Effect of different mixtures of some bioagents and Rhizobium phaseoli on bean damping-off under field condition

Mona M.M. Ragab*,K. A. Abada*,Maisa L. Abd-El-Moneim** and Yosra Z. Abo-Shosha**
*Plant Pathol. Dept. Fac. of Agric., Cairo Univ. Giza, Egypt
**Central Lab. of Organic Agriculture. Agricultural Research Center,Giza, Egypt.

ABSTRACT—Bean is considered a very common vegetable crop in many countries worldwide. Beans are used as green pods or as dry beans. Egypt cultivates beans either for local consumption or for exportation especially during winter and spring seasons. Root rot and damping-off during early stages of bean growth, cause great losses for growers. Growers usually use highly toxic chemical fungicides to protect their investment and get rid of damping-off problem. Using these toxic chemicals led to rejection of exported shipment. The present work offers biological control as a substitute for the toxic chemicals. In this work three different biocontrol mixtures were used. These mixtures consist of *Trichoderma* spp. isolates (T1+T2) or mixture of *Bacillus subtilis* isolates (B1+B2) or combination between *T. harzianum* isolate plus *B. subtilis* isolate (B2+T2) to illustrate their effect on plant protection against damping-off and also their side effect on beneficial bacterium *Rhizobium phaseoli*. Data obtained showed that the mixture of the different bioagents either formulated as powder or suspension were varied in their effect on controlling damping-off disease in beans under field condition. A mixture consists of *B.subtilis*+ *T.harzianum* (B2+T2) either in form of powder or suspension showed the highest effect in disease control compared with control treatment and the other two mixtures (T1+T2) and (B1+B2). Using of the mixture (B2+T2) as powder or suspension led to increase in fresh and dry weight of shoot and root system of treated plants compared with all other treatments. Using of the same mixture (B2+T2) also led to an increase in the number of pods/plant, yield and percentage of dry weight/ 100(g) pods. Regarding effect of biological treatment on chemical components of the treated plants, it was clear that using the mixture of (B2+T2) led to clear increase in free phenols, total phenols, reduced sugars, amount of chlorophyll in addition to percentage of protein compared with control treatment and the other used mixtures(T1+T2) or (B1+B2). When *Rh. phaseoli* was considered in plant protection process, obtained data indicated that *Rh.phaseoli* alone has very slight effect against incidence of damping-off disease. This slight effect increased in presence of any used biological mixtures. The highest synergistic effect was recorded when *Rh. phaseoli* was in combination with the mixture (B2+T2). Obtained results also revealed that the presence of *Rh. phaseoli* with biocontrol mixtures led to changes in some chemical components in the treated plants compared with the control and the other mixtures and *Rh.phaseoli* alone. The highest increase in these components (free phenol- total phenol- reduced sugars- chlorophyll- protein) was noticed when *Rh.phaseoli* was used in presence of (B2+T2) mixture. Effects of adding *Rh.phaseoli* with biocontrol mixtures on yield were also studied under field conditions. Data obtained indicated that all mixtures of biocontrol agents in combination with *Rh.phaseoli* led to increase number of rhizobium nodules, fresh and dry weight of roots and shoots, in addition to increase in number of pods/plant, yield/plant and weight of 100 pods.

Key words— *Bacillus subtilis*, bean, Biocontrol agents, damping-off, *Rhizobium phaseoli*, *Trichoderma harzianum*.

1 INTRODUCTION

Bean is considered as a very important vegetable crop worldwide. Bean is consumed as green pods or dry bean seeds. In year 2012 about 115,750 Feddan were cultivated with different bean varieties. This area produced about 251,279 ton of green beans and 69,486 ton of dry seeds (**Agriculture Statistics, 2012**). Exported amount of green bean during 2013 reached about 35,881 ton with cash value about 392,881 L.E. (**Anonymous, 2013**). This shows the economic importance of bean as vegetable crop either for local consumption or exportation. Beans are attacked by numerous diseases. These diseases can attack all plant parts i.e. roots, leaves, stems and pods. (**Graham *et al.*, 1997 and Mukankusi *et al.*, 2010**). Soil borne pathogens play a very important role in quantity and quality of bean production process. *Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii* are considered the most aggressive and destructive pathogens causing high losses in bean under field conditions (**Abeysinghe, 2007**; **Abd-El-Khair *et al.*, 2011** and **Pena *et al.*, 2013**). In general using chemical fugicides in vegetable production lead to toxic residues in the produced vegetable product. These residues have negative effects on consumer health and also our environment and biological balance in the soil. Countries import Egyptian green bean ask for nearly free of chemicals in the imported beans. Presence of these chemicals in bean may be lead to rejection of all shipment. Biological control of soil borne pathogens attack bean were successfully tested and used before (**Elad *et al.*,1980; Estevez de Jensen *et al.*, 2002 and Ahmadzadeh *et al.*,2009**).

