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Field Evaluation of Beneficial and Deleterious Effects of *Trichoderma* spp. on the Incidence of Flax Seedling Blight and on some Agronomic Traits

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ABSTRACT

The beneficial and deleterious effects of five isolates of *Trichoderma harzianum* and seven isolates of *T. longibrachiaturn*, on the stand and some agronomic traits of flax, were evaluated under field conditions in 2013/2014 and 2014/2015 growing seasons. In each season, seeds of flax cultivar Giza 10 were treated with a dry powder of *Trichoderma* - sorghum mixture at a rate of 10 g/kg. seeds. Field evaluation revealed poor and inconsistent performance among the tested isolates from one season to another. *Thus*, while certain isolates effectively controlled seedling blight and improved some agronomic traits in one season, they were ineffective or even increased the disease in the other season. Due to the unstable and poor performance of *Trichoderma* isolates, they were considered either ineffective or at the most part effective in controlling flax seedling blight and improving agronomic traits. In each season, grouping the isolates by cluster analysis, based on their effect pattern, was not related to their morphological taxonomy.

INTRODUCTION

Linseed or Flax (*Linum usitatissimum* L.), one of the earliest crops and significant cash fiber crop in Egypt, it positions second following cotton plants in terms of financial importance and production. There is a regular boost in flax production due to the developing tendency back again to healthy fibres for textiles (El-Hawary, 2008). Cultivation of Flax plants is presently enclosed to the Nile Delta governorates. Seedling blight and root rot are prevalent disease in flax growing areas across the Nile Delta. On the other hand, if affected seedlings are destroyed quick in the season, they could turn into wind-blown or rained out and their damage is rarely observed (Aly *et al.,* 2011). Flaxseed is sensitive and their external layer is quickly broken within the threshing. Small cracks, which usually may perhaps not be apparent except when the seed is examined under a magnifying glass, allow easy transmission of fungal pathogens except if the seed is guarded with a fungicide. Untreated damaged seeds may possibly rot soon without germination, or they may germinate, generating weak seedlings that fail quickly to attack by different pathogens that induce seedlings blight. Flax plants infected by seedling blight might happen singly or in areas. The patches may comprise of simply a couple of plants in a row, or they may include various plants and cover a significant location. Influenced seedling becomes yellow, wilt, and die. Seedlings roots of recently infected plants exhibit red to brown lesions, but during a few days, they wilt and turn brown (Martens *et al.,* 1984). Root rot symptoms generally appear on older plants soon after the blooming stage. Plants turn brown too early and commonly set few or no seeds. The underground part of the stem and the roots are discoloured and the root system could possibly be stunted (Martens *et al.,* 1984). The main causal organism associated with diseased flax roots including *Rhizoctonia solani* and *Fusarium* spp. among the high frequently fungal microflora associated with seedling blight and root rot in the Nile Delta (Aly *et al.,* 2011 and Aly *et al.,* 2013).The genus Trichoderma is most important biocontrol agent, its species are among the most commonly encountered soil fungi (Roiger *et al.,* 1991). Trichoderma may be proven to act as a mycoparasite against a range of economically essential aerial and soil-borne fungi (Saba *et al.,* 2012).

Regarding soil-borne fungi pathogenic on flax, a number of reports demonstrated that *Trichoderma* isolates could be effectively used for controlling these fungi (Cariou-Pham and Bonnan, 2006; Pristchepa *et al.,* 2006; Amin *et al.,* 2010; Kumar and Tripathi, 2018). Alternatively, *Trichoderma* has been reported to be pathogenic to at least 32 genera of plants (Aly *et al.,* 2014). The current investigation aims to estimate the positive and harmful effects of *T. harzianum* and *T. longibrachiatum* on the stand and some agronomic traits of flax under field conditions.

