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EFFECT OF BIOAGENTS, FUNGICIDES AS WELL AS NITROGEN AND PHOSPHORUS FERTILIZERS ON: A-CONTROLLING OF WHITE MOULD DISEASE OF SNAP BEAN PLANTS (*Phaseolus vulgaris* L.) AND ITS EFFECT ON VEGETATIVE GROWTH AND CHEMICAL COMPOSITION.

BY

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ABSTRACT

This study was conducted at the experimental Farm of the Faculty of Agriculture, Moshtohor to investigate the effect of two bioagents, one of the fungicides as well as nitrogen and phosphorus fertilization on the percentage of white mould infection, growth and chemical composition of snap bean plants, (*Phaseolus vulgaris* L.) c.v. Bronco .

The first part of the experiment was carried out at the laboratory and greenhouse, both the two bioagents (*Tricoderma harzianum* and *Bacillus subtilis*) and fungicide (Benlate- 50) reduced mycelial growth of *Sclerotinia Sclerotiorum*. In the greenhouse, the results indicated that the percentage of infected bean plants with Sclerotinia has been reduced by inoculation the seeds before sowing by any one of two studied bioagents or the fungicide.

The second part of the experiment was carried out at the field. Seed inoculation by the same two bioagents and fungicide each alone (which were used in the first part of this study) before sowing as well as fertilizing with low amounts of some macro-elements (N and P) showed that inoculated seeds with *Tricoderma* followed by Benlate-50 and than Bacillus decreased the percentage of natural infection of *S. sclerotorum*. In this concern, the results indicate that these treatments were the most effective, which resulted in the highest values of growth parameters of bean plants (plant height, number of leaves and the fresh and dry weight of both leaves and branches of plant) and its minerals content (N and K content and their uptake by plant). Such results were true at both seasons of this study. In addition, Bacillus treatment resulted in the highest values of P concentration in plants.

Using the level 20kg N/fed. combined with 31 kg P_2O_5/fed. produced the highest values of different studied characteristics of vegetative growth and its chemical composition.

The interactional effect of *Tricoderma* or Benlate-50 within NP fertilizers (20 N + 31 P_2O_5 kg /fed.) resulted in the highest values of most studied characters.

INTRODUCTION

Snap bean (*Phaseolus vulgaris*, L.) is one of the most important leguminous vegetable crops grown around the year in Egypt. It is subjected to a group of fungal diseases under field conditions. *Sclerotinia sclerotiorum* (Lib.) de Bary is a widely distributed and destructive pathogen on several legume and vegetable crops as well as many other host plants. The incidence of *Sclerotiorum* disease ranged from a trace to 100% in bean plants (Tu, 1997). *Sclerotinia sclerotiorum* is a soil borne plant pathogen and can survive in the soil for a long period in the form of sclerotia. In addition, Ghanim (1993) reported that, ascospores, mycelium and sclerotia are the sources of the fungal inoculums in the soil. The need for chemical fungicides usage critically increased to eliminate fungal pathogens. This chemical control has been recommended as alternative and quick means of disease control by several investigators, among them was Ghanim (1993). On the other hand, traditionally, N and P fertilizers have been used to correct the deficiency problems of both, but these application are not entirely satisfactory solution because of the limited availability, its high cost as well as it is considered as a factor of pollution for the environment and human health (Hong *et al.*, 2001) .

Nowadays, bioagents such as *Tricoderma harzianum* or *Bacillus subtilis* can be used as biological control to control the diseases such as white mould caused by *Sclerotinia sclerotiorum* (Mazen, 1995 and Abdel-Ghafar *et al.*, 1996). The other benefit of these bioagents *Tricoderma* and *Bacillus)* is that they are used as biofertilizers beside mineral fertilizers for the plant nutritional requirements in agriculture and this is carried out to minimize the use of chemical fertilizers.

On the other hand, using *Tricoderma harzianum* or *Bacillus subtilis* as bioagents or *Benlate*-50 as fungicide in combination with low amounts of chemical fertilizers such as nitrogen in the form of ammonium sulphate (20.5 %N) and or phosphorus as calcium superphosphate (16% P_2O_5) fertilizers has been reported to be one of the important factors that affect growth vigor, chemical composition and control the white mould disease of bean plants.

Several microorganisms have been reported to be parasite on antagonists to *Sclerotinia spp.* such as *Tricoderma harzianum* and *Bacillus subtilis* (Abdel-Moity *et al.*, 1993 and Abdel-Ghafar *et al.*, 1996).

Many investigators reported that Benomyl inhibited germination of sclerotia and subsequent mycelial growth (Mazen, 1995).

Also, agricultural practices such as fertilization influence on *S. sclerotiorum*. Soil supplement with essential elements as N, P and K increased apothecial formation. These results were observed by (Mazen, 1995).

Moreover, the manipulation which used to control sclerotinia disease appeared good results on bean plants growth and chemical composition.

In this respect, many investigators reported that inoculation with *Tricoderma harzianum* as a biological seed or soil treatment enhanced the vegetative growth of plant and increased its dry matter content. Abdel-Kader, 1997 on bean; Sayed-Ahmed, 1988 on soybean and El-Gamal, 2000 on tomato.

It has been reported also that *Bacillus subtilis* application increased plant growth (Ibrahim *et al.*, 1995 on broad bean, Patel *et al.*, 1998 and Srivastava *et al*,, 1998 on peas and chemical composition (Ibrahim *et al.*, 1995 on broad bean and Mahmoud and Amara, 2000 on tomato).

