International Journal of Agricultural Technology 2017 Vol. 13(5): 771-788
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Seed Bio priming as Biological Approach for Controlling - Root Rot Soil Born Fungi on Soybean (*Glycine max* L.) Plant

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Mona, M.M. Ragab, Ashour A.M.A, R.S.R. El-Mohamedy, Morsy, A.A. and Hanafy, E.K. (2017). Seed Bio priming as Biological Approach for Controlling Root Rot Soil Born Fungi on Soybean (*Glycine max* L.) Plant. International Journal of Agricultural Technology 13(5): 771-788.

Fusarium solani, Rhizoctonia solani , Sclertium rolfsii and Macrophomina phaseolina proved to be the most soil borne fungi isolated from soybean plants showing root rot and wilt symptoms and collected from different five Governorates in Egypt. Pathogenicity test provided that the most aggressive fungi causing root rots on soybean were *F. solani, R. solani, S. rolfsii* and *M. phaseolina* followed by *F. oxysporum.* In greenhouse trails, Bio-priming seed treatments (seed primed and coated with bio control agents *Trichoderma harzianum, T. viride , Bacillus subtilus and Pseudomonas flouresences*) as well as fungicide (Rizolex-T) treatment were significantly reduced root rot diseases caused by such pathogens on soybean plants .Meanwhile, coated soybean seeds with either *T. harzianum, T. viride* , *B. subtilus* and *P. flourecencse* without priming caused a considerable effect. Under field conditions, bio-priming (primed seed and coated with *T. harzainum* and/or *B. subtilus*) treatments as well as fungicide seed treatments successfully suppressive root rot disease incidence during 2015 and 2016 seasons. Such treatments cause improvements in both vegetative growth and yield parameters of soybean. The present study suggested that bio-priming could be safety used commercially as substitute of traditional fungicide seed treatments for controlling seed and soil borne plant pathogens.

Key Wards: Bio –Priming – Soybean—Control- *T. harzainum* . *B. subtilus*.

Introduction

Soybean (*Glycine max* L.) plant is one of the world's most important sources of oil (20%) and protein (40%). It has the highest protein content among leguminous crops (Prévost *et al*. 2010; Lakshmeesha *et al*., 2013). Soil borne fungal pathogens are causal agents of legume diseases of increasing economic importance such as root rots, seedling damping-off and vascular wilts. Soil-borne fungal diseases are among the most important factors limiting the yield production of many crops as well as soybean in many countries, resulting in serious economic losses (Mihajlović *et al*., 2017). Several soil borne fungi have been reported to affect soybean plants

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such as *Fusarium oxysporium, F.solani ,Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotium rolfsii* (El-Baz 2007. Sweets, 2008; Abd EL-Hai *et al* 2016). Synthetic fungicides are useful in sustaining the production of crops and protecting the plants from fungal pathogens, but the use of these fungicides can cause several negative effects, such as: (a) causes hazards to humans and environmental pollution, (b) development of pathogen resistance, and (c) expensive and pose serious threat to human health (d) damage to beneficial organisms (Hungria *et al.,* 2005).

Recent efforts have targeted on developing environmentally safe, long-lasting, and effective bio control approaches for the management of plant diseases. Bio-priming of seeds with microbial antagonists is gaining importance in the recent times which not only improves the vigor and seedling establishment but also minimizes the risk of several plant diseases .In this respect , bio –priming a new technique of seed treatment that integrate biological and physiological aspects of disease control was recently used as alternative method for controlling many seed and soil borne pathogens (Callan *et al.,* 1990 and 1991; Jahn and Puls , 1998 , Jensen *et al.,* 2001 ;El- Mohamedy , 2004 , El- Mohamedy and Abd Elbaky , 2008; El-Mohamedy *et al.,* 2006) . Bio priming a new novel technology was considered as a safe, cheap and easily applied bio control method against these soil-borne plant pathogens. Besides, bio-priming also improves seed germination, seedling establishment and vegetative growth. Thus, bio-priming can be exploited by seed companies and organic farmers in the sustainable agriculture, which would be more economical and environmental-friendly (El-Mohamedy *et al.,* 2006; El- Mohamedy and Abd El-Baky, 2008; El-Mougy and Abdel Kader, 2008; Begum *et al.,* 2011 and El-Mohamedy and AbdAlla, 2013).