MATERIALS AND METHODS

***Trichoderma* isolates used in the present study:**

Five isolates of *Trichoderma harzianum* and seven isolates of *T.longibrachiatum* were randomly selected from the *Trichoderma* culture collection of Cotton and Fiber Crops Disease Research Section; Plant Pathology Research Institute., Agricultural Research Center, Giza, Egypt. All isolates were originally isolated from flax roots.

Preparation of *Trichoderma* inocula:

Media substrate used for growing of Trichoderma isolates was well prepared in 500-ml glass bottles, every bottle included 50g of sorghum grains and 40 ml of tap water. Contents of each bottle were sterilized in autoclave for 30 minutes. Isolate inoculums, obtained from one-week-old culture on PDA, was aseptically inserted into the bottle and allowed to fungal colonize sorghum for 21 days. Trichoderma -sorghum combination was air-dried in the greenhouse. The dry Trichoderma -sorghum mixture was homognized to a fine powder in a mixer. (Paparizas and Lewis, 1981).

Field trials:

Experiments were conducted over two successive growing seasons on heavy clay soil at El-Gemmaiza Agricultural Research Station (El-Gharbiya Governorate, Middle Delta Region), beginning in the fall of 2013. Each season, experimental plots were established in a field known to be infested with flax seedling blight pathogens.

Experiments consisted of a randomized complete block design of four replications (blocks). Plots were 3×2 ($6m^2$). Powdered inocula of *Trichoderma* isolates were added to slightly moist seeds of flax cultivar Giza

10 at a rate of 10 g/kg. seeds. Seeds were shaken thoroughly in flasks for 5 min. and allowed to dry before being dispensed in paper bags for storage until the time of use. In the control treatment, no *Trichoderma* isolates were added to seeds. Within a week after treatment, plots were manually planted with the treated seeds at a rate of 85.7g/plot, which is equivalent to 60 kg/ feddan. Planting dates were 10 November 2013 and 20 November 2014. Stand (number of healthy surviving plants) was recorded 60 days after planting in a 25×25-cm. randomly selected area in each plot. At harvest, a random sample of 10 plants was taken from each plot and observations were recorded on individual plants for each of the following characters:

1.Total plant height (cm): plant height from the cotyledonary node to the apical bud of each plant.
2.Straw yield/plant (g): Wight of the mature air-dried straw per plant after removing the capsules.
3.Straw yield/plot (kg.): The weight of the mature air-dried straw per plot after removing the capsules.
4.Seed yield/plant (g): Weight of harvested seeds per plant.
5.Seed yield/plot (kg.): The weight of the harvested seeds per plot.

Fungal isolation from the experimental plots

Flax seedlings infected with soil-borne fungi, which revealed standard seedling blight symptoms or order plants, which usually showed root rot had been collected from the field and cleaned under tap water to eradicate any adhering soil. Tiny parts of necrotic tissues were surface sanitized with 10% Clorox solution for 2 min. and rinsed many times with sterilized water. The sterilized pieces were dried up on sterilized filter papers and distributed on potato dried dextrose agar (PDA) medium modified with streptomycin sulfate and Rose Bengal to remove bacterial contamination. Petri plates have been incubated at 26±3ºC for 3-7 days. The fungal colonies were developed and diagnosed according to Gilman (1966), Barnet and Hunter (1979). Colonies of each fungus were indicated as percentages of the total developing colonies.

Statistical analysis of the data:

The experimental model of field trials was a randomized complete block with four repeats. A least factor (LSD) was applied to assess treatment means. Analysis of variance (ANOVA) was completed by MSTAT-C statistical package. Data of every season were put through ANOVA independently due to variances in ecological conditions and control practices of every season. Cluster analysis and correlation evaluates were achieved with the program package SPSS 10.0.

RESULTS

Aspergillus spp., *Fusarium* spp., and *Rhizoctonia solani* were isolated from the infected seedlings in both seasons, while *Alternaria* spp., *Macrophomina., phaseolina* and *Rhizopus* were only isolated in the first season (Table 1). *Fusarium* spp. was the predominant fungus in the first season, while *R. solani* and *Aspergillus* spp. were the predominant ones in the second season.

