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ORIGINAL ARTICLES

Bio-Priming Seed Treatment For Suppressive Root Rot Soil Borne Pathogens and Improvement Growth and Yield of Green Bean (*Phaseulas Vulgaris L.,*) In New Cultivated Lands.

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ABSTRACT

Fusarium solani, Rhizoctonia solani and *Fusarium oxysporum* proved to be the most soil born fungi isolated from green been roots infected with root rot diseases collected from different Governorates in Egypt . *Macrophomina phaseolinae*, *Pythium* spp and *Sclerotium rolfsii* show less frequent. Pathogenicity test provided that the most aggressive fungi on green bean were *F. solani* and *R. solani* followed by *F. oxysporum* . In greenhouse trails, Bio-priming seed treatments significantly reduced root rots diseases incidence .Coated green bean seeds with either *T. harzianum* or *T. viride* as well as fungicide (Rizolex-T) treatment caused a considerable effect in reducing root rots diseases incidence. Under field conditions, bio-priming and fungicide seed treatments successfully suppressive root rot incidence at pre-and post emergence stages during 2010 and 2011 seasons. Such treatments cause significant increase vegetative growth parameters, early and total pods yield, and improve the quality of green pods such as T.T.S., total sugars, total protein and total nitrogen of green bean pods. So, the present study suggested that bio- priming could be safely used commercially as substitute of traditional fungicide seed treatments for controlling seed and soil borne plant pathogens.

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

Introduction

Green bean **(*Phaseulas vulgaris* L.)** is one of the important leguminous crops in many countries including Egypt .High quality of green pods and mature seeds are used for fresh meals and food industries Common beans are a favorite commodity in export markets and local consumption. The wide area and great advances in techniques and methods of common bean production in A.R. Egypt during the last decade perhaps open an enormous pathway for exportation. The successful production and good marketability of this crop depend on maintaining high quantity and quality. After the construction of high dam, concentration of the micronutrients in Nile water greatly depressed and soil ph of most Egyptian fields are relatively high. For increasing bean production to meet the progressive increasing in human population and exportation, increasing yield and quality should be achieved in winter season during low temperature under low tunnels which is expensive. However, higher plant growth may not be totally interpreted in the form of yield (Nabban, 1966). Damping –off and root rot diseases of bean are caused by single or combination of soil borne fungi i.e., *Fusarium solani* Mart. Sacc , *F. oxysporium, Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc (Begum, 2010 , Lewis and Lumsden 2001, Abdel-Kader, 1997 and Nayaka, *et al*., 2008).

An investigation for controlling green bean root rot diseases is consider important , epically in view of its prevalence in Egypt , particularly in new reclaimed land in the desert . Fungicides were the essential trial as seed treatment for controlling damping-off and root rot diseases for along time (El-Mougy, 2001). However, fungicidal treatments cause hazards to human health and increase environmental pollution. Therefore there are needed to alternative fungicidal seed treatments. Application of biological control using antagonistic microorganisms against seed and root rot pathogens proved to be sussccfully and its efficiency in controlling root rot pathogens and improving growth and yield quality of many crops (Rao, *et al*.,2009). Coating seeds of many crops with bio control agents such *Trichoderma* spp. , *Bacillus subtilus*, *Psedomonas fluorescens* was the most effective treatments for controlling seed and root rot pathogens (Begum *et al*., 2010, Ferreira, *et al*.,1991and El-Mohamedy and Abd-El-Baky, 2008).

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 seeds and soil borne pathogens (Ferreira, *et al*., 1991; El- Mohamedy, 2004). Seed priming, osmo-priming and solid matrix priming (Harman. *et al*., 1998). Technique developed by Rojo *et al*., 2007) were used commercially in many horticultural crops ,

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as a tool to increase speed and uniformity of germination and improve final stand (Rashid and Singh, 2000) . However, if seeds are infected or contaminated with pathogens, fungal growth can be enhanced during priming, thus resulting in undesirable effects on plants (Conway, *et al*., 2001). Therefore, Seed priming alone or in combination with low dosage of fungicides and/or bio control agents have been used to improve the rate and uniformity emergence of seed and reduce damping off disease (Callan *et al*., 1991 and Conway *et al*., 2001).

The present work was conducted to study causal organisms of damping- off and root rot diseases of green bean in different Governorates in Egypt , and evaluate different alternatives fungicidal seed treatment *i.e*. bio-priming, priming and seed coating with bio control agents as well as seed dressing with Rizolex-T (Fungicide) as comparison treatment in control of green bean root rot diseases as well as the efficiency of such seed treatment on vegetative growth, early yield and green pod yield quality parameters was also investigated during two seasons 2010 and 2011.

Materials and Methods

Causal organisms:

Samples of green bean plants showing root rot disease symptoms were collected from different regions of four 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) and Nelson *et al* (1983). The number of each isolated fungus was recorded and the percentage of frequency of each fungus in each location was calculated.

