# Management of Root Rot Diseases and Improvement Growth and Yield of Green Bean Plants Using Plant Resistance Inducers and Biological Seed Treatments

# El-Mohamedy, R. S. R.<sup>1</sup>\*, Shafeek, M. R.<sup>2</sup> and Fatma, A. R.<sup>2</sup>

<sup>1</sup>Plant Pathology Department, National Research Center, Cairo, Egypt.; <sup>2</sup>Vegetable Research Department, National Research Center ,Cairo, Egypt.

El-Mohamedy, R. S. R., Shafeek, M. R. and Fatma, A. R. (2015). Management of root rot diseases and improvement growth and yield of green bean plants using plant resistance inducers and biological seed treatments. International Journal of Agricultural Technology 11(5):1219-1234.

Abstract This study was carried out to evaluate the efficiency of using Trichoderma harzianium (bio control agent) in combination with sorbic and benzoic acid (chemical resistance inducers) in management of root rot diseases on green bean plants caused Rhizoctonia solani and Fusarium solani. In green house, combination between T. harzianium and sorbic and/or benzoic acid at treatments were more effective in control Rhizoctona and Fusarium root rots on bean plants, than each of them separately. Seed soaking in Sorbic acid 5% and /or Benzoic acid at 5% + Seed coating with T. harzianum treatments cause reduction in Rhizcotonia root rot reach to 70.2%; 68.4% and 63.6%; 61.2%, and reach to 68.2; 65.8% and 70.4;68.8 % of Fusarium root rot at pre and of post emergence stages respectively. In field trails, seed coating *T. harzianum* combined with sorbic acid and/or benzoic acid at 5.0% as well as fungicide were the most effective treatment, they reduce root rot diseases by 74.7, 70.9,72 .2% and 70.7, 70.4,72.4% of pre emergence damping off and root rot respectively. Moreover, such treatments improve plant growth, total yield and its components as well as green pod nutritional value parameters of green bean superior to fungicides seed treatment. The use of biological seed treatment combined with chemical plant resistance inducers might be considered as safe, cheap and easily applied method for controlling such soil-borne plant pathogens considering the avoidance of environmental pollution and the side effect of pesticide application.

# Introduction

Green Bean (*Phaseolus vulgarus* L.) is one of the most important leguminous crops in Egypt. Root rot disease caused by *Fusarium solani* and *Rhizoctonia solani*is a serious and persistent diseases problem of bean plants during growing season (Abdel-Kader ,1997;El-Mougy , 2001; Harveson , *et al.*, 2005; Wen, *et al.*, 2005). An investigation for controlling green bean root rot

<sup>\*</sup>Corresponding author: El-Mohamedy, R. S. R.; Email: riadelmohamedy@yahoo.com

diseases is consider important, epically in view of its prevalence in Egypt, particularly in new reclaimed land in the desert. There is a growing need to develop alternative approaches for controlling plant diseases. An alternative to fungicidal application, it may be possible to utilize a scheme of inducible plant defenses, which provides protection against broad spectrum of disease-causing organisms. Induction of resistance in plants to overcome pathogen infection is a promising approach for controlling plant diseases. This induced resistance to pathogens can be achieved by the application of various abiotic agents (chemical inducers) such as potassium salts (Abdel-Monaiem, 2010, El-Mohamedy et al., 2013). Conversely, application of these chemical inducers under field conditions have increased growth parameters, yield components and quality of many vegetable plants (El-Mohamedy et al., 2014). Among synthetic chemical inducers, salicylic, sorbic and benzoic acids have been found to be active as antimicrobial agents in various trials as disease resistance inducers .Also, they have been reported for inducing resistance against several plant pathogens (Abd-el-Kareem ,1998; El-Kallal, 2007; Abdel-Monaiem, 2010; Abdel-Monaiem, et .al., 2010; Abdel-Kader et al., 2012).

Biological control is proposed to be an effective and non-hazardous strategy to reduce crop damage caused by plant pathogens. Application of biological control using antagonistic microorganisms against seed and root rot pathogens proved to be successfully and its efficiency in controlling root rot pathogens and improving plant growth, total yield and nutritional values of many vegetable crops (El-Mohamedy *et al.*, 2014).Coating seeds of many crops with bio control agents such *Trichoderma* spp. , *Bacillus subtillus* , *Psedomonas fluorescens* was the most effective treatments for controlling seed and root rot pathogens (Nayaka *et al.*, 2008; Begum *et al.*, 2011). However, El-Mohamedy and Abd-Alla (2013) 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.

Biological control of soil borne pathogens is often attributed to improved nutrition that boosts host defenses or to direct inhibition of pathogen growth and activity. Amendment with certain a biotic factor (inducers) appears to stimulate the disease resistance by indirectly stimulating indigenous populations of microorganism that are beneficial to plant growth and antagonistic to pathogens. For example chitin amendment of soil has been found to stimulate the growth of chitinolytic microorganisms (De Boer *et al.*, 1999), increase the biocontrol activity and stimulate the expression of plant defense proteins (Roby *et al.*, 1987). All these effects may culminate in enhancing plant protection. Similarly, SA and H2O2 amendment was tested in combination with bio control agents. Saikia *et al.* (2003) tested the efficiency of *Pseudomonas fluorescence* with or without SA amendment in chickpea against *Fusarium* wilt infection .The application of *P. fluorescence* (pf4-92) with SA recorded highest protection of chickpea seedlings against wilt disease.