Concerning the effect of fungicides Gaafar *et al.* (1989) indicated the enhancing influence on growth of bean plants.

Respecting the effect of NP fertilizers, it increased leguminous plant growth (Fekry, 1994, Abou-El-Salehin and Ahmed, 1998, Hong *et al.*, 2001 and Ahmed *et al.*, 2003 on bean and Abd-Alla *et al.*, 2000 on pea) and chemical composition (El-Afifi *et al.*, 1995, Abou El-Salehein and Ahmed, 1998 and Ahmed *et al.*, 2003 on bean and Abd-Alla *et al.*, 2000 on pea).

Regarding the interaction effect of bioagents or fungicides inoculation combined with mineral NP fertilizers on plant growth and chemical composition, Mahmoud and Amara (2000) showed that *Bacillus subtilis* combined with 50% of NP from the recommended dose had a beneficial effect in this respect.

This investigation consists of two parts, the first one was carried out under greenhous conditions by using two bioagents (*Tricoderma harzianum* and *Bacillus subtilis)*, one fungicide (Benlate-50), seed inoculation to determine the effective treatment for controlling the infection of *Sclerotinia sclerotiorum* caused the white mould in bean plants. The second part contains all these treatments beside used low amounts than the recommended dose (Average 25% and 50%) of nitrogen and phosphorous fertilizers each alone or in combination with the other treatments which conducted in field to study their effect on the percentage of white mould infection, vegetative growth and minerals composition of snap bean plants.

MATERIALS AND METHODS

Isolation of the pathogen:

Snap bean plants showing white mould were collected from the Farm of Faculty of Agriculture Moshtohor. Samples were washed with tap water, then surface sterilized with sodium hypochlorite 3% for 2 min. and dried between

sterilized absorbent paper. Plant pieces were placed on potato-dextrose agar plates (PDA) and incubated for 5 days at 20± 2°C.

Sclerotinia sclerotiorum (Lib) de Bary was identified according to the keys of plant pathogenic species of *Sclerotinia* described by Kohn (1979).

Bioagents:

Tricoderma harzianum and *Bacillus subtilis* were isolated from the rhizophere of bean plants and identified by mycology and plant disease survey (Dept. Plant Path., Res. Inst. Of Agric. Res. Cent., Giza, Egypt) and examined for antagonistic effects against *Sclerotina* pathogen.

Tricoderma harzianum were grown on malt extract liquid medium for 15 days at 25°C. Spore suspension was adjusted by hemicytometer slide 4 x 10^8 conidia/ml.

Bacillus subtilis were grown on nutrient broth medium over night at 28°C and cell suspension adjusted to 10^7 cfu/ml.

I. Disease control in the laboratory and greenhouse:

A. Laboratory experiment

1. Bioagents

Discs (4 cm in diameter) of *Sclerotinia sclerotiorum* and the antagonistic fungus *Tricoderma harzianum* or *Bacillus subtilis* were taken from the activity 2-days – old cultures and placed in one half of PDA plates. The plates incubated at 20 ±2 ^{0}C. Four replicates were used in this experiment.

2. Fungicide

Benlate 50 (Benomyl) (methyl 1- butyl-carbamoyl) was used as three concentrations(25, 50 and 75 ppm) for the inhibitory effect on a linear growth of *S. sclerotiorum*. Tested fungicide was added to known amount of sterilized PDA medium immediately before pouring in petri disches. After solidification, petri dischs were inoculated with 4 cm diameter discks taken from the 7- days –old culture of *S. sclerotiorum* and incubated at 20 ±2 ^{0}C. Three replicated for each concentration were used. After that growth reduction was recorded.

$$\text{Growth reduction (\%)} = \frac{\text{Growth diameter in control- Growth diameter in treatment}}{\text{Growth diameter in control}} \times 100$$

B- Greenhouse experiment:

This investigation was conducted at the Experimental greenhouse of the Faculty of Agriculture, Moshtohor, Zagazig University, during the two successive seasons of 2001 and 2002. Sterilized 25 cm diameter pots containing sterilized clay soil was used. Soil was infested with fungus (*Sclerotinia sclerotiorum*) which grown on bottles containing autoclaved sorghum medium.

Some of bean seeds Bronco cv. were treated with each of bioagents, *T. harzanium* or *B. subtilis* (one ml of spore suspension of each were added to 1% ml Arabic gum solution as an adhesive material mixed with 5 gm seeds and shaking them for ten min. The other seeds some of it chaked in solution consists of the fungicide Benlate-50 at the rate 3 g/kg mixed with Arabic gum for the same time. The uninoculated seeds were chaked in tap water.

Five seeds were sown per pot in the treated soil and untreated one as a control on April 5th in 2001 and April 2nd in 2002. A randomized complete block design with four replicates was used each year. The percentage of infection was recorded 45 days after sowing.

II. Disease control and its effect on growth and chemical composition of bean plants in field:

Two field experiments were carried out during 2001 and 2002 summer growing seasons at the Farm of Faculty of Agriculture, Moshtohor, Zagazig University.

Bean seeds of Bronco cv. were sown on April 5th in 2001 and April 2nd in 2002. Physical and chemical analysis on the investigated soil were carried out according to Black *et al.* (1982) and the values were tabulated in Table (1).

Table (1) : Physical and chemical analysis of soil used in the current study.

