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Effect of Seed Treatment on Control of Root Rot Disease and Improvement of Growth and Yield of Pea Plants

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ABSTRACT

The efficiency of bio-priming, seed coating with bio-control gents (*Trichoderma harzianum*, *Bacillus subtilis* or *Psedomonas fluorescens*), seed priming and seed dressing with Rizulex–T (a fungicide) to control pea root rot disease, and improve the growth and yield of pea plants were investigated. *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* spp. were isolated from roots of pea plants infected by root rot disease in Nobaria province, Egypt. All isolated fungi were able to induce root rot on pea plants, *F. solani* and *R. solani* being the most severe fungi. In greenhouse trials seed priming enhanced the effectiveness of *T. harzianum*, *B. subtilis* and *P. fluorescens* to control root rot pathogens as the highest percentage disease reduction was recorded with bio-primed seed treatments. Seed coating with bio-control agents was superior to fungicide seed treatment by decreasing pea root rot disease caused by *F. solani*, *R. solani* and *S. rolfsii*. Under field conditions bio-priming treatments strongly reduced pea root rot disease over two seasons. There was no significant difference between seed coating with bio-control agents and fungicide seed treatment in decreasing the incidence of root rot. Bio-priming and seed coating with *T. harzianum* or *B. subtilis* most effectively stimulated vegetative growth, observed by plant height, number of leaves/plant and number of branches/plant, and significantly increasing the yield of early and total green pods. Moreover, these treatments resulted in the highest values of pea pod quality, namely pod length, pod diameter, number of seeds/pod and chemical contents of pods (i.e., TSS, total carbohydrate and protein) over both seasons.

Keywords: bio-priming, seed treatment

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important leguminous crops in many countries, including Egypt. High quality green pods and mature seeds are used for fresh meals and for the food industry.

Damping-off and root rot diseases in pea are caused by a single or a combination of soil-borne fungi i.e., *Fusarium solani* Mart. Sacc., *F. oxysporium*, *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc., *Pythium* spp. Tower and *Phytophthora cactorum* (Sm. et. Sm.) Leonion (Abda *et al*. 1992; Persson *et al*. 1997; Ragab *et al*. 1999; El-Kareem 2002; Xue 2003). These pathogens attack roots during the growing season causing substantial losses in yield (Moheshwari *et al*. 1983; Hwang *et al*. 1991; Tu 1991; Rauf 2000). Fungicides were the traditional seed treatment for controlling damping-off and root rot diseases for a long time. However, fungicidal treatments are hazardous to human health and increase environmental pollution. Therefore alternative fungicidal seed treatments are needed. Application of biological control using antagonistic microorganisms against seed and root rot pathogens have proved to be successful by efficiently controlling many diseases and improving vegetative growth and yield quality of many crops (Adams 1990; Farzana and Ghaffar 1991; Callan *et al*. 1997). Coating seeds of many crops with bio-control agents such *Trichoderma* spp., *Bacillus subtilis*, or *Psedomonas fluorescens* were the most effective treatments for controlling many seed- and soil-borne pathogens (Harman *et al*. 1989; Adams 1990; Lacicowa and Pieta 1996; Ragab *et al*. 1999; El-Kareem 2002; El-Mohamedy 2004; El-Mohamedy *et al*. 2006).

However, biological seed treatments may not provide adequate seed protection under all conditions as bio-protection may fail to establish on seed or in the rhizosphere at a sufficient level for disease control (Osburen and Scharoth 1988, 1989; Baird *et al*. 1994).

Seed priming, osmo-priming and solid matrix priming were used commercially in many horticultural crops as a tool to increase the speed and uniformity of germination and to improve the final stand (Osburen and Scharoth 1989; Charles 1991; Rowse 1996; Conway *et al*. 2001). However, if seeds are infected or contaminated with pathogens, fungal growth can be enhanced during priming, thus resulting in undesirable effects in plants (Nascimento and West 1998). Therefore, seed priming alone or in combination with a low dosage of fungicides and/or bio-control agents have been used to improve the rate and uniform emergence of seed and to reduce damping-off disease (Taylor *et al*. 1985; Callan *et al*. 1990, 1991; Baird *et al*. 1994; Conway *et al*. 2001; Jensen *et al*. 2001).

