NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 31, 2014
Print ISSN 1222-4227; Online ISSN 2067-5720

BENEFICIAL AND DELETERIOUS EFFECTS OF *TRICHODERMA HARZIANUM* AND *T. LONGIBRACHIATUM* ON GROWTH OF COTTON SEEDLINGS AND THEIR BIOCONTROL CAPACITY AGAINST SEEDLING DAMPING-OFF

Aly A. Aly[1], Ezzat M. Hussein[1], Ahmed A. EL-Awamri[3], Marian M. Habeb[1], and Kamel A. Abd-Elsalam[1, 2*]

[1]Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[2]Unit of Excellence in Nano-Molecular Plant Pathology Research (ARC), Egypt
[3]Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt
*Corresponding author. E-mail: abd-elsalam.k.a.@post.com

ABSTRACT

***Trichoderma* spp. were isolated from roots of cotton plants showing the typical symptoms of seedling damping-off or adult plants root rot. Identification of 15 randomly selected isolates of *Trichoderma* to species level revealed that eight isolates (53.3%) were belonging to *T. longibrachiatum*, while seven isolates (46.7%) were belonging to *T. harzianum*.**

Geographic origins of *T. harzianum* in the sample were restricted to the Nile Delta, while isolates of *T. longibrachiatum* were isolated from widely separated geographic origins in the Nile Delta, Middle Egypt, and Upper Egypt.

The beneficial and deleterious effects of *Trichoderma* isolates on growth of cotton seedlings were evaluated by planting cotton seeds in autoclaved soil infested with *Trichoderma* isolates. Each of *T. longibrachiatum* and *T. harzianum* included both beneficial and deleterious isolates.

Moreover, the same isolate may exhibit both the beneficial and deleterious effects depending on the variable under consideration. Biocontrol capacity of *Trichoderma* spp. against soil-borne fungi involved in cotton seedling damping-off was evaluated by planting cotton seeds in autoclaved soil infested with a mixture of the soilborne fungi commonly involved in the disease. Before planting, seeds were treated with a fine powder consisted of a mixture of sorghum and *Trichoderma* spp. Grouping the isolates by cluster analysis, based on their beneficial effects, deleterious effects, and biocontrol capacity, was neither related to their geographic origin nor their species.

Key words: biocontrol capacity, beneficial and deleterious effects, *Trichoderma* spp., cotton seedlings**.**

INTRODUCTION

Trichoderma Pers. is a genus of hyphomycetes. Its species are among the most commonly encountered soil fungi (Roiger et al., 1991). *Trichoderma* has been shown to act as a mycoparasite against a range of economically important aerial and soilborne plant pathogens. Different factors involved in the antagonistic properties of *Trichoderma* have been identified, including antibiotics (Dennis and Webster, 1971a, b) and hydrolytic enzymes, such as β-(1, 3) glucanases, proteases and chitinases (Elad et al., 1984; Geremia et al., 1993). The initial interaction between *Trichoderma* and its host is characterized by the chemotrophic growth of hyphae of the mycoparasite towards the host (Chet and Elad, 1983). When the mycoparasite reaches the host, its hyphae often coil around it or are attached by hook-like structures (Elad et al., 1983a). Following these interactions, the mycoparasite penetrates the host mycelium, apparently by partially degrading its cell wall. Susceptible host mycelia show rapid vacuolation, collapse and disintegration (Elad et al., 1983b; Benhamou and Chet, 1993).

Trichoderma spp. exert their beneficial effect on plant growth by producing a growth-promoting factor that increases the rate of seed germination and dry weight of shoots and roots (Baker et al.,

Received 28 May 2013; accepted 03 February 2014. First online: 20 April 2014. DII 2067-5720 RAR 2014-282

1984; Windham et al., 1986; Chang et al., 1986; Menzies, 1993; Hanson, 2000). *Trichoderma* spp. play an important role in biological control thanks to their advantageous ecological and physiological properties: a good environmental fitness, which includes the ability to exploit competitively many different nutritional sources and high antagonistic ability against soil microorganisms (Howell, 2003; Sariah et al., 2005, Vergara et al., 2006; Abd-Elsalam et al., 2010*;* Saba et al., 2012.).

Regarding soil-borne fungi pathogenic on cotton, a number of reports demonstrated that isolates belonging to *Trichoderma* spp. could be effectively used for controlling these fungi (Aly et al., 2000a; Hanson 2000; Mathivanan et al., 2000; Haq and Khan, 2000; Xueling et al., 2003; Howell and Puckhaber, 2005; Asran et al., 2005).

