Effects of A-Mycorrhizal Fungi, Bacteria, And Yeast as A Biological Control of *Sclerotinia sclerotiorum*, on The Growth of Common Bean (*Phaseolus vulgaris* L.)

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ABSTRACT

A pot experiment was carried out in a greenhouse at the Faculty of Agriculture (Saba-Basha)-Alexandria University in order to evaluate the effect of two species of mycorrhizae (*Glomus intraradiaces* (M1) and *Glomus macrocarpium* (M2)), two genera of bacteria (*Bacillus subtilis* (B1) and *Pseudomonas fluorescens* (B2)) and two genera of yeast (*Cryptocooccus neoformans* (Y1) and *Candida albicans* (Y2)) as a biological control of white mold of bean disease (*Phaseolus vulgaris* L. with two varieties Bronco and Contender) which infected by *Sclerotinia sclerotiorum*. Two isolates of *Sclerotinia sclerotiorum* (S4 and S6) were selected. The results of this study suggested that the difference microorganisms which used as bioagents especially AMF first specie (*Glomus intraradiaces*) was sufficient to inhibit the *Sclerotinia* isolates. On the other hand, Contender common bean variety was more sufficient than the other variety (Bronco). Also, the first isolate of *Sclerotinia* (S4) was less dangerous compared with the other isolate (S6). In future studies, the interaction between the bioagents and the host plant are needed to develop much more efficient biological control agents of the related diseases.

ADDITIONAL INDEX WORDS: common bean varieties, biological control, *Sclerotinia sclerotiorum*

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is considered one of the most important leguminous crops cultivated not only in Egypt but also in many other countries all over the world for the human consumption. It is considered an economical crop due to its high protein content, balanced amino acid composition and good digestibility.

Bean plants are commonly exposed to attack by many serious damping-off pathogens, i.e. *Py.hium debaryanum*, *Pseudomonas ultimum*, *Pseudomonas dissotocum*, *Pseudomonas oligandrum*, *Pseudomonas violae*, *Rhizoctonia solani*, *Fusarium solani*, *F. semitectum*, *Aphanomyces euteiches*, *Sclerotinia sclerotiorum* and many species of *Verticillium* and *Cladosporium* (King and Parke, 1993 and Chen and McBeath, 1993). Most of them cause damping-off and root rot diseases (Abada *et al.*,1992), leading to great economic losses in crop yield and quality.

Sclerotinia sclerotiorum (Lib.) de Bary is an important fungal pathogen of many plant hosts and is responsible for substantial losses in crop production in Egypt and worldwide. Symptoms include stem, leaf, root and fruit rot or cancer. Pesticide application is currently the primary way to control crop disease, but it has raised an array of environmental problems. Achieving sustainable agriculture will require avoiding a heavy reliance on pesticides. Also, the high cost of fungicides and the difficulties in the obtaining resistant cultivars make biological control a more interesting alternative for the suppression of this fungus (Baker and Paultiz, 1996).

Biological control is one of several strategies used to control pests to avoid economic damage on crop plants. Biological control has been defined a number of times. A recent definition by Eilenberg *et al.* (2001) is: "the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be"

Arbuscular mycorrhizal fungi (AMF) can act as a biological control agent due to its effect on reducing damage to plants caused by soil-borne pathogens (Murphy *et al.* 2000). Decreased pathogen development in mycorrhizal and non-mycorrhizal parts of inoculated roots is associated with accumulation of phenolics and plant cell defense response. The protective effects induced by AM fungi against a phytoplasma are reported in tomato (Lingua *et al.*,2002). Inoculation of onion with *Glomus* sp. Zac-19 delayed onion white rot epidemics caused by *Sclerotium cepivorum* Berk by two weeks and increased the yield by 22% under field conditions (Torres-Barragan *et al.*,1996).

Punja (1997) Showed that the most extensively studied bacterial organisms, including *Pseudomonas spp.* *Bacillus subtilis*, and *Enterobacter cloacae*, have been reported to reduce many seedling diseases and root rots on several vegetable crop species. The bacteria appear to

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Received October 19, 2010, Accepted December 21, 2010.

protect plants against a wide range of pathogens. That could be due to enhance plant growth and reduce disease by utilizing a number of different mechanisms: a) Production of antibiotics, toxins and lytic enzymes. B) Direct parasitism of hyphae or propagates of the pathogen. C) Competitive exclusion by occupation of infection sites and/or depletion of nutrients. D) Plant growth enhancement and induction of host resistance mechanisms. E) Reduction of aggressiveness or virulence through mycovirus transmission.