Bio-priming, a seed treatment that integrates biological and physiological aspects of disease control, has been used as an alternative method for controlling many seed- and soil-borne pathogens (Harman and Taylor 1988; Harman *et al*. 1989; Callan *et al*. 1990, 1991; Jahn and Puls 1998; Jensen *et al*. 2001; El-Mohamedy 2004; El-Mohamedy *et al*. 2006).

This study was conducted to study the causal organisms of damping-off and root rot diseases of pea in the Nobaria region, Egypt, and to evaluate different alternative fungicidal seed treatments i.e. bio-priming, priming and seed coating with bio-control agents as well as seed dressing with Rizolex-T (a fungicide) in the control of pea root rot pathogens and to improve plant growth and yield.

MATERIALS AND METHODS

Causal organisms

Samples of pea plants showing root rot symptoms were collected

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from different pea fields at three locations in Nobaria province, Egypt. All samples were subjected to isolation trials for the causal organisms. The purified isolated fungi were identified according to cultural and microscopically characters (Gilman 1957; Barnet and Hunter 1972; Nelson *et al*. 1983). The pathogenic ability of isolated fungi i.e., *Fusarium solani*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolsfsii*, and *Pythium* spp. was tested under greenhouse conditions. Surface-sterilized pea seeds of cv. 'Master-B' obtained from the vegetable research Department, National Research Center, were sown in plastic pots (20 cm diameter) containing sterilized sand loam soil artificially infested with individual inocula of each tested isolate, which was previously grown for two weeks on sand barley medium (1:1 (w/w) and 40% water). Ten pots, each containing five seeds, were used as replicates for each isolate as well as for the control treatment. Root rot disease incidence was recorded after 15 and 45 days from sowing date as the percentage of pre- and post-emergence damping off.

Greenhouse experiment

This experiment was carried out to evaluate the efficiency of different seed treatments (i.e., seed coating with bio-control agent, seed priming, seed bio-priming, as well as seed dressing with a fungicide, Rizolex-T) in the control of pea root rot pathogens under artificially infested soil.

Seed coating

Pea seeds were immersed for 30 min in 1% CMC (carpoxy methyl cellulose) suspension of *Trichoderma harzianum*, *Psedomonas fluorescens* and *Bacillus subtilis*. These biocontrol agents were previously isolated from the rhizosphere soil of healthy pea plants and the antagonistic ability against some root rot pathogens was recorded. Spore suspension of *T. harzianum* (3×10^4 cfu/ml) was prepared from 7-day old cultures grown on PDA medium as well as bacterial suspensions at 10^7 cells/ml prepared from 3 days-old culture grown on broth nutrient medium according to Sallam *et al*. (1978).

Seed priming

Pea seeds were primed according to methods described by Osburen and Scharoth (1989). Seeds which were initially washed with tap water to remove soluble exudates were primed in polyethylene glycol 8000 (PEG) (30.2 g/100 ml) in an Erlenmeyer flask on a rotary shaker set at 150 rpm. PEG was subsequently added (1:5 w/v) to seeds for 30 min to osmoticum. Seeds were shaken at 150 rpm for 72 h. Then seeds were rinsed twice with tap water, then dried at room temperature and used as primed seeds.

Seed bio-priming

A spore suspension of *T. harzianum* as well as cell suspensions of *P. fluorescens* and *B. subtilis* previously supplemented in CMC 1% solution were subsequently added individually to pea seeds during the priming process. These were then dried at room temperature and used as bio primed seeds.

Seed dressing

Pea seeds were dressed with Rizolex-T 50% WP at a recommended dose of 3 g/kg seed and used as the control treatment.

Plastic pots containing artificially infested soil with individual pathogenic fungi i.e., *F. solani*, *R. solani* and *S. rolfsii* were used. Ten pea seeds were sown in each pot, and 10 pots were used as replicates for each particular seed treatment.

The following seed treatments were prepared:

1) Seed bio-priming: primed seed were coated with *T. harzianum*, *P. fluorescens* and/or *B. subtilis*;
2) Seed coating: non-primed seeds were coated with *T. harzianum*, *P. fluorescens* and/or *B. subtilis*;
3) Seed dressing: pea seeds were dressed with fungicide (Rizolex-T 3 g/kg seed);
4) Seed priming: pea seeds were primed with PEG;
5) Control: untreated seeds.

The percentage root rot infection at pre-emergence, damping-off and post-emergence stages were recorded after 15 and 45 days from sowing. The percentage survival of plants was also calculated.

Field experiment

The most promising treatments against pea root rot pathogens under artificially infested soil in pot experiment were applied under field condition.

