Suppression of *Rhizoctonia* Damping-off of Cotton by Combining Some Fungal and Bacterial Isolates with Organic Composts

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ABSTRACT
Application of different types of agricultural and animal manure composts was tested for their efficiency to suppress *Rhizoctonia solani* cotton damping-off incidence. Moreover, the effect of addition of some biocontrol agents to these types of composts on their efficiency was also investigated. The results revealed various inhibition response of *R. solani* linear growth *in vitro* with tea composts (30-70% growth reduction). The horse manure compost tea was proved to be the most effective one in suppressing growth of *R. solani* followed by sheep manure compost. In addition, mature composts were more effective than immature ones. The greenhouse studies showed that horse manure compost was the most effective one in reducing the disease incidence followed by sheep manure compost, whereas both of wheat straw and mushroom compost showed the least control values (infection % were 21.44, 27.33, 29.63 and 40.31%, respectively). The improvement in disease suppression was achieved by introducing one or more of the biocontrol agents for each compost. Animal manure composts highly suppressed the disease. Addition of *Trichoderma harzianum*, to the compost types resulted in higher performance for the composts compared with their separate application, where infection % were 12.91, 18.49, 21.02 and 40.16%, for horse manure, sheep manure, mushroom and wheat straw compost, respectively. The results also showed significant improvement of disease suppression by combining *Bacillus subtilis* in addition to *T. harzianum* for each compost (infection % were 10.38, 15.44, 17.82 and 36.68%, respectively). In general, the raw compost applications were more effective than tea compost applications on reducing the incidence of *Rhizoctonia* damping-off of cotton seedlings.

Different forms of infection cushions of *R. solani* were obtained and proved to be varied from simple to very complicated shape with different combinations of biocontrol agents and tested composts. The least values of the frequency of complicated IC types were obtained with the application of wheat straw and spent mushroom compost. Microscopic examination indicated that the penetration of the pathogen through open stomata and formation of early stage of lobate appressorium on the inoculated cotton seedling hypocotyls were observed on the first horse of inoculation. Cross sections of cotton hypocotyls, after several hours of inoculation, showed different behaviors of *R. solani* throughout the colonization of the layers of hypocotyls cotton seedlings. Complete destruction of epidermal layer and all cortical cells were clearly showed after 96 hours of inoculation.

INTRODUCTION
Cotton seedlings are vulnerable to attack by a number of soil-borne pathogens, including *Rhizoctonia solani* Kuhn (Rothrock, 1996), which can be quite serious in the world (Brown and McCarter, 1976) and often results

in a substantial stand loss. The widespread use of chemicals has become a subject of public concern and scrutiny, mainly due to their harmful effect on environment, human and animal health (Zaki *et al.*, 1998).

Biocontrol with beneficial microorganisms seems to be a promising approach to managing cotton seedling damping-off (Howell, 1982; Hagedorn *et al*, 1989; Lewis and Papavizas, 1991). Among the promising biocontrol agents are *Trichoderma* spp. and *Gliocladium virens* in the field (Lewis and Papavizas, 1991; Cliquet and Scheffer, 1996), *G. virens* (Howell, 1982), *Pseudomonas fluorescens* (Howell and Stipanovic, 1979), *Bacillus cereus* (Pleban *et al.*, 1995) in the greenhouse. *Burkholderia cepacia* (Mcloughlin *et al.*, 1992), *Bacillus subtilis* (Asaka and Shoda, 1998). A nonpathogenic binucleate *Rhizoctonia* (Villajuan *et al.*, 1996) have been reported to suppress *R. solani* induced damping-off in other crops in the greenhouse.

Composted organic material such as plant debris and animal manure add nutrient to the soil thereby increasing the soil fertility. This improves plant growth and makes the plant less prone to infection by pathogens (Muhammad *et al.*, 2001 and Ayodele, 1997). Schueter (1989) found that various types of agricultural/municipal wastes suppress different types of soil-borne plant diseases by making plants more vigorous and better able to withstand attack. Muhammad *et al.* (2001) also observed that sawdust composted soil reduced incidence of seedling blight of *Parkia biglobosa* caused by *Fusarium solani* ranged from 30% to 74.2%. While rice-husks composted soil reduced the incidence of wilting of *P. biglobosa* caused by *F. solani* ranged from 31.4% to 70.3%.

This study was, therefore, undertaken to : (i) examine the effects of different types of waste composts on suppression of pre-, and post-emergence damping-off of cotton caused by *R. solani* in vitro and in greenhouse; (ii) observe the infection cushions of *R. solani* formed in relation to disease suppression in potting mixes amended with composted soil treated with the different biocontrol agents; (iii) investigate histological reactions in hypocotyls of cotton seedlings treated with different combinations of composts and the tested biocontrol agents

MATERIALS AND METHODS
Compost and compost tea preparation
The composts used in these experiments were spent mushroom, sheep manure, horse manure compost and chicken manure compost. The substrates of the different compost used were prepared according to standard protocols (Wuest, 1992). Raw compost materials used with peat were retained in production for 1 to 4 months for maturation. The quantities

of compost needed for particular applications were mixed with water (1:2 w/v, compost/water) in plastic containers and incubated without agitation in the laboratory or a storage shed (15 to 25° C) for 7 to 8 days. After incubation, the containers contents were stirred and the compost extracts were obtained by filtration through a single layer of cheesecloth.

Isolation and inoculum preparation

R. solani isolate

Isolation trials were carried out from diseased hypocotyls and root samples of cotton seedlings. Developing fungal isolate was picked out, subcultured several times, purified using hyphal tip technique. The purified fungal isolate was maintained on PDA slants. Identification was carried out according to Parmeter and Whitney (1970). Inocula were prepared by growing the tested isolate of R. solani on PD medium in 250 ml conical flasks, each containing 50 ml of the medium. Inoculated flasks were incubated at 27° C for one week, after which fungal mats were collected, blended with tap water and used as inocula at rate of one mycelial mat/pot (8 cm diameter).