Many researchers used seed bio priming to control of some diseases such as Carrot Damping Off (Jensen *et al.* 2004) , Cowpea Root Rot (El-Mohamedy *et al.* 2006), Faba Bean Root Rot (El-Mougy and Abdel- Kader 2008) , Pea Root Rot (El-Mohamedy and Abd El-Baky 2008) , Okra root rot (El-Mohamedy, 2004) , cow pea root rot (El-Mohamedy *et al.,* 2006) , green bean root rot (El-Mohamedy and Abd Alla, 2013) , Soybean Damping Off (Begum *et al.* 2010) , Pearl Millet Downy Mildew (Raj *et al.* 2004) , Sun flower Leaf Blight (Rao *et al.* 2009) , Rape Oil Seed Blackleg (Ruba *et al.* 2011) , Sesamum Charcoal Rot (Elad *et al.* 1983) and (Sankar and Sharma 2001). Rafi and Dawar (2015) Seeds bio priming with *Trichoderma harzianum* and *Rhizobium melilotii* and different concentrations of *Acacia nilotica* and *Sapindus mukorossi* leaves extracts most effective for enhancing the growth and suppression of root rot fungi like *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* sp on leguminous and non-leguminous crops.

The present work was conducted to evaluate bio- priming seed treatment as well as seed dressing with Rizolex-T (Fungicide) as comparison treatment in controlling soil borne root rot fungi , improving plant growth and yield quality of soybean (*Glycine max* L.) plants under in vitro , green house and field conditions

Material and Methods

Survey of the causal organisms and pathogenicity test

Samples of soybean plants showing root rot/wilt symptoms were collected from five governorates in Egypt. 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 governorate were calculated.

Pathogenic ability of selected ten fungal isolates from the total isolated fungi were tested for their Pathogenicity to induce root rot diseases on soybean plant (cv. Giza 21and Giza 22) in pots under greenhouse condition .The tested fungal isolates were *Fusarium solani* isolates 123 and 222 , *Fusarium oxysporium* isolates 213 and 113, *Macrophamina phaseolina* isolates 134 and 223, *Rhizoctonia solani* isolates 113 and 213, *Sclerotium rolfsii* isolates 212 and 324. Surface sterilized seeds of each cultivar of soybean were sown in plastic pots (20 cm diameter) containing sterilized sandy loam soil ,artificially infested with individually inoculum of each tested isolate ,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 soybean root rot pathogens in vitro

The inhibitory effects of three isolates of each bio agents i.e., *Trichoderma harzianum* isolates 532, 131and 311, *T viride* isolates 412 , 411and 522, *Bacillus subtilis* isolates 523,111and 412 and *Psedomonas florescence* isolates 522,122and 421, previously isolated from rhizospheric soil of healthy soybean plants during survey studies by the authors were tested against the most sever root rot pathogenic fungi on soybean plants i.e., *F. solani* 222 , *R. solani* 212 , *S.rolfsii* 324 and *M. phaseolina* 223 using dual culture technique (Ferrari *et al*., 1991). Mycelia disks (0.5 cm diam.) of 7 day old cultures of each fungi 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 were used . Reduction of linear growth and spore/sclerotia production were calculated.

Management of soil borne root rot fungi of soybean in greenhouse.

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 of soil borne root rot fungi of soybean in artificially infested soil in greenhouse at plant pathology department , National Research Center , Cairo , Egypt .

Type of Seed treatments

Seed coating with bio agents

Soybean seeds cultivars Giza 21and Giza 22 were immersed for 30 min in suspension of spore and /or cell suspension of each *Trichoderma harzianum* 131, *T. viride* 412, *Bacillus subtilis* 111 and*/or Psedomonas florescence* 122. These bio control agents were previously isolated from rhizosphere soil of healthy soybean plant and the antagonistic ability against root rot pathogens of soybean 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 on nutrient broth medium and incubated at 30 Co for 48 h. and then cell suspension were adjusted to 2.8 X 10^8 cuf (Kiraly *et al*., 1970).

Seed priming

Soybean seeds cvs. Giza 21and Giza 22 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* 131 *and/or T. viride* 412 *as* well as cell suspensions of *B.subtilis 111* and *P. fluorecens 122* previously supplemented in CMC1% solution were subsequently added individually to soybean seeds during priming process. Then dried at room temperature and used as bio primed seeds.