A field experiment was carried out during two successive seasons: 2005/2006 and 2006/2007 in a pea field with a history of damping off and root rot disease on an experimental farm of the National Research Center at Nobaria province, Beheria Government. Each experiment consisted of 48 plots (plot 3 m × 7 m). Each plot comprised 10 rows and 50 pits/hole/row, which were conducted in randomly complete block design with eight replicates (plots) for each particular treatment as well as a check treatment (control). The percentage pre-emergence damping-off after 20 days from sowing date as well as root rot infection after 45 and 60 days was recorded and the percentage of surviving plants in each particular treatment was calculated. Moreover, the beneficial effects of different types of seed treatments on vegetative growth and yield quality of pea plants were investigated.

A) Vegetative growth

Plants were randomly chosen at 45 days after sowing and the following data was recorded: Plant height (cm); number of leaves/plant; number of branches/plant; dry weight of shoots g/plant.

B) Green pods yield parameters

The harvest period was started on the 20th and 25th of January in 2006 and 2007, respectively. The total fresh pods from each plot were harvested, 3 times weekly and the first early yield (yield of the two weekly harvestings) per feddan (1 feddan (fed) = 4200 m^2) were calculated.

Average number of pods/plant and the average pod weight (g) were calculated.

C) Pod quality

Samples of twenty pods were taken from each experimental plot. The following were recorded: average pod length (cm); average pod diameter (cm); average number of seeds per pod; percentage of green seeds to total pod weight.

D) Nutritional value of seeds

1) Total soluble sugar (T.S.S.) percentage was determined with a hand rafractometer.
2) Total carbohydrates were determined using the method of Dubois *et al*. (1960).
3) Protein percentage was determined using the method of Yenu and Follard (1952).

Statistical analyses

All the data were statistically analyzed using one-way analysis of variance (ANOVA). ANOVA was performed according to Steel and Torrie (1980) using a general linear procedure using SAS software. Significant treatment differences were evaluated by using Duncan's multiple range test at $P = 0.05$.

RESULTS

The causal organisms

Sixty-five fungal isolates representing five species belonging to four genera, i.e., *Fusarium*, *Rhizoctonia*, *Sclerotium* and *Pythium* were isolated (**Table 1**). The most dominant pathogen was *Fusarium solani* (38.4%, 25 isolates) followed by *R. solani* (26.1%, 17 isolates) and *S. rolfsii* (26.1%, 17 isolates). *F. oxysporum* and *Pythium* spp. were less frequent

Table 1 Frequency (%) of fungi isolated from roots of pea plants showing root rot infection at Nobaria region.

Nobaria location	*Fusarium solani*	*Rhizoctonia solani*	*Sclerotium rolfsii*	*Fusarium oxysporum*	*Pythium* spp.	Total
El-Bostan	38.4 (25*)	26.1 (17)	18.5 (12)	10.8 (7)	6.1 (4)	100.0 (65)
El-Essraa	38.4 (25)	26.1 (17)	18.5 (12)	10.8 (7)	6.1 (4)	100.0 (65)
El-Emam Malek	38.4 (25)	26.1 (17)	18.5 (12)	10.8 (7)	6.1 (4)	100.0 (65)
Total %	38.4 (25)	26.1 (17)	18.5 (12)	10.8 (7)	6.1 (4)	100.0 (65)

*Total number of isolates

Table 2 Pathogenic ability of isolated fungi to induce root rot incidence on pea plants sown in artificially infested soil in greenhouse.

Fungal isolate	Root rot disease incidence (%)		Plant survival (%)
	Pre-emergence stage	Post- emergence stage	
Fusarium solani (1)	57.5 ab	82.3 d	17.6
Fusarium solani (2)	65.0 a	100.0 a	0.0
Rhizoctonia solani	50.0 cd	80.0 b	20.0
Sclerotium rolfsii	55.5 bc	55.5 c	44.4
Fusarium oxysporum	40.0 e	41.7 cd	58.3
Pythium spp.	45.0 ed	36.3 d	63.6
Control	5.0 f	10.5 e	89.4

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

Table 3 Pre-emergence damping-off on pea plants as affected by different types of seed treatment under artificially infested soil in greenhouse.

Type of seed treatment	% Pre-emergence damping-off 15 days after sowing					
	F. solani	% Reduction	*R. solani*	% Reduction	*S. rolfsii*	% Reduction
Seed bio-priming						
T. harzianum	17.5 f	58.8	25.0 cd	50.0	17.5 d	50.0
P. fluorescens	15.0 fg	64.7	20.0 d	60.0	15.0 d	57.1
B. subtilis	10.0 h	76.5	12.5 e	75.0	10.0 e	71.4
Seed-coating						
T. harzianum	30.0 c	29.4	35.0 b	30.0	25.0 bc	28.5
P. fluorescens	22.5 de	47.1	27.5 c	45.0	25.0 bc	28.5
B. subtilis	20.0 ef	52.9	29.0 c	45.0	20.0 cd	42.8
Seed dressing						
Rizolex-T (3 g/kg seed)	25.0 d	42.0	20.0 d	60.0	20.0 cd	42.8
Seed priming	37.5 b	11.7	45.0 a	10.0	30.0 ab	14.2
Control (non-treated seeds)	42.5 d	0.0	50.0 a	0.0	35.0 a	0.0

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

Table 4 Root rot disease and survival of pea plants as affected by different types of seed treatment under artificially infested soil in greenhouse.

Type of seed treatment	% Root rot disease 45 days after sowing						Survival plants (%)		
	F. solani	% Red.	*R. solani*	% Red.	*S. rolfsii*	% Red.	*F. solani*	*R. solani*	*S. rolfsi*
Seed bio-priming									
T. harzianum	48.4 e	46.9	40.0 d	47.3	36.3 de	41.1	42.5	45.0	52.5
P. fluorescens	41.1 ef	54.9	31.2 e	58.9	32.3 ef	47.4	50.0	55.0	57.0
B. subtilis	36.1 f	60.4	25.7 e	66.1	30.5 f	50.4	57.5	65.0	62.5
Seed-coating									
T. harzianum	71.4 b	21.7	53.8 c	29.2	46.6 c	24.2	20.0	30.0	40.0
P. fluorescens	67.7 bc	25.8	48.2 c	36.5	40.0 d	34.9	25.0	37.5	45.0
B. subtilis	56.2 d	38.4	41.3 d	45.6	31.2 ef	49.2	35.0	42.5	55.0
Seed dressing									
Rizolex-T (3 g/kg seed)	63.3 cd	30.6	40.6 d	46.5	31.2 ef	49.2	27.5	47.5	55.0
Seed priming	84.0 a	7.4	63.1 b	10.3	53.5 b	13.0	10.0	17.5	32.5
Control (non-treated seeds)	91.3 a	0.0	76.0 a	0.0	61.5 a	0.0	5.0	12.5	25.0

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test. Red. = Reduction

(10.8 and 6.1%, respectively).

The ability of *F. solani* (isolates no. 1 and 2), *R. solani*, *F. oxysporum* and *Pythium* spp. to induce root rot of pea plants was studied. All fungal isolates were able to cause root rot infection to different degrees at both pre- and post-emergence stages of pea plants (**Table 2**). *F. solani* isolate 2 caused a highly significantly effect at pre- and post-emergence stages with a 65.0 and 100% rate, respectively while *R. solani* and *S. rolfsii* cause 50.0-80.0 and 55.5% root rot disease in pea plants at pre- and post-emergence stages, respectively. Meanwhile, a lower root rot disease incidence was recorded with *F. oxysporum* and *S. rolfsii*, 40.0 and 45.0%, and 41.7 and 36.0% at pre- and post-emergence stages, respectively.

Greenhouse experiment

All types of seed treatments suppressed the incidence of root rot at the pre-emergence stage (15 days after sowing) compared with priming and control treatments (**Table 3**). The most effective type of seed treatment was bio-primed seeds that were coated with *B. subtilis*, those coated with the same bio-agent as well as seed dressed with fungicide treatment. The three treatments reduced pre-emergence caused by *F. solani*, *R. solani* and *S. rolfsii* by 76.5, 52.9 and 42.0%, 75.0, 45.0 and 60.0% and 71.4, 42.8 and 42.8%, respectively. Coated primed and non-primed seeds with *T. harzianum* reduced root rot disease caused by the same pathogens estimated at 64.7 and 47.1%, 60.0 and 45.0%, and 57.1 and 28.5%, respectively. Meanwhile, primed and non-primed pea seeds coated with *P. fluorescens* had a marked effect on disease control at the pre-emergence stage. The highly protective effect of all test treatments was recorded in the case of *F. solani* and/or *R. solani* followed by *S. rolfsii*.

The same trend was observed 45 days after sowing when bio-priming seed treatments were applied, leading to a highly significantly control of root rot disease caused by *F. solani*, *R. solani* and *S. rolfii* (**Table 4**). Moreover, high levels of healthy, surviving plants were observed following the application of these treatments. Coating seeds with either *B. subtilis* or *T. harzianum*, i.e. bio-primed seeds, was the most effective seed treatment in reducing root rot disease incidence caused by *F. solani*, *R. solani* and *S. rolfii* 45 days after sowing by 60.4 and 54.9%, 66.1 and 58.9%, and 50.4 and 47.4%, respectively. Seed coating with *B. subtilis* or *T. harzianum* treatments reduced root rot disease incidence by 38.4 and 25.8%, 45.6 and 36.5% and 49.2 and 34.9% caused by the same three pathogens, respectively. Some, but not all, bio-priming and seed coating treatments were superior to seed treatment with a fungicide (Rizolex-T) (**Tables 3, 4**).

Since greenhouse pot trials provided promising results the same treatments were applied to field conditions during two successive seasons, 2005/2006 and 2006/2007, to assess the control of pea root rot disease in a practical environment.

Field experiments

The effects of different types of seed treatments i.e., seed bio priming, seed coating with either *B. subtilis* or *T. harzianum* as well as seed dressing with fungicide treatment on the control of root rot disease of pea under field conditions was studied. Moreover, the effect of these treatments on vegetative growth and yield quality of pea was also investigated.

1. *Influence on pea root rot disease control*

Coating primed seeds with either *B. subtilis* or *T. harzianum* strongly reduced root rot incidence at pre- and post emergence stages, resulting in high survival percentages (**Table 5**). These treatments reduced root rot at the pre-emergence stage reach to 83.3 and 72.7% during the 2005/2006 season for *B. subtilis* or *T. harzianum*, respectively and 84.5 and 77.1% during 2006/2007 season. These values decreased further when seed coating and seed dressing with Rizolex-T treatments were applied (**Table 5**). After 45 and 60 days from sowing pea root rot was further reduced during both seasons. No significant differences were recorded between seed coating treatments and fungicide seed treatment.

2. *Influence on pea vegetative growth*

Bio priming, seed coating with either *T. harzianum* or *B. subtilis* and fungicide (Rizolex-T) treatments clearly stimulated the vegetative growth of pea plants during both seasons when compared with priming and control treatments (**Table 6**). Coated primed seeds with *B. subtilis* was the most superior treatment, recording the greatest plant height, average number of leaves/plant, average number of bran-

Table 5 Root rot disease and survival plants of pea plants as affected by different types of seed treatments under field conditions in Nobaria region during 2005/2006 and 2006/2007 seasons.

Season	Type of seed treatment	% Root rot disease incidence						% Plant survival
		Pre-emergence after 15 days	% Reduction	Post-emergence after				
				45 days	% Reduction	60 days	% Reduction	
2005/2006 Season	**Seed bio-priming**							
	T. harzianum	9.0 d	72.7	7.5 c	72.2	6.0 c	67.6	77.5
	B. subtilis	5.5 e	83.3	5.5 c	79.6	4.5 c	75.7	84.5
	Seed coating							
	T. harzianum	17.0 b	48.4	14.5 b	46.3	10.5 b	43.2	58.0
	B. subtilis	14.0 bc	57.5	11.5 b	57.4	8.5 b	54.1	66.0
	Seed dressing							
	Rizolex-T (3 g/kg seed)	13.0 c	60.6	12.5 b	53.7	10.0 b	45.9	64.5
	Seed priming	30.0 a	9.0	25.0 a	7.4	18.0 a	2.7	27.0
	Control (non-treated seeds)	33.0 a	0.0	27.0 a	0.0	18.5 a	0.0	21.5
2006/2007 Season	**Seed bio-priming**							
	T. harzianum	6.5 c	77.1	5.5 c	76.6	6.0 c	70.0	82.0
	B. subtilis	4.5 c	84.5	4.0 c	82.9	4.0 c	80.0	87.5
	Seed coating							
	T. harzianum	12.5 b	56.5	11.5 b	51.1	11.0 b	45.0	65.0
	B. subtilis	11.0 b	61.4	10.0 b	57.4	9.0 b	55.0	70.0
	Seed dressing							
	Rizolex-T (3 g/kg seed)	10.5 b	63.1	10.0 b	57.4	8.5 b	57.5	71.0
	Seed priming	25.5 d	10.5	22.0 a	6.4	19.0 a	5.0	33.5
	Control (non-treated seeds)	28.5 a	0.0	23.5 a	0.0	20.0 a	0.0	28.0

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

Table 6 Effect of type of seed treatment on vegetative growth of pea plants under field condition during 2005/2006 and 2006/2007 seasons at Nubaria region.

Season	2005/2006 Season				2006/2007 Season			
Growth parameter / Type of seed treatment	Plant height (cm)	Av. № of leaves /plant	Av. № of branches/ plant	Dry weight of shoots/plant (g)	Plant height (cm)	Av. № of leaves/ plant	Av. № of branches/ plant	Dry weight of shoots/plant (g)
Seed bio-priming								
T. harzianum	69.0 a	29.3 a	4.5 b	16.5 ab	58.6 a	33.0 a	4.5 a	15.0 a
B. subtilis	73.3 a	33.3 a	5.3 a	18.2 a	60.3 a	34.7 a	5.0 a	15.2 a
Seed coating								
T. harzianum	48.3 c	23.3 c	2.7 c	14.5 bc	54.7 b	26.0 b	3.5 b	13.2 d
B. subtilis	57.0 b	27.3 b	4.2 bc	15.3 b	57.3 ab	28.0 b	4.0 b	14.4 b
Seed dressing								
Rizolex-T (3 g/kg seed)	45.3 cd	25.7 cd	3.8 c	15.5 b	53.7 bc	26.7 b	3.7 b	14.0 b
Seed priming	47.3 cd	22.7 cd	2.2 d	14.2 bc	51.7 c	22.0 c	3.5 e	12.8 de
Control (non-treated seeds)	43.0 d	21.0 d	1.8 d	11.5 e	49.7 e	18.7 e	3.0 e	12.3 e

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

Table 7 Effect of type of seed treatment on some yield parameters of pea plants under field condition during 2005/2006 and 2006/2007 seasons at Nubaria region.

Season	2005/2006 Season					2006/2007 Season				
Growth parameter / Type of seed treatment	Av. № of pods /plant	Av. pod weight (g)	Early yield Kg/Fed	Total pods yield		Av. № of pods /plant	Av. pod weight (g)	Early yield Kg/Fed	Total pod yield	
				Ton/ fed	Increase %				Ton/fed	Increase %
Seed bio-priming										
T. harzianum	7.8 a	5.1 a	1126 a	4.7 b	51.2	9.3 ab	4.0 a	590 a	5.4 a	42.1
B. subtilis	8.0 a	5.3 ab	1180 a	5.3 a	70.9	9.7 a	4.2 a	625 a	6.2 a	63.2
Seed coating										
T. harzianum	6.0 b	4.7 c	535 c	3.8 d	22.6	7.7 d	3.5 bc	513 b	4.5 cd	18.4
B. subtilis	6.8 bc	5.1 bc	895 b	4.1 c	32.2	9.0 b	3.8 ab	553 bc	4.8 b	26.3
Seed dressing										
Rizolex-T (3 g/kg seed)	6.7 c	4.9 de	884 b	4.1 c	32.3	8.2 c	3.7 bc	523 bc	5.0 bc	31.6
Seed priming	6.0 d	4.6 d	520 c	3.3 d	6.5	7.3 e	3.5 bc	428 c	4.0 de	5.2
Control (non-treated seeds)	5.5 e	4.2 d	500 c	3.1 e	0.0	7.0 e	3.3 c	400 e	3.8 e	0.0

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

Table 8 Parameters of pods yield quality of pea plants as affected by different types of seed treatment under field condition during 2005/2006 season at Nubaria region.

Yield quality / Type of seed treatment	Av. length of pod (cm)	Av. pod diameter (cm)	Av. № of seeds/pod	% green pod seeds to pod weight	TSS %	Total carbo-hydrates %	Total protein %
Seed bio-priming							
T. harzianum	7.0 a	1.7 a	5.4 a	54.3 a	6.82 a	3.22 a	4.3 a
B. subtilis	7.3 a	2.0 a	5.4 a	55.8 a	7.08 a	3.40 a	4.7 a
Seed coating							
T. harzianum	7.0 b	1.7 a	5.0 a	48.5 b	5.77 c	2.83 b	3.9 b
B. subtilis	7.0 b	1.8 a	5.3 a	52.3 a	6.49 b	2.97 b	4.1 a
Seed dressing							
Rizolex-T (3 g/kg seed)	7.0 a	1.7 a	5.1 a	49.2 b	6.24 b	2.91 b	3.9 b
Seed priming	6.7 b	1.5 b	4.6 b	46.7 c	5.62 c	2.78 c	3.6 c
Control (non-treated seeds)	6.5 b	1.4 b	4.2 b	45.5 c	5.50 c	2.70 c	3.6 c

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

Table 9 Parameters of yield quality of pea plants as affected by different types of seed treatment under field condition during 2006/2007 season at Nubaria region.

Yield quality / Type of seed treatment	Av. pod length (cm)	Av. pod diameter (cm)	Av. № of seeds/pod	% of green pod seeds to pod weight	TSS %	Total carbo-hydrates %	Total proteins %
Seed bio-priming							
T. harzianum	7.0 a	1.9 a	5.4 a	53.2 a	6.97 a	3.1 a	4.5 a
B. subtilis	7.0 a	2.0 a	5.5 a	54.7 a	7.29 a	3.3 a	4.9 a
Seed coating							
T. harzianum	6.5 a	1.6 a	5.1 a	48.5 b	6.93 a	3.0 a	4.2 a
B. subtilis	6.8 a	1.8 a	4.9 a	51.2 b	6.69 a	3.1 a	4.1 a
Seed dressing							
Rizolex-T (3 g/kg seed)	6.7 a	1.7 a	5.4 a	49.0 b	6.30 a	3.0 a	3.9 b
Seed priming	6.0 b	1.4 b	4.3 b	46.2 c	5.63 b	2.7 b	3.8 b
Control (non-treated seeds)	5.8 b	1.4 b	4.1 b	44.5 c	5.34 b	2.7 b	3.6 b

Values in a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

ches/plant as well as dry weight of shoots/plant in both seasons.

3. *Influence on early and total green pods of pea plants*

All seed treatments, except for priming, significantly increased the early and total green pods as well as average number of pods/plant and average pod weight (**Table 7**). Bio-priming treatments gave significantly higher values than all other treatments in both seasons. These treatments consequently resulted in an increase in total pod yield in both seasons.

4. *Influence on characters and chemical content of green pod of pea plant*

Bio-priming and seed coating with either *T. harzianum* or *B. subtilis* treatments significantly enhanced the quality and chemical contents of green pods (**Tables 8, 9**) during both seasons, specifically TSS, total carbohydrates and total protein. These treatments also resulted in an increasing in values of pod quality such as length, pod diameter, and number of seeds/pod. Fungicide (Rizolex-T) treatment enhanced both pod quality and the chemical content of green pods compared with priming and control treatments.

DISCUSSION

Root rot is the most important disease affecting pea plants during the growing season, causing substantial yield losses (Moheshwari *et al.* 1983; Abda *et al.* 1992; Persson *et al.* 1997). Results in the present study proved that four genera of fungi i.e., *Fusarium* spp. (*Fusarium solani* and *F. oxysporum*) *Rhizoctonia solani*, *Sclerotoium rolfsii* and *Pythium* spp. were isolated from roots of pea plants showing symptoms of root rot infection in Nobaria province, Egypt. All isolated fungi were able to induce root rot on pea plants in greenhouse experiments with *F. solani* and *R. solani* being the most frequent and severe fungi. These results are similar to those reported by Ragab *et al.* (1999) and Abd El-Kareem (2002), who noted that *F. solani* and *R. solani* were the most severe pathogens of pea plants in Egypt. Meanwhile, Abda *et al*. (1992), Persson *et al*. (1997) and Xue (2003) attributed the incidence of pea root rot to many soil-borne fungi, namely *F. oxysporum*, *F. solani*, *S .rolfsii* and *Pythium* spp.

Recently many types of seed treatments such hydration pre-sowing seed priming (Khan *et al.* 1992; Rowse 1996), seed coating with bio-control agents (Ragab *et al*. 1999; We 2000; Conway *et al*. 2001; Abd El-Kareem 2002) and bio-priming seed treatments (El-Mohamedy 2004; Jensen *et al.* 2004; El-Mohamedy *et al*. 2006) have been considered as environmentally acceptable alternatives to existing fungicide seed treatments.