The objective of the research work performed was to evaluate different alternatives fungicidal seed treatment *i.e.* biological seed coating with *Trichoderma harzianum* and chemical plant resistance inducers as well as seed dressing with Rizolex-T (Fungicide) as comparison treatment against root rot disease incidence of green bean under field conditions. The efficiency of such seed treatments on vegetative growth, total yield and its components as well as green pod nutritional value parameters are investigated.

#### Materials and methods

#### Source of bean seeds, pathogenic fungi and bio agent

Bean seeds cv. Giza 3 were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt. *Fusarium solani and Rhizoctonia solani* were isolated from bean showed typical root rot disease symptoms. Bio control agent *Trichoderma harzianum* was isolated previously from rhizospher soil of healthy bean plant and antagonistic ability against most soil borne pathogenic fungi was recorded in previous studies (El-Mohamedy *,et al.*, 2013)

#### Types of Seed treatments

**Biological seed coating:** Bean seeds cv. Giza 3 were immersed in 1% CMC suspension of *Trichoderma harzianum* for 60 min. Spore suspension of *T.harzianum* ( $3x10^4$ cfu /ml) as prepared from 7-dayeold cultures grown on PDA medium *T. harzianum* was previously isolated from rhizosphere soil of healthy green bean plants and the antagonistic ability against some root rot pathogens was recorded..

*Seed soaking*: Disinfected bean seeds were soaked for 2 h in the tested essential oils at the rate of Sorbic acid and Benzoic acid at 2.5 and 5.0% (v/w), and then left to air dry before sowing. Stock solutions (4000 ml) of each of the tested inducers were prepared in concentrations of 2.5 % by dissolving in sterilized distilled water and used for foliar spray.

*Fungicide seed dressing*: Bean seeds was dressed with Rizolex-T50 % 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.

## Management of root rot diseases of green bean udder greenhouse conditions

This experiment was carried out to evaluate the efficiency of different seed treatments i.e., seed coating TH ,(applying *T. harzianum* to green bean seeds), sorbic acid at rate 2.5% and 5% (w/v), benzoic acid at rate 2.5% and 5% (w/v), Seed coating TH + sorbeic acid 2.5%, Seed coating TH + sorbeic acid 5%, Seed coating TH + benzoicacid2.5%, Seed coating TH + benzoic acid 5% as well as seed dressing with fungicide (Rizolex-T) as comparison treatment in controlling green bean root rot pathogens under artificially infested soil .Plastic pots (20 cm diameter) containing artificially infested soil of individually pathogenic fungus *i.e.*, *F. solani* and *R. solani* were used. The following seed treatments used as follow:

(A) Single treatments: 1-Seed coating TH .2 -Sorbicacid 2.5%. 3-Sorbic acid 5%. 4-Benzoic acid 2.5%. 5-Benzoic acid 5%.

(B) Combined treatments: 6- Seed coating TH + Sorbic acid 2.5%. 7-Seed coating TH Sorbic acid 5%. 8-Seed coating TH + Benzoic acid 2.5%. 9- Seed coating TH + Benzoic acid 5%. 10-Fungicide (Rizolex-T 3g/kg seed). 11-Control (untreated seeds). Treated and untreated bean seeds (Giza, 3 cv.) were sown in plastic pots (25 cm diameter) in artificially infested loamy soil with inocula of either *R. solani* or *F. solani* at the rate of 5% of soil weight (Abdel-Kader, 1997).Comparison treatment included a set of bean seeds coated with fungicide Rizolex-T at the rate of 3 g/kg of seeds.Untreated plastic pots were left for general check. Five bean seeds were sown in each pot, and ten pots were used as replicate for each particular treatment. Foliar spray with chemical inducers was applied at the second true leaves stage of emerged bean seedlings.

Percentages of root rot incidence of bean at pre- and post-emergence stages were calculated after 10 and up to 45 days of the experimental period, respectively.

Pre-emergence (%) was based on the number of un-emerged seeds in relation to the number of sown seeds, while Post-emergence (%) was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings.