Seed dressing

Soybean seeds cvs. Giza 21and Giza 22 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 as mentioned above were used. Five soybean 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: T 1, 2 ,3 and 4 =-Seed bio priming (primed seed were coated individually with *T. harzianum 131* (BP-TH) *, T. viride 412* (BP-TV) *, P. fluorecens 122*(BP-PF) *and B. subtilis* (BP-BS) *111*. T 5, 6, 7 and 8 =Seed coating (non-primed seeds were coated individually with *T. harzianum* (SC-TH), *T.viride* (SC-TV) *, P fluorecens.* (SC-PF) *and B. subtilis* (SC-BS) .T 9= Seed priming (seeds were primed with PEG) .T 10 = Seed dressing (seeds were dressed with a fungicide Rizolez –T 3g/kg seed. T 11= Control 1 (none treated seeds and infested soil). T 12 = Control 2 (none treated seeds and non-infested soil) .After 15 and 45days of seed sowing percentage of root rot disease infection (DI) at pre-and post-emergence damping off stages were recorded and the percentage of survival plants were also calculated in each treatment.

Management of soil borne root rot fungi of soybean under field condition.

Field experiments were carried out during seasons of 2015 and 2016 at the Experimental Research Station of National Research Centre at El-Noubaria region, Behera Governorate, Egypt. The highly effective treatments that mentioned above in controlling root rot pathogenic fungi under greenhouse conditions were evaluated under field conditions. The field trail conducted in naturally heavily infested soil with root rot pathogens with 24 plots. The evaluated treatments were applied as follows:
T 1 and 2 = Seed bio priming (primed seed were coated individually with *T. harzianum* 131 (BP-TH) and/or *B. subtilis* 111 (BP-BS). T3 = seed dressing with Fungicide (Rizolex-T 50% at rate of 3g/Kg seeds). 4- Control (untreated seeds). All seed treatments were applied in a complete randomized block design with six replicates (plots) for each treatment. Soybean seeds were sown at rate of 3 seeds per hill and 30 cm distance between hills on one side of ridge with 70 cm width and 4 m length.

The percentage of root rot incidence at pre-emergence stage was calculated as the number of absent emerged seedlings in relative to the total number of sown seeds after 15 days from sowing date. Meanwhile, the percentage of root rot disease incidence (DI) at post-emergence stage was calculated as the number of soybean plants showed disease symptoms in relative to the total number of soy bean plants, periodically every 10 days starting from 20 up to 90 days after sowing date. In the same time the effect of different seed treatments on vegetative growth and yield parameter were investigated.

Statistically analysis

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

Results and discussion

Causal organisms

Soybean plants showing root rots and wilt disease symptoms were collected from five different governorates in Egypt were subjected for isolation the causal organisms.

Results in Table 1 show 174 fungal isolates belonging to four fungal genera representing six species *i.e*. *Macrophomina phaseolina* (44 isolates), *Rhizoctonia solani* (40 isolate), *Fusarium* solani (37 isolate), *Sclerotium rolfsii* (28 isolate) , *Fusarium oxysporum* (12 isolate), and *Fusarium.* spp. (7 isolate) were isolated .The most dominant fungi were *M. phaseolina* (25.3%) frequent and *R. solani* (23.0 %) followed by *F. solani* (21.3%) and *S. rolfsii* (16.7%). Meanwhile, *F. oxysporum* show the least frequency (6.9 %).Many investigators have reported that *Fusarium oxysporum*, *F.solani* , *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii* is the causal agents of root rot /wilt disease of soy bean plants (Hashem *et al* 2009; Fayzall *et al* 2009 ; Abd EL-Hai *et al.*, 2010, 2016) .

Table 1. Frequency (%) of fungi isolated from roots of soybean plants showing root rot / wilt symptoms under field conditions from five governorates in Egypt.

Governorate	**Frequency of isolated fungi %**														**Total**
	M. phaseolina		*R. solani*		*F. solani*		*S. rolfsii*		*F .oxysporium*		*F.* spp		others		
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	
BeniSuef	11	25.0	9	20.5	10	22.7	7	15.9	4	9.1	1	2.3	2	4.5	44
El Behera	6	25.0	7	29.2	4	16.7	2	8.3	3	12.5	1	4.2	1	4.2	24
El Minia	12	26.1	10	21.7	13	28.3	6	13.0	2	4.3	2	4.3	1	2.2	46
El Qalubia	7	21.2	8	24.2	6	18.2	9	27.3	1	3.0	2	6.0	0	0.0	33
El Sharkia	8	29.6	6	22.2	4	14.8	5	18.5	2	7.4	1	3.7	1	3.7	27
Total	44	25.3	40	23.0	37	21.3	29	16.7	12	6.9	7	4.0	5	2.9	174

Pathogenicity test

Ten fungal isolates of isolated fungi were selected and tested for their Pathogenicity to soybean plant (cv. Giza 21and Giza 22) in pots under greenhouse condition .The tested fungal isolates were *Fusarium solani* isolates 123 and 222, *Fusarium oxysporium* isolates 213 and 113, *Macrophamina phaseolina* isolates 134 and 223, *Rhizoctonia solani* isolates 113 and 213, *Sclerotium rolfsii* isolates 212 and 324. Percentages of root rot incidence of both pre – and post emergence damping of was recorded after 15, 30 and 45 days of sowing.

