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Trichoderma harzianum in combination with sheep manure amendment enhances soil suppressiveness of Fusarium wilt of tomato

Radwan M. BARAKAT* and Mohammad I. AL-MASRI[1]

Faculty of Agriculture, Hebron University, P.O box 40, Hebron- Palestinian Authority

Summary. The effect that the biocontrol agent *Trichoderma harzianum* (isolate Jn14) in combination with an amendment of sheep manure has on the soil suppressiveness of Fusarium wilt of tomato was investigated over a 28-month period. A combination of *T. harzianum* and organic amendment at concentrations (w:w) of 6 and 10% reduced tomato wilt by 21–36 % and 29–36% respectively, after 0–28 months of soil incubation. When the amendment was added at concentration of 2%, the wilt was suppressed only after 18–28 months. A combination of *T. harzianum* and the amendment at 6% also increased tomato plant fresh weights by 52% after 28 months, and the 10% amendment increased fresh weights by 56, 40, and 63%, after 18, 24, and 28 months respectively, compared to the experimental controls. Organic amendment at the higher concentrations further stimulated *T. harzianum* populations, enhanced microbial activity against *Fusarium oxysporum* in the soil and reduced pathogen populations. Without *T. harzianum*, the organic amendment at a concentration of 10% reduced disease by only 22, 24, and 23% and only after 18, 24 and 28 months of soil incubation respectively, compared with the controls. However, tomato wilt was not reduced at a 2% manure concentration in less than 12 months of incubation. Organic amendment alone at 6 and 10% reduced the pathogen population by 25% and 37% respectively after 28 months of soil incubation compared with the control. *T. harzianum* produced fungitoxic metabolites that reduced mycelial growth of *Fusarium* by 37% and conidium germination by 55% when the pathogen was grown on potato dextrose agar amended with a *T. harzianum* culture filtrate.

Key words: biological control, organic amendment, *Fusarium oxysporum*.

Introduction

Tomato wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (W.C. Snyder & H.N. Hansen) is a serious disease in greenhouses and open fields in the temperate regions of the world. Fungicides and soil fumigants are used to control the disease. The toxicity of these compounds has led to a general trend to reduce their application in the soil. Several alternatives to these products have been suggested to control the disease, including organic amendments (compost and manure) and biological control.

The effectiveness of antagonistic micro-organisms against the pathogen has been thoroughly investigated; nonpathogenic strains of *F. oxysporum* and fluorescent *Pseudomonas* spp. are some examples (Lemanceau and Alabouvette, 1993; Larkin and Fravel, 1998; Duijff *et al*., 1999; Fuchs *et al.*, 1999). In addition, *Trichoderma* spp. are well documented as effective biological control agents against many soilborne pathogens (Papavizas, 1985; Coley-Smith *et al.*, 1991; Sivasithamparam and Ghisalberti, 1998). *Trichoderma* spp., used alone or in combination with organic amendments against *F. oxysporum* f. sp. *lycopersici* on tomato plants has been shown to suppress tomato wilt in contained soil and to improve the efficacy of biocontrol against the pathogen (Cotxarrera *et al.*, 2002; Noble and Coventry, 2005; Spadaro and Gullino, 2005). Organic amendments such as compost, animal manures or industrial by-products are all well-documented to be suppressors of soilborne plant pathogens (Noble and Coventry, 2005; Cook and Baker, 1983; Hoitink and Boehm, 1999; Ryckeboer, 2001; Barakat, 2008). Composts prepared from wastes and used in container media or as soil amendments have been found to be highly suppressive of diseases caused

Corresponding author: R.M. Barakat
Fax: + 972 2 5859586
E-mail: rbarakat@netvision.net.il

by a wide variety of soilborne pathogens such as *Pythium* spp. (Mandelbaum and Hadar, 1990; Pascual *et al.*, 2000), *Phytophthora* spp. (Hoitink and Boehm, 1999; Widmer *et al.*, 1999), *Rhizoctonia* spp. (Kuter *et al.*, 1983; Tuitert *et al.*, 1998; Barakat, 2008) and *Fusarium* spp. (Chef *et al.*, 1983; Trillas-Gay *et al.*, 1986, Cotxarrera *et al.*, 2002).

The objective of the present study was to evaluate the role of a naturally occurring local biocontrol agent, *Trichoderma harzianum* Rifai (isolate Jn14) in combination with a sheep manure soil amendment in improving the soil suppressiveness of *F. oxysporum* f. sp. *lycopersici*.

Materials and methods

Soil preparation

Soil samples were collected from Al-Aroub Agricultural Research Station (10 km north of Hebron, Palestinian National Authority) and the soil was analyzed for its physical and chemical properties. The soil texture was clayey (sand 19.4%, silt 20.6%, clay 60%), and its chemical properties were: pH 7.29, EC 1.5 ms, organic matter 2.5%, nitrate 45 ppm, ammonium 15.4 ppm, potassium 179.4 ppm and phosphorus 45.4 ppm. Soil samples were passed through a 4 mm mesh sieve to remove plant residues and gravel. Sieved samples were amended with mature air-dried, sieved (4 mm) sheep manure at 0 (experimental control), 2, 6 and 10% (w:w). The experimental design was completely randomized with eight treatments and three replicates for each treatment. The treatments were: organic matter in the soil at concentrations of 0 (CK-T), 2, 6 and 10%, treated with the pathogen (*F. oxysporum* f. sp. *lycopersici*) alone at the rate of 10^3 conidia g^{-1} soil of *Fusarium*-talc inoculum. The same soil amendments were also applied in other containers in combination with the biocontrol agent *T. harzianum* isolate Jn14 at a concentration of 10^6 colony forming units (cfu) g^{-1} of soil. Each replicate was a 20 L container filled with 20 kg of soil. The soil moisture in the containers was maintained at 60–80% of field capacity and the soil was incubated at 25°C for a period of 28 months. The variously treated soils in the containers were later used to determine the incidence of tomato wilt, the tomato fresh weights (g $plant^{-1}$), the *F. oxysporum* populations (cfu g^{-1}), the total fungal populations (cfu g^{-1}), the *T. harzianum* populations (cfu g^{-1}), and dehydrogenase activity (µL H g^{-1} $24h^{-1}$) after 0, 6, 12, 18, 24, and 28 months of incubation.

Preparation of *Fusarium* inoculum

The *F. oxysporum* f. sp. *lycopersici* isolate used in the study was originally isolated from the stem of a diseased tomato plant from Halhul (North of Hebron, Palestinian Authority). The isolate was purified and designated as *F. oxysporum* f. sp. *lycopersici* isolate P. FoL2. Inoculum was prepared by inoculating four autoclaved Erlenmeyer flasks (500 mL capacity) each containing 100 mL potato dextrose broth (PDB; Difco, Detroit, MI, USA) with mycelium discs of 10-day-old single conidium cultures. Inoculated flasks were fitted on a shaker set at 200 rpm and incubated at 25ºC for 14 days to allow the mycelium to grow. Mycelial/conidial suspensions from these cultures were filtered through a sterile sintered glass funnel (pore size: 100 µm) to separate the mycelia from the conidia. The filtrate suspension containing the conidia was then centrifuged at 2308 ×*g* (RCF) for 15 min. to form a pellet of conidia and the supernatant was discarded. The conidium pellets were then washed twice with sterile water, vortexed each time to ensure homogeneous suspension and re-centrifuged. The pellets were recovered in sterile water and the final volume was adjusted to 100 mL. Each 100 mL conidial suspension was then added to 200 mL of talcum powder (Sigma, Steinheim, Germany) and mixed with a sterile spatula. The mixture was placed in small heaps on aluminium foil in a sterile tray in a disinfected aerated oven at 20ºC for 48–72 h. When the heaps dried into cakes, they were crushed and sieved (200 µm) in a sterile environment. The talcum-formulated inoculum was stored dry in a tightly closed flask at 4ºC. Inoculum density was evaluated by dilution plating to determine the cfu g^{-1} talc. The quantity of inoculum to be used was calculated to give 10^3 conidia g^{-1} soil/manure mixture.

Preparation of *T. harzianum* inoculum

The *T. harzianum* isolate Jn14 used was isolated in 2004 (Barakat *et al*., 2006). The isolate was grown on 90 mm Petri dishes containing potato dextrose agar (PDA, Difco) at 25°C for 10 days. Autoclaved Erlenmeyer flasks (500 mL capacity) each containing 100 mL potato dextrose broth (PDB, Difco) were inoculated with 5 mm mycelium plugs of the isolate. The flasks were then incubated under shaking (200 rpm) at 25°C in a growth chamber for 12 days. The content of the flasks was filtered through a sterile glass funnel (pore size, 100 µm). The filtrate suspension containing the conidia was centrifuged at 2308 ×*g* (RCF) for 15 min. to obtain a pellet of conidia and the supernatant was discarded. The pellets were twice washed with sterile distilled water, vortexed to ensure a homogeneous suspension and re-centrifuged. After washing, the pellets were recovered in sterile water. The inoculum was applied to the manure-amended soil to achieve a final concentration of 10^6 conidia g^{-1} of soil.

Assessment of Fusarium wilt and tomato fresh weights

Fusarium wilt and tomato plant growth in manure-amended soils with or without *T. harzianum* were assessed after 0, 6, 12, 18, 24, and 28 months. The experiments were conducted using a completely randomized design with 8 treatments and 36 replicates (pots). In each pot, a plastic grid was placed at the bottom to prevent the soil from escaping; 20 mL of autoclaved perlite was also added to allow water drainage. After that, 100 mL of *Fusarium*-infested soil amended with only manure or with manure plus *T. harzianum* was added and covered with a 2 mm layer of autoclaved perlite to prevent pre-emergence damping off. At each assessment date (0, 6, 12, 18, 24, 28 months), each pot was seeded with 3–5 tomato seeds and a second layer of autoclaved perlite was added as a cover. After emergence, one seedling was maintained per pot. The pots were placed in plastic boxes on three layers of mesh and the control-treatment pots were protected from contamination from splashing irrigation water. Plants were incubated in a growth chamber at 25°C, with a 15 h photoperiod. Plants were irrigated with deionized water each day. Wilted plants were identified by observing the discoloration in the vascular systems, which was done by making horizontal sections in the tomato stem. The numbers of wilted plants were recorded twice a week after sowing from week 3 to week 8, and were removed. The fresh weight of the unwilted plants was measured at the end of each assessment date.

Fungal populations

The total fungal populations and the populations of *F. oxysporum* and *T. harzianum* (cfu g^{-1}) were evaluated in treated soils after incubation for 0, 12, 18, 24, and 28 months. The total fungal population was evaluated using the dilution plate technique on PDA. The *F. oxysporum* population was determined using the method of Komada (1975). Air-dried soil samples were ground with a mortar and pestle and sieved at 200 µm. Each treated soil sample in the containers was divided into three subsamples; 10–20 mg soil was taken from each subsample and spread on 150 mm diameter glass Petri dishes. Each sample consisted of three dishes. A basal medium containing 1 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.01 g L^{-1} Fe-Na-EDTA, 2.0 g L^{-1} L-asparagine, 20 g L^{-1} D-galactose and 20 g L^{-1} agar was autoclaved at 121°C for 15 min and cooled to 55°C, after which the antimicrobial agents were added. The antimicrobial agents were prepared in 10 mL sterile distilled water by adding 1 g L^{-1} pentachloronitrobenzene (PCNB, Sigma), 0.5 g L^{-1} Oxgall, 1 g L^{-1} $Na_2B_4O_7 \cdot 10H_2O$ and 0.3 g L^{-1} streptomycin sulfate. The pH of the medium was adjusted to 3.8 with 10% phosphoric acid. The selective medium was then poured (20–30 mL) into each 150 mm glass dish and carefully shaken to mix with the sieved soil in the dish. The dishes were incubated at 25°C in the dark for 24 h, and under natural light for 3 days, after which they were examined and the number of colonies counted. The experimental design was completely randomized with eight treatments and 27 replicates (dishes).

The *T. harzianum* population (cfu g^{-1}) in the amended soil was evaluated using the dilution plate technique on a *Trichoderma*-selective medium (TSM) (Elad *et al*., 1981), according to the following procedure: a 25 g air-dried soil sample was suspended in 225 mL of 0.1% water agar and shaken for 20–30 min. in a rotary shaker at 200 rpm. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were made of each soil sample, and 0.1 mL of a 10^{-5} soil suspension was plated onto 90-mm diam. Petri dishes containing TSM. The plates were then incubated for 5–7 days at 25°C, and the colonies were counted. The experimental design was completely randomized with 15 replicates (plates) for each treatment.

Dehydrogenase enzyme assay

Dehydrogenase activity was measured to indicate respiration and hence the activity of the soil microflora. Soil dehydrogenase activity was determined by measuring the rate at which 2, 3, 5- triphenyltetrazolium chloride (TTC, Sigma) was reduced to 2, 3, 5- triphenyltetrazolium formazan (TTF, Sigma) (Smith and Pugh, 1979). Enzyme activity in treated soils was evaluated by weighing 1.2 g soil samples and placing them in test tubes that were sealed. The experimental design was completely randomized with nine replicates (tubes) per treatment. Soil samples were saturated with 400 µL of a 1% solution of TTC and mixed thoroughly in a vortex shaker. The sealed tubes were incubated in a covered shaking water bath at 30°C for 24 h. After incubation, 2.4 mL of methanol **(**Sigma**)** was added to each tube and the contents were vortexed and allowed to stand at room temperature for 10 min. The tube contents were then centrifuged at 2308 ×*g* (RCF) for 15 min. Aliquots were removed and the intensity of the red color, produced by the reduction of TTC to TTF, was determined spectrophotometrically at 485 nm. The amount of TTF was calculated by comparison with absorbance for known standard amounts of TTF freshly prepared in methanol. Dehydrogenase activity was assessed in µL hydrogen; the formation of 1 mg of TTF required 150.35 µL hydrogen. The data presented were calculated as µL H g^{-1} $24h^{-1}$. The dehydrogenase enzyme was assessed after four periods of soil incubation (0, 12, 18, and 28 months).

Assessment of *T. harzianum* antagonistic potential

Hyphal interaction on thin films of agar

Hyphal interaction was examined on sterile glass cover slips coated with 2% water agar (20 g Difco agar L^{-1} distilled water). Each cover slip was immersed for 1–2 s in autoclaved melted water agar at 45°C, allowed to drain and placed on 2% solidified water agar in a 90 mm diameter Petri dish. Five-mm disks were cut from the margins of 1-week-old colonies of *F. oxysporum* and of *T. harzianum* and were placed 3 cm apart on the agar surface and incubated at 25°C. Colony margins in each dish met across the coated cover slips in less than 5 days. The cover slip was removed carefully without damaging mycelial contact and was inverted on a sterile microscopic slide. A microscopic examination was carried out through the coated cover slip using fresh direct mounts in lactophenol cotton blue at ×200 and ×400 magnification. Specimens were always sealed with nail polish to prevent drying (Laing and Deacon, 1991). Fungal interactions, including coiling and penetration, were examined for possible mycoparasitism.

