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Effect of some Bioagents on Root-Rot Disease Incidence on Bean Plants

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Root-rot of beans is one of the most dangerous diseases which reduce the number of plant stands and consequently the seed yield. Different methods are used to control the disease however, the biological method is the most safe and economic one. In this research work the effect of four bioagents, *i.e. Trichoderma harzianum, T. hamatum, Bacillus subtilis* and *Pseudomonas fluorescens* were *in vitro* tested against *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. All bioagents significantly reduced the mycelial growth of the causal organisms. *T. hamatum* gave more significant results in reducing the linear growth of the tested pathogenic fungi. These beneficial microorganisms in addition to a mixture of *T. harzianum* + *T. hamatum* were also used as seed treatment to study their effect (s) on bean seedling root-rot disease and their effect on nutrition status of plants under commercial green house condition, in seasons, 2008/09 and 2009/10. *B. subtilis* gave more significant effect as single bioagent against root-rot disease and only 2% disease incidence was recorded compared to 54% in the control treatment. Reinoculation using one of these bioagents as soil treatment after 5 days from sowing treated seeds with same bioagent led to more significant increase in plant protection. Percentage of survived plants in these treatments ranged from 96-100% compared with the control treatment where the recorded percentage of survived plants ranged from only 46-48% in the two seasons. Positive correlation was observed between the tested doses (5, 10 and 20 gm/kg seed) of bioagent and their efficacy against root-rot disease. Bean plants treated with different bioagents showed higher percentages in contents of N, P and K due to the effect of the different bioagents on roots, compared to plants in control treatment. Treated plants also showed vigour growth and more yield when compared to the control plants.

Keywords: *Bacillus subtilis*, beans, biological control, nutritional status, *Phaseolus vulgaris*, *Pseudomonas fluorescens* and *Trichoderma* spp.

Biological control of plant diseases using microorganisms is a very promising alternative to the use of chemical fungicides. Biocontrol is cheaper and has no accumulating effects comparing with chemical pesticides. *Trichoderma* spp. are known for their abilities in controlling plant pathogens. Modes of action of these

fungi include direct effects upon target fungi via competition, mycoparasitism and antibiosis (Abd El-Moity, 1981; Chet, 1987 and Harman, 2006). In addition, using these fungi increased plant growth (Chang *et al*, 1986) due to the production of some growth regulators. These antagonists also show abilities to solubilize fix plant nutrients and increase plant nutrient uptake (Altomare *et al*., 1999; Yedidia *et al*., 2001 and Harman *et al*., 2004). These fungi also can grow in the rhizosphere of treated plant and support populations of rhizosphere as a first line of defence against soil borne plant pathogens as stated by Cook *et al*., (1995).

Several *Bacillus* spp. include *B. subtilis* are antagonistic to plant pathogenic fungi and bacteria. *Bacillus* spp. are known to produce at least 66 different antibiotic compounds **(**Ferreria *et al*., 1991). Several strains of *B. subtilis* are used to suppress take all disease and Rhizoctonia root-rot in wheat (Ryder *et al*., 1999). The mechanism of these bacteria against plant pathogens include antibiosis, competition for nutrients or space also it leads to enhance of root and plant development, induction of plant resistance, solubilization complex and fixed nutrients and/or inactivation of the pathogens enzymes (Intana *et al*., 2008). Some bacteria show beneficial effects on plants such as some of *Pseudomonas fluorescens* and *B. subtilis* these bacteria are known as plant growth promoting rhizobacteria (PGPR). The positive effects of PGPR are normally divided into two categories: growth promotion and biological control (Kloepper, 1997). Root colonizing bacteria *P. fluorescens* can protect plants against soil borne pathogens (Ashnaei *et al*., 2008). Strains of *P. fluorescens* that produce the antibiotic 2, 4 diacetylphoroglucinol (2, 4 DAPG) are among the most effective rhizobacteria controlling diseases caused by soil borne pathogens (Landa *et al*., 2003 and Desuze *et al*., 2003).

The objectives of this study were to evaluate the effect of different bioagents (microorganisms) against root-rot disease and nutrition status of bean (*Phaseolus vulgaris).*

Materials and Methods

The present work was carried out during the period of 2008-2010 at the Central Lab. of Organic Agric., ARC, Giza, Egypt and in greenhouse of the Ministry of Agric., Giza, Egypt. The greenhouse research work was carried out in two winter seasons of 2008/2009 and 2009/2010.