Table 1. Frequency of fungi isolated from flax seedlings infected with postemergence damping – off in the experimental plots of Gemmaiza Agricultural Research Station.

	Isolation Frequency (%)[a] in	
Fungus	**2013/2014[b]**	**2014/2015[c]**
Alternaria spp.	10.19	0.00
Aspergillus spp.	9.53	36.01
Fusarium spp.	48.00	21.31
Macrophomina phaseolina	6.67	0.00
Rhizoctonia solani	2.86	42-68
Rhizopus spp.	22.76	0.00
LSD ($P \leq 0.05$)	15.63	8.56

Colonies of each fungus were expressed as the percentage of the total developing colonies. Each value was the mean of five (b) or three (c) replicates.

Certain *Trichoderma* isolates did effectively control seedling blight or improve agronomic traits in one season; however, there was a lack of consistency among seasons, that is, they were ineffective in controlling the disease or improving agronomic traits in the other season. *Thus*, isolates 8 and 12 significantly increased stand in the second season (Table 3), while they did not show any effect on the stand in the first seasons (Table 2). Isolate 7 had no effect on stand count in the first season (Table 2), while it significantly reduced it by 26.83% in the second seasons (Table 3). In the first seasons, total height was significantly increased by isolates 4,9,10 and 12. Similarly, straw yield/plant was significantly increased by isolates 2, 4, 5, and 11 (Table 2). In the second season, each of total height and straw yield/plant was not affected by any of the tested isolates (Table 3). In the first season, none of the tested isolates was able to affect seed yield/plant (Table 2); however, in the second season, isolates 1, 8, and 9 significantly increased it (Table 2). In the first season, seven isolates (2, 4, 5, 9, 10, 11, and 12) showed beneficial effects on some agronomic traits (Table 2); however, in the second season, the number of beneficial isolates decreased to 4 (1, 8, 9, and 12) as shown in Table 3. Straw yield/plot and seed yield/plant were not affected by any isolate in both seasons (Tables 2 and 3). Isolate 7 was the only isolate, which caused a significant reduction in the stand in the second season, while it showed no effect on the stand in the first season (Tables 2 and 3).

Effect of *Trichoderma* isolates on the stand and agronomic traits were not correlated in the two seasons (Table 4). In each season, almost all agronomic traits were not correlated when they were evaluated under the effect of *Trichoderma* isolates (Table 5).

In the First season, three groups of similar isolates (isolates 5, 7, 4, 12; isolates 8, 11, 2, 3; and isolates 1, 6, 9, 10) were identified by cluster analysis based on their effect patterns (Fig. 1). In the second season, the isolates were placed in the other three groups of similar isolates. These groups were isolates 1, 12, 2, 8; isolates 5, 9, 4, 11, 6, 10, and isolates 3, 7 *(*Fig. 2). In any season, grouping the isolates by cluster analysis was not related to their morphological taxonomy.

Table 2. Effects of treating flax seeds with *Trichoderma* isolates on some agronomic traits under field conditions in 2013/2014

		Agronomic traits					
N0.	**Treatment**	**Stand (in 0.06m^2)**	**Total height (cm.)**	**Straw Yield / plant (g.)**	**Straw Yield / plot (Kg.)**	**SeedYield / plant (g.)**	**Seed Yield / plot (Kg.)**
1-	*T. longibrochiatum*	81.75	111.50	2.37	6.48	0.37	1.54
2-	*T. longibrochiatum*	102.50	115.88	3.20*(+)	7.77	0.65	1.80
3-	*T. harzianum*	98.25	109.13	1.90	6.20	0.45	1.45
4-	*T. longibrochiatum*	101.75	118.75*(+)	3.55*(+)	6.90	0.41	1.44
5-	*T. harzianum*	92.25	110.00	2.85×(+)	7.18	0.43	1.57
6-	*T. longibrochiatum*	78.00	102.88	2.43	6.53	0.42	1.52
7-	*T. harzianum*	95.25	112.75	2.56	6.88	0.34	1.59
8-	*T. harzianum*	95.25	109.38	1.77	7.63	0.36	1.67
9-	*T. longibrochiatum*	93.00	118.00×(+)	2.49	6.33	0.39	1.69
10-	*T. harzianum*	80.00	118.25×(+)	2.58	7.63	0.52	1.58
11-	*T. longibrochiatum*	99.00	112.75	2.81×(+)	8.48	0.54	1.76
12-	*T. longibrochiatum*	97.00	118.13×(+)	2.32	7.83	0.59	1.67
13-	Control	89.50	110.13	2.12	6.53	0.40	1.48
LSD (P ≤ 0.05)		N. S.	8.23	0.82	N. S.	N. S.	N. S.
LSD (P ≤ 0.10)		N. S.	6.93	0.69	N. S.	N. S.	N. S.

