International Journal of Agricultural Technology 2013 Vol. 9(3): 589-599
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Bio-priming seed treatment for biological control of soil borne fungi causing root rot of green bean (*Phaseolus vulgaris* L.)

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R.S.R. El-Mohamedy and M.A. Abd Alla (2013). Bio-priming seed treatment for biological control of soil borne fungi causing root rot of green bean (*Phaseolus vulgaris* L.). International Journal of Agricultural Technology 9(3):589-599.

Abstract Bio-priming seed treatment that integrates the biological and physiological aspects of disease control was used as alternative method for controlling soil borne pathogens of green been. Results indicate that *Fusarium solani , Rhizoctonia solani* and *Fusarium oxysporum* proved to be the most dominant isolated fungi from roots of green bean been plants infected with root rot disease in Noubaria province. Meanwhile, *Pythium* spp. , and *Sclerotium rolfsii* show less frequent. Pathogenicity test provided that the most aggressive fungi of bean were *F. solani* and *R. solani* followed by *F. oxysporum* . In vitro *Trichoderma harzianum, Psedomonas florescence and Bacillus subtilis* cause complete reduction of the linear growth of *F. salami, R. solani, F. oxysporum ,Pythium spp.* and *S. rolfsii* . In greenhouse trails, Bio-priming seed treatments successfully controlled green bean root rots caused by *F. solani , R.solani* and *F. oxysporum* ,as these treatments reduced root rot diseases up to 73.9 and 68.5 % at pre- and post -emergence stages. Meanwhile, coated bean seeds with *T. harzianum* or dressed with Rizolex-T caused a moderate effect in reducing root rots diseases incidence. It could be noted that practical using of bio-priming seed treatment to control root rot soil borne plant pathogens as a substitute of chemical fungicides is possible without any risk to human, animal and the environment.

Key wards: Bean-Bio-priming - Root rot- *Trichoderma harzianum* .Seed treatment

Introduction

Bean (*Phaseolus vulgaris* L.) is one of the most important leguminous crops due to high nutritional value .Green bods and seeds are used for fresh meal and food ,plant shoot system and low quality seeds are used for animal feeding .Green bean is attacked by certain soil borne pathogens i.e.,*Fusarium solani* Mart sacc., *Rhizoctonia solani* Kuhn ,*Fusarium oxysporum* , *Sclerotium rolfsii* and *Pythium spp* which attack roots causing damping-off and root rot diseases (Abdel-Kader, 1997; El-Mougy, 2001). These diseases cause

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substantial losses to beans crop, yield losses in severely infested areas may be as high as 50% (Estevez deJensen *et al*., 2001).

Control of soil borne pathogens depends mainly on fungicidal applications, that causing hazards to the human health and environmental pollution. Therefore, there are needed to alternative fungicidal treatments. Biological seed coating and bio-priming seed treatments are gaining importance in management of many plant pathogens as another alternative to chemical fungicides in recent times. Seed treatment with bio control agents along with priming agents may serve an important means of managing many soil borne diseases, this process often known as bio priming .The bio priming seed treatment developed for control of Pythium seed root of Ch2 sweet corn combines microbial inoculation with pre plant seed hydration (Callan *et al*., 1990, 1991; Conway *et al*., 2001).

Bio-priming as seed treatment that integrates the biological and physiological aspects of disease control was recently used as alternative method for controlling many seed and soil borne pathogens (Nayaka *et al*., 2008; Rao, 2009; El-Mougy and Abdel-Kader, 2008; El-Mohamedy, 2004; El-Mohamedy *et al*., 2006; El-Mohamedy and AbEl-Baky, 2008; Begum *et al*., 2010). Also, seed coating with bio-control agents was the most effective treatment for controlling root rot diseases (Abdel-Kader and Ashour, 1999; El-Mougy *et al*., 2007; El-Mohamedy and Abd-Elbakey, 2008; Sallam *et al*., 2008; Rojo *et al*., 2007). The present study aimed to study the causal agents of damping -off and root rot pathogens of green bean in Nobaria province, and to evaluate the integration between biological and physiological seed treatments into system termed bio priming in controlling soil borne fungi causing root rot of green bean under artificially infested soil in green house.

