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Biological Control of Green Bean Damping-off Disease Caused by *Rhizoctonia solani* by *Streptomyces parvulus* Strain 10d

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THIS STUDY aimed to evaluate the efficacy of applying spores and chitinase enzyme of *Streptomyces parvulus* strain 10d in biological control of damping-off disease caused by *Rhizoctonia solani* in green beans (*Phaseulus vulgaris*) and compare it with Rhizolex fungicide application. Five seeds of green bean were sown in plastic pots, filled with either sterilized or non-sterilized soils, infested with *R. solani* inoculum (5g/kg soil). Pots were kept in the greenhouse for 45 days from sowing. Pre-emergence damping-off was recorded 15 day from planting and post-emergence damping-off and survival rate were recorded 30 days from planting. Plants growth characteristics were recorded 45 days of sowing.

Results showed that Rhizolex treatment had the best survival rate and lowest root–rot severity (79 and 25%), followed by crude enzyme treatment (76 and 25%), then spores' treatment (75 and 27%, respectively), in infested and non-sterilized soils.

For plant growth characteristics, in infested and non-sterilized soil, spores' treatment had the best effect on plant height (41.5cm), followed by crude enzyme (39.5cm) and Rhizolex treatments (34.6cm). Numbers of leaves/plant were the highest in Rhizolex (17), followed by spores (15) and enzyme treatments (14). Numbers of pods/plant were 14 in Rhizolex treatment and 11 in crude enzyme and spores' treatments. The highest dry weight was recorded in spores' treatment followed by Rhizolex treatment (10 and 7 g/plant, respectively).

Conclusions: results suggest that using chitinolytic *Streptomyces* strain 10d for the biological control of *R. salani* and damping-off disease of green bean plants can be an attractive alternative for pesticides in organic agriculture.

Keywords: Biological control, Chitinase, Damping-off, Green beans, *Phaseulus vulgaris*, *Rhizoctonia solani*, Rhizolex, *Streptomyces parvulus*.

Introduction

Damping-off disease can occur on any herbaceous crops grown from seed, including vegetables, ornamentals, and field crops. Seeds, seedlings and young plants may be affected, resulting in poor stands in home gardens, greenhouses, and commercial fields. Losses due to damping-off can be severe, especially when cool, wet weather prevails at seeding or seed emergence. The disease is mostly caused by fungi, and most commonly by *Rhizoctonia* or *Pythium, Botrytis*, *Sclerotinia,* and *Alternaria* are also occasionally responsible for damping-off (Chung et al., 2003).

The cell wall structure of these fungi, like most of true fungi, is composed largely (~22 to 44%) of chitin, a polymer of unbranched chains of β-1,4-linked sugar (*N*-acetylglucosamine, GlcNAc) residues, in addition to other polysaccharides (Patil et al., 2000). It is associated with glucan molecules in form of microfibrils, which are embedded in an amorphous matrix and provide the framework in cell wall morphology (Ravikumar & Perinbam,

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2016). Glucan and chitin form intrachain hydrogen bonds and can assemble into fibrous microfibrils that form a basket-like scaffold around the cell. This branchedβ-(1,3): β-(1,6) glucan is bound to proteins and/or other polysaccharides, whose composition may vary with the fungal species (Gow et al., 2017). Degrading chitin in the cell wall of plant pathogenic fungi, e.g. by using chitinase enzymes, has been adopted as biological control procedure to control these pathogens (Iqpal & Anwar, 2019).

Chitinase enzymes occur in a wide range of organisms including viruses, bacteria, fungi, insects, plants and animals. The physiological functions of chitinases in these organisms are diverse. In plants, most chitinases are induced by stress factors such as infection by pathogens containing chitin and are considered pathogens related proteins (Krishnaveni et al., 1999; Regalado et al., 2000). Moreover, the insect molting enzyme, chitinase, has been described from *Bombyx mori* (silkworm), *Manduco sexta* (Tobacco hawk moth) and several other species (Patil et al., 2000). The role of these enzymes in antifungal activity and biocontrol has been the subject of studies (Mathivanan et al., 1998; Zhu et al., 2008; Thiagarajan et al., 2011).

In bacteria chitinases play a role in nutrition by degrading chitin, which is used as a source of both carbon and nitrogen by the cell (Wiwat et al., 2002). Actinomycetes, particularly *Streptomyces* sp., including *S. antibioticus, S. griseus, S. plicatus, S. lividans, S. aureofaciens, S. halstedii,* and *S. thermoviolaceus*, possess potential production of chitinolytic enzymes (Taechowisan et al., 2003; Joo, 2005; Narayana & Vijayalakshmi, 2009).