Significant different from the control at P ≤ 0.10 (×) or P ≤ 0.05 (*). The tested isolate caused significant increase (+).

Table 3. Effects of treating flax seeds with *Trichoderma* isolates on some agronomic traits under field conditions in 2014/2015

		Agronomic traits					
N0.	**Treatment**	**Stand (in 0.06m^2)**	**Total height (cm.)**	**StrawYield / plant (g.)**	**Straw Yield / plot (Kg.)**	**Seed Yield / plant(g.)**	**Seed Yield / plot(Kg.)**
1-	*T. longibrochiatum*	62.75	93.48	1.58	3.47	0.54	2.82*(+)
2-	*T. longibrochiatum*	63.50	97.65	1.43	2.99	0.27	1.24
3-	*T. harzianum*	42.25	96.28	1.67	3.45	0.44	1.45
4-	*T. longibrochiatum*	52.75	95.30	1.53	4.50	0.32	1.00
5-	*T. harzianum*	49.75	89.20	1.55	3.28	0.25	1.83
6-	*T. longibrochiatum*	61.00	99.18	1.22	3.03	0.28	1.98
7-	*T. harzianum*	37.25×(-)	99.70	1.79	2.13	0.34	1.25
8-	*T. harzianum*	64.25×(+)	97.48	1.56	3.74	0.23	2.28*(+)
9-	*T. longibrochiatum*	50.50	91.98	1.49	2.86	0.31	2.23×(+)
10-	*T. harzianum*	56.75	96.23	1.57	2.78	0.23	1.78
11-	*T. longibrochiatum*	55.50	97.60	1.27	3.93	0.37	1.40
12-	*T. longibrochiatum*	64.00×(+)	94.15	1.42	2.90	0.41	2.10
13-	Control	51.52	94.43	1.32	2.83	0.29	1.48
LSD (P ≤ 0.05)		14.91	N. S.	N. S.	N. S.	N. S.	0.79
LSD (P ≤ 0.10)		12.55	N.S.	N. S.	N. S.	N. S.	0.67

Significant different from the control at P ≤ 0.10 (×) or P ≤ 0.05 (*). The tested isolate caused significant increase (+)or significant decrease (-).

Table 4. Correlation between effects of years on agronomic traits used for evaluating the performance of 12 *Trichoderma* isolates used for treating flax seeds.

	Agronamic trait	**Correlation**
1-	Stand	-0.222[a] (0.488)[b]
2-	Total height	-0.262 (0.411)
3-	Straw yield/plant	-0.204 (0.524)
4-	Straw yield/plot	0.128 (0.691)
5-	Seed yield/plant	-0.085 (0.793)
6-	Seed yield/plot	0.043 (0.894)

[a] **Linear correlation coefficient, which measures the degree of association between effects of two years on the designated agronomic trait.**