*Isolation and identification of the causal organisms***:**

Samples of bean plants showing identical symptoms of root-rot were collected from naturally infected plants grown in commercial green house of the Ministry of Agric., Giza, Egypt. The collected root samples were washed, dried and then surface sterilized using 5% chlorine solution for three minutes. Sterilized surface plant materials were then washed several times with sterilized distilled water and dried between two sterilized filter papers. Dried sterilized plant materials were cut into small pieces with sterilized scalped and cultured on plane agar medium in Petri plates (9-cm-diam.). Plates were incubated at 28^{o}C. Incubated plates were examined periodically and the developed mycelia were transferred to nutrient glucose agar (NGA) medium. The isolated fungi were purified using hyphal tip and/or single

spore techniques. Isolated fungi were identified according to their culture and morphological characteristics according to keys developed by (Gilman, 1957, Barnett and Hunter, 1972 and Singh, 1982). The purified isolated pathogens were identified as *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Sclerotinia sclerotiorum.*

Antagonistic microorganisms:

Different bioagents, *i.e. Trichoderma harzianum, T. hamatum, Bacillus subtilis,* and *Pseudomonas fluorescens*, were kindly obtained from the Central Lab. of Organic Agric., ARC, Giza, Egypt. *T. harzianum* and *T. hamatum* were grown for 9 days on liquid gliotoxin fermentation medium (GFM) developed by Brain and Hemming (1945) under complete darkness to stimulate toxin production (Abd El-Moity and Shatla, 1981).

Bacillus subtilis and *P. fluorescens* were grown on nutrient glucose broth (NGB) developed by (Dowson, 1957) for 48 h. Mixture of *T. harzianum* and *T. hamatum* were mixed at the rate of 1:1 (v/v).

Different bioagents were formulated as suspension or powder. Prepared suspension or powder were adjusted to be contain thirty millions colony forming units/1ml or 1gm (30×10^6 cfu/1ml or 1gm) either for all used single antagonist or their mixture (Abd El-Moity, 1985).

Effect of different bioagent isolates on the linear mycelial growth of pathogenic soil borne fungi under laboratory condition:

Different bioagents were evaluated under laboratory conditions for their antagonistic effect against pathogenic fungi *(Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Sclerotinia sclerotiorum*). Petri dishes 9.0cm in diameter each contains 15ml of GFM were used to determine the antagonistic effect between used antagonists and isolated pathogenic fungi. On the other hand, plates contain NGB medium were used to determine the effect of antagonistic bacteria against pathogenic fungi. Different plates were inoculated with discs (6mm in diameter) of pathogenic fungi obtained from the periphery of 4 days old colony. The pathogenic fungus was inoculated at one side where as the opposite side was inoculated with either disc of bioagent fungus (6-mm-diameter), obtained from 3 days old culture or with loop full of antagonistic bacteria grown on liquid NGB medium for 48 hours. Five plates were used for each treatment. Plates inoculated only with the pathogenic fungi served as a control treatment. Inoculated plates were incubated at 25^{o}C. When mycelial growth covers all medium surfaces in control treatment, all plates were examined and percentages of reduction in mycelial growth of pathogenic fungi were calculated using the next formula:

$$X = 100 - [G2 / G1 \times 100]$$

Whereas: X : Reduction (%)
G1: linear growth in mm of pathogenic fungi in control plates.
G2: linear growth in mm of pathogenic fungi in treated plates.

Green house experiments:

This study was carried out in two seasons (2008/09 and 2009/10) under greenhouse conditions, in high natural infested soil (60m X 9m = 540 m^2) belongs to Central Lab. for Agric. Climate, Agric. Res. Centre, Ministry of Agric. and Land Reclamation, Giza, Egypt. Selected bioagents, *i.e. T. harzianum*, *T. hamatum*, Mixture of *T. harzianum+T. hamatum*, *B. subtilis* and *P. fluorescens*, were evaluated for their efficacy in controlling root-rot disease of beans.

Bean (*Phaseolus vulgaris*) cv. Polista H. F1. was used. Experiments were designed in complete randomize plots. Each treatment contained 5 replicates and each replicate (2mX2m) contained 20 bean seeds. Pre- and post-emergence damping-off were determined according to disease index developed by Ismail (2011) (Categories 3 and 4 include pre- and post-emergence damping-off). The percentages of pre-emergence were determined 8 and 11days after sowing, meanwhile percentages of post-emergences were determined 12 and 30 days after sowing. Percentages of survived plants were determined one month from sowing. Efficacy of treatments was calculated using the following formula:

$$\text{Efficacy (\%)} = 100 - [D2 / D1 \times 100]$$

Whereas: D1: Root-rot (%) in control.
D2: Root-rot (%) in treatment.

To determine the effect of these bioagents on plant vigour, some agronomic characteristics were also determined at the end of growing season, these characteristics included:

1- Plant height (cm).
2- Plant fresh weight: The average fresh weight of plants was determined in (g) using Sartorius digital electrical balance.
3- Plant dry weight: the average dry weight (g) of plants sample was dried at 70^{o}C for overnight to get rid of all water in plant.
4- Average number of leaves and branches per plant.
5- Nitrogen and phosphorus contents, as g / 100g dry weight was assayed according to Jackson (1973) where as Potassium content was determined using atomic absorption spectrophotometer (Barkin Elmer, 3300) according to (Chapman and Pratt, 1978), the results calculated as g/100g dry weight. The following experiments were carried out:

1. *Evaluation of some bioagents as seed dresser against root-rot disease incidence on beans under commercial greenhouse condition:*

In this study the antagonists previously mentioned were used as powder. Powders were prepared as described by (Abd El-Moity, 1985). Bean seeds were wetted using 5% solution of Arabic gum then 10g of prepared powder were added per each 1kg of seeds and mixed thoroughly. All treated seeds were sown in heavily infested soil. Seeds only received water act as control treatment. Pre- and post-root-rot disease, in addition to agronomic characteristics were determined as described before.