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

Fungal isolate	Cod	Soybean cultivar(cv)	pre - emergence (15day)	Root rot/wilt disease incidence %		Survived plants (%)
				Post - emergence (30 day)	Post- emergence (45 day)	
F. solani	123	Giza 21	24 m	13.5 k	34.3 i	36 h
		Giza 22	26 l	26.3 g	36.4 h	34 i
F. solani	222	Giza 21	30 k	25.4 h	42.1 f	32 j
		Giza 22	35 i	26.5 g	54.3 a	22 n
F. oxysporium	213	Giza 21	11 r	10.3 n	18.3 p	62 c
		Giza 22	12 q	16.2 j	16.4 q	54 d
F. oxysporium	113	Giza 21	22 o	12.5 l	20.5 o	46 f
		Giza 22	26 l	15.6 j	22.3 n	42 g
M. phaseolina	134	Giza 21	20 p	28.4 f	44.2 e	32 j
		Giza 22	33 j	33.5 c	46.2 d	24 m
M. phaseolina	223	Giza 21	23 n	32.1 d	50.3 c	26 l
		Giza 22	40 f	41.3 a	52.5 b	16 p
R. solani	113	Giza 21	36 h	12.2 l	14.5 r	48 e
		Giza 22	38 g	19.5 i	16.3 q	42 g
R. solani	212	Giza 21	58 b	19.3 i	23.3 m	26 l
		Giza 22	62 a	31.0 e	30.4 k	18 o
S. *rolfsii*	212	Giza 21	40 f	11.5 m	23.3 m	34 i
		Giza 22	44 e	26.4 g	26.0 l	26 l
S. *rolfsii*	324	Giza 21	48 d	26.7 g	31.3 j	30 k
		Giza 22	54 c	35.7 b	39.2 g	22 n
control		Giza 21	6 t	4.3 o	0.0 s	90 a
		Giza 22	8 s	4.7 o	0.0 s	86 b

Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level of probability.

Results in table 2 show that pathogenicity test proved that all tested fungal isolates were able to cause root rot infection on soybean cultivar(Giza 21and 22), but with various degrees of infection .*F. solani* 222, *F. oxysporium* 113, *M. phaseolina* 223, *R. solani* 212 and *S. rolfsii* 324 were the most aggressive isolates showing highest percentages of damping of and root rot infection on soybean c.v. Giza 21 and 22. *R. solani* 212 and *S.rolfsii* 324 followed by *M. phoseolina* 223 and *F. solani* 222 cause high percentage at pre –emergence stage on soybean cv.G22 (62.0,54.0% and 40.0,35.0 %), however *F. oxysporium* 113 cause least percentage (26%) after45 of sowing.

F. solani 222 and *M.phaseolina* 223 followed by *R. solani* 212 and *S.rolfsii* 324 caused highest percentages of root rot infection on soybean cultivar 22 (54.3,52.5 and 46.2, 39.2%). Theses fungal isolates were sever fungi causing damping -off and root rots of soybean plants as the least percentages of survival plant were recorded with *F. solani* (222), *M.phaseolina* (223), *R.solani* (212) and *S.rolfsii* (324).These isolates were chosen for fur them investigation as they were the most virulent to induce root rots to soybean.

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 soybean plants (Abd El-Hai *et al*. 2010, Mishra *et al*. 2011, Dalal and Kulkarni 2014 and Abd EL-Hai *et al*. 2016).In Egypt, El-Hai *et al*. (2010) reported that *Fusarium oxysporum, F. solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* were the main causal pathogens of soy bean plants

Testing of antagonistic agents against linear growth and sporulation of soybean root rot pathogens in vitro.