Source of bioagents isolates

The isolates of T. harzianum, Bacillus subtilis and Pseudomonas fluorescens as biocontrol agents used in this study were kindly obtained from the Research Institute of Phytopathology, Ministry of Agric., Giza, Egypt.

Trichoderma isolate preparation

T. harzianum isolate used in this study was prepared according to Hadar et al. (1979) by autoclaving a wheat bran and tap water mixture (1:2 v/v) for one hr a day at 121° C on two successive days. Flasks containing this medium were inoculated with T. harzianum, incubated for 3 days at 30o C, illuminated for 12 hrs and then incubated for 5 days. A preparation of T. harzianum was used at the rate of 8 g/kg soil.

Bacterial isolates preparation

The bacterial bioagents used in this study were grown on nutrient broth medium in flasks 25 ml. Bacterial suspension concentration applied was 10^{-7}/ml.

Effect of compost on infection cushions morphology

In order study the role of composts tested (sheep manure, spent mushroom, and wheat straw compost) amended with tested biocontrol agents on the types of infection cushions developed by R. solani as a response of root exudates, plastic pots 8 cm diameter, containing autoclaved sandy loam soil were infested with R. solani isolates. The tested treatments included the study of the following interactions:

1. *R. solani* + compost.
2. *R. solani* + *T. harzianum* + compost.
3. *R. solani* + T. *harzianum* + *B. Subtilis* + *compost*.
4. *R. solani* + T. *harzianum* + *B. Subtilis* + P. *fluorescens* compost.
5. *R. solani* + *T. Harzianum* (control 1).
6. *R. solani* (control 2).

Cotton (*Gossypium barbadense*) cv Giza 83 seed samples were obtained from the Agriculture Research Center, Giza, Cairo, and used throughout this study. According to the method of El-Samra *et al.* (1981) to study the morphology of the infection cushions, one week later, 8 cotton seedlings, one week old were wrapped in cellophane sheets, and transplanted in the infested pots. Seedlings were, then irrigated every other day for a period of one week. Cellophane sheets were, then lifted and outer surface, facing the soil of each sheet was gently cleaned using soft brush. Areas of (2 X 4 cm) of each sheet were cut, at random, stained with lactophenol cotton blue, mounted in glycerine-gel on glass slide and examined microscopically for the incidence of the infection cushions. Infection cushions were categorized according to the complexity of branching and size as defined by El-Samra *et al.* (1981). Frequency of each type of infection cushions was estimated as a mean percentage of cushions per microscopic field for each treatment.

Greenhouse experiments

In order to determine the efficacy of different types of composts and compost extracts (i.e., mushroom ; sheep ; horse; wheat compost) alone or plus some biocontrol agents (*Trichoderma* sp., *Pseudomonas* sp., *Bacillus* sp.) to suppress cotton seedlings damping-off, the soil treatments were as follows:

a. Pots infested with *Rhizoctonia solani* and inoculated with compost or compost extracts (tea compost).
b. Pots infested with *R. solani* and inoculated with compost or tea compost and *T. harzianum*.
c. Pots infested with *Rhizoctonia solani* and inoculated with compost or with tea compost; *T. harzianum* and *Bacillus subtilis*.
d. Pots infested with *Rhizoctonia solani* and inoculated with compost or with tea compost; *T. harzianum*; *Bacillus subtilis* and *Pseudomonas fluorescens*.
e. Pots infested with *R. solani* (control 1).
f. Pots infected with *R. solani* and inoculated with *T. harzianum* (control 2).

Cotton Seeds of (Giza 83) were planted in pots of 25 cm diameter (10 seeds/pot). At the end of expermints (2 weeks), pre- and post-

emergence damping-off of pots for all treatments were detected.

Effect of compost and compost tea on the growth rate of *R. solani*

Composts were stored in a shed for different time of incubation and the compost extract (tea) were obtained at the different degree of incubation periods according to the method described above. The compost extract (tea) were centrifuged at 4000-5000 rpm for 15 min and then sterilized by Seitiz filter. The filtration of compost extract was used to determine the effect of filtrate compost (10 ml/100ml medium) on the growth rate of *R. solani.*

100 ml hand-warm medium with compost tea was filled in 5 Petri plates (9 cm diameter). Three Petri plates were inoculated each with four disks (8 mm diameter) of fungal mycelium taken at the edge of an active colony incubated at 22°C. The inoculated medium was also poured into plates as a control. The average diameter growth was measured after 4-7 days.

Histopathological investigations

(A) Microtome sections technique

Cotton seedlings hypocotyls of Giza 83 cultivar at the age of two weeks after planting, were inoculated with *R. solani.* Serial microtome sections were prepared on cotton hypocotyls (5-8 mm) after 24, 48, 72 and 96 hours of inoculation. The technique used in this study, described in detail by Waked (1979), is considered a modification of the procedures recommended by Berlyn and Miksche (1976); Brooks *et al.* (1950) and Sass (1958). Sections were examined using a bright field microscope. Results of extensive microscopic observations of different stages of infection were presented in many selected photographs.

(B) Stripping technique

In order to investigate changes directly occurred after inoculation with *R. solani*, hand stripping of the inoculated hypocotyl epidermis was also made, fixed and, then stained. Strippings were examined using a bright field microscope and photographed.

Statistical analysis

Statgraphic package was used for analysis of variance (ANOVA) to evaluate the effect of different types of compost and some biocontrol agents or their combinations on controlling of *R. solani* damping-off disease. Fisher's least significant difference (LSD) was used to compare the means. Results with a different letter are significantly different from each other for $\alpha=0.05$ (Fry, 1993). LSD = t * [(2 * Error Mean Square)/repetitions]$^{1/2}$.

