomato plants grown in sandy soil amended with CM during 3-6 days after inoculation with *F. oxysporum*, then declined, relative to these found in tomato plants grown in sandy soil alone (Fig. 1). Similar trend to PAL activities was found in terms of accumulation of total soluble phenolic compounds. It was evident that challenge inoculation with *F. oxysporum* significantly increased the enzymatic activities, as well as total soluble phenolics, when tomato plants were grown in soil amended with CM, relative to the control.

DISCUSSION

In the field of plant protection, induction of disease resistance via biotic and abiotic agents has been reported to provide protection against invasion of phytopathogens in several plant species (Van Loon *et al.* 1998). In this investigation, plant disease severity (Table 1) was significantly decreased and plant fresh weight was significantly increased in soil amended with unsterilized CM, relative to the control plants grown in sandy soil alone. Thermal sterilized CM also exhibited a potential role in reducing the disease severity and increasing plant fresh weight, suggesting that manure's abiotic agents also have a capability to protect tomato plants against *F. oxysporum*. Our results, in terms of manure's biotic and abiotic agents, are in agreement with Zhang *et al.* (1996), Hoitink and Boehm (1999), Erhart *et al.* (1999), Szczech (1999), Reuveni *et al.* (2002), and Kavroulakis *et al.* (2005) who reported that compost amendments have been shown to suppress plant diseases caused by several soilborne plant pathogens.

In general, defense related enzymes and phenolic content are often associated with disease resistance in various plant species (Harborne, 1994; Sticher *et al.* 1997). The results of the present investigation indicated that sandy soil amended with CM induced higher activities of ß-1,3-glucanase, PER, PPO, PAL, as well as total soluble phenolics in tomato plants upon challenge with *F. oxysporum* compared with tomato plant grown in sandy soil without CM. The present results also indicated that the early increases of ß 1,3-glucanase, PER, PPO, PAL, as well as total soluble phenolics in tomato plants are correlated with a reduction in disease severity (Table 1 and Fig. 1A, B, C, & D). The obtained results are in agreement with Hammerschmidt *et al.*(1982), Pellegrini *et al.* (1994), Zhang *et al.* (1998), Reuveni *et al.* (2002) who reported that ß 1,3-glucanase, PER, PPO, PAL, as well as total soluble phenolics are associated with induced resistance in many plant species. Our results are also in accordance with (Zhang *et al.*, 1996; Pharaud *et al.*, 2002; Krause *et al.*, 2003) who reported that compost amendments may also induce systemic resistance, via inducing defense-related reactions, in plants to plant diseases.