The antagonistic ability of three isolates for each selected antagonistic fungi and bacteria i.e., *Trichoderma harzianum* isolates 532,131 and 311, *T. viride* isolates 412,411and 522, *Bacillus subtilis* isolates 523,111 and 412 *and Psedomonas florescence* 522,122 and 421, previously isolated from rhizospheric soil of healthy soybean plants during survey studies by the authors were tested against root rot pathogens of soybean on PDA medium. .Meanwhile the tested pathogenic fungi were *F. solani* 222, *R. solani* 212, *S.rolfsii* 324 and *M. phaseolina* 223 .The linear growth (mm)and spore/scloratia production (cm2) were assessment and calculated and printed in table 3.

Data presented in Table 3 show that the linear growth and spore/scloratia production of all tested pathogenic fungi were highly affected in response to the inhibitor action of all tested antagonistic agents. *T.harzianium* isolates 131, *T.viride* isolates 412, *B. subtillis* isolates 111and *P.flroceances* isolates 122 had greatest effect on the linear growth and spore/scloratia production of all tested pathogenic fungi.

The reduction percentages of both linear growth and spore/scloratia production of the tested pathogens were observed with these bio agents. The records of reduction calculated as arrange of 53.0 -77.0 % & 89.1- 95.5 %

with *F. solani*, 48.9-79.3&50.6-79.3 with *S. rolfsii*, 47.4-73.7 and 30.0-73.8with *R. solani* and 40.0-72.2 and 42.5-73.7 with *M.phaseolina* .

Many researchers used microorganisms as antagonistic to plant pathogens as an alternative control method to fungicides as the seed or soil treatment, (Huang and Erickson 2002; Smolinka and Kowalska 2006; Naffa *et al.* 2010 and Raspor *et al.* 2010),

Table 3. The percentage of reduction on linear growth and sporulation of soybean root rot pathogenic fungi affected by antagonistic fungi and bacteria isolated from rhizospheric soil of healthy soy bean plant on PDA medium in vitro.

Bio agent	Pathogenic fungi							
	F. solani		*S. rolfsii*		*R.solani*		*M. phaseolaina*	
	LG	S	LG	S	LG	S	LG	S
***T. harzianum* 532**	69.7	92.1	65.2	72.2	67.8	62.3	65.2	64.0
***T. harzianum* 131**	77.0	95.5	74.1	79.3	73.7	73.8	72.2	73.7
***T. harzianum* 311**	70.0	93.7	68.2	73.5	68.1	71.7	65.9	66.1
***T. viride* 412**	71.1	94.9	70.0	74.8	68.9	73.3	67.8	66.4
***T. viride* 411**	62.6	90.1	56.7	61.6	61.1	54.0	52.6	54.3
***T. viride* 522**	56.7	90.9	51.1	57.1	48.1	30.9	45.9	46.0
***B. subtilis* 523**	64.1	91.6	62.2	63.1	62.2	57.1	55.2	56.5
***B. subtilis* 111**	64.8	92.1	64.1	65.3	64.1	61.3	60.0	62.1
***B. subtilis* 412**	60.0	90.9	54.1	58.6	57.0	41.9	47.8	48.7
***P. fluorescens* 522**	53.0	89.1	48.9	50.6	47.4	30.0	40.0	42.5
***P. fluorescens* 122**	64.4	91.6	64.1	63.8	63.7	59.2	58.9	57.3
***P. fluorescens* 421**	60.4	91.2	58.9	58.9	59.3	50.8	50.4	49.5

Control of soil born root rot pathogenic fungi on soybean plant in green house.

This experiment was carried out in plastic pots (20 cm) containing individually artificially infested soil with soybean root rot pathogens i.e., *F. solani* 222, *R. solani* 212, *S. rolfsii* 324 and *M. phaseolina* 223. Bio priming and seed coating treatments as well as fungicide seed treatment were applied to evaluate their efficacy in controlling root rot disease pathogens under artificially infested soil.

Results in Tables 4 indicate that all types of seed treatments have reduced significantly the percentage of root rot diseases caused by *F. solani* 222 , *R. solani* 212 , *S. rolfsii* 324 and *M. phaseolina* 223 except seed priming treatment The most effective treatments were bio-priming and fungicide treatment followed by seed coating treatments. The highest records of root rot disease incidence were obtained with BP-TH, BP-BS (bio–priming treatments) and fungicide seed treatments, as the percentages of reduction in disease incidence at pre –emergence damming -off ranging from 66.7 to 75.0%; 57.1% 62.2% and 71.4% to 81.3%, and from 66.7%

to 73.6 %, 48.0 % to 67.2 % and 68.7% to 79.7% at post emergence after 45 days of all tested pathogenic fungi respectively.