#### Management of root rot diseases of green bean under field conditions

Field experiments was carried out during 2013 and 2014 seasons at the Experimental Research Station of National Research Centre at El-Noubaria region, Behera Governorate, Egypt. This field is well known by the authors as naturally heavily infested with soil borne pathogens. The efficacy of the promising treatment obtained under greenhouse trail for controlling root rot diseases of bean were applied. The fungicide Rizolex-T was used in this study as a comparison to the other treatments. Benzoic acid and sorbic acid chemical inducers were used as bean seed soaking before sowing; next, the emerged plants (at 2-3 true leaves age) were sprayed with the same inducers at concentration of 2.5% as stated above. The field trail conducted in 35 plots, each 12m<sup>2</sup>, established in naturally heavily infested soil with root rot pathogens. The evaluated treatments applied as follows: (A) Single treatments: Seed coating. Sorbic acid 5%. Benzoic acid 5%.(B) Combined treatments: Seed coating + Sorbic acid 5%. Seed coating +Benzoic acid 5%. Fungicide (Rizolex-T 50% at the rate of 3g/Kg seeds as seed dressing).Control (untreated seeds). All treatments applied in Complete Randomized Block Design with five replicates (plots) for each particular treatment. The Bean seeds cv. Giza 3 were sown as 3 seeds per hill and 30cm distance between hills. Percentage of root rot incidence at the pre-emergence stage calculated as the number of absent emerged seedlings in relative to the total number of sown seeds. Meanwhile, percentage of post- emergence root rot calculated as the number of bean plants showing disease symptoms in relative to the total number of emerged seedlings. The percentages of pre-emergence root rot incidence was calculated after 20 days from sowing, meanwhile post-emergence bean root rot incidence was recorded periodically every 10 days starting from 20 up to 60 days of plant growth. The beneficial effect of different seed treatments on vegetative growth, yield and pod nutritional values. The normal cultural practices i.e. irrigation, fertilizer and pest control for the bean productions were followed.

#### 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, leaf area plant  $cm^2$  as well as fresh and dry weight of leaves and stems.

#### 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 yield per fed. and the following data were recorded: average number of pods per plant, pods weight(g), total yield of green pods/  $m^2$  (kg) and total green pods (ton / fad.) 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 and Weight of pods per pant (g).

#### Nutritive Value of pods

A random sample of 50 green pods at 2- picking were taken and the following data were recorded: N % in pod was determined according to the method of Pregl (1945). In addition, protein percentages in pod was calculated by multiplying nitrogen content by 6.25. Potassium was assayed using flame spectrophotometer according to Allen *et al.* (1984). Phosphorous was extracted and measured spectrophotometer according to Jackson (1965). As well as dry matter % was determined according to the method of Dubois *et al.* (1960).

#### Statistical Analysis

All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1982).

#### **Results and Disscusion**

#### Control of root rot disease of green bean in greenhouse

Different seed treatments *i.e.*, seed coating TH (seed of green bean coated with *T. harzianum*), seed soaking in sorbic acid and/or benzoic acid at rate 2.5% and 5%(w/v), seed soaking in sorbic acid and/or benzoic acid at rate 2.5% and 5%(w/v)+ Seed coating TH and seed dressing with fungicide (Rizolex-T) as comparison treatment were applied to control the main root rot pathogens (*Rhizoctonia solani and Fusarium solani*) of green bean plants under artificially infested soil in green house.

Results in Table (1) show that all seed treatments significantly decreased Rhizoctonia root rot on been at pre and post emergence stages if compared with control treatment. Fungicide(Rhizolex-T3g/kg seed) treatment cause reduction of root rot disease incidence reach to72.0% and68.2% at pre and post emergence stages respectively. Meanwhile, combined treatments between bio agent T. harzianum and chemical inducers (sorbic and benzoic acids) cause superior effective in control of bean root rot disease caused by R. solani .As, seed coating TH + seed soaking in Sorbic acid 5% and /or Benzoic acid 5% treatments cause reduction in bean root rot incidence reach to 70.2% and 68.4% of pre emergence and 63.6% and 61.2% of post emergence after 45 days of seed sowing, the highest records of survival plants 77.3% and 75.7% were recorded with these treatments . Seed coating TH and Sorbic acid 5.0 % cause considerable effect, as these treatments cause reduction of root rot incidence at pre and post emergence stages reach to 54.8 % and 55.0% respectively, however single treatments of Sorbic acid (2.5% and 5.0 %) and Benzoic acid 5.0 % cause less effect, as the root rot disease reduction was less than 50%. These results are agreement with those reported by many researchers.

Treatments		Pre-emer	rgence	Root	ro	Survival
ST	FA	damping off		incidence	%	plants %
	•			after 45 day		
		Infection	Reduction	Infection	Reduction	
Seed coating TH		10.9c	54.8	19.2c	55.0	69.9c
Sorbic acid 2.5%	2.5%	14.5b	40.0	25.1b	41.2	60.4b
Sorbic acid 5.0 %	2.5%	11.8b	51.8	22.6b	47.0	65.6b
Benzoic acid 2.5%	2.5%	13.9b	42.4	25.3b	40.8	60.8b
Benzoic acid 5%	2.5%	12.5b	48.2	23.5b	45.0	64.0b
Seed coating TH + Sorbic acid	2.5%	9.9c	58.8	16.9c	60.4	73.2c
2.5%						
Seed coating TH + Sorbic acid	2.5%	7.2d	70.2	15.5c	63.6	77.3d
5%						
Seed coating TH + Benzoic acid	2.5%	10.4c	57.0	17.9d	58.0	71.7c
2.5%						
Seed coating TH + Benzoic acid	2.5%	7.7d	68.4	16.6d	61.2	75.7d
5%						
Fungicide (Rhizolex-T 3g/k)		6.8d	72.0	13.6de	68.2	79.6d
seed)						
Control		24.2a	0.0	42.8a	0.0	33.0a

**Table 1.** Effect of different treatments on root rot disease incidence of green bean plants grown in artificially infected soil with *Rhizoctoniasolani* under greenhouse conditions.