The present research was carried out to study the role of *Streptomyces parvulus* strain and culture filtrate as a biological agent in controlling *Rhizoctonia solani*, the causative agent of damping-off of green bean plants (*Phaseulus vulgaris*).

Materials and Methods

Microorganism

Streptomyces parvulus strain 10d was previously isolated and identified by the authors of this work (Korayem et al., 2012) and chosen for its high chitinase activity. Fungal pathogen *Rhizoctonia solani* was obtained from the department of Plant Pathology, Faculty of Agriculture, Ain Shams University.

Seeds

Green bean seeds (*Phaseolus vulgaris* var. *valentino*) were provided from Agricultural Research Center, Giza Governorate, Egypt.

Phytotoxicity effect of cultural filtrate of Streptomyces parvulus strain 10d

To detect if the *Streptomyces parvulus* strain 10d has any phytotoxicity for green bean seeds germination, twelve seeds of green bean plants were put in Petri dishes (9.0cm diameter) between two sterile filter papers (No.1), moistened with several dilutions of cultural filtrate (described below) in distilled water, i.e. 50.0, 25.0, and 12.5%, The control was moistened with sterile distilled water. All treatments were incubated in dark at 30±2°C for 4 days, after which, numbers of germinated seeds were counted (Bordoloi et al., 2001).

Fungal filtrate was prepared as follows: Fresh culture of *Streptomyces parvulus* strain 10d was used to inoculate 250ml conical flask containing 100ml Starch-nitrate liquid medium (Waksman & Lechevalier, 1961), incubated at 25±2°C for 14 days (Hajieghrari, 2010). After incubation, the culture filtrates were filtered into pre sterilized conical flasks using Whatman no. 1 filter paper. The filtrates were stored in a refrigerator at 4±2°C

Use of S. parvulus strain 10d for the biocontrol of damping-off disease in green bean plants

A pot experiment was carried out during spring season in the greenhouse of Faculty of Agriculture, Ain Shams University, to test the efficacy of applying *Streptomyces* spores or crude enzyme for the biocontrol of green bean plants damping-off caused by *Rhizoctonia solani*.

Plastic pots (25cm diameter) were filled with either sterilized or non-sterilized soil and infested with *R. solani*-inoculum (at 5g/kg soil). Pots with infested soil were irrigated and kept for 3 days before seed sowing.

Green bean seeds were surface-sterilized by soaking in 2% sodium hypochlorite for 1min. before sowing. Five seeds were sown in each pot, with 5 replicates for each treatment. Pots were kept in the greenhouse for 45 days of sowing.

Treatments

Each treatment had five replicates:

1. Non-infested soil(control).
2. Non-infested soil + crude enzyme (50 ml/ pot).
3. Non-infested soil+ *Streptomyces* spores (50ml/ pot).
4. Infested soil with *R. solani* (1% w/w). (control).
5. Infested soil with *R. solani* + Rhizolex (50ml/ pot at the rate of 1.5g L^{-1}).
6. Infested soil with *R. solani* + crude enzyme.
7. Infested soil with *R. solani* + *Strept.* spores (50ml/ pot).
8. Infested soil with *R. solani* + 1/2 Rhizolex + curd enzyme.
9. Infested soil with *R. solani* + 1/2 Rhizolex + *Strept*. spores (50ml/ pot).

Preparation of Rhizoctonia solani inoculum

Inoculum of *R. solani* was prepared, according to the method of Dhingera & Sinclair (1995), on moistened barley grain in 500ml glass bottle (each bottle contained 50g of barley grains and 40ml of tap water), autoclaved twice at 24hrs intervals for 30min. Bottles were aseptically inoculated with one-week old inoculum, grown on Potato dextrose agar medium (PDA), and incubated at 30±2°C for 2 weeks to colonize grain.

Disease assessment

Disease incidence

Pre-emergence damping-off was recorded 15 day after planting, while post-emergence damping-off and survival rate were recorded 30 days after planting. Percentage of pre-emergence damping-off was calculated according to the following formula:

Percentage of pre-emergence damping- off= (No. of non-emerged/ No. of sown seeds) X 100

The percentage of post-emergence damping-off of infected plants with root-rot was recorded 30 days after sowing according to the following formula:

Percentage of post emergence damping off= ((No. of emerged plants-No. of survived plants)/ No. of emerged plants) X 100

Survival rate

Survival rate was calculated as follows= 100 – (pre-emergence damping- off % + post-emergence damping-off %).

