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EVALUATION OF SOME FUNGAL BIOCONTROL EFFICIENCY IN RELATION TO THEIR CHITINASE INDUCTION POTENTIAL

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ABSTRACT: Six fungal biocontrol agents (FBAs) were tested for their efficiency against some cucurbitaceous damping-off pathogens, *in vitro* and *in vivo* (under greenhouse condition). The highest infection reduction percetage was realized by the FBA *Trichoderma harzianum* and *Trichoderma koningii*. *Alternaria alternata, Fusarium solani, Pythium ultimum* and *Macrophomina phaseolina* were more sensitive to FBAs treatment, especially with *T. harzianum* than the other tested damping-off pathogens, where infection reduction rates were 51.72 to 68%, compared with control. On the other hand, *Rhizoctonia solani* was the most tolerant among the tested pathogens against the effect of any FBAs applied *in vitro* and *in vivo* (infection reduction rate was 28.13% of control).

Amendment of the untreated culture with 2% chitin resulted in significant chitinase activities, particularly with *T. harzianum* (44.96 unit/cm^3). The presence of the tested pathogens in the growth media, chitinase activities significantly increased in all FBA/pathogen combinations, except for *P. ultimum*. The highest chitinase activities were induced by *T. harzianum* against *M. phaseolina, F. solani* and *Sclerotinia sclerotiorum* (56.48 – 59.89 unit/ml).

Electrophotometric analysis of protein of the tested FBAs using SDS-PAGE technique revealed 6 bands from *T. harzianum* and *T. viride*, 5 bands for *T. koningii*, 4 bands for *T. aureoviride* and only three bands for *Gliocladium roseum*. SDS-PAGE polypeptide analysis indicated the presence of 7 bands in *T. harzianum* and *T.*

***viride*, 6 bands from *T. koningi*, 5 bands from *T. aureoviride* and *G. roseum* and only 4 bands from *T. longibrachiatum*. The results obtained suggested high correlation between mycoparasitic effeciences of the tested FBAs and their chitinase activities against the tested pathogens and as well as their molecular weight of protein.**

Key Words: Biological control - *Trichoderma* sp. - *Gliocladium roseum* - damping-off - cucurbitaceous.

INTRODUCTION

The potential of some bacteria and fungi as biocontrol agents was reported since 1932 by Weindling. *Trichoderma* spp., the most common FBAs, are avirulent plant symbiants, as well as being parasites of other phytopathogenic fungi (Harman *et al.*, 2006).

The most effective use of biocontrol agents to control plant diseases depends essentially on the deep understading of the mechanisms employed by biocontrol agents to affect disease control (Howell, 2003). Mechanisms of biocontrol include competition for nutrients iron, infection sites, production of antibiotics, secondary metabolites and induced systemic resistance (Cook *et al.*, 1995 and Buchenauer, 1998), and promotion of plant growth (van Loon *et al.*, 1998). The antagonistic effect of fungal biocontrol agents, according to the available literature, is mainly attributed to the production of specific antibiotics and enzymes.

Many *Trichoderma* species are capable of suppressing the growth and development of different plant disease pathogens through the production of antibiotic, gliotoxin (Howell and Stepahovic, 1983 & 1995; Haraguchi *et al.*, 1996),) and some terpenoid compounds (Haraguchi *et al.*, 1996).

A correlation between the activity of endo-, and exochitinases produced by fungal biocontrol agents, i.e. *T. harzianum*, *T. koningii* and *T. virens*, and suppression of diseases incited by chitin-containing plant pathogenic fungi was established and confirmed by many researchers (De La Cruz *et al.*, 1992; Haran *et al.*, 1996; Lorito, 1998; Elad and Kapat, 1999 and Metcalf and Wilson, 2001). Most of these researches were carried out on cotton/ *R. solani* interactions, however, the correlation was also found in other

transgenic tobacco, potato (Lorito, 1998) and apple /scab interactions (Bolar *et al.*, 2000).

A mixture of several enzymes might be necessary for breaking down polysaccharides, chitin and glucans that are responsible for rigidity of fungal cell walls, thereby destroying cell wall integrity (Migheli *et al.*, 1998 and Metcalf and Wilson, 2001).

Production of proteases by some FBAs, *T. harzianum* resulted in significant suppression of *Botrytis cinerea* on bean leaves (Kapat *et al.*, 1998). Sharon *et al.* (2001) reported that, the protease enzymes break down hydrolytic enzymes into peptide chains and/or their constituent amino acids and thereby destroy their capacity to act on plant cells.