ST –seed treatment FA- foliar application TH – *Trichoderma harzianum*. For each column, means followed by the same letter are not significantly different according to Duncan's multiple range test ( $P \le 0.05$ ).

In this respect, many researchers used chemical resistance inducers for controlling root rot and wilt diseases under greenhouse and field conditions (Abdel-Kareem, 1998). These results were in agreement with these which obtained by many investigators. As, the tested chemical inducers might stimulate some defense mechanisms such as phenolic compounds, oxidative enzymes and other metabolites (Amel *et al.*, 2010; Abdel-Monaim *et al.*, 2011; El-Mohamedy *et al.*, 2013).The results reported here suggest that the presence of *T. harzianum* in the rhizosphere significantly reduces the root rot caused by *F. solani* in bean plants. The reduction might be related to the decline of the population density of *F. solani* in soil and also due to alterations caused by *T. harzianum* in the *F. solani* .On the other hand, it should be mentioned that some chemical inducers may also have a direct antimicrobial effect and are, thus, involved in cross inking in cell walls, induction of gene expression, phytoalexin production and induction of systemic resistance against root rot pathogen (Abdel-Monaim , *et al.*, 2011; Abdel-Kader *et al.*, 2012).

Concerning to Fusarium root rot disease control, the same trends of results are shown in Table (2). As, all biological and inducers seed treatments significantly reduce Fusrium root rot disease incidence on bean plants up to 45 of seed sowing. Combined treatments between seed coating with T.harziaunm and seed soaking in chemical inducers sorbic or benzoic acids as well as fungicide seed treatment were the most effective in control Fusarium root rot of green bean plants compared with single and control treatments. Seed coating TH + Sorbic acid 5%, Seed coating TH + Benzoic acid 5% and Fungicide (Rhizolex-T 3g/kg seed) treatments cause reduction of Fusarium root rot on bean plants reach to 68.2, 65.8, 70.0 % and 70.4, 68.8, 75.7 % at pre and post emergence stages respectively. The highest records of survival plants 70.4,68.8, and 75.7 % were also observed with these treatments compared with 21.8% of non-treated seeds (control). Single treatments of seed coating TH and Sorbic acid 5% cause considerable effect in control bean Fusarium root rot disease, as the disease reduction was up to 53.0 and 51.4 % of pre and post emergence stages respectively. Meanwhile, Sorbic acid (2.5% and 5.0%) and Benzoic acid 5.0 % cause less effect ,as the root rot disease reduction was less than 50% and the least records of survival plants were recorded compared with others treatments . Integrated between soaking green bean seeds in sorbic acid or benzoic acid at 5.0% and coating seeds with bio control agent T.harzianum cause significantly reduction in root rot diseases of green bean and were superior to fungicide seed treatment effect. 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). Many

researchers have demonstrated the potential of *Trichoderma* spp in controlling damping off and root rot diseases of crop plants caused by *Rsolani* and *Fusarium spp*. (Lewis and Lumsden, 2001; El-Mohamedy, 2004; Rojo *et al.*, 2007). Most likely, the enhanced growth of root system by *T. harzianum* as evidenced by increased biomass may be positively acted in diseases control. Moreover, *T. harzianum* is a well-known producer of cell wall-degrading enzymes and the antibiotics thus could act synergistically with other mechanisms (Vinale *et al.*, 2006).

Plants respond to chemical elicitor treatments by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading. The defense mechanisms include the fast production of reactive oxygens pieces DeGara *et al.* (2003) alterations in the cell wall constitution; accumulation of antimicrobial secondary metabolites known as phytoalexins. Agrios (2005) activation and/or synthesis of defense peptides and proteins Castro and Fontes (2005).

Treatments	Pre-emergence		Root		Surviva	
ST		damping off		incidence %		plants %
	_			after 45 day		_
		Infection	Reduction	Infection	Reductio	1
Seed coating TH		14.1c	53.0	23.4c	51.4	62.5c
Sorbic acid 2.5%	2.5%	17.0b	41.3	29.9b	32.2	53.3b
Sorbic acid 5%	2.5%	14.9c	50.2	26.0b	46.0	59.1c
Benzoic acid 2.5%	2.5%	17.8b	40.6	28.9b	40.0	53.3b
Benzoic acid 5%	2.5%	15.4c	48.8	26.1b	45.8	58.5b
Seed coating TH + Sorbic acid	2.5%	12.0d	60.0	23.6c	51.0	64.4c
2.5%						
Seed coating TH + Sorbic acid	2.5%	9.4e	68.2	19.7d	59.0	70.4d
5%						
Seed coating TH + Benzoic acid	2.5%	12.4d	58.4	23.7c	50.8	63.9c
2.5%						
Seed coating TH + Benzoic acid	2.5%	10.2e	65.8	21.0d	56.4	68.8d
5%						
Fungicide (Rhizolex-T 3g/ks		9.0e	70.0	15.3e	68.2	75.7e
seed)						
Control		30.0a	0.0	48.2a	0.0	21.8a

**Table 2.** Effect of different seed treatments on root rot disease incidence of green bean plants artificially infected with *Fusariumsolani* under greenhouse conditions

ST –seed treatment FA- foliar application TH – *Trichoderma harzianum*. For each column, means followed by the same letter are not significantly different according to Duncan's multiple range test ( $P \le 0.05$ ).