Meanwhile, coated soybean seeds with *the same bio agents* showed considerable results in reducing the incidence of roots of soybean caused by the same pathogens if compared with priming and control treatments.

Table 4. Effect of bio- priming seed treatment on damping -off disease incidence of soybean plants sowing in artificially infested soil by *F.solani, R.solani* , *S.rolfsii* and *M.phaseolina* in green house

Plant growth stage	**Pathogens**	**Root rot**	**Type of seed treatment**										
			BP-TH	BP-TV	BP-BS	BP-PF	SC-TH	SC-TV	SC-BS	SC-PF	Priming	Rizolex-T	control
% Pre-emergence damming off after 15 day	*F. solani*	DI	9.0	11.0	12.0	14.0	13.0	15.0	17.0	19.0	25.0	7.0	31.0
		R	71.0	64.5	61.3	54.8	58.1	51.6	45.2	38.7	19.4	77.4	0.0
	R. solani	DI	15.0	17.0	20.0	22.0	21.0	23.0	25.0	27.0	35.0	13.0	42.0
		R	64.3	59.5	52.4	47.6	50.0	45.2	40.5	35.7	16.7	69.0	0.0
	S. rolfsii	DI	11.0	13.0	14.0	15.0	14.0	16.0	17.0	20.0	28.0	9.0	34.0
		R	67.6	61.8	58.8	55.9	58.8	52.9	50.0	41.2	17.6	73.5	0.0
	M. phaseolina	DI	8.0	10.0	12.0	14.0	13.0	15.0	16.0	18.0	24.0	6.0	30.0
		R	73.3	66.7	60.0	53.3	56.7	50.0	46.7	40.0	20.0	80.0	0.0
% post-emergence damming off after 45 day	*F. solani*	DI	12.1	15.3	15.8	16.9	16.2	17.1	18.1	21.2	31.1	10.5	44.7
		R	72.9	65.8	64.7	62.2	63.8	61.7	59.5	52.6	30.4	76.5	0.0
	R. solani	DI	5.1	7.7	10.5	13.7	10.8	16.5	17.5	18.6	29.1	4.6	32.5
		R	84.3	76.3	67.7	57.8	66.8	49.2	46.2	42.8	10.5	85.8	0.0
	S. rolfsii	DI	5.0	7.8	10.7	11.4	11.1	12.3	12.9	13.4	13.7	4.7	15.3
		R	67.3	49.0	30.1	25.5	27.5	19.6	15.7	12.4	10.5	69.3	0.0
	M. phaseolina	DI	12.1	15.0	15.7	16.6	16.2	17.1	18.2	21.7	30.2	9.3	45.8
		R	73.6	67.2	65.7	63.8	64.6	62.7	60.3	52.6	34.1	79.7	0.0
% survival plant	*F. solani*		76	72	70	67	68	64	58	56	42	83	29
	R. solani		76	72	68	62	66	60	56	52	38	80	28
	S. rolfsii		77	71	67	63	64	58	55	53	44	80	35
	M. phaseolina		72	69	65	60	63	58	55	51	41	79	28

BP-TH = seed primed and coated with *T. harzianum* BP-TV= seed primed and coated with T,viride BP-BS=seed primed and coated with *B. subtilus* BP-PF = seed primed and coated with P.flourecense SC-TH =seed coated with *T.harzaium* SC-TV = seed coated with *T.viride* SC-BS = seed coated with *B. subtilus* SC-PF = seed coated with *P.,flourecense* Priming =seed primed in PEG Rizolex-T =seed dressed with fungicide Rizolex –T 50% 3g/kg seed

Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level of probability.

Applied of bio control agents such *T. harzianum, and /or B,subtilis* to soybean seeds during priming process (bio-priming) resulted in highly suppression in root rots disease incidence under artificially infested soil under greenhouse conditions. The observed improvements due to bio priming of soybean 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)

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 soybean 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.*, 1986; Jahm and Puls, 1998, Warren and Bennett, 1999; Abdel-Kader and Ashour, 1999. In this respect, 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.

Management of root rots diseases of soybean under field conditions

Since greenhouse pot experiments provided promising results, the same treatments were applied under field conditions to review the control of soybean root rot diseases in practical environment. The effects of promising seed treatments i.e. bio-priming BP-TH; bio-priming PB-BS and fungicide seed treatments on the control of root rot diseases of soybean under field conditions was studied. Moreover, the beneficial effects of these treatments on vegetative growth and yield quality were also investigated.