On the other hand, *Trichoderma* spp. have been shown to induce deleterious effects on plant growth. Watkins (1981) reported that *Trichoderma* contains many species of moulds living as saprophytes or scavengers in the soil. They are unimportant as cotton disease agents, except when the seedlings are weakened. Many seeds simply rot without sprouting, others sprout, but the seedlings decay before emerging. Chilly, damp weather increases losses. *Trichoderma* has been reported to be pathogenic to at least 32 genera of plants (Powell et al., 1971; McFadden and Sutton (1975; Yang et al., 1976; Farr et al., 1989; Menzies, 1993; Howell et al., 1997b; Seaby, 1998; Howell and Pukhaber, 2005; Komon-Zelazowska et al., 2007).

All strains of *T. virens* are capable of producing the steroid phytotoxin, viridiol, on substrates with high C/N ratios. Preparations containing viridiol, when placed in close proximity to cottonseed, can have devastating effects on the emerging cotton radicle (Howell et al., 1997a). The main objectives of the present study were to (1) detect the deleterious and beneficial effects of *Trichoderma* spp. on growth of cotton seedlings, (2), to evaluate their biocontrol capacity against cotton seedling damping-off.

MATERIAL AND METHODS

Isolation, purification, and identification of *Trichoderma* spp. from cotton (*Gossypium barbadense* L.) roots

Isolation was made from samples collected from several locations in cotton-producing areas of Daqahliya, Minufiya, Gharbiya, Giza, and Assiut in Egypt. Each sample included from 10 to 15 seedlings affected with a variety of damping-off symptoms or rotted roots of 5 adult plants. Seedlings or roots of adult plants were removed from the field and washed thoroughly under running tap water to remove any adhering soil. Small pieces (approximately 0.5 cm long) of necrotic root tissues were surface sterilized with 10% Chlorox solution for 2 minutes, and washed several times with sterilized water. The surface sterilized pieces were then blotted dry between sterilized filter paper and plated on potato dextrose agar (PDA) medium amended with streptomycin sulphate or penicillin G and Rose Bengal to eliminate bacterial contamination. The plates were incubated at 26±3°C for 3-7 days. The developing colonies were identified to genus level according to Barnett and Hunter (1979). A random sample of 15 *Trichoderma* isolates, purified by the single spore technique, was identified to species level according to Rifai (1969). Identification of isolates to species level was kindly provided by Mycological Center, Assiut University.

Beneficial and deleterious effects of *Trichoderma* spp. on growth of cotton seedlings

Substrate for growth of each *Trichoderma* isolate was prepared in 500-ml glass bottle; each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of bottle were autoclaved for 30 minutes. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for 3 weeks. The present test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each isolate at a

rate of 50 g kg^{-1} of soil. Infested soil was dispended in 15-cm-diameter clay pots and these were planted with 10 seeds per pot (cultivar Giza 89). In the control treatment, no fungal inoculum was added to the autoclaved soil. Pots were randomly distributed on a greenhouse bench under a temperature regime ranged from 24±6°C to 34±4°C. Preemergence damping-off was recorded 15 days after planting, postemergence damping-off, survival, plant height (cm), and dry weight (mg $plant^{-1}$) were recorded 45 days after planting. The test was repeated once with almost the same results.

Biocontrol capacity of *Trichoderma* spp. against soilborne fungi involved in cotton seedling damping-off

Autoclaved clay loam soil was placed on a greenhouse bench and infested with a fungal mixture consisting of *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Pythium* sp., and *Sclerotium rolfsii*. These fungi were chosen for soil infestation because they are commonly involved in damping-off of cotton seedlings (Aly et al., 2000a). After thoroughly mixing, infested soil was dispended into 15-cm-diameter clay pots. *Trichoderma*-sorghum mixtures were air-dried in the greenhouse. The dry mixtures were triturated to fine powders in a blender (Papavizas and Lewis, 1981). Slightly moist seeds (cultivar Giza 89) were treated with the powdered inoculum of each isolate at a rate of 10 g kg^{-2} seeds, and thoroughly shaken in plastic bags before being planted in the infested soil at a rate of 10 seeds per pot. Untreated seeds were planted in the control treatments (uninfested and infested soils). The pots were randomly distributed on a greenhouse bench under a temperature regime ranging from 19.5±1.5°C to 34±4°C. Preemergence damping-off was recorded 15 days after planting. Postemergence damping-off, survival, plant height (cm), and dry weight (mg $plant^{-1}$) were recorded 45 days after planting. The test was repeated once with almost the same results.