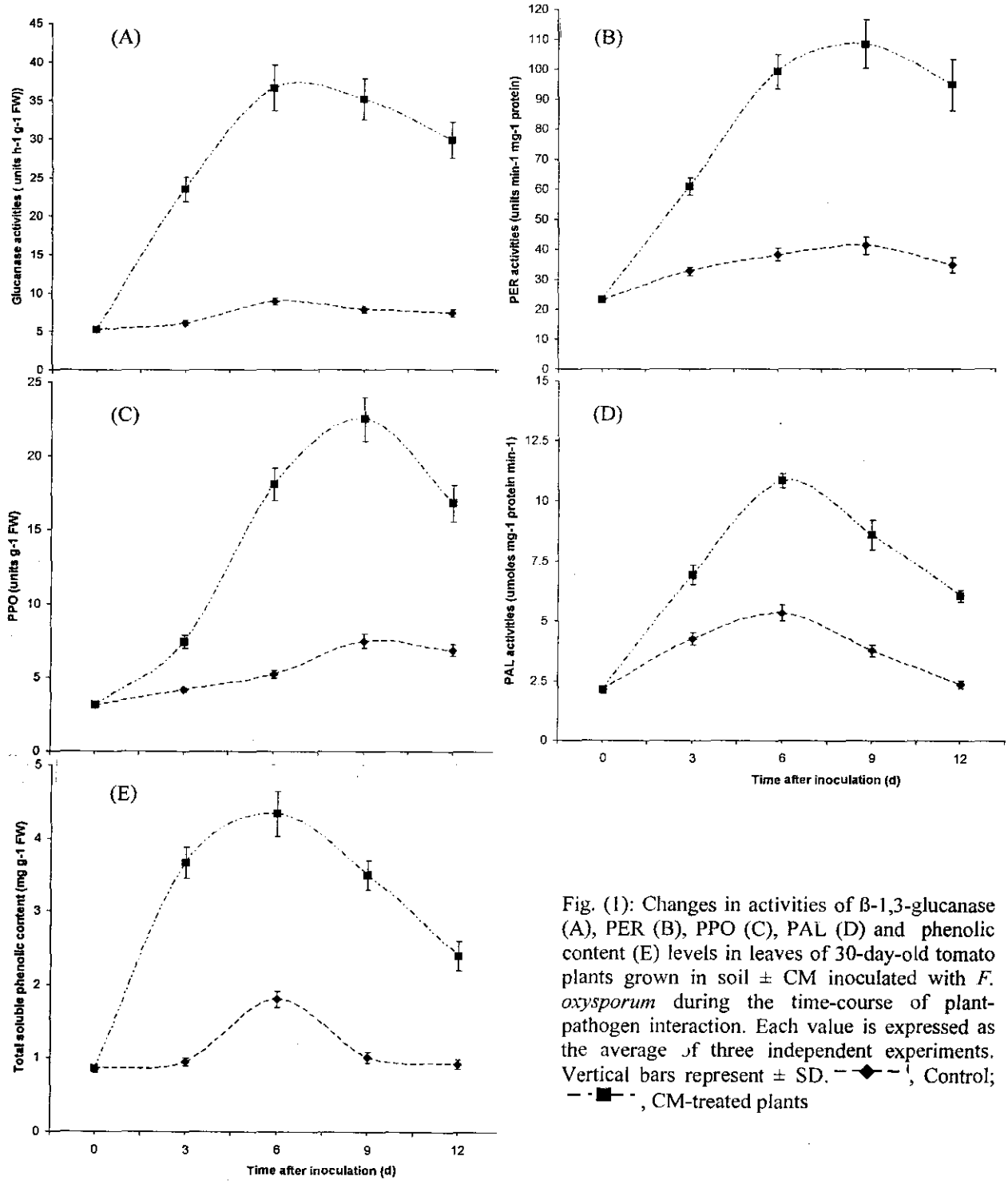

Fig. (1): Changes in activities of ß-1,3-glucanase (A), PER (B), PPO (C), PAL (D) and phenolic content (E) levels in leaves of 30-day-old tomato plants grown in soil ± CM inoculated with *F. oxysporum* during the time-course of plant-pathogen interaction. Each value is expressed as the average of three independent experiments. Vertical bars represent ± SD. –◆–, Control; –■–, CM-treated plants

ß-1,3-Glucanases are able to partially degrade fungal cell walls by catalyzing the hydrolysis of ß-1,3-glycosidic bonds in glucans, which are, together with chitin, the major cell wall components and are thought to be involved in plant defense mechanisms against fungal infection (Van Loon, 1997). PERs have been implicated in multiple functions of plant disease resistance, including reinforcement of cell wall, lignin biosynthesis and hydrogen peroxide generation, cross-linking of phenolics and glycoproteins, suberization and phytoalexin production (Milosevic and Slusarenko, 1996; Wojtaszek, 1997). In addition, phenolic compounds can be oxidized by PER and PPO and produce antimicrobial phenolic substances, such as quinones, which are more toxic to pathogens than the former (Sivaprakasan and Vidhyasekaran, 1993). PAL is the first enzyme in the phenylpropanoid and flavonoid pathways and its increased activity has been associated with increases in lignin, phytoalexins, salicylic acid and other defense compounds ((Lee *et al.*, 1995; Whetten and Sederoff, 1995; Mauch-Mani and Slusarenko, 1996; Shadle *et al.*, 2003), which were proposed to reduce incidence of plant disease through antifungal activity and stimulation of plant defense responses (Reymond and Farmer, 1998; Jeandet *et al.*, 2002). Phenolics are well known antifungal, antibacterial and antiviral compounds occurring in plants (Sivaprakasan and Vidhyasekaran, 1993). According to Matern and Kneusal (1988), the first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen.