Characteristics	Values
Physical analysis:	
Coarse sand (%)	3.41
Fine sand (%)	16.47
Silt (%)	34.86
Clay (%)	40.89
Textural class	**Clay loam**
Chemical analysis:	
Organic matter (%)	1.6
Available N ppm	82.55
Available P ppm	20.06
Exchangeable K ppm	291.90
E.C. Mmhos/cm at 25°C	4.15
pH	7.8

This experiment was investigated to study

1- Two bioagents, *Tricoderma harzianum* at the concentration of 4 $x10^8$ and *Bacillus subtilis* at the concentration of 10^7 cfu/ml.
2- Benlate-50, as a fundicide at the recommended dose 3g/kg seeds.
3- Six treatments of both nitrogen and phosphorus fertilizers as follows:

	N	P_2O_5
	Kg/fed.	
1	0	0
2	10	0
3	20	0
4	0	15.5
5	0	31.0
6	20	31.0

4- The interaction between each of both used bioagents and fungicide Benlate-50 treatments with the six treatments of NP fertilization forming 18 combinations was tested.

These treatments were arranged in split-plot design with four replicates. The used two bioagents in addition to the fungicide Benlate –50 were assigned in the main plots and the six levels of NP fertilizers were randomly distributed in the sub plots. The area of sub-plot was 8.4 m^2 (4 ridges of 3.5 m long and 0.6 m width). One guard row was left without planting between each two plots.

Seeds were inoculated, at sowing time, with *Tricoderma harzianum*, *Bacillus subtilis* or Benlate-50 and sown on one side of the ridges.

Nitrogen fertilizer was applied in the form of ammonium sulphate (20.5 % N) and that of phosphorus in the form of calcium superphosphate (16.0 % P_2O_5).

In addition, potassium fertilizer was applied in the form of potassium sulphate (48 % K_2O) at a rate of 100 kg K_2O/fed. for all treatments.

The different amounts of fertilizers were added at two equal doses, three and eight weeks after sowing.

The other cultural treatments of growing beans were practiced as usually followed in the commercial production of green pod yield of bean.

Data collected of this experiment were as follows:

1-Percentage of natural infection with *S. sclerotiorum*:

The naturally infected bean plants were recorded in each plot 65-70 days after planting. Disease incidence was calculated on the base of:

$$\text{Percentage of infection} = \frac{\text{No. of infected plants X 100}}{\text{Total plants}}$$

2- Vegetative growth characteristics:

Two weeks after the second addition of fertilizers, when plants were 70 days age, a random sample of 5 plants was chosen from each experimental plot for evaluating the following characteristics:

Plant height (cm), number of both leaves and branches per plant, fresh and dry weight per plant (gm).

3- Chemical composition:

Random samples from the dried leaves and stems were ground and wet digested and the concentration and uptake of N, P and K were determined by Cottenie *et al.* (1982).

4- Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cochran (1980) to compare treatments by the least significant differences (L. S. D.) at 5% probability.

RESULTS AND DISCUSSION

I. Disease control in the laboratory and greenhouse:

A. Laboratory experiment

The effect of bioagents (*Tricoderma harzianum* or *Bacillus subtilis)* and fungicide (Benlate-50 at different concentrations) on mycelial growth of *S. sclerotiorum* were recorded in Table (2). The obtained data showed the interaction between both bioagents or the fungicide and *S. sclerotiorum* in the laboratory test, which the fungus gave a very zone inhibition of linear growth after five days. Also, effect of the fungicide Benlate-50 on the mycelial growth was increased by increasing the concentration of it.

In this respect, Mazen (1995) showed that biological control revealed that *Trichoderma sp* (as antagonistic agent) covered growth of *S. sclerotiorum* in the laboratory.

Concerning the effect of *B. subtilis*, Abdel-Ghafar *et al.* (1996) reported that, it can be used as antagonistic bacteria in this respect which recorded the fungal infection.

Regarding the effect of Benlate-50, Ghanim (1993) and Mazen (1995) indicated that, fungicides reduced mycelial growth of *S. sclerotiorum* in the laboratory test.

Table (2): Effect of *Tricoderma harzianum, Bacillus subtilis* and different concentrations of Benlate-50 on the linear growth of *S. sclerotiorum.*

Tested fungus	Antagonists		Fungicide (concentration ppm) Benlate-50			L.S. D. 0.05
	T.harzianum	*B. subtilis*	25	50	75	
S. sclerotiorum.	1.1	1.2	1.0	0.0	0.0	0.1

B- Greenhouse experiment:

Results in Table (3) show the effect of *Tricoderma harzianum* and *Bacillus subtilis* as bioagents and *Benlate*-50 as fungicide which used as seed dressing on controlling *S. sclerotiorum* on bean plants grown in greenhouse.

These results reveal that the percentage of infected bean plants with *S. sclerotiorum* has reduced by inoculation the seeds by any of these treatments in the two growing seasons compared with the control treatment. In this respect, the percentage of infected plants was decreased by application of *Tricoderma harzianum* followed by Benlate-50 and then *Bacillus subtilis*.