Fungal biocontrol agents were found to enhance the induction of systemic resistance as one of the major mechanisms that suppress plant pathogens. Extensive work was carried out on induced systemic resistance as another mechanism explaining fungal biocontrol activity, especially by *Trichoderma*. Mechanism of induced systemic resistance by *Trichoderma* includes induction of PR-proteins, phytoalexins and xylanases has been studied by Shoresh and Harman (2008).

Therefore, the present work was carried out to evaluate the efficacy of some fungal biological control agents against some cucurbit seed-borne pathogens both *in vitro* and under greenhouse conditions as well as determination of some biochemical parameters that characterizing antagonist-pathogen interactions such as total proteins and the activity of chitinases.

MATERIALS AND METHODS

In Vitro Experiments

Detection of the tested FBAs on growth of the tested pathogens

The tested pathogens included *Rhizoctonia solani* (RS), *Pythium ultimum* (PU), *Fusarium solani* (FS) and *Alternaria alternate* (AA). These pathogens were isolated from infected seeds of cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*) and tested for their pathogenic capability by Abou-Shaala (2008).

Biocontrol fungal agents used in this experiment were

Trichoderma harzianum, T. viride, T. koningii, T. aureoviride, T. longibrachiatum and *Gliocladium roseum*. The fungal isolates were kindely obtained from the Mycological Center, Assiut University, Egypt.

Through a preliminary study carried out byAbou-Shaala (2008), two methods were applied to determine the efficacy of the antagonistic potential of the tested FBAs against the tested pathogens, the linear growth and the inhibition zone diameter (IZD) method. The late method proved to be more efficient.

The multipoint-inoculation technique (Poppe, 1991) was used to inoculate PDA medium in Petri dishs with the tested pathogens (*R. solani, S. sclerotiorum, P. ultimum, M. phaseolina, F. solani, F. oxysporum, F. moniliforme* and *A. alternata*). Drenched sterile filter paper disks (13 mm in diameter) in culture filtrate for 5 min for each individual biocontrol agent then placed on the inoculated PDA plate. Paper-disks soaked in sterilized water were used as a control. The plates of *S. sclerotiorum* were incubated at 18°C, whereas the other tested pathogens were incubated at 25°C. Inhibition zone diameters (IZD) were measured when the growth of each fungus in control treatment reached to the dish's edge.

Measurment of Some Biochemecal Properties of the Fungal Bioagents

Activity of chitinases

Determination of chitinases activities of the tested FBAs were carried out using their cultural filterates and expressed as reduction percentages in viscosity(Miller, 1959). One unit of chitinase activity is defined as the amount of enzyme that is required to release 1 µM of N acetyl-B-D glucosamine per min from 0.5 % dry colloidal chitin solution under assay conditions.

Total protein content

Cultures of *G. roseum, T. aureoviride, T. harzianum, T. koningii, T. longibrachiatum* and *T. viride* total proteins were prepared according to Mousa (1992). Electrophotometric analysis of the sample treatments were executed after staining the fractionated proteins with commassie blue R 250. The gel was then destained with destaing solution until the bands become clear.

The protein content of *G. roseum, T. aureoviride, T.*

harzianum, T. koningii, T. longibrachiatum and *T. viride* was measured using the modified Lowry's method (Lowry *et al.*, 1951). Protein molecular weights of the six bioagents: *G. roseum, T. aureoviride, T. harzianum, T. koningii, T. longibrachiatum* and *T. viride* were determined using SDS-PAGE according to Laemmli (1970).

In vivo (Greenhouse) Experiments

Inoculation tests were carried out in 16 cm diameter plastic pots. Pots were sterilized by submerging in 7% formalin solution for a one hr and left for aeration. Inocula were prepared by growing each of the tested pathogens (*R. solani, S. sclerotiorum, P. ultimum, M. phaseolina* and *F. solani*) on PD medium in 250 cm^3 conical flasks, each containing 50 cm^3 of medium and incubated at 25°C for 15 days, except for *S. sclerotiorum*, which was incubated at 18-20° C. After 15 days incubation, fungal mats were collected, blended with sterile water. The inocula were used at the rate of 3g/Kg autoclaved soil. Autoclaved clay and sandy soil (1:1 v) was inoculated with the tested cucurbitaceous damping-off pathogens individually in 16 cm plastic sterilized pots. Certified seeds of the tested Alpha cucumber and Eskandarani squash cultivars were sterilized by dipping in 3% sodium hypochlorite for three minutes, rinsed for several times in sterilized tap water, then dried with filter paper.

