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Research Article

EFFECT OF FUNGAL BIOCONTROL AGENTS AGAINST SEED-BORNE PATHOGENS *IN VITRO* AND UNDER GREENHOUSE CONDITIONS

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ABSTRACT

The aim of this study was to check the efficiency of some biocontrol agents on controlling damping-off disease incited by the tested pathogens both in laboratory and under greenhouse conditions. Results obtained by radial growth technique indicated that all the tested FBA's significantly reduced growth of the seed-born pathogens. The highest reduction rates were obtained by the FBA *T. harzianum* (86.55%) followed by *T. viride* (81.33%). *F. semitictum* was the most sensitive compared with the other tested pathogens (79.77%), whereas *M. phaseolina* was the least sensitive to FBA treatments, where reduction rate attained (61.11%). *T. koningii* induced the lowest reduction rates (77.66%). Generally, treatment soil with the tested FBA's significantly reduced TIP values in cantaloupe cvs. *T. harzianum* was more efficient in reducing TIP than *T. viride* against all the tested seed-born pathogens, except with *F. moniliforme*. The highest reduction rates were detected in *T. harzianum* / *F. semitictum* in CREDO F1 treatment (81.81%). *F. solani* was the least sevsitive to FBA soil treatment, compared with the other tested cucurbit seed-borne pathogens. In CREDO F1 cv. Reduction rates ranged from 65.62% to 77.27%. Both of FBA's soil treatment significantly reduced PRD values. Moreover, *T. harzianum* treatment realized the highest PRD reduction rates ranged from 57.14% (*F. moniliforme*) in CREDO F1 cv. to 83.33% (*F. moniliforme*) in ISI 54139 F1 cv. In ISI54139 F1 cv., PRD reduction rates were 75.0% in *T. harzianum* / *F. semitictum* soil treatment to 83.33% in *T. harzianum* / *F. moniliforme*. *T. viride* was the most efficient in reducing PRD incited by *F. semitictum* with ANANAS cv., and *M. phaseolina* with ISI54139 F1 cv. (80.0%). Soil treatment with the tested FBA's, in general, significantly reduced PTD incidence. In addition, *T. harzianum* was more efficient in reducing PTD by *F. semitectum* (100%) in CREDO F1 cv., where reduction rates in *T. harzianum* / *F. moniliforme* and *F. semitictum* were 62.38% and 72.62%, respectively compared with control. In *T. harzianum* / *F. solani* or *M. phaseolina* treatments, equal rates were obtained (64.44%). *T. viride* was the most efficient in reducing PTD values in ISI 54139 F1 cv. inoculated with *F. moniliforme* (83.33%).

Keywords: *Fungal Biocontrol Agent, Green House, Trichoderma*

INTRODUCTION

Fungal biocontrol agents (FBA's) for plant diseases are currently being tested as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. Among the FBA commonly used are fungal agents those related to the genera *Trichoderma* spp.. More investigations are still required to study different aspects concerning application of fungal biocontrol agents against pathogens attacking cucurbitaceous crops, furthermore evaluation of the efficacy of different fungal FBA's, behavior of the antagonists against the target fungal pathogens and biochemical changes characterizing the antagonist-pathogen interactions, especially production of chitinases and changes host proteins. Therefore, the objective of this work was to evaluating the efficacy of some fungal biological control agents against cucurbit seed-borne pathogens both *in vitro* and under greenhouse conditions.

MATERIALS AND METHODS

In Vitro Experiments

The bioreaction between the Fungal Bioagents and the Tested Pathogenic Fungi: Three species of *Trichoderma* (*T. viride*, *T. hamatum* and *T. harzianum*) were kindly obtained from Plant Mycological