The results from greenhouse trials indicate that pea seeds coated with *T. harzianum*, *B. subtilis* and/or *P. fluorescens* decreased pea root rot caused by *F. solani*, *R. solani* and *S. rolfsii* more than fungicide seed treatment (Rizolex-T at 3 g/kg seed, the recommended dose). In addition, combined treatments, i.e. seed coating with these bio agents and bio-priming resulted in even higher levels of inhibition of root rot incidence at pre- and post-emergence stages of all tested pathogens compared with other treatments (**Tables 3, 4**). These results are similar to those reported by Callan *et al.* (1990, 1991), Farzana and Ghaffar (1991), Baird *et al.* (1994), Laciowa and Pieta (1996), Conway *et al*. (2001) and Xue (2003), who used bio priming as a technique of seed treatment to control many seed and soil-borne plant pathogens. Suppression of seed- and soil-borne pathogens of bio-primed seeds is related to the rate of reduction of the incidence of seed colonization by the pathogens due to reduced seed exudation of nutrients from primed seeds, thus overcoming chilling injury (Khan 1992), reducing the germination time and increasing thiol protease that is needed for germination (We 2000); bio agents also show a direct antagonistic ability against pathogens by eliminating pathogens that colonize seed or roots of plants (Taylor *et al*. 1985; Osborn and Scharoth 1988; Waller 1988).

Under field conditions during two seasons, bio-primed treatments of pea seed caused a highly significant reduction in root rot disease incidence (**Table 5**) compared with non-primed pea seeds that were coated with either *T. harzianum* or *B. subtilis*. This may be due to the failure of bio agents to bio-protect at specific levels of disease control; moreover, non-primed seeds might release high level of exudates during germination that stimulates pathogen growth (Osborn and Scharoth 1988, 1989; Harman *et al*. 1989; Nascimenta and West 1998; Conway *et al*. 2001). In contrast bio-priming has great promise for enhancing the efficiency, shelf-life and mass multiplication of bio-control agents in the rhizosphere soil (Callan *et al*. 1990, 1991; Jensen *et al.* 2004; El-Mohamedy *et al*. 2006).

In this study, bio-priming seed treatments and seed coating with either *T. harzianum* or *B. subtilis* caused a significant increase in vegetative growth parameters of pea plants (**Table 6**), early green pod yield, total green pod (**Table 7**) and resulted in high values of yield quality such as pod length, pod diameter, number of seeds/pod and high values of chemical content of green pods such as TTS, total carbohydrate and total protein (**Tables 8, 9**) compared to other treatments during two successive seasons. These results are supported by those of several groups (Harman *et al*. 1989; Callan *et al.* 1990, 1991; We 2000; Shegand and Huang 2001; El-Mohamedy 2004; El-Mohamedy *et al*. 2006), who noted that *B. subtillus* caused an increase in growth and nutrient uptake; this may also be related to its ability to produce hormones, especially IAA and auxins. The increasing in plant growth parameters due to bio-priming and seed coating treatments may be due to the effect of the bio-priming process on physiological and metabolic activities of pea plants. The enhancing effect of bio-priming on vegetative growth parameters of pea plants might be attributed to its efficiency in supplying growing plants with biologically fixed nitrogen, dissolved immobilized induce exudates of some hormonal substances such as gibberellic acid, cytokinins and auxins which can stimulate nutrient absorption as well as photosynthetic processes, which subsequently increased plant growth (Benhamou *et al*. 1996; Xi *et al*. 1996; Xue 2002). Moreover, these treatments suppress root rot disease, leading to healthier plants (and a greater survival; **Table 5**). Moreover, these treatments resulted in increasing in the quality values of green pods such average of length, diameter, number of seeds/pod and the percent of green pod seeds to pod weight as well as chemical content of green pods such T.S.S, total carbohydrates ant total protein. These results may be due to high vegetative growth and a reduction of disease incidence led to high plant vigor's that gave high green pod with high yield quality. These results in accordance with (Loeffez *et al*. 1986; Windham *et al*. 1986; Adams 1990; Khan *et al*. 1992; Callan *et al*. 1997; Abd El-Kareem 2002).

CONCLUSIONS

Bio-priming seed treatments can provide a high level of protection against root rot disease of pea plants. This protection was generally equal or superior to the control provided with fungicide seed treatment. It can thus be concluded that bio-priming (combined treatments between seed priming and seed coating with bio control agents) may be safely used commercially as a substitute for traditional fungicide seed treatments for controlling seed- and soil-borne plant pathogens.

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