Statistical analysis of greenhouse studies

The experimental design of all greenhouse studies was a randomised complete block with five replications. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Least significant difference (LSD) was used to compare isolate means. Percentage data were transformed into arc sine angles before carrying out ANOVA to produce approximately constant variance. Correlation analysis was performed with the same statistical package. Isolates were clustered by the average linked technique (unweighted pair-group method) and the results were expressed as a phenogram. Cluster analysis was performed with the SPSS 13 statistical package.

RESULTS

Isolation and identification of *Trichoderma* spp. from cotton roots

Identification of 15 randomly selected isolates of *Trichoderma* to species level (Table 1) revealed that 8 isolates (53.3%) belonged to *T. longibranchiatum*, while 7 isolates (47.7%) belonged to *T. harzianum*.

Ten of the isolates (66.7%) were isolated from the Nile Delta (Daqahliya, Minufiya, and Gharbiya governorate), two isolates (13.3%) were isolated from each of Middle Egypt (Giza governorate) and Upper Egypt (Assiut governorate), and one isolate (6.7%) was isolated from unknown location (isolate T9). Geographic origins of *T. harzianum* in the sample were restricted to the Nile Delta (Daqahliya and Gharbiya), while isolates of *T. longibranchiatum* were isolated from widely separated geographic origins in the Nile Delta (Minufiya governorate), Middle Egypt (Giza governorate), and Upper Egypt (Assiut governorate).

Table 1. Sources and geographic origins of *Trichoderma* spp. used in the present study

Isolate code	Source	Geographic origin	Identification
T3	Cotton seedlings	Daqahliya, Simbellawain	*T. harzianum*
T4	Cotton seedlings	Assiut, Assiut	*T. longibrachiatum*
T5	Cotton seedlings	Assiut, Assiut	*T. longibrachiatum*
T6	Cotton seedlings	Daqahliya, Simbellawain	*T. harzianum*
T9	Cotton seedlings	Unknown	*T. longibrachiatum*
T10	Cotton seedlings	Daqahliya, Simbellawain	*T. harzianum*
T14	Cotton seedlings	Giza, Giza	*T. longibrachiatum*
T18	Cotton seedlings	Minufiya, Minouf	*T. longibrachiatum*
T23	Cotton seedlings	Gharbiya, El-Mahalla El-Kobra	*T. harzianum*
T27	Cotton seedlings	Gharbiya, El-Mahalla El-Kobra	*T. harzianum*
T29	Cotton seedlings	Daqahliya, Simbellawain	*T. harzianum*
T31	Cotton seedlings	Daqahliya, Simbellawain	*T. harzianum*
T38	Cotton seedlings	Minufiya, Shibeen El-Kom	*T. longibrachiatum*
T39	Cotton seedlings	Minufiya, Shibeen El-Kom	*T. longibrachiatum*
T42	Cotton seedlings	Giza, Giza	*T. longibrachiatum*

Beneficial and deleterious effects of *Trichoderma* spp. on growth of cotton seedlings

The *Trichoderma* isolates did not show significant effects on preemergence damping-off (Table 2). Isolates T5, T9, T10, T14, T23, T27, T29, T39, and T42 significantly increased postemergence damping-off. Three of three isolates (T23, T27, and T39) also significantly reduced the percentage of surviving seedlings. Isolates T27, T29, and T38 did not show significant effects on plant height, while the remaining isolates significantly increased it. Isolates T3 and T42 were the only isolates, which significantly affected dry weight; however, they showed a striking contrast in their effects. Thus, T3 increased dry weight by 26.10%, while T42 decreased it by 22.14%.

Table 2. Beneficial and deleterious effects of *Trichoderma* isolates on cotton seedlings (cultivar Giza 89) under greenhouse conditions