Pathogenicity test:

Fusarium solani, Rhizoctonia solani and *Macrophomina phaseolina* the high frequency isolated fungi were selected to study their pathogenic ability to induce root rot on green bean plants as follow: Plastic pots (20 cm diameter) containing sterilized sandy loam soil infested individually with inoculums of each fungus, which was grown on sandy-barley medium (1:1 w/w of soil and 40 % water) for two weeks at 25 ± 1°C. Ten pots were used for each fungus. Check treatment (control) was prepared without addition the tested fungi .Surface sterilized green bean seeds (Giza, 3 cv.) were sown at the rate of five seeds/pot. The percent of pre- and post-emergence damping -off incidence after 15 and 45 days from sowing was calculated. Pre –emergence(%)was based on the number of un-emerged seeds in relation to the number of sown seeds , while post –emergence (%0 was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings

Disease control in the greenhouse:

The efficacy of bio-priming seed treatments (applying *T. harzianum* and/or *T.viride* to green bean seeds during priming process) ,seed coating with the same bio agents , seed dressing with fungicide and seed priming treatment in controlling root rot pathogens were evaluated in greenhouse experiments.

Seed Priming and bio-priming:

Green bean seeds (Giza, 3 cv.) were initially washed with tap water to remove soluble exudates. Seeds were primed according to methods described by Osburn and Scharoth, 1989 and Harman and Taylor, 1989 in 1% CMC (Carboxyl methyl cellos) in Erlenmeyer flask on a rotary shaker set at 150 rpm. CMC 1% or supplemented 1% CMC with spore suspension of each *T. harzianum* or *T .viride* ($3x10^6$ spore /ml) were subsequently added to seed during priming process for 30 minutes to primed and bio-primed seeds respectively. Primed and bio-primed seeds were shaken at 150 rpm for 12 hour, then dried at room temperature and placed in polyethylene bags for further studies. Seed coating with bio agents*:* Fungal spores of *T. harzianum* and *T.viride* were gently scraped from PDA cultures in water and filtered through nylon mesh (38 Mm). All spores solution were adjusted with sterile water to density concentration of $1x10^7$ cfu/ml. Seeds were coated by shaking 1 g of seeds per treatment with 4 ml of the adjusted conidial suspension on a shaker (1 KA vibrax, 1 KA works, Wilmington Ncl for 10 min. at 130 rpm. Subsequently, the seeds were air-dried on filter paper for 1 h in a laminar flow hood before planting.

Loamy soil was artificially infested with the inoculums of each *F. solani, R. solani* and *M. phaseolinae* as maintained before in plastic pots (20 cm diameter) and relevant to each treatment as follow: Seed priming and bio-priming: Primed and/or bio-primed bean seeds were sown in infested soil. Seed coating with *Trichoderma spp:* Bean seeds were soaked (15 min) in spore suspension of each *T. harzianum*a or *T.viride* ($3 X 10^6$ spore/ml) and then sown in artificially infested soil. Fungicide seed dressing: Bean seeds was dressed with Rizolex-T 50 % at the recommended dose (3 g/kg seeds) then sown in infested soil and served as a comparison treatment.

Control treatment Non-treated (healthy seeds) of bean were sown in infested soil with pathogenic fungi..Five green bean seeds were sowing in each pot and ten pots were used as replicates for each particular treatment. The percentage of damping- off and root rot incidence during 45 days of sowing date was calculated.

Control of root rot diseases of green bean under field conditions:

Two field experiments were carried out during 2010 and 2011 seasons under field condition in naturally heavily infested soil with bean root rot pathogens at Noubaria province. The highly effective treatment that maintained before in controlling root rot pathogens under greenhouse were chosen to evaluated under field conditions. Field experiment consisted of 20 plots (3 m x 7 m) each comprised of 10 rows and 60 pits/holes/row, which were conducted in randomly complete block design with five replicates (plots) for each particular treatment as well as control (check treatment). Green bean seeds (Giza 3 cv.) were sown in all treatment at the rate of 3seeds/pit. .Cultivated plots were received the traditional agricultural practices. Percentages of root rot disease incidence at pre- and post-emergence stages of green bean plants was recorded after 20, 40 days and 60 days from sowing date. The beneficial effects of different seed treatments on vegetative growth and yield quality as well as early and total green pods yield was investigated during two successive seasons 2010 and 2011.

Plant growth measurements:

A representative sample of 8 plants was taken by random 45 days after sowing (flowering stage), from each experimental plot for measuring the plant growth characters, as follows: Plant height from soil surface to the highest point of the plant, number of leaves and branches per plant, total fresh weight and dry weight of plant (determined at 65ºC for 72 hours using the standard methods as illustrated by A.O.A.C, (1990).

Green pod yield parameters:

At harvest stage (60 days from seeds sowing), the total green pods from each plot were collected along the harvesting season (40 days) and the first early yield (yield of the two weekly harvestings) per fedden (1 / fedden (fed.) =2400m^2) and the following data were recorded: early and total green pod yields per fedden, were calculated average number of pods per plant, pods weight(g) and total green pods (ton / fadden) were calculated.