Conidia of *G. roseum, T. aureoviride, T. koningii, T. longibrachiatum, T. harzianum* and *T. viride* were harvested from a culture grown on PDA plate medium by adding 10 ml of sterile distilled water to the plate and gently rubbing the surface with a soft brush. The conidial suspension was adjusted to 10^6 conidia/cm^3 by the aid of haemocytometer slide. Talc powder formula was prepared by adding 0.5 g carboxymethyl cellulose (CMC) to 50 ml of conidial suspension and mixed with 100 g of talc powder. One ml of this formula was used to treat 2g seeds. Seeds treated with the biocontrol agents were then inocubated for one day at 25°C. Commercial *Trichoderma* spp. of promot was used at the rate of 4 g/kg weigh seeds. Ten coated seeds were sown in each pot. Surface sterilized seeds of cucumber and squash cultivars were sown each in infested plastic

pot (16 cm) according to the previous treatments and placed in the greenhouse at approximately 20°C. Four replicates (4 pots) of each treatment were used. Four pots infested with tested pathogens only and others untreated pots served as controls for this experiment.

Damping-off incidence was calculated out 14 days following planting. Completely randomized design with 4 replicates was used. Percentage data were transformed into arcsine angles before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Least significant difference (LSD) at 5% level of probability was applied for comparing treatment means (Snedecor and Cochran, 1981).

RESULTS AND DISCUSSION

In Vitro Experiments

Efficacy of the tested fungal biological control agents (FBA's)

Results obtained in Fig. 1 showed that cultural filterates of all the tested FBAs showed significant antagonistic effect against the tested pathogens, however, the potential of the antagonistic effect significantly differed according to the applied FBA and the tested pathogen. *T. harzianum* followed by *T. koningii* and *T. viride* were the most efficient in suppressing the growth of the tested pathogen (IZD = 31.42 mm as a global estimation). On the other hand, treatment with *T. longibrachiatum* and *T. aureoviride* showed the least antagonistic effect (less than 18.33 mm IZD). *M. phaseolina* was the most sensitive to all FBA treatments, particularly with *T. harzianum*, *T. viride* and *T. koningii*.

Most of the tested FBA's including *T. harzianum*, *T. viride*, *T. koningii*, *T. aureoviride*, *T. longibrachiatum* and *G. roseum*, proved to be antagonistic to all the tested pathogens, at both *in vitro* and *in vivo* trials. Many researchers confirmed the significant role of FBA's in controlling damping-off diseases, particularly *T. harzianum* (Singh *et al.*, 2007 and Rojo *et al.*, 2007), *T. viride* (Hamed, 1999; Kumar and Kumar, 2000), *T. koningii* (Soltan, 1998), *Gliocladium* sp. ((Burgess and Keane, 1997; Lacicowa and Pieta, 1997, Soltan, 1998 and Tarantino *et al.*, 2006). 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*