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

The effects of promising seed treatments, i.e. seed coating TH; Sorbic acid 5%, Benzoic acid 5% .combined treatments of seed coating TH + sorbic acid or benzoic acid at 5.0% on control of root rot diseases of green bean under field conditions studied during two seasons. Moreover, the beneficial effects of these treatments on vegetative growth and yield quality of green bean also investigated.

#### Influence on control of green bean root rot diseases

Seed coating with *T. harzianum* treatment alone or in combined with seed soaking in benzoic or sorbic acid strongly reduced root rot incidence at pre- and post-emergence stages of green bean plants , resulting in high survival healthy plants Table(3). The highest records of survival plant 88.8 , 82.4 , 83.3 % were recorded with these treatments respectively .Meanwhile , single treatments of Seed coating with *T. harzianum* , benzoic acid 5.0% and sorbic acid 5.0% cause reduction of pre emergence reach to 61.2, 53.4, 51.2% at pre emergence stage and reach to 63.4 , 60.0 , 58.2 % of root rot after 60 days of seed sowing respectively .Under field condition soaking bean seeds in benzoic acid 5.0% and/orsorbic acid 5.0% then coated with *T. harzianum* successfully controlled root rot disease of green bean and showed effects superior to fungicide seed treatment.

These results are confirmed with other investigators. They reported that incidence of several soil-borne plant pathogens have also been reduced by using bio control agents and chemical inducers. 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; Ahed *et al.*, 2003 Warren and Bennet, 2004; Rojo *et al.*, 2007,). Seed coating with bio-control agents was the most effective treatment for controlling root rot diseases on bean (El-Mohamedy *et al.*, 2013), pea (El-Mohamedy and Abd–El-Baky 2008), cowpea (Abdel-Kader and Ashour, 1999; El-Mohamedy *et al.*, 2006), Okra (El-Mohamedy, 2004) and Tomato (El-Mohamedy *et al.*, 2014).

The combination between bio control agents and chemical inducers were more effective to reduce damping-off, root rot/wilt severity and increased fresh and weights of survival plants than used of them individually. In this respect, Saikia *et al.* (2003) tested the efficiency of *P. fluorescence* with or without SA amendment in chickpea against Fusarium wilt infection. The application of *P. fluorescence* (pf4-92) with SA recorded highest protection of

chickpea seedlings against wilting. Rajkumar *et al.* (2008) found that pepper seeds treated with inducers (SA and chitin) alone showed a moderate degree of plant protection against *R. solani*. However, the reduction in disease was more pronounced when inducers were applied with fluorescent pseudomonades (SE21 and RD41) Amendment with chitin alone enhanced bio control efficiency of both SE21 and RD41. However, amendment with SA alone or in conjunction with chitin showed a moderate effect on bio control efficiency of the antagonists.

**Table 3.** Effect of biological seed treatment and chemicals inducers on damping off and root rot diseases incidence of green bean under field conditions (average of two seasons 2013 and 2014).

Treatments		Pre-emergence		Root rot incidence %		Root rot incidence %		Survival
SI	FA	damping off		after 40 day		after 60 day		plants
		Infection	Reduction	Infection	Reduction	Infection	Reduction	%
Seed coating TH		6.3b	61.2	8.5b	58.0	8.0b	63.4	77.2b
Sorbic acid 5%	2.5%	7.5b	53.4	8.8b	56.4	8.8b	60.0	74.4b
Benzoic acid 5%	2.5%	7.9b	51.2	9.2b	54.6	9.2b	58.2	73.7b
Seed coating TH +	2.5%	4.1c	74.7	5.7c	71.8	6.4c	70.7	88.8c
Sorbic acid 5%								
Seed coating TH +	2.5%	4.7c	70.9	6.4c	68.6	6.5c	70.4	82.4c
Benzoic acid 5%								
Fungicide		4.5c	72.2	6.1c	70.0	6.1c	72.4	83.3c
Rhizolex-T 3g/kg se	eed)							
Control		16.2a	0.0	20.4a	0.0	22.0a	0.0	41.4a
~ .								

ST –seed treatment FA- foliar application TH – *Trichoderma harzianum*. For each column, means followed by the same letter are not significantly different according to Duncan's multiple range test ( $P \le 0.05$ ).