Isolate code	Identification	Preemergence damping-off		Postemergence damping-off		Survival		Plant height (cm)	Dry weight (mg/plant)
		%	Trans-formed[a]	%	Trans-formed	%	Trans-formed		
T3	*T. harzianum*	4.0	7.38	4.0	7.38	92.0	77.31	16.81	172.00
T4	*T. longibrachiatum*	4.0	7.38	0.0	0.00	96.0	82.62	18.05	139.60
T5	*T. longibrachiatum*	12.0	20.06	12.0	20.06	76.0	60.78	16.56	122.60
T6	*T. harzianum*	8.0	14.75	2.0	3.69	90.0	73.62	16.76	133.00
T9	*T. longibrachiatum*	8.0	12.69	8.0	14.75	84.0	66.69	16.94	130.80
T10	*T. harzianum*	8.0	14.75	8.0	12.69	84.0	66.98	17.34	125.80
T14	*T. longibrachiatum*	6.0	11.06	16.0	21.25	78.0	64.76	16.98	135.20
T18	*T. longibrachiatum*	10.0	11.95	2.0	3.69	88.0	74.36	16.59	127.80
T23	*T. harzianum*	6.0	9.00	28.0	31.75	66.0	54.73	16.20	120.40
T27	*T. harzianum*	10.0	14.02	56.0	49.16	34.0	34.97	13.29	114.00
T29	*T. harzianum*	14.0	21.69	8.0	14.75	78.0	62.40	14.05	124.20
T31	*T. harzianum*	12.0	17.71	0.0	0.00	88.0	72.29	15.70	120.20
T38	*T. longibrachiatum*	8.0	12.69	0.0	0.00	92.0	77.31	15.17	136.00
T39	*T. longibrachiatum*	8.0	12.69	24.0	28.93	68.0	55.76	16.77	116.40
T42	T. longibrachiatum	0.0	0.00	18.0	22.28	82.0	67.72	16.23	106.20
Control[b]		14.0	19.33	0.0	0.00	86.0	70.67	13.30	136.40
LSD (P < 0.01)		NS		14.77		19.26		2.84	31.11
LSD (P < 0.05)		NS		11.19		14.60		2.15	23.57

[a]Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.
[b]Autoclaved soil.

Correlations among variables used for evaluating beneficial and deleterious effects of Trichoderma isolates are shown in Table 3. A significant positive correlation was observed between preemergence damping-off and postemergence damping-off. A highly significant negative correlation was found between survival and each of preemergence damping-off and postemergence damping-off. Survival was negatively correlated with plant height, while it was positively correlated with dry weight. Plant height and dry weight were negatively correlated.

Table 3. Correlation coefficients among variables used for evaluating beneficial and deleterious effects of *Trichoderma* spp. on growth of cotton seedlings (cultivar Giza 89)

Variable	Variable			
	2	3	4	5
1. Preemergence damping-off	0.635*[a]	-0.723**	0.354	-0.753**
2. Postemergence damping-off		-0.792**	0.024	-0.590*
3. Survival			-0.613*	0.911**
4. Plant height				-0.723**
5. Dry weight				

[a] Linear correlation coefficient (r) is significant at P<0.01 (**) or P<0.05 (*).

Biocontrol capacity of *Trichoderma* spp. against soilborne fungi involved in cotton seedling damping-off

The lack of significant difference in preemergence damping-off between control 1 and control 2 (Table 4) indicated that the fungal mixture used for soil infestation was nonpathogenic during the preemergence stage - that is, there was no disease pressure during this stage. Isolate T5 was the only isolate, which significantly affected preemergence damping-off, as it increased it by 150%. Isolates T5 and T27 significantly reduced postemergence damping-off by 90.48 and 71.43%, respectively; however, the other isolates had no significant effects on postemergence damping-off.

Table 4. Effects of *Trichoderma* isolates on incidence of cotton seedlings damping off (cultivar Giza 89) under greenhouse conditions

Isolate code	Identification	Preemergence damping-off		Postemergence damping-off		Survival		Plant height (cm)	Dry weight (mg/plant)
		%	Transformed[a]	%	Transformed	%	Transformed		
T3	T. harzianum	20.0	23.49	18.0	24.64	62.0	52.20	8.52	325.40
T4	T. longibrachiatum	20.0	23.91	16.0	23.02	64.0	53.82	10.37	422.60
T5	T. longibrachiatum	80.0	72.00	4.0	7.37	16.0	12.69	2.05	128.00
T6	T. harzianum	10.0	16.37	14.0	19.63	76.0	63.56	11.46	392.00
T9	T. longibrachiatum	26.0	27.13	52.0	46.45	22.0	21.69	6.29	130.00
T10	T. harzianum	24.0	28.33	18.0	24.22	58.0	49.84	11.48	341.20
T14	T. longibrachiatum	14.0	19.33	74.0	59.57	12.0	15.64	5.77	108.00
T18	T. longibrachiatum	36.0	36.00	46.0	39.56	18.0	21.86	8.75	186.00
T23	T. harzianum	60.0	54.00	34.0	30.01	6.0	9.0	4.80	60.00
T27	T. harzianum	28.0	31.11	12.0	18.00	60.0	51.04	11.87	301.00
T29	T. harzianum	44.0	40.89	48.0	41.31	8.0	12.69	5.30	36.00
T31	T. harzianum	52.0	46.80	34.0	34.85	14.0	14.49	6.43	134.00
T38	T. longibrachiatum	60.0	53.95	38.0	34.85	2.0	3.69	2.40	4.00
T39	T. longibrachiatum	48.0	44.02	36.0	36.47	16.0	18.0	4.98	100.00
T42	T. longibrachiatum	20.0	26.27	18.0	24.94	62.0	52.02	10.05	315.00
[b] Control 1		32.0	33.79	42.0	40.30	26.0	30.60	10.55	164.00
[c] Control 2		19.0	25.56	0.0	0.00	81.0	62.82	12.61	412.00
LSD (P < 0.01)			33.85		27.78		25.31	7.06	240.45
LSD (P < 0.05)			25.45		20.76		18.92	5.28	179.71