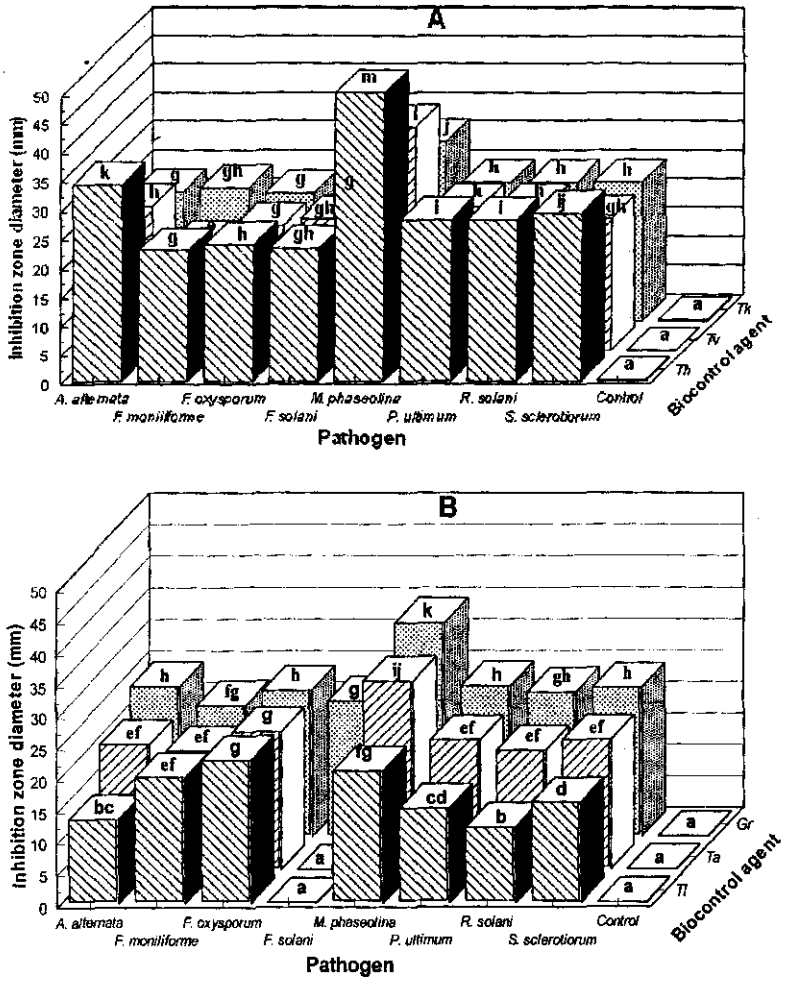

Fig. 1. Inhibition zones (mm) induced by culture filtrates of the tested biocontrol agents on cucurbits seed-borne pathogens

Where: A= indicate the reaction of *Th*, *Tv* and *Tk*.

B= indicate the reaction of *Gr*, *Ta* and *Tl*.

Tk= T. koningii, *Tv= T. viride*, *Th= T. harzianum*, *Gr= Gliocladium roseum*, *Tl= T. longibrachiatum* and *Ta= T. aureoviride*.

Similar letters indicate non-significant between trearments.

and *F. semitectum*. Most of these FBA/pathogen combinations were covered in the present study especially in laboratory experiments. *G. roseum* was one of the least effective FBA's in present study; however, Tarantino *et al.* (2006) considered *G. roseum* as one of the most powerful biocontrol agents. This discrepancy might be explained by the possible variations in the applied isolate of *G. roseum* and the host pathogen. Tarantino *et al.* (2006) controlled *R. solani* in tobacco by the application of low doses of fungicide combined with a resistant strain of *G. roseum*.

Measurment of Some Biochemecal Properties of the Fungal Bioagents

Chitinase activities in different FBA-pathogen interactions

Data presented in Table 1 indicated that, in control experiment, chitinase activities of FBA's in cultural filterates in the absence of the pathogen and free of chitin substrate were almost very limited or insignificant. However, growing any of the tested biocontrol agents in PD media amended with chitin at conc. 2% significantly stimulated the activity of chitinases, compared with control treatment without chitin amendment. Moreover, enzyme activity in amended control was significantly differed among the tested biocontrol agents, where *T. harzianum* realized an exclusive high chitinase activity (44.96 unit/ml), compared with that of the other tested FBAs (4.5 to 19.42 unit / ml).

In dual cultures of FBAs and pathogens, activity of chitinases was significantly stimulated. This was true for all FBA/pathogen combinations, except FBA's /*P. ultimum* which did not stimulate such activities (1.68-5.02 unit/ml). Chitinase activity values attained the highest levels in *T. harzianum* as stimulated by *S. sclerotiorum*, *M. phaseolina* and *F. solani*. *Rhizoctonia solani* induced the least chitinase activities with all the tested FBAs, compared with those of the other tested pathogens. The highest chitinase activity values were found to be induced by both *T. harzianum* and *T. viride*, compared with the other tested FBA's. However, differences in chitinase activity values between *T. harzianum* and *T. viride* were, generally, insignificant.

The obtained results confirmed the previous findings on the role of chitinases in biocontrol. High chitinase activities were induced by *T. harzianum*, *T. viride* and

Table 1. Activity of chitinases in different fungal biocontrol agents (FBA) and cucurbitaceous seed-borne pathogens interaction

Fungal biocontrol isolates	Chitinase activity (Unit /cm^3)							LSD
	Rhizoctonia solani	*Sclerotinia sclerotiorum*	*Pythium ultimum*	*Macrophomina phaseolina*	*Fusarium solani*	Control *PD without chitin	Control PD amended with chitin 2%	
Trichoderma harzianum	50.98	56.84	2.02	59.89	59.12	1.88	44.96	6.05
T. viride	48.66	52.18	1.84	54.00	54.14	0.00	19.42	4.97
T. koningii	39.10	40.93	3.21	44.18	44.79	3.52	13.53	4.51
T. aureoviride	36.15	38.40	1.68	40.17	42.35	0.00	8.58	4.42
T. longibrachiatum	25.75	29.52	3.29	30.32	31.82	3.05	7.53	3.95
G. roseum	10.00	10.23	5.02	10.08	12.22	4.44	4.50	3.15
LSD	4.32	4.87	1.69	6.17	6.19	1.49	2.32	

* PD = Potato dextrose.