RESULTS AND DISCUSSION

1. Compost suppressiveness of *R. solani* growth *in vitro*

Different composted materials were tested for the suppression of *R. solani*, Cotton damping-off agent (Table 1). Several composts were consistently suppressive to *Rhizoctonia* damping-off in laboratory bioassays. The data indicated that horse manure compost tea can be considered as the most effective compost in suppressing growth of *R. solani* as well as sheep manure compost, while the others were less effective. The tea compost prepared from *Untricularia* sp. was completely non effective against *R. solani*. Higher reduction in growth rates were, generally, observed by increasing the maturity of compost. Data in Table 1 also indicated that cultural filtrate of *Trichoderma* significantly inhibited the growth of *R. solani*. It was also found that hyphal density of *R. solani* was affected more than radial growth. The data obtained from these tests were carefully analyzed and used to select optimal protocols for the greenhouse trials.

Mature composts conferred suppressiveness to *R. solani* to tea compost added to PDA medium. Radial growth of *R. solani* was significantly decreased in the substrates with 30 to 70% of the long-matured (4 months) in case of horse manure compost. However, short-matured (1 month) compost even stimulated pathogen growth. This stage was preceded by an initial suppressiveness of the freshly delivered compost. These results on long-matured compost are in line with those obtained on horse manure compost (Schüler *et al.*, 1989), Sheep compost (Nelson *et al.*, 1983) and chicken compost (Kuter *et al.*, 1988). The effect in this *in vitro* test was smaller than that in the bioassay. *In vivo*, parasitism might have been involved or the antagonistic potential based on the other interactions is not fully expressed in the agar ring method.

Mature composts are more effective than immature ones in the suppression of soilborne pathogens, especially *Pythium* and *Rhizoctonia* spp. (Chen *et al.*, 1988; Boehm and Hoitink, 1992; Cabrera and Beare, 1993). This may be due to the high level of microbial activity that induce intense microbial competition leading to microbiostatis and disease suppression (Boehm *et al.*, 1997).

2. Improving biological activity of composts to suppress disease incidence

In order to maximize and improve compost suppressiveness, the biocontrol agents such as *B. subtilis*, *P. fluorescens* and *T. harzianum* were added each individual or in combination with each compost to infested soil with *R. solani*. In general, the biocontrol agents had a beneficial effect when

Table (1): Effect of maturity of tea composts on suppression of *R. solani* linear growth.

Age of compost (week)	Linear growth (cm/24 hrs)						L.S.D $_{0.05}$
	Compost tea type						
	HCT	SCT	MCT	WCT	CCT	UCT	
4 weeks	1.00*	1.20	1.60	1.95	1.00	2.0	0.42
6 weeks	0.90	1.00	1.50	1.90	1.96	1.90	0.47
8 weeks	0.82	0.90	1.40	1.80	0.90	1.85	0.47
12 weeks	0.66	0.84	1.35	1.75	0.90	1.80	0.49
20 weeks	0.60	0.75	1.30	1.75	0.88	1.80	0.52
Control							
a) Untreated				2.9			
b) Treatment with cultura filtrate of *Trichoderma* spp.				1.2			
L.S.D $_{0.05}$	0.14	0.09	0.08	0.10	0.06	0.10	

Whereas:

HCT: Horse compost tea.   SCT: Sheep compost tea.
MCT: Mushroom compost tea.   WCT: Wheat compost tea.
CCT: Chickens compost tea.   UCT: *Untricularia* Sp. ورد النيل
* Values are average of three replicates.

applied with compost for suppresion damping-off of cotton caused by *R. solani*. Table (2) shows the influence of two forms of sheep compost (raw and tea compost) on the reduction of cotton damping-off incidence and in the presence of fungal and bacterial biocontrol agents. The data clearly indicated that *Rhizoctonia* cotton damping-off highly suppressed in soil amended with sheep manure compost (27.55%) than those amended with tea compost (38.60%), compared with disease incidence in soil nonamended with compost (83.33%) and nonamended soil treated with *T. harzianum* (41.14%). The data also indicated that sheep manure compost amended soil and infested with *Rhizoctonia* and mixed with the biocontrol agents were varied in their affect. The disease infection percentage was reduced when *T. harzianum* was added (18.49% with raw compost and 31.05% with tea compost). Addition of *B. subtilis* to the previous mixture, highly enhanced disease suppression (15.44% for raw compost and 22.97% for tea compost). Whereas, the addition of the antagonistic bacteria *P. fluorescens* to compost plus *T. harzianum* increased *Rhizoctonia* cotton seedlings suppression (15.68 and 22.77%) more than *T. harzianum* (21.02 and 31.05% for both raw and tea compost) apart. The addition of *P. fluorescens* to the mixture plus *B. subtilis* did not significantly enhance the disease suppression in case of raw compost, while the infection percentages were 17.5% and 25.13% for both raw and tea compost

Table (2): Influence of biocontrol agents in different combinations with sheep manure compost on infection percentage (PI).

Treatments	Raw compost		PI*** (%)	Tea compost		PI (%)
	Pre	Post		Pre	Post	
	emergence			emergence		
R** + compost	14.80*	12.75	27.55	20.80	17.80	38.60
R + compost + T	12.80	5.69	18.49	13.80	15.83	29.63
R + compost + T + B	8.22	7.22	15.44	10.22	11.75	22.97
R + compost + T + P	8.82	6.82	15.68	10.13	12.64	22.77
R + compost + T + B + P	9.75	8.91	17.5	14.33	10.80	25.13
Control	Pre-emergence		Post-emergence		PI (%)	
R (alone)	45.83		37.50		83.33	
R + T	19.76		10.38		41.14	
L.S.D $_{0.05}$	2.15	2.10	2.05	4.43	2.46	5.51

* Values are average of three replicates.
** R= R. solani   T= T. harzianum   B = B. subtilis;   P = P. fluorescens
*** PI% = percentage of infection

Statistically significant effects of two forms of spent mushroom compost were occurred and appeared in suppressiveness to *Rhizoctonia* damping-off shown in Table (3). Results indicated that both forms of spent mushroom compost highly enhanced their biological activities when mixed with other biological control agents in different combinations and reflected as a good suppression of *R. solani* on cotton seedlings. The data clearly indicated that *Rhizoctonia* cotton damping-off highly suppressed in soil amended with sheep manure compost (29.63%) than that amended with tea compost (31.58%) in comparison with infested nonamended soil (83.33%) and nonamended soil treated with *T. harzianum* (41.14%). Data also showed that spent mushroom composts amended soil, infested with *Rhizoctonia* and mixed with the biocontrol agents varied in their effiency.