[a]Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.
[b]Autoclaved soil infested with a mixture of soilborne fungi involved in cotton seedling damping-off. [c]Autoclaved soil.

Isolates of *Trichoderma* spp. showed variable effects on survival. Thus, isolated T5, T9, T14, T18, T29, T31, and T39 did not significantly affects survival, isolates T3, T4, T6, T10, T27, and T42 significantly increased it, while isolates T23 and T38 significantly decreased it.

The majority of *Trichoderma* isolates did not show significant effects on plant height or dry weight; however, isolates T5, T23, T38, and T39 significantly reduced plant height, while isolates T4 and T6 significantly increased dry weight.

Correlation among variables used for evaluating biocontrol capacity of *Trichoderma* isolates are shown in Table 5. A highly significant negative correlation was found between preemergence damping-off and each of survival, plant height and dry weight. Postemergence damping-off was negatively correlated with each of survival ($P<0.01$) and dry weight ($P<0.05$). A highly significant positive correlation was observed between survival and each of plant height and dry weight. Plant height and dry weight were positively correlated ($P<0.01$).

Table 5. Correlation coefficients among variables used for evaluating biocontrol capacity of *Trichoderma* spp. against soilborne fungi involved in cotton seedling damping-off

Variable	Variable			
	2	3	4	5
1. Preemergence damping-off	-0.084[a]	-0.705**	-0.790**	-0.691**
2. Postemergence damping-off		-0.647**	-0.376	-0.618*
3. Survival			0.872**	0.969**
4. Plant height				0.882**
5. Dry weight				

Cluster analysis of *Trichoderma* isolates

The results of cluster analysis of *Trichoderma* isolates, based on their beneficial effects, deleterious effects, and biocontrol capacity are shown in Figure 1. Three groups of similar isolates (isolates T4, T10, T6, T42, T3, T27; isolates T9, T14, T18, T31, T39, T5; isolates T29, T38, T23, respectively) were identified by cluster analysis. Grouping the isolates was neither related to their geographic origin nor their species.

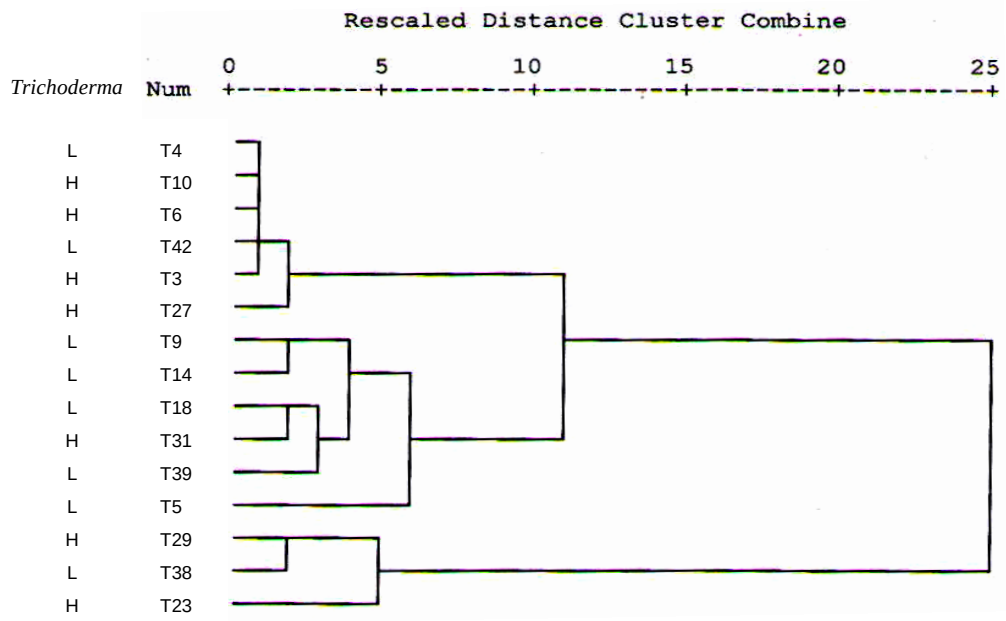

Figure 1. Phenogram based on average linkage cluster analysis of beneficial effects, deleterious effects, and biocontrol capacity of 15 isolates of *Trichoderma longibranchiatum* (L) and *T. harzianum* (H)