Arbuscular mychorrhizal (AM) fungi and bacteria can interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition and inhibition of fungal plant pathogens (Artursson *et al.*,2006). For example, Citernesi *et al.* (1996) studied bacteria isolated from the mycorrhizosphere of *Glomus mosseae* kept in pot cultures for 17 years, and found that several of those were actively antagonistic against *Fusarium* and *Phytophtora* growing *in vitro*. On the other hand, Filion *et al.*,(1999) tested the crude extract obtained from the growth medium of the AM fungus *Glomus intraradices*, on the growth of two bacteria and on the sporulation of two pathogenic fungi. Their results indicated that growth of *Pseudomonas chlororaphis* (a biocontrol agent) and conidial germination of *Trichoderma harzianum* (a mycoparasite) were stimulated in the presence of the AM fungal extract, whereas growth of *Clavibacter michiganensis* (a plant pathogen) was not affected and germination of *Fusarium oxysporum* (a plant pathogen) was reduced. Also, Aysan and Demir (2009) reported that AMF and *Rhizobium* as the most important symbionts of rhizosphere have shown stimulating (Edwards *et al.*,1998) or inhibiting effects on pathogens (Van der Heijden *et al.*, 2006). This could be due to impact on the composition of bacterial communities. This impact may be relayed through the plant root because has been shown to change the chemical composition of root exudates and these are often a source of nutrients to associated bacteria in the mycorrhizosphere (Linderman, 2000 and Artursson, *et al.* 2006). However, changes in composition and activity of bacteria communities by AM fungi have also been ascribed to more direct interactions, including competition for inorganic nutrients (Christensen and Jakobsen, 1993).

Classical biological control of fungal plant diseases by yeasts and yeast-like organisms has been amply described in the literature, yet few commercial products have arisen out of those research efforts. Yeasts are one of the important sources of Vitamin B12which may not be required directly for plant growth, yeasts in the root zone may influence plant growth indirectly by encouraging the growth of other plant growth promoting rhizomicroorganisms (PGPRs) (Boby, *et al.*,2006). Most yeast identified to date has been categorized as exerting their activity through the manifestation of one or more of four modes of action: competition, parasitism, antibiosis and induced resistance (Bélanger and Avis, 2002).

All the yeasts had a synergistic interaction with the mycorrhizal fungus and dual inoculation improved plant growth compared to single inoculation with *Glomus mosseae* alone. Nitrogen and phosphorus uptake of plants was also enhanced significantly in *Glomus mosseae* and soil yeasts combinations. Growth, N, P, chlorophyll content, phenolic content in leaves and yield of cowpea were highest in plants treated with *Glomus mosseae* + R. *mucilaginosa*. Mycorrhizal root colonization, spore number and population of yeasts in the root zone soil were also highest in the treatment *Glomus mosseae*+ R. *mucilaginosa* and least in the uninoculated plants (Boby, *et al.*,2006). Also, Fracchia *et al.*, (2003) reported enhanced AM colonization of soybean and red clover when the yeast R. *mucilaginosa* was applied to the soil.

MATERIALS AND METHODS

A pot experiment was carried out in the greenhouse at the Faculty of Agriculture (Saba-Basha)-Alexandria University, in order to evaluate the effect of two species of mycorrhiza (*Glomus intraradices* and *Glomus macrocarpium*), two genera of bacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*) and two genera of yeast (*Candida albicans* and *Cryptococcus neoformans*) as a biological control of white mold disease of two bean varieties (*Phaseolus vulgaris* L.) which infected by *Sclerotinia sclerotiorum*.

1- Isolation and inoculum preparation

1.1. Pathogen isolates preparation

Two isolates of *Sclerotinia sclerotiorum* (S4 and S6) were selected according to their highly virulence. Inocula were prepared by growing each of the tested isolates in 250 ml conical flask, each containing 50 ml of PDA (potato dextrose agar) medium and incubated at 18-25° C for 15 days until sclerotia formed.

1.2. Bacterial isolates

The two bacterial isolates (*Bacillus subtilis* and *Pseudomonas fluorescens*) were obtained from Laboratory of Phytopatholgy, Agricultural Botany, Faculty of Agriculture ,Saba Basha, University of Alexandria, Egypt.

1.3. Yeasts isolates

The tested yeasts (*Candida albicans* and *Cryptococcus neoformans*) used in this study were obtained from Department of Microbiology, Faculty of Sciences, University of Tanta, Egypt.

1.4. Arbuscular Mycorrhiza Fungi (AMF)

Two mycorrhizal species (*Glomus intraradices* and *Glomus macrocarpium*) were used in this experiment were obtained from German Universities, Hanover and Göttingen and activated in the Soil Microbiology Lab - Soil and Agriculture Chemistry Dep, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt. The first specie of mycorrhizal fungi (*Glomus intraradiaces*) was obtained from Hanover University, Germany; and the second specie of mycorrhizal fungi (*Glomus macrocarpium*) was obtained from Göttingen University, Germany.

2. Soil preparation

The used soil was prepared by mixing sand and peat with a ratio 3:1. The soil was sieved through a 2 mm sieve to homogenize and separated roots from soil. The soil was autoclaved at 1.5 Kg/cm^2 for 90 minutes and, then left to aerate for 7 days. The main soil physical and chemical properties are shown in Table (1):

Basal applications of N,P, k and Mg fertilizers were corporate with each Kg soil at a rate of 150 mg N as NH_4NO_3, 100 mg P as KH_2PO_4, 150 mg K as K_2SO_4 and 40 mg Mg as $MgSO_4$ per Kg soil.

3. Experimental

Autoclaved soil samples each weighing 500 g were separately filled into plastic pots (Ø= 11 cm) and compacted to the bulk density of about 1.4 g cm^{-3}. The weight of 500 g soil in each pot corresponded to a volume of 350 cm^3.The pots filled with soil were placed in a greenhouse. One week before adding *Sclerotinia sclerotiorum*, the soil in each pot was watered to the volumetric moisture content 0.20 cm^3cm^{-3}.