Center, Assiut University, Egypt to study their effect on growth and development of the tested cucurbits seed-borne pathogens (*F. solani, F. moniliforme, F. semitectum, S. sclerotiorum* and *M. phaseolina*). Petri-dishes (9 cm in diameter) each contains PDA medium were used. All *Trichoderma* spp. and/or tested pathogenic fungi were grown on PDA medium for 7 days at 25 ± 2°C and then two discs (4mm in diameter) of 7 days old *Trichoderma* spp. were placed at periphery of the plates then, one disc (4 mm in diameter) from each of the pathogenic fungi was placed at the center of each plate. In the control treatments, the plates were inoculated only with each of the pathogenic fungi. Three Petri dishes were used for each particular treatment. All inoculated dishes were incubated at 25ºC ± 2. After the pathogenic fungi almost covered the medium surface in control treatment, the main diameter of the pathogenic fungi was measured in each treatment and percentage of growth reduction due to the presence of different *Trichoderma* spp., Data were obtained or % of growth reduction was calculated according to the above mentioned formula.

In Vivo Experiments

Certified seeds of the tested cantaloupe cultivars were sterilized with dipping in 3% sodium hypochlorite for three minutes, rinsed for several times in sterilized water, then dried with filter paper. Inoculation tests were carried out in 12 cm diameter pots. Pots were sterilized by submerging in 7% formaldehyde solution for a few hours and left for aeration. The soil was autoclaved at 1.5 Kg/cm2 for 90 minutes, and then left to aerate for 7 days before adding the inoculum. Inocula were prepared by growing each of the tested pathogens (*F. solani, F. moniliforme, F. semitectum* and *M. phaseolina*) on PD medium in 250 ml conical flasks, each containing 50 ml of medium and incubated at 25^{o}C for 15 days. After 15 days incubation, fungal mats were collected, blended with sterile water. The inocula were used at rate of 3g/Kg autoclaved soil. On the other hand, *T. viride* and *T. harzianum* were grown on a wheat bran: sawdust: tap water (3 : 1 : 4 v/v), autoclaved for 30 minutes at 121^{o}C on 2 successive days (Hadar *et al*., 1979 and Elad *et al.*, 1981).

The medium was inoculated then incubated for 10 days. Different combinations from the inocula of the tested pathogens and the antagonistic bioagents were added to the infested soil at the rate of 5 g inoculum of bioagents per Kg soil. Surface sterilized cantaloupe seeds cultivars were sawn each in plastic pot (12 cm) containing 250 gm of infested soil and placed in the greenhouse at approximately 20^{o}C. Four replicates (4 pots) of each treatment were used. Four pots infested with the tested pathogen only and others untreated pots served as controls. Pre- and post-emergence damping-off were calculated out 14 days after planting. The following treatments represented different interactions between cucurbits seed-borne pathogens and biocontrol fungal agents were tested:

1. *F. solani* alone
2. *F. solani* and *T. viride*
3. *F. solani* and *T. harzianum*
4. *F. moniliforme* alone
5. *F. moniliforme* and *T. viride*
6. *F. moniliforme* and *T. harzianum*
7. *F. semitectum* alone
8. *F. semitectum* and *T. viride*
9. *F. semitectum* and *T. harzianum*
10. *M. phaseolina*alone
11. *M. phaseolina* and *T. viride*
12. *M. phaseolina* and *T. harzianum*

RESULTS AND DISCUSSION

Experimental Results

The aim of this investigation was to check the inhibitory effect of three fungal bioagents (*Trichodermaviride, T. harzianum* and *T. koningii*) against the five tested cucurbitaceous seed-borne pathogens, i.e. *F. solani*, *F. moniliforme*, *F. semitectum*, *M. phaseolina* and *S. sclerotiorum* both under laboratory and greenhouse conditions.

In Vitro Experiments

Bioreaction between the fungal bioagents and cucurbitaceous seed-borne pathogens in vitro

According to the method described in detail in the section of "Materials and Methods. Linear growth method was determined for all the tested cucurbitaceous seed-borne pathogens grown in Petri dishes along with the tested fungal bioagents. Data were then statistically analyzed, presented in Table 1.

According to the obtained data, generally, it was evident that all the tested fungal bioagents significantly reduced growth of mycelial growth of all the tested cucurbitaceous seed-borne pathogens. However, reduction rates differed according to the bioagent or the pathogen tested.