This work illustrates the efficacy of some bioagents, *i.e.* (*Bacillus subtilis* **(Schleifer, 2001)** and *Trichoderma* spp. (**Rifai, 1969)** in presence or absence of *Rh. phaseoli* on controlling these pathogens using different formula to figure out correlation between formula and efficacy of the treatment. Also, studying the effect of combination between the bioagents and *Rh. phaseoli,* as beneficial microorganism in the soil. The work was expanded to evaluate the effect of *Rh. phaseoli,* on protein content and fresh and dry weight of treated plants.

2 Materials and Methods

In all field experiments, unless otherwise indicated, experiments were carried out during season 2011/ 2012 at farm located at Badr district Behera governorate. Natural infested soil in this farm belongs to light sandy soil with

alkaline reaction pH 7.5. Paulista bean cultivar was used. All experiments were designed in complete randomize plots. Five plots were used for each treatment. Each plot (4 x 0.6 m) was used as replicate and 30 Paulista seeds (25 cm apart) were sown in each replicate. This means 150 seeds were used for each treatment.

Preparing and use of different bioagents

Different bioagents *T.viridi* (T1) and *T.harzianum*(T2) were grown separately on Gliotoxin Fermentation medium (GFM) (Brian and Hemming, 1945) for 15 days under complete darkness condition to stimulate toxin production (Abd El-Moity,1985). *B. subtilis* isolate(B1) and *B. subtilis* isolate(B2) were grown separately on Nutrient glucose (NG) medium (Dowson,1957) for 2 days. Different bioagents were blended in electric blender for 2 minutes. Suspension of different biocontrol agents was prepared as powder using Talc powder as a method developed by Abd El Moity (1985). The same antagonists were also prepared as suspension. Numbers of colony forming units (cfu) per each one gram of prepared powder or per each ml of prepared suspension of different antagonists were adjusted to be contain 30 x106 cfu/ 1ml or 1 g of formula. *Rhizobium* inoculum was prepared by inoculating pure identified *Rhizobium phaseoli* isolate on yeast mannitol(YM) medium (Allen,1959) for 3 days at 25^{o}C. Obtained culture was adjusted, to be contain 30 x 10^{6} cfu/ml. Adjusted *Rh. phaseoli* suspension was used, as suspension at the rate of 1L/ 100 L water as soil drench after one week from sowing time.

Antagonistic mixtures were prepared as powder or liquid suspension. Powder and suspension for were used as seed coating at the rate of 10 g or 10 ml/1 kg of seeds. Plots with only bean seeds and pathogenic inocula, without antagonists, were act as control treatment. All treatments received the same amount of fertilizers and irrigation water.

Collecting data and statistical analysis

Pre- emergence damping-off was recorded 15 days after sowing. Whereas post-emergence was recorded 30 days after sowing. Percentage of damping-off was calculated according to next form:

$$\text{Percentage of damping-off} = \frac{N1 - N2}{N1} \times 100$$

N1 = Number of sown seeds

N2 = Number of seedlings

All data were subjected to statistical analysis using the standard procedures including general linear model (GLM) available in SAS (1996). Duncan's multiple- range test was also used to illustrate significances among different means**.**

Effect of using different bioagents on controlling damping-off disease in beans under field conditions

The efficacy of the mixture of different biocontrol agents in form of powder or suspension, this experiment was conducted.

Paulista bean seeds were sown in natural infested soil, as described before, different antagonistic mixtures were used in two forms as powder at the rate of 10 g/ 1kg or as suspension at the rate of 10 ml/ 1kg of bean seeds as seed coating treatment. Plots only received pathogens without biocontrol treatments were act as control treatment. Data were collected 15 and 30 days after sowing for pre-emergence and post-emergence damping-off. Survived plants were recorded 45 days after sowing. Data were subjected to statistical analysis as mentioned before.