Two isolates of *Sclerotinia sclerotiorum* (S4 and S6) were added to each two hyphal growth with sclerotia obtained from two flasks to the pots on 7/4/2009. Two genera of bacteria and yeasts were added to the infested soil 10 days later at the rate of two flasks (250 ml) of inoculum for each bioagents per pot. The pots were covered with paper sheet and left for 3 days before planting common bean seeds and used mycorrhizae. The soil was mixed with 20 ml mycorrhizae one week before planting as described by Malibari *et al.*,(1990). Also, 10 ml inoculums were added with the seeds at sowing time, (in total, the rate of 500 spores per pot).

Five seeds from each common bean variety (*Phaseolus vulgaris*) were sown in each hill on 21/4/2009. The nitrogen fertilizer was added at two equal closes (2 and 20 days after sowing) at the rate of 75 mg N/20 ml water for each pot. The treatments were replicated five times in a randomized complete block design. The bean plants were harvested 37 days after sowing. At harvest, shoots were separated from roots. The shoots were washed with tap water , distillated water , air dried , and oven dried at 70°C for 48 hours (Steyn, 1959) to constant weight and the shoot dry weights were recorded , then grounded in a mill and stored for analysis. Furthermore, root length was measured (Tennant, 1975).

Available phosphorus was extracted with 0.5 N $NaHCO_3$ procedures according to Olsen *et al.*,(1954). The phosphorus in $NaHCO_3$-extract was colorimetrically determined using ascorbic acid-molybdenum blue method at wave length of 406 mm as described by Murphy and Riley (1962).

Powder of plant material was wet-digested with H_2SO_4-H_2O_2 (Lowther, 1980) and the following determinations were carried out in the digested solutions:-

- Total N was determined colorimetrically by Nessler method (Chapman and Pratt, 1978).
- Total P was determined using vanadomolybdophosphoric method (Jackson, 1967).
- Total micro nutrients (Fe, Mn, Cu and Zn) were determined using the atomic absorption spectrophotometer (Jackson, 1967).

- Root length

Root length was estimated by the line intersect method of Tennant (1975) as follows:

$$RL=11/14 \times N \times G$$

Where: RL = root length, N= sum of horizontal and vertical crossing, G= length of the grid unit (2 cm or 1 cm)

The sample root length (0.1 g) was converted to total root length per pot based on total fresh mass of the root in the pot.

Table 1. The main physical and chemical properties of the used soil

Sand%	Silt %	Clay %	Textural class	$CaCO_3$ %	pH (1:1)	EC (1:1) (ds/m)	Av. P(1) mg/kg soil	OM %
83.70	4.30	12.0	sandy loam	5.6	7.5	1.55	7.10	1.88

(1) Av. P = available P in soil

Root surface area

Surface area of a 1 cm root cylinder (SAC) was calculated as follows:

$$SAC = 2\pi \times r_0$$

Where: SAC= surface area of the root cylinder , r_0 = root radius

Mean half distance between roots

Mean half distance between neighboring roots (r1) was calculated according to the following formula (Schenk and Barber, 1979).

$$r_1 = \sqrt{\frac{V}{\pi \ RL}}$$

Where: V= volume of the soil in the pot (cm^3). , RL= root length per pot.

Statistical analysis

The collected data were arranged in a randomized complete block design and replicated five times. Data were statistically analyzed for ANOVA and means compares to fulfill the significance according to Steel and Torrie (1982). A significance level of $\alpha = 0.05$ was used in all analysis.

RESULTS AND DISCUSSION

1- Infection percentage

Figure (1) showed that the two tested isolates (S4 and S6) significantly increased the disease incidence comparing with non infected soil. The difference between both tested isolates was not significantly (33.39 and 33.75 total infection percentages for S4 and S6, respectively). The isolate S6 was more virulent one.

2. Growth Attributes

2.1. Shoot growth

2.1.1. Main effect of biological control agents

Table (2) indicated that there was a highly significant increase in the plant growth (shoot fresh weight (g) and shoot dry weight (g)) as affected by different biological control agents compared with the control. The increases in shoot dry weight (g) which inoculated with different bioagents species of AMF (*Glomus intraradiaces* and *Glomus macrocarpium*) , two genera of bacteria (*Bacillus subtilis* and of *Pseudomonas fluorescens*); and two genera of yeasts (*Cryptocococcus neoformans* and *Candida albicans*) were about 107%, 87%,73%, 60%, 27% and 47% respectively compared with the other plants without inoculation control. Similarly, Punja (1997) reported that the most biological control agents as bacterial, fungi and yeasts reduced many seedling diseases and root rots on several vegetable crop species. The bioagents have been postulated to enhance plant growth and reduce disease by utilizing a number of different mechanisms. These include the production of antibiotics and toxins that reduce pathogen growth and infection potential (Handelsman and Stabb, 1996), competition for infection sites (Zhou and Paulityz, 1993), or nutrients (Elad and Chet, 1987) required by the pathogens to penetrate the host, stimulation of plant growth and vigor (Rankin and Paulitz, 1994) and induction of resistance mechanisms in the plant (Wei *et al* ., 1996) that prevent or slow down pathogen ingress (Punja, 1997). In the same line, Aysan and Demir (2009), reported that, all biological control agents increased plant growth in treated plants compared with the control. This result was in agreement with Trillas *et al*., (2006); Amer and Abou-El-Seoud, (2008).