T. koningii, particularly if grown with *S. sclerotiorum*, *M. phaseolina* and *F. solani*. On the other hand, insignificant induction of chitinases was obtained with *P. ultimum*. This might be explained by the fact that *P. ultimum*, an oomycetes lacking significant amounts of chitin in its cell wall. *G. roseum* was proved to be the least effective in chitinase activities, compared with the other tested FBAs. Production of chitinase by *Trichoderma* spp. and the possible role of chitinolytic enzymes in biocontrol are further supported by many researches (Elad and Kapat, 1999; Bolar *et al.*, 2000; Metcalf and Wilson, 2001 and Bolwerk, 2005). Metcalf and Wilson (2001) pointed out that hyphae of the biocontrol agent penetrated into infected epidermal and cortical tissue of the root to destroy the hyphae of the pathogen, with a little or no damage to uninfected plant tissue. The author ascribed this biocontrol phenomenon to the production of endo- and exo-chitinase by the antagonist.

Total protein content

Electrophoretic analysis of bioagents tested proteins was prepared after staining with commassie blue. This analysis demonstrated obvious differences in the number and molecular weight of the tested FBA proteins (Fig. 2 and Table 2).

The SDS-PAGE protein analysis of *T. harzianum* culture yielded 6 bands, including 5 specific bands with molecular weight ranged from 2.67 to 48.02 kDa, whereas *T. viride* analysis yielded 5 bands, including two specific bands (23.75-125.83 kDa), The analysis also yielded 5 bands in *T. koningii*, including one specific band (28.61-118 kDa), 4 bands in *T. aureoviride*, including two specific bands (28.61-110.15 kDa), 3 bands in *T. longibrachiatum* with no specific bands (35.38-76.78 kDa) and 3 bands in *G. roseum*, including two specific bands (50.51-113.3 kDa). SDS-PAGE analysis of the antagonist protein revealed that the highest number of bands was isolated from *T. harzianum* and *T. viride* followed by 5 bands from *T. koningii*, 4 bands from *T. aureoviride*. The least number of bands (3 bands) was obtained from *T. longibrachiatum* and *G. roseum*.

Polypeptide analysis in FBA mycelium using SDS-PAGE technique Table 3, indicated the presence of 7 bands in *T. harzianum* and *T. viride*, 6 bands

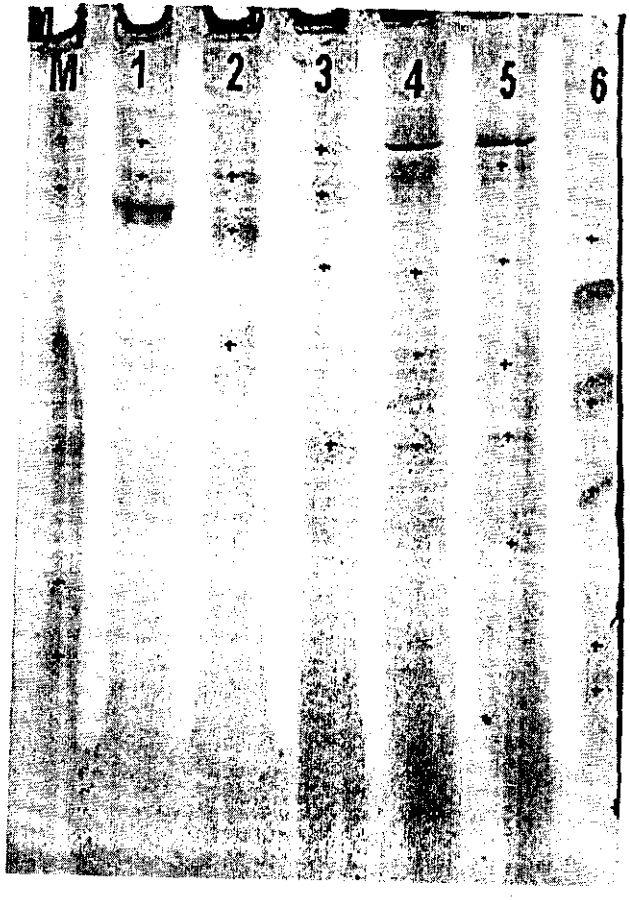

Fig. 2. SDS-PAGE of FBA's, showing different total Protein

MMarker

1.*G. roseum*
2.*T. longibrachiatum*
3.*T. aureoviride*
4.*T. koningii*
5.*T. viride*
6.*T. harzianum*

