Journal

J. Biol. Chem. Environ. Sci., 2010, Vol. 5(1):461-472 www.acepsag.org

EFFECT OF BIOFERTILIERS ON SNAP BEAN ROOT-ROT DISEASE

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ABSTRACT

Root-rot disease is considered among the most serious diseases affecting snap bean in Egypt. *Rhizoctonia solani, Fusarium solani* and *Macrophomina phasolina* were more prevalent than all the isolated fungi. The most effective biofertilizers in controlling snap bean root-rot disease were MicroPen and Phosphoren followed by Rhizobacterin and NitroPen while Biogen were less effective against the disease, in the field. The cultivar Bronco is more resistance than cv. Polesta. However, snap bean shoot length, shoot fresh weight, root length and root fresh weight were significantly promoted after biofertilizer application. The study showed also a high increase in the activity of polyphenoloxidase and peroxidase enzymes, in plants treated with the tested biofertilizers.

Key words : Biofertilizers, MicroPen, Rhizobacterin, Phosphoren, NitroPen, Biogen, Root-rot, *R. solani, F. solani* and *M. phasolina* , Peroxidase, Polyphenoloxidase, Snap bean.

INTRODUCTION

Snap bean (*Phaseolus vulgaris*) is one of the most popular vegetable crops in Egypt, either for local consumption or for exportation. Root- rot diseases affect the yield qualitatively and quantitatively especially in heavy soils (Abawi and Widmer 2000). *Rhizoctonia solani, Macrophomina phaseolina* and *Fusarium solani* were the main causal organisms of snap bean root-rot disease (Pedroza-Sandoval, 1994, Otsyula, *et al* 1998, Siddiqui, *et al* 1999 and Sallam, *et al* 2008).

Application of biofertilizers, either as seed dressing or as soil drenching, has shown a significant suppression of root infecting pathogens (Zaki and Ghaffar, 1987, Ehteshamul-Haque *et al*., 1990, Shahzad and Ghaffar, 1992 and Sheikh *et al*., 2006). *Rhizobium* spp., the plant growth promotor rhizobacteria, have beneficial effects on plants including biological control of soil borne pathogens, induce systemic resistance in plants against plant pathogens and improvement of plant nutrient uptake (Yeoung *et al*., 2000). The genus *Rhizobium* has an ability of nitrogen-fixation in leguminous plants (Hynes and Connel, 1990) and in depressing of soil-borne root-infecting fungi (Ehteshamul-Haque and Ghaffar, 1993 and Siddiqui *et al*., 1998).

Siddiqui *et al*. (1999) used the plant growth promotor rhizobacterium (PGPR) *Pseudomonas aeruginosa*, alone or in combination with urea to reduce root-rot, of mung bean cused by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani*. Nasima *et al*. (2001) also found that *Pseudomonas aeruginosa* (strain 78) significantly promoted growth and increased *Bradyrhizobium*-nodules.

Gray *et al*. (2006) stated that microbial antagonists, viz. *Bacillus subtilis, B. thuringiensis, B. cereus* and *Rhizobium meliloti* were used in the control of root infecting fungi. Also he found that seed germination, shoot length, shoot weight, root length and root weight in okra and mung bean showed promising results after application (1% w/v) with the bacterial antagonists *B. subtilis, B. thuringiensis, B. cereus* and *R. meliloti.* The same treatment completely suppressed the infection of *R. solani* and *M. phaseolina* on mung bean roots (Tariq *et. al* 2007).

Liang *et al*. (2004) reported that the number of *Rhizoctonia solani* infected spots on turfgrass roots decreased after inoculation with bacterial fertilizers. Furthermore the contents of peroxidase, polyphenol oxidase [catechol oxidase] and phenylalanine ammonia-lyase were higher in treated tissues than those of not treated with the biofertilizers Liang *et al*. (2004). The resistance of turfgrass to *R. solani* was also improved.

This study aimed to evaluate the influence of five biofertilizers, as soil treatment, on incidence of root-rot disease of snap bean and the impact of this treatment on growth promotion, and activity of oxidase enzymes in the treated plants.

MATERIALS AND METHODS

1-Frequency of fungi isolated from snap bean rotted roots:

Samples of snap bean rotted roots were obtained from fields of Dkahliya governorate, Egypt during 2008 season. Snap bean seedlings showed ideal symptoms of root-rot disease were washed throughly with tap water for two minutes. The diseased tissues were cut into small pieces and surface sterilized with 3% sodium hypochorite solution for two minutes and washed twice with sterile distilled water, and finally dried between two sterilized filter papers towels. Small pieces of sterilized samples (0.5 mm) were plated on potato dextrose agar (PDA) and incubated for 5-7 days at 25ºC and observed daily for fungal growth. The growing fungi were purified either with single spore (Keitt, 1915) or hyphal tip methods (Brown, 1924). Identification of isolated fungi was verified in Fungal Taxonomy Dep., Plant. Pathol. Inst., Agric. Res. Center, Egypt.

