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EFFECTIVENESS OF BIO-GEL BASED POWDER FORMULATIONS OF BACTERIAL BIOCONTROL AGENTS IN CONTROLING ROOT ROT DISEASE OF BEAN CAUSED BY *Sclerotium rolfsii*

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ABSTRACT

Natural polymers(biogel) along with inert carriers were used to formulate two antagonistic bacterial isolates of *Pseudomonas fluorescens* and *Bacillus subtilis*. Four different formulations i.e. biogel- vermiculite based powder (F1), biogel-talc based powder (F2), biogel- charcoal based powder (F3) and biogel wettable powder (F4) were developed. These formulations were tested for their shelf life efficiency as storage substrates of the two bioagents for 24 month and their ability to control root-rot disease of bean caused by *Sclerotium rolfsii*. Viability of *P. fluorescens* propagules in biogel- vermiculite based powder and biogel wettable powder was stable for up to 16 months and decreased sharply after 20 months .The populations in biogel-talc based powder and biogel- charcoal based powder were decreased beyond 1 year of storage. *B.subtilis* populations of viable propagules were stable for up to 2 years with minor differences in populations either in formulation types or the storage periods tested. Active colonization of bean rhizosphere was occurred by *P. fluorescens* and *B. subtilis* following seed coating or soil amendment with different formulations. The highest population of *Ps. fluorescens* and *B. subtilis*, was found in seed coating treatment than soil amendment. Biogel- vermiculite based powder gave the highest rhizosphere population followed by biogel wettable powder for both bacteria. In general, seed coating treatment gave an obvious results than soil amendment in reducing root rot disease incidence. Seed coating with *B. subtilis* applied in biogel-vermiculite based powder was the most effective one in reducing the disease incidence . Soil treatment was more effective than seed coating in reducing the sclerotia formed by *Sclerotium rolfsii*. Soil treatment with *B. subtilis* applied in biogel-vermiculite based powder was the most one in reducing the number of sclerotia formed. However, Soil amendment with *B, subtilis* in biogel- vermiculite based powder was the most effective treatment than other formula.

Keywords: Bean, root rot*, P. fluorescens* , *B. subtilis,* formulation

INTRODUCTION

Biological control using natural antagonistic microorganisms i.e. *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* sp. has become a very important alternative strategy for plant disease control (Cook and Baker, 1983; Weller, 1988; Kumar, 1998 and Schisler *et al*., 2004,Masoud and Abbas,2009). These strategies has been used especially to overcome many problems such as chemical hazardous, environmental pollution and resistance to chemicals fungicides (Lumsden and Vaughn, 1993 and Koch, 1999), Recently, successful production of effectiveness formulations has become essential for the stable and economical development of biopesticides (Burges, 1998 and Jayaraj, *et al*.,2005).

Microorganisms and other biocontrol agents are not very resistant to harsh natural conditions, has weak bioactivities and short shelf life compared to synthetic agrochemicals and very fragile to hostile conditions (Burges,

1998). Development of suitable formulations techniques can stabilizes the product for storage by providing an adequate shelf life (Wiyono *et al.*, 2008). It makes a biological control agent both convenient to use and safe to handle. Lewis, (1991) and Amer and Utkhede, (2000) stated that commercial suitable formulations of bio- control agents can stable, posses adequate shelf-life for at least one year but more practically 18-24 months.

Different carrier materials have been used to optimize favorable conditions for antagonists who sustain long lasting survival and improve the bio-control activity (Vidhyasekaran *et al.*, 1997; Ali *et al.*, 2001and Schmidt *et al.*, 2001). The polysaccharides starch and cellulose used to encapsulate pesticides for controlled release. Biocontrol agents have been encapsulated in chemical polymer matrices such as polyvinyl pyrollidone and polyvinyl alcohol (Baker, *et al.,* 1987). The bio-gel matrix serves as a protective coating and provides enough nutrients for reproduction and production of bioactive compounds by cells inside the biogel matrix (Amer, 2007) *Sclerotium rolfsii* is a soil-borne plant pathogen of almost unlimited host range including bean and sunflower (Punja, 1985). This work was carried out to:

1-Use of natural biopolymer to formulate two bacterial biocontrol agents in biogel-based powder formulations

2- Test the shelf life efficiency of the formulated biocontrol agents.