Influence on soybean root rot disease control

Coating primed soybean seeds with either *T. harzainum* or *B. subtilis* strongly reduced root rot incidence at pre- and emergence stages of soybean plants, resulting in high survival healthy plants Table 5. Coated primed soybean seeds with either *T. harzainum* and/or *B. subtilis strongly* reduced root rot by 67.6 & 65.9% and 62.2 and 61.5 % at pre emergence stage and by 78.7 and 73.3% and 69.8 and 75.3 at post emergence after 60 days of sowing during 2015 and 2016 seasons. These treatments showed high records of survival plants if compared with control treatments. Meanwhile, dressed soy bean seeds with Rixolex-T decreased pre-emergence and root rot incidence by 89.2%, 84.0 % and 88.9%, 83.5% during the same seasons respectively.

Many researchers have demonstrated the potential of *Trichoderma* spp and *B. subtlis* in controlling damping off and root rot diseases of crop plants caused by *Rhizoctonia solani* and *Fusarium* spp. (Lewis and Lumsden, 2001; Warren and Bennett, 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* 1991; Loeffez *et al* 1986; Jahm and Puls 1998, Warren and Bennett, 1999 .

Table 5. Effect of different seed treatments on root rot disease incidence (%) of soybean plants under field condition during 2015 and 2016 seasons.

Treatment	**% Pre-emergence damping-off**		**% Root rot disease incidence DI after**				**Survival plants %**
Seed treatment	DI	R%	45 day		90 day		
			DI	R%	DI	R%	
			First season 2015				
Bio -priming TH (BP - TH)	6.0 b	67.6	5.0 c	83.2	4.8 b	78.7	82.0 c
Bio -priming BS (BP - BS)	6.3 b	65.9	7.3 b	75.4	6.0 b	73.3	77.6 b
Rizolex-T 3g/kg seed	4.5 c	75.7	4.1 d	86.2	4.3 c	80.9	88.0 d
Control	18.5 a	0.0	29.7 a	0.0	22.5 a	0.0	51.5 a
			Second season 2016				
Bio -priming TH (BP - TH)	7.5 b	62.5	7.0 b	77.4	7.3 b	69.8	81.2 c
Bio -priming BS (BP - BS)	7.7 b	61.5	8.2 b	73.5	8.0 b	66.9	75.3 b
Rizolex-T 3g/kg seed	6.5 c	67.5	5.7 c	81.6	6.4 c	73.6	86.0 d
Control	20.0 a	0.0	31.0 a	0.0	24.2 a	0.0	47.5 a

Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level of probability.

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

Blum, *et al*., (1991), Harman, *et. al*., (1989); Jensen *et al*, (2002); Jahn and Puls , (1998) . The observed decrements in *Fusarium* and *Rhizoctonia* root rot due to seed bio priming of soybean may be due to that priming induced quantitative changes in biochemical content of the seeds and improved membrane integrity (Callan *et al.,* 1991). This may be also due to the proliferation of the bio agent in the primed medium (El-Mohamedy *et al.,* 2006; El-Mougy and Abdel-Kader, 2008; Akram and Anjum, 2011).

Influence on soybean vegetative growth

Bio priming and seed dressed with Rixolex-T (fungicide) treatments clearly stimulating the vegetative growth of soybean plants during both seasons when compared with control Table 6. Coating primed soybean seeds with either *T. harzainum* or *B. subtilis were* superior treatments recoding the greatest plant height plant branches during and fresh and dry weight of root and shoot plant during the two seasons .Fungicide seed treatment showed approximately effects like bio priming treatments on increasing such records. Bio priming TH treatment was the best treatments in increasing all tested growth parameters of soybean plants, as the highest records of plant height, average of branches /plant as well as fresh and dry weight of roots and shoots/plant were observed during two seasons.