Effect of using different bioagents on some plant characteristics of bean

To confirm that there are no negative effect due to using any of the used antagonists either in powder or suspension forms, yield component, fresh and dry weight (g)/plant, number of pods, yield/plant and dry matter/100g pods were determined in mature plants. Fresh weight was, also, determined using calibrated digital balance. Dry weight was determined by drying plant parts on 70^{o} C for 5 days.

Effect of using different bioagents on some chemical components of bean plants

The effect of different bioagents on the chemical composition of the treated plants was carried out. Paulista mature plants were collected randomly from each treatment. Collected plants were cleaned up and extracted to determine free, conjugated and total phenols according to the method developed by Simons and Ross (1971). Amount of reduced and non reduced sugars were also determined using the method mentioned by Thomas and Dutcher (1924). Protein content in addition to amount of chlorophyll were also assessed using the methods described by Bradford (1976) and Nornai (1982), respectively.

Reaction between *Rhizobium phaseoli* and mixture of the bioagents

Effect of combination between biocontrol mixture as suspension and endo-nitrogen fixer bacterium *Rh. phaseoli* on damping-off disease incidence and on capacity of *Rh. phaseoli* in nitrogen fixation, expressed as percentage of protein content, in the treated plants were studied. The following two experiments were designed and conducted to illustrate the effect of the presence of *Rh. phaseoli* on capacity of different bioagents on controlling damping-off. On the other hand, the effect of different bioagents on capacity and activity of *Rh. phaseoli* expressed as protein content in different plants received different biocontrol treatment. *Rh. phaseoli* was grown on yeast mannitol (YM) medium for 3 days at 25^{o}c. *Rh. phaseoli* was added to the soil at the rate of 1L/ 100 L water as soil drench after one week from sowing time. Bioagent mixtures were added as described before.

Effect of using different bioagents in presence or absence of *Rh. phaseoli* on incidence of damping-off diseases

The different mixtures of the bioagents were used on bean seeds alone or in combination with *Rh. phaseoli* inoculum to test their effect on the incidence of damping-off. Data of pre- and post-emergence damping-off in addition to percentage of the survived plants were determined as described before

to figure out if any negative or positive effect due to the presence of *Rh.phaseoli* on the efficacy of the bioagents.

Effect of using the mixture of biofertilizer bacterium *Rh.Phaseoli* with the different bioagents on some chemical components of bean plants

To illustrate and compare effect of *Rh.phaseoli* on protein content of the treated plants compared with the effect of the same nitrogen fixer bacterium in combination with different bioagent mixtures, this experiment was carried out. Bean seeds Paulista variety, were treated separately using different biocontrol mixtures (B1+B2), (T1+T2) or (B2+T2) at the rate of 10 ml/1kg of bean seeds, at sowing time. *Rh.phaseoli* was added as soil drench at the rate 1:100 ml water after one week from sowing time. Plots only received *Rh. phaseoli*, without biocontrol agents, in addition another group of plots without any treatment, neither biocontrol agents nor *Rh. phaseoli* were used as control treatments. All plots received the same amount of fertilizers and irrigation water.

Effect of using the mixture of biofertilizer bacterium *Rh. phaseoli* with the different bioagents on bean plant characteristics

To compare the effect of *Rh. phaseoli* alone with the effect of *Rh. phaseoli* in combination with different bioagent mixtures on characteristics of the treated plants, this experiment was carried out. Another aim of this experiment was to be sure that using different bioagent mixtures have no negative effect against the beneficial bacterium *Rh. phaseoli*.

Bean seeds were treated with different bioagent mixtures, separately, and then *Rh. phaseoli* was added to soil at the rate of 1L / 100 L water as soil drench after one week from sowing time. Seeds were sown in plots at the rate of 30 seeds per plot. Five plots were used for each treatment. At ripening stages, randomize plants were collected from each treatment to determine fresh weight of root and shoot system. Roots and shoots were then dried using electric oven at 70^{o}C for 5 days and dry weight was determined. Yield and number of pods/plant were also determined in addition to dry matter of 100 g of pods. Data were statistically analyzed and means were compared using Duncan's multiple- range test.

3 Results and Discussion

The aim of this work is to find out a biological method can replace using toxic chemical fungicides to protect bean plants against some soil borne pathogens attack bean at early stages of growth causing damping-off. At the same time to be sure about if there is any side effect of these bioagent treatments on *Rhizobium* as beneficial soil borne biofertilizer.