Table 2. Molecular weights (kDa) of fungal biocontrol agent's (FBA) proteins fractionated with SDS-PAGEFungal

Molecular wt. of standard proteins (Marker) kDa	Biocontrol isolates					
	Gliocladium roseum	*Trichoderma longibrachiatum*	*Trichoderma aureoviride*	*Trichoderma koningii*	*Trichoderma viride*	*Trichoderma harzianum*
-	-	-	-	-	125.83	-
118	-	-	-	118	-	-
-	113.3	-	-	-	-	-
-	-	-	110.17	-	-	-
-	-	-	-	94.5	94.5	-
-	71.78	71.78	-	-	-	-
52.2	-	-	52.2	-	-	-
-	50.51	-	-	-	-	-
-	-	48.04	-	-	-	48.04
-	-	-	44.62	44.62	44.62	-
-	-	-	-	-	-	42.2
35.38	-	35.38	-	35.38	35.38	-
-	-	-	-	-	-	33.23
29.61	-	-	29.61	29.61	29.61	-
-	-	-	-	-	-	27.06
-	-	-	-	-	23.75	-
20.8	-	-	-	-	-	-
-	-	-	-	-	-	10.93
6.8	-	-	-	-	-	-
-	-	-	-	-	-	2.67

Table 3. Amounts of polypeptide bands appeared in SDS-PAGE analysis of fungal biocontrol agents (FBA)

Marker	Fungal biocontrol isolates					
	Gliocladium roseum	*Trichoderma longibrachiatum*	*Trichoderma aureoviride*	*Trichoderma koningii*	*Trichoderma viride*	*Trichoderma harzianium*
-	-	-	-	-	3.48	-
2.26	-	-	-	2.78	-	-
-	3.37	-	-	-	-	-
-	-	-	1.92	-	-	-
-	-	-	-	2.04	1.73	-
-	1.79	2.05	-	-	-	-
3.79	-	-	3.61	-	-	-
-	6.25	-	-	-	-	-
-	-	4.25	-	-	-	2.57
-	-	-	3.69	2.82	2.29	-
-	-	-	-	-	-	9.2
3.36	--	4.81	-	3.17	5.63	-
-	-	-	-	-	-	3.82
4.04	-	-	4.58	1.79	2.1	-
-	-	-	-	-	-	1.48
-	-	-	-	-	3.04	-
4.75	-	-	-	-	-	-
-	-	-	-	-	-	4.51
4.71	-	-	-	-	-	-
-	-	-	-	-	-	3.19
22.9	11.4	11.1	13.8	12.6	18.3	24.8
Total no. of bands	4	4	5	6	7	7

from *T. koningi*, 5 bands from *T. aureoviride* and *G. roseum* and 4 bands from *T. longibrachiatum*. The higher number of bands and presence of special bands might explain the higher pathogenicity of *T. harzianum* against the tested pathogens. The similarity of *T. harzianum*, *T. viride* and *T. koningii* in the molecular weights of the three bands might also be explained in correlation with the higher antagonistic effect of those antagonists compared with the other tested bioagents. This explanation could be confirmed by the higher chitinase content in these three antagonists, obtained throughout this research. This explanation could be confirmed by De La Cruz *et al.* (1992) who isolated chitinases of 37 kDa and 33 kDa from some effective biocontrol agents. Moreover, Haran *et al.* (1996) isolated chitinase from *T. harzianum* with molecular weight ranged from 28 to 73 kDa. Although no specific bands were detected in *T. longibrachiatum*, however it was relatively antagonistic to some of the tested pathogens. This might be attributed to its high content of protein (2.05, 4.25 and 4.81 kDa).

The present research may be partially realizing the high correlation between the mycoparasitic efficiencies of the tested antagonists, their chitinase activities and the protein bands of the applied FBAs. However, it is believed that, more sophisticated biochemical analysis should be carried out to give better understanding about this phenomen and to throw more light on the nature of this suggested correlation.

Greenhouse experiment

This experiment was performed to check the effiency of treatment with the tested FBAs on reducing damping-off incidence, caused by the tested seed-borne pathogens, in both squash and cucumber seedlings. Application was carried out by seed-treatment, which proved in preminarly trails to be more efficient than soil treatment. Results in Figure (2-A and 2-B) showed that seed treatment with the tested FBAs significantly reduced total infection percentage (TIP) values incited by the tested seed-borne pathogens in both squash and cucumber. However, degree of damping-off suppression significantly differed according to the FBA used, the tested pathogen and host.

T. harzianum and *T. viride* were more efficient in reducing TIP values than the other tested FBAs.

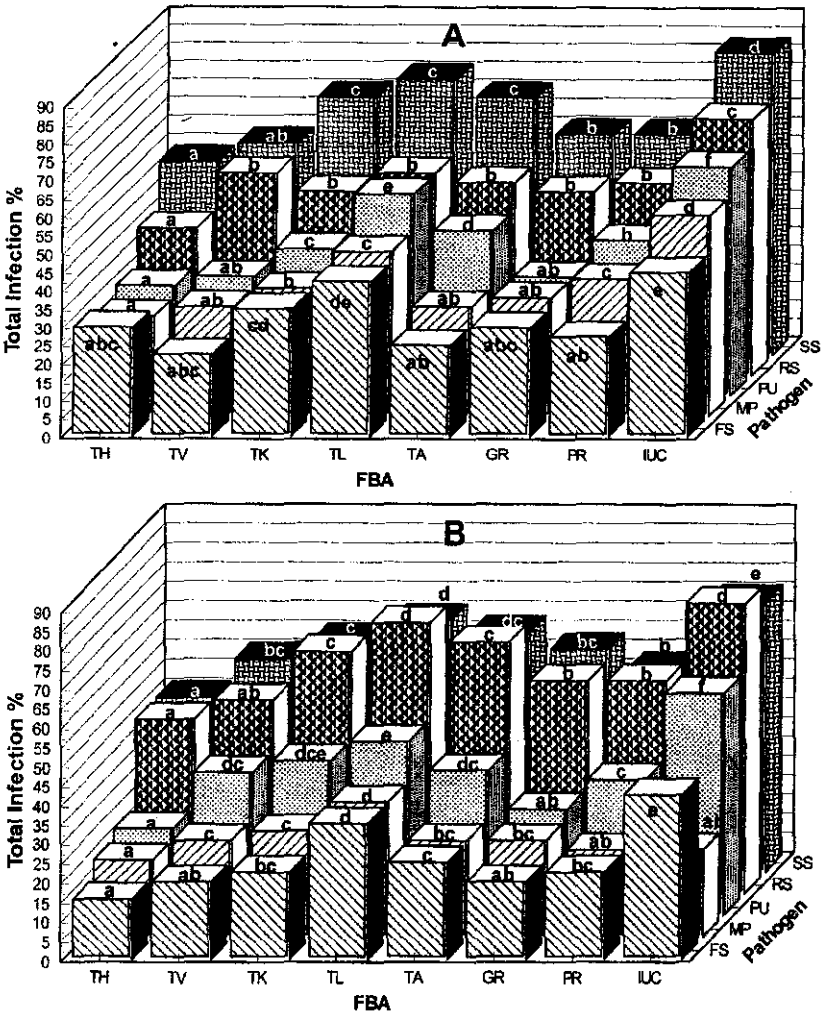

Fig. 2. Effect of seed treatment with the tested FBAs on controlling squash (A) and cucumber (B) damping-off incited by the tested pathogens

Where: TK= *T. koningii*, TV= *T. viride*, TH= *T. harzianum*, GR= *Gliocladium roseum*, TL= *T. longibrachiatum* and TA= *T. aureoviride*, PR= Promot, IUC= infected uninoculated control, RS= *R. solani*, SS= *S. sclerotiorum*, PU= *P. ultimum*, MP= *M. phaseolina* and FS= *F. solani*.

Similar letters indicate non-significant between trearments.

The highest infection reduction rate (68%, compared with infected control) was detected in *T. harzianum/F. solani*/cucumber treatment, followed by *T. harzianum/P. ultimum* and *M. phaseolina* (51.72%). Meanwhile, the least reduction rate (28.13% of infected control) was realized in *T. harzianum/R. solani* and *S. sclerotiorum* treatments.

Seed treatment with biocontrol agents was proved to be effective measure in controlling damping-off pathogens. This was in agreement with many other investigations (Howell *et al.*, 2000 and El-Farnawany (2006). Mathre *et al.* (1995) pointed out that bio-priming is considered a seed treatment system that integrates the biological and physiological aspects of disease control. Efficiency of seed treatment by biocontrol agents might be the result of coating the seed with the bioagents which prevents seed-borne and soil-borne pathogens from getting in contact with seed and hence gives more protection against soil-borne pathogens.