Green Pod Quality:

A random sample of 100 green pods at 2-picking were taken, from each experiment plots ,and the following parameters were recorded average pod weight (g), average No. of pods per plant, Weight of pods Per pant(gm), Weigh of 100 seeds (gm) Weigh of seeds per plant (gm). No. of seeds per plant.

Nutritive Value of seeds:

A random sample of 50 green pods at 2-picking were taken and the following data were recorded: The total soluble solids (T. S. S. %): it was obtained by using the hand refractometer and total protein percentage in pods: a factor of 6.25 was used for conversion of total nitrogen to protein percentage according to method described by A.O.A.C, (1990). Total sugar and total nitrogen were determined according to methods of Dobois *et al*., 1960 and Fackson, (1962)

Statistical Analysis:

All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1982) means were compared by Yenu and Follard, (1952) multiple range tests at the 5 % level of probability in the two seasons of experimentation.

Results And Disscusion

Causal organisms:

Different soil borne fungal isolates representing six species belonging to four genera, *i.e*. *Fusarium* spp., *Macrophomina phaseolinae*, *Rhizoctonia solani* ,*Pythium* spp and *Sclerotium rolfsii* were isolated from bean plants showing root rot disease symptoms. Results in Table (1) indicate that the most dominant of isolated fungi were *R. Solani* and *F. solani* (42.8 % and 32.4 %) followed by *F. oxysporum*(10.5%) . Meanwhile, *M.*

Phaseolinae, *S. rolfsii* and *Pythium* spp were less frequency, as the mean of their frequency were 6.8, 5.0 and 2.1% respectively.

Table 1: Frequency occurrence (%) of fungi isolated from green bean plants showing root rots infection under field conditions in different governorates in Egypt.

Location (governorate)	*Rhizoctonia solani*	*Fusarium solani*	*Fusarium oxysporium*	*Macrophomina phaseolinae*	*Sclerotium rolsfii*	Others
Giza	37.4	29.1	12.8	6.1	5.5	3`0
Kalubeia	46.1	33.4	10.8	5.1	3.5	2`0
Ismaelia	43.4	36.1	9.8	6.1	6.5	2`5
Beheira	44.4	31`0	8`8	6.1	4.5	1.0
Mean	42.8	32.4	10.5	6.8	5.0	2.1

* Each figure represents the percentage of isolates in relative to the whole isolated fungi

Pathogenicity test:

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. Results in Table (2) show that *F. solani, R. solani* and *F .oxysporium* were the most fungi caused root rot disease of green bean plants. *F. solani, R. solani* and *F .oxysporium* the main pathogens of root rot disease on green bean, as the least percent of survival plants were recorded with these pathogens (20.0,23.0 and 45.0%) compared with the highest records of survival plants in control (non infested soil), *S. rolfsii* and *M. phaseolina* (100, 74.0 and 60.0% respectively). *F. solani* and *R. solani* caused a highly significantly effect at pre- and post-emergence stages 36.4; 31.6 % and 80.0; 77.0% respectively, followed by *F. oxysporum* ,they caused 24.2 and 55.0 % pre and post emergence of green been. Meanwhile, *M. phaseolinae* and *S. rolfsii* show the least effect if compared with the other tested fungi. As, the least percent of pre and post emergence damping off were recorded (16.0; 14.4 % and 40.0; 26.0%). Similar results were reported by many investigators (Abdel-Kader 1997 and 2001 ; El-Mougy *et al*., 2007).They 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

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

Fungal isolate	Root rot incidence %		
	Pre-emergence (15 day)	Post-emergence (45 day)	Survival plants (%)
Rhizoctonia solani	36.4 d	80.0 e	20.0 e
Fusarium solani	31.6 d	77.0 e	23.0 e
Fusarium oxysporum	24.2 c	55.0 d	45.0 d
Macrophomina phaseolinae	16.0 b	40.0 c	60.0 c
Sclerotium rolfii	14.4 b	26.0 b	74.0 b
Control	3.0	0.0a	100a

Figures with the same letter or not significantly differed (P = 0.05). Figures with the same letters are not significant (P = 0.05).

Control of root rot disease of green bean in green house:

Efficacy of bio primed seeds with *T. harzianum* (TH) and *T.viride* (TV), seed coating with the same bio agents, seed priming and seed dressing with fungicide as comparative treatment in control of *R. solani* , *F.solani* and *F. oxysporum* the main pathogens of green bean root rot disease was evaluated under artificially infested soil in green house.

Bio priming and seed coating with bio agents (*T. harzainum and /or T. viridi*) as well as seed dressing with fungicide (Rizolex-T) suppressed green bean root rot at pre - emergence damping off stage (15 days after sowing) compared with priming and control treatment (non treated seeds) Table(3) .The most effective seed treatment was primed seeds that coated with *T. harzainum* (bio priming TH) followed by seed dressed with fungicide and primed seeds that coated with *T. viridi* (bio priming TV), these treatments decreased pre-emergence damping off caused by *R. solani* , *F.solani* and *F. oxysporum* by 78.2. 73.7% and 70.5%; 73.8, 71.0and 69.0%; 78.2, 73.0and 67.0% respectively. Meanwhile, coated non primed seeds with *T. harzainum and /or T. viridi* reduced pre emergence damping off by a rang 55.6-60.9% and 48.8-53.3% of the al pathogens respectively. 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 (Callan, *et al*., 1997) .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.