Data presented in Table (1) indicate that using the mixture of *T.viride*+*T.harzianum* (T1+T2) or mixture of both *B. subtilis* isolates (B1+B2) or the combination between B2+T2, either formulated as powder or suspension led to clear significant reduction in damping-off disease in beans compare with control treatment. In all cases, when bioagents were used, no diseased plants were recorded during post emergence stage compare with 20% post- emergence damping-off in control treatment. Percentage of survived plants was increased in all biocontrol treatments compare with control. Table (1) also shows that the mixture that contained *B. subtilis* isolate2 + *T. harzianum* isolate2 (B2+T2) resulted in the best effect on reducing damping-off , which only 16.7% damping-off was recorded either when this mixture was used as powder or suspension.

When the mixture of both *B. subtilis* isolates was used (B1+B2), significant different was noticed between using the same mixture as powder or suspension, where 20% damping-off was recorded in case of using the suspension, meanwhile, 30% was recorded when the same mixture was used as powder. (Abo-Shosha, 2006).

These data could be explained in the light of facts that *T. harzianum* can grow very fast and produces Gliotoxin and Trichodermin (Abd-El-Moity,1981, Abd El-Moity,1985, Balode, 2010, Abdollahi et al., 2012), whereas *B. subtilis* produces 66 different antibiotics (Ferreira et al.,1991). The combination between the two different bioagents increase spectrum and duration of the establishment of the antagonists in the rhizosphere of the treated plants. This reflected directly as an increase in the percentages of survived plants ,where 83.3% survived plants was recorded when B2+T2 was used compare with 80 and 76.7% when (B1+B2) or (T1+T2) were used as suspension, respectively. The same bioagent mixtures (B1+B2) or (T1+T2) were of less efficacy, being 70.0 and 73.4% survived plants when these bioagent mixtures were used as powder compared with 80.0 and 76.7% in case of using the same mixtures as suspension. This could be justified according to facts that the suspension can spread very quickly in the court of infection and occupied the area around root system preventing the pathogen to come close and negatively affected on the root. Antagonists as powder need some time to elaborate and spread out surround the root. As a result, suspension showed better results.

Table 1: Effect of using different bioagents, formulated as powder or suspension, on controlling damping-off disease in beans under field conditions.

Bioagents*	% Damping-off				Survived plants (%)	
	Pre-emergence		Post-emergence			
	Suspension 10ml/kg seeds	Powder 10 g/kg seeds	Suspension 10ml/kg seeds	Powder 10 g/kg seeds	Suspension 10ml/kg seeds	Powder 10 g/kg seeds
T_1+T_2	23.3^{d}	26.6^{c}	0.0^{b}	0.0^{b}	76.7^{c}	73.4^{d}
B_1+B_2	20.0^{e}	30.0^{b}	0.0^{b}	0.0^{b}	80.0^{0}	70.0^{e}
B_2+T_2	16.7^{f}	16.7^{f}	0.0^{b}	0.0^{b}	83.3^{a}	83.3^{a}
Control	56.7^{a}		20.0^{a}		23.3^{f}	

Means with the same letter are not significantly different.

* T_1+T_2= the mixture of *T.viride+T.harzianum* (T1+T2) , B_1+B_2 = the two *B. subtilis* isolates and B_2+T_2= *B. subtilis* isolate2 + *T. harzianum* isolate2.

High quality and quantities of the yield always the main target for most of research works. To figure out effect of different bioagents on yield components (shoots- roots and pods) either on fresh or dry weight, this work was carried out. Data shown in Table (2) indicate that using any of the antagonist mixture led to significant increase in all the determined parameters included fresh or dry weight of root, shoot and pods, in addition to number of pods / plant compared with control treatment. Data also revealed that the mixture of (B2+T2) showed the highest effect in all measured parameters compared with all the other treatments or control. No or slight significant differences were noticed in percentage of dry matter when effect of different biocontrol mixtures as suspension were compared with effect of the same mixture in the form of powder.

The other two mixtures (T1+T2) and (B1+B2) came in the second rank after (B2+T2), with no significant differences, regarding number of pods / plant, fresh and dry weight of yield. Regarding other parameters, root, shoot weight either fresh or dry indicated that no or slight difference were recorded when these parameters were determined in case of using T1+T2 or B1+B2 either as suspension or powder.
These results can be explained in light of facts, that *T. harzianum*+ *B.subtilis* provide good protection for root system (Yobo *et al.*,2011). *T. harzianum* produces group of plant growth promoting (PGP) compounds, which stimulate and improve root and shoot system and of course increase yield (Harman *et al.*,2004 and Elsayed,2005).