The mechanism by which composted CM enhance the resistance against plant diseases is not completely understood. Generally, antifungal hydrolytic enzymes, *i.e.* ß-1,3-glucanase, and oxidative enzymes, *i.e.* PER and PPO are considered potentially important in host resistance mechanism, as also suggested by Schneider and Ullrich (1994). In addition, PPO, ß-1,3-glucanase, and PAL also showed an important role in induced resistance of plants (Sharan *et al.*, 1998; Li and Steffens, 2002). Activation of PAL in tomato could directly affect accumulation of secondary toxic compounds, such as phytoalexins, which might be released in root exudates and on root segment surfaces from the inoculated plants to inhibit fungal spore germination and growth. As ß-1,3-glucanase has been proven to hydrolyze the ß-1,3-linked glucans, major components of the cell wall of fungi, and synergistically acts with chitinase to inhibit fungal growth (Schneider and Ullrich, 1994; Kim and Hwang, 1997). PAL is the first enzyme of the phenylpropanoid pathway and is involved in the biosynthesis of phenolics, phytoalexins, and lignins (Pellegrini *et al.*, 1994). Therefore, the increased PAL activity will contribute to the reduced percentage of incidence. Phenolics are known to be involved in disease resistance in plants (Harborne, 1994). Many phytoalexins produced by plants as a direct *in vivo* response to fungal infection are specific antifungal agents and belong to the phenolic class.

The results of the present investigation suggest that treatment with composted CM triggers the induction of defense-related reactions in tomato plants upon challenge with *F. oxysporum*, the causal agent of tomato Fusarium wilt. The modes of CM action to suppress plant diseases caused by plant pathogens required further work to fully elucidate disease suppression by compost The effect of CM on other enzymatic and non-enzymatic plant defense mechanisms may also participate in disease resistance.

REFERENCES

Aryantha I. P., Cross, R., and Guest D. I. 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. Phytopathol. 90: 775-782.

Beaudoin-Eagan L. D., and Thorpe T. A. 1985. Tyrosine and phenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. Plant Physiol. 78: 438-441

Berger F., Li, H., White, D., Frazer R., and Leifert C. 1996. Effect of pathogen inoculum, antagonist density, and plant species on biological control of Phytophthora and Pythium damping-off by *Bacillus subtilis* Cot1 in high humidity fogging glasshouses. Phytopathol. 86: 428-433.

Borrero C. Ordovás J., Trillas M. I., and M. Avilés M. 2006. Tomato Fusarium wilt suppressiveness. The relationship between the organic plant growth media and their microbial communities as characterised by Biolog. Soil Biol. & Biochem. 38: 1631–1637.

Bradford M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Canal M. J., Tamès R, S., and Fernández B. 1988. Peroxidase and polyphenoloxidase activities in *Cyperus esculentus* leaves following glyphosate application. Physiol. Plant. 74: 125-130.

Hammerschimidt R., Knuckles E. M., and Kuc J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20: 73-82.

Harborne H. B. 1994. Do natural phenols play a role in ecology?. Acta Hort. 381: 36-43

Hardy, G. E. S. J., and K. Sivasithamparam. 1989. Microbial, chemical and physical changes during composting of an *Eucalyptus* (*Eucalyptus calophylla* and *Eucalyptus diversicolor*) bark mix. Biol. Fert. Soil 8: 260-270.

Hoitink H. A. J., and Boehm M. J. 1999. Biocontrol within the context soil microbial communities: A substrate-dependent phenomenon. Annu. Rev. Phytopathol. 37: 427-446.

Erhart E., Burian K., Hartl W., and Stick K. 1999. Suppression of *Pythium ultimum* by biowaste composts in relation to compost microbial

biomass activity and content of phenolic compounds. J. Phytopathol. 147: 299-305.

Jeandet P., Douillet-Breuil A. C., Bessis R., Debord S., Sbaghi M., and M. Adrian. 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity and metabolism. J. Agric. and Food Chem. 50: 2731-2741.

Karaoulanis N., Ehaliotis C., Ntougias S., Zervakis G., and Papadopolou G.. 2005. Local and systemic resistance against fungal pathogens of tomato plants elicited by a compost derived from agricultural residues. Physio. and Mol. Pl. Pathol. 66: 163–174.

Kavroulakis N., Ehaliotis C., Ntougias S., Zervakis G. I., and Papadopoulon K. K. 2005. Local and systemic resistance against fungal pathogens by a compost derived from agricultural residues. PMPP 66: 163-174.

Kim Y. J., and Hwang B. K. 1997. Isolation of a basic 34-kilodalton ß-1,3-glucanase with inhibitory activity against *Phytophthora capsici* from pepper stems. Physiol. Mol. Pl. Pathol. 50: 103-115.

Krause M. S., De Ceuster T. J. J., Tiquia S. M., Michel F. C., Jr., Madden L. V., and Hoitink H. A. J. 2003. Isolation and characterization of rhizobacteria from composts that suppress the severity of bacterial leaf spot of radish. Phytobathol. 93: 1292-1300.