These results are in agreement with those of Budge and Whipps (1991), Abdel-Moity *et al.* (1993) and Mazen (1995) who found that sclerotia of *S. sclerotiorum* were reduced in the presence of *Tricoderma spp*. The mechanism by which *Trichoderma* affected *S. sclerotiorum* was investigated by Lorito *et al.* (1993 a and b) and El-Gamal (2000) who suggested that compitability between the bioagents and plant kind is important in the success of biological control as some plant root exudates encourage the growth of these bioagents and some others do not and this determines the kinds of microorganisms that dominate the rhizosphere microflora and hence antagonist the pathogenic fungi and the antagonistic ability of the fungal bioagents depended on the enzymatic producing ability and antibiotic production, such as *Trichoderma harzianum* which active in chitinase production specially in neutral medium and this chitinolytic enzymes act synergistically to inhibit growth of a variety of plant pathogenic fungi and this may be by release nutrients from hyphae of target fungi.

Table (3): Effect of *T. harzianum*, *Bacillus subtilis* and Benlate-50 seed dressing on disease incidence caused by *S. sclerotiorum* on snap bean plants grown in greenhouse.

Treatments	Percentage of diseased plants	
	2001	2002
Control	11.9	12
Tricoderma	6.1	6.3
Bacillus	7.2	7.6
Benlate-50	6.2	7.4
L.S.D. (0.05)	0.223	0.340

With regard to the effect of *Bacillus subtilis* on controlling *S. sclerotiorum*, El-Gamal (2000) stated that this kind of bacteria is consider among the best antagonistic bacteria in this respect. such results may be due to bacterial biocontrol agents could utilize the nutrients for proliferation and the subsequent increase in bacterial populations should enhance the ability of these bacteria to act as biocontrol agents.

Concerning the effect of Benlate-50 as fungicide, obtained results are in harmony with those of Mazen (1995) who found that in the greenhouse, seed dressing by fungicides (i.e. Benlate-50) reduced percentage of *Sclerotinia* infection compared with untreated chickpea seeds.

II. Disease control and its effect on growth and chemical composition of bean plants in field:

1-Percentage of natural infection with *S. sclerotiorum*:

1.a. Effect of bioagents and fungicides:

Data presented in Table (4) show that inoculated seeds by either *Tricoderma harzianum, Bacillus subtilis* (as bioagents) or Benlate-50 (as fungicide) reduced the reduced the percentage of infection with *S. sclerotiorum* in the two growing seasons compared with the control treatment. Moreover, the percentage of natural infection decreased in the presence of *Tricoderma harzianum,* followed by Benlate-50 and then *Bacillus subtilis.*

In this concern, the effect of the first bioagent *T. Harzianum* may be due to its mycelium invaded the inner content of sclerotia causing complete destruction of it, moreover, the second bioagent *B. subtilis* may be inhibited pathogens by producing antibiotic and fluorescent siderophores (Abdel-Moty *et al.*, 1993 and Abdel-Ghafar *et al.*, 1996). On the other hand, the fungicide Benlate-50 gave a good protection against the *Sclerotinia* disease and this may be due to its capable for inhibiting germination of sclerotia and subsequent mycelial growth (Ghanim, 1993 and Mazen, 1995).

Table (4): Effect of *T. harzianum, Bacillus subtilis,* Benlate-50 and NP fertilization levels on disease incidence caused by *S. sclerotiorum* on snap bean plants in field.

Treatments		Percentage of diseased plants	
		2001	2002
Control		15.67	17.67
Tricoderma		4.23	4.42
Bacillus		5.18	5.53
Benlate-50		4.95	4.93
L.S.D. (0.05)		0.110	0.110
N	P		
kg/fed.			
0	0	5.40	5.00
10	0	5.75	6.30
20	0	7.98	8.20
0	15.5	7.58	6.50
0	31.0	8.65	8.50
20.0	31.0	9.70	11.00
L.S.D. (0.05)		0.159	0.188

1.b. Effect of NP fertilization:

Data tabulated in Table (4) indicate that soil fertilized by NP singly or in combination affected the infection by *S. sclerotiorum.* The same data show also that increasing of N-fertilizer from 10 to 20 N and P-fertilizer from 15.5 to 31.0 P_2O_5 kg/fed. increased the percentage of diseased plants, but the highest percentage of infection achieved at 20 N+ 31 P_2O_5 kg/fed. in the two growing seasons.

Obtained results are going in agreement with those reported by Boland and Hall (1988) and Mazen (1995) who reported that soil supplement of essential elements as N and P increased apothecial formation and subsequently the amount of *Sclerotinia* stem rot disease likely to occur. Moreover, manipulation of fertilizer applications might help in controlling *Sclerotinia* disease.

1.c. Effect of bioagents, fungicides and NP fertilization interaction:

Data presented in Table (5) indicate that the percentage of diseased plants were significantly affected by the interaction between the bioagents (*Tricoderma harzianum* or *Bacillus subtilis)* or fungicides (Benlate-50) and NP fertilizer levels in both growing seasons compared with control treatment. Moreover, the percentage of infected plants was decreased by application of *Tricoderma harzianum* with low level either with N or P fertilizers (10 N or 15.5 P_2O_5 kg /fed.). Similar trend was observed by the same treatments of N or P fertilizers in combination with Benlate-50 followed by *Bacillus subtilis*.

Table (5): Effect of the interaction between *T. harzianum*, *Bacillus subtilis*, Benlate-50 and NP fertilization levels on disease incidence caused by *S. sclerotiorum* on snap bean plants in field.