The same trend of results was observed 30 and 45 days after sowing when bio –priming seed treatment were applied, leading to a highly significantly control of root rot disease caused by the same pathogens Table

(3). Moreover, high level of healthy, surviving plants was observed following the application of such treatments. Bio primed and seed coated with either *T. hazianum* or *T. viridi* as well as seed dressed with fungicide treatment were the most effective in controlling root rots caused by *R. solani* , *F. solani and F. oxysporum* 45 days after sowing. As, these treatments decreased root rot caused by such pathogens by 76.0,76.0 and 70.8%; 73.6, 71.2 and 67.4%; 71.2, 68.8,and 50.6% respectively . Bio –primed and seed coated treatments were superior to seed dressed with fungicide (Rizolex-T). Since green house pot experiments provided promising results the same treatments were applied to field conditions during two successive seasons 2010and 2011 to assess the control of green bean root rot diseases in practical environment

Table 3: Effect of bio- priming seed treatment on the incidence of pre - emergence damping - off and root rot of green bean plants in artificially infested soil in green house .

Seed treatment	Pre-emergence damping-off %		Root rot incidence % after				Survival plants %
			30 day		45 day		
	Infection	Reduction	Infection	Reduction	Infection	Reduction	
Rhizoctonia solani							
Bio-Priming TH	3.4 d	78.2	4.4 d	74.2	4.0 d	76.0	88.2 d
Bio-Priming TV	4.6 d	70.5	5.8 d	66.0	4.8 d	71.2	84.8 d
Seed coating TH	6.1 c	60.9	7.2 c	57.8	7.0 c	58.1	79.7 c
Seed coating TV	7.2 c	53.3	8.4 c	50.8	8.2 c	50.9	76.2 c
Fungicide dressing	4.1 d	73.7	5.0 d	70.7	4.4 d	73.6	86.5 d
Priming	12.1 b	22.4	14.0 b	18.1	14.2 b	14.9	59.7 b
Control	15.6 a	0.0	17.1 a	0.0	16.7 a	0.0	50.6 a
Fusarium solani							
Bio-Priming TH	4.6 d	73.8	5.8 d	69.8	5.2 d	70.8	84.4 d
Bio-Priming TV	5.4 d	69.3	6.8 d	64.5	6.4 d	64.0	81.4 d
Seed coating TH	7.8 c	55.6	10.2 c	46.8	8.8 c	50.6	73.2 c
Seed coating TV	9.0 c	48.8	11.8 c	38.5	10.0 c	43.8	69.2 c
Fungicide dressing	5.1 d	71.0	6.4 d	66.6	5.8 d	67.4	82.7 d
Priming	13.8 b	21.5	16.2 b	15.6	15.2 b	14.6	61.2 b
Control	17.6 a	0.0	19.2 a	0.0	17.8 a	0.0	45.4 a
Fusarium oxysporium							
Bio-Priming TH	3.4 d	78.2	4.4 d	74.2	4.0 d	76.0	88.2 d
Bio-Priming TV	5.0 d	67.9	5.2 d	69.5	5.2 d	68.8	84.6 d
Seed coating TH	6.1 cd	60.9	7.2 c	57.8	7.0 c	58.0	79.7 c
Seed coating TV	7.8 c	50.0	8.8 c	48.5	8.2 c	50.8	75.2 c
Fungicide dressing	4.2 d	73.0	4.6 d	73.0	4.8 d	71.2	86.4 d
Priming	12.1 b	22.4	14.0 b	18.1	14.2 b	14.9	59.7 b
Control	15.6 a	0.0	17.1 a	0.0	16.7 a	0.0	50.6 a

Bio priming TV: primed seeds that coated with *T. viridi* . Bio -priming TH: primed seeds that coated with *T. harzainum* . Seed coating TH : seed coated with spores of *T. harzainum* . Seed coating TV: seed coated with spores of *T. virid* . Fungicide dressing: seed dressed with Riezolex-T(3 g/Kg seed) . Figures with the same letters are not significant (P = 0.05).

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 *Fusarium oxysprum* 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 (Bambaras and Ndakidemi 2010). This may be also due to the proliferation of the bio agent in the primed medium (Lewis and Lumsden 2001 El-Mougy and Abdel-Kader 2008 ; Jahn and Puls 1998)

Control of root rot diseases of green bean under field conditions:

The effects of different seed treatments, i.e. bio-priming TH; bio-priming TV and fungicide treatment on the control of root rot diseases of green bean under field conditions was studied. Moreover, the beneficial effects of these treatments on vegetative growth and yield quality of green bean were also investigated.