2. *study the effect of reinoculation with bioagents as soil drenches on root-rot diseases under commercial greenhouse condition :*

Five days after sowing bean seeds treated with bioagent, another dose of the same antagonist (diluted l/100 l water) was added at the rate of 100ml/seed (plant). Seeds (plants) in control treatment received only water. The effect of this treatment on root-rot disease incidence was determined by calculation percentages of root-rot in different treatments compare with control. Moreover, agronomic characteristics were also determined, as described before.

3. *Effect of different doses of bioagents as seed dresser on root-rot disease of bean under commercials green house condition:*

Different antagonists were used as powder in different doses. Used doses were 5, 10, 20 g/kg seeds. The aim of this experiment is to study effect of these doses on pathogenic fungi and their effect on up take of N.P.K. Pre- and post- root-rot disease were determined and agronomic characteristics were also determined, as described before.

Statistical analysis was carried out using the procedures ANOVA (Snedecor and Cochran, 1980).

Results and Discussion

Effect of different bioagents isolates on the linear mycelial growth of pathogenic soil borne fungi under laboratory conditions:

Data in Table (1) indicated that *Trichoderma hamatum* was the most effective antagonist against the four test soil borne pathogenic fungi. *Trichoderma harzianum* showed less significant effect compared to *T. hamatum.* This high potentiality in antagonism might be due to that *Trichoderma* spp. act through different mechanisms including mycoparasitism (Abd El-Moity, 1976; Benhamou and Chet, 1993 and Abd El-Khair *et al*., 2010). Also, through production of anti-fungal substances (Turner, 1971; Abd El-Moity, 1981 and Hayes, 1992). *Trichoderma* spp. also act through production of destructive enzymes, *i.e.* chitinase (Bolar *et al*., 2000) which attack and destroy pathogenic fungi structures.

Table 1. Effect of different bioagents on the linear mycelial growth of four pathogenic soil borne fungi under laboratory conditions

Tested bioagent	Reduction in the linear growth (%)			
	R. solani	*S. rolfsii*	*S. sclerotiorum*	*F. solani*
T. harzianum	80.5	81.2	82.1	82.4
T. hamatum	83.1	86.5	85.6	89.9
B. subtilis	53.0	53.5	52.4	54.5
P. fluorescens	50.0	52.0	50.0	52.5
Control	0.0	0.0	0.0	0.0
L.S.D. at 5%	1.156	0.427	1.710	1.488

Bacillus subtilis significantly reduce the growth of pathogenic fungi under test but at degree less than *T. harzianum* and *T. hamatum*. This might be due to that *B. subtilis* act through production of number of antibiotics (Ferreria *et al*., 1991 and Asaka and Shoda, 1996). *Pseudomonas fluorescens* showed the least significant effect compare with the other three antagonists previously mentioned before. This might be due to that *P. fluorescens* act through production of some antibiotics (Sarniguet *et al*., 1995; Duffy and Defago, 1997 and Sharifi *et al*., 1998).

Evaluation of some bioagents as seed dressing against root-rot disease incidence on beans under commercial greenhouse conditions:

Data in Table (2) showed that all tested bioagents or mixture of Trichoderma isolates significantly reduced the disease incidence compared to the control treatment. *Bacillus subtilis* showed the highest significant effect in decreasing percentage of disease incidence (98% healthy surviving and 96.29% of efficacy in first season and 99% healthy surviving and 98.07% of efficacy in second season). This might be due to that bacteria produce more than one antibiotic (bacteriosin and subtilisin) which act as inhibitors to pathogenic fungi (Ferreria *et al*., 1991; Asaka and Shoda, 1996 and Abd El-Moneim *et al*., 2006). *Bacillus subtilis* also grown very fast and occupied the court of infection and consumed all available nutrients and prevent nutrients utilization by pathogens, thus these pathogen cannot establish and invade plant tissues (Wolk and Sorkar, 1994).

Table 2. Evaluation of some bioagents as seed dressing against root-rot disease incidence on beans cv. Polista in naturally infested soil under commercial greenhouse conditions

Tested bioagent	First season (2008/09)				Second season (2009/10)			
	Damping-off (%)		Survived plants (%)	Efficacy (%)	Damping-off (%)		Survived plants (%)	Efficacy (%)
	pre	post			pre	post		
B. subtilis	2	0.0	98	96.29	1	0.0	99	98.07
T. harzianum	13	7	80	62.96	14	5	81	63.46
T. hamatum	12	6	82	66.66	14	4	82	65.38
P. fluorescens	7	3	90	81.48	7	3	90	80.76
Mix. of Trichoderma isolates	8	2	90	81.48	8	1	91	82.69
Control	43	11	46	0.00	44	8	48	0.00
L.S.D. at 5%	0.797	0.233	1.70	2.366	0.586	0.124	1.62	2.354