[b] **Probability level and n = 12.**

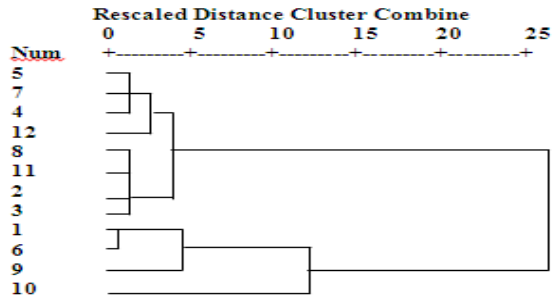

Fig. 1. Phenogram based on average linkage cluster analysis of beneficial and deleterious effects of 12 *Trichoderma* isolates on flax stand and some agronomic traits under field conditions in 2013/2014 growing season. The tested isolates belonged to *T.harzianum* (isolates no$_s$. 3, 5,7,8, and 10) or *T.longibrachiatum* (isolates no$_s$. 1,2,4,6,9,11, and 12).

Table 5. Correlation among agronomic traits in two years under the effects of 12 *Trichoderma* isolates used for treating flax seeds.

		Agronomic traits				
Year	**Agronomic traits**	**1**	**2**	**3**	**4**	**5**
2013/2014	Stand					
	Total height	0.378[a](0.226)[b]				
	Straw yield/plant	0.298(0.347)	0.455(0.137)			
	Straw yield/plot	0.310(0.327)	0.290(0.361)	0.218(0.497)		
	Seed yield/plant	0.295(0.352)	0.369(0.238)	0.312(0.324)	0.636(0.026)*	
	Seed yield/plot	0.310(0.327)	0.267(0.402)	0.068(0.834)	0.680(0.015)*	0.538(0.071)*
2014/2015	Stand					
	Total height	-0.003(0.992)				
	Straw yield/plant	-0.609(0.035)*	-.094(0.772)			
	Straw yield/plot	0.247(0.438)	-0.140(0.665)	-0.263(0.408)		
	Seed yield/plant	-0.064(0.842)	-0.105(0.744)	0.145(0.652)	0.115(0.722)	
	Seed yield/plot	0.482(0.113)	-0.391(0.209)	-0.085(0.793)	-0.116(0.719)	0.281(0.377)

[a]**Linear correlation coefficient.** [b]**Probaility level and n = 12** ***Significant Correlation**

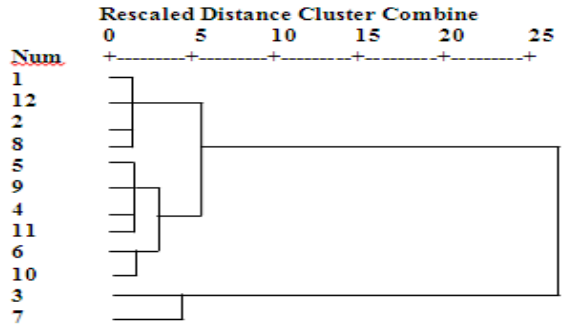

Fig. 2. Phenogram based on average linkage cluster analysis of beneficial and deleterious effects of 12 *Trichoderma* isolates on flax stand and some agronomic traits under field conditions in 2014/2015 growing season. The tested isolates belonged to *T.harzianum* (isolates no$_s$. 3, 5,7,8, and 10) or *T.longibrachiatum* (isolates no$_s$. 1,2,4,6,9,11, and 12).

DISCUSSION

Fusarium spp. In the first season and *Rhizoctonia solani* in the second season showed the highest isolation frequencies. These fungi are considered major causes of flax seedling blight (Aly *et al.*, 2011 and Aly *et al.*, 2013). On the other hand, the other isolated fungi, except *Macrophomina phaseolina.* are not reported as root pathogens of flax (Aly *et al.*, 2011). Therefore, the disease pressures in the experimental plots were due mainly to *Fusarium* spp., and *Rhizoctonia solani* in the first and in the second seasons, respectively. Plant stands of the control provided the basis for determining the disease pressure estimate, which was low in the first season and high in the second season.

An effective biocontrol agent should meet two requirements. First, it should significantly reduce disease development. Second, it should have stable performance when it is evaluated under different environmental conditions. Most of the tested isolates in the present study did not meet these requirements. Therefore, there were considered either ineffective or at the most part effective in controlling flax seedling blight and improving agronomic traits.