The disease infection percentage showed a satisfactory disease suppression when *T. harzianum* was added (21.02% with raw compost and 29.63% with tea compost). On the other hand, adding *B. subtilis* to the previous mixture highly enhanced the disease suppression by 17.82% for raw compost and 26.6% for tea compost. Addition of the antagonistic bacteria *P. fluorescens* to the compost along with *T. harzianum* increased *Rhizoctonia* cotton seedlings suppression (18.74%) over than *T. harzianum* (21.02%) apart. Addition of *P. fluorescens* to the compost mixture along with *B. subtilis* did not significantly enhance the disease suppression in

case of raw compost (20.63%), compared to that of *T. harzianum* added to spent mushroom compost (21.02%). Addition of all bioagents to compost tea led to relatively higher infection values (20.63%).

Table (3): Influence of biocontrol agents in different combinations with spent mushroom compost on infection percentage (PI).

Treatments	Raw compost		PI (%)	Tea compost		PI (%)
	Pre-	Post-		Pre-	Post-	
	emergence			emergence		
R** + compost	15.83*	13.80	29.63	14.75	16.83	31.58
R + compost + T	11.80	9.22	21.02	15.22	15.83	31.05
R + compost + T + B	10.60	7.22	17.82	12.80	13.80	26.60
R + compost + T + P	9.83	8.91	18.74	11.26	14.12	25.48
R + compost + T + B + P	8.80	11.83	20.63	15.83	14.8	30.63
Control	Pre-emergence		Post-emergence		PI (%)	
R (alone)	45.83		37.50		83.33	
R + T	19.76		10.38		41.14	
L.S.D $_{0.05}$	3.85	2.11	3.59	3.05	2.34	3.95

* Values are average of three replicates.
** R= *R. solani*   T= *T. harzianum*   B = *B. subtilis*;   P = *P. fluorescens*
*** PI% = percentage of infection

Amendment of horse manure compost induced high suppressiveness for *Rhizoctonia* damping-off cotton disease. Moreover, suppression was highly enhanced by the addition of the tested biocontrol agents (Table 4). Infection percentages were 21.44, 23.02 and 41.14% for raw compost, tea compost and *T. harzianum*, respectively, in the absence of compost, compared with infested nonamended soil (83.33%). The presented data showed that the disease highly suppressed in soil amended with horse manure compost when biocontrol agents were added. Addition of *T. harzianum* to compost significantly reduced infection % (12.91% with raw compost and 17.49% with tea compost). Moreover, addition of *B. subtilis* to the previous mixture highly enhanced the disease suppression by 10.38% for raw compost and 18.60% for tea compost. Addition, of the antagonistic bacteria *P. fluorescens* to the compost in the presence of *T. harzianum* has improved *Rhizoctonia* damping-off suppression (13.65% and 15.56% for raw and tea compost). Data obtained also indicated that the highest values of disease suppression were recorded in soil amended with horse manure compost along with the all tested biocontrol agents (9.91% and 12.63% for both raw and tea horse manure compost, respectively).

Table (4): Influence of biocontrol agents in different combinations with horse manure compost on infection percentage (PI).

Treatments	Raw compost		PI** (%)	Tea compost		PI (%)
	Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
R** + compost	8.69*	12.75	21.44	13.80	9.22	23.02
R + compost + T	5.22	7.69	12.91	10.80	6.69	17.49
R + compost + T + B	5.69	4.69	10.38	8.80	9.80	18.60
R + compost + T + P	5.90	7.75	13.65	8.22	7.32	15.56
R + compost + T + B + P	5.22	4.69	9.91	6.83	5.8	12.63
Control	Pre- emergence		Post-emergence		PI (%)	
R (alone)	45.83		37.50		83.33	
R + T	19.76		10.38		41.14	
L.S.D $_{0.05}$	4.72	3.85	4.57	2.87	2.40	3.37

* Values are average of three replicates.
** R= R. Solani, T= T. Harzianum, B = B. Subtilis, and P = P. fluorescens
*** PI% = percentage of infection.

Results of straw wheat compost and tea compost (Table 5) showed a significant reduction cotton damping-off incidence compared with that of control (45.13, 53.83 and 83.33%, respectively). Treatment of composted soil infested with *R. solani* and *Trichoderma* spp. induced a pronounced reduction in percentages of infection (38.13%, 50.25% for both straw and tea compost, respectively).

Addition of the bacterial bioagents *B. subtilis* with *T. harzianum* reduced PI% for both raw (36.68%) and tea compost (40.29%), whereas the addition of the antagonistic bacteria *P. fluorescens* to the compost plus *T. harzianum* increased *Rhizoctonia* cotton seedlings suppression (30.55%) over than *T. harzianum* (40.16%) apart. The addition of *B. subtilis* and *P. fluorescens* enhanced the biological activity of tested composts in addition to *Trichoderma* spp, where infection % were 30.55 & 32.58% for compost and tea compost, respectively. Generally, application of compost alone or along with other bioagents in the present study, significantly suppressed *R.* damping-off of cotton. Similar results were obtained on the suppression effect of different types of compost on different pathogens and crops (Lumsden *et al.*, 1983; Dick and McCoy, 1993; Hoitink and Grebus, 1994;; Hoitink and Boehm. 1999; Aryantha *et al.*, 2000 and Diab *et al.*, 2003).