Influence on green bean root rot disease control:

Coating primed green bean seeds with either *T. harzainum* or *T. veridi* strongly reduced root rot incidence at pre- and post emergence stages of green bean plants , resulting in high survival healthy plants Table(4) . Coated primed green bean seeds with either *T. harzainum* or *T. veridi* strongly reduced root rot at pre emergence stage by 78.2, 60.9%and 73.8.55.6% during 2009and 2010 seasons .These treatments caused reduction in root rot incidence percentage after 40 and 60 days from sowing reach to 76.0, 58.1% and 70.8, 50.6% during the same seasons. Meanwhile, dressed green bean seeds with Rixolex-T decreased pre-emergence and root rot incidence by 71.8, 63.6 % and 60.4, 60.0 during 2009 and 2010 seasons respectively. 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; Warren and Benne t, 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* 1996; Jahm and Puls 1998, Warren and Bennett, 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 Blum, *et al*.,1991 Harman, *et. al*., 1989; ; Jensen *et al*, 2002; Jahn and Puls ,1998.

Table 4: Effect of bio- priming seed treatment on the incidence of pre -emergence damping - off and root rot of bean plants during 2010 and 2011 seasons

Seed treatment	Pre-emergence damping-off %		Root rot incidence % after				Survival plants %
			40 day		60 day		
	Infection	Reduction	Infection	Reduction	Infection	Reduction	
First season 2010							
Bio-Priming TH	3.4 c	78.2	4.4 c	74.2	4.0 c	76.0	88.2 c
Bio-Priming TV	6.1 b	60.9	7.2 b	57.8	7.0 b	58.1	79.7 b
Fungicide dressing	4.4 c	71.8	6.0 bc	64.9	6.6 b	60.4	83.0 c
Control	15.6 a	0.0	17.1 a	0.0	16.7 a	0.0	50.6 a
Second season 2011							
Bio-Priming TH	4.6 c	73.8	5.8 c	69.8	5.2 c	70.8	84.4 d
Bio-Priming TV	7.8 b	55.6	8.2 b	46.8	8.8 b	50.6	73.2 b
Fungicide dressing	6.4 c	63.6	7.2 bc	62.5	7.0 bc	60.0	79.4 c
Control	17.6 a	0.0	19.2 a	0.0	17.8 a	0.0	45.4 a

Bio priming TV: primed seeds that coated with *T. viridi* . Bio -priming TH : primed seeds that coated with *T. harzainum* . Fungicide dressing: seed dressed with Riezolex-T(3 g/Kg seed). Figures with the same letters are not significant (P = 0.05).

Influence on green bean vegetative growth:

Bio priming and seed dressed with Rixolex-T(fungicide) treatments clearly stimulating the vegetative growth of green bean plants during both seasons when compared with control and priming seed treatments Table(5). Coating primed green bean seeds with either *T. harzainum* or *T. veridi* were the most superior treatments recoding the greatest plant height and plant branches during two seasons .Fungicide seed treatment cause considerable effects on increasing records of plant height and number of branches of plants if compared with primed and control treatments . Bio priming TH treatment was the best treatments in increasing all tested growth parameters of green bean plants, as the highest records of plant height (59.2 and 60.4 cm), average of leaves /plant (22.8 and 23.0), average of branches /plant (8.8 and 9.4) as well as fresh and dry weight of shoots/plant (83.8, 89.2 gm and 12.6, 13.2 gm) were observed during two seasons respectively. 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 pea 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*[11989; 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.).

Table 5: Effect of seed treatments on vegetative growth parameter of green bean plants during 2010 and 2011 seasons.

Seed treatment	Average vegetative growth parameter				
	Plant Height (cm)	No. of branches per plant	No. of leaves/plant	Fresh weight of plant (g)	Dry weight of plant (g)
First season 2010					
Bio-Priming TH	59.2b	8.8b	22.8b	83.8b	12.6b
Bio-Priming TV	57.0b	8.2b	23.0b	80.0b	11.2b
Fungicide dressing	56.4a	7.8b	21.0b	72.6b	9.4a
Control	45.0a	6.4a	19.2a	66.4a	8.5a
Second season 2011					
Bio-Priming TH	60.4b	9.4b	23.0b	89.2b	13.2b
Bio-Priming TV	58.2b	8.0b	23.4b	82.0b	10.6b
Fungicide dressing	55.0a	8.4b	22.8b	75.8b	10.2b
Control	54.6a	7.0a	21.0a	70.0a	9.0a

Bio priming TV: primed seeds that coated with *T. viridi* . Bio -priming TH : primed seeds that coated with *T. harzainum* . Fungicide dressing: seed dressed with Riezolex-T(3 g/Kg seed) . Figures with the same letters are not significant (P = 0.05).