Materials and methods

Survey of the causal organisms and pathogenicity test

Samples of green bean plants showing root rot symptoms were collected from different green beans field of three locations in Nobaria region. All samples were subjected to isolation trials for the causal organisms. The purified isolated fungi were identified according to cultural and microscopically characters described by Gilman, 1957; Barnet and Hunter, 1971; Nelson *et al*., 1983. Number of each isolated fungus was recorded and the percentages of frequency of each location were calculated .Pathogenic ability of isolated fungi i.e., *Fusarium solani, Rhizoctonia solani, Fusarium oxysporium, Sclerotium rolsfsii,* and *Pythium* spp was tested under greenhouse conditions. Surface sterilized of green bean were sown in plastic pots (20cm diameter) containing

sterilized sand loam soil artificially infested with individually with the inoculums of each isolate tested ,which was previously grown for two weeks on sand barley medium (1:1w.w and 40% water). Ten pots each containing five seeds were used as replicates for each isolate as well as control treatment. Root rot disease incidence was noticed and recorded after 15 and 45 days from sowing date as percentage of pre- and post emergence damping off.

The effect of bio agents on growth of green bean root rot pathogens in vitro

The inhibitory effects of three bio agents i.e., *Trichoderma harzianum, Psedomonas florescence and Bacillus subtilis*, previously isolated from rhizospheric soil of healthy green bean plants during survey studies by the authors were tested against root rot fungi of green bean ,using dual culture technique (Ferrari *et al*., 1991). Mycelia disks (0.5 cm diam.) of 7 day old cultures of *Fusarium solan, Rhizoctonia solani, Fusarium oxysporium, Sclerotium rolfsii and Macrophomina phaseolinnae* were transferred singly to the center of Petri dishes (10 cm diam.) containing PDA medium. Four loop growth of each antagonistic bacteria from two days old nutrient broth cultures were placed at four corners of the plate in perpendicular positions. Disks of *T. harzianum* and disks of pathogenic fungi were placed were placed on opposite sides of Petri dishes containing PDA medium .Inoculated plates were incubated for 7 days at 25 C. Five plates for each replicate and four replicates for each test were used . Reduction of growth was calculated for pathogenic green bean root rot fungi using the following formula:

$$\text{Growth reduction \%} = \frac{\text{Growth in control – growth in treatment}}{\text{Growth in control}} \times 100$$

Control of root rot pathogens of green bean in green hose

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

Type Seed treatments

Seed coating with bio agents

Green bean seeds Giza 3 cv. were immersed for 30 min in suspension of

spore and /or cell suspension of each *Trichoderma harzianum, Psedomonas florescence* and *Bacillus subtilis*. These bio control agents were previously isolated from rhizosphere soil of healthy green bean plant and the antagonistic ability against some root rot pathogens was recoded . Spore suspension of *T. harzianum* ($3x10^4$ cfu /ml) was prepared from 7-daye old cultures grown on PDA medium as well as bacterial suspensions at concentration 10^7 cell/ml prepared from 3-days old culture grown on broth nutrient medium.

Seed priming

Green bean seeds Giza 3 cv. were primed according to methods described by Osbern and Scharuth 1989 .seeds were initially washed with tap water to remove soluble exudates . seeds were primed in polyethylene glycol 8000 (PEG)30.2 g/ $100ml^{-1}$ in Erlenmeyer flask on a rotary shaker set at 150 rpm. PEG was subsequently added (1:5 w/v) of seeds during 30 minutes to osmoticum. Seeds were shaken at 150 rpm for 72 hours. Then seeds were rinsed twice with tap water, then dried at room temperature and used as primed seeds.

Seed bio priming

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

Seed dressing

Green bean seeds were dressed with Rizolex –T 50% wp at recommended dos 3 g/ kg seed and used as comparison treatment.

Plastic pots containing artificially infested soil with the individually pathogenic fungus i.e., *F. solani, R. solani* and *F. oxysporum* at were used. Five green bean seeds were sowing in each pot, and ten pots were used as replicate for each particular seed treatment. The following different seed treatment used as follow. T1=-Seed bio priming (primed seed were coated individually with *T. harzianum , P. fluorecens and B. subtilis*). T2 =Seed coating(non primed seeds were coated individually with *T. harzianum ,P fluorecens. and B. subtilis*) .T3= Seed dressing(seeds were dressed with a fungicide Rizolez –T 3g/kg seed) T4 = Seed priming (seeds were primed with PEG). T5= Control (none treated seeds). After 15 and 45days from seed sowing percentage of root rot infection

at pre-and post-emergence damping off stages were recorded and the percentage of survival plants were also calculated in each treatment.