DISCUSSION

The beneficial and deleterious effects of *Trichoderma* isolates from cotton were evaluated by planting cotton seeds in autoclaved soil infested with *Trichoderma* isolates. The isolates did not show significant effects during the preemergence stage - that is, seed germination and seedling growth were not significantly affected during the first 15 days after planting, which may indicate that pathogenicity of the isolates or their growth-promoting effects did not appear early in the growth of cotton seedlings and were not as pronounced as effects reported on other crops caused by *Trichoderma* spp. (Harman, 2000). Our results demonstrated that each of *T. longibrachiatum* and *T. harzianum* included both beneficial and deleterious isolates. Thus, of the 9 isolates, which were pathogenic during the post emergence stage, 5 belonged to *T. longibrachiatum* and 4 belonged to *T. harzianum*. Similarly, of the 12 isolates that significantly promoted seedling height, 7 belonged to *T. longibrachiatum* and 5 belonged to *T. harzianum*. Moreover, the same isolate may exhibit both the beneficial and deleterious effects depending on the variable under consideration. For example, isolates T5, T9, T14, and T42 of *T. longibrachiatum* as well as isolates T10 and T23 of *T. harzianum* were pathogenic during postemergence stage; however, the same isolates significantly increased the height of the surviving seedlings.

A significant negative correlation was observed between postemergence damping-off and dry weight. This correlation implies that the higher the disease pressure caused by *Trichoderma* isolates during the postemergence stage, the less vigorous the surviving seedlings would be in their growth that is, even the seedlings, which survived postemergence damping-off, suffered from a subtle weakness, which reduced dry weight. Evidently, such seedlings would develop into unthrifty and less productive plants (Watkins, 1981; Minton and Garber, 1983). This interpretation holds true for the significant positive correlation between survival and dry weight.

The tested isolates of *Trichoderma* spp. could be classified into 3 distinct groups based on their biocontrol capacity. The first group included T3, T9, T6, T10, T27, and T42, which were effective biocontrol agents. Isolates of this group significantly increased the percentage of the surviving seedlings. Moreover, isolates T4 and T6 significantly increased dry weight of the surviving seedlings. The second group included T23, T38, and T39, which significantly reduced seedling height. Isolates T23 and T38 also reduced the percentage of the surviving seedlings. The effects of the isolates in this group were consistent with a previous report, in that some *Trichoderma* isolates were stimulatory for pathogenicity of some *Macrophomina phaseolina* isolates on cotton seedlings (Omar, 2005). These effects are also in agreement with results of Khan and Gupta (1998) who demonstrated that *T. polysporum* was stimulatory for radial growth of *M. phaseolina* on PDA. The third group included T9, T14, T18, T29, and T31, which were neither biocontrol agents nor pathogens. Each of these groups included isolates of both *T. longibrachiatum* and *T. harzianum*. It is worth noting that isolates T5 showed a peculiar behaviour because it showed both pathological effects (increase in preemergence damping-off and reduction in seedling height) and biocontrol activity (reduction in postemergence damping-off).

Based on the results of ANOVA of the greenhouse studies, it seems reasonable to conclude that morphological variation among species (Rifai, 1969), the basis of the genus taxonomy, does not provide sufficient explanation for the differences in the beneficial or deleterious effects on the growth of cotton seedlings or for the variation in biocontrol capacity against the soilborne fungi involved in cotton seedling damping-off. Grouping the isolates by cluster analysis was also neither related to their geographic origin nor their species.

Acknowledgement

This work was supported by grant from Agricultural Research Centre, Giza, Egypt.

REFERENCES

Abd-Elsalam, K.A., Almohimeed, I., Moslem, M.A. and Bahkali, A.H., 2010. *M13-microsatellite PCR and rDNA* sequence markers for Identification of *Trichoderma species in soil from Saudi Arabia.* Genetics and Molecular Research, 9: 2016-24.