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Influence on early and total yield of green pods of green bean plants:

Bio priming and seed dressing with Rixolex-T(fungicide) treatments significantly increase the early and total green pods yield as well as their parameters Table (6). Coating primed green bean seeds with either *T. harzainum* or *T. veridi* were the most superior treatments recoding the greatest No. of pods /plant (17.1-18.8 and 18.0-18.3), weight pods /plant (35.3-38.3 and 34.1-37.5 gm), no of seeds /plant (47.2-52.2 and 50.1-51.6) weight of seed/plant(16.5-18.2and 17.2-18.6 gm), weight of 100 seeds /plant (32.2-35.1 and 35.8-38.7 gm), early green pods yield /fed(2.2-2.3 and 2.6-2.8 t/fed) and total yield/fed (5.6-6.3 and 5.8-6.5 t/fed) during 2010and 2011 season respectively. Meanwhile, fungicide treatment (Rhizolex-T) show increasing in early and total yield of green pods reach to 2.1, 5.1 and 2.5 , 5.3 t/fed during two seasons compared with 1.2, 4.2 and 1.4, 4.4 t/fed of control treatment during two seasons respectively. Bio- priming cause increase in early green pods yield, total green yield, total pods/plant and average weight of pods if compared with fungicide treatment and control treatments. Moreover, increasing the vegetative growth (Table, 5) turn on increasing pods yield and gave the best quality of green pods of green bean. The results are in harmony with those reported by Sharif *et al.* (2000), Din *et al.* (2003), Kon *et al.* (2007), Hamideldin (2010), Khalil *et al.* (2010) and Mengistu andmoah (2010) who reported that bio –priming seed treatments caused increasing in the vegetative growth, pod yield as well as gave the best quality yield parameters of many crops . 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 6: Effect of seed treatment on yield parameter of green bean plants during 2010 and 2011 seasons

Seed treatment	No. of pods per plant	Weight of pods Per pant(gm)	No. of seeds per plant	Weigh of seeds per plant (gm)	Weigh of 100 seeds (gm)	Early green pods yield(t/fed)	Total green pods yield(t/fed)
First season 2010							
Bio-Priming TH	18.8 c	38.3 d	52. 2 d	18.2 c	35.1c	2.3b	6.3c
Bio-Priming TV	17.1 bc	35.3 c	47.6 c	16.5 b	32.2b	2.2b	5.6b
Fungicide dressing	16.4 b	32.2b	48.2 b	15.8 b	33.0b	2.1b	5.1b
Control	16.3 a	30. 6 a	40.8 a	13.7 a	30.0a	1.2a	4.2a
Second season 2011							
Bio-Priming TH	18.3 c	37.5c	51.6c	18. 6c	38.7c	2.8c	6.5c
Bio-Priming TV	18.0 c	34.1b	50.1b	17.2b	35.8b	2.6b	5.8b
Fungicide dressing	17.4 b	35.0b	50.0b	16.5a	35.2b	2.5b	5.3b
Control	16.6 a	30.2 a	46.3a	16.4a	32.5a	1.4a	4.4a

Bio priming TV: primed seeds that coated with *T. viridi* . Bio -priming TH: primed seeds that coated with *T. harzainum* . Fungicide dressing: seed dressed with Riezolex-T(3 g/Kg seed) . Figures with the same letters are not significant (P = 0.05).

Influence on nutritional value of green bean seeds:

Chemical parameters of green bean seeds are improved du to all seed treatments Table (7).Total protein, total sugars, total nitrogen content and TSS were significantly increased with seed bio priming treatments, the highest values were recorded with bio priming TH and bio priming TV treatments, in both growing seasons. Bio –priming TH and Bio Priming TV treatments were significantly enhance the quality and chemical contents of green pods of green bean plants during two seasons. Where, the high values of total nitrogen (4.38, 3.8 and 4.1, 3.8 g/100g), total sugars (1.43, 1.40and1.45. 1.40 %), total protein (21.0, 20.0 and 20.8, 20.2 %) and total T.S.S (4.8, 4.7 and 4.7, 4.6 %) were recorded during 2010 and 2011 seasons respectively .Fungicide seed treatment show considerable effect in increasing the same chemical parameters of green bean pod during the same seasons. 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 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.

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

Seed treatment	Total nitrogen g / 100g(f.w)	Total sugars g / 100g(f.w)	Total protein %	T.S.S
First season 2010				
Bio-Priming TH	4.38 b	1.43b	21.0c	4.8b
Bio-Priming TV	3.85b	1.40b	20.0c	4.7b
Fungicide dressing	3.25 a	1.40a	18.2 b	4.2a
Control	3.11 a	1.39a	14.8a	4.1a
Second season 2011				
Bio-Priming TH	4.10b	1.45b	20.8c	4.7b
Bio-Priming TV	3.80 b	1.40b	20.2c	4.6b
Fungicide dressing	3.41 a	1.38b	19.0 b	4.6b
Control	3.50a	1.38b	15.0a	4.1a

Bio -priming TH : primed seeds that coated with *T. harzainum* . Bio priming TV: primed seeds that coated with *T. viridi* . Fungicide dressing: seed dressed with Riezolex-T(3 g/Kg seed) . Figures with the same letters are not significant (P = 0.05).

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 green bean seeds with either bio control agents such as T .*harzaium* and *T. veridi* 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 pea 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. 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. This application of Bio-priming as seed treatment that integrates the biological and physiological aspects of disease control has been widely used in order to improve product quality and to overcome some soil problems related to ground fertilization. In this study, Bio-priming as seed treatment application aimed at not only improving plant growth pares, but also improving the economic yield in terms of quantity and quality.

References

A.O.A.C., 1990. Official Methods of Analysis of Association of Official Agricultural Chemists. 15th: 1045-1106.

Abdel-Kader, M.M., 1997. Field application of *Trichoderma harzianum* as biocide for control bean root rot disease .Egypt J. Phytopatholo., 25: 19-25.

Bambara, S., P.A. Ndakidemi, 2010. Changes in selected soil chemical properties in the rhizosphere of *Phaseolus vulgaris* L. supplied with *Rhizobium* inoculants, molybdenum and lime. Sci Res Essays, 5(7): 679- 684.

Begum, M.M., M. Sariah, A.B. Puteh and M. Siddiquiy, 2010. Field performance of bio primed seeds to suppress *Colletotrichum truncotum* causing damping off and seedling stand of soybean . Biological Control., 53(1): 18-23.

Begum, M.M., M. Sariah, A.B. Puteh and M. Siddiquiy, 2010. Field performance of bio primed seeds to suppress *Colletotrichum truncotum* causing damping off and seedling stand of soybean . Biological Control., 53(1):18-23.

Bhattacharyya, P. and N. Mukherjee, 1990. *Rhizobium* challenges the root rot pathogen (*Sclerotium rolfsii*) on groundnut surfaces. *Indian Agriculturist.,* 34: 63-71.

Blum, L.K., S.D. Frey and G. Soto, 1991. Effect of fluorescent-pigment producing *Rhizobium* on the severity of *Rhizoctonia solani* seed and root rot of *Phaseolus vulgaris.* A paper presented at a symposium held at the Beltsville Agricultural Research Center (BARC), held on 8-11 May, 1989.

Blum, L.K., S.D. Frey and G. Soto, 1991. Effect of fluorescent-pigment producing *Rhizobium* on the severity of *Rhizoctonia solani* seed and root rot of *Phaseolus vulgaris.* A paper presented at a symposium held at the Beltsville Agricultural Research Center (BARC), held on 8-11 May, 1989.

Callan, N.W., D.E. Mathre and I.B. Miller, 1991. Field performance of sweet corn seed bio-primed and coated with *Pseudomonas fluoresens* AB 245 Hort science, 26: 1163-1165.

Callan, N.W., D.E. Mathre, I.B. Miller and C.S. vavrina, 1997. Biological seed treatments, factors affecting their efficacy.Hort. Science, 32: 197-183.

Callen, N.W., D.T. Mathre and J.B. Miller, 1991. Yield performance of sweet corn seed bio-primed and coated with Pseudomonas fluorescence AB 254. Hort. Science, 26: 1163-1165.

Conway, K.E., R. Mereddy, A.B. kahan, Y. Wa and L. Wa, 2001. Beneficial effects of solid matrix chem.-priming on okra .Plant Dis., 85: 535-537.

Dobois, D., 1960. Dubois, M.K., A. Gilles, J.H. Hamilton, R. Rebers and F. Smith, 1960. Colorimetric method for determination of sugars and related substances. Ann. Chem., 28(3): 350-356.

Duncan, D.B., 1955. Multiple ranges and multiple F test J. Biometrics, 11: 1-42.

El-Mohamedy, R.S.R. and M.M.H. Abd –El-Baky, 2008. Effect of seed treatment on control of root rot disease and improvement growth and yield of pea plants. *Middle Eastern and Russian Journal of Plant Science and Biotechnology,* 2(1-2): 84-90.

El-Mohamedy, R.S.R, M.A. Abd –Alla and R.I. Badiaa, 2006. Soil amendment and bio - priming treatments as alternative fungicides for controlling root rot diseases on cowpea plants in Nobria province. Research J. Agric and Biological Sci. (Pakistan) 2(6): 391-398.

El-Mohamedy, R.S.R., 2004. Bio-priming of okra seeds to control damping off and root rot diseases. Annals Agric. Sci. Ain Shams Univ. Cairo, 49(1): 339-356.

El-Mougy, N.S and M.M. Abdel-Kader, 2008. Long –term activity of bio priming seed treatment for biological control of faba bean root rot pathogens .Australasian plant pathology, 37(5): 464-471.

Estevez de Jensen, C., J.E. Kurle and A. Pereich, 2001. Management of dry bean root rot in Minmesota .Northarvest Grower ,6. fluorescent *Pseudomonas* and *Rhizobium*. *Plant Soil.*, 229: 25-34.

Ferreira, J.H.S., F.N. Matthee, A.C. Thomas, 1991. Biological control of *Eutypa lata* on Grapevine by an antagonistic strain of *Bacillus subtilis.* Phytopathology, 81: 283-287.

Gilman, J.C., 1957. A manual of soil fungi 450 pp. The Iowa State College Press, U.S.A.

Haque, S.E. and A. Ghaffar, 1993. Use of Rhizobia in the control of root rot disease of sunflower, okra, soybean and mungbean. *J. Phytopathol.*, 138: 157-163.