3- Assessment their ability to control root rot disease of bean caused by *Sclerotium rolfsii* as model of soil borne pathogens.

MATERIALS AND METHODS

Biocontrol agent's production and harvest:

The biocontrol agents used were, two bacterial isolates i.e. *Pseudomonas fluorescens* (Pf-5) and *Bacillus subtilis* (BS-2) .These biocontrol agents were obtained from Department of Agricultural Botany, Faculty of Agriculture, Minufiya University, Egypt. Cultures were maintained on king's B (KMB) medium for Pf-5and nutrient glucose agar (NGA) for BS-2 and it had been developed as an exhibited antibiotic-resistant strains and the methodology was described in detail by Vidhyasekaran and Muthamilan (1995). The marked strains grew well in KMB containing rifampicin (190 ug /ml^{-1}) and streptomycin(30ug /ml^{-1}) for *P. fluorescens*, and on NGA containing ampicillin (100ug mL^{-1})and erythromycin(30ug mL^{-1})for *B. subtilis* which at these concentrations inhibited all wild strains.

The biomass of each biocontrol agent was prepared after inoculation of 2L batches of early mentioned broth medium for each, supplemented with selective antibiotic, prepared in 500 ml flasks each contain 150 ml broth medium and incubated in 250-rpm shaker at 30°C for 72 hrs. When the cultures were fully turbid, the cells were collected by centrifugation at 5000 rpm for 10 min, the supernatant discarded. The resulting pellet suspended in 100 ml batches of phosphate buffer as final volumes for each and stored in refrigerator until use.

Preparation of formulations:

To generate various formulations of *P.fluorescens* or *B.subtilis*, the phosphate buffers suspensions containing around $7x10^{12}$ colony-forming

units (CFUs) mL^{-1} were used to prepare various formulations as described in Table (1).

The biogel matrix was prepared by mixing the followed materials: soybean powder 100 gm, dasheen peel (taro) 50 gm, durum wheat flour 50 gm, , whey 15 gm, shimmed milk 10 gm, glucose 2 gm, $CaCO_3$ 1gm, Fe $SO_4.7H_2O$ 50 mg, Mn $Cl_2.4H_2O$ 10 mg and corn oil 10 ml. The ingredients used in biogel formulation were selected based on availability, cost effective, functionality and almost are agricultural products. All ingredients were mixed together in 1L of deionized water and boiled at 100°C for 1 hr with stirring to make a uniform biogel matrix, the mixture was autoclaved at 121°C for 30 min. The carrier materials were steam sterilized for 30 min of two consecutive days, and dried aseptically for 24 h in laminar flaw .Mixing of bacterial suspensions with the carrier materials and biogel was conducted under sterile conditions .The pH of all formulations was adjusted to pH7.0 by adding appropriate quantities of calcium carbonate. The powder and wettable powder formulations were dried for 48hrs.Dried formulations were mixed in a blender for 30s at low speed and passed through an 18-mesh screen. The dry mixtures were packed in polypropylene bags and heat sealed. All the formulations were prepared in the same manner but without the bacterial cells, and used as control in the subsequent treatment experiments.

Table (1): Production of *P. fluorescens* (Pf5) and *B. subtilis* (BS2) formulations.