Table 6. Effect of seed treatments on vegetative growth parameter of soybean plants during 2015 and 2016 seasons

Seed treatment	Growth parameters					
	Plant height (cm)	Number of branches / Plant	Fresh weight (g)		Dry weight (g)	
			Root	Shoot	Root	Shoot
		First season 2015				
Bio -priming TH (BP - TH)	53.0 b	6.0 b	13.4 b	154.1 b	4.5 b	55.0 b
Bio -priming BS (BP - BS)	50.6 b	5.6 b	10.5 b	152.0 b	4.0 b	54.3 b
Rizolex-T 3g/kg seed	54.3 c	6.5 c	14.5 c	159.5 c	5.3 c	58.1 c
Control	37.5 a	1.9 a	4.0 a	96.4 a	1.6 a	35.6 a
		Second season 2016				
Bio -priming TH (BP - TH)	54.1 b	6.4 b	14.0 b	155.0 b	5.0 b	55.6 b
Bio -priming BS (BP - BS)	52.0 b	6.3 b	11.0 b	153.0 b	4.5 b	55.0 b
Rizolex-T 3g/kg seed	56.5 c	6.7 c	15.2 c	162.0 c	6.0 c	59.0 c
Control	39.0 a	2.3 a	5.3 a	97.0 a	2.0 a	36.0 a

Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level of probability.

Many investigators noted that coated seeds by bio agents and seed bio priming cause significant increase of vegetative growth of many crops (El-Mohamedy *et al*., 2006). The enhancing effect of bio –priming on increasing vegetative growth parameter of soybean plants might be attributed to its efficiency in supplying the growing plants with biologically fixed nitrogen, dissolved immobilized induce exudates of some hormonal substances like geberlic acid,cytokinins and ouxins which could stimulate nutrients absorption as well as photosynthesis process which subsequently increased plant growth Moreover, these treatments suppress root rot disease led to increasing of healthy plants (survival plants) Table 3.

These results are in accordance with, Harman *et al* 1989; Callan *et al* 1991; Hhque and Ghaffar, 1993, they noted that *Bacillus subtillus* cause increasing in high growth and branches led to the promotion of plant uptake and growth they added that growth enhancement by *Bacillus* spp may also relate to its ability to produce hormones especially IAA and oxines. The increasing in plant growth parameter due to bio priming and seed coating treatments may be due to the effect of bio priming process on the physiological and metabolic activities of pea plants.

Influence on yield parameters of soybean plants

Bio priming and seed dressing with Rixolex-T (fungicide) treatments significantly increase the soybean yield as well as their parameters Table 7. Coating primed soybean seeds with either *T. harzainum* or *B. subtillus* were the most superior treatments recoding the greatest No. of pods /plant, weight pods /plant, no of seeds /plant ,weight of seed/plant , weight of 100 seeds /plant and total yield/fed during 2015 and 2016 season respectively. Meanwhile, fungicide treatment (Rhizolex-T) show superior increasing in such yield parameters during the same seasons.

Bio- priming cause superior increasing in pods yield, total yield, total pods/plant and average weight of pods if compared with fungicide treatment .Moreover, increasing the vegetative growth (Table, 6) turn on increasing no of pods /plant , total yield and gave the high quantity of yield. This can be explained on the basis of the above explanation where increasing plant growth promoters and nutrients reduce fiber contents in addition to increasing assimilate production which means higher carbohydrates going to the pods and less stress on the growing pods (Lewis and Lumsden, 2001).

Table 7. Effect of seed treatment on yield parameter of green bean plants during 2010 and 2011 seasons

Seed treatment	Yield parameters				
	Number of pods/plant	Number of seeds/plant	Hundred seed weight(gm)	Seed yield (ton/fedan)	Increase (%)
			First season 2014		
Bio - priming TH (BP -TH)	53.8	187.7	28.1	1.536	55.3
Bio - priming BS (BP - BS)	51.3	185.5	26.5	1.487	50.4
Rizolex-T 3g/kg seed	54.2	189.3	30.9	1.553	57.0
Control	22.6	66.2	10.1	0.989	0.0
			Second season 2015		
Bio - priming TH (BP -TH)	54.3	188.0	28.5	1.559	51.9
Bio - priming BS (BP - BS)	52.0	186.0	25.7	1.529	49.0
Rizolex-T 3g/kg seed	56.0	190.0	31.3	1.598	55.8
Control	23.0	67.0	10.5	1.026	0.0

Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level of probability.

Conclusion

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. Results of the present study indicated that coating or bio priming of soybean seeds with either bio control agents such as *T .harzaium* and *Bacillus subtillus* caused highly decrease in root rot disease incidence and provides protection to seedlings against soil borne infections. Bio- priming seed treatments can provide a high level of protection against root rot disease of soybean plants. This protection was generally equal or superior to the control provided with fungicide seed treatment. So, It could suggested that bio- priming (combined treatments between seed priming and seed coating with bio control agents) may be safely used commercially as substitute of traditional fungicide seed treatments for controlling seed and soil borne plant pathogens.

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(Received: 15 July 2017, accepted: 10 August 2017)