Lee H, Leon J., and Raskin I. 1995. Biosynthesis and metabolism of salicylic acid. Proc. Natl. Acad. Sci. USA 92: 4076-4079.

Lewis J. A., lumsden R. D., Millner P. D., and Keinath A. P. 1992. Suppression of damping-off of peas and cotton in the field with composted sewage sludge. Crop Prot. 11: 260-266.

Li L., and Steffens J. C. 2002. Overexpression of polyphenol oxidases in transgenic tomato plants results in enhanced bacterial disease resistance. Planta 215: 239-247.

Lima L. H. C., Ulhoa C. J., Fernandes A. P., and Felix. C. R. 1997. Purification of a chitinase from *Trichoderma* sp. and its action on *Sclerotium rolfsii* and *Rhizoctonia solani* cell walls. J. Gen. Appl. Microbiol. 43: 31-37.

Matern U., and Kneusal R. E. 1988. Phenolic compounds in plant disease resistance. Phytoparasitica 16: 153-70.

Mauch-Mani B., and Slusarenko A. J. 1996. Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *Perenospora parasitica*. Plant Cell 8: 203-212

Mckellar M. E.,, and Nelson E. B. 2003. Compost induced suppression of Pythium damping-off is mediated by fatty-acid-metabolizing seed-colonizing microbial communities. Appl. Environ. Microbiol. 69: 452-460.

Milosevic N., and Slusarenko A. J. 1996. Active oxygen metabolism and lignification in the hypersensitive response in bean. Physiol. and Mol. Pl. Pathol. 49: 143-157.

Métraux J. P., Nawrath C. and Genoud T. 2002. Systemic acquired resistance. Euphytica 124, 237–43.

Nash S. M., and Snyder W. C. 1962. Quantitative estimation by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathol. 52: 567-572.

Pascual J. A., Hernandez, T., Garcia C., Lerma, S., and Lynch J. M. 2002. Effectiveness of municipal waste compost and its humic fraction in suppressing *Pythium ultimum*. Microbial Ecol. 44: 59-68.

Pharaud B., Carisse, O., and Benhamou N. 2002. Cytological aspects of compost mediated induced resistance against Fusarium crown and root rot in tomato. Phytopathol. 92:424-438.

Pellegrini L., Rohfritsch O., Fritig B., and Legrand M. 1994. Phenylalanine ammonia-lyase in tobacco. Plant Physiol. 106: 877-886.

Reymond P., and Farmer E. E. 1998. Jasmonate and Salicylate as global signals for defense gene expression. Curr. Opin. Plant Biol. 1: 404-411.

Reuveni R., Raviv M., Krasnovsky, A., Freiman L., Medina S., Bar A., and Orion D. 2002. Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop Prot. 21: 583-587.

Roiger D. J., and S. N. Jeffers. 1991. Evaluation of *Trichoderma* spp. for biological control of Phytophthora crown and root rot of apple seedlings. Phytopathol. 81:910-917.

Schneider S., and Ullrich W. R. 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol. Mol. Pl. Pathol. 45: 291-304.

Shadle G. L., Wesley V., Korth K. L., Chen F., Lamb C., and Dixon R. A. 2003. Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of L-phenylalanine ammonia-lyase. Phytochem. 64: 153-161.

Sharan M., Taguchi G.K., Jouke T., Shimosaka M., Hayashida N., and Okazaki M. 1998. Effects of methyl jasmonate and elicitor on the activation of phenylalanine ammonia-lyase and the accumulation of scopoletin and scopolin in tobacco cell cultures. Plant Sci.132: 13-19.

Sid Ahmad A., Perez-Sanchez, C., Egea C., and Candela M. E. 1999. Evaluation of *Trichoderma harzianum* for controlling root rot caused by *Phytophthora capsici* in pepper plants. Plant Pathol. 48:58-65.

Singleton V. L., and Rossi. I. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult 16: 144-158.

Sivaprakasan K, and Vidhyasekaran P. 1993. Phenylalanine ammonia lyase gene for crop disease management. In: Vidhyasekaran P, ed. Genetic Engineering, Molecular Biology and

Tissue Culture for Crop Pest and Disease Management. Delhi, India: Daya Publishing House, pp. 113-122.

Smith, V. L., Wilcox, W. F., and G. E. Harman. 1990. Potential for biological control of Phytophthtora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathol. 80: 880-885.