Table 2: Effect of using different bioagents formulated as powder or suspension on some plant characteristics of bean plants.

Bioagents*	Formulas	Fresh Weight (g)/plant		Dry weight(g)/plant		No. of Pods/ plant	Yield(g)/ plant	%Dry matter/100g pods
		shoots	roots	shoots	roots			
T1+T2	suspension	85.5^{c}	4.4^{c}	17.9^{cd}	1.2^{b}	40^{d}	200^{d}	13.9^{b}
	powder	80.3^{d}	4.3^{c}	16.8^{d}	0.9^{c}	39^{d}	195^{d}	13.5^{b}
B1+B2	suspension	85.1^{c}	4.5^{c}	18.7^{c}	1.5^{a}	43^{c}	215^{c}	14.0^{b}
	powder	80.1^{d}	4.3^{c}	17.3^{cd}	1.0^{bc}	40^{d}	200^{d}	13.4^{b}
B2+T2	suspension	95.9^{a}	5.6^{a}	24.6^{a}	1.6^{a}	53^{a}	265^{a}	16.7^{a}
	powder	92.3^{b}	5.1^{b}	21.1^{b}	1.2^{b}	50^{b}	250^{b}	16.0^{a}
Control		35.8^{e}	2.1^{d}	6.8^{e}	0.2^{d}	18^{e}	81^{e}	8.3^{c}

Means with the same letters are not significantly different.

* T_1+T_2= the mixture of *T.viride+T.harzianum* (T1+T2) , B_1+B_2 = the two *B. subtilis* isolates and B_2+T_2= *B. subtilis* isolate2 + *T. harzianum* isolate2.

To figure out any physiological change due to using of the tested bioagent, phenols, sugars, chlorophyll and protein content in treated plants were measured compare with control treatment.

Results presented in Table (3) show that using any of bioagents led to high increase in the amount of free phenols compared with control treatment. This could be explained by the work of Mahmoud *et al.*(1995), Attia et al.(2011) and

Elsayed (2013). They stated that adding spores or mycelia fragments to plant led to stimulate free phenol production in treated plant as a self mechanism against pathogens. Increase percentage of healthy plant appears as a result to increase free phenol in treated plants. Data also show that conjugated phenols were reduced in all treated plants compare with control treatment. Reduced sugar also was increased compare with control. This is due to increase biological activity in treated plants. This increase in activity needs reduced sugar to be used in energy production. These data also in harmony with (Abd-El-Khair et al., 2011).

As a direct result for healthy root system and absorption of adequate amount of nutrients and availability of needed energy, amount of protein and chlorophyll were increased in treated plants compare with control treatment. These results are confirmed by Shoresh and Harman (2008); Akladious and Abbas (2012) and Karlidag et al.(2012).

Results presented in Table (3) also revealed that the highest values of free phenols, reduced sugars, chlorophyll and protein were recorded when (B2+T2) was used in form of suspension. This confirm previous results were obtained in the same work regarding high capacity of this mixture (B2+T2) as suspension in control root rot diseases and protect bean roots against pathogens under test. Data in the same Table also show that the effects of other two mixtures (T1+T2) or (B1+B2) were very close to each others with slight differences in the measured parameters.

Table 3: Effect of using different bioagents formulated as powder or suspension on some chemical components in treated plants.

Treatments*		Phenols (Mg/g)			Sugars(Mg/g)		Chlorophyll (Mg/g)	Protein (Mg/g)
		Free	Conj	Total	Reducing	Non-red.		
T1+T2	S**	6.5	1.9	8.4	4.1	0.9	16.1	7.0
	P***	6.2	1.6	7.8	3.8	0.9	16.0	6.9
B1+B2	S	6.5	2.1	8.6	4.02	0.4	15.9	7.1
	P	6.1	1.4	7.5	3.5	1.1	15.3	6.8
B2+T2	S	9.9	5.0	14.9	4.7	1.4	17.0	7.9
	P	8.2	3.4	11.6	4.5	1.6	17.0	7.3
Control		4.8	7.5	12.3	0.9	1.3	9.0	2.1

* T_1+T_2= the mixture of *T.viride*+*T.harzianum* (T1+T2) , B_1+B_2 = the two *B. subtilis* isolates and B_2+T_2= *B. subtilis* isolate2 + *T. harzianum* isolate2 , **suspension and *** powder.