Formulations	Ingredients/Method
1-Biogel-vermiculite based powder(F1)	Bacterial suspension(Pf5 or Bs2) (100ml)containing 10^{12}CFUsml^{-1} was mixed with fine grade vermiculite (150gm); biogel (100gm) and carboxy methyl cellulose (CMC) (3gm).
2-Biogel-Talc based Powder(F2)	Bacterial suspension (Pf5 or Bs2) (100ml) containing 10^{12}CFUs ml^{-1} was mixed with talc powder (150gm); biogel (100gm) and CMC (3gm).
3-Biogel charcoal based powder(F3)	Bacterial suspension (Pf5 or Bs2) (100) containing 10^{12}CFUs ml^{-1} was mixed with charcoal (150gm);biogel(100gm) and CMC (3gm).
4-Biogel wettable powder(F4)	Bacterial suspension (Pf5 or Bs2)(100) containing 10^{12}CFUs ml^{-1} was mixed with biogel(100gm);corn starch(30gm);talc powder(100);tween-80(5ml) and (CMC)(3gm).

Survival of *P. fluorescens* (Pf5) and *B. subtilis*(Bs2) in different formulations:

Formulations prepared and packed as described above have been stored at room temperature ($28c^0$) for two years. Samples (1gm) of each formulation were drown at intervals of 1, 2, 4, 8, 12, 16, 20 and 24 months of storage at room temperature. Dilutions were prepared and 0.1 ml aliquots were plated on specified medium of each biocontrol agent in Petri plates. Plates were incubated for 24 hrs at $28c^0$. The population was assessed as colony forming units (CFUs). The independent samples were analyzed with

three replicates. This experiment was set as a completely randomized design.

Colonization of bean rhizosphere by *P. fluorescens* and *B. subtilis*.

Three parts of field soil and one part of well decomposed farm yard manure (FYM) were mixed together and filed in plastic pots of 25cm diameter .The antibiotic resistant strains of *Ps.fluorescens* (Pf5) and *B.subtilis* (BS2) in different formulations were used. Bean cv (Giza-6) seeds were treated with the powder formulations of exhibited antibiotic resistant strains at the rate of 10gm/kg seed following the method of Weller and Cook(1986) shade dried overnight in laminar flow hood. The treated seeds were sown in pots containing unsterilized soil. Soil application of the power formulations was carried out, on which 25gm of the power formulation of each isolate was mixed individually with 2.5kg farm yard manure at the time of sowing. .Seeds and soils without the power formulations treatment served as controls. Four seeds were sown for each pot and five pots were kept as one replicate, there were three replicates. The pots were arranged in randomized block design. The rhizosphere populations of both biocontrol agents were assessed at intervals of 30,60 and 90 days after sowing. One plant from each pot was pulled out gently with roots intact and soil adhering to the roots was gently removed by tapping the roots. The roots portions were cut and transferred to 100ml of sterile water in a 250 ml Erlenmeyer flask. After shaking, the population of Pf5 and Bs2 in the suspensions was estimated by dilution plate technique on specified agar medium containing the specified antibiotics for each. The plates incubated at 30c^{o}, and fluorescent colonies were viewed under UV light and counted. Five Petri plates for each dilution and three replications were maintained. The root samples were weighed and the bacterial population was expressed per gram of root sample.

Efficacy of *P. fluorescens* (Pf5) and *B. subtilis* (BS2) formulations on bean root rot incidence under potted condition.

Sclerotium rolfsii was isolated from diseased plants grown in Minufiya Governorate. Cultures of the pathogen were maintained on PDA.

The pathogen inoculua:

The pathogen was grown on wheat bran-vermiculite (1:1 w/w) in a polyethylene bags for 10 days at 28°C. Ten grams of pathogen inoculum containing actively growing mycelium along with sclerotia (approx. 3 gm of sclerotia/ 10 gm of inoculum) was used for each pot. Three parts of field soil and one part of well decomposed farm yard manure (FYM) were mixed together and filled in plastic pots of 25 cm diameter.