Pseudomonas fluorescens and mixture of Trichoderma isolate showed significant effect after *B. subtilis* in this respect, in the two seasons. Effect of *P. fluorescens* might be due to cleat available iron in the rhizosphere area. Under this iron starvation conditions, the pathogens can't grow (Loper, 1988). Also, *P. fluorescens* act through production of some antibiotics (Duffy and Defago, 1997 and Sharifi *et al*., 1998). Mixture of Trichoderma isolates led to sever reduction in disease incidence this reduction might be due to that *Trichoderma* spp. act through different mechanisms including mycoparasitism and production of antifungal substances include gliotoxin, viridin (Turner, 1971, Abd El-Moity, 1976 and 1981 and

Abd El-Moneim, 2005). These antifungal materials inhibit growth of pathogenic fungi consequently they become more susceptible to the effect of other microorganisms naturally exist in the soil (Hader and Gorodecki, 1991). On the other hand, both *T. harzianum* and *T. hamatum* as single isolates show slight significant result after *B. subtilis*, *P. fluorescens* or mixture of both Trichoderma isolates.

Data in Table (3) and (4a & b) showed the effect of applying bioagents on macro elements, *i.e.* nitrogen, phosphorus, and potassium (NPK), in bean plants and their agronomic characteristic as dry or fresh weight / plant, number of leaves / plant, number of branches ⁄ plant and plant height during the two successive growing seasons. Data show that all treatments led to increase in NPK content in treated plants. All other parameters were also increased, compared with control treatment.

Table 3. Nitrogen, phosphorus, and potassium uptake by bean plants cv. Polista as affected by different microorganisms

Tested bioagent	First season (2008/09)				Second season (2009/10)			
	Total yield/ plant (g)	N%	P%	K%	Total yield/ plant (g)	N%	P%	K%
B. subtilis	76.7	4.2	0.4	2.5	78.0	4.3	0.5	2.9
T. harzianum	61.4	3.0	0.3	2.2	61.7	3.1	0.3	2.3
T. hamatum	63.0	3.0	0.3	2.4	64.4	33	0.3	2.4
P. fluorescens	65.1	3.6	0.3	2.7	66.1	3.6	0.4	2.8
Mix. of Trichoderma isolates	67.3	4.0	0.4	2.7	67.6	4.0	0.4	2.9
Control	36.4	2.7	0.3	1.9	36.0	2.5	0.3	1.7
L.S.D. at 5%	0.88	0.44	0.06	0.08	0.14	0.15	0.07	0.01

Table 4a. Agronomic characteristics of bean plants cv. Polista as affected by different bioagents during 2008/09 season

Tested bioagent	First season (2008/09)								
	Dry weight/plant (g)			Fresh weight/plant(g)			Mean No. of leaves / plant	Mean No. of branch / plant	Plant height (cm)
	Plant	Root system	Shoot system	Plant	Root system	Shoot system			
Bacillus subtilis	3.8	1.4	2.4	14.8	3.0	11.8	12.5	4.3	28.7
Trichoderma harzianum	2.7	1.0	1.7	9.5	1.9	7.6	9.9	3.3	23.0
T. hamatum	2.8	1.2	1.6	9.9	2.0	7.9	10.0	3.3	23.9
Pseudomonas fluorescens	3,3	1.3	2.0	11.7	2.3	9.4	11.3	3.9	23.9
Mix. of Trichoderma isolates	3.5	1.6	1.9	12.9	2.6	10.3	11.3	3.9	28.4
Control	1.9	0.5	1.4	6.1	1.2	4.9	7.6	2.7	21.3
L.S.D. at 5%	0.03	0.01	0.02	0.05	0.01	0.04	0.02	0.01	0.05

Table 4b. Agronomic characteristics of bean plants cv. Polista as affected by different bioagents during 2009/10 season

Tested bioagent	Second season (2009/10)								
	Dry weight/plant (g)			Fresh weight/plant (g)			Mean No. of leaves / plant	Mean No. of branches / plant	Plant height (cm)
	Plant	Root system	Shoot system	Plant	Root system	Shoot system			
Bacillus subtilis	3.8	1.5	2.3	14.9	3.0	11.9	13.7	3.5	31.4
Trichoderma harzianum	2.9	1.0	1.9	12.6	2.5	10.1	10.3	3.6	23.3
T. hamatum	2.8	1.2	1.6	9.8	2.0	7.8	10.0	3.5	24.2
Pseudomonas fluorescens	3.5	1.4	2.1	12.1	2.4	9.7	11.7	4.3	24.6
Mix. of Trichoderma isolates	3.6	1.6	2.0	13.0	2.6	10.4	11.6	3.9	29.8
Control	1.0	0.4	0.6	5.1	1.0	4.1	5.1	2.2	20.7
L.S.D. at 5%	0.03	0.01	0.02	0.04	0.01	0.03	0.01	0.07	0.02

Total yield of plant and agronomic characteristics or plant contents of NPK were highly increased when plants were treated with *B. subtilis*. Mixture of *Trichoderma* spp. or *P. fluorescens* follow *B. subtilis* and showed less significant effect compared with *B. subtilis* or Mixture of *Trichoderma* spp. Single isolates of *T. hamatum* and *T. harzianum* showed slight significant differences compared with Mixture of *Trichoderma* spp. or *P. fluorescens*. This increase in yield may be due to that, these microorganisms occupied the rhizosphere and produced amounts of organic acids, as tartaric, citric acid and lactic acid which improve soil fertility due to increase availability of some complex and fix elements in addition to improve plant productivity due to increase in the root system (Abd El-Moneim and Seddik, 2006).