Application of the three biocontrol agents to maximize the performance of composts not only resulted in better suppression of *R. solani* but also reduced the variability of disease control. In the present study, the three biocontrol agents were applied under constant conditions in combination with composts and we tested the hypothesis that using multiple interactions between the tested biocontrol agents and compost. Data obtained proved that application of a mixture of bioagents in suppressing R. damping-off was markedly more effective than that of each

biocontrol agent alone. These findings were in line with those of Guetsky *et al.* (2001 & 2002) who recommended the application of several biocontrol agents simultaneously, provided that they possess different ecological requirements for survival, growth, and activity as one important strategy to imporove efficacy and reduce variability of biological control. Most of the studies dealing with biocontrol mechanisms focused on single biocontrol agent and single mechanism of disease suppression. Nevertheless, there are a few examples of biocontrol agents exhibiting more than one mechanism of control (Bèlanger *et al.*, 1995).

From the obtained data, long-mature compost was more effective in suppressing R. damping-off of cotton than the less mature compost. These findings were consistent with those of Tuitert *et al.* (1998), who found a higher population density of bioagent in long-mature compost. Sheep manure compost in both applied forms proved to be highly effective in suppressing R. damping-off in the present study. Diab *et al.* (2003) successfully controlled many vegetable diseases caused by soil-borne pathogens by the application of sheep manure compost.

The suppressive effect of compost and other tested bioagents obtained in our research could be explained on the basis of antibiosis, parasitism and competition (Arras and Arru, 1997). However, individual specific mechanisms may characterized individual biocontrol agents (Guetsky *et al.*, 2002).

Accordingly, application of many biocontrol interactions among the tested bioagents and compost throughout the present work was carried out in order to get full use of the additive activities of such bioagents, in which several means of disease suppression are applied concurrently. When one or more means/mechanisms is not effective, the others may compensate for the former absence.

Table (5): Influence of biocontrol agents in different combinations with wheat straw compost on cotton damping-off infection percentage (PI).

Treatments	Raw compost			Tea compost		
	Pre	Post	PI[***] (%)	Pre	Post	PI (%)
	emergence			emergence		
R[**] + compost	28.33[*]	14.80	45.13	31.50	28.33	53.83
R + compost + T	20.75	17.38	40.16	38.13	23.49	50.25
R + compost + T + B	19.41	17.27	36.68	20.80	23.49	40.29
R + compost + T + P	13.80	16.25	30.55	18.60	21.83	35.43
R + compost + T + B + P	16.75	13.80	30.55	16.75	19.83	32.58
Control	Pre-emergence		Post-emergence		PI (%)	
R (alone)	45.83		37.50		83.33	
R + T	19.76		10.38		41.14	
L.S.D $_{0.05}$	4.01	2.95	2.99	3.25	3.40	4.21

[*] Values are average of three replicates.
[**] R= R. Solani, T= T. Harzianum, B = B. Subtilis, and P = P. fluorescens
[***] PI% = percentage of infection.

3. Infection cushions (IC) induction in relation to compost and some bioagents treatments

This study aimed to determine the frequency of infection cushion (IC) types induced by *R. solani* as response to the presence of the tested biocontrol agents (*T. harzianum, B. subtilis* and *P. fluorescens*) and as affected with the tested different types of compost (manure compost). Therefore, cotton seedlings (Giza 83) were used as a test host. Types of infection cushions were determined using the illustrated scheme recorded by El-Samra *et al.* (1981).

A. Treatment of soil inoculated with *R. solani*

The formation of more simple types of infection cushion (IC) with little or no formation of more complicated IC types was clearly showed. The formation of simple IC types (tree secondary branches-like structure) was more frequently (Fig. 1-A, B and C), with a little formation of early stage of complicated forms (Penicillium-like structure) (Fig. 1-D).

B. Treatment of soil infested with *R. solani* and amended with *T. harzianum*

The formation of early and middle stage of complicated types of IC was clearly showed (Fig. 2-A &B). Little induction of simple IC types was noticed. In general, *T. harzianum* enhanced the formation of complicated types.

C. Treatment of soil infested with *R. solani* and amended with compost and *T. harzianum*

The addition of sheep and horse manure compost to the inoculated soil with the pathogen amended with *T. harzianum* enhanced and improved the formation of very complicated types of IC (Fig. 2-C & D). Table (6) clearly indicated that the frequency of complicated types of IC formed was 75% in the treatment of *R. solani* and *T. harzianum*. The data also showed that the application of the tested compost amended with soil infested with *R. solani* played a great role on disease suppression which resulted in induction of more complex IC types. The application of compost in the soil infested with *R. solani* and amended with some bioagents increased the suppressive effect against *R. solani* and induced the formation of complicated types of IC. Data recorded in Table (6) indicated that the high values of complicated types of IC were recorded when sheep and horse manure compots were applied (85 & 80%, respectively). The least values of complicated IC types were obtained on the application of wheat straw and spent mushroom compost (45 & 55%, respectively).