Treatments	N	P_2O_5 Kg/fed.	Percentage of diseased plants	
			2001	2002
Control	0	0	12.0	14.0
	10	0	11.0	13.0
	20	0	18.0	19.0
	0	15.5	16.0	19.0
	0	31.0	18.0	20.0
	20.0	31.0	19.0	21.0
Tricoderma	0	0	2.9	3.1
	10	0	3.2	3.5
	20	0	4.0	3.9
	0	15.5	4.3	4.5
	0	31.0	5.0	5.3
	20.0	31.0	6.0	6.2
Bacillus	0	0	3.5	3.7
	10	0	4.4	4.6
	20	0	4.9	5.3
	0	15.5	5.2	5.8
	0	31.0	6.1	6.5
	20.0	31.0	7.0	7.3
Benlate-50	0	0	3.2	3.2
	10	0	4.0	4.4
	20	0	4.5	5.0
	0	15.5	4.4	4.8
	0	31.0	5.2	5.5
	20.0	31.0	6.3	6.8
L.S.D. (0.05)			0.319	0.375

2. Vegetative growth characteristics:

2.a . Effect of bioagents and fungicides:

Data presented in Table (6) showed clearly that inoculated seeds either with bioagents, i.e. *Tricoderma harzianum, Bacillus subtilis* or fungicides, i. e. Benlate-50 significantly increased most of the growth parameters expressed as plant height, number of leaves, fresh and dry weight per snap bean plant compared with the control treatment . On the other hand, number of branches per plant was not significantly affected . Obtained results are true during both seasons of study. In addition, *Tricoderma harzianum* scored the highest values for growth characteristics followed by Benlate-50 and then *Bacillus subtilis.*

In this respect, Windeham *et al.* (1986), Sayed-Ahmed (1988) and Abdel-Kader (1997) reported that *Tricoderma harzianum* enhanced growth directly by production of Indole Acetic Acid (IAA) as growth regulator, also, fungi contained some of vitamin B group (Niacin, Pantothenic acid and B_6) with a highest amount which may be play a role in the control of the stem rot disease of leguminous crops by making the plant more healthy and strong in growth and gives it a chance to escape from the disease.

Regarding the effect of *Bacillus subtilis* on soil and plant, many investigators indicated that Bacillus posses the ability to bring insoluble phosphate in the soil into soluble forms by secreting phosphatase enzymes and organic acids such as formic, acetic, propionic, lactic, fumaric and succinic acids (Illmer *et al.*, 1995 and Forssard *et al.*. 2000) . Moreover, Bally *et al.* (1983) reported that some Bacillus strains had indirect effect on nitrogen fixation increasing the absorption of zinc and cupper, as well as, increasing some growth hormones.

Concerning the effect of fungicides, such superiority of chemical fungicide (Benlate-50) may be due to its direct effect through preventing diseases infection especially during the vegetative stage of growth earlier than those treated by bioagents. Furthermore, the activity and effect of bioagents may be due to the effect of prevailing environmental conditions.

Obtained results are in agreement with those by Gaafar *et al.* (1989) on common bean, Ibrahim *et al.* (1995) on broad bean and Patel *et al.* (1998) and Srivastava *et al.* (1998) on pea.

2.b. Effect of NP fertilization:

Data in Table (6) indicate that the addition of NP fertilizers to snap bean plants significantly increased bean plant growth over the control one. These results were true for all studied plant growth aspects i.e., plant height, number of both leaves and branches and fresh and dry weight of bean plant. The same data show also that increasing of N-fertilizer from 10 to 20 N and P-fertilizer from 15.5 to 31.0 P_2O_5 kg /fed. increased plant growth characters, but the highest values were achieved at 20 N + 31 P_2O_5 kg/fed. in the two growing seasons.

Table (6). Effect of *Tricoderma harzianum*, *Bacillus subtilis*, Benlate-50 and NP fertilization levels on vegetative growth of snap bean plants.

Treatments		Plant height (cm)		No. of leaves/plant		No. of branches/ plant		Fresh weight/ plant (gm)		Dry weight /plant (gm)	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Control		54.50	44.11	15.28	14.51	4.36	3.83	88.26	62.06	18.27	15.26
Tricoderma		57.72	48.44	17.72	17.01	4.58	4.32	103.70	83.34	21.53	18.69
Bacillus		55.50	47.98	15.69	14.82	4.44	3.96	102.47	77.92	19.64	16.94
Benlate-50		56.14	48.27	17.19	15.12	4.52	4.14	103.39	82.16	20.32	17.41
L. S. D. (0.05)		0.63	0.39	0.83	0.37	N. S.	N. S.	1.05	0.34	0.65	0.31
N	P										
kg/fed.											
0	0	52.08	42.29	12.54	12.29	3.75	3.39	77.25	59.66	15.40	14.77
10	0	55.90	45.62	15.99	14.68	4.42	3.74	93.96	67.91	19.01	15.64
20	0	58.36	49.08	17.94	16.57	4.50	4.23	106.73	79.63	20.21	17.75
0	15.5	53.42	45.91	15.90	14.97	4.52	4.02	93.67	73.65	19.27	16.53
0	31	54.39	48.28	17.40	16.00	4.81	4.33	110.01	84.71	21.64	18.33
20	31	61.11	52.03	19.06	17.68	4.87	4.68	115.11	92.66	23.42	19.43
L.S.D. (0.05)		0.60	0.38	0.60	0.40	0.41	0.28	0.56	0.51	0.42	0.23

As for the role of NP elements in plant, Edmond *et al.* (1981) concluded that nitrogen is an indispensable elementary constituents of numerous organic compounds of general importance (amino acids, protein, nucleic acids) and also it is needed in the formation of protoplasm and new cells, as well as, encouragement for cell elongation. Furthermore, they also added that, phosphorus is a part of molecular structure of several vitally important compounds notably nucleic acids (DNA, the two forms of RNA). In addition, it plays indispensable role in the enzyme system necessary for the energy transform in photosynthesis and respiration, it is also a constituent of cell nucleus and essential for cell division and for the development of meristem tissues, all this in turn increased bean growth and its dry matter.