Statistically analysis

Tukey test for multiple comparisons among means was utilized. (Neler *et al*., 1985).

Results and disscusions

Causal organisms and pathogenicity test

Green bean plants showing root rot disease symptoms collected from different location at Nobaria province were used to isolate pathogenic fungi.

Results in Table (1) show that fifty fungal isolates representing belonging five species *i.e*. *Fusarium* solani (22 isolate), *Rhizoctonia solani* (12 isolate), *Fusarium oxysporum* (7 isolate), *Sclerotium rolfsii* (5 isolate) and *Pythium spp.* (4 isolate) were isolated .The most dominant fungi were *F. solani* (44.0 %) frequent and *R. solani* (24.0 %) followed by *F. oxysporum* (14.0 %). Meanwhile, *S. rolfsii* and *Pythium* spp. were less frequency (10.0 and 8.0%).

Table 1. Total* and Frequency (%) of fungi isolated from roots of green bean plants showing root rot infection in different lacation at Nobaria region

Nobaria /location	***Fusarium solani***	***Rhizoctonia solani***	***Sclerotium rolfsii***	***Fusarium oxysporium***	***Pythium spp***	**Total**
El-Bostan	45.4 (10)*	22.7 (5)	9.1 (2)	13.6 (3)	9.1 (2)	100 (22)
El-Essraa	38.4 (5)	23.1 (3)	15.3 (2)	15.3 (2)	7.6 (1)	100 (13)
El-Emam Malek	46.6 (7)	26.6 (4)	6.6 (1)	13.3 (2)	6.6 (1)	100 (15)
Total	44.0 (22)	24.0 (12)	10.0 (5)	14.0 (7)	8.0 (4)	100 (50)

Pathogenicity test proved that all tested fungal isolates were able to cause root rot infection on green bean plants with different degrees at both pre- and post-emergence stages. Root rot disease on green bean caused by *F. solani, R. solani* and *F. oxysporum* were significantly decrease under artificially infested soil in greenhouse. Results in Table (2) show that, *F. solani* and *R. solani* were the sever fungi in causing damping-off and root rot disease of green bean plants. *F. solani* and *R. solani* caused a highly significantly effect at pre- and post-emergence stages (55.0, 90.0 % and 40.0, 84.0%) respectively). While, *F.oxysporum, S. rolfsii* and *Pythium spp.* cause least percentages of root rot

incidence if compared with *F. solani*. As the least percent of survival plants were recorded with *F. solani*(10.0%), meanwhile, these records 93.0, 68.8, 64.0 and 46.4% with control (non infested soil) , *S .rolfsii* , *Pythium spp.* and *F. oxysporum* respectively .Many researchers noted that *Fusarium solani ,Rhizoctonia solani, and F. oxysporum* were the most dominant isolated fungi from infected roots of green plants collected from different cultivation region in Nobaria province. Meanwhile, *Sclerotium rolfsii* and *Macrophomina phaseolina* were less frequent. The most pathogenic fungi on green bean plants were *F. solani and R. solani* followed by *F.oxysporum*. Many investigators noted that *F. solani, R. solani, M. phaseolina, F. oxysporum* and *S. rolfsii* are considered among the main pathogens causing root rot diseases of green bean plants (Abdel-Kader, 1997; El-Mougy, 2001; El-Mougy *et al*., 2007).

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

Fungal isolate	**Root rot disease incidence %**		**Survival plants %**
	Pre-emergence stag	Post-emergence stage	
Fusarium solani	55.0 e	90.0 d	10.0 d
Rhizoctonia solani	40.0 d	84.0 d	16.0 d
Fusarium oxysporum	35.5 d	53.6 c	46.4 c
Sclerotium rolfsii	20.0 b	31.2 b	68.8 b
Pythium spp.	28.0 c	36.0 b	64.0 b
Control	5.0 a	7.0 a	93.0 a

Figures with the same letters are not significant (P = 0.05).