Sticher, L., Mauch-Mani, B., and Metraux J. P.1997. Systemic acquired resistance. Annu. Rev. Phytopathol. 35: 235–70.

Stone, A. G., Traina, S. J., and Hoitink H. A. 2001. Particulate organic matter composition and Pythium damping-off of cucumber. Soil Sci. Soc. Am. J. 65: 761-770.

Szczech M. M. 1999. Suppressiveness of vermicompost against Fusarium wilt of tomato. J. Phytopathol. 147: 155-161.

Van Loon L. C., Bakker P. A., Pieterse C. M. 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phyropathol. 36: 453-483.

Van Loon L. C., 1997. Induced resistance in plants and the role of pathogenesis related proteins. Eur. J. Plant Pathol. 103: 753-765.

Wojtaszek, P. 1997. The oxidative burst: a plant's early response against infection. Biochem. J. 322: 681-692.

Whetten R., and Sederoff R. 1995. Lignin biosynthesis. Plant Cell 7: 1001-1014

Youssef S. A. 2007. Evaluation of composted chicken manure in biocontrolling Fusarium wilt on tomato. Egypt. J. Phytopathol. 35: 61-72.

Zhang W., Han D. Y., Dick W. A., Davis K. R., and Hoitink H. A. J. 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and *Arabidopsis*. Phytopathol. 88: 450-455.

Zhang, W., Dick, W. A., and H. A. Hoitink. 1996. Compost-induced systemic acquired resistance in cucumber to Pythium root rot and anthracnose. Phytopathol. 86: 1066-1070.

تأثير كومبوست مخلفات الدواجن على حث تفاعلات النبات الدفاعية ضد الذبول الفيوزاريومى في الطماطم

سحر على جمال الدين يوسف - كامل أحمد حسين طرطورة

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اجري هذا البحث بهدف دراسة تأثير كومبوست مخلفات الدواجن على حث التفاعلات الدفاعية لنباتات الطماطم ضد الذبول الفيوزاريومى المتسبب عن فطر *Fusarium oxysporum* . تم زراعة بذور نباتات الطماطم للحصول على شتلات عمر ٣٠ يوما من الزراعة والتي تم نقلها وزراعتها في أصص بها مخلوط من تربة رملية معقمة حراريا وكومبوست مخلفات الدواجن غير المعقم والمعقم حراريا كل بنسبة ٤ : ١ (حجم / حجم، تربة إلى كومبوست، على التوالي). تم عدوى النباتات المنقولة بمعلق الجراثيم الكونيدية بالمسبب المرضى بعد ١٤ يوما من نقل الشتلات وزراعتها. وقد أظهر هذا الخليط سواء كان الكومبوست غير معقم أو معقما حراريا تقليل شدة الإصابة المرضية وأعراض الذبول في نباتات الطماطم النامية ببيئة معدية صناعيا بالمسبب المرضى مقارنة بنظيرتها المنزرعة في تربة رملية فقط مع ملاحظة أن الكومبوست الغير معقم كان تأثيره كبيرا في خفض الإصابة المرضية وأعراض الذبول عن نظيرة المعقم. أظهرت النتائج أيضا أن الكومبوست المضاف إلى التربة الرملية قد شجع نباتات الطماطم عقب عدواها بالمسبب المرضى في رفع محتواها من الإنزيمات الدفاعية تحت الدراسة وأيضا من الفينولات الكلية مقارنة بنظيرتها النامية في تربة رملية وبدون الكومبوست (الكونترول) . فقد زادت مستويات كل من أنشطة إنزيمات التحلل المائي hydrolytic enzyme بينا ٣،١-جلوكانيز، إنزيمات الأكسدة والاختزال oxidoreductases (البيروكسيديز، والبولى فينول اكسيديز) وأيضا نشاط الإنزيم المسئول بدرجة أساسية عن بناء المركبات الفينولية phenylalanine ammonia-lyase (PAL) أثناء المراحل الأولى من تفاعل النبات مع المسبب المرضى . يستنتج من تلك الدراسة أن كومبوست مخلفات الدواجن قد شجع نباتات الطماطم على رفع مستويات الأنشطة الإنزيمية ذات العلاقة بمقاومة المسبب المرضى للذبول الفيوزاريومى والتي من المحتمل أن يعزى إليها خفض شدة الإصابة المرضية وأعراض الذبول على النباتات.