Extensive work was carried out on antagonistic mechanisms of *Trichoderma* spp. against phytopathogenic fungi, including (i) competition through rhizosphere competence and replacement of endogenous fungi on the root surface, leading to their suppression and therefore mask their presence (Harman, 2000 & 2001 and Howell, 2003), (ii) production of antibiotics such as gliotoxin (Howell and Stipanovic, 1995 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 chitinases (Elad and Kapat, 1999, Bolar *et al.*, 2000 and Metcalf and Wilson, 2001), Glocanases (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), (v) toxic compounds, i.e. viridian, sesquiterpene, gliocladic acid, heptolidic acid, viridio and valinotricin (Smith *et al.*, 1990). Many objectives were realized throughout our study on the application of FBAs for controlling damping-off and root rot pathogens. *T. aureoviride*, *T. longibrachiatum* and *G. roseum* significantly reduced damping-off and root rot incidence, however, they were still less effective than the other traditional FBAs, i.e. *T. harzianum*, *T. viride* and *T. koningii*.

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تقييم فعالية بعض عوامل المكافحة البيولوجية الفطرية وعلاقتها بإستحثاث إنزيم الكيتينيز

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تم إختبارفعالية ستة من عوامل المكافحة البيولوجية الفطرية ضد بعض مسببات أمراض موت البادرات فى القرعيات تحت ظروف المعمل و الصوبة الزراعية. كانت أعلى نسبة إنخفاض فى الإصابة مع إستخدام فطريات المكافحة الحيوية وهى ترايكودرما هارزيانم و ترايكوديرما كوننجاى (أقل من ٧٦,٧% بالمقارنة بالكنترول المصاب والغير معامل). أظهرت النتائج أن ألترناريا ألترناتا، فيوزاريوم سولانى، بيثيوم ألتيمم و ماكوفومينا فاصيولينا كانت أكثر المسببات المرضية حساسية لمصادر المكافحة البيولوجية الفطرية وبصفة خاصة ترايكوديرما هارزيانم، حيث كانت نسب خفض الإصابة مابين ٥١,٧٢ – ٦٨% بالمقارنة بالكنترول. وعلى الجانب الآخر، كان ريزوكتونيا سولانى أكثر المسببات المرضية ومتعادلاً مع تأثير أى من مصادر المكافحة البيولوجية الفطرية المستخدمة فى كل من المعمل والصوبة الزراعية (نسبة إنخفاض الإصابة كانت أقل من ٢٨,١٣ مقارنة بالكنترول).

لم يتم رصد أى نشاط لإنزيمات الكيتينيز لأى من مصادر المكافحة البيولوجية فى غياب الكيتين. أظهرت النتائج فروق معنوية فى أنشطة إنزيم الكيتينيز فى مصادر المكافحة البيولوجية المنماة على بيئة محتوية على الكيتين بتركيز ٢% وخاصة مع الترايكوديرما هارزيانم (٤٤,٩٦ وحدة/ مل). حدث زيادة معنوية فى نشاط أنزيم الكيتينيز فى وجود المسببات المرضية فى بيئة النمو بإستثناء البيثيوم ألتيمم.تم الحصول على أعلى نشاط إنزيمى للكيتينيز مع الترايكوديرما هارزيانم ضد كل من الماكروفومينا فاسيولينا، الفيوزاريوم سولانى وإسكليرتينيا إسكليروشيورم (٥٦,٤٨ – ٥٩,٨٩ وحدة/ مل).

نتج عن تحليل البروتين لمصادر المكافحة البيولوجية الفطرية المختبرة بإستخدام تكنيك (SDS-PAGE) ستة حزم لكل من الترايكوديرما هارزيانم و الترايكوديرما فيردى، ٥ حزم من الترايكوديرما كوننجاى، ٤ حزم من الترايكوديرما أوريوفيريدى وثلاث حزم فقط من الجليوكلاديوم روزيام .أظهر تحليل البوليبتيد بإستخدام تكنيك (SDS-PAGE) عن وجود ٧ حزم من التارايكوديرما هارزيانم والترايكوديرما فيردى، ٦ حزم من الترايكوديرما كوننجاى، ٥ حزم من الترايكوديرما أوريوفيردى والجليوكلاديوم روزيام و أربع حزم فقط من الترايكوديرما لونجيبراكياتم.

تشيرالنتائج المتحصل عليها على وجود علاقة قوية بين كفاءة التطفل الفطرية لعوامل المكافحة البيولوجية المختبرة ونشاطها للكيتينيز ضد المسببات المرضية المختبرة والوزن الجزيئى لحزم الأنتيجين البروتينية.