2. Effect of different biofertilizers on snap bean root-rot :

A green house experiment was conducted during two seasons, 2008 and 2009, to evaluate the effect of five commercial biofertilizer on incidence of root-rot disease of snap bean.

Inoculum of fungal pathogens was prepared by inoculating maize meal-sand medium in 500 ml glass bottles with 5 mm. disks of a 7-day-old culture of each pathogen (*Rhizoctonia solani, Fusarium solani* and *Macrophomina phasolina*); then incubated at 25± 1 °C for 15 days. Throughout all greenhouse trials, clay pots (25 cm in diameter) were used. Pots were always sterilized by immersing in 5% formalin for 15 minutes and then air dried for 5 days. Soil infestation was carried out by adding of the fungal inoculum of *R. solani, F. solani* and *M. phasolina* (at the ratio 1:1:1 w/w) to the sterilized soil (at the rate of 3% of soil weight). The fungal inocula were thoroughly mixed with the soil, one day before planting. Soil mixed alone with the same amount of autoclaved maize meal-sand medium served as a check treatment. Each pot was planted by five seeds of cv. Polesta and cv. Bronco snap bean and four pots were used for each treatment. Five biofertilizers (Table 1) were applied under greenhouse conditions.

The biofertilizers were obtaind from Agriculture Research Center (ARC), Giza, Egypt. These biofertilizers agents individually added to the soil at the rate of 3 g /pot after 15, 30 and 45 days of

planting. Plant fresh weight (shoots and roots) and infection percentage were recorded 60 days after planting.

Disease assessments:

Root-rot was recorded after 60 days from planting on a scale of 0-5 as described by Emara (1995) as the following :

(0) = No symptoms (apparently healthy).

(1) = Slight browning of roots, but no symptoms in the top.

(2) = Browning of the root, with slight chlorosis of the leaves.

(3) = Browning of the roots, with medium chlorosis of the leaves.

(4) = Browning of the root system, with strong chlorosis of the leaves.

(5) = Necrosis and root system completely rotted and plant death.

Then calculated as formula:

$$\% \text{ of root rot} = \frac{(0 \times n0) + (1x\ n1) + (2 \times n2) + (3 \times n3) + (4 \times n4) + (5 \times n5)}{\text{Total No. of plants} \times 5}$$

Where N = number of the diseased plants in each scale.

The root systems of treated snap bean were removed from infested and control soil, washed in tap water, then the disease severity was calculated using the disease scale values described previously. The obtained data were statistically analyzed as completely randomized experimental design and then tabulated.

Table (1): The biofertilizers tested with Snap bean root-rot disease.

Biofertilizer	Active bioagent (ARC)
MicroPen	*Azotobacter* sp., *Azosprillum* sp., *Pesudomonas* sp., *Bacillus magatherium, Rhizobium phasolina*
Rhizobacterin	*Azotobacter chroococum, Azosprillum* sp., *Rhizobium phasolina*
NitroPen	*Azotobacter* sp. , *Azosprillum* sp.
Phosphoren	*Bacillus magatherium*
Biogen	*Azotobacter chroococum, Azotobacter vineiaudii*

3. Determination of oxidative enzymes

Treated and untreated roots were used for determining the activity of peroxidase and polyphenol oxidase enzymes. Enzyme substrate was extracted from the roots of treatment and interment plants as recommended by Maxwell and Bateman (1967). The root tissues were crushed with 0.1 M sodium phosphate buffer at pH 7.1 (2 ml. buffer /g fresh root tissues), in a mortar. These triturated tissues were strained through four layers of cheesecloth and filtrates were centrifuged at 3000 rpm for 20 min at 6°C. The supernatant fluids were used for enzyme assays. Peroxidase and polyphenol oxidase assays were measured in (Spectronic 601) spectronictor. The control cuvette contained the same solution except that the substrate solution was replaced by distilled water. Readings were recorded every 30 sec for 5 minutes in case of peroxidase and polyphenol oxidase.

3-1- Peroxidase assay

Peroxidase activity was determined according to the method described by AlIam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm. The sample cuvette contained 0.5 ml 0.1 M potassium phosphate buffer al pH 7, 0.3 ml enzyme extract, 0.3 ml 0.05 ml pyrogallol and 0.1 ml 1 % H_2O_2. The mixture was completed to 3 ml with distilled water. Peroxidase activity was expressed as the change in absorbance / minute / g fresh weight.