Disease severity

Rhizoctonia root-rot development was rated using an arbitrary scale described by Agrios (1997) as follows: 4= Plant dead, 3= 51 to 75% of leaves with symptoms; 2= 26 – 50% of leaves with symptoms; 1= < 25% of leaves with symptoms; and 0= No symptoms.

The disease rating was calculated by the following formula:

Disease index = ((Rating No. x No. of plants in the rating)/ (Total No. of plants X highest rating)) X 100.

Growth characteristics of green bean plants

After 45 days of sowing, growth characteristics [plant height (cm), number of leaves/plant, number of pods/plant and dry weight (g/plant)] of green bean plants were recorded.

Statistical analysis

Results were analyzed using Costat computer program V 6.303 statistical analysis (2004), according to Snedecor & Cochran (1991). LSD at 5% level as significance was used to differentiate between means.

Results and Discussions

Biological control of damping-off disease in green bean plants, caused by Rhizoctonia solani

Before investigating the efficacy of *Streptomyces* spores or its crude enzyme as biocontrol agent of green bean plants damping-off caused by *Rhizoctonia solani*, the fungal filtrate was tested for having phytotoxicity effect on the green bean seeds germination. Results of this experiment showed that the fungal cultural filtrate had no detectable phytotoxicity, that germination rate was 100% in both cultural filtrate and control treatments. This indicates that culture filtrate has no phytotoxicity effect on green bean seeds.

Streptomyces have been described as potent antagonists of fungal pathogens due to their abilities to produce extracellular chitinase enzymes, targeting the fungal cell wall for degradation (You et al, 1996; Mahadevan & Crowford, 1997). In this context, *Streptomyces parvulus* strain 10d (previously isolated by the same authors of this work and chosen for its high chitinolytic activity) and its culture filtrate were tested for their abilities to control *R. solani*, the causative agent of damping-off disease of green

bean plants. The incidence of pre- and post-emergence damping off, survival plants and root-rot severity as well as growth parameters of plants were recorded.

Data in Table 1 reveal that pre- and post-emergence damping-off in the three non-infested soil treatments (control + crude enzyme and +*Streptomyces* spores were significantly lower than those of other treatments.

In the *R. salani*-infested treatments, pre- and post-emergence damping-off were significantly higher in *R. salani*-infested treatment than all other treatments. Rhizolex-treated plants, in non-sterilized soil, had lower pre-emergence damping-off incidence (12%) than +crude enzyme (20%) and +spores' treatments (16%). On the other hand, there was no significant difference in post-emergence damping-off incidence between spores and Rhizolex treatments, while crude enzyme treatment gave much lower post-emergence incidence than the other two treatments.

These results were similar to those found by Sadeghi et al. (2006) who illustrated that treatment with *Streptomyces* formulation increased seed germination and inhibit *Rhizoctonia* damping-off completely.

Results also showed that *Streptomyces* spores was less effective in non-sterilized than sterilized soil. For instance, pre-emergence incidence in spores' treatment in non-infested soil was 16 and 4% in non-sterilized and sterilized soils, respectively. This might be due to the interaction between soil organisms that, which were absent in sterilized soil.

Moreover, results of post-emergence damping-off in infested non-sterilized soil showed that crude enzyme gave significantly low percentage (4%) followed by the 1/2 dose-Rhizolex + spores treatment (5%), while in infested sterilized soil, the lowest percentage (8%) was recorded for treatments of Rhizolex and *Streptomyces* spores.

Results of survival rate and root-rot severity (Table 2) are almost parallel to the results of pre- and post-emergence damping-off %. Rhizolex treatment gave the best survival rate and lowest root-rot severity (79 and 25%, respectively) in infested non-sterilized soil followed by crude enzyme treatment, giving 76 and 33%, respectively and *Streptomyces* spores treatment, giving 75 and 27%, respectively. In infested sterilized soil, *Streptomyces* spores treatment resulted in the highest survival rate and lowest root-rot severity, being 88 and 28%, respectively, followed by Rhizolex treatment, giving 80% survival rate and 32% severity.

TABLE 1. Incidence of damping-off disease (*R. solani*) of green bean plants in response to Rhizolex, *Streptomyces* spores and crude chitinase treatments.

Treatments	Disease incidence (%)			
	Pre-emergence damping-off (%)		Post emergence damping-off (%)	
	Non-sterilized soil	Sterilized soil	Non-sterilized soil	Sterilized soil
Non-infested soil	8^{g}	0^{g}	8^{d}	4^{f}
+Crude enzyme[2]	4^{h}	-	4^{f}	-
+ *St*. spores	4^{h}	-	9^{c}	-
Infested soil (*R.solani)*	40^{b}	56^{a}	12^{a}	17^{a}
+ Rhizolex[1]	12^{f}	12^{e}	9^{c}	8^{e}
+Crude enzyme	20^{d}	20^{b}	4^{f}	9^{d}
+ *St*. spores	16^{e}	4^{f}	9^{c}	8^{e}
+1/2 Rhizolex+ crude enzyme	32^{c}	20^{b}	10^{b}	10^{c}
+1/2 Rhizolex+ *St*. spores	44^{a}	16^{c}	5^{e}	14^{b}

1. The fungicide Rhizolex (1.5 g L^{-1}) was added at a rate of 50ml/pot.
2. Culture filtrate was added at a rate of 50ml/pot.

Means with same letters are insignificantly different ($P< 0.05$).

TABLE 2. Survival rate (%) and root-rot severity (%), as a result of damping-off disease (*R. solani*), of green bean plants in response to Rhizolex, *Streptomyces* spores and crude chitinase treatments.

Treatments	Survival rate (%)		Root-rot severity (%)	
	Non-sterilized	Sterilized	Non-sterilized	Sterilized
Non-infested soil	84^{c}	96^{a}	22^{g}	8^{g}
+Crude enzyme[2]	92^{a}	-	8^{i}	-
+ *St*. spores	87^{b}	-	12^{h}	-
Infested soil (*R.solani)*	48^{i}	27^{f}	81^{a}	77^{a}
+ Rhizolex[1]	79^{d}	80^{c}	25^{f}	32^{e}
+Crude enzyme	76^{e}	71^{d}	33^{d}	39^{d}
+ *St*. spores	75^{f}	88^{b}	27^{e}	28^{f}
+1/2 Rhizolex+ crude enzyme	58 ^{g}	70^{e}	49^{c}	50^{c}
+1/2 Rhizolex+ *St*. spores	51^{h}	70^{e}	52^{b}	61^{b}

1. The fungicide Rhizolex (1.5 g L^{-1}) was added at a rate of 50ml/pot.
2. Culture filtrate was added at a rate of 50ml/pot.

Means with same letters are insignificantly different (P< 0.05).

These results were similar those obtained by Sabaratnam & Traquair (2002) and Sadeghi et al. (2006) who reported that*Streptomyces* spores and enzyme treatment enhanced survival of tomato seedling and reduced damping off disease in infested soil. Another study revealed that a bioformulation containing *Streptomyces* sp. MOST-1 effectively controlled damping-off of Chinese Cabbage, providing 100 % suppression when it was applied at the rate of 10 g per 1 kg of potting material (Siripornvisal & Suddee, 2016).

The effect of *Streptomyces* spores and its crude enzyme on growth characteristics of green bean plants grown in sterilized and non-sterilized soils, infested with *Rhizoctonia solani,* are given in Tables 3 and 4. Data revealed that *Streptomyces* spores had the best effect on plant height in both non-sterilized and sterilized soil (41.5 and 38.08cm, respectively) followed by crude enzyme (39.52 and 34.22%, respectively), which also had the same effect as with non-infested plants. Number of leaves was recorded high in Rhizolex treatment (17 leaves/plant), followed by *Streptomyces* spores treatment (15 leaves/ plant) in non-sterilized soil, while in sterilized soil *Streptomyces* spores gave 19 leaves/plant followed by Rhizolex which gave 15 leaves/plant.

TABLE 3. Plant height and numbers of leaves as affected by damping-off disease in response to Rhizolex, *Streptomyces* spores and crude chitinase treatments.