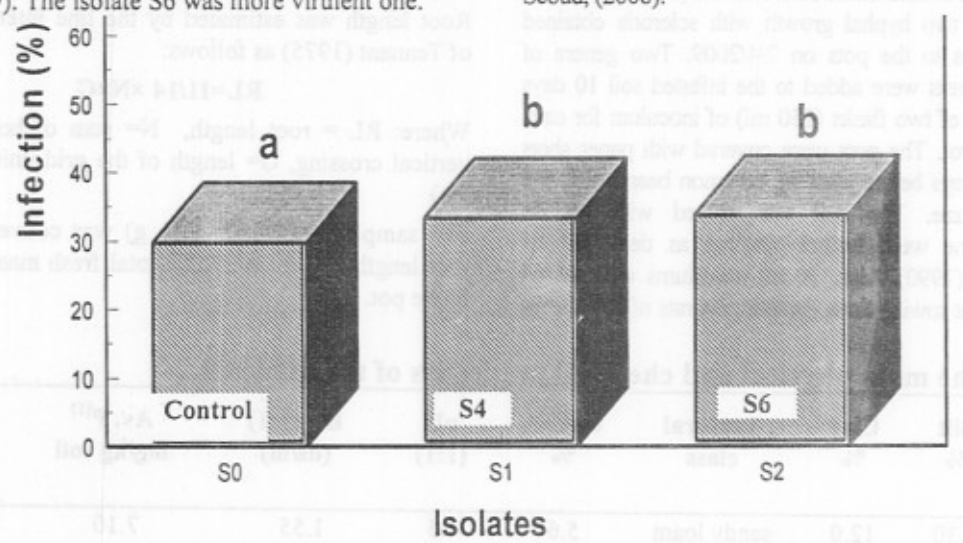

Figure 1. Effect of two isolates of *Sclerotinia sclerotiorum* on incidence of damping-off on bean seedlings (global of all means)

Table 2. Main effect of *Sclerotinia* isolates, common bean varieties and bioagents on plant growth (g/pot)

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)
Biological control agents (Bio)		
Control	1.3 e	0.15 e
B1	2.1 bc	0.26 bc
B2	1.8 cd	0.24 cd
Y1	1.64 de	0.19 de
Y2	1.75 cd	0.22 cd
M1	2.91 a	0.31 a
M2	2.43 b	0.28 b
Common bean varieties (V)		
Bronco	1.24 b	0.17 b
Contender	2.43 a	0.30 a
***Sclerotinia sclerotiorum* isolates (S)**		
S0	2.25 a	0.31 a
Isolate 1 (S4)	1.89 b	0.23 b
Isolate 2 (S6)	1.84 c	0.17 c

On the other hand, the shoot fresh and dry weights of common bean plants inoculated with AMF species were increased significantly compared with the other bioagents. This could be reduced by root colonization of AMF via several mechanisms including increasing the mineral absorption (Davies and Linderman, 1991; Smith and Read, 1997), phenolic compounds (Devi and Reddy, 2002) and pathogenesis-related proteins (Dassi *et al.*, 1998). Also, Aysan and Demir (2009) found that inoculation with AMF significantly increased shoot fresh and dry weights, in comparison to the other treatments. In the same line, Ozgonen *et al.*,(2010) showed that AMF could effectively be used against stem rot caused by *Sclerotinia rolfsii* Sacc. Also, they showed that AMF inoculation increased the yield and gave best results for control of *Sclerotinia rolfsii.* On the other hand, the AMF *Glomus intraradiaces* was significantly higher than the other AMF *Glomus macrocarpium*. Similarly, Ozgonen *et al.*,(2010) reported that *Glomus caledonium* showed the highest effect on shoot dry weight by 84% compared with the other AMF species.

2.1.2. Main effect of common bean varieties

Table (2) revealed that, the shoot fresh and dry weights of the second variety of common bean plants (Contender) increased by about 12% and 77% respectively, in comparison to the first variety of common bean (Bronco). The difference between the two common bean varieties could be due to different mechanisms such as root growth (root branches, root length, and root radius) and root exudates which lead to increase the nutrient availability and increase the nutrient depletion zone. These mechanisms developed the plant growth. Similarly, many studied reported that, the response to beneficial microorganisms such as AMF and bacteria can vary within a plant species and cultivars (Gryndler *et al.*,2002; Abou-El-Seoud, 2005). In the same line, Abou-El-Seoud and Wafaa (2010) reported that, the shoot dry matter was significantly difference between the maize genotypes under the same P level.

2.1.3. Main effect of *Sclerotinia sclerotiorum* isolates:-

Table (2) clearly showed that shoot fresh and dry weights of common bean plants decreased significantly with infecting by the two *Sclerotinia* isolates, compared with the other plants without infection. The shoot dry weight of common bean plants infected by first and second isolates of *sclerotinia* (S4 and S6) reduced by about 35% and 82% respectively in comparison to the other common bean plants without infection. Similarly, Aysan and Demir (2009) showed that, pathogenic infection by *Sclerotinia sclerotiorum* reduced plant growth of the common bean. On the other hands, there was significant difference between the two *Sclerotinia* isolates in shoot fresh and dry weights of common bean plants. On other words, the plants infected by the first isolate of *sclerotinia* (S4) was significantly higher than the other *sclerotinia* isolate (S6) in both shoot fresh weight and shoot dry weight of common bean plants.