Effect of reinoculation with bioagents as soil drenches on root-rot diseases under commercial greenhouse condition using cv. Polista of beans:

Plants developed from treated seeds, using different antagonist were reinoculated using the same antagonist after five days from sowing seeds. These treatments were carried out for two successive growing seasons. Data in Table (5) revealed that all bioagents significantly reduced the disease incidence compared to control treatment. Data also indicated that mixture of *Trichoderma* spp. or *B. subtilis* were the most effective bioagents against the tested pathogens and gave very significant effect compared to the control treatment. This high potentiality in antagonism may be due to the compatible and synergistic relation between selected *Trichoderma* spp. The selected isolates in the mixture acted through certain mechanisms including mycoparasitism (Benhamou and Chet, 1993 and Harman, 2006), where as the other acted through production antifungal substances (Hayes, 1992*). Trichoderma* spp. may also acted through production destructive enzymes, *e.g.* chitinase (Bolar *et al*., 2000) so, by mixing more than one spices this led to combination of different mode

Table 5. Effect of reinoculation with bioagents on root-rot diseases of beans cv. Polista in naturally infested soil under commercial greenhouse conditions

Tested bioagent	First season (2008/09)				Second season (2009/10)			
	Damping-off (%)		Survived plants (%)	Efficacy (%)	Damping-off (%)		Survived plants (%)	Efficacy (%)
	pre	post			pre	post		
Bacillus subtilis	0	0	100	100.0	0	0	100	100.0
Trichoderma harzianum	3	1	96	92.59	2	1	97	94.23
T. hamatum	2	0	98	96.29	2	0	98	96.15
Pseudomonas fluorescens	1	1	98	96.29	2	0	98	96.15
Mix. of Trichoderma isolates	0	0	100	100.0	0	0	100	100.0
Control	43	11	46	0.0	44	8	48	0.0
L.S.D. at 5%	0.397	0.12	1.445	0.039	0.13	0.10	1.432	0.039

of actions and improve efficacy of the mixture. Action of *B. subtilis* is due to that this bacterium grown very fast and occupied the court of infection in addition to produce high level of antibiotics (Ryder *et al*., 1999) this fast growth and production of antibiotics acted, as barrier surround roots tissues and prevent pathogen to invade these healthy tissues. *Pseudomonas fluorescens* and *T. hamatum* showed significant effect compared to control treatment, and gave 96.3% in controlling root-rot disease. *T. harzianum* gave significant effect compared to treatment, and gave 92.6% in efficacy controlling of root-rot disease. Action of *P. fluorescens* may be due to produce some antibiotics, *i.e.* pyrrolnitrin, pyoluterin and 2, 4 diacetylploroglucinol. These antibiotics suppressed root-rot disease (Sarniguet *et al*., 1995). These results also can be explained in the light of data obtained by (Wolk and Sorkar, 1994). They stated that the efficacy of antagonists depend on their capacity of number and potential comparing with other microorganisms that occupied rhizosphere area.

Data in Tables (6, 7a & b) show that when bean plants developed from treated seeds with bioagents were reinoculated using the same bioagents as soil drench, this led to increase in NPK content as well as plant vigority. Parameters of vigority were expressed as dry, fresh weight ⁄ plant number of leaves, and branches ⁄ plant, plant height and yield/plant. *Bacillus subtilis* was the most effective treatment compared to others. These treatments can be explained in the light of fact that *B. subtilis* grown very fast and occupied the court of infection and competed for spaces and nutrients so prevent pathogens to invade plants (Wolk and Sorkar, 1994). As a result of this action strong root system was build and spread in healthy condition and increased uptake elements by plants. Also, *B. subtilis* produced some growth regulators that increased all growth parameters compared with control treatment (Asaka and Shoda, 1996; Grosch and Grote, 1998 and Ryder *et al*., 1999).