Harman, G.F., A.G. Taylor and T.E. Stasz, 1989. Combining effective strains of *Trichoderma harzanum* and solid matrix priming to improve biological control seed treatment. Phytopathology, 73: 631-637.

Harman, G.F., A.G. Taylor and T.E. Stasz, 1989. Combining effective strains of *Trichoderma harzanum* and solid matrix priming to improve biological control seed treatment. Phytopathology, 73: 631-637.

Jahn, M. and A. Puls, 1998. Investigations for development of a combined biological physical method to control soil borne and seed borne pathogens in carrot seed. J. Plant Dis. Prot., 105: 359-375.

Jensen, B., T.M.B. Knudsan and D.S. Jensen, 2002. Survival of conidia of *Clonostachys rosea* coated on barley seeds and their bio-control efficacy against seed borne *Bipolaris sorokiniana*. Biocontrol Sci. Technol., 12: 427-441.

Khalequzzaman, K.M., M.K. Uddin, M.A. Rahman, M.A. Rouf and A.K.M.Z. Rahman, 2003. Effect of fungicides in controlling foot and root rot of French bean by seed treatment. *Bangladesh J. Agric. Sci*., 30: 9-14.

Kibria, M.G. and I. Hossain, 2002. Effect of biofertilizer and *Rhizobium* on foot and root rot disease and seed yield of mungbean. *Bangladesh J. Seed Sci. & Tech.,* 6(1&2): 41- 45.

Kirkby, E.A. and V. Römheld, 2004. Micronutrients in plant physiology: functions, uptake and mobility. Proceedings No. 543, International Fertilizer Society.

Lewis, J.A and R.D. Lumsden, 2001. Bio control of damping off of greenhouse grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp Crop Protection., 20: 49-56.

Lewis, J.A. and R.D. Lumsden, 2001. Bio control of damping off of greenhouse grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp Crop Protection., 20: 49-56.

Loeffez, R., T. windhand and R. Baken, 1986. Mechanisms of biological of pre. emergence damping-off of pea by seed treatment with *Trichoderma spp*. Phytopatho Loqy, 76: 720-725.

Nabban, H.M., 1966. Studies on the suspended matter in Nile water, with special references to is physical and chemical properties. M. Sc. Thesis Fac. Agric., Cairo Univ

Nayaka, S.C., S.R. Nironjana, A.C. Shankar and C.N. Mortensen, 2008. Seed bio-priming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisin in maiza . Archives of phytopathology and plant protection., pp: 1-19.

Nayaka, S.C., S.R. Nironjana, A.C. Shankar and C.N. Mortensen, 2008. Seed bio-priming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisin in maiza . Archives of phytopathology and plant protection., pp: 1-19.

Nayaka, S.C., S.R. Nironjana, A.C. Shankar and C.N. Mortensen, 2008. Seed bio-priming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisin in maiza . Archives of phytopathology and plant protection., pp: 1-19.

Nelson, P.E., T.A. Tousoun and W.F.O. Marason, 1983. *Fusarium* spp. An illustrated Manual of identification 218 pp. The Pennsylvenina Univ. Press, Park. USA.

Osburn, R.M. and M.N. Scharoth, 1989. Effect of osmo priming sugar beet seed on germination rate and incidence of *Pythium ultimum* damping-off. Plant disease, 73: 21-24.

Persson, L., L. Badker and M. Larsson, 1997. Prevalence and pathogenicity of foot and root-rot pathogens of pea in southern Scandinavia. Plant Dis., 81: 171-174.

Rao, M.S.L., Y.H. Kulkarmi, S. Lingaraju and H.L. Nadaf, 2009. Bio priming of seeds : Potential tool in the integrated management of Alternaria blight of sunflower .HELTA 32(50): 107-114.

Rashid, M.A. and D.P. Singh, 2000. A Manual on Vegetable Seed Production in Bangladesh. AVRDC—USAID--Bangladesh Project, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh., pp: 73.

Rojo, F.G., M.M. Reynoso, M.F. Sofa, N. Chuluzel and A.M. Torres, 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions Crop Protecion., 78: 153-156.

Sallam(Nashaw), M.A., K.A.M. Abo-Elyouser and M.A.E. Hassan, 2008. Evaluation of Trichoderma species as biocontrol agents for daming-off and wilt diseases of Phaseolus vularia L.and efficacy of suggested formula. Egypt J. Phytopathol., 36(1-2): 81-93.

Snedecor, C.W. and W.G. Cochran, 1982. Statistical Methods. 7th Ed.The lowa state Univ. Press.Ames. lowa, USA, pp: 325-330.
Warren, J.E. and M.A. Bennett, 1999. Bio-osmo-priming of tomato (*Lycopersicon esculentum* Mill.) Seeds for improved stand establishment. Seed Sci. Technol., 27: 489-499.
Yenu, S.H. and A.G. Follard, 1952. The determination of nitrogen in agricultural by Nessler reagent. J. Sci. Food Agric., 3: 442.