## Influence on green bean growth characters

Data recorded in Table (4) shows clearly that all both mixed of seed coating with sorbic or benzoic acid and fungicide (Rhizolex 3 g/kg seed) significantly increased snap bean plant growth characters expressed as (plant length, number of leaves and branches as well as fresh and dry weight of leaves and branches and leaf area plant) compared to all treatments and untreated (control). Moreover, the maximum significantly values for all plant growth characters were obtained by mixed of seed coating with sorbic acid followed in descending order by snap bean plants by mixed of seed coating with benzoic acid and followed fungicide (Rhizolex 3 g/kg seed) and followed as individually seed coating or sorbic or benzoic acid followed as control treatment. The tested chemical inducers might stimulate some defense mechanisms such as phenolic compounds, oxidative enzymes and other metabolites (El-Khallal, 2007, Amel, *et al.* 2010 and Abdel-Monaim, *et al.* 2011). Also, it should be mentioned that some chemical inducers may also have 1229

a direct antimicrobial effect and are, thus, involved in crosslinking in cell walls, induction of gene expression, phytoalexin production and induction of systemic resistance (Abdel-Monaim, 2010). These results were in agreement with these which obtained by (Nayaka, *et al.*, 2008, Begum *et al.*, 2011 and El-Mohamedy and Abd-Alla, 2013).

0-	(							
Treatments	Plant	Number of		Fresh weight (g)		Dry weight (g)		LA
	length	Leaves	Branches	Leaves	Branches	Leaves	Branches	/plant
	(cm)							$(\mathrm{cm}^2)$
Seed coating TH +	50.45*	10.99*	7.72*	17.04*	17.58*	6.90*	7.61*	157.15*
Sorbic acid 5%								
Seed coating TH +	45.34*	10.87*	7.66*	16.03*	17.36*	6.67*	7.33*	155.63*
Benzoic acid 5%								
Fungicide Rhizolex	50.38*	10.28*	7.20*	16.36*	16.56*	6.17*	7.39*	156.63*
T 3g/kg seed)								
Seed coating TH	42.68	8.75	6.13	14.50	12.57*	5.23	6.09	149.38*
Sorbic acid 5%	41.52	8.27	6.20	13.33	11.53	5.33	5.65	148.07
Benzoic acid 5%	39.36	8.26	5.70	13.33	10.82	5.38	5.85	146.74
Control	38.86	8.00	5.07	11.45	9.42	4.63	5.01	144.05
LSD 5%	6.43	1.22	1.68	3.61	2.15	1.07	1.17	5.19

**Table 4.** Effect of seed coating and sorbic or benzoic acid on growth characters of green bean plants (average of two seasons).

#### Influence on total pods yield and its components

Concerning to the effect of seed coating with sorbic or benzoic acid individual or mixture on the total yield of green bean pods (Table 5), the resulted data showed that all treatments of mixture had an enhancement in green pods yield and its components if compared with that plant no treatment (control). The presented data revealed that, seed coating with sorbic acid resulted the heaviest pods yield (3703.33 kg/fed.) compared (3431.33 kg/fed.) with that plant no treatment (control). The response of number of pods/plant, weight of pods/ plant, average of pod weight and total yield of green pods/  $m^2$ to the seed coating with sorbic acid or benzoic acid nearly followed the same pattern of change which mentioned before. These increases in yield quantity and quality may be attributed to elicitors effect on physiological processes in plant such as ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis (Gharib and Hegazi, 2010). Some chemical inducers are also endogenous growth regulators of phenolic nature, which influence a range of diverse processes in plants, including ion uptake and transport, membrane permeability, photosynthetic and growth rate (Khan et al. 2003).

green bean plants (average of the beabons).									
Treatments	Number of pods / plant	Weight of pods/ plant (g)	Average of pod weight (g)	Total yield of green pods/ m <sup>2</sup> (kg)	Total yield of green pods/ fed. (kg)				
Seed coating TH + Sorbid									
acid 5%	21.50*	48.55*	2.14*	10.22*	3703.33*				
Seed coating TH + Benzoid									
acid 5%	21.17*	46.90*	2.00	10.07*	3693.67*				
Fungicide Rhizolex-T 3g/kg									
seed)	20.42*	45.41*	1.93	9.67*	3613.33*				
Seed coating TH	17.90	43.17	1.92	9.00	3531.67				
Sorbic acid 5%	17.83	42.87	1.88	8.85	3497.33				
Benzoic acid 5%	17.57	42.42	1.88	8.66	3486.67				
Control	15.62	40.51	1.79	8.49	3431.33				
LSD 5%	2.49	3.92	0.31	0.71	133.62				

**Table 5.** Effect of seed coating and sorbic or benzoic acid on total yield of green bean plants (average of two seasons).