Table 6. Nitrogen, Phosphorus, and Potassium content in bean plants cv. Polista affected by reinoculation with different microorganisms as soil drench treatment

Tested bioagent	First season (2008/09)				Second season (2009/10)			
	Total yield/plant (g)	N%	P%	K%	Total yield/plant (g)	N%	P%	K%
Bacillus subtilis	80.0	4.3	0.4	2.6	81.5	4.4	0.5	2.9
Trichoderma harzianum	65.0	3.1	0.3	2.4	67.0	3.2	0.4	2.4
T. hamatum	67.0	3.2	0.3	2.5	69.0	3.2	0.3	2.5
P. fluorescens	73.0	3.5	0.3	2.5	74.0	3.6	0.3	2.6
Mix of Trichoderma isolates	73.5	3.6	0.4	2.5	75.0	3.9	0.4	2.7
Control	36.4	2.7	0.3	1.9	36.0	2.5	0.3	1.7
L.S.D. at 5%	0.18	0.04	0.08	0.02	0.12	0.04	0.01	0.03

Table 7a. Effect of reinoculation using different bioagents on agronomic characteristics of bean plants cv. Polista during 2008/09 season

Tested bioagent	Dry weight/plant(g)			Fresh weight/plant (g)			Mean No. of leaves \ plant	Mean No. of branches \ plant	Plant height (cm)
	Plant	Root system	Shoot system	Plant	Root system	Shoot system			
Bacillus subtilis	3.6	1.6	2.0	16.4	3.3	13.1	15.0	4.4	29.9
Trichoderma harzianum	2.8	1.2	1.6	12.0	2.4	9.6	11.5	4.0	26.8
T. hamatum	2.9	1.3	1.6	12.7	2.5	10.2	12.6	4.1	28.6
Pseudomonas fluorescens	2.9	1.4	1.5	13.1	2.6	10.5	12.5	4.2	28.7
Mix. of Trichoderma isolates	3.5	1.5	2.0	16.5	3.3	13.2	14.0	4.3	29.5
Control	1.9	0.5	1.4	6.1	1.2	4.9	7.6	2.7	21.3
L.S.D. at 5%	0.06	0.02	0.04	0.08	0.02	0.06	0.04	0.01	0.01

Table 7b. Effect of reinoculation using different bioagents on agronomic characteristics of bean plants cv. Polista during 2009/10 season

Tested bioagent	Dry weight/plant(g)			Fresh weight/plant(g)			Mean No. of leaves \ plant	Mean No. of branches \ plant	Plant height (cm)
	Plant	Root system	Shoot system	Plant	Root system	Shoot system			
Bacillus subtilis	3.7	1.7	2.0	17.0	3.4	13.6	14	4.5	30.1
Trichoderma harzianum	3.0	1.3	1.7	12.7	2.5	10.2	12.0	4.2	27.4
T. hamatum	2.9	1.4	1.5	12.8	2.6	10.2	13.0	4.2	27.9
Pseudomonas fluorescens	2.8	1.4	1.4	12.9	2.6	10.3	11.0	4.3	27.8
Mix. of Trichoderma isolates	3.6	1.6	2.0	16.9	3.4	13.5	17.0	4.5	30.0
Control	1.0	0.4	0.6	5.1	1.0	4.1	5.1	2.2	20.7
L.S.D. at 5%	0.05	0.01	0.03	0.02	0.01	0.01	0.06	0.08	0.03

Effect of different doses of bioagents as seed dressing on root-rot disease of bean cv. Polista under commercial green house conditions:

Data in Table (8) showed that using different concentrations (doses) of bioagents led to different degrees of protection against soil borne pathogens. Positive correlation was observed between levels (doses) of treatments and percentage of survived plants. This may be due that treatment with bio preparation at high dose led to increase in secondary metabolites (include antifungal, antibiotics, enzymes, and growth regulators) compared to lower doses. (Aly *et al*., 1995; Rodriguez and Cotes, 1999 and Abd El-Moneim, 2005).

Table 8. Effect of different doses of bioagents as seed dressing on root-rot disease of beans cv. Polista in naturally infested soil under commercial green house conditions

Tested bioagent	Dose g/kg seeds	First season (2008/09)				Second season (2009/10)			
		Damping-off (%)		Survived plants (%)	Efficacy (%)	Damping-off (%)		Survived plants (%)	Efficacy (%)
		pre	post			pre	post		
Bacillus subtilis	20	0.0	0.0	100	100	0.0	0.0	100	100
	10	2	0.0	98	96.29	1	0.0	99	98.07
	5	5	3	92	85.18	7	0.0	93	86.53
Trichoderma harzianum	20	6	0.0	94	88.88	5	0.0	95	90.38
	10	13	7	80	62.96	14	5	81	63.46
	5	20	8	72	48.14	20	8	72	46.15
T. hamatum	20	4	0.0	96	92.59	3	0.0	97	94.23
	10	12	6	82	66.66	14	4	82	65.38
	5	15	7	78	59.25	17	3	80	61.53
Pseudomonas fluorescens	20	4	0.0	96	92.59	3	1	96	92.30
	10	7	3	90	81.48	7	3	90	80.76
	5	20	0.0	80	62.96	19	0.0	81	63.46
Mix. of Trichoderma isolates	20	0.0	0.0	100	100	0.0	0.0	100	100
	10	8	2	90	81.48	8	1	91	82.69
	5	16	4	80	62.9.6	16	4	80	61.53
Control		43	11	46	0.0	44	8	48	0.0
L.S.D. at 5%		0.270	0.012	0.522	1.150	0.22	0.011	0.560	1.161

Data in Table (9) and (10a & b) showed the effect of different doses of bio-preparations on macronutrients (N P K) content in bean plants cv. Polista and their vegetative growth and productivity including fresh and dry weight / plant, number of leaves, number of branches plant and plant height on two successive growing seasons 2008-09 and 2009-10. A positive correlation among weight of dose and NPK in bean plants and growth parameters was observed.