Similar findings were obtained by Fekry (1994), Abou El-Salehein and Ahmed (1998), Hong *et al.* (2001) and Ahmed *et al.* 2003 on bean and Abd-Alla *et al.* (2000) on pea.

2.c. Effect of bioagents, fungicides and NP fertilization interaction:

Data in Table (7) show that the interaction between bioagents, i.e., *Tricoderma harzianum* and *Bacillus subtilis,* fungicides, i.e. Benlate-50 and NP fertilization were significant with regard to plant growth except number of branches/plant. In this respect, the highest values of plant growth parameters were obtained by application of *Tricoderma harzianum* with 20 N+ 31 P_2O_5 kg/fed. followed by the other treatments Benlate-50 and Bacillus, respectively. On the other hand, the lowest values were obtained by the control treatment. Similar trend was observed during both growing seasons. In this concern, some investigators showed that, fertilizers interact with microbial communities in soils in a number of ways either promoting growth directly by providing nutrients or indirectly by stimulating plant growth and enhancing root C flow (Buyanovsky and Wagner, 1987). Alternatively, fertilizer inputs may result in soil acidification limiting microbial growth and activity in soils (Macrae *et al.*, 1999 and Anthony *et al.*, 2001). Obtained results agree also with those of Mahmoud and Amara (2000) on Tomato.

3. Chemical composition:

Mineral content

3.a. Effect of bioagents and fungicides:

With regard to the effect of bioagents and fungicides on minerals content of bean plant (stems + leaves), the data presented in Table (8) show that *Tricoderma harzianum* followed by Benlate –50 gave the highest values for both N and K content as concentration or uptake in two growing seasons.

On the other hand, Bacillus treatment significantly increased P content as concentration but *Tricoderma* followed by Bacillus and Benlate-50 resulted the highest values for P uptake in plants in two growing seasons.

The enhancing effect of Bacillus in this concern may be due to that it posses the ability to bring insoluble phosphate in the soil into soluble forms (Subba Rao, 1984).

Table (7). Effect of the interaction between *Tricoderma harzianum*, *Bacillus subtilis*, Benlate-50 and NP fertilization levels on vegetative growth of snap bean plants.

Treatments	N	P_2O_5 kg/fed.	Plant height (cm)		No. of leaves /plant		No. of branches/plant		Fresh weight/plant (gm)		Dry weight/plant (gm)	
			2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Control	0	0	50.7	41.5	11.2	11.3	3.33	3.23	72.7	51.7	13.8	13.7
	10	0	53.3	42.8	15.0	13.6	4.00	3.67	87.0	55.3	18.3	13.9
	20	0	56.8	45.3	16.6	15.3	4.33	4.00	93.4	62.7	19.5	15.0
	0	15.5	51.3	42.3	14.3	14.0	4.57	3.83	88.5	57.5	17.5	14.5
	0	31	52.7	45.2	15.5	15.1	4.67	3.97	97.2	68.0	19.8	16.9
	20	31	58.6	47.5	18.3	16.6	4.68	4.17	100.5	77.2	20.7	17.4
Tricoderma	0	0	53.7	43.3	14.2	13.2	4.00	3.67	83.8	66.8	16.4	16.7
	10	0	58.6	47.9	16.0	16.3	4.67	3.78	97.7	73.0	19.4	17.1
	20	0	61.7	50.3	19.0	18.8	4.67	4.73	115.9	87.0	22.7	19.3
	0	15.5	55.3	47.4	18.5	16.4	4.69	4.33	105.8	80.6	21.9	17.4
	0	31	54.5	49.8	18.7	18.0	4.97	4.78	117.7	96.3	23.3	19.7
	20	31	64.5	55.1	21.0	19.3	5.07	4.93	121.8	103.8	24.5	22.6
Bacillus	0	0	52.3	41.5	11.7	12.2	3.83	3.33	75.8	55.9	15.7	14.2
	10	0	54.3	46.5	15.3	13.8	4.50	3.73	95.5	70.4	19.4	15.3
	20	0	57.7	50.3	17.2	16.0	4.60	4.10	108.4	79.8	20.5	17.8
	0	15.5	52.7	47.3	15.0	15.2	4.67	3.87	90.2	82.5	17.5	17.6
	0	31	55.3	48.8	17.3	15.4	4.70	4.21	113.2	85.5	21.3	18.1
	20	31	60.0	51.7	18.3	17.1	4.83	4.77	118.7	93.4	23.4	18.7
Benlate-50	0	0	51.7	42.8	13.2	12.5	3.83	3.33	76.7	64.3	15.7	15.0
	10	0	57.9	45.2	17.7	15.0	4.50	3.77	95.7	72.9	18.9	16.2
	20	0	57.3	50.3	19.0	16.1	44.67	4.17	109.2	89.1	20.9	18.9
	0	15.5	54.3	46.5	15.8	14.2	4.67	4.07	100.0	80.6	20.1	16.7
	0	31	55.1	49.8	18.2	15.5	4.83	4.33	114.0	89.3	22.1	18.6
	20	31	61.3	53.8	18.6	17.7	5.00	4.83	120.4	96.3	24.1	19.0
L.S.D. (0.05)			1.21	0.77	1.19	0.81	N. S.	N. S.	1.12	1.02	0.84	0.46

Table (8). Effect of *Tricoderma harzianum*, *Bacillus subtilis*, Benlate-50 and NP fertilization levels on nitrogen , phosphorus and potassium concentration and their uptake in snap bean plants.