From results presented in Table 6, it could be concluded that the addition of microbial bioagents to all different types of compost enhanced the pathogen to form the complicated types of IC. On the other hand, complicated IC types tended to be formed more frequently in the treatment of compost amended with both *T. harzianum* and *B. subtilis*. These findings were similar to the data obtained by El-Faham and Aboshosha (1987), El-Farnawany (1991) and (1996) who found that the more compatible the host-isolate interaction, the more simple type of infection cushions formed. Moreover, El-Samra *et al.* (1981) found simple infection cushions were more commonly formed on cotton cultivars susceptible to *R. solani*, whereas more complex forms predominated on resistant cultivars.
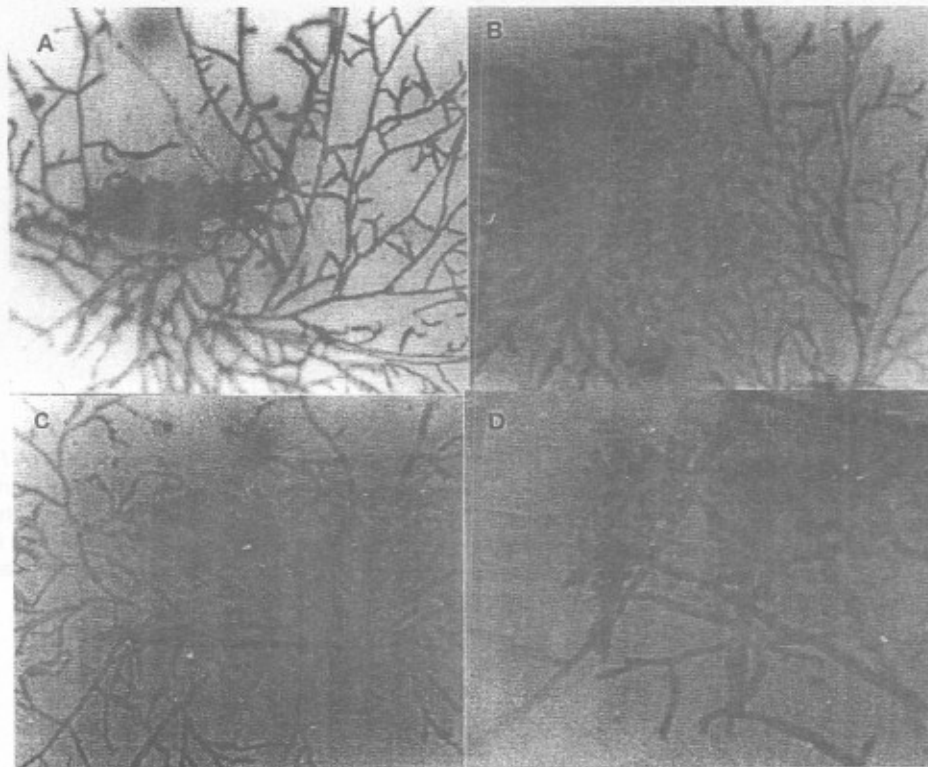

Figure (1): Light microscopy micrograph of different forms of infection cushions (IC) induced in soil infested with *R. solani*. (A), (B) and (C) simple types of infection cushions (tree secondary branches like structure) (TSB). (D) Early stage of compact form of IC (*Penicillium* like structure).
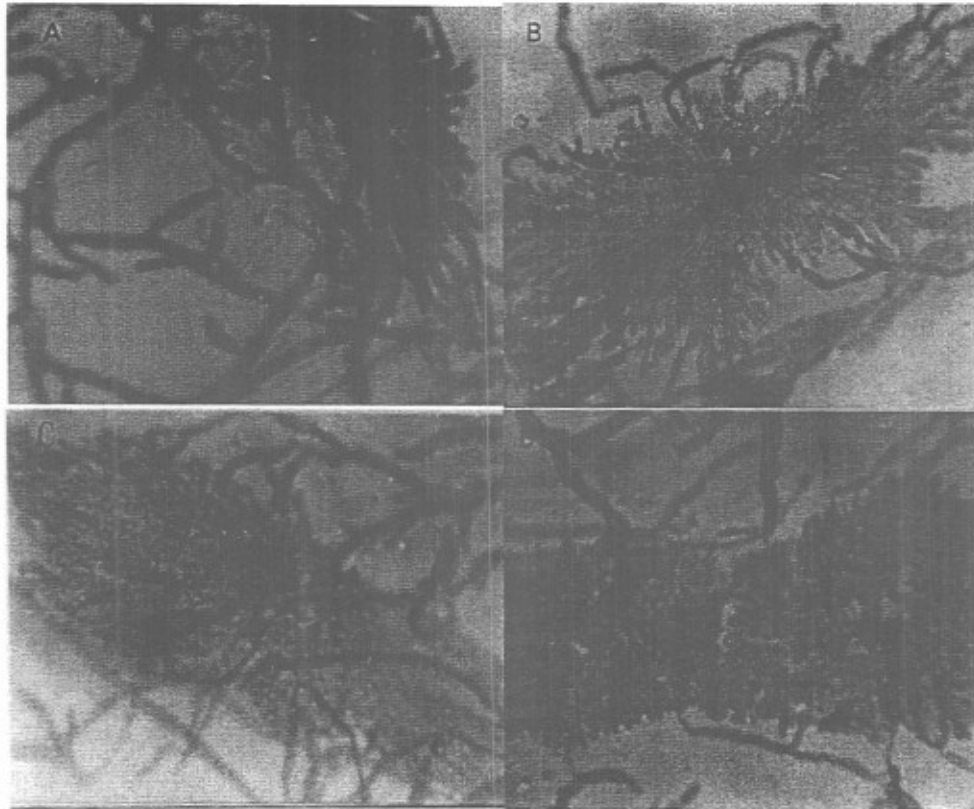
Figure (2): Light microscopy micrograph of different forms of infection cushions (IC) of *R. solani* induced in soil infested with *R. solani* and amended with *T. harzianum*. (A) Early stage of compacted type of IC; (B) Spider like structure SLS) a middle stage of the complicated type of IC; (C) and (D) are the complicated type of IC of *R. solani* formed in presence of sheep manure compost. Notice the type of IC (D) was more complicated than the type of (CA).

Table (6): Effect of different types of compost on the frequency of infection cushions (IC) types (%) induced by *Rhizoctonia solani* as a response to root exudates of cotton seedlings (Giza 83).