Results revealed that at high dose 20 g/kg seed. (NPK) content significantly increased in both seasons compared with other doses of the same bioagent. This increase can be explained in the light of fact that treatment using bioagents at high dose may increase amount of growth regulators produced by antagonist which increase plant nutrients uptake and plant growth (Chang *et al*., 1986; Altomare *et al.*, 1999; Yedidia *et al.*, 2001 and Harman *et al.*, 2004).

Table 9. Nitrogen, Phosphorus, and Potassium content in bean plants cv. Polista as affected by different doses of various microorganisms

Tested bioagent	Concentration (g/kg seed)	First season 2008/09				Second season 2009/10			
		Total yield/ plant (g)	N%	P%	K%	Total Yield /plant (g)	N%	P%	K%
Bacillus subtilis	5	61.3	4.5	0.4	2.4	61.5	4.5	0.4	3.2
	10	69.5	4.7	0.5	2.8	69.2	4.7	0.5	3.4
	20	80.7	5.1	0.6	3.1	81.2	5.4	0.6	3.4
Trichoderma harzianum	5	45.2	2.9	0.3	2.1	45.2	2.7	0.3	2.4
	10	59.3	3.1	0.4	2.3	59.6	3.2	0.4	2.5
	20	66.1	3.4	0.5	2.6	65.5	3.2	0.5	2.5
T. hamatum	5	51.5	2.6	0.3	2.2	51.9	2.7	0.3	2.4
	10	63.8	3.3	0.4	2.6	63.8	3.2	0.4	2.7
	20	65.1	3.4	0.5	2.7	66.9	3.4	0.5	2.7
Pseudomonas fluorescens	5	60.0	3.8	0.3	2.7	60.2	3.8	0.3	3.1
	10	65.1	3.9	0.4	3.2	66.2	3.9	0.4	3.3
	20	70.8	4.1	0.5	3.0	70.5	3.9	0.5	3.3
Mix. of Trichoderma isolates	5	48.1	4.5	0.4	2.6	49.4	4.2	0.4	2.7
	10	67.1	4.6	0.5	3.1	67.5	4.9	0.5	3.2
	20	72.8	4.2	0.6	3.2	71.3	4.6	0.6	3.4
Control		36.4	2.7	0.3	1.9	36.0	2.5	0.3	1.7
L.S.D. at 5%		0.04	0.01	0.01	0.06	0.45	0.04	0.02	0.07

Table 10a. Effect of different microorganisms doses on agronomic characteristic of bean plants cv. Polista during 2008/09 season

Tested bioagent	Dose (g/kg seed)	First Season 2008/09								
		Dry weight/plant (g)			Fresh weight/plant (g)			Mean No. of leaves \ plant	Mean No. of branches \ plant	plant height (cm)
		Plant	Root system	Shoot system	Plant	Root system	Shoot system			
Bacillus subtilis	5	2.5	1.1	1.4	12.8	2.6	10.2	11.0	4.1	31.1
	10	3.9	1.5	2.4	17.2	3.4	13.8	14.1	4.6	31.7
	20	5.1	2.1	3.0	18.7	3.7	15.0	15.1	5.3	33.8
Trichoderma harzianum	5	2.5	1.0	1.5	12.5	2.5	10.0	9.8	3.0	22.8
	10	2.7	1.1	1.6	12.8	2.6	10.2	10.9	3.3	23.9
	20	3.8	1.4	2.4	15.1	3.0	12.1	11.3	3.7	25.8
T. hamatum	5	2.9	1.2	1.7	13.7	2.7	11.0	10.1	3.0	22.7
	10	2.9	1.2	1.7	14.2	2.8	11.4	11.1	3.4	24.8
	20	3.3	1.3	2.0	15.7	3.1	12.6	11.2	3.8	24.9
Pseudomonas fluorescens	5	2.7	1.3	1.4	12.4	2.5	9.9	11.0	3.8	21.7
	10	3.0	1.3	1.7	15.1	3.0	12.1	12.1	4.2	24.1
	20	4.1	1.6	2.5	17.2	3.4	13.8	13.3	4.2	24.9
Mix. of Trichoderma isolates	5	3.1	1.4	1.7	15.7	3.1	12.6	10.5	3.2	29.8
	10	4.0	1.6	2.4	16.8	3.4	13.4	13.7	4.3	31.5
	20	4.9	2.0	2.9	17.9	3.6	14.3	14.4	4.6	31.9
Control		1.9	0.5	1.4	6.1	1.2	4.9	7.9	2.7	21.3
L.S.D. at 5%		0.04	0.01	0.03	0.03	0.01	0.02	0.05	0.06	0.02

Table 10b. Effect of different microorganisms doses on agronomic characteristic of bean plants cv. Polista during 2009/10 season