Treatments		Minerales of plants (% on D. W. basis)						Minerales uptake (mg/plant)					
		N		P		K		N		P		K	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Control		2.534	2.597	0.596	0.629	3.537	3.507	462.9	396.3	108.8	95.9	646.2	535.1
Tricoderma		2.688	2.758	0.599	0.640	3.745	3.702	578.7	515.4	128.9	119.6	806.2	691.9
Bacillus		2.577	2.656	0.630	0.646	3.608	3.640	506.1	449.9	123.7	109.4	708.6	616.6
Benlate-50		2.634	2.681	0.584	0.627	3.699	3.612	535.2	466.7	118.6	109.1	751.6	628.8
L. S. D. (0.05)		0.006	0.005	0.008	0.001	0.009	0.009	16.892	8.415	3.937	1.970	22.820	11.211
N	P												
kg/fed.													
0	0	2.371	2.395	0.543	0.574	3.278	3.326	365.1	353.7	83.6	84.7	504.8	491.2
10	0	2.557	2.643	0.568	0.615	3.610	3.490	486.0	413.3	107.9	96.1	686.2	545.8
20	0	2.714	2.791	0.586	0.637	3.772	3.623	548.4	495.4	118.4	113.0	762.3	643.0
0	15.5	2.549	2.609	0.616	0.648	3.648	3.689	491.1	431.2	118.7	107.1	702.9	609.7
0	31	2.644	2.709	0.638	0.664	3.740	3.735	609.5	496.5	138.0	121.7	809.3	684.6
20	31	2.817	2.891	0.661	0.674	3.837	3.828	659.7	561.7	154.8	130.9	898.6	743.7
L.S.D. (0.05)		0.009	0.010	0.009	0.001	0.009	0.011	10.536	6.454	2.471	1.499	15.400	8.787

These results are in agreement with those reported by Ibrahim *et al.* (1995) on broad bean.

3.b. Effect of NP fertilization:

Data tabulated in Table (8) show that the highest increments regarding plants content of N, P and K as concentration and uptake in all cases were observed by increasing nitrogen fertilizer from 10 to 20 kg N/fed. and phosphorus fertilizer from 15.5 to 31 P_2O_5 kg /fed. but the highest values were obtained at 20 N+ 31 P_2O_5 kg/fed. at both growing seasons of the experiment.

The enhancing effect of nitrogen and phosphorus fertilizers in this concern may be due to the available N and P in soil and /or the high absorbing efficiency of bean roots.

Similar findings were demonstrated by El-Afifi *et al.* (1995), Abu El-Salehein and Ahmed (1998) and Ahmed *et al.* (2003) on bean and Abd –Alla *et al.* (2000) on pea.

3.c. Effect of bioagents, fungicides and NP fertilization interaction:

Data presented in Table (9) clearly indicate that the concentration of N, P and K and their uptake were significantly affected by the interaction between *Tricoderma harzianum,* and *Bacillus subtilis* as bioagents or Benlate-50 as a fungicide and NP fertilizers level in both growing seasons.

In addition, there was an increase in N and K concentration and their uptake in plant by inoculated seeds with *Tricoderma* or Benlate-50 and fertilized the plants with 20 kg N+ 31 kg P_2O_5/fed., but the first treatment was the best (*Tricoderma* + 20 kg N +31 kg P_2O_5/fed.) In spite of that, P concentrations in plant were increased by the interaction of Bacillus with 20 kg N+ 31kg P_2O_5/fed., but P uptake was increased by used *Tricoderma* combined with 20 kg N +31 kg P_2O_5/fed.

These results are in agreement with those reported by Mahmoud and Amara (2000) on tomato.

Table (9). Effect of the interaction between *Tricoderma harzianum*, *Bacillus subtilis*, Benlate-50 and NP fertilization levels on nitrogen, phosphorus and potassium concentration and their uptake in snap bean plants.