Types of compost	Frequency of infection cushions (%)				
		Complicated forms			
	Simple forms (SF)	Early complicated form (ECF)	Complicated form (CF)	Very complicated form (VCF)	Total
Sheep compost					
R**+compost	15.0*	25.0	35.0	25.0	85
R+T +compost	15.0	35.0	30.0	20.0	85
R+T+B+compost	10.0	15.0	50.0	25.0	90
R+T-	15.0	30.0	35.0	20.0	85
B+P+compost					
Horse compost					
R+compost	20.0	25.0	30.0	25.0	80
R+ T +compost	15.0	25.0	25.0	35.0	85
R+T+B+compost	10.0	30.0	20.0	40.0	90
R+T-B+P+compost	15.0	25.0	25.0	35.0	85
Wheat compost					
R+compost	55.0	15.0	15.0	20.0	45
R+ T +compost	25.0	30.0	20.0	25.0	75
R+T-	25.0	25.0	35.0	15.0	75
B+P+compost	25.0	25.0	30.0	20.0	75
Spent mushroom compost					
R+compost	45.0	15.0	25.0	15.0	55
R+ T +compost	20.0	20.0	30.0	30.0	80
R+T+B+compost	15.0	25.0	30.0	30.0	85
R+T-B+P+compost	20.0	30.0	20.0	30.0	80
Control					
R+T	25.0	20.0	25.0	20.0	75
R (alone)	65.0	15.0	5.0	15..0	35

* Values are average of three replicates.
** R = *R. solani*; T = *T. Harzianum*; B = *Bacillus subtilis*; and P = *P. Fluorescens*

4. Histology of cotton hypocotyls inoculated with *R. solani*

Stripping and cross sections of cotton seedlings (14 day-old) prepared 15 hours after inoculation with the pathogen showed extensive hyphal growth over the surface of the epidermis, but without the development of any lesions. However, slight discoloration was frequently observed on some areas of contact between the inoculum and the hypocotyls surface. Penetration through opening stomata was recorded (Fig. 3-A). The penetration hyphae grew over the surface of the hypocotyls toward the stomatal opening. This finding was in agreement with Christou (1962)and Dodman *et al.* (1968) on bean and Selim (1985) on cotton
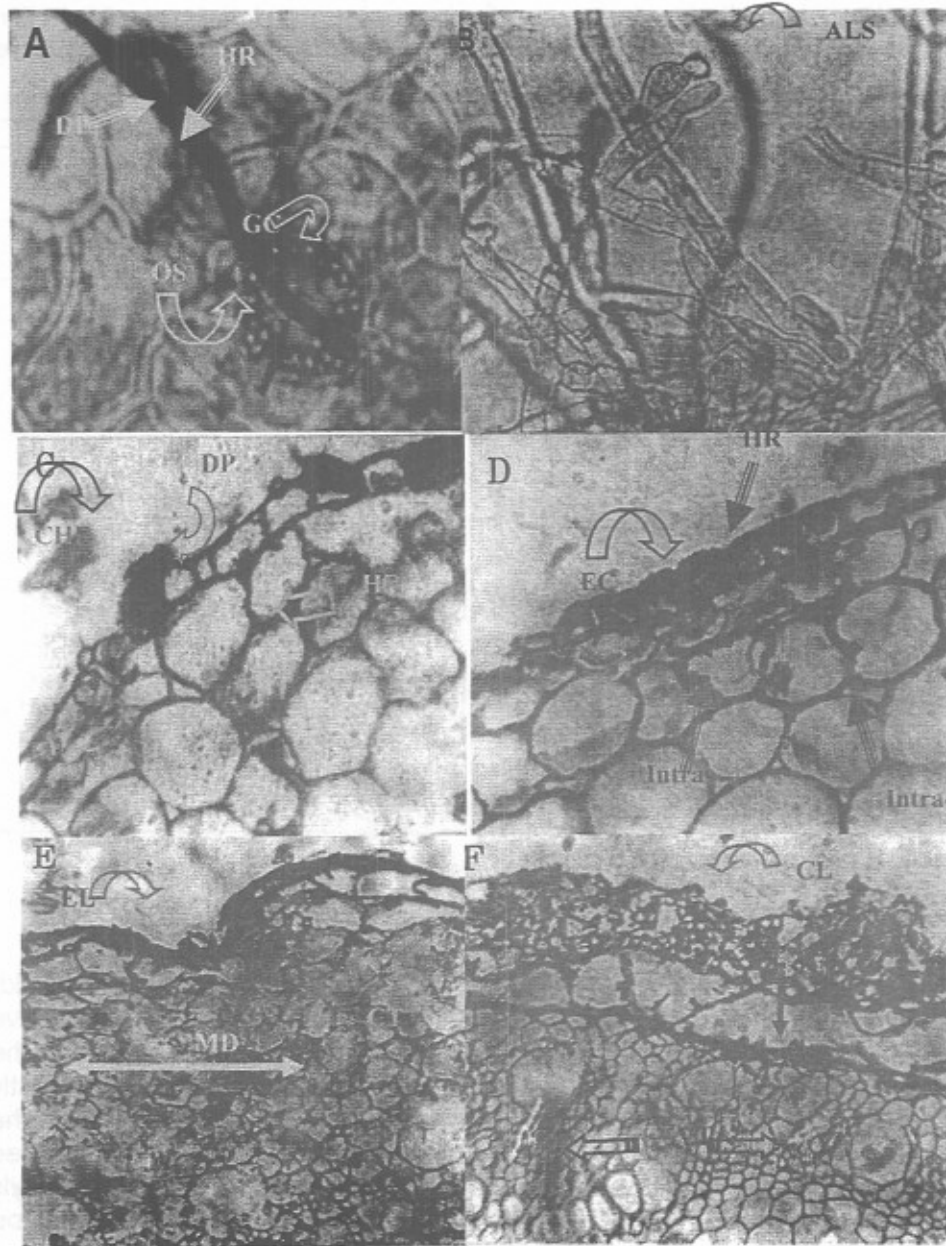
Figure (3) :

However, the latter authors considered stomata a secondary mode of penetration. Direct penetration occurred also through the formation of appressorium over the surface of cotton hypocotyl epidermis (Fig. 1-B). Formation of lobate appressorium laterally from the main hyphae was recorded also by Dodman *et al.* (1968) on bean and Marshall and Rush (1980) on rice. Dodman *et al.* (1968) concluded that lobate appressorium were commonly formed by the bean and cotton foliage isolates of *R. solani*.