3-2- Polyphenol oxidase assay

The activity of polyphenol oxidase was measured by the colorimetric method of Maxwell and Bateman (1967). The reaction mixture contained 0.5ml-enzyme extract, 0.5-ml sodium phosphate buffer at pH 7 and 0.5 ml of catechol brought to a final volume of 3 ml with distilled water. The activity of phenol oxidase was expressed as the change in absorbance /minute /g fresh weight at 495 nm.

RESULTS AND DISCUSSION

1-Frequency of fungi associated with snap bean root- rot:

Isolation trials from the rotted rots yielded 25 fungal isolates. The isolated fungi were purified and identified as : *Rhizoctonia solani, Fusarium solani*, *Macrophomina phaseolina, Pythium* spp, *Sclerotnia sclerotiorum* and *Helminthosporium satevium*.

Data in Table (2) clearly show that the fungus *Rhizoctonia solani* occurred than other fungl isolated from Snap bean rotted roots. It was followed by, *Fusarium solani, Macrophomina phaseolina, Pythium spp.* The least frequents were *Sclertium sclerotnia* (2%) and *Helminthosporium satevium* (1%).

Rhizoctonia solani, *F. solani* and *M. phaseolina* were the most pathogenic on snap bean roots. This was confirmed by Siddiqui *et al*., 1999, Pedroza-Sandoval *et al*., 1994, Otsyula *et al*., 1998 and Sallam *et al*., (2008)

Table (2): Frequency of fungi isolated from snap bean rotted roots obtained from Dkahliya governorate fields during 2008.

Fungi	Number of isolates*	Frequency%
Rhizoctonia solani	8	33.2
Fusarium solani	6	25.5
Macrophomina phaseolina	5	23.8
Pythium sp.	3	14.5
Sclerotnia sclerotiorum	2	2.0
Helminthosporium satevium	1	1.0
Total	25	100

* Number of each fungi isolated from all collected samples.

2. Effect of different biofertilizers on incidence of root-rot snap bean:

Data in Table (3) demonstrated that snap bean cv. Polista was more susceptible to root-rot infection than cv Pronko. According to the effect of different biofertilizers on snap bean root-rot under greenhouse conditions in seasons 2008 and 2009, it is clear that MicroPen (commercial formulation of the bacterium, *Pesudomonas* sp., *Bacillus magatherium, Rhizobium phasolina*) was the most effective biofertilizer to reduce root-rot disease. It was followed by Phosphoren (commercial formulation of the bacterium, *Bacillus magatherium*). The least effective biofertilizers was NitroPen and Biogen, in both growing seasons, 2008 and 2009.

The results of this study demonstrate. The efficacy of biofertilizer treatments in suppression of root-rot disease on snap bean. Such results have been reported also by Siddiqui *et. al*. (1999) used of *Pseudomonas aeruginosa*, the plant growth promotor

rhizobacterium (PGPR) alone or in combination with urea to reduce mungbean root-rot. Guetsky *et. al*. (2002) reported that mixture of *Pichia guilermondii* and *Bacillus mycoides* resulted in additive activity compared with their separate application. The combined activity was due to the summation of biocontrol mechanisms of both agents. On the other hand, Guetsky *et. al*. (2001) suggested that application of more than one biocontrol agent as a reliable mean of reducing of pathogen activity. Tariq, Marium *et. al*. (2007) reported that microbial antagonists viz., *Bacillus subtilis, B. thuringiensis, B. cereus* and *Rhizobium meliloti* were used in the control of root infecting fungi of mash bean and okra.

Table (3): Effect of different biofertilizers on snap bean root-rot disease incidence % under greenhouse conditions in seasons of 2008 and 2009.

Biofertilizer	Root- rot severity %							
	cv. Polista				cv. Pronko			
	2008	2009	mean	*E%	2008	2009	mean	*E%
MicroPen	22.00	24.00	23.00	61.02	21.50	23.00	22.00	60.36
Rhizobacterin	30.00	33.00	31.50	47.46	35.00	32.00	33.50	39.64
NitroPen	40.00	40.00	40.00	32.20	41.00	38.00	39.50	28.83
Phosphoren	28.00	31.00	29.50	50.00	25.00	33.00	29.00	47.75
Biogen	48.00	56.00	52.00	11.86	45.00	52.00	48.50	12.61
Control	53.00	65.00	59.00	------	50.00	61.00	55.50	------