Effect of combination between the tested bioagents and *Rh.phaseoli* on incidence of damping-off. *Rh.phaseoli* is a beneficial bacterium for bean plants. The present experiment was conducted to study effect of the interaction between the tested bioagents and *Rh.phaseoli* on the incidence of damping-off.

Data in Table (4) indicate that all the tested bioagent mixtures either at the presence or absence of *Rh.phaseoli* led to significant reduction in incidence of damping-off. *Rh.phaseoli* alone shows very slight effect in disease control and 43.3% pre-emergence damping-off was recorded compare with 56.7 % in control treatment. Using different bioagents in presence of *Rh.phaseoli* led to increase efficacy of the biocontrol treatment. When these bioagents were used in combination with *Rh.phaseoli*, the percentages of damping-off were reduced to be 13.3, 16.7 for Rh+B1+B2 and Rh+T1+T2, respectively, compared with 43.3% in case of using *Rh.phaseoli* alone. The highest reduction in damping-off was recorded to be 6.7% when mixture of Rh+B2+T2 was used. This may be due to the presence of *Rh.phaseoli* increased nitrogen uptake by the treated plants and in the rhizosphere. This increase act as stimulator to other antagonistic microorganisms to grow and spread out within court of infection preventing from any other pathogens to invade plant tissue.

Table 4: Effect of using different bioagents as suspension in presence or absence of *Rh.phaseoli* on incidence of bean damping-off diseases.

Treatments*	% Damping-off		
	Pre-emergence	Post-emergence	Survived plants (%)
Rh+T1+T2	16.7^{d}	0.0^{b}	83.3^{c}

Rh+B1+B2	13.3[d]	0.0[b]	86.7[b]
Rh+B2+T2	6.7[e]	0.0[b]	93.3[a]
Rh.phaseoli	43.3[b]	0.0[b]	56.7[d]
Control	56.7[a]	20.0[a]	23.3[e]

Means with the same letters are not significantly different.

* Rh+T_1+T_2= the mixture of ***Rh.phaseoli*** *+T.viride+T.harzianum* , Rh+B_1+B_2 = ***Rh.phaseoli*** + the two *B. subtilis* isolates and Rh+ B_2+T_2= ***Rh.phaseoli*** *+B. subtilis* isolate2 + *T. harzianum* isolate2.

To be sure that there is no antagonistic effect from different used biocontrol agents against *Rh.phaseoli* as beneficial bacterium, this experiment was carried out. Data obtained were tabulated in table (5). Data in table (5) show that capacity of *Rh.phaseoli* on protein production (nitrogen fixation) was increased at presence of any used antagonistic mixtures. Plants received *Rh.phaseoli*+B2+T2 showed the highest amount of protein (8.51 mg/g) and chlorophyll (18.3 mg/g) compare with plants received *Rh.phaseoli* alone (3.4, 12.1 mg/g) or the other two antagonistic mixtures. The other two mixtures showed very close results to each others in their effect regarding all measured parameters. As noticed before amount of free phenols, reduced sugars were also positively correlated with protein and chlorophyll contents. This means that plants with high amount of protein and chlorophyll show high amount in phenols and reduced sugars and vise versa. These data can be explained in the light of facts that *Rh.phaseoli* penetrates root system and works inside plant, far away from any outside root effect where different antagonists are working (Elkoca et al., 2010). Also good healthy root system means increase root exudates which stimulate and increase number of *Rh. phaseoli* consequently increase number of nodules and percentage of protein.

Table 5: Effect of using the mixture of biofertilizer, bacterium *Rh. phaseoli*, and different bioagents on some chemical components of bean plants.