Chitinase and β-1,3- glucanase is key enzymes involved in the biocontrol activity of *Trichoderma* isolates. Production of chitinase and activity of β-1,3- glucanase are markedly affected by pH. *Thus*, the optimum pH is 6 for the production of chitinase (EL-Katatny *et al.*, 2000). Similarly, the optimal activity of β-1,3- glucanase is usually in the range of pH 4-6 (Noronha and Ulhoa, 2000). The pH values of the Egyptian soil range from7.92 to 9.15 (Aly and Kandil, 1999). Therefore, this range is unfavourable for the production of chitinase or activity of β-1,3- glucanase of *Trichoderma* isolates applied into the soil. This may explain, at least partially, the poor performance of the tested isolates.

Another possibility for interpreting the poor performance of the isolates is the differential effects of host cultivar and/or pathogen isolates on the performance of *Trichoderma* isolates. That is, a single isolate of a pathogen can be highly sensitive to the application of a single isolate of *Trichoderma* but may be exhibit minimal sensitivity to the application of another *Trichoderma* isolate (Asran *et al.*, 2005). Similarly, a host cultivar may be highly responsive to the application of a *Trichoderma* isolate but may show minimal response to the application of another isolate of *Trichoderma* (Asran, 2007).

These interpretations for the isolates poor performance do not rule out the possibility that the exclusive reliance on *Trichoderma* isolates may be responsible for such a poor performance. In the present study, *Trichoderma* isolates were evaluated as an end in themselves rather than as synergistic components within the integrated disease management system. *Thus*, the value of *Trichoderma* isolates as biocontrol agents are likely to be underestimated until we are more adequately explore ways of using them in the integrated disease management system. In the future, high priority should be given to developing the extensive use of *Trichoderma* isolates as components of the integrated disease management system (Klassen, 1981).

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التأثيرات الضارة والمفيدة لفطريات التريكوديرما على حدوث مرض لفحة بادرات الكتان وعلى بعض الصفات المحصولية تحت ظروف الحقل

شوقي محمد المتولي زايد ، على عبد الهادي على ، أمل عبد المنجي عسران و محمود توفيق محمود منصور

معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، الجيزة ، مصر

قيمت خمس عزلات لفطر تريكوديرما هارزياتم وسبع عزلات لفطر تريكوديرما لونجيبراكياتم من حيث تأثيراتها المفيدة والضارة على حدوث مرض لفحة بادرات الكتان ، وعلى بعض الصفات المحصولية ، وذلك تحت ظروف الحقل ، خلال موسمي 2013/ 2014 و 2014/ 2015. في كل موسم ، عوملت بذرة الكتان صنف جيزة 10 بمسحوق جاف يتكون من خليط لعزلات الترايكوديرما والذرة الرفيعة بمعدل 10 جم / كجم بذرة. أظهرت عزلات التريكوديرما ، عند تقييمها حقليا ، أداءً ضعيفا إتسم بعدم الثبات في مقاومة المرض وفي تحسين الصفات المحصولية ، ففي حين كانت بعض العزلات فعالة في مقاومة المرض وفي تحسين الصفات المحصولية في أحد الموسمين ، فإنها كانت غير فعالة في الموسم الآخر ، بل وقد تحدث زيادة في مستوى حدوث المرض. وعلى ذلك فقد خلصت الدراسة الحالية إلي أن عزلات التريكوديرما كانت غير فعالة ، أو على الأكثر فعالة جزئيا في مقاومة المرض وفي تحسين الصفات المحصولية. أمكن في كل موسم تقسيم العزلات إلى مجموعات ، بإستعمال التحليل العنقودي المبني على نمط تأثيرها ، إلا أن المجموعات المتحصل عليها لم ترتبط بالوضع التقسيمي للعزلات المبني على صفاتها المورفولوجية.