2.2. Root growth

2.2.1. Main effect of biological control agents:-

Table (3) clearly showed that, a highly significant increase in root fresh weight, root dry weight, root length, and root surface area of common bean plants infected by bioagents in comparison to the other plants without infection (control). The increase in root fresh and dry weights which infected by bioagents (*Glomus intraradiaces* and *Glomus macrocarpium*); *Bacillus subtilis*; *Pseudomonas fluorescens*,

Table 3. Main effects of *Sclerotinia* isolates, common bean varieties and bioagents on root growth

Treatments	Root fresh weight (g)	Root dry weight (g)	Root length (RL) (cm)	Root surface area (SA) (cm^2)	Mean half distance between roots (mm)
Biological control agents (Bio)					
Control	0.16 e	0.04 d	40.28 e	10.85 e	1.83 a
B1	0.80 c	0.15 c	87.67 c	28.62 c	1.2 c
B2	0.70 c	0.12 c	81.72 c	26.63 c	1.22 c
Y1	0.28 de	0.05 d	62.67 d	14.37 d	1.4 b
Y2	0.38 d	0.07 d	68.78 d	16.38 d	1.35 b
M1	1.43 a	0.29 a	136.72 a	50.28 a	0.97 e
M2	1.23 b	0.22 b	117.39 b	44.59 b	1.08 d
Common bean varieties (V)					
Bronco	0.63 b	0.11 b	81.19 b	25.93 b	1.32 a
Contender	0.20 a	0.16 a	88.87 a	22.86 a	1.27 b
***Sclerotinia sclerotiorum* isolates (S)**					
S0	0.85 a	0.16 a	100.86 a	30.14 a	1.16 b
Isolate 1 (S4)	0.72 b	01.3 b	78.21 b	27.44 b	1.35 a
Isolate 2 (S6)	0.57 c	0.11 c	76.03 b	24.60 c	1.36 a

and *Cryptocooccus neoformans* and *Candida albicans* were about (8-fold and 6.25-fold); (6.7-fold and 4.5-fold); (4-fold and 2.75-fold); (3.4-fold and 2-fold); (75% and 25%); and (1.4-fold and 75%) respectively compared with the plants without bioagents infection (control). Also, the increase in root length and root surface area of plant with bioagents (M1, M2, B1, B2, Y1 and Y2) were about (2.4-fold and 3.6-fold); (1.9-fold and 3.1-fold); (1.2-fold and 1.6-fold); (1.1-fold and 1.5-fold); (56% and 32%); and (71% and 51%) respectively compared with the common bean plants without bioagents. In the same line, Artursson *et al.*,(2006) reported that, the treatments inoculated with AMF and bacteria significantly increased root growth, compared with their controls which were not inoculated. Similarly, Amer and Abou-El-Seoud (2008) found that the root length of tomato plants with bioagents had increased significantly compared with their control. In contrast, the mean half distance between roots (rl, mm) of common bean plants with bioagents decreased significantly compared with the other plants without bioagents. Abou-El-Seoud (2005) reported that when r_l is larger than the depletion zone around roots, part of this nutrient would be unavailable. On the other words, when the root length density increased, the r_l decreased. This result leads to increase the nutrient depletion zone of the soil, and then, the plant with less r_l, it will get more yields compared with the other plant with high r_l value. The amount of nutrients taken up from soil depends on the size of the root system and its distribution in the soil profile. The amount of nutrients in each root segment will depend on the soil volume it can exploited which is measured by the average distance between the root segment and any neighboring root segment (r_l) and the morphological and physiological properties of the root (Abou-El-Seoud, 2005).

From table (3), it could be seen that, the root fresh weight; root dry weight; root length and root surface area of common bean plants inoculated with AMF species increased significantly compared with the other plants and with other bioagents. These results are in the same line with Abou-El-Seoud (2005 and 2008). Similarly, Giri *et al.*, (2005) found that root dry weight and root length of *Cassia siamea* were higher in mycorrhiza than non-mycorrhizal plants. Also, Neumann and George (2005) reported that the root length of tomato was increased with inoculations with mycorrhizal fungi. In contrast, Bonanomi *et al.*, (2001) reported that no significant difference in total root length or number and length of lateral roots was noticed with and without mycorrhizal fungi inoculation. In contrast, the mean half distance between roots of A-mycorrhizal species plants decreased significantly in comparison to the other plants with other bioagents. On the other hand, there was significant difference between the two AMF species in root fresh and dry weights, root length, root surface area and mean half distance between roots. Similarly, Drew *et al.*, (2003) observed that plants grown with *Glomus intraradiaces* contained more root growth than those grown with *Glomus mosseae.*

2.2.2. Main effect of common bean varieties:-

From table (3) can notice that, the root fresh weight, root dry weight; RL and SA of second variety (Contender) increased by about 27%, 46%, 89% and 11% respectively compared with Bronco. In contrast, r_1 of Contender was decreased by about 4% compared with Bronco. Nutrient uptake efficiency of a plant depends on morphological root characteristics such as root length, root surface area, root architecture, and cluster roots (Jungk, 2001; and Alves *et al.*,2001). The ability of plant varieties to adapt their morphological root characteristics to variable nutrient availability is genetically demonstrated (Fransen *et al.*,1999). Similarly, Abou-El-Seoud and Wafaa (2010) reported that, significant difference in root length among the maize genotypes was observed. Plants having larger root systems are able to absorb higher amounts of P from the soil and achieve greater fields than those having smaller root system (Abou-El-Seoud, 2008).