Treatments	N	P_2O_5 kg/fed.	Minerals of plant (%on D.W.basis)						Minerals uptake (mg/plant)					
			N		P		K		N		P		K	
			2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Control	0	0	2.310	2.340	0.536	0.552	3.150	3.237	318.7	320.5	73 9	75.6	434.7	443.4
	10	0	2.460	2.510	0.560	0.601	3.540	3.313	450.1	348.8	102.4	83.5	647.8	460.5
	20	0	2.640	2.720	0.581	0.630	3.610	3.573	514 8	408.0	113.2	94.3	703 9	535.9
	0	15.5	2.463	2.563	0.602	0.651	3.540	3.610	431.0	371.6	105.3	94.5	619.5	523.4
	0	31	2.613	2.650	0.636	0.665	3.640	3.640	517.8	447.8	125.9	112.3	720.7	615.1
	20	31	2.720	2.800	0.660	0.672	3.743	3.670	563.0	487.2	136.6	116.9	774.8	638.5
Tricoderma	0	0	2.433	2.480	0.552	0.580	3.360	3.407	399.0	414.1	90.5	96.8	551.0	568.9
	10	0	2.700	2.767	0.556	0.629	3.620	3.610	523.8	473.1	107.8	107.5	702.2	617.3
	20	0	2.797	2.890	0.571	0.641	3.940	3.680	634.9	557.7	129.6	123.7	894.3	710.2
	0	15.5	2.610	2.653	0.617	0.651	3.720	3.793	571.5	461.6	135.1	113.2	814.6	659.9
	0	31	2.680	2.760	0.631	0.666	3.850	3.823	624.4	543.7	147.0	131.2	897.0	753.1
	20	31	2.910	3.000	0.665	0.672	3.980	3.897	742.0	678.0	169.5	151.8	975.1	880.7
Bacillus	0	0	2.350	2.360	0.572	0.596	3.283	3.310	368.9	335.1	89.8	84.6	515.4	470.0
	10	0	2.510	2.653	0.611	0.620	3.640	3.600	486.9	405.9	118.5	94.8	706.1	550.8
	20	0	2.700	2.783	0.630	0.642	3.730	3.667	553.5	495.3	129.1	114.2	764.6	652.7
	0	15.5	2.513	2.580	0.642	0.660	3.550	3.710	439.7	454.0	112.3	116.1	621.2	652.9
	0	31	2.630	2.710	0.655	0.672	3.670	3.693	560.1	490.5	139.5	121.6	781.7	668.4
	20	31	2.760	2.847	0.670	0.686	3.773	3.860	645.8	532.3	156.7	128.2	882.8	721.8
Benlate-50	0	0	2.390	2.400	0.540	0.569	3.320	3.350	375.2	360.0	84.7	85.3	521.2	502.5
	10	0	2.557	2.640	0.546	0.611	3.640	3.437	483.2	427.6	103.1	98.9	687.9	556.7
	20	0	2.720	2.770	0.563	0.634	3.807	3.573	568.4	523.5	117.6	119.8	795.6	675.2
	0	15.5	2.610	2.640	0.605	0.629	3.780	3.043	524.6	440.8	121.6	105.0	759.7	508.1
	0	31	2.653	2.717	0.630	0.651	3.800	3.783	586.3	505.3	136.2	121.0	839.8	703.6
	20	31	2877	2.917	0.650	0.666	3.850	3.883	693.3	554.2	156.6	126.5	927.8	737.7
L.S.D. (0.05)			0.017	0.019	0.018	0 002	0.018	0.021	14.899	12.907	4.943	2.999	29.800	17.575

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تاثير عوامل المقاومة الحيوية والكيماوية وكذلك التسميد النتروجيني والفوسفاتي علي : أ- السيطرة علي الأصابة بمرض العفن الأبيض علي نباتات الفاصوليا وكذلك تأثيرها علي النمو الخضري والتركيب الكيماوي

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أجريت هذه الدراسة بمزرعة التجارب بكلية الزراعة بمشتهر لبحث تأثير أثنين من العوامل الحيوية وأحد المبيدات الفطرية وذلك بجانب التسميد النتروجيني والفوسفاتي علي نسبة الإصابة بالعفن الأبيض ، النمو والتركيب الكيماوي لنباتات صنف الفاصوليا برونكو.

الجزء الأول من هذه التجربة تم إجرائه بالمعمل و الصوبة وقد أظهر اختبار المعمل أن كلا العوامل الحيوية (التريكودرما هرزينيم والباسلس سبتلس) والمبيد الفطري (البنليت-٥٠) أدي إلي نقص نمو هيفات الأسكلروتينيا اسكلروشيم. أما في الصوبة فقد أشارت النتائج إلي نقص نسبة نباتات الفاصوليا المصابة بالأسكلروتينيا عند تلقيح البذور قبل الزراعة بأي من العوامل الحيوية أو المبيد الفطري التي تم دراستها. وبالإضافة إلي ذلك فقد حققت المعاملة بالترايكودرما أفضل النتائج في هذا المجال.

الجزء الثاني من التجربة فقد تم إجرائه بالحقل وقد أظهر تلقيح البذور بالعوامل الحيوية والمبيد الفطري كل بمفردة (و التي سبق استخدامها بالجزء الأول من التجربة) قبل الزراعة او مع التسميد بكميات صغيرة ، من السماد النتروجيني والفوسفاتي، أن معاملة البذور بفطر التريكودرما تليها المعاملة بالبنليت-٥٠ ثم الباسلس قد أدت إلي نقص الإصابة بفطر الأسكلروتينيا ، كما أشارت النتائج إلي أن هذه المعاملات هي الأكثر فعالية حيث أدت إلي أعلي القيم لصفات النمو لنباتات الفاصوليا خلال موسمي النمو (طول النبات ، عدد الأوراق ، الوزن الغض و الوزن الجاف للنبات) كذلك والمحتوي المعدني (المحتوي من النيتروجين والبوتاسيوم وامتصاصهما في النبات) إضافة إلي ذلك فقد حققت المعاملة ببكتريا الباسلس أعلي القيم لمحتوي النبات من الفوسفور.

أعطي استخدام المعدلات ٢٠ كجم ن مع ٣١ كجم فو٢أه /فدان أعلي القيم لمختلف الصفات التي تم دراستها لنمو النبات ومحتواه الكيماوي.

أدي التأثير المتداخل بين فطر الترايكودرما أو البنليت-٥٠ مع ٢٠ كجم ن و ٣١ كجم فو٢أه /فدان إلي أعلي القيم لمعظم الصفات المدروسة.