Aly, A.A., Hussein, E.M., Allam, A.D.A., Amein, A.M. and El-Samawaty, A.M.A., 2000a. *Use of Trichoderma spp., Aspergillus spp., and Penicillium spp. to suppress damping-off of cotton seedlings*. Journal of Agricultural Science Mansoura Univ., 25: 7611-7619.

Asran, A.A., Abd-Elsalam, K.A., Omar, M.R. and Aly, A.A., 2005. *Antagonistic potential of Trichoderma spp. against Rhizoctonia solani and use of M13 microsatellite-primed PCR to evaluate the antagonist genetic variation. J*. Plant Dis. and Protec., 112: 550-561.

Baker, R., Elad, Y., and Chet, I., 1984. *The controlled experiment in the scientific method with special emphasis on biological control*. Phytopathology, 74: 1019-1021.

Barnett, H.L. and Hunter, B.B., 1979. *Illustrated Genera of Imperfect Fungi*. 3rd Ed. Burgess Publishing Company Minneapolis, Minnesota, 241 p.

Benhamou, N. and Chet, I., 1993. *Hyphal interactions between Trichoderma harzianum and Rhizoctonia solani: ultrastructure and gold cytochemistry of the mycoparasitic process.* Phytopathology, 83: 1062-1071.

Chang, Y.C., Chang, Y.C., Baker, R., Kleifeld, O. and Chet, I., 1986. *Increase growth of plants in the presence of the biological control agent Trichoderma harzianum.* Plant Dis., 70:145-148.

Chet, I. and Elad, Y., 1983. *Mechanism of mycoparasitism*. In Colloq. I'INRA Institute National de la Researche Agronomique, Dijon, France, 18: 35-40.

Dennis, G. and Webster, J., 1971a. *Antagonistic properties of species groups of Trichoderma.* I. Production of non-volatile antibiotics. Trans. Brit. Mycol. Soc., 57: 25-39.

Dennis, G. and Webster, J., 1971b. *Antagonistic properties of species groups of Trichoderma. II. Production of volatile antibiotics*. Trans. Brit. Mycol. Soc., 57: 41-48.

Elad, Y., Chet, I., Boyle, P. and Henis, Y., 1983a. *Parasitism of Trichoderma spp. On Rhizoctonia solani and Sclerotium rolfsii. Scanning electron microscopy and fluorescent microscopy*. Phytopathology, 73: 85-88.

Elad, Y., Barak, R., Chet, I. and Henis, Y., 1983b. *Ultrastructural studies of the interaction between Trichoderma spp. And plant pathogenic fungi*. Phytopathol. Z., 107: 168-175.

Elad, Y., Barak, R. and Chet, I., 1984. *Parasitism of sclerotia of Sclerotium rolfsii by Trichoderma harzianum*. Soil Biol. Biochem., 16: 381-386.

Farr, D.F., Bills, G.F., Chamuris, G.P. and Rossman, A.Y., 1989. *Fungi on plants and plant products in the United States*. APS Press, St. Paul, Minnesota, USA, 999 p.

Geremia, R., Goldman, G.H., Jacobs, D., Ardiles, W., Vila, S.B., Van Montagu, M. and Herrera-Estrella, A., 1993. *Molecular characterization of the proteinase encoding gene, prb1, related to mycoparasitism by Trichoderma harzianum*. Mol. Microbiol., 8: 603-613.

Hanson, L., 2000. *Reduction of Verticillium wilt symptoms in cotton following seed treatment with Trichoderma virnes*. J. Cotton Sci., 4: 224-231.

Haq, I., and Khan, S.M., 2000. *Antagonistic reaction of ten fungal isolates from root rot affected cotton plants*. Pakistan J. Phytopathol., 12: 109-111.

Harman, G.E., 2000. *Myths and dogmas of biocontrol: Changes in perceptions derived from research in Trichoderma harzianum T-22*. Plant Dis., 84: 377-393.

Howell, C.R., DeVay, J.E., Garber, R.H. and Batson, W.E., 1997a. *Field control of cotton seedling diseases with Trichoderma virnes in combination with fungicide seed treatment*. J. Cotton Sci., 1: 15-20.

Howell, C.R., DeVay, J.E., Garber, R.H. and Weir, B.L., 1997b. *Field efficacy of viridiol(-) mutants of Trichoderma virnes for biocontrol of cotton seedling diseases.* Pp.: 87-88. In: Beltwide Cotton Prod. Res. Conf.

Howell, C.R., 2003. *Mechanisms employed by Trichodermaspecies in the biological control of plant diseases: the history and evolution of current concepts*. Plant Dis., 87: 4-10.