2.2.3. Main effect of *Sclerotinia* isolates:-

Table (3) showed that, there were significant difference in the root growth parameters (root fresh and dry weights, RL, SA and r_1) between common bean plants without *Sclerotinia* infection and the two *sclerotinia* isolates. The plants without *Sclerotinia* infection was increased significantly at all root growth parameters except mean half distance between roots (r_1) compared with the other plants infected with the two *sclerotinia* isolates. These results are in the same line with Amer and Abou-El-Seoud (2008) who reported that the root length of tomato plants infected with *Rhizoctonia solani* was shorter than the other plants without infection. *Sclerotium rolfsii* Sacc. disease causes damage on root system of plant. In contrast, Aysan and Demir (2009) found that, dry and fresh weights of roots were significantly lower in control plants than these infected with *Sclerotinia sclerotiorum*. On the other hand, the first isolate (S4) increased significantly in root fresh and dry weights and SA compared with the second *Sclerotinia* isolate (S6). In contrast, there was insignificant difference in root length (RL) and mean half distance between roots (r_1) between the two *Sclerotinia* isolates.

3. Elemental composition:

3.1. Main effect of biological control agents:-

From table (4) can notice that, P concentration, P uptake, N concentration, N uptake of common bean plants with bioagents were increased significantly compared with the control plant. Except, the N uptake of plants treated with both yeast genera were insignificant difference compared with control. The P concentration and uptake of *Glomus intraradices* and *Glomus macrocarpium*; *Bacillus subtilis* and*Pseudomonas fluorescens*, *Cryptococcus neoformans* and *Candida albicans* were increased by about (1.3-fold and 2-fold); (1.25-fold and 1.6-fold); (1.1-fold and 1.2-fold); (100% and 97%); (44% and 18%), and (80% and 62%) respectively in comparison to the control (without bioagents). The N concentration and uptake of first and second species of AMF;*Bacillus subtilis* and *Pseudomonas fluorescens*, and *Cryptococcus neoformans* and *Candida albicans* were increased by about (2-fold and 5.2-fold); (1.9-fold and 4.2-fold); (1.24-fold and 2.7-fold); (93% and 1.9-fold); (9% and 35%), and (27% and 78%), respectively compared with the control. In the same line, Artursson *et al.* (2006) demonstrated that, the treatments inoculated with AMF or bacteria alone significantly increased the nitrogen (N) and phosphorus (P) accumulation in plant tissues, compared with their control. Also, Aysan and Demir (2009) reported that all biological control agents increased total contents of P and N in treated plants compared to the control.

The macronutrient contents (N and P) of common bean plants inoculated with both AMF species increased significantly compared with other bioagents and untreated plants (control). This could be due to that AMF increase the surface area of plant roots by increasing branches of first order lateral roots (Aguin *et al.* 2004), which led to increase acquisition of nutrients by increasing the extension of depletion zone around the root. The mycorrhizal fungi extend a network of hyphal several centimeters out into the surrounding soil, thereby expanding the effective volume of soil that plant can exploit (Franke, 2002). The hyphal mycelium increases the total absorption surface of infected plants and this improves its access of immobile elements such as P (Ortas *et al.*,2001; Giri *et al.*,2005; and Grant *et al.*,2005) in areas beyond the root's depletion zone. Also, the production of extra cellular phosphates by mycorrhizal fungi could increase the ability of host plants to obtain P directly from organic sources (Aysan and Demir, 2009). Mycorrhizal fungi can also absorb N from NH_4-N mineral fertilizers and transport it to the host plant (Johanseu *et al.*, 1993). Similarly, Abou-El-Seoud (1998) showed that, the inoculation with AM fungi produced a highly significant increase in the concentration of N and P of cotton leaves. On the other hand, AMF have an important role in the absorption of N by plants in many crops such as *Medicago sativa* (Nielsen and Jensen, 1983), Soybean (Vejsadova *et al.*, 1992). Also, AMF increased N uptake of 24 tropical forage legumes and grasses (Saif, 1987).

The present data in table (4) indicated that, there was insignificant difference in P concentration, P uptake and N concentration between the AMF species. In contrast, there was a significant difference between the AMF species in nitrogen uptake. The macronutrient contents (N and P) increased significantly by treated with both bacteria genera (as bioagents) compared with the control. Similarly Aysan and Demir (2009) reported that the nitrogen content of shoot treated with bacteria (as

bioagents) was higher than the control. On the other hand, organic P may be mineralized by bacteria that secrete phosphates whereas inorganic P may be related by bacteria that excrete organic acid (Smith and Read, 1997).