Microscopic examination of cross sections of cotton hypocotyls 24 hours after inoculation showed hyphae growing over the epidermis layer forming compacted mycelium and then invaded the epidermal cells through direct penetration. Moreover, an active progress of the fungus through cortex both inter-, and intracellulary was also clearly showed (Fig. 3-C & D). After 48 hours of inoculation, damage was progressed on the epidermal layer and all cortical layers (Fig. 3-E). Complete destruction of epidermal layer and all cortical cells occurred after 96 hours of inoculation (Fig. 3-D). This confirms the finding of previous studies of *R. solani* on bean (Van Etten *et al.*, 1967; Dodman *et al.*, 1968; Kenning and Hanchey, 1980) and cotton (Selim, 1985). Ruppel (1973) found that invasion by *R. solani* was limited by the epidermis or the outer layers of secondary cortex in resistant sugar beet plants, but in susceptible plants, the fungus transected several vascular rings.

Figure (3) : Stripping of epidermal layers from cotton hypocotyls 15 hours following inoculation showing a long branched hyphae of *R. solani* grew toward the opening stomata (OS) – Notice that the stomatal guard cell appeared curved under the pressure of the branched hyphae (A). Direct penetration was also observed by growing of the hyphae for some distance to develop germination hyphae (GH) (B). Stripping of cotton hypocotyl 15 hours after inoculation showing the formation of appressorium-like structure (ALS). Cross section through cotton hypocotyls 24 hours after inoculation showing hyphal invasion of epidermal cells and subsequent inter-, and intracellular development through cortical layers (C & D). More damage was progressed on the epidermal layer and all cortical layers after 48 hours of inoculation (E). Cross section through cotton hypocotyls showing much damage of epidermal layer and all cortical layers (F). After 96 hours of inoculation complete destruction of epidermal layer and all cortical cells were occurred.

EC = Epidermal cells   DP = Direct penetration   GC = Guard cells
MD = Most distruction   CT = Cortical tissue   EI = Epidermal layer
VT = Vascular tissues   Inter- = Intercellular   Intra- = Intracellular
CHR = Complicated hyphae of *Rhizoctonia*   OS = Open stomata
HR = Hyphae of *Rhizoctonia*   ALS = Appressorium lik-structure

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الملخص العربــــى

زيادة فاعلية نشاط المكافحة الحيوية للكومبوست بإضافة بعض العزلات الفطرية و البكتيرية لمقاومة مرض الذبول الطرى الرزيكتونى فى القطن

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تم دراسة تأثير إضافة بعض أنواع من الكومبوست (المخلفات الزراعية والحيوانية) على مقاومة مرض الذبول الطرى الريزوكتونى فى القطن. بالإضافة إلى تأثير إضافة بعض مصادر المقاومة الحيوية لأنواع الكومبوست المختبرة لتحسين فعاليته فى وقف حدوث المرض. ولقد أوضحت الدراسة المعملية أن مستخلص كومبوست مخلفات الخيل ذات تأثير مرتفع فى تثبيط نمو ريزوكتونيا سولانى المسبب للمرض عند إضافته لبيئة النمو ويتبعه فى التأثير كومبوست مخلفات الأغنام ، حيث وصل معدل نقص نمو الفطر بصفة عامة إلى ٣٠-٧٠% كما زادت فاعلية الكومبوست بزيادة الفترة الزمنية لنضجه. أظهرت نتائج دراسة مقاومة المرض فى الصوبة الزجاجية أن مخلفات الخيول كانت أكثر أنواع الكومبوست فاعلية فى خفض نسبة إصابة المرض يتبعها كومبوست مخلفات الأغنام ، وكان كومبوست قش القمح ومخلفات زراعة عيش الغراب أقل القيم فى نسبة الإصابة (٢١,٤٤ ، ٢٧,٦٣ ، ٢٩,٦٣ ، ٤٠,٣١% على الترتيب). وكان وضحاً أن إضافة فطر التريكودرما هيرزيانم (فى بيئة الردة ونشارة الخشب) قد حسنت معنوياً من كفاءة أنواع الكومبوست المستخدمة (نسب الإصابة كانت ١٢,٩١، ١٨,٤٩، ٢١,٠٢، ٤٠,١٦% لكومبوست الخيول، كومبوست الأغنام ، كومبوست مخلفات زراعة عيش الغراب و كومبوست القمح ، على الترتيب)، بينما زادت الكفاءة بإضافة بكتيريا باسيلس سانلس مع فطر التريكودرما فى تقليل نسب الإصابة بالمرض حيث بلغت نسب الإصابة ١٠,٣٨ ، ١٥,٤٤ ، ١٧,٨٢ ، ٣٦,٦٨% ، على الترتيب. وبصفة عامة أظهرت الدراسة أن فاعلية الكومبوست الخام كانت أكثر قدرة على خفض نسب الإصابة بالمرض مقارنة بمستخلصات أنواع الكومبوست المختلفة.

أظهرت الدراسة تكون أشكال مختلفة من الوسائد الهيفية للمسبب للمرضى تتراوح مابين الشكل البسيط والشكل المعقد وذلك تبعاً للتوليفات المختلفة بين مصادر المكافحة الحيوية و أنواع الكومبوست المختبرة. وكانت أقل نسبة لتكون الأشكال الهيفية المعقدة مع كومبوست كل من مخلفات عيش الغراب وقش القمح ، بينما كانت أعلى نسبة لتكون الأشكال المعقدة فى حالة كومبوست مخلفات كل من الخيول والأغنام. كما أوضحت الدراسة الميكروسكوبية حدوث الإختراق من خلال الثغور المفتوحة وكذا تكوين أطوار مبكرة من عضو الإلتصاق (أبريسوريم) وذلك خلال الساعات الأولى من عدوى السويقة الجنينية السفلى لبادرات القطن المعداه بالفطر ريزوكتونيا سولانى. كما أظهر الفحص الميكروسكوبى مقدرة المسبب المرضى على الإتلاف الكامل لكل من طبقتى البشرة والقشرة بعد ٩٦ ساعة من الإصابة.