Bioagents*	Phenols (Mg/g)			Sugars(Mg/g)		Chlorophyll (Mg/g)	Protein (Mg/g)
	Free	Conj	Total	Reducing	Non-red.		
Rh+T1+T2	11.0	2.3	13.3	5.3	0.8	17.0	7.6
Rh+B1+B2	11.7	2.0	13.7	5.4	0.7	17.4	7.8
Rh+B2+T2	13.9	1.8	15.7	6.1	0.7	18.3	8.5
Rh.phaseoli	9.5	2.5	12.0	2.8	0.5	12.1	3.4
Control	4.8	7.5	12.3	0.9	1.3	9.0	2.1

* Rh+T_1+T_2= the mixture of ***Rh.phaseoli*** *+T.viride+T.harzianum* , Rh+B_1+B_2 = ***Rh.phaseoli*** + the two *B. subtilis* isolates and Rh+ B_2+T_2= ***Rh.phaseoli*** *+B. subtilis* isolate2 + *T. harzianum* isolate2.

Different bioagents were used in presence of *Rh.phaseoli* to be sure that no negative effect can be occurred due to biological treatment. To confirm this effect, plant weights either on fresh or dry basis were determined in addition to yield component (No. of Pods/plant, yield(g)/plant, and % dry matter/100 g pods).

Data in Table (6) show that use any of biocontrol preparation led to improve in capacity of *Rh. phaseoli*. This improvement was reflected on plant parameters either fresh or dry weights of treated plants in addition to yield component. *Rh.phaseoli*+(B2+T2) was the best combination. The highest fresh weight of shoot and root were reached 119.5 g and 7.7 g /plant compare with only 35.8g and 3.5 g/plant for shoots and roots in control treatment. When *Rh. phaseoli* was used alone without any biocontrol treatment only 78.5 and 4.2 g/plant were recorded for fresh shoots and roots, respectively. The same results were obtained when shoots and roots were determined on dry basis and compare with control or only *Rh. phaseoli* treatments. As logic, healthy good shoot and root systems reflects in increase the number of pods/plant, yield (g)/plant and % dry matter/100 g pods were increased.

In *Rh.phaseoli*+B2+T2 mean numbers of pods/plant reach 78 pods/plant compare with only 18 in control and 40 in only *Rh.phaseoli* treatment. Yield and percentage dry matter also increased compare with any other treatment. This work confirms results obtained by Estevez de Jensen et al.(2002); Abd-El-Moneim et al.(2006) and Parikka et al.(2009).

Results can be explained as follow; using effective bioagents protect root system and allow these roots to grow and establish and absorb high amount of NPK converted in plants to green leaves and increase number of pods and yield. Other two mixtures show different effects.

Rh.phaseoli+B1+B2 show slight better effect compare with *Rh.phaseoli*+T1+T2 and number of pods reach 64/plant compare with 60/plant in *Rh.phaseoli*+T1+T2 where yield in *Rh.phaseoli*+B1+B2 reach 320.3 g/plant compare with 300 g/plant in

Rh.phaseoli+T1+T2. Also percentage of dry matter was increased from 16.9 in case *Rh.phaseoli*+T1+T2 to be 17.6 in *Rh.phaseoli*+B1+B2.

Table 6: Effect of the mixture of the biofertilizer bacterium *Rh. phaseoli* and different bioagents on bean plant characteristics.

Bioagents*	No. of nodules/ plant	Fresh weight(g)/plant		Dry weight(g)/plant		No.of pods/plant	Yield(g)/ plant	%Dry matter/100g pods
		shoots	root	shoots	root			
Rh+T1+T2	25^{c}	91.6^{c}	6.5^{b}	19.9^{c}	2.5^{b}	60^{b}	300.0^{c}	16.9^{b}
Rh+B1+B2	30^{b}	97.1^{b}	5.8^{c}	23.5^{b}	2.9^{a}	64^{b}	320.3^{b}	17.6^{b}
Rh+B2+T2	38^{a}	119.5^{a}	7.7^{a}	26.6^{a}	3.0^{a}	78^{a}	395.0^{a}	19.3^{a}
Rh.phaseoli	18^{d}	78.5^{d}	4.2^{d}	15.0^{d}	1.3^{c}	40^{c}	195.7^{d}	11.4^{c}
Control	0^{e}	35.8^{e}	3.5^{e}	6.8^{e}	0.2^{d}	18^{d}	81.8^{e}	8.3^{d}

Means with the same letters are not significantly different.

* Rh+T_1+T_2= the mixture of ***Rh.phaseoli*** *+T.viride+T.harzianum* , Rh+B_1+B_2 = ***Rh.phaseoli*** + the two *B. subtilis* isolates and Rh+ B_2+T_2= ***Rh.phaseoli*** *+B. subtilis* isolate2 + *T. harzianum* isolate2.

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