Table (5) show that, all micronutrients (Fe, Cu, Mn, and Zn) of common bean plants treated with bioagents were increased significantly compared with the control (without bioagents). On the other hand, the micronutrients which present in table (5) of common bean plants inoculated with the mycorrhizal species were highly significant in comparison to the plants treated by other bioagents. In the same line, Artursson *et al.*,(2006) discussed that, the uptake of essential micronutrients from the soil by the AM fungal hyphae might also play a role in general plant growth improvement. The same results were recorded by (Abou-El-Seoud, 1998, and Ortas *et al.*, 2001). The first specie of AMF (*Glomus intraradices*) significantly increased in the micronutrients (Fe, Cu, Mn, and Zn) by about 35%; 12%; 14%; and 21% ,respectively compared with the second AMF specie (*Glomus macrocarpium*).The common bean plants treated with both bacteria genera were increased significantly in micronutrients (Fe, Cu, Mn, and Zn) in comparison to the other plants without bioagents or treated with both genera of yeast.

3.2. Main effect of common bean varieties:-

From tables (4 and 5) can notice that, the second common bean variety (Contender) were increased significantly in P concentration, P uptake, N uptake, and micronutrients concentration (Fe, Cu, Mn and Zn) by about 7%, 107%, 90%, 51%, 10%, 21%, and 28% respectively compared with the first common bean variety (Bronco). In the same line, Abou-El-Seoud and Wafaa (2010) reported that, the efficient maize genotypes were higher in nutrient uptake than the other maize genotypes. Similarly, Eticha (2000) found that the efficient cabbage genotypes took up more P from the soil under deficient P supply. In contrast, it was insignificant difference in N concentration between the two common bean varieties.

3.3. Main effect of *Sclerotinia* isolates

The result present in tables (4 and 5) clearly show that, the macronutrients concentration and uptake of P and N and micronutrients concentrations (Fe, Cu, Mn and Zn) of common bean plants decreased significantly with infecting by the two *Sclerotinia* isolates, compared with the other plant without infection. The P and N content of common bean plants infected by first and second isolates of *Sclerotinia* reduced by about 26% P and 10% N (first isolate (S4)) and 66% P and 24% N (second isolate (S6)) compared with the other common bean plants without infection. Similarly, Aysan and Demir (2009) reported that pathogenic infection by *Sclerotinia sclerotiorum* reduced nutrient uptake by the common bean.

On the other hand, there was significant difference between the two isolates of *Sclerotinia* in nutrients content of plants. On the other words, the plants infected by the first isolate of *Sclerotinia* were increased significantly in nutrients content of plants (P, N, Fe, Cu, Mn, and Zn) compared to the other isolates of *Sclerotinia*.

Table 4. Main effect of *Sclerotinia* isolates, common bean varieties and bioagents on macronutrient content

Treatments	Phosphorus concentration (mgP/ g D.m.)	Phosphorus uptake (mg P /plant)	Nitrogen concentration (mg N/ gD.m.)	Nitrogen uptake (mg N/ plant)
Biological control agents (Bio)				
Control	1.33 e	0.34 f	17.87 e	2.87 d
B1	2.82 b	0.76 bc	40.05 b	10.58 c
B2	2.69 b	0.67 cd	34.53 c	8.42 c
Y1	1.91 d	0.40 ef	19.43 de	3.86 d
Y2	2.39 c	0.55 de	22.67 d	5.10 d
M1	3.08 a	0.02 a	54.42 a	17.64 a
M2	2.99 a	0.88 ab	51.7 a	14.92 b
Common bean varieties (V)				
Bronco	2.38 b	0.43 b	33.50 a	6.24 b
Contender	2.54 a	0.89 a	35.26 a	11.87 a
***Sclerotinia sclerotiorum* isolates (S)**				
S0	3.08 a	0.98 a	37.99 a	12.33 a
Isolate 1 (S4)	2.44 b	0.61 b	34.59 b	9.13 b
Isolate 2 (S6)	1.86 c	0.39 c	30.56 c	5.70 c

Table 5. Main effect of *Sclerotinia* isolates, common bean varieties and bioagents on micronutrient content

Treatments	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
Biological control agents (Bio)				
Control	166.6 f	23.87 d	42.18 f	58.7 f
B1	290.13 c	37.7 c	66.13 c	77.86 c
B2	242.95 d	35.86 c	56.73 d	73.07 cd
Y1	193.01 ef	28.23 d	44.43 f	67.15 e
Y2	210.28 e	33.69 c	51.86 e	71.12 de
M1	525.15 a	54.39 a	85.94 a	101.38 a
M2	388.03 b	48.77 b	75.18 b	84.16 b
Common bean varieties (V)				
Bronco	229.24 b	35.76 b	54.55 b	66.68 b
Contender	346.82 a	39.25 a	66.1 a	85.67 a
***Sclerotinia sclerotiorum* isolates (S)**				
S0	339.94 a	55.19 a	68.72 a	78.28 a
Isolate 1 (S4)	275.10 b	32.11 b	57.27 b	76.14 ab
Isolate 2 (S6)	249.06 c	25.21 c	54.98 c	74.11 b