T. viride

T. viride in vitro was very effective in inhibiting the growth of all the tested pathogens. It was more effective against *S. sclerotiorum*, *F. solani* and *F. semitectum* (86.66%, 85.55% and 85.55%, less than control). Less inhibition rates were induced against *M. phaseolina* (64.44%). *F. solani* and *F. semitectum* were give equal rates (85.55%), while *M. phaseolina* the least sensitive to the effect of *T. viride*, compared with control (64.44%).

T. harzianum

The inhibitory efficacy of the bioagent *T. harzianum* against the tested pathogens was, to great extent, similar to that of *T. viride*. Both *F. solani* and *F. semitectum* were the most sensitive to *T. harzianum*, realizing higher reduction rates (87.77% and 87.22%, respectively) than obtained in *F. moniliforme* (84.44%). The equal rates were obtained in *S. sclerotiorum* and *M. phaseolina* (86.66%).

T. koningii

The inhibitory efficacy of the bioagent *T. koningii* against the tested pathogens was similar effect on all tested pathogens (ranged 81.66% to 84.44% less than control) except *F. solani*. *F. solani* was the most sensitive to *T. koningii*, realizing higher reduction rates compared with control (56.11%).

Therefore, from data obtained in Table 1 the following could be concluded:

- All tested fungal bioagents significantly reduced the mycelial growth of the tested tuber rot pathogens. Reduction rates compared with control ranged from 56.11% to 87.77%.
- Generally, *T. harzianum* was the most effective among the other tested fungal bioagents in suppressing the mycelial growth of all the tested cucurbitaceous seed-borne pathogens. Moreover, *F. solani* and *F. semitectum* were the most sensitive to *T. harzianum*, where reduction rates compared with control attained 87.77% and 87.22%, respectively.
- *T. viride* bioagent was more inhibitory against *S. sclerotiorum*, *F. solani* and *F. semitectum* (86.66%, 85.55% and 85.55%, less than control, respectively). *T. viride* was also significantly effective against growth of *F. moniliforme* and *M. phaseolina* but at less reduction rates (84.44% and 64.44%, respectively).

Table 1: Antagonistic Effect of some Fungal Bioagents on the Mycelial Growth of the Tested Cucurbitaceous Seed-Borne Pathogens

B.C.A	**Reduction %**					
	The Tested Pathogen					**Mean**
	F. Solani	***F. Moniliforme***	***F. Semitectum***	***S. Sclerotiorum***	***M. Phaseolina***	
T. viride	85.55*	84.44	85.55	86.66	64.44	81.33
T. harzianum	87.77	84.44	87.22	86.66	86.66	86.55
T. koningii	56.11	81.66	84.44	82.22	83.88	77.66
Control	0.00	0.00	0.00	0.00	0.00	0.00
Mean	76.48	83.51	85.74	85.18	78.33	

**Percentage of reduction in the mycelial growth.*

L.S.D. at 5% for:	*Bioagent (B)*	*Fungi (F)*
	1.48	*0.934*

In Vivo Experiments:

Efficiency of BCA's in controlling damping-off disease in greenhouse

This study aimed to check the effect of soil inoculation with different bioagents, namely: *T. viride*, *T. harzianum* for controlling pre- and post-emergence damping-off caused by some cucurbitaceous seed-borne pathogens, i.e. *F. solani*, *F. moniliforme*, *F. semitectum* and *M. phaseolina* on cantaloupe (CEREDO F1, ISI 54139 F1 & ANANAS cultivars) under greenhouse conditions.

In order to achieve such target, pots were inoculated individually with the four tested pre-emergence damping-off (PRD), post-emergence damping-off (PTD) pathogens under greenhouse conditions. The Two tested bioagents were applied through soil inoculation. Data were calculated as percentages, statistically analyzed, and then presented in Tables (2-5).

F. solani (Table 2):

Data in Table (2) indicate the following points:-

(1) Soil treatment with *T. harzianum* significantly reduced PRD incidence (68.75% to %77.77 less than control). The highest PRD suppression was detected in *T. harzianum* x ISI 54139 F1 cv. (77.77%), while, *T. viride* significantly reduced PRD incidence (60.00% to 77.45% less than control), The lowest PRD suppression was detected in *T. viride* x CEREDO F1 cv. (60.00%).