LSD at 0.05	2008	2009
V = Varieties	n.s	1.516
B = Biofertilizer	1.972	1.852
VB = Interaction	2.788	2.619

*E % = Efficiency = [(control- treatment)/ control] x100

3. Effect of different biofertilizers on snap bean growth parameters:

Results presented in Table (4) show that, snap bean plants treated with MicroPen, Rhizobacterin and Phosphoren, increased the shoot and root fresh weight were significantly promoted after biofertilizer application in the two tested cultivars. MicroPen is

recorded as the best results in shoot and root fresh weight in Pronko (cv.) while Biogen showed the least effect in shoot and root fresh weight. Such results have been reported also by Gray *et al*. (2006) who found that seed germination, shoot length, shoot weight, root length and root weight in okra and mung bean showed promising results after application with the bacterial antagonists *B. subtilis, B. thuringiensis, B. cereus* and *R. meliloti.*

Table (4): Effect of different biofertilizers as growth promoters on two snap bean cultivars under greenhouse conditions.

Biofertilizer	Growth promoting					
	cv. Polista			cv. Pronko		
	Shoot fr. wt.	Root fr. wt.	Total	Shoot fr. wt.	Root fr. wt.	Total
MicroPen	6.20	1.81	8.01	8.10	3.00	11.10
Rhizobacterin	5.80	1.20	7.00	7.30	1.80	9.10
NitroPen	5.00	1.10	6.10	5.10	1.80	6.90
Phosphoren	5.30	1.30	6.60	6.80	1.90	8.70
Biogen	5.00	1.00	6.00	4.90	1.50	6.40
Control	4.60	0.82	5.42	4.80	1.00	5.80
LSD at 5%	0.3	0.17	---	0.4	0.23	---

4-Effect of biofertilizers on oxidative enzymatic activity :

Data in Table (5) showed that peroxidase and polyphenoloxidase activities were higher in the untreated roots of cv. Pronko than in cv. Polista. It was also indicated that peroxidase and polyphenol-oxidase activities were increased greatly in plants treated with MicroPen followed by Phosphoren, while Biogen showed the least effect.

Table (5) : Peroxidase and polyphenolaxidae activity in roots of two snap bean cultivars after treatment with biofertilizers.

Biofertilizers	Peroxidase*		polyphenolaxidae*	
	cv. Polista	cv. Pronko	cv. Polista	cv. Pronko
MicroPen	0.908	1.528	0.082	0.092
Rhizobacterin	0.387	0.767	0.071	0.061
NitroPen	0.367	0.734	0.042	0.051
Phosphoren	0.606	1.074	0.074	0.086
Biogen	0.293	0.510	0.042	0.047
Control	0.104	0.050	0.012	0.039

- Absorbance / minute / g fresh weight.

These results are in agreement with those obtained by Liang *et al*. (2004) found that the contents of peroxidase, polyphenol oxidase [catechol oxidase] and phenylalanine ammonia-lyase were higher in treated tissues than those of not treated with the biofertilizers.

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تأثير التسميد الحيوي على مرض عفن جذور الفاصوليا الخضراء

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يعتبر مرض عفن الجذور من أهم الأمراض التي تصيب الفاصوليا في مصر، وقد وجد أن أكثر الفطريات تكرارا في العزل من جذور النباتات المصابة هي رايزوكتونيا سولانى و فيوزاريم سولانى يليهما فطر مكروفومينا فاصولينا. تم استخدام خمسة أسمدة حيوية وهى مكروبين وفوسفورين ورايزوبكترين ونتروبين وبيوجين وذلك لدراسة تأثيرها على خفض الاصابة بالمرض فى تجاب الصوب خلال موسمى نمو عام 2008-2009. أظهرت المعاملة بالسمادان الحيويان مكروبين وفوسفورين كفاءة عالية في خفض شدة الاصابة بعفن الجذور على صنفى الفاصوليا برونكو وبولستا يليهما الرايزوبكترين والنتروبين بينما كان البيوجين أقل فاعلية فى خفض الاصابة بالمرض.

أظهر الصنف برونكو مقاومة ضد مرض عفن الجذور أكبر من الصنف بوليستا وقد زادت تلك المقاومة عند المعاملة بالتسميد الحيوي. أدى معاملات التسميد الحيوي إلى زيادة الوزن الرطب للنبات وكذلك زيادة نشاط أنزيمات البولي فينول أوكسيديز والبيروكسيديز خاصة عند استخدام مركبى المكروبين والفوسفورين ، ومن المعروف أن هذه المركبات تتميز بأنها آمنة الاستخدام بالنسبة للإنسان والبيئة وقد تعتبر بديلا عن استخدام المبيدات فى المكافحة.