4. Available Phosphorus in soil

4.1. Main effect of biological control agents

From table (6) can notice that, the available P in soil of plants treated with bioagents was increased significantly compared with the available P in soil of control (without bioagents). The amounts of available P in soil cultivated with common bean plants treated with bioagents (M1; M2; B1; B2; Y1, and Y2) increased by about 91%; 81%; 69%; 59%; 29%, and 44% ,respectively compared to available P of unrated plants. Available P of soil cultivated with common bean plants inoculated with the two AMF species increased significantly compared with the other bioagents (Table 6). In the same line, Abou-El-Seoud (1998) showed that available P significantly increased as a result of inoculation common bean plants with AMF, that could be due to the hyphae of the A-mycorrhizal fungi produce organic acids and phosphates which catalyze the release of P from organic complexes which tend to improve the available P in soil (Bucking and Heyser, 2003 and Aono *et al.*,2004). In addition, in alkaline soils, mycorrhizal hypae lead to decrease in alkalinity of the rhizosphere soil from 8.5 to 7.4 by organic acids exudation, which due to solubilizing immobile elements such as P (Giri *et al.*,2005). On the other hand, there was insignificant difference in the amounts of available P in soil between the two AMF species. The available P in soil of plants treated with both genera of bacteria had increased significantly compared with the control and plants treated with yeast, that could be due to the bacteria play an important role in releasing P through producing chelating substrate and various organic acids (Kapoor, *et al.*,1999); inorganic acids, as well as CO_2 (Zayed, and Zeid, 1998); and acid phosphates which play a major role in the mineralization of organic phosphorus in soil (Rodriguez and Fraga, 1999).. Also, there was a significant difference between the two yeast genera.

4.2. Main effect of common bean varieties

Table (6) show that, the amounts of available P in soil cultivated with the second common bean variety (Contender) was increased significantly by about 6% compared with the first common bean variety (Bronco). Several studies done on P efficiency of different crops (Schenk and Barber, 1979; Gahoonia *et al.*,1999) indicated that variability exists among the existing crop species as well as genotypes with the same species in P efficiency are attainable. The efficient variety of root plant can exude a wide range of organic and inorganic compounds, which may increase available P in soil to absorb by root plants (Marschner, 1995); acid phosphates produced by roots of higher plants and fungi (Waski *et al.*,2000).

4.3. Main effect of Sclerotinia isolates

From table (6) can notice that, the amounts of available P from the soil cultivated with common bean plants without infection with *Sclerotinia* was significantly higher than the first and second isolates of *Sclerotinia* by about 3% and 5% respectively. In the other hand, there was insignificant difference in available P from the soil cultivated with common bean plants infected with the two *Sclerotinia* isolates (S4 and S6).

The results of this study suggested that the difference microorganisms which used as bioagents especially

Table 6. Main effect of *Sclerotinia* isolates, common bean varieties and bioagents on available P in soil

Treatments	Available P in soil (mg P/kg soil)
Biological control agents (Bio)	
Control	7.00 e
B1	11.80 b
B2	11.11 b
Y1	9.05 d
Y2	10.06 c
M1	13.38 a
M2	12.64 a
Common bean varieties (V)	
Bronco	10.41 b
Contender	11.02 a
***Sclerotinia sclerotiorum* isolates (S)**	
S0	10.97 a
Isolate 1 (S4)	10.68 b
Isolate 2 (S6)	10.49 b

AMF first specie (*Glomus intraradiaces*) was sufficient to inhibit the *Sclerotinia* isolates. On the other hand, Contender common bean variety was more sufficient than the other variety (Bronco). Also, the first isolate of *Sclerotinia* (S4) was less dangerous compared with the other isolate (S6). In future studies, the interaction between the bioagents and the host plant are needed to develop much more efficient biological control agents of the related diseases.

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الملخص العربي

تأثير فطر الميكوريزا والبكتريا والخميرة كمكافحة حيوية لفطر إسكلروتينيا إسكلروشيورم وتأثيرهم على النمو لنبات الفاصوليا

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أجريت تجربة أصص بصوبة كلية الزراعة- سابا باشا-جامعة الإسكندرية بهدف تقييم تأثير نوعين من الميكوريزا (*Glomus intraradiaces* (M1) and *Glomus macrocarpium* (M2)) وسلالتين من البكتريا *Bacillus subtilis* (B1) و(B2) *Pseudomonas fluorescens* وسلالتين من الخمائر *Cryptococcus neoformans (Y1)* و *Candida albicans (Y2)* كمكافحة حيوية لمرض العفن الأبيض القاعدى لصنفين من الفاصوليا (برونكو وكونتندر) مصابين بفطر *Sclerotinia sclerotiorum* . تم إختيار عزلتين من *Sclerotinia sclerotiorum* (S4 and S6).

تشير نتائج هذه الدراسة إلى أن إستخدام الكائنات الحية الدقيقة كعوامل حيوية خاصة النوع الأول من فطر الميكوريزا *Glomus intraradiaces* كان كافياً لتثبيط نشاط فطر الأسكلروتينيا بعزلتيه. ومن ناحية أخرى، الصنف الثانى من الفاصوليا (كونتندر) أكثر كفاءة من الصنف الأخر (برونكو). وأيضاً العزلة الأولى من الأسكلروتينيا (S4) أقل خطورة مقارنة بالعزلة الأخرى (S6). وفى الدراسات المستقبلية، هناك حاجة إلى دراسة التداخل بين الكائنات الحية الدقيقة المستخدمة كمكافحة حيوية مع العائل النباتى لتطوير كفاءة المكافحة البيولوجية للأمراض المرتبطة.