Testing of bio agents on growth of green bean root rot pathogens in vitro

The antagonistic ability of three bio agents i.e., *Trichoderma harzianum, Psedomonas florescence and Bacillus subtilis* , previously isolated from rhizospheric soil of healthy green bean plants during survey studies by the authors were tested against root rot pathogens of green bean PDA medium . Results in Table (2) show that al the tested bio agents were antagonistic to all tested pathogens with different degrees of inhibition .The complete reduction in the linear growth was found after 8 days of incubation with *F. solani R. solani and F. oxysporum*. Where the least reduction was recorded with *S. rolfsii and Pythium spp. T. harzainum* inhibited the linear growth of all tested pathogens by overcoming their growth in Petri-dishes .The inhibition in the growth of the pathogen could be attributed to antibiosis, hyperparasitism or production of chitinase and ß-1,3 glucanase enzymes which degrade the cell wall leading to lyses of mycelium of the pathogen (Windhan *et al*., 1986; Adams, 1990).

Table 3. The percentage of reduction in fungal growth of green bean root rot pathogens affected by three bio agents on PDA medium

Bio agent / Pathogenic isolate	*T.harzianum*		*B.subtillis*		*P.flouresences*	
	% Reduction in linear growth after					
	4 days	8 days	4 days	8 days	4 days	8 days
Fusarium solani	58.0 b	100	61.2 b	100	68.0 b	100
Rhizoctonia solani	46.0 a	100	53.4 a	100	52.2 a	100
Fusarium oxysporum	56.0 b	100	68.0 b	100	70.0 b	100
Sclerotium rolfsii	40.0 a	100	53.2 a	100	50.0 a	100
Pythium spp.	42.0 a	100	55.0 a	100	52.0 a	100

Figures with the same letters are not significant (P = 0.05).

Greenhouse trials

This experiment was carried out in plastic pots (20 cm) containing individually artificially infested soil with green bean root rot pathogens. Different seed treatments maintained before, were applied to evaluate their efficacy in controlling root rot disease pathogens under artificially infested soil.

Results in Tables (4 and 5) indicate that all different types of seed treatments have reduced significantly the percentage of root rot diseases caused by *F. solani, R. solani* and *F .oxysporum* except seed priming treatment The most effective treatments were bio-priming and seed coating treatments followed by seed dressing with fungicide . Coated Primed green bean seeds by *T. harzianum, B. subtillus* and/ or *P. floresence* (bio priming) reduced damping of caused by *F. solani, R. solani* and *F .oxysporum* by 58.1- 69.8% , 58.7 – 73.9% and 51.4 – 64.2 % respectively . These treatments also caused reduction in root rot incidence caused by the same pathogens after 45 days of seed sowing by 59.9 – 68.5 %, 58.3 – 62.2 % and 55.1 – 60.4 % (Table 5). Meanwhile, coated green bean seeds with *T. harzianum* was the best seed coating treatments, as the reduction in damping-off and root rot incidence caused by *F. solani, R. solani* and *F .oxysporum* reaching to 53.1,50.0,48.5% and 56.4,55.2 ,51.1 % .Also, coated seeds with *B. subtillus* and *P. floresence* treatments gave considerable results in reducing the incidence of root rot of green bean caused by the same pathogens if compared with priming and control treatments.

Applied *T. harzianum* to green bean seeds during priming process (bio-priming) resulted in highly reduce in root rots incidence caused by *F. solani, R. solani* and *Pythium spp* under greenhouse conditions. The observed improvements due to bio priming of green bean seeds may be due to priming induced quantitative change in biochemical content of the seeds and improved membrane integrity (Sung and Chang, 1993) .This may be also due to the proliferation of the bio agent in the primed medium .Callan *et al*., 1990 and El-

Mohamedy *et al*., 2006; El-Mougy and Abdel-Kader (2008) also stated that the bio control agent may multiply substantially on seed during bio priming.

Meanwhile, coated primed green bean seeds with *T. harzianum* or dressed with fungicide (Rizolex-T) caused a highest reduction in root rots incidence if compared with other seed treatments. This may be due to the fail bio-protection on seed or in rhizosphere at sufficient level for disease control and releasing high level of exudates during germination. Coating seeds of many crops with bio control agents such *Trichoderma* spp., *Bacillus subtilus*, *Psedomonas florocense* was the most effective treatments for controlling seed and root rot pathogens. However , biological seed treatments may not provide adequate seed protection under all condition as bio-protection may be fail to establish on seed or in rhizospher at sufficient level for disease control . (Harman *et al*., 1998; Nascimenta and West, 1998; El- Mohamedy, 2004 and El- Mohamedy *et al*., 2006). Many researchers have demonstrated the potential of *Trichoderma* spp in controlling damping off and root rot diseases of crop plants caused by *Rhizoctonia solani* and *Fusarium spp*. (Lewis and Lumsden, 2001; El-Mohamedy, 2004; Rojo *et al*., 2007). seed coating with bio-control agents was the most effective treatment for controlling root rot diseases as shown by Callan *et al*., 1990, 1991; Loeffez *et al*., 1996; Jahm and Puls, 1998 ,Warren and Bennett, 1999; Abdel-Kader and Ashour, 1999.