Howell, C.R. and Puckhaber, L.S., 2005. *A study of the characteristics of "p" and "Q" strains of Trichoderma virnes to account for differences in biological control efficacy against cotton seedling diseases*. Biological Control, 33: 217-222.

Khan, M.R. and Gupta. J., 1998. *Antagonistic efficacy of Trichoderma species against Macrophomina phaseolina on eggplant*. J. Plant Dis. and Protec., 105: 387-393.

Komon-Zelazowska, M., Bissett, J., Zafari, D., Hatvani, L., Manczinger, L., Woo, S., Lorito, M., Kredics, L., Kubicek, C.P. and Druzhinina, I.S., 2007. *Genetically closely related but phenotypically divergent Trichoderma species cause green mold disease in oyster mushroom farms worldwide*. Appl. Environ. Microbiol., 73 (22): 7415-26.

Mathivanan, N., Srinivasan, K., and Chelliah, S., 2000. *Biological control of soil-borne diseases of cotton, eggplant, okra and sunflower by Trichoderma viride*. J. Plant Dis., and Protec., 107: 235-244.

McFadden, A.G. and Sutton. J.C., 1975. *Relationships of populations of Trichoderma spp. in soil to disease in maize.* Can. J. Plant Sci., 55: 579-586.

Menzies, J.G., 1993. *A strain of Trichoderma viride pathogenic to germinating seedlings of cucumber, pepper and tomato*. Plant Pathology, 42: 784-791.

Minton, E.B. and Garber, R.H., 1983. *Controlling the seedling disease complex of cotton.* Plant Dis., 67: 115-118.

Omar, M.R., 2005. *Pathological and biochemical studies on Macrophomina phaseolina pathogenic on cotton.* Ph.D. Thesis, Suez Canal Univ., Ismailliya, 173 p.

Papavizas, G.C. and Lewis. J.A., 1981. *Introduction and augmentation of microbial antagonists for the control of soilborne plant pathogens*. Pp.: 305-322. In: Biological Control in Crop Production (BARC Symposium No. 5-Goerge C. Papavizas, ed.), Allanheid, Osmun, Totowa.

Powell, N.T., Meléndez, P.I. and Batten, C.K., 1971. *Disease complexes in tobacco involving Meloidogyne incognita and certain soil-borne fungi*. Phytopathology, 61: 1332-1337.

Rifai, M.A., 1969. *A review of the genus Trichoderma.* Mycol. Inst. Mycol. Pap., 116: 1-56.

Roiger, D.J., Jeffers, S.N. and Caldwell, R.W., 1991. *Occurrence of Trichoderma species in apple orchard and woodland soils.* Soil Biol. Biochem., 23: 353-359.

Saba, H., Vibhash, D., Manisha, M., Prashant, K.S., Farhan, H., Tauseef, A., 2012. *Trichoderma – a promising plant growth stimulator and biocontrol agent*. Mycosphere, 3(4): 524-531

Sariah, M., Choo, C.W., Zakaria, H. and Norihan, M.S., 2005. *Quantification and characterization of Trichoderma spp. from different ecosystems*. Mycopathologia, 159: 113-117.

Seaby, D., 1998. *Trichoderma as a weed and pathogen in mushroom cultivation*. In: Kubicek, C.P. & Harman, G.E. (eds.), *Trichoderma* and *Gliocladiu*m. Vol. 2. Enzymes, Biological Control and Commercial Applications: 267-283, Taylor and Francis Ltd., London, UK.

Vergara, M., Flacone, G., Pecchia, S., and Vannacci, G., 2006. *Isolation and sequencing of an endopolygalacturonase gene in Trichoderma virens.* Pp 33. In: 9th International Workshop on Trichoderma and Glocladium, Book of Abstracts. April 6-8, 2006, Vienna, Austria.

Watkins, G.M. (ed.), 1981. *Compendium of Cotton Diseases*. The Am. Phytopathol. Soc., St. Paul, Minnesota, 87 p.

Windham, M.T., Elad, Y., and Baker, R., 1986. *A mechanism for increased plant growth induced by Trichoderma spp.* Phytopathology, 76: 518-521.

Xueling, L., Yun, L., Yu, Z.T., and Jian, W., 2003. *Application of Trichoderma spp*. In : The control of cotton *Verticillium wilt.* Acta Phytophylactica Sinica, 30: 284-288.

Yang, H., Powell, N.T. and Baker, K.R., 1976. *The influence of Trichoderma harzianum on root-knot Fusarium wilt complex in cotton*. J. Nematol., 8: 81-86.