Treatments	Plant height (cm)[1]		Number of Leaves[1]	
	Non sterilized	Sterilized	Non sterilized	Sterilized
Non-infested soil	34.92^{e}	27.60^{e}	14^{e}	16^{b}
+Crude enzyme[4]	**39.4**^{b}	-	19^{b}	-
+ *St*. spores[3]	**39.2**^{c}	-	20^{a}	-
Infested soil (*R.solani)*	**18.4**^{g}	23^{g}	7^{g}	6^{g}
+ Rhizolex[2]	**34.6**^{f}	33^{c}	17^{c}	15^{c}
+Crude enzyme	**39.52**^{b}	34.22^{b}	14^{e}	12^{f}
+ *St*. spores	**41.5**^{a}	38.08^{a}	15^{d}	19^{a}
+1/2 Rhizolex+ crude enzyme	**35.1**^{d}	30.9^{d}	12^{f}	13^{e}
+1/2 Rhizolex+ *St*. spores	**34.94**^{e}	24.9^{f}	12^{f}	14^{d}

1. Plant height and numbers of leaves were measured 30days after cultivation.
2. The fungicide Rhizolex(1.5 g L^{-1})was added at a rate of 50ml/pot.
3. *Streptomyces* spores were added at concentration of 15x10^{9}spore/plant.
4. Culture filtrate was added at a rate of 50ml/pot

TABLE 4. Number of pods and dry weight as a result of damping-off disease (*R. solani*) in response to Rhizolex, *Streptomyces* spores and crude chitinase treatments.

Treatment	Number of pods[1]		Dry weight (g/plant)[2]	
	Non sterilized	Sterilized	Non sterilized	Sterilized
Non-infested soil	12[c]	11[b]	4[g]	6.39[e]
+Crude enzyme[5]	12[c]	-	11[a]	-
+ *St*. spores	13[b]	-	10[b]	-
Infested soil (*R.solani)*	7[f]	4[g]	3[h]	4.48[f]
+ Rhizolex[3]	14[a]	12[a]	7[d]	6.91[d]
+Crude enzyme	11[d]	10[c]	6[e]	7.98[c]
+ *St*. spores[4]	11[d]	9[d]	10[c]	9.32[b]
+1/2 Rhizolex+ crude enzyme	9[e]	7[f]	5[f]	7.91[c]
+1/2 Rhizolex+ *St*. spores	7[f]	8[e]	6[e]	10.13[a]

1. Number of pods was measured after 45 days after cultivation.
2. Dry weight was determined after 45 days after cultivation.
3. The fungicide Rhizolex (1.5g L^{-1}) was added at a rate of 50ml/pot.
4. *Streptomyces* spores were added at concentration of $15x10^{9}$spore/plant.
5. Culture filtrate was added at a rate of 50ml/pot.

These results were similar to those recorded by Sadeghi et al. (2006) who reported that treatment with *Streptomyces* increased shoot and root dry weight and root density of sugar beet compared with control.Another study reported that application of a bioformulation containing *Streptomyces* sp. MOST-1 led to plant growthpromotion of Chinese cabbage (Siripornvisal & Suddee, 2016).

Regarding number of pods (Table 4), plants grown in non-sterilized soil and treated with Rhizolex alone had the largest number (14 pods /plant) followed by crude enzyme treatment (11 pods/plant). similarly, plants grown in sterilized soil had the largest number of pods/plant when treated with Rhizolex (12pods/plant) followed by crude enzyme and spore's treatments.

Infested plants, grown in non-sterilized soil, had the highest dry weight when treated with *Streptomyces* spores followed by Rhizolex (10 and 7g/plant, respectively), while in sterilized soil, plants treated with half-dose Rhizolex+ *Streptomyces* spores had the highest dry weight (10g/plant) followed by those treated with *Streptomyces* spores (9.3g/plant).

Generally, in non-sterilized soil, treating green bean plants with *Streptomyces* spores produced the highest plant height (41.5cm) and best dry weight (10g/plant), the second-best numbers of pods/plant (11 plant) and leaves/plant (15/plant). In sterilized soil, also *Streptomyces* spores gave the highest plant height (38cm), highest leaves number (19 leaves/plant), second best dry weight (9.3g/plant) and third order in number of pods (9 pods/plant).

Conclusions

The present study revealed the Rhizolex pesticide had better results in many aspects, e.g., pre- and post-emergence damping-off, survival rate, root-rot severity and number of pods/plant, over spores and crude enzyme treatments. However, it should be noted that applying chemical fungicides, although is an effective method to control *R. solani,*has some side effects, such as creating resistance and increasing contamination of environment, in addition to having adverse high toxicity on microbial communities and a degradative effect on the ozone layer. Therefore, we could say that using chitinolytic *Streptomyces* strain 10d or its crude chitinase for the biological control of *R. salani* may offer a possible alternative for pesticides, especially in organic agriculture for the control of plant diseases, particularly after enhancing its efficiency through biotechnological techniques.

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