Bio-priming in which specific biological control agents are incorporated into the seed priming process, can be very effective in suppressing many disease caused by seed and soil borne pathogens. Moreover, bio-priming has great promise for enhancing the efficacy, shelf life and consistent performance of biological control agents as shown by Harman *et al*., 1989; Callan *et al.*, 1990, 1991; Jensen *et al.*, 2001and 2002; Jahn and Puls, 1998.

Table 4. Pre- emergence damping- off on green bean plants as affected by different seed treatment under artificially infested soil in greenhouse

Seed treatment		**% Pre-emergence damping-off after 15 day from sowing**					
		F. solani	R*	*R. solani*	R*	*F oxysporum*	R*
Seed bio-priming	*T.harzainum*	11.8 d	69.8	11.0 d	73.9	12.4 e	64.2
	B. subtillus	16.4 c	64.7	14.0 d	66.8	15.0 d	57.1
	P.floresence	13.0 d	58.1	17.4 c	58.7	17.0 d	51.4
Seed coating	*T .harzainum*	18.4 c	53.1	21.0 c	50.0	18.0 d	48.5
	B. subtillus	17.5 c	47.1	23.5 c	44.3	22.0 c	37.1
	P. floresence	23.0 b	41.3	25.0 c	40.7	23.4 c	33.1
Seed dressing	Rizolex-T(3 m/kg seed)	15.0 c	61.7	13.0 d	69.1	20.0 c	62.8
Seed priming		32.5 a	17.1	35.0 b	17.1	30.0 b	14.2
Control (non treated seeds)		39.2 a	0.0	42.2.0 a	0.0	35.0 a	0.0

Figures with the same letters are not significant (P = 0.05).
R*:% Reduction

Table 5. Root rot disease incidence and survival plants of green bean plants as affected by different seed treatment under artificially infested soil in greenhouse

Seed treatment	% Root rot disease incidence (after 45 day)						Survival plants %		
	F. solani	R*	*R . solani*	R*	*S. rolfsii*	R*	*F. solani*	*R. solani*	*S. rolfsii*
					Seed bio-priming				
T .harzaium	26.2 e	68.5	30.2 d	62.2	29.0 d	60.4	73.8a	69.8a	71.0a
B. subtillis	29.2 d	64.9	31.2 d	60.9	31.2 d	57.4	70.8a	68.8a	68.8a
P. floresecence	33.4 d	59.9	33.3 d	58.3	32.9 d	55.1	6.6g	66.7a	67.1a
					Seed –coating				
T .harzainum	36.3 d	56.4	35.8 d	55.2	35.8 d	51.2	63.7b	64.2a	64.2b
B. subtillis	43.5 c	47.8	42.8 c	46.5	41.9 c	42.9	56.5c	57.2b	58.1b
P floresence	50.2 c	39.8	46.7 c	41.6	40.2 c	45.2	49.8d	53.3b	59.8b
					Seed dressing				
Rizolex-T (3 gm/kg seed)	31.1 d	62.6	31.6 d	60.5	32.1 d	56.2	68.9a	68.4a	67.9a
Seed priming	75.5 b	9.4	69.3 b	13.3	65.3 b	11.0	24.5e	30.7c	34.7c
Control (non treated seeds)	83.4 a	0.0	80.0 a	0.0	73.4 a	0.0	16.6f	20.0d	26.6d

Figures with the same letters are not significant (P = 0.05).
R*:% Reduction

Conclusion

Results of the present study indicated that coating or bio priming of green bean seeds with either bio control agents such as *T .harzaium ,B. subtillis* and *P. floresecence* caused highly decrease in root rot disease incidence and provides protection to seedlings against soil borne infections. So,it can be represented an environmentally eco friendly strategy seed treatment with chemicals fungicides ,which is economically ,eco friendly for controlling seed and soil borne pathogens as substitute of chemical fungicides.

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(Received 10 January 2013; accepted 30 April 2013)