Seed coating treatment:

Seeds of bean cultivar Giza-6 were coated with a thin layer of 1% carboxyl methyl cellulose (CMC) then mixed with 4gm/kg seed of each individual formulation for each biocontrol agent. Ten coated seeds were sown per pot; some seeds were dressed with the fungicide Rovral (Iprodione) at the rate of 3 gm/kg seeds and used for comparative analysis. Sterilized seeds were sown in pots inoculated with pathogen which served as control.

Soil treatment:

Formulation of each biocontrol agent was added individually to the infested soil pots at the rate of 3 gm/pot before sowing. 10 surface sterilized

seeds were sown per pot. Four replicates were used for Each treatment. The percentage of plants exhibited root rot symptoms were recorded after 30, 60 and 90 days after sowing.

Effect of *P. fluorescens* and *B. subtilis* formulations on the number of sclerotia formed:

Ten gm of soil samples were taken from each of the above-mentioned treatments when the plants reached 60 days old. The number of sclerotia was counted by washing the soil samples with running tap water on an 850um mesh screen. Sclerotia were picked from the screen and counted.

RESULTS AND DISCUSSION

The described formulations supported the growth and survival of *P. fluorescens* and *B. subtilis* and improved their shelf-life for 2 years. Also, they were useful for protection of biocontrol agent cells inside the biogel –based powder formulations. Most spore forming biocontrol agents survived very well during the formulations process at room temperature.

During storage at room temperature, spore forming biocontrol agents such as *B. subtilis* (BS-2)) survived very well for two years period. However, non-spore forming *P. fluorescens* (Pf-5) poorly survived during storage period.

Shelf-life of formulated biological control agents was assessed over a period of two years at room temperature. Data in Table (2) showed that population of *B. subtilis* was increased within biogel –based powder formulations up to 4 months(55.33 x10 ^{8} cfu/gm in Formulation 4), then showed insignificant decrease up to 24 months (47.96 and 47.53 x10 ^{8} cfu/gm in F1 and F2, respectively). Initial population of of *P. fluorescens* started by 54.33 - 57.1x10^{8} cfu/gm in biogel –based powder formulations, then decreased to the average of 33.76 - 48.36x10^{8} cfu/gm after 8 months of storage, and to 22.43-43.96x10^{8} cfu/gm after one year of storage. After two years of storage, the survival was decreased sharply to the average of 1.03-11.83x 10^{8} cfu/gm. It could be noticed that, F4 recorded the highest survival number followed by F1.The formulations F2 and F3 gave the lowest survival numbers during storage period.

For any commercial formulation the long shelf-life of the product is one of the pre-requisites. Vidhyasekaran and Muthamilan (1995) found that, *Ps. fluorescens* can survive well in talc powder or peat-based formulations for more than 8 months. *P. putida* population declined after 45 days of storage at different carriers (Amer and Utkhede, 2000). In pre-gelatinized corn flour powder formulation ,the survival of *Ps. fluorescens* was prolonged up to one year with 59% survival, also survived in semolina-tale powder up to 4 months of storage and declined after 6 months (Bashan and Gonzalez, 1999 and Das et.al,2008). *Ps. fluorescens* a non-spore former survived poorly during storage, it could be due to formulation and drying process.

Bacillus. subtilis shelf-life in the biogel- based powder formulations was prolonged for 2 years of storage. The initial population was 54.16x10^{8} cfu/gm for F1 to 50.80 x10^{8} cfu /gm for F3 of biogel-based powder formulations (Table 2) .The population recorded more survival stability

during two years of storage period. The population was 55.9x10^{8} cfu/ gm for F4 to 48.66x10^{8} cfu /gm for F2after 8 months of storage. After one year of storage the survival number was 54.76 x10^{8} cfu/gm for F4 and 45.36x10^{8} cfu/gm for F2.After two years of storage, the survival number was 47.53x10^{8} cfu/gm for F4 and 39.20x10^{8} cfu/gm for F2.

Bacillus subtilis cells entrapped in biogel-based powder formulation seems to multiply in the biogel matrix utilizing food sