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Characteristics of Three *Trichoderma* Species in Peanut Haulms Compost Involved in Biocontrol of Cumin Wilt Disease

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ABSTRACT

Peanut haulms compost as a carrier of three *Trichoderma* species (*T. harzianum, T. hamatum* and *T. koningii*) exhibited different morphological and chemical characteristics and its ability to control *Fusarium* wilt of cumin plants caused by *Fusarium oxysporum* compared with peat/vermiculite. The peanut haulms carrier was able to increase the population size, numbers, viability, survival, microbial biomass and activity during twelve months experiment while population from other commercial peat/vermiculite was decreased. Detecting *Trichoderma* biochemical activity in compost by High Performance Liquid Chromatography (HPLC) analyses revealed that relative amounts of antibiotics as gliotoxin, trichodermin and gliovirin as well as total phenols production increased. At the same time, spectrophotometric determination of the enzymatic hydrolysates revealed that chitinase, protease, cellulase, β- glactosidase and β-1,3-glucanase were also increased. In infested and natural soil with *Fusarium oxysporum,* amendment with peanut haulms compost inoculated with *Trichoderma spp.* were found to be effective in reducing wilt disease incidence and pathogen population. Addition of peanut compost increased the population counts of all *Trichoderma spp* in soil. Plant growth yield, nitrogen content and seeds oil content were improved. Peanut compost inoculated with *T. harzianum* was effective in reducing disease incidence and increased yield components. Similarity between levels and specificities of biological activity and the morphological and chemical characterization of biocontrol agents in compost were found in relation to antagonist. Peanut haulms compost, in general, exhibited high stimulation of biocontrol activity.

Key Words: Cumin; *Fusarium oxysporum;* Peanut; Waste compost; *Trichoderma spp;* Biological control

INTRODUCTION

Cumin (*Cuminum cymium* L.) of family Apiaceae (Umbelliferae); is one of the most important condiments consumed in Egypt. Essential oils of fruits have been reported to have antimicrobial activity. *Fusarium* wilt disease, caused by *Fusarium oxysporum* f. sp. *cumini* is a destructive disease and reduces the production of crops considerably in Egypt (Haggag, 1993; Omer *et al.*, 1997) and in other countries (Purohit & Bohra, 1999).

Chemical and physical methods being less in use owing to their obvious limitation against *Fusarium* wilt. Biological control is another option. *Trichoderma spp* .are species aggregates which includes a plethora of strains that can be used as biological control agents of plant pathogenic fungi for over 70 years (Samuels, 1996; Harman & Björkman, 1998; Godwin & Arinze, 2000; Hermosa *et al.,* 2000), but it is only recently that these strains become commercially available. Knowledge concerning the behaviour of these fungi as antagonists is essential for their effective use since they can act against target organisms in several ways (Howell, 1998; Haggag & Saber, 2000; Cutler *et al*., 2001; Monte, 2001). Strains of *Trichoderma spp* .can produce extracellular enzymes and antifungal antibiotics and also may be competitors to fungal pathogens and promote plant growth. The commercial use of biocontrol agents requires inoculum that retains high inoculum viability and can easily be transported and applied. Compost materials represent ideal base for the proliferation of biocontrol agents and offer a great opportunity to sustainable clean farming system (Hermosa *et al*., 1999; Mekki *et al*., 1999; Saber, 2000; Hoitink *et al.,* 2001a**;** Abbasi *et al*., 2002). Such preparations might also be useful as a carrier for biocontrol agents to stimulate metabolite production, reduce plant disease and enhance plant growth (Sampangiramaiah, 1997; Thornton & Gilligan, 1999; Haggag & Saber, 2001). At the same time, extension of the use of compost to the horticultural industry as a substitute for part of the peat in potting mixtures would reduce the amount of peat and the use of fertilizers in container–grown crops (Hoitink *et al.,* 2001b; Krause *et al*., 2001; Stone *et al*., 2001).

Due to the greater concern about the use of synthetic and toxic chemicals of *Trichoderma* in agriculture and the viability of certain potential new "biological" more and more growers consider the shift to biological approaches. The following study was conducted to evaluate the effect of peanut haulms compost as a carrier on the activity of three *Trichoderma* species included *T. harzianum, T. hamatum* and *T. koningii* for controlling of *Fusarium* wilt disease of cumin plants caused by *Fusarium oxysporum*, which will be eco-friendly in its approach. Effects were explained using quantification of different morphological and biochemical characteristics.

MATERIALS AND METHODS

Strains. Three *Trichoderma* species used for the investigation were *T. harzianum* Rifai*, T. hamatum* Bon and *T. koningii* Ouden. *Trichoderma* species were isolated from the soil rhizosphere of cumin plants grown in Sharkia governorate. *Fusarium oxysporum* was isolated from diseased cumin plants grown in Sharkia governorate using the standard dilution series on Malt Extract Agar medium (MEA). *Trichoderma* strains were tested against *Fusarium* pathogen *in vitro*. Cultures were maintained at 24°C on Potato Dextrose Agar (PDA; Difco) for four days, and liquid Potato Dextrose broth (PDB) for 72 h at a speed of 120 rpm for sporulation. Spores were collected on filter paper in a Buchner funnel, washed with distilled water and adjusted with sterilized distilled water in 10^4/mL colony forming units (cfu).

Compost. It was prepared from the haulms of peanut plants as described by Abo- Sedera (1995), Badr El-Din and Abo-Sedera (2001). The chemical analysis of compost is shown in (Table I) .The compost was oven dried (at 60°C for 24 h), grinded to powder form and enriched with calcium carbonate (4.0%) and 0.01% molasses. Compost was inoculated with 10 mL/100 g on even dry basis, suspension of each of the biocontrol agents [4 x 10^4 /mL (cfu)] and incubated in plastic containers (100 g/m^3) at 22-24°C and 25-30% humidity. Commercial growth medium (peat and vermiculite, 1:1, w/w) was used as control treatment. One and twelve months after storage, one hundred –gram portions of fortified compost were taken from each treatment and examined for:

Morphological features of *Trichoderma spp*. Chlamydospores and conidiospores production, size and colony numbers of *Trichoderma spp*. in compost were recorded on MEA medium after 14 days incubation at 25°C. The biomass and its viability were also determined by the method of Ineson and Anderson (1982) under the same conditions. Microbial activity was assessed by measuring the rate of hydrolysis of fluorescein diacetate (FDA) at 490 nm as proposed by SchnÜrer and Rosswall (1982). Morphological results are the mean values determined from at least 10 plants for 1 g /dry weight compost.

Biochemical features of *Trichoderma spp*. Metabolites assembled by *Trichoderma spp*. were assessed in the various treatments. Dry carrier were extracted for one hr. at 4°C with 20 mL 0.1% w/v aqueous Tween 80. After centrifugation (15,000 *g* for 15 min), 10 mL portions of the supernatant were used for metabolites assay. Both commercial peat and vermiculite and liquid growth medium (MEA) were used as control.

Enzymes. Hydrolysis enzymes were assessed according to the method given by Elad *et al.* (1985) using a Labsystems Uniskan II microtiter plate spectrophotometer. The activity of chitinases (Boller *et al.*, 1983), was measured as the release of N-acetylglucoseamine from chitin and one unit (U) of enzyme activity was deliberated as the amount of enzyme that release/M mol of reducing groups min^{-1} mL^{-1} of the filtrate. Protease activity was measured in dimethylcasein (5mg mL^{-1} in 20 mM phosphate buffer pH 7.0) as substrate using autoanalyser. The release of alanine was measured and used as a basis for the expression of protease avtivity (I U = 1 µmol alanine min^{-1} g^{-1}). The activity of cellulase (Chernolazov *et al*., 1989) was measured as the release of sugar from carboxymethyl cellulose and one unit (U) of enzyme activity was expressed as the amount of enzyme that catalyzed the release/M mol of galacturonic acid min^{-1} mL^{-1} of the filtrate. The activities of β-galactocidase (Antal *et al.*, 2000) was measured as the release of sugar from *p*-nitro-phenyl-β-D-glucopyranoside and one unit (U) of enzyme activity was expressed as the amount of enzyme that catalyzed the release /M mole of *p*-nitro-phenol min^{-1} mL of the filtrate. The activities of β-1,3-glucanase (Elad *et al*., 1983) was measured as the release of sugar from leminarian and carboxymethyl cellulase and one unit (U) of enzyme activity was expressed as the amount of enzyme that catalyzed the release/U mole of galacturonic acid min^{-1} ml of the filtrate.

Antibiotics and total phenols assay. Antibiotics and total phenols were identified using High Performance Liquid Chromatography (HPLC). Extracts were injected into a Beckman Ultraspece ODS 5 µm column (4.6 x 250 mm) that was mounted in a Beckman HPLC system comprising a 421A controller, a 427 integrator, and a 165 variable wavelength detector set at 254 nm. The elegant was a mixture of double distilled water (65%), HPLC-grade acetonitrile (20%), and HPLC-grade methanol (15%), adjusted to pH 4.0 with acetic acid. Retention times of the major compounds had been determined and compared with standard (Sigma, Chemicals).

Biological Activity

Pot experiments. The effect of the peanut haulms compost as a carrier of *Trichoderma spp*. on control of *Fusarium* wilt was determined under infested soil. Five seeds were sown per pot -25 cm in diameter- filled with sand and 2% fertified compost. Pots were inoculated with 5% of a suspension of *F. oxysporum*, 5 x 10^4 cfu g^{-1} sand soil compost mixture. The other pots (control) received sterile tap water. Plants were grown for 60 days in the greenhouse under controlled conditions of light, humidity and temperature. Pots were irrigated to 75% of the water–holding capacity. The wilt disease incidence was calculated during growth periods. The disease incidence was compiled by adopting 0-5 scale to cover all the broad symptoms. The percent disease incidence was calculated using the following formula:

$$Percent\ disease\ incidence = \frac{Sum\ of\ the\ total\ scores}{Maxmum \times total\ number\ of\ plant\ assessed} \times 100$$

Population counts of both *Fusarium* and *Trichoderma spp*. were counted during growth periods using dilution plate techniques and expressed as colony forming units (cfu). All treatments were repeated four times.

Field studies. In order to confirm the efficacy of peanut haulms compost as a carrier of *Trichoderma spp*., a field trail was carried out in a farm with sandy loam soil in Sharkia (Belbes) during 2003 and 2004 seasons with natural infested conditions. Briefly, cumin seeds were sown in rows inoculated with fortified compost at 2%. The experiment included five replicates, each comprising 5 rows distributed in a randomized complete block design (200 seeds per replicate). Wilt disease incidence were recorded as mentioned above at 30 days interval. Population counts of both *Fusarium* and *Trichoderma spp*. in soil rhizosphere were counted during growth periods as pervious described.

At the harvested stage, growth parameters i.e. plant height, dry weight, yield and nitrogen content were determined per plant. Volatile oils of cumin seeds of all treatments were extracted and determined with hydro distillation according to Guenther (1961). The oil of each treatment was subjected for Gas Liquid chromatography analysis adapting the conditions mentioned by Masada (1976).

Statistical analysis. The data were subjected to analysis of variance (ANOVA) and form which L.S.D was computed for comparison between treatments (Snedecor & Cockran, 1972).

RESULTS

Morphological feature. The parameters with reference to sporulation, biomass, viability and microbial activity of three *Trichoderma spp*. on the culture media obtained after storing in peanut compost were recorded. Mean values and standard deviation for the three *Trichoderma* species, separated in different characteristics are shown in Table II. During twelve months of incubation, abundant *Trichoderma spp*., growth, biomass and activity were visible in the peanut haulms compost in comparison with commercial peat and vermiculite. The results recorded significant differences in the population counts and size of three *Trichoderma spp.* between peanut compost and in peat/ vermiculite. Conidiospores and chlamydospores of all *Trichoderma spp*. were formed. The largest population number and size could be detected of both conidiospores and chlamydospores during incubation in peanut compost in comparison with peat. After one month of inocubation , high conidiospores production was recorded by *T. koningii* and *T. harzianum* in peanut compost in comparison with peat/ vermiculite, then decline at 12 months . Meanwhile, after 12 months of incubation *T. harzianum* and *T. koningii* showed the highest numbers of chlamydospores in peanut compost and decline in peat/ vermiculite. Overall, colony diameter of all *Trichodema spp*., on MEA medium, were higher after 12 months in peanut compost than in peat. The same results were obtained in fungus biomass and viability, which increased in peanut compost during 12 months of incubation when compared with peat/ vermiculite. Microbial activity also remained higher up to 12 months of incubation in peanut compost, but it decreased in peat/vermiculite. Higher biomass and viability in general, were observed in *T. harzianum* inoculated in compost.

Biochemical feature. To study the influence of peanut compost as a carrier of three *Trichodema spp.* on its activity, antifungal analysis was included. Results displayed in (Table III) imply that *T. harzianum, T. koningii* and *T. hamatum* produced chitinase, protease, cellulase, β –glactosidase and β -1,3-glucase when grown in commercial peat/vermiculite and in peanut compost media. Data also revealed that peanut compost exhibited the highest enzymes activities by the different *Trichoderma spp* after one and 12 months of storage. The results accentuated the production of higher amounts of chitinase and protease by *T. harzianum* when inoculated in peanut compost. It reached 0.34 and 0.55 µg/g dry weight and folded up to 0.38 & 0.56 µg/g dry weight, respectively, after one and 12 months of storage in peanut compost. On the other hand, sole peat/vermiculite formulation of *T. harzianum* revealed the chitinase and protease production were not exceeding 0.23 and 0. 12µg/g dry weight and 0.09 and 0.09 µg/g dry weight, respectively, at the end of the one and 12 months of storage. The same trend hold true for cellulase production in peanut compost by different *Trichoderma spp.* The highest enzymes was found by *T. hamatum* in compost by 0.30 &0.40 µg/g dry weight compare with peat and vermiculite by 0.24 &0.12 µg/ g dry weight; respectively at the end of one and 12 months of storage. For β –glactosidase and β -1, 3-glucase, the highest enzymes activity was conferred in peanut compost in compared with peat. Also, compost yield higher amounts of β –glactosidase and β -1,3-glucase by other *Trichoderma spp*. After 12 months of incubation in compost, a height enzymes was visible by *T. hamatum* and *T. harizanum*.

Results of antibiotics and total phenols assay are as illustrated in Table III. Gliotoxin, glioviridni and trichodermine antibiotics as well as total phenols were more frequent in peanut compost formulation until 12 months compared to a sole peat and vermiculite of the fungi. A much higher concentratoin of trichodermine and gliotoxin antibiotics were found to be produced by *T. harzianum* in peanut compost. It reached 0.387 and 0.122 µg/g dry weight and increased to 0.489 and 0.330 µg/g dry weight, after one and 12 months of storage in peanut compost, respectively. On the other hand, sole peat / vermiculite formulation of *T. harzianum* revealed the antibiotics production were not exceeding 0.130 and 0.012 µg/g dry weight and 0.036 and 0.021 µg/ g dry weight at the end of the one and 12 months of storage, respectively. Meanwhile, the highest amounts of antibiotics gliovirin were found to be produced by *T. hamatum* in peanut compost untill 12 months. It reached 0.38 µg/g dry weight and folded up to 0.46 µg/g dry weight, respectively after one and 12 months of storage in peanut compost. On the other hand, sole peat/vermiculite formulation of *T. hamatum* revealed that antibiotics production were not exceeding 0.25 and 0.18 µg/g dry

Table I. Chemical analysis of compost prepared from peanut plant haulms

Constituents	Organic matter (%)	Organic- C (%)	N (%)	P (%)	NH_4-N (%)	NO_3-N (%)	Organic-N (%)	Humus (%)	C/N Ratio
Compost	50.40	30.40	2.5	0.15	0.2	0.0015	2.37	21.4	12.80

Table II. Morphological characteristics of *Trichoderma spp*. after one and twelve months of inoculated in peanut waste compost and peat/vermiculite.

Characteristics	*T. harzianum*				*T. hamatum*				*T. koningii*			
	Peat/vermiculite		Peanut compost	waste	Peat/vermiculite		Peanut compost	waste	Peat/vermiculite		Peanut compost	waste
	Months after inocubation											
	One	Twelve	One	Twelve	One	Twelve	One	Twelve	One	Twelve	One	Twelve
Conidiospores												
- number*	10.2±2.3*	2.1±0.6	14.3±1.2	11.3±1.4	8.0±0.6	2.0±0.3	11.6±1.7	8.3±0.4	11.3±1.2	4.3±0.4	19.4±2.4	13.4±3.1
-diam> 2.5 µm	2.6±0.2	0.8±0.2	3.3±0.4	9.3±1.0	2.8±0.7	0.7±0.1	3.6±0.4	10.5±0.8	4.6±0.5	2.3±0.3	8.3±0.8	14.4±3.5
Chlamydospores												
-number	2.3±0.9	5.3±0.8	2.6±0.6	35.2±5.2	2.1±0.2	5.0±0.5	2.3±0.3	27.3±3.4	2.3±0.3	5.3±1.0	2.3±0.5	30.2±4.6
- diam.> 10 µm	0.3±0.2	2.1±0.5	0.6±0.2	22.3±2.4	0.3±0.1	2.0±0.4	0.3±0.03	20.1±3.2	0.3±0.1	2.3±0.6	0.6±0.02	21.5±4.3
Colony diam >8mm on MEA medium	1.8±0.3	0.70±0.01	2.0±0.4	5.3±0.8	2.1±0.4	0.1±0.03	2.2±0.2	4.8±0.7	3.4±0.7	1.1±0.6	3.6±0.7	5.0±0.7
Biomass	40.3±4.5	54.3±4.6	43.2±5.6	742.5±15.6	36.5±4.6	53.0±4.7	41.5±3.3	453.0±11.2	42.5±5.7	48.2±4.9	34.2±6.5	635.2±15.3
Viability (%)	96.6±6.3	42.3±4.1	96.2±7.6	95.0±5.8	95.1±8.4	32.3±4.6	95.3±6.5	91.2±8.3	97.2±7.7	53.2±6.3	97.4±9.8	94.1±11.8
Microbial activity (µg FDA min^{-1} g^{-1} D.M.)	2.11±0.4	1.74±0.3	5.23±1.2	5.53±0.6	1.46±0.5	0.87±0.3	4.32±0.4	4.65±0.7	2.03±0.5	1.06±0.4	4.48±0.7	5.21±0.8

Each value is the mean ± S.E. of six replicates; * Number of *Trichoderma spp*. in log ^{5} cfu /g compost.

Table III. Chemical characteristics of *Trichoderma spp*. after one and twelve months of inoculated in peanut waste compost and in peat

Compost	Month after inocubation	Hydrolytic enzymes					Antibiotics*			Total phenols
		Chitinase	Protease	Cellulase	ß-glactosidase	ß-glucanase	Trichodermine	Gliotoxin	Gliovirin	
		Concentration (µg / g dry weight)								
Peat/vermiculite + *T. harzianum*	One	0.23	0.12	0.18	0.17	0.18	0.130	0.012	0.017	0.013
	Twelve	0.09	0.09	0.07	0.10	0.07	0.036	0.021	0.08	0.011
Peanut compost + *T.harzianum*	One	0.34	0.55	0.22	0.21	0.22	0.387	0.122	0.024	0.037
	Twelve	0.38	0.56	0.24	0.25	0.25	0.489	0.33	0.022	0.039
Peat/vermiculite + *T. hamatum*	One	0.12	0.10	0.24	0.29	0.24	0.05	0.034	0.25	0.010
	Twelve	0.05	0.07	0.12	0.15	0.12	0.08	0.023	0.18	0.012
Peanut compost + *T. hamatum*	One	0.24	0.33	0.30	0.56	0.30	0.09	0.051	0.38	0.027
	Twelve	0.26	0.41	0.40	0.32	0.40	0.28	0.063	0.42	0.030
Peat/vermiculite + *T. koningii*	One	0.21	0.09	0.20	0.15	0.20	0.12	0.011	0.010	0.011
	Twelve	0.08	0.07	0.10	0.09	0.12	0.20	0.008	0.09	0.010
Peanut compost + *T. koningii*	One	0.31	0.31	0.25	0.16	0.25	0.30	0.031	0.026	0.016
	Twelve	0.34	0.36	0.39	0.26	0.39	0.42	0.064	0.075	0.015
Untreated control	One	nd	0.04	0.05	0.02	0.03	nd	nd	nd	0.009
Peat/vermiculite	Twelve	nd	0.03	0.11	0.05	0.08	nd	nd	nd	0.009
Untreated control	One	nd	0.10	0.12	0.06	0.05	nd	nd	nd	0.013
Peanut compost	Twelve	nd	0.13	0.15	0.09	0.12	nd	nd	nd	0.015

*Antibiotics and total phenols were quantified by HPLC analysis and normalized to the dry weight of the collected sample. nd = not

weight; at the end of one and 12 months of storage, respectively. For phenols production, all *Trichoderma spp*. were found to be capable of producing high detectable phenol content in peanut compost during one month of storage and increased after 12 months of storage. On the other hand, peat/vermiculite formulation of *Trichoderma spp*. displayed the lowest level of phenols. A higher increase of phenol was attained by *T. harzianum*.

Biological Activity

Pot experiments. Effects of peanut compost fortified with *Trichoderma spp*. on disease incidence and *Fusarium* counts under artificial infested soil with *F. oxysporum* are summarized in Table IV. The results show that, the wilt disease incidence was significantly lower when peanut compost added to the soil (22.5%) as compared with that amended with peat/vermiculite (58.2%) and unamended control (69.7%). At the same time, a progressive decrease in the disease was evident with peanut compost fortified with *Trichoderma spp* amendment. A lower percentage of wilted plants was recorded in peanut fortified with *T. harzianum* (1.0%) than in peanut compost and *T. harzianum* in peat/vermiculite (15.6%). Also, wilted plants were significantly lower in peanut fortified with *T. koningii* (2.3%) than in *T. koningii* in peat/vermiculite (17.5%).

Similarly, the number of *Fusarium* propagules in soil rhizosphere was significantly lower in soil amended with peanut fortified with *Trichoderma spp*. in comparison with then unamended compost and untreated control. Amendment of soil with peanut compost fortified with either *T. harzianum* or *T. koningii* caused a significant reduction in the number of propagation of *F. oxysporum* by (1.0 & 1.3 cfu x 10^3 g^{-1} soil) compared with unamended by (14.3 & 16.9 cfu x 10^3 g^{-1} soil), respectively. However, amendment with compost fertified with *T. hamatum* also had a significant reduction in the propagules of *F. oxysporum*. At the same time, amendment of peanut compost in soil resulted in greater population counts of *Trichoderma spp*. in soil rhizosphere. The greatest increase was observed for *T. koningii* (515.5 cfu x 10^3 g^{-1} soil) and *T. harzianum* (494.8 cfu x 10^3 g^{-1} soil) in comparison with unamended compost (33.7 & 26.3 cfu x 10^3 g^{-1} soil), respectively.

Field trails. The effects of peanut compost fortified with *Trichoderma spp*. on disease incidence and *Fusarium* counts under natural infested soil with F. oxysporium are reported in Fig. 1. In The control soil, percentage of disease plants increased during the experiment and reached 40.3 and 44.0% at the end of six months of growth in both seasons

Table IV. Influence of peanut compost fortified with *Trichoderma spp*. on cumin wilt disease incidence and *Fusarium* count in soil infested with *Fusarium oxysporum*.

Treatments	Wilt disease (%)	Number of propagules g^{-1} dry soil (cfu x 10^3)	
		Fusarium	Trichoderma
Peat/vermiculite + *T. harzianum* + *F. oxysporum*	5.4	14.3	26.3
Peanut waste compost+ *T. harzianum* + *F. oxysporum*	0.1	1.0	494.8
Peat/vermiculite + *T. hamatum*+ *F. oxysporum*	9.2	17.2	18.2
Peanut waste compost+ *T. hamatum*+ *F. oxysporum*	2.6	2.0	301.2
Peat/vermiculite + *T. koningii*+ *F. oxysporum*	7.5	16.9	33.7
Peanut waste compost+ *T. koningii*+ *F. oxysporum*	0.3	1.3	515.5
Peat/vermiculite + *F. oxysporum*	28.2	27.2	0.0
Peanut waste compost+ *F. oxysporum*	10.5	19.2	0.0
F. oxysporum	49.7	28.0	0.0
Untreated control	19.3	0.0	0.0
LSD (5%)	5.4	4.8	14.5

Table V. Plant growth, yield and nitrogen content of cumin plants cultivated in reclaimed sandy soil amended with peanut waste compost fortified with *Trichoderma spp*.

Treatments	2003				2004			
	Plant height (cm)	Dry weight (g)/plant	Plant yield (g)	Nitrogen content %	Plant height (cm)	Dry weight (g)/plant	Plant yield (g)	Nitrogen content %
Peat/vermiculite + *T. harzianum*	39.3	5.11	1.18	0.47	41.3	5.23	1.20	0.46
Peanut waste compost+ *T. harzianum*	49.8	5.96	1.94	0.60	51.3	5.98	1.99	0.62
Peat/vermiculite + *T. hamatum*	36.1	5.10	1.08	0.45	39.5	5.24	1.10	0.45
Peanut waste compost+ *T. hamatum*	42.5	5.73	1.78	0.55	45.2	5.84	1.87	0.57
Peat/vermiculite + *T. koningii*	36.8	5.11	1.10	0.45	39.2	5.23	1.21	0.47
Peanut waste compost+ *T. koningii*	44.8	5.84	1.77	0.58	47.2	5.88	1.84	0.59
Peat/vermiculite	31.6	4.99	0.84	0.40	33.3	4.97	0.97	0.41
Peanut waste compost	35.5	5.32	0.98	0.48	35.1	5.10	1.02	0.49
Untreated control	28.5	4.89	0.83	0.35	30.2	4.88	0.95	0.36
LSD (5%)	5.3	0.41	0.28	0.09	5.5	0.46	0.30	0. 10

Table VI. Relative percent of the main constituents of the volatile oil of cumin Plants cultivated in reclaimed sandy soil amended with peanut waste compost fortified with *Trichoderma spp*.

Treatments	Seeds oil content (%)	Main constituents of the volatile oil					
		α-pinene	β-pinene	Phellandrene	Limonene	γ-terpinene	Cumin aldehyde
Peat/vermiculite + *T. harzianum*	0.60	59.2	32.1	41.2	17.2	10.2	40.2
Peanut waste compost+ *T. harzianum*	0.84	198.2	179.2	141.0	89.2	92.0	197.2
Peat/vermiculite + *T. hamatum*	0.58	42.3	21.0	40.2	11.2	10.2	38.2
Peanut waste compost+ *T. hamatum*	0.75	154.3	153.2	117.2	57.2	89.3	179.0
Peat/vermiculite + *T. koningii*	0.55	47.2	30.2	36.2	11.2	9.3	38.2
Peanut waste compost+ *T. koningii*	0.80	197.2	153.3	134.2	63.2	83.2	187.3
Peat/vermiculite	0.45	25.7	13.2	14.3	11.6	0.63	11.8
Peanut waste compost	0.49	71.0	61.2	47.2	23.2	19.3	54.3
Untreated control	0.42	20.2	11.5	13.6	11.5	0.68	10.6

(Fig. 3). The disease incidence was decreased by peanut compost fortified with *Trichoderma spp*. to 19.5 and 20.3% in comparison with peat/ vermiculite by 25.2 and 29.3% in both seasons, respectively. The reduction in the disease incidence was maximum in peanut compost fortified with *T. harzianum*, that cause significantly the highest ability to delayed disease incidence to four months and reduced wilt disease to 0.6% compared with 8.8% and 9.3 when fortified in peat/vermiculite in both seasons, respectively. Also, peanut compost fortified with either *T. koningii* or *T. hamatum* could manage to delay disease incidence to 4 months and reduced the wilt disease to (0.8%) and (1.6 & 2.6%) compared to (11.3 & 13.2%) and (9.8 & 10.8%) when fertified in peat/ vermiculite in both seasons, respectively. Addition of peanut compost to the soil decreased the initial populations of *Fusarium* (18.3 cfu x 10^3 dry soil) as comparison with untreated control (28.6 cfu x 10^3 dry soil) (Fig. 2). Addition of compost fortified with *Trichoderma spp*. in soil, resulted in high decreasein the population counts of *Fusarium*. Peanut compost fortified with *T. harzianum* was found most effective in reducing the inoculum of the fungus pathogen after 6 months of sowing (2.4 cfu x 10^3 dry soil) in compared with peat/vermiculite (9.5 cfu x 10^3 dry soil). Also, peanut compost fertified with either *T. koningii* or *T. hamatum* was effective in reducing *Fusarium* counts to 3.0 and 3.2 cfu x 10^3 dry soil) as compared with peat/vermiculite (12.3 & 13.5 cfu x 10^3 dry soil), respectively. Overall, *Trichoderma* population was greatly increased during the growth periods when fortified in peanut compost than in peat/vermiculite (Fig. 3). The greatest increase was observed for *T. koningii* (1423.3 cfu x 10^3 dry soil)and *T. harzianum*(861.2 cfu x 10^3 dry soil) in comparison with peat/ vermiculite (325.3 and 49.2 cfu x 10^3 dry soil), respectively.

Growth parameters and yield of cumin plants as affected by peanut compost fortified with *Trichoderma spp*. are shown in Table V. Soil amendment with peanut compost significantly increased plant height, dry weight, nitrogen content and yield in both seasons when compared with non-amended soil. Soil amended with peanut compost fortified with *Trichoderma spp.* resulted in the longest plants, maximum dry weight and yield (g/plant) in both seasons. The highest growth and yield were achieved with peanut compost fortified with *T. harzianum* followed by *T. koningii* and *T. hamatum*. Application of peanut compost fortified with *Trichoderma spp*. positively promoted the oil content (%) of cumin plants compared with untreated control (Table VI). Addition of peanut compost to soil increased oil content in seeds. The best results were obtained in the soil amended with compost fortified with *T. harzianum* followed by *T. koningii.* Gas liquid chromatographic analysis of essential oil of cumin seeds revealed that soil amendes with peanut compost and fortified with *Trichoderma spp.* also, increased oil composition compared with untreated control. Data also exhibited the highest effect in increasing the percentage of the main compound, cumin aldehyde when

Fig. 1. Influence of peanut compost fortified with *Trichoderma spp.* Amended to reclaimed sandy soil on the incidence of *Fusarium* wilt of cumin

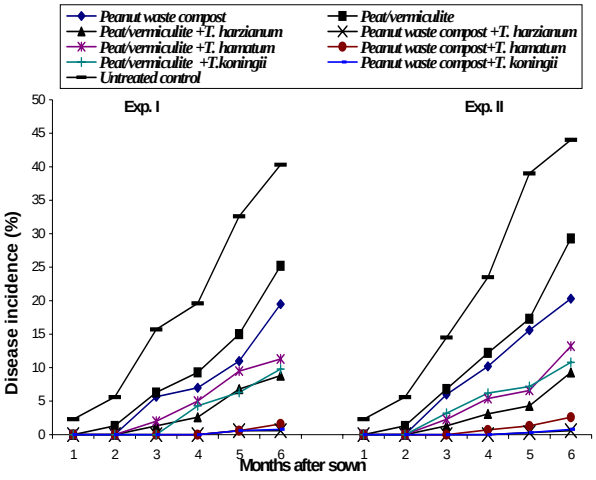
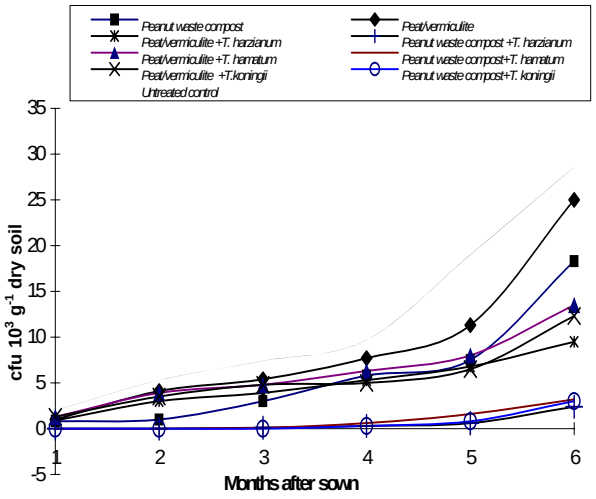


Fig. 2. Population dynamics of *Fusarium oxysporum* in soil rhizosphere of cumin plants cultivated in reclaimed sandy soil amended with peanut waste compost fortified with *Trichoderma spp.*



used peanut compost fortified with *T. harzianum, T. koningii* and *T. hamatum*, respectively. The same trend was also found in other compounds as compared with uninoculated compost. Still, addition of compost fortified with *T. harzianum* in soil resulted in strong increased oil compounds. Similar results have been achieved by *T. koningii* in fortified peanut compost.

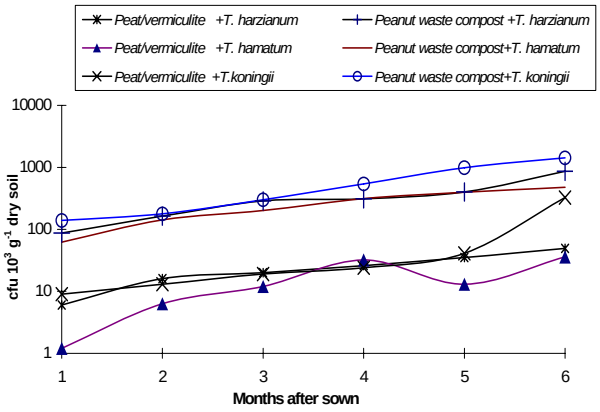
DISCUSSION

Development of agricultural production is more important for several countries than sustainability, rational

use of resources or even environmental quality (Saber, 2000). He also added that clean farming directly given rise to enriching the soil colloidal fraction through composting and heavy organic manuring Reports on the fertilizer effect of compost have been published by many authors (Abo Sedera, 1995; Mekki *et al*., 1999; Badr El-Din & Abo-Sedera, 2001). The compost not only provided an environment favorable for plant development (Mekki *et al*., 1999) but also offered suitable conditions for plant protection and stimulation of biocontrol agent activity (Haggag & Saber, 2000; 2001). *Trichoderma* species can act as biocontrol agents (BCAs) through different synergistic mechanisms. However, it is difficult to predict the degree of synergism and the behavior of a BCAs in a natural pathosystem. Considering that environmental conditions are important, the right selection of BCAs, with a potential characterization, is equally important (Hermosa *et al*., 2000). This latter point was evident in the results of the present study where it was observed that the use of peanut haulms compost as a carrier of *Trichoderma spp.* imptoved different morphological and chemical characteristics. These characteristics, have served to establish biocontrol agents, which can be related to different levels of biological activity and be directed against pathogen. Population densities of bioagents did not decline in the compost after being stored for 12 months. The presence of high population of chlamydospores may be of primary importance for survival of the biological antagonists in the soil and proved to be of use for establishing *Trichoderma* in this study. The production and establish ment of chlamydospores of *Trichoderma* are characteristics that have been used by other worken (Samuels, 1996) was show that even without additional energy sources the basic composted organ. Mineral carrier has a good physicochemical properties and sufficient nutrients to ensure the survival, viability and perforation of fungi (Hermosa *et al*., 1999; Haggag & Saber, 2000; Saber, 2000; Hoitink *et al*., 2001a & b). The rate of hydrolysis of fluorescein diacete (FDA) is another parameter to assay the microbial activity of antagonistic microorganisms in compost. Microbial activity was positively influence with compost amendment, which is very high after 12 months of storage.

Also, our results, using HPLC and spectrophotometer, indicated that after 12 months, the treatment with peanut waste compost showed higher metabolites production such as antibiotics (gliotoxin, gliovirin and trichodemin) , phenols and hydrolytic enzymes (chitinase, protease, cellulase , β – galactosidase and β -1,3-glucase) compared with cocedr. This high metabolic activity was indicative of high microbial activity, further supporting the idea that *Fusarium* establishment was inhibited. A direct relationship between antagonist capacity and enzymatic activities of *Trichoderma spp*. has been reported previously by Godwin and Arinze (2000), and Haggag and Saber (2000) that the levels of hydrolytic enzymes and antibiotics produced by *T. harzianum* differ when different substrates and composts are involved.

Fig. 3. Population dynamics of *Trichoderma spp.* in soil rhizosphere of cumin plants cultivated in reclaimed sandy soil amended with peanut waste compost fortified with *Trichoderma spp.*



Under greenhouse and field conditions, the use of peanut waste compost as a carrier of *Trichoderma spp.* significantly control cumin wilt disease incidence. Soil amendment with compost fortified with *Trichoderma spp*. resulted in a significant increase in the number of colony forming units produced by BCAs and decreased the pathogen count in general. The pronounced effects of compost carriers in controlling wilt disease in cumin might be ascribed to the well furnished medium for the proliferation of *Trichoderma spp.* and decreased the pathogen .Hoitink *et al*. (2001a & b) notes that success or failure of any compost treatment for disease control depends on the nature of the raw product from which it was prepared, the maturity of the compost, as well as the compost quality that contain disease-suppressive organisms such as beneficial microorganisms (*Trichoderma, Gliocladium spp. , Bacillus spp., Pseudomonas spp*., and others).

At the same time plant growth, yield, seeds oil content and its compounds increased compared to untreated control under field condition. Cumin aldehyde, the main compound of oil was increased when cumin plants grown in soil amended with peanut compost as a carrier of *Trichoderma spp*. The result also revealed that peanut compost fortified with *T. harzianum* exhibited the highest effect in decreasing the disease incidence and increasing plant growth and yield as well as oil content. Thus, it could be concluded that peanut compost have effectively reduced the disease incidence and /or could possibly be due to enhanced activity of biocontrol agents providing antagonism to pathogen and /or decomposition products of compost being favorable for the multiplication of the inoculum (Abbasi *et al*., 2002)

The presented results support the use of morphological, physical and biochemical characteristics for

the selective choice of the carrier for biocontrol agents activities to induce consistent levels of inhibition to pathogen. In conclusion, the study indicated that 12 months after fortified the peanut compost, microbial activity was enhanced leading to improved biological control activity against *Fusarium*. Therefore, incorporation of organic compost fortified with *Trichoderma spp*. products to agricultural soils not only benefits morphological and chemical soil characteristics, but also enhances plant growth.

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