(2) PTD values were significantly higher, in general, compared with inoculatedcontrol; however, soil treatment with *T. harzianum*, since it induced the highest reduction in PTD incidence attaining maximum percentages in tested cantaloupe cultivars (60.00%-66.67% less than control). The most effective of *T. viride* treatment came next to *T. harzianum*, particularly on tested cantaloupe cultivars (33.33-66.67% less than control).

(3) In genaraly TIP values in control inoculated with *F. solani*, untreated with any oftested FBA's, i.e. *T. harzianum* and *T. viride*, ranged from (69.71% and 62.13%. respectively). Both FBA's treatments significantly reduced TIP of damping-off incited by *F. solani*. The highest reduction in disease incidence (75.00% in ISI 54139 F1 cv., compared with control) was detected by *T. harzianum*. While, the lower reduction in disease incidence (57.69% in ANANAS cv., compared with control) was detected by *T. viride*.

Table 2: Efficiency of some Biocontrol Agents on Controlling Damping-Off of Cantaloupe cvs., Incited by *F. Solani*

Treatment	Percentage of Seedling Infection (PSI)									Mean Values TIP
	Verities									
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
F. solani + *T. viride*	10.0	5.0	15.0	10.0	10.0	20.0	15.0	12.5	27.5	20.83
F. solani + *T. harzianum*	7.5	5.0	12.5	10.0	5.0	15.0	12.5	10.0	22.5	16.66
Control (1) (*F. solani* alone)	25.0	15.0	40.0	45.0	15.0	60.0	40.0	25.0	65.0	55.0
Control (2) (Untreated)	0.0	0.0	0.0	5.0	0.0	5.0	10.0	0.0	10.0	5.0
L.S.D	2.67	2.45	2.51	2.64	2.29	3.81	1.98	1.87	3.92	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

F. moniliforme (Table 3):

(1) PRD incited by *F. moniliforme* significantly reduced as a result of soil treatment with all the tested fungal biocontrol agents, compared with control. Soil treated with *T. harzianum* proved to be the most efficient treatment in reducing PRD incidence by 57.14%, 83.33% and 66.67% less than control in CEREDO F1, ISI 54139 F1 and ANANAS cultivars, respectively. Soil treatment with *T. viride* was also effective in controlling PRD in all tested cultivars, 71.43%, 75.00% and 75.00% less than control in CEREDO F1, ISI 54139 F1 and ANANAS cultivars.

(2) PTD values by *F. moniliforme* in the tested treatments. Significant reductions in PTD were obtained CEREDO F1 cv. by *T. harzianum*or *T. viride* (80.00%). On the other hand, ISI 54139 F1 cv. was responsed more actively by *T. viride* (83.33%). In ANANAS cv, significant reductions in PTD values were induced by *T. viride*or *T. Harzianum* (57.14%, less than inoculated control).
(3) Both FBA's treatments significantly reduced TIP of damping-off incited by *F. moniliforme*. The highest reduction in disease incidence by *T. viride* with ISI 54139 F1 and CEREDO F1 cvs, (77.78% and 75.00%, respectively, less than control) While, the lower reduction in disease incidence (53.33% in ANANAS cv., compared with control) was detected by *T. harzianum*.

Table 3: Efficiency of some Biocontrol Agents on Controlling Damping-Off of Cantaloupe cvs., Incited by *F. Moniliforme*

Treatment	Percentage of Seedling Infection (PSI)									Mean Values TIP
	Verities									
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	**PTD**	**TIP**	**PRD**	**PTD**	**TIP**	**PRD**	**PTD**	**TIP**	
F. moniliforme + *T. viride*	5.0	2.5	7.5	7.5	2.5	10.0	7.5	7.5	15.0	10.83
F. moniliforme + *T. harzianum*	7.5	2.5	10.0	5.0	7.5	12.5	10.0	7.5	17.5	13.33
Control (1) (*F. moniliforme*alone)	17.5	12.5	30.0	30.0	15.0	45.0	30.0	17.5	47.5	40.83
Control (2) (Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	5.0	1.66
L.S.D	2.41	2.67	2.83	2.54	2.62	3.44	2.88	3.11	3.97	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

F. semitectum (Table 4):
(1) All the tested FBA's against PRD in cantaloupe cvs., caused by *F. semitectum*, were significantly efficient in reducing PRD incidence attaining maximum reduction in *T. viride* with ANANAS cv., *T. harzianum* with ISI 54139 F1 and *T. viride* or *T. harzianum* with CEREDO F1 (80.00%, 75.00% and 66.67%, compared with control, respectively).
(2) All PTD values resulted from soil treatment of cantaloupe cvs. with the tested FBA's were significant compared with control. The highest reduction rate was obtained by *T. harzianum* with CEREDO F1 cv. (100%), follwed by, *T. viride* with CEREDO F1 cv. (80.00%), while, reduction of *T. harzianum* or *T. viride* with ISI 54139 F1 cv. was equal rate (75%). On the other hand, the lower reduction rate was obtained by *T. viride* with ANANAS cv. (28.57%).
(3) The highest reductions in TIP values disease incidence was induced by *T. harzianum*, *T. harzianum* significantly reduced TIP in CEREDO F1, ISI 54139 F1 and ANANAS cvs. (81.81%, 75%, and 52.94%, respectively), compared with control, whereas *T. viride* reductions were (72.72%, 66.67% and 58.82, respectively). In generaly, rates reduction TIP obtained by *T. harzianum* and *T. viride* (69.62% and 66.07% less than control, respectively).
M. Phaseolina (Table 5):
Data in table (5) suggested the following finding :-
(1) All the tested PRD values in all the tested FBA/cultivars combinations were significant, compared with control inoculated alone with *M. phaseolina*. Moreover, *T. harzianum* was the most effective in reducing PRD values,followed by *T. viride*, since the treatment induced the highest reduction in PRD values in ISI 54139 F1 cv. with *T. harzianum* or *T. viride*, CEREDO F1 cv. with *T. harzianum* and

ANANAS cv. with *T. harzianum* or *T. viride* (80.00%, 75.00% and 71.43%, less than control, respectively).

(2) All the tested PTD values in all the tested FBA/cultivars combinations were significant, compared with control inoculated alon with *M. phaseolina*. Significant reduction in PTD values were obtained as a result of soil inoculation with *T. harzianum* x CEREDO F1 cv. or ANANAS cv. or*T. viride* x CEREDO F1 cv. (66.67%).While, the lower reduction in PTD value was obtained by *T. viride* withISI 54139 F1 cv. (40.00%).

(3) Soil treatment with *T. harzianum* gave the most significant reductions TIP in CEREDO F1, ISI 54139 F1 and ANANAS cvs. (71.43%, 75.00% and 69.56% respectively less than control), followed by, *T. viride* (64.28%, 70.00% and 60.87% respectively less than control). In generaly, rates reduction TIP obtained by *T. harzianum* and *T. viride* (71.99% and 65.05% less than control, respectivelty).

Table 4: Efficiency of some Biocontrol Agents on Controlling Damping-Off of Cantaloupe cvs., Incited by *F. Semitectum*

Treatment	Percentage of Seedling Infection (PSI)									Mean Values TIP
	Verities									
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
F. semitectum + *T. viride*	5.0	2.5	7.5	7.5	2.5	10.0	5.0	12.5	17.5	11.66
F. semitectum + *T. harzianum*	5.0	0.0	5.0	5.0	2.5	7.5	10.0	10.0	20.0	10.83
Control (1) (*F. semitectum*alone)	15.0	12.5	27.5	20.0	10.0	30.0	25.0	17.5	42.5	33.33
Control (2) (Untreated)	5.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	1.66
L.S.D	1.92	2.14	2.61	2.30	2.46	2.72	2.22	2.63	2.96	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

Table 5: Efficiency of some Biocontrol Agents on Controlling Damping-Off of Cantaloupe cvs., Incited by *M. Phaseolina*

Treatment	Percentage of Seedling Infection (PSI)									Mean Values TIP
	Verities									
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
M. phaseolina + *T. viride*	7.5	5.0	12.5	7.5	7.5	15.0	10.0	12.5	22.5	16.66
M. phaseolina + *T. harzianum*	5.0	5.0	10.0	7.5	5.0	12.5	10.0	7.5	17.5	13.33
Control (1) (*M. phaseolina* alone)	20.0	15.0	35.0	37.5	12.5	50.0	35.0	22.5	57.5	47.5
Control (2) (Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	5.0	1.66
L.S.D	1.93	1.85	2.38	2.18	2.59	2.55	2.95	3.26	3.53	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

Discussion

In Vitro:

Fungal biocontrol agents (FBA's) *viz*., *T. harzianum, T. viride,* and *T. koningii* have significantly reduced linear growth of all tested fungi compared to control. In the present work, *T. harzianum* proved to be more effective in reducing growth of the tested damping-off pathogens *in vitro* than other FBA's followed by *T. viride* where they recorded the highest percentages of reduction. Growth of *Fusarium solani* was inhibited by *T. harzianum, T. viride*and *T. koningii* that corroborates well with the results reported by Akrami *et al*., (2012) and Dwivedi and Dwivedi (2012). Suppression in growth of *F. solani* by *T. harzianum, T. viride* and*T. koningii* during the present studies supports the findings of Haggag and El-Gamal (2012). The varying level of growth inhibition of *F. solani* by the antagonist might indicate the existence of the different mechanisms of antibiosis exhibited by different antagonistic. Abushaala (2008).

Resuls of experiments were conducted to evaluate the role of fungal bioagent which proved a good antagonistic activates against *F. moniliforme* under laboratory, *T. harzianum*wasmore effect followed by *T. viride,* however, the lowest reduction rate was obtained by *T. koningii*. Similar results were obtained by Abushaala (2008).

F. semitectum was the most sensitive among all the pathogens tested to FBA's. Antagonistic capability of *T. harzianum, T. viride,* and*T. koningii*, were tested *in vitro* against *F. semitectum*. The percentage of linear growth of the pathogen was recorded when its growth covered the plate surface in control treatment. Results of this study revealed that all tested FBA's inhibited growth of *F. semitectum*. *T. harzianum* and *T. viride* gave the greatest percentage of growth inhibition. Such results are in agreement with those reported by Rose *et al.,* (2004). *Trichoderma viride* (isolate no. 17), *T. harzianum* (isolate no. 19) and *Fusarium concolar* (isolate no.4) showed significant percentage of inhibition against *F. semitectum* (Sallam *et al.*, 2014). Antagonistic effect may be due to direct influence of antagonistic fungi against the pathogens through cloning their hyphae around the hyphae of the pathogens to prevent their continued growth **(**Chu and Wn, 1981; Adekunle *et al.,* 2006) and/or produce antagonistic substance which can play an important role in lyses of cell wall components of the pathogenic fungi to help the antagonists to penetrate the host hyphae and grown on it as hyper parasite (Papavizas *et al.,* 1984). Similar results have been reported by Mathew and Gupta (1998).

Sclerotinia sclerotiorum was high sensitive to tested BCA's, while decrease growth and not produce scloricia. FBA's were inhibited *in vitro* the mycelial growth of *S. sclerotiorum*. Similar results *in vitro* have been reported by Levy *et al.,* (2004), Rama *et al.,* (2004), Abdullah *et al.,* (2008) and Abushaala (2008). Mohamed and Gomaa (2001) reported that *Trichoderma harzianum* destroyed sclerotia of *Sclerotinia sclerotiorum* converting them to spores of *T. harzianum* within 12 days under laboratory conditions. *T. viride* isolated from decayed sclerotia caused a high level of decay in *S. sclerotiorum.* On the other hand, Levy *et al*., (2004) reported that biological control using *Trichoderma harzianum* isolate T39 controlled several foliar pathogens like *B. cinerea*, powdery mildews and *Sclerotinia sclerotiorum* by inducing the resistance locally or systemically through decreasing the reactive oxygen species (ROS) in uninfected leaf tissue or enhancing the formation of antioxidant enzymes as well as formation of phytohormones.

Growth of *Macrophomina phaseolina* was found to be inhibited by *T. harzianum*, *T. viride* and *T. koningii.* Similar results have been reported by El-Mougy and Abdel-Kader (2008), Abushaala (2008), Hajieghrari *et al.,* (2008), Bandopadhyay *et al.,* (2011) and Gajera *et al.,* (2012). Ramzan *etal.,* (2014) reported that, growth of *M. phaseolina* was inhibited by 11 fungi *viz., A. fusispora*, *Aspergillus flavus A. fumigatus, A. niger, Drechslera hawaiiensis, Emericella nidulans*, *Penicillium chrysogenum*, *P. citrinum, S. atra, T. harzianum* and *T. virens,* and sevenbacteria *viz*., *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, M. varians* and *P. fluorescens*. The growth inhibition of pathogen may be due to production of chitinase and B-1,3-glucanase enzymes which degrade the cell wall of the pathogens (Ahmed and Baker, 1987).

Trichoderma generate many enzymes that are used against cell walls of fungi to utilize the fragment of pathogens (Grosch *et al.,* 2006).

In Vivo:

Biocontrol agents (FBA's) including *T. harzianum, T. viride* and *T. koningii,* proved to be antagonistic to all the tested pathogens, both *in vitro* and *in vivo*. Many researches confirmed the significant role of FBA's in controlling damping-off diseases, particularly *T. harzianum* (Amer and El-Desouky, 2000; Elad *et al.,* 2000; Aly, 2005; Singh *et al.,* 2007 and Rojo *et al.,* 2007), *T. viride* (Hamed, 1999), *T. koningii* (Soltan, 1998). Most of these researches dealt with controlling damping-off diseases incited by *R. solani, F. solani, M. phaseolina, P. ultimum, A. alternata, S. sclerotiorum, F. oxysporum, Sclerotium rolfsii, Cladosporium* sp., *F. moniliforme* and *F. semitectum*. To improve biological control of the disease, antagonistic fungal isolates of *T. harzianum, T. viride* and *T. koningii,* with different carriers (talc based powder and wheat bran) were tested on incidence of cantaloupe damping-off caused by of the tested pathogens in greenhouse and field on conditions. Under greenhouse conditions, application of antagonistic FBA's one week before planting showed higher percentage of survival plant of pre and post emergence damping-off compared control. In pre emergence damping-off formulation of isolate *T. harzianum* gave the highest number of survival plants precentage followed by *T. viride*. Several researchers have reported that *T. viride* and *T. harzianum* were superior as antagonistic fungi against several soil and seed borne plant pathogens (Poddar *et al.,* 2004; Lee *et al*., 2008; 2011). The potentiality of *Trichoderma* spp. as biocontrol agents of phytopathogenic fungi in several crops is well known especially to *Fusarium* spp. The two primary mechanism of action associated with nonpathogenic *Fusarium* spp. are induced systemic resistance and competition for nutrients in the soil and parasitic competition for infection sites on the roots (Kaur *et al*., 2010). Under field conditions in the two growing seasons, applied formulations of antagonistic fungi into infested soil with *F. semitectum* at the time of planting showed the higher percentage of survival plants in the case of pre and post emergence damping-off than applied two weeks before planting (Sallam *et al.,* 2014). Such results agree with those reported by Lewis and Lumsden (2001). This may be due to that application of biocontrol formulations at the time of planting has avoided the spread of the pathogen in soil. Coley-Smith *et al.,* (1991) reported that formulations of biocontrol against soil-borne fungi were more effective when added at the time of planting compared to those applied two weeks before planting**.** Lewis *et al.,* (1998) reported that, the ability of a fangal biocontrol agent in formulation to inhibit the spread of the pathogen is perhaps more important than the effectiveness of the formulation to eliminate pathogen propagules evenly distributed in the soil. Also, application of *Trichoderma* spp. as powder formulation into soil provides nutrient sources for other soil microorganisms such as growth promoting rhizobacteria (Sallam *et al.,* 2008).

Extensive work was carried out on antagonistic mechanisms of *Trichoderma* spp. against phytopathogenic, including (i) competition through rhizosphere competence and replacement of endogenous fungi on the root surface, leading to their suppression and therefore mask their presence (Zangh *et al*., 1996; Harman, 2000 and 2001 and Howell, 2003), (ii) production of antibiotics such as gliotoxin (Lumsden *et al*., 1992; Wilhite *et al*., 1994 and Haraguchi *et al*., 1996) and gliovirin (Howell and Stipanovic, 1983), which inhibit acetolactate synthase, responsible of catalyzing the production of branched chain aminoacids, (iii) production of enzymes such as chitinase (De La Cruz *et al.*, 1992; Elad and Kapat, 1999 and Metcalf and Wilson, 2001), Glocanase (Migheli *et al.,* 1998), Chitinases and glucanases (Metcalf and Wilson, 2001 and Inglis and Kawchuk, 2002), Proteases (Kapat *et al*., 1998 and Sharon *et al*., 2001), (iv) acetaldehyde and other acidic volatiles (Dennis and Webster, 1971 a & b), (v) toxic compounds, such viridian, sesquiterpene, gliocladic acid, heptolidic acid, viridio and valinotricin (Turner, 1971 and Smith *et al*., 1990).

It is believed that, the modes of action of antibiotics and enzymes against pathogens are scarcely clarified. A better understanding of the mode of action is essential to allow for the prediction of the likelihood of resistance of the target pathogens to the antibiotics. In addition, a better understanding of the mode of action might allow for the development of more effective synthetic antibiotic analogues. Many objectives were realized throughout our study on the application of FBA's in controlling damping-off and root rot pathogens. Some of the tested untraditional FBA's such., *T. harzianum* proved to be more effective than *T. viride*.

The important goals of this study was to check the efficiency of some biocontrol agent on controlling damping-off disease incited by the tested pathogens both in laboratory and under greenhouse conditions. Results obtained by radial growth technique indicated that all the tested FBA's significantly reduced growth of the seed-born pathogens. In addition, the highest reduction rates were obtained by the FBA *T. harzianum* (86.55%) followed by *T. viride* (81.33%). *F. semitictum* was the most sensitive compared with the other tested pathogens (79.77%), whereas *M. phaseolina* was the least sensitive to FBA treatments, where reduction rate attained (61.11%). *T. koningii* induced the lowest reduction rates (77.66%). Generally, treatment soil with the tested FBA's significantly reduced TIP values in cantaloupe cvs. *T. harzianum* was more efficient in reducing TIP than *T. viride* against all the tested seed-born pathogens, except with *F. moniliforme*. The highest reduction rates were detected in *T. harzianum* / *F. semitictum* in CREDO F1 treatment (81.81%). *F. solani* was the least sevsitive to FBA soil treatment, compared with the other tested cucurbit seed-borne pathogens. In CREDO F1 cv. Reduction rates ranged from 65.62% to 77.27%. Both of FBA's soil treatment significantly reduced PRD values. Moreover, *T. harzianum* treatment realized the highest PRD reduction rates ranged from 57.14% (*F. moniliforme*) in CREDO F1 cv. to 83.33% (*F. moniliforme*) in ISI 54139 F1 cv.. In ISI54139 F1 cv., PRD reduction rates were 75.0% in *T. harzianum* / *F. semitictum* soil treatment to 83.33% in *T. harzianum* / *F. moniliforme*. *T. viride* was the most efficient in reducing PRD incited by *F. semitictum* with ANANAS cv., and *M. phaseolina* with ISI54139 F1 cv.(80.0%). Soil treatment with the tested FBA's, in general, significantly reduced PTD incidence. In addition, *T. harzianum* was more efficient in reducing PTD by *F. semitectum* (100%) in CREDO F1 cv., where reduction rates in *T. harzianum* / *F. moniliforme* and *F. semitictum* were 62.38% and 72.62%, respectively compared with control. In *T. harzianum* / *F. solani* or *M. phaseolina* treatments, equal rates were obtained (64.44%). *T. viride* was the most efficient in reducing PTD values in ISI 54139 F1 cv. Inoculated with *F. moniliforme* (83.33%).

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