Tested bioagent	Dose (g/kg seed)	Second season 2009/10								
		Dry weight/plant (g)			Fresh weight/plant (g)			Mean No. of leaves \ plant	Mean No. of branches \ plant	plant height (cm)
		Plant	Root system	Shoot system	Plant	Root system	Shoot system			
Bacillus subtilis	5	2.8	1.2	1.6	14.3	2.9	11.4	11.0	4.4	31.1
	10	4.0	1.5	2.5	17.7	3.5	14.2	15.0	4.7	35.5
	20	5.3	2.1	3.2	18.9	3.8	15.1	16.0	5.4	35.9
Trichoderma harzianum	5	2.5	1.0	1.5	12.3	2.5	9.8	10.2	3.1	23.3
	10	2.9	1.2	1.7	12.8	2.6	10.2	10.8	3.2	24.6
	20	3.8	1.4	2.4	14.2	2.8	11.4	11.0	3.6	27.7
T. hamatum	5	3.0	1.2	1.8	14.0	2.8	11.2	10.0	3.1	22.8
	10	3.2	1.3	1.9	14.1	2.8	11.3	10.2	3.5	25.7
	20	3.5	1.4	2.1	16.2	3.2	13.0	11.7	3.8	25.9
Pseudomonas fluorescens	5	2.7	1.3	1.4	12.5	2.5	10.0	11.1	3.7	21.9
	10	3.2	1.3	1.9	14.8	3.0	11.8	11.5	4.2	24.8
	20	4.1	1.6	3.5	17.6	3.5	14.1	14.0	4.5	26.0
Mix. of Trichoderma isolates	5	3.2	1.4	1.8	15.3	3.1	12.2	10.9	3.4	31.9
	10	3.9	1.6	2.3	16.5	3.3	13.2	13.5	4.1	32.8
	20	5.0	2.1	2.9	18.1	3.6	14.5	17.7	4.8	34.0
Control		1.0	0.4	0.6	5.1	1.0	4.1	5.1	1.9	20.7
L.S.D. at 5%		0.03	0.01	0.02	0.04	0.01	0.03	0.04	0.08	0.02

In conclusion**,** this research can be utilized in process of bean production to improve quantity and quality of produced green beans. Using data obtained in this research can lead to reduction in amounts of highly toxic substances are used in food production chain. Chemical fertilizers also can be reduces by increasing uptake of plants throughout increase the root system and its ability to absorb nutrient substances.

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تأثير بعض العوامل الحيوية على حدوث مرض عفن الجذور في نباتات الفاصوليا

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يعتبرعفن جذور الفاصوليا واحد من اخطر الامراض التي تسبب نقص في عدد النباتات ومحصول البذور. واستخدمت طرق عديدة لمقاومة المرض والتي من ضمنها طرق المقاومة الحيوية وهي تعتبر من اكثر الطرق الآمنة والاقتصادية، وفي هذه الدراسة تم دراسة التأثير التضادي لأربعة كائنات حيوية، وهى: *Trichoderma harzianum, T. hamatum, Bacillus subtilis,* *Pseudomonas fluorescens* ضد أربعة من الفطريات الممرضة، وهي *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum* وذلك تحت ظروف المعمل (*In vitro*) وادى ذلك إلى خفض النمو الميسليومى للفطريات الممرضة بواسطة الكائنات المضادة انخفاضا معنويا – واعطى فطر الترايكودرما هاميتم اعلى تثبيط فى نمو الفطريات الممرضة الاربعة.

كما تم دراسة تأثير هذه الكائنات النافعة بالإضافة إلى خليط من فطر *T. harzianum* و *T. hamatum* وذلك في معاملة البذور لدراسة تأثيرها على مقاومة مرض موت البادرات وتأثيرهم على الحالة الغذائية لنباتات الفاصوليا لمدة موسمين متتاليين وهما ٢٠٠٨/٢٠٠٩ – ٢٠٠٩/٢٠١٠ تحت ظروف الصوبة التجارية. أعطت بكتريا *B. subtilis* احسن النتائج كمعاملة مفردة فى مقاومة مرض موت البادرات وكانت نسبة المرض ٢% فقط مقارنة بنسبة ٥٤% في معاملة المقارنة.

وأدى اعادة المعاملة بنفس الكائنات المضادة على هيئة معلق أضيف للتربة بعد ٥ أيام من المعاملة الاولى والزراعة إلى زيادة حماية النباتات وتراوحت نسبة النباتات السليمة بين ٩٦-١٠٠% مقارنة بمعاملة المقارنة حيث كانت ٤٦-٤٨% وذلك في الموسمين.

كما لوحظ ان هناك علاقة طردية بين الجرعات المستخدمه وهي (٥، ١٠، ٢٠ جم/كجم بذرة) من كائنات المقاومة الحيوية وزيادة فاعلية المعاملة في مقاومة موت البادرات.

اظهرت النباتات التي عوملت بكائنات المقاومة المختلفة زيادة في محتواها من العناصر الكبرى (NPK) وزيادة النمو الخضري والمحصول وذلك مقارنة بمعاملة المقارنة.