## Influence on chemical quality of green bean pods

The mixture of seed coating and sorbic or benzoic acid and fungicide (Rhizolex 3 g/kg seed) on chemical quality of green bean pods resulted more nutritional values of the percentage of, i.e., N, Protein, P, K and dry matter in pods tissues if compared with the individual seed coating, sorbic and benzoic acid and control treatment (Table 6). Moreover, that plants which treated with the mixture of seed coating and sorbic acid resulted the best chemical properties followed in descending order by snap bean plants by mixed of seed coating with benzoic acid and followed fungicide (Rhizolex 3 g/kg seed) and followed as individually seed coating or sorbic or benzoic acid followed as control treatment. This might be attributed to the role of seed coating and sorbic acid or benzoic acid 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. In addition, coating seeds with bio control by bacteria inhabiting the rhizosphere and beneficial to plants are termed plant growth promoting rhizobacteria (Kloepper et al., 1980). Seed soaking application of bio control agents and chemical inducers individually or combination in both seasons showed a significant increase in faba bean growth parameters, yield components and protein content in seeds. The combination between biocontrol agents and chemical inducers were recorded highly increased in all growth and yield parameters more than in used of them individually, especially seed coating TH + sorbic and/or benzoic acid inducers. These increases may be attributed to biotic and abiotic elicitors effect on 1231

physiological processes in plant such as ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis (Abd-el-Kareem, 1998; Gharib and Hegazi, 2010).

Treatments Chemical quality % Ν Protein Dry matter Ρ Κ Seed coating TH + Sorbic acid 5% 3.167\* 19.770\* 0.352\* 2.167\* 7.120\* 19.737\* Seed coating TH + Benzoic acid 5% 3.150\* 0.355\* 2.157\* 7.150\* Fungicide Rhizolex-T 3g/kg seed) 3.150\* 19.653\* 0.355\* 2.143\* 7.030\* 19.230 Seed coating TH 3.100 0.333 2.127 6.677 Sorbic acid 5% 3.117 19.460 0.332 2.120 6.562 Benzoic acid 5% 3.093 19.337 0.332 2.117 6.349 Control 3.040 19.020 0.302 2.070 6.377 LSD at 5% 0.079 0.505 0.028 0.049 0.592

**Table 6.** Effect of seed coating and sorbic or benzoic acid on chemical quality of green bean pods (average of two seasons).

#### Conclusion

The combination between biocontrol agents and chemical inducers were better than used of them individually especially seed coating with *T. harzianum* + seed soaking in sobic or benzoic acids 5.0% (v/w). Soaking green bean seeds in chemical inducers and coated with biocontrol agent *T. harzianum* in dividedly or in combination treatments significantly reduced root rot diseases and increased survival green bean plants under either green house or field conditions. In addition, these treatments increased plant growth, yield quantity and quality.

#### References

- Abdel-Kader, M. M. (1997). Field application of *Trichoderma harzianum* as biocide for control bean root rot disease. Egyptian Journal of Phytopathology 25:19-25.
- Abdel-Kader, M. M. and Ashour, A. M. A. (1999). Biological control of cowpea root rot in solarized soil. Egyptian Journal of Phytopathology 27:9-18.
- Abdel-Kader, M. M., El-Mougy, N. S., El-Gammal, N. G., Abd-El-Kareem, F. and Abd-Alla, M. A. (2012). Laboratory evaluation of some chemicals affecting pathogenic fungal growth. Journal of applied sciences research 8:523-530.
- Abdel-Kareem, F. I. (1998). Induction of resistance to some disease of cucumber plants grown under greenhouse condition. (Doctor of Philosophy's Thesis). Ain Shams University.
- Abd-El-Kareem, F. (2007). Induced resistance in bean plants against root rot and Alternaria leaf spot diseases using biotic and abiotic inducers under field conditions. Research journal of agriculture and biological sciences 3:767-774.
- Abdel-Monaim, M. F., Ismail, M. E. and Morsy, K. M. (2011). Induction of systemic resistance of benzothiadiazole and humic acid in soybean plants against Fusarium wilt disease. Mycobiology 39:290-298.