Production of antifungal metabolites

The capacity of the *T. harzianum* isolate Jn14 to reduce mycelium growth of *F. oxysporum* by producing fungitoxic metabolites was evaluated using the method of Dennis and Webster (1971). Fifty mL of PDB, (pH 6) in a 250 mL Erlenmeyer flask was inoculated with a 7-mm agar disk from a 7-day-old PDA culture of *T. harzianum* and incubated at 25°C. After incubation for 10 days, cultures were filtered through Millipore membrane filters (0.45 µm) and were autoclaved at 121°C for 15 min. The culture filtrates were added to PDA medium at 10 and 25% v:v, and the mixture was poured into Petri dishes. The filtrate-amended PDA dishes were then centrally inoculated with 7 mm mycelium plugs of *F. oxysporum*. Petri dishes were incubated at 25°C; unamended PDA dishes served as the experimental controls. The mycelium growth rates were measured after 48 h and calculated as $cm^2\ day^{-1}$. In addition, the conidium germination of *F. oxysporum* on filtrate-amended PDA dishes was evaluated; 200 µL of conidial suspension (10^{-5} conidia mL^{-1}) was spread onto each plate. The plates were incubated at 25°C for 3 days in the dark and for 4 days under natural light. The number of germinated conidia was counted and percent germination was calculated as compared with the controls. The experimental design was completely randomized with four replicates (dishes) for each concentration.

Statistical analyses

Data from the experiments were statistically analyzed using one-way repeated measurement analysis of variance (ANOVA). Fisher LSD tests ($P \leq 0.05$) were used to separate the means (Sigma Stat® 2.0 program, SPSS Inc., Chicago, IL, USA).

Results

Fusarium wilt and tomato plant fresh weight

The combination of *T. harzianum* and the organic amendment (OA) at concentrations of 6 and 10% reduced (LSD=18.1) the disease significantly (P=0.05) by 21–36% and 29–34% respectively, after soil incubation for 0–28 months compared to the control at the corresponding assessment date (Figure 1a). When *T. harzianum* was introduced at the lowest OA concentration (2%), the disease reduction became significant only after 18, 24, and 28 months at 19, 28 and 24% respectively; the reduction was not significant after 6 and 12 months of incubation. There was a general trend for wilt to be reduced with increasing OA concentration and with increasing time of incubation.

The combination of *T. harzianum* and 6% OA increased tomato plant fresh weight significantly (LSD=3.2) by 52% after only 28 months, while 10% OA increased tomato fresh weight by 56, 40, and 63% after 18, 24, and 28 months soil incubation respectively, as compared with the corresponding controls. With 2% OA, differences in the fresh weights were not significant at any of the incubation periods (Figure 1b).

The OA treatment alone at the highest concentration (10%) reduced wilt significantly by 22, 24, and 23% but only after 18, 24, and 28 months of soil incubation respectively, as compared with the control. At lower OA concentrations (2 and 6%), however, reductions in disease and plant fresh weight were not significant at all incubation assessment dates as compared with the control (Figure 1c). OA at a concentration of 10% increased plant fresh weights by 40 and 19% after 18 and 28 months respectively, as compared with the control (Figure 1d).

Trichoderma harzianum with 10% OA increased tomato plant fresh weights by 14–44% compared to OA alone after 18–28 months of soil incubation respectively (Figure 1b).

Fungal population

The total fungal population (10^7 cfu g^{-1}) was greater (LSD=3.26, P=0.05) with all OA treatments after 12–28 months of soil incubation compared to the population

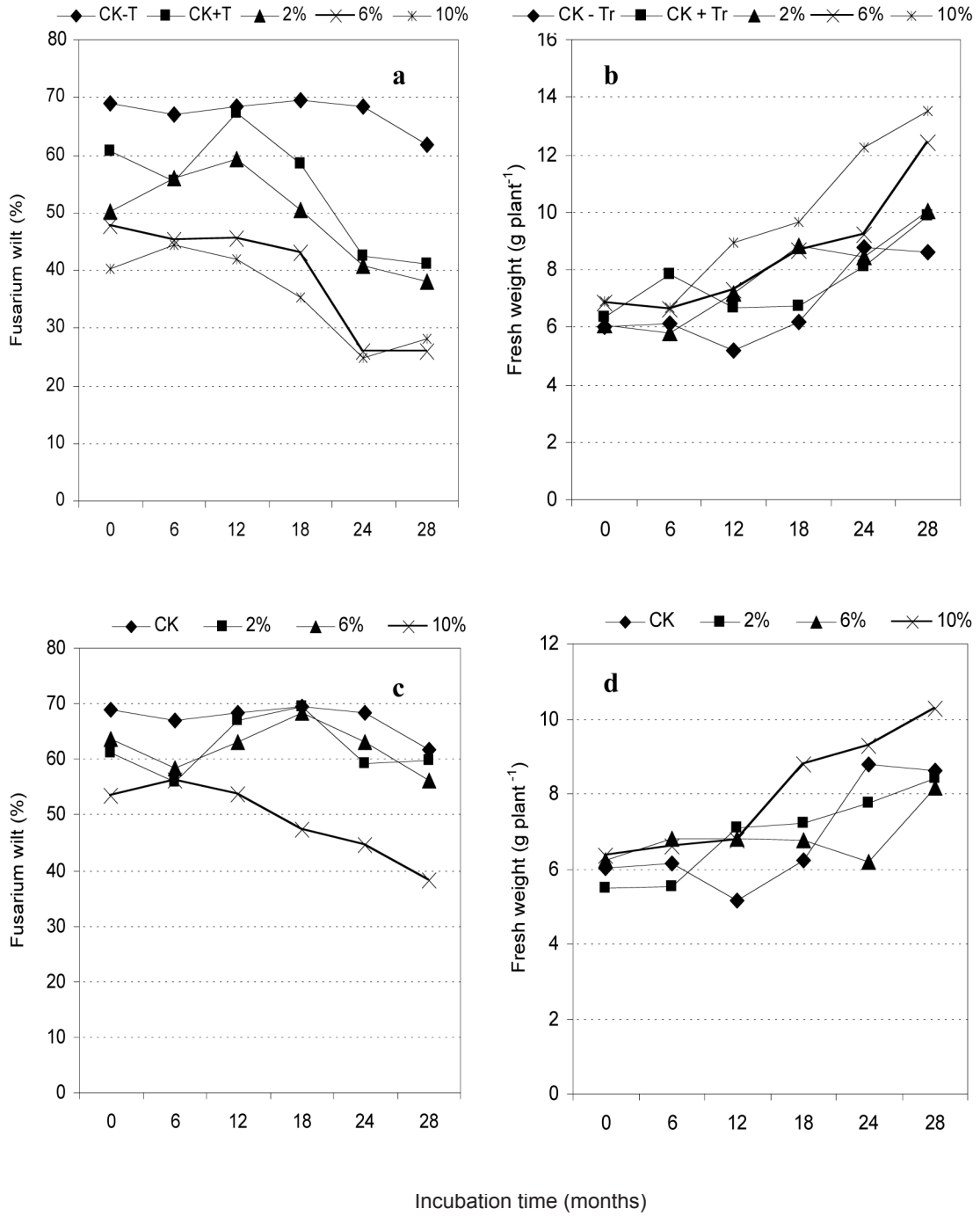

Figure 1. Effect of combinations of *Trichoderma harzianum* and organic amendment on wilt of tomato caused by *Fusarium oxysporum* (a) and on tomato plant fresh weight (b), and effect of organic amendment alone on wilt (c) and on plant fresh weight (d) after 0, 6, 12, 18, 24 and 28 months of soil incubation at 25°C in a growth chamber at 25°C. (Each point is the average of 36 replicates); CK -T, control without *T. harzianum*; and CK+T, control with *T. harzianum*. Fisher LSD of disease=18.1 and LSD of fresh weight=3.2.

at the start, irrespective of whether or not *T. harzianum* was added. A combination of *T. harzianum* and OA at a concentration of 10% decreased the total population by 37 and 55% after 18 and 28 months of soil incubation respectively, as compared with the corresponding controls. With 6% OA, the total fungal population was reduced by 21 and 37% after 18 and 28 months of soil incubation respectively (Figure 2a). OA treatments alone at a concentration of 10%, however, increased the total population by 42, 61, and 90% after 12, 18 and 28 months of soil incubation respectively. OA at 6% increased the population by 53% after 28 months of soil incubation (Figure 3a).

However, a combination of *T. harzianum* and OA at a concentration of 6 and 10%, reduced (LSD=0.17, *P*=0.05) the *F. oxysporum* population after 18 and 28 months of soil incubation compared to the corresponding controls (Figure 2b). The *F. oxysporum* population was reduced by 28% after 18 months at OA concentrations of 6 and 10%. After 28 months at these OA concentrations, the *F. oxysporum* population was reduced by a further 39 and 54% as compared with the control (Figure 2b).

Organic amendment alone at concentrations of 6 and 10% reduced the *F. oxysporum* population by 25 and 37% respectively after 28 months of soil incubation, compared with the control after the same incubation period. Furthermore, the *T. harzianum* populations (10^6 cfu g^{-1}) greatly increased (LSD=1.26, *P*=0.05) with time and increasing OA concentration (Figure 2c). The population was increased (*P*=0.05) 10, 20, and 42 fold at an OA concentration of 6% and 13, 27, and 54 fold at an OA concentration of 10% after 12, 18 and 28 months of soil incubation respectively, as compared with the control at the start. The population of *T. harzianum* in the control treatment and with 2% OA increased over time, but to a less extent than with the higher OA concentrations (Figure 2c).

Microbial activity in the soil

Microbial activity expressed as dehydrogenase activity increased (LSD=5.54, *P*=0.05) with increasing duration of soil incubation, especially after 28 months of incubation (Figures 2d and 3c). The statistically significant effect of a combination of *T. harzianum* and OA at 10% was very clear after 28 months (Figure 2d). Microbial activity with OA alone without *T. harzianum* followed the same trend and increased greatly after 28 months of incubation (Figure 3c). The introduction of *T. harzianum* increased microbial activity at all levels (Figure 3d). The length of incubation had the greatest impact on microbial activity, with or without *T. harzianum*.

Assessment of *Trichoderma* antagonistic potential

In vitro examination of the interaction between *T. harzianum* and *F. oxysporum* did not show any interactions between the antagonist and the pathogen. *T. harzianum* hyphae did not coil around or penetrate *F. oxysporum* hyphae. However, the antibiosis study indicated that *T. harzianum* produced antifungal metabolites (AFM) in PDB. These metabolites significantly reduced (Fisher LSD= 0.8, *P*=0.05) the growth rate of *F. oxysporum* mycelium, by 37% on PDA amended with 25% *T. harzianum* culture filtrate. Furthermore, the antifungal metabolites significantly reduced (Fisher LSD=18.4, *P*=0.05) the conidial germination of *F. oxysporum,* by 33 and 55% on PDA amended with *T. harzianum* culture filtrate at 10 and 25% respectively (Figure 4).

Discussion

Sheep manure amendment at a high concentration (10%) in the soil tended to reduce Fusarium wilt over time. Introducing a *T. harzianum* isolate into the system greatly enhanced disease reduction with higher organic amendment concentrations (6 and 10%). The reduction of disease in soils with OA was accompanied by a reduction in the *F. oxysporum* population and an increase in the *T. harzianum* population.

Organic amendment alone increased the total fungal population and the microbial activity in soil over time, probably because the amendment provided nutrients for all these organisms to grow and proliferate. However, the situation was altered when *T. harzianum* was introduced to the soil. The population of other fungi, including *F. oxysporum*, declined as the population of *Trichoderma* increased, probably due to competition for nutrients and space. This was reflected in a reduction in Fusarium wilt. The study also found that the antifungal metabolites produced by *T. harzianum* reduced the conidium germination and mycelium growth of *F. oxysporum*. Similar results were obtained by Cotxarrera *et al.* (2002), who reported that a combination of organic amendments and *Trichoderma asperellum* suppressed Fusarium wilt of tomato and potentially controlled the disease. Several investigators have attempted to explain why this combination reduces disease severity (Hoitink and Boehm, 1999; Noble and Conventry, 2005). It has also been suggested that the reduction in disease achieved is due to the mixtures of volatile fatty acids produced by the system, which may include acetic, propionic, butyric, isobutyric and valeric fatty acids. These are produced during the decomposition of manure and are considered potential plant pathogen suppressors (Tenuta *et al*., 2002).

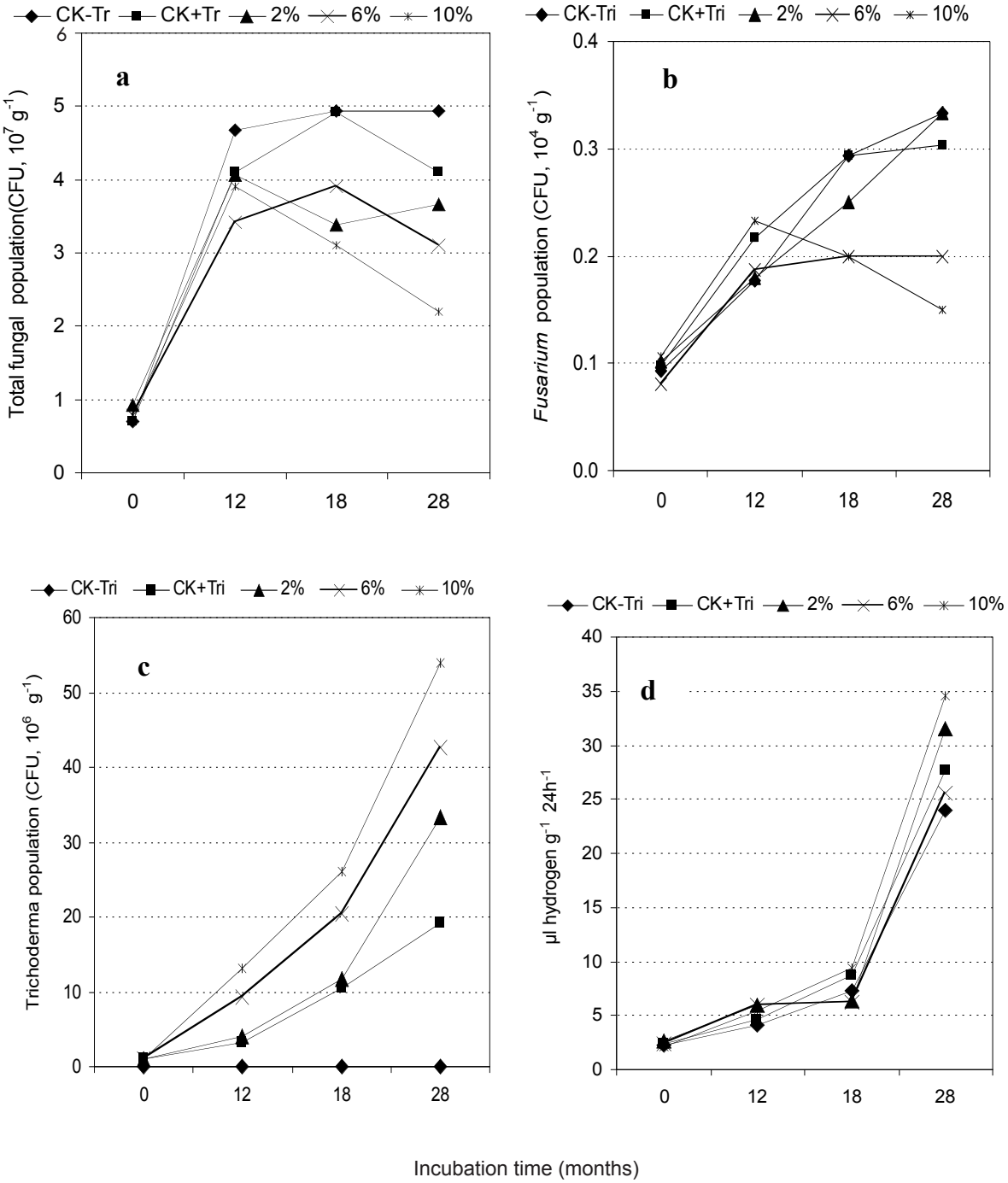

Figure 2. Effect of combinations of *Trichoderma harzianum* and organic amendment on total fungal population (a), *Fusarium oxysporum* population (b), *T. harzianum* population (c), and dehydrogenase activity (d) after 0, 12, 18, and 28 months of soil incubation at 25°C. CK-T, control without *T. harzianum*; and CK+T, control with *T. harzianum*. Fisher LSD of total population=3.26, LSD of *Fusarium* population=0.17, LSD of *Trichoderma* population=1.26, and LSD of dehydrogenase=5.54.

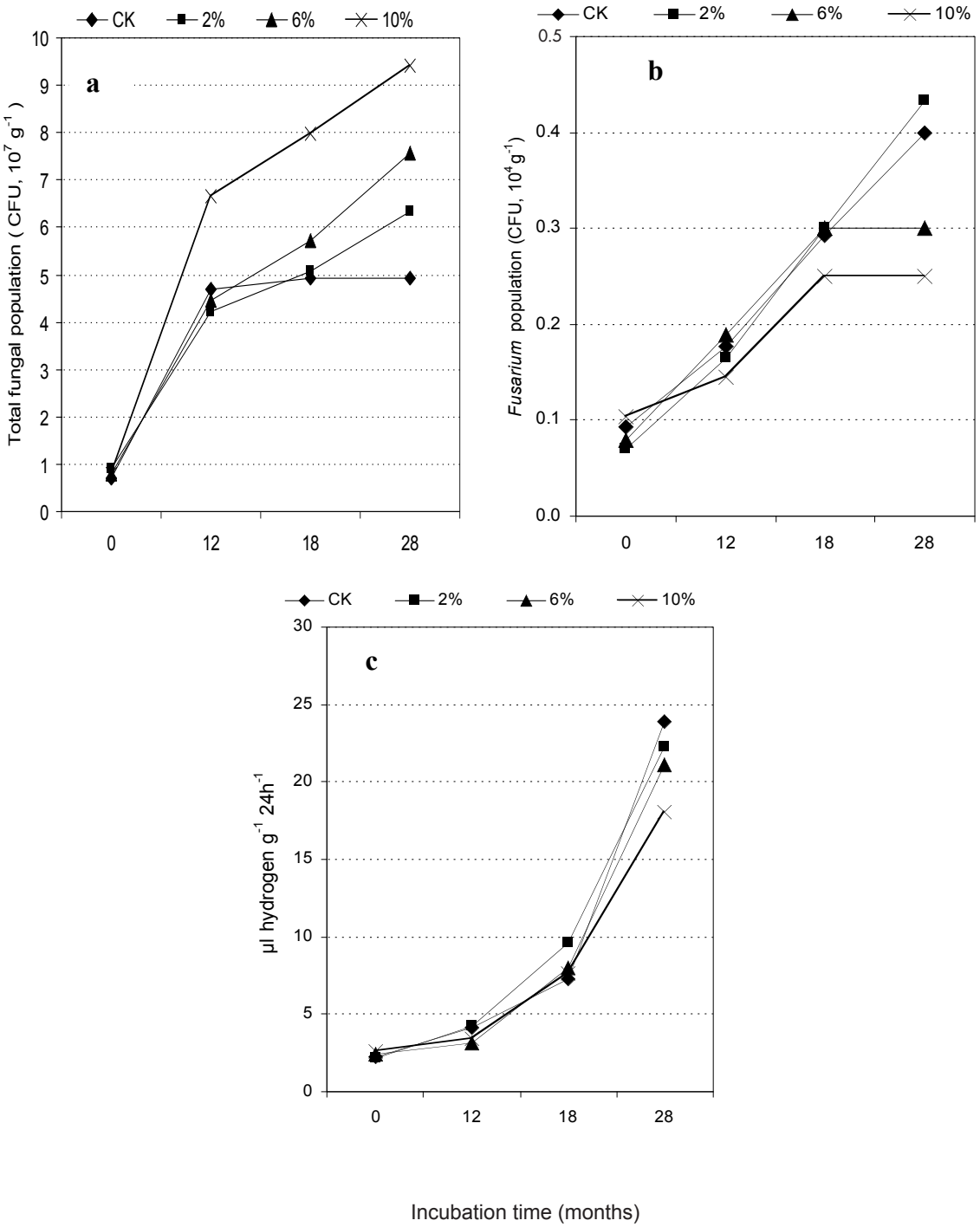

Figure 3. Effect of sheep manure amendments on total fungal population (a), *Fusarium oxysporum* population (b), and dehydrogenase activity (c) after 0, 12, 18, and 28 months of soil incubation at 25°C. CK, control without *Trichoderma harzianum*. Fisher LSD of total population=3.26, LSD of *Fusarium* population=0.17, and LSD of dehydrogenase=5.54.

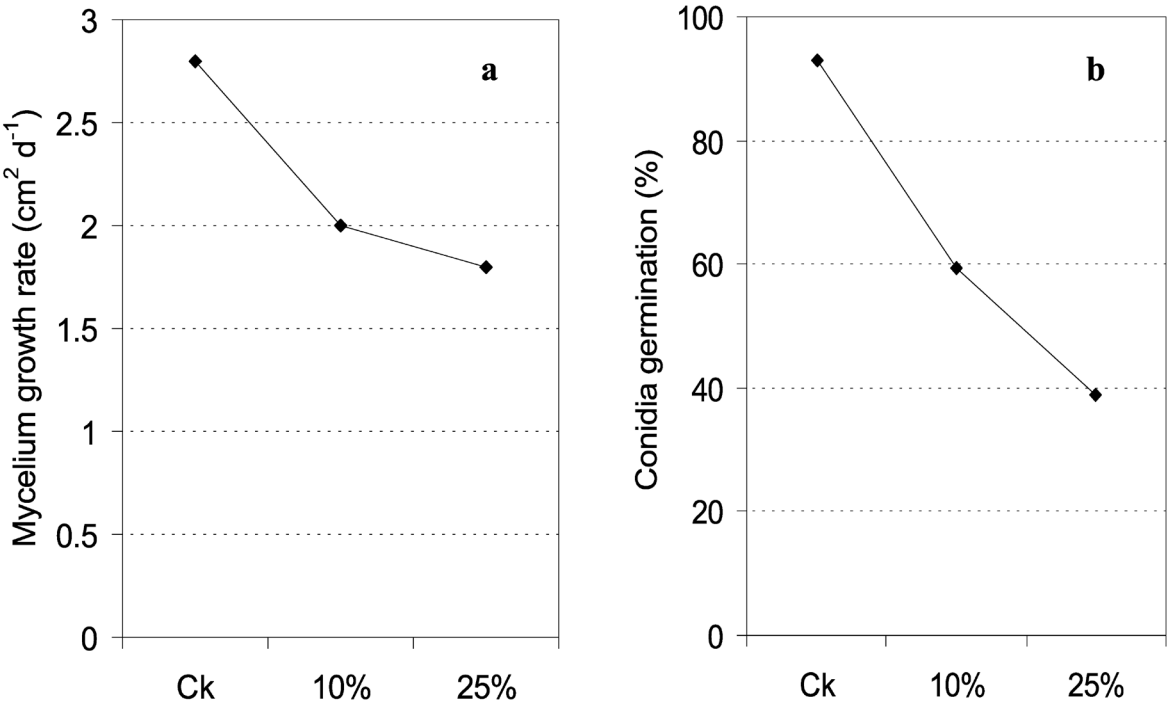

Figure 4. Effect of *Trichoderma harzianum* culture filtrates (10 and 25%, v:v amended PDA) on the mycelium growth rate of *Fusarium oxysporum* (a) and on conidial germination (b) at 25°C. Fisher LSD of mycelium growth rate=0.8 and LSD of conidial germination=18.4.

Organic amendments alone suppressed Fusarium wilt at high OA concentrations (10%), and OA effectiveness increased over time. Similar findings have been reported in numerous container-based studies in greenhouses or growth rooms (Noble and Coventry, 2005), which have consistently demonstrated that compost and organic amendments suppress soilborne plant pathogenic fungi, including *Rhizoctonia solani*, *Pythium ultimum*, *Phytophthora* spp., *F. oxysporum* and *Verticillium dahliae*. In addition, Cheuk *et al*. (2005) reported that plant waste compost reduced Fusarium root and crown rot and improved yield of tomato grown under greenhouse conditions.

The increase of *Trichoderma* populations in soils rich in organic matter or organically amended has been well documented (Papaviza, 1985; Coley-Smith *et al.*, 1991). In the present study, the *T. harzianum* population increased in all organically amended soils over time. The fungus probably used the organic amendment as a nutrient base for growth and proliferation.

Vegetative growth of tomato plants increased in soils with high concentrations of organic amendment (6 and 10%). This is likely to be due to the abundant nutrients (mainly nitrate and ammonia) released during manure decomposition (Tuitert *et al.*, 1998). In addition, a number of studies reported that plants grown in *Trichoderma*-amended soils exhibit increased growth (Coley-Smith *et al*., 1991; Sivasithamparam and Ghisalberti, 1998; Harman, 2000; Barakat, 2008). In the present study, the introduction of *T. harzianum* to the soil increased tomato plant growth by 14–44% compared with plants grown in soils treated only with organic amendments.

Increased growth of plants following application of *Trichoderma* to the soil has been well documented (Baker, 1989; Kleifeld and Chet, 1992; Anusuya and Jayarajan,

1998; Altomare *et al*., 1999; Harman, 2000; Yedidia *et al*., 2001, Barakat, 2008). A number of mechanisms have been suggested to explain this phenomenon. Kleifeld and Chet (1992) suggested that *Trichoderma* made host plants more resistant to the pathogen. Altomare *et al*. (1999) reported that *T. harzianum* solubilizes insoluble tricalcium phosphate *in vitro,* which may contribute to increased plant growth. Yedidia *et al*. (2001) found that the increased tomato growth caused by *Trichoderma* also increased the root area, which may explain the increased root dry weight, shoot length and leaf area compared with that of untreated plants. They also suggested that *T. harzianum* enhanced the mineral uptake of plants at a very early stage of the fungus-plant association. Harman (2000) reported that *Trichoderma* spp. are opportunistic plant colonizers that stimulate plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones.

In conclusion, the study demonstrated that Fusarium wilt of tomato is reduced and tomato growth is increased when *T. harzianum* is applied to the soil in combination with a sheep manure amendment at concentrations (above 6%, v:v), and when soil incubation is extended over longer periods.

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