- Abdel-Monaim, M. F. (2010). Induced systemic resistance in tomato plants against Fusarium wilt disease. Proceedings of the 2<sup>nd</sup> Minia Conference for Agriculture and Environmental Science, Minia, Egypt. pp. 253-263.
- Agrios, G. N. (2005). Plant Pathology. 5th Ed. San D iego, USA: Academic Press.
- Ahed A. H. M. and Juber, K. S. (2003). Biological control of bean root rot disease caused by *Rhizoctonia solani* under green house and field conditions. Agriculture and Biology Journal of North America 4:512-519.
- Allen, S. F., Grimshaw, H. F. and Rowl, A. B. (1984). Chemical Analysis. In: Methods in plant Ecology, Moor, PD and S.B. Chapman (Eds.), Blackwell. Oxford. pp. 185-344.
- Amel, A., Soad, H., Ahmed, M. and Ismail, A. A. (2010). Activation of tomato plant defense response against Fusarium wilt disease using *Trichoderma harzianum* and salicylic acid under greenhouse conditions. Research journal of agriculture and biological sciences 6:328-338.
- Begum, M. M., Sariah, M., Puteh, A. B. and Siddiquiy. (2011). Field performance of bio primed seeds to suppress *Collectrichum truncotum* causing damping off and seedling stand of soybean. Biological Control 53:18-23.
- Castro, MS. And Fontes, W. (2005). Plant defense and antimicrobial peptides. Protein and Peptide Letters 12:11-6.
- De Boer, W., Gerards, S., Klein Gunnewiek P. J. A. and Modderman R. (1999). Response of the chitinolytic microbial community to chitin amendments of dune soils. Biology and Fertility of Soils 29:170-7.
- De Gara, L., de Pinto, M. C. and Tommasi, F. (2003). The antioxidant systems vis- à vis reactive oxygen species during plant pathogen interaction. Plant Physiology and Biochemistry 41:863-870.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Robors, P. A. and Smith, F. (1960). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.
- El-Khallal, S. M. (2007). Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid and salicylic acid): 1- changes in growth, some metabolic activities and endogenous hormones related to defense mechanism. Australian Journal of Basic and Applied Sciences 1:691-705.
- El-Mohamedy, R. S. R. (2004). Bio-priming of okra seeds to control damping off and root rot diseases. Annals of Agricultural Sciences 49:339-356.
- El-Mohamedy, R. S. R., Abd–Alla, M. A. and Badiaa, R. I. (2006). Soil amendment and biopriming treatments as alternative fungicides for controlling root rot diseases on cowpea plants in Nobria province. Research journal of agriculture and biological sciences 2:391-398.
- El-Mohamedy, R. S. R. and Abd–El-Baky, M. M. H. (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 2:84-90.
- El-Mohamedy, S. R., Abdel-Kader, M. M., Abd- El-Kareem, F. and El-Mougy, N. S. (2013). Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens *in vitro*. Journal of Agricultural Technology 9:1507-1520.
- El-Mohamedy R. S. R. and AbdAlla, M. A. (2013). Bio-priming seed treatment for biological control of soil borne fungi causing root rot of green bean (*Phaseolus vulgaris* L.). Journal of Agricultural Technology 9:589-599.

- El-Mohamedy, R. S. R., Jabnoun-Khiareddine, H. and Daami-Remadi, M. (2014). Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers. Tunisian Journal of Plant Protection 9:45-55.
- El-Mougy, N. S. (2001). Field application of certain biological and chemical approaches on controlling bean wilt disease. Egyptian Journal of Phytopathology 29:69-78.
- El-Mougy, N. S., Nadia, G. and Abdel-Kader, M. (2007). Control of wilt and root rot incidence in *Phaseolus vulgaris* L. by some plant volatile compounds. Journal of Plant Protection Research 47:255-265.
- Gharib, F. A. and Hegazi, A. Z. (2010). Salicylic acid ameliorates germination, seedling growth, phytohormone and enzymes activity in bean (*Phaseolus vulgaris* L.) under cold stress. Journal of American Science 6:675-683.
- Harveson, R. M., Smith, J. and Stroup, W. W. (2005). Improving Root Health and Yield of Dry Beans in the Nebraska Panhandle with a New Technique for Reducing Soil Compaction. Plant Disease 89:279-184.
- Jackson, M. L. (1965). Soil chemical analysis advanced course. USA: Wisconsin.
- Khan, W., Prithivira, B. and Smith, A. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology 160:485-492.
- Kloepper, J. W., Schroth, M. N. and Miller, T. D. (1980). Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
- Lewis, J. A. and Lumsden, R. D. (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.
- Nayaka, S. C., Nironjana, S. R., Shankar, A. C and Mortensen, C. N. (2008). Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisin in maiza. Archives of Phytopathology and Plant Protection 43:264-282.
- Rajkumar, M., Lee, K. J. and Freitas, H. (2008). Effects of chitin and salicylic acid on biological control activity of *Pseudomonas* spp. against damping off of pepper. South African Journal of Botany 74:268-73.
- Roby, D., Gadelle, A. and Toppan, A. (1987). Chitin oligosaccharides aselicitors of chitinase activity in melon plants. Biochemical and Biophysical Research Communications 143:885-92.
- Rojo, F. G., Reynoso, M. M., Sofa, M. F., Chuluzel, N. and Torres, A. M. (2007). Biological control by Trichoderma species of *Fusarium solani* causing peanut brown root rot under field conditions Crop P rotection 78:153-156.
- Saikia, R., Singh, T., Kumar, R., Srivastava, J., Srivastava, AK, Singh, K. and Arora. DK. (2003). Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri*in chickpea. Microbiological Research 158:203-13.
- Snedecor, G. W. and Cochran, W. G. (1982). Statistical methods. USA: Iowa State University Press. pp. 215-237.
- Vinale, F., Marra. R., Scala, F., Ghisalberti, E. L., Lorito, M. and Sivasithamparam, K. (2006). Letters in Applied Microbiology 43:143-148.
- Wen, K., Seguin, P., Arnaud, M. S. and Jabaji-Hare, S. (2005). Real-Time Quantitative RT-PCR of Defense-Associated Gene Transcripts of *Rhizoctonia solani*-Infected Bean Seedlings in Response to Inoculation with a Nonpathogenic Binucleate Rhizoctonia Isolate. Phytopathology 95:345-353.

(Received: 1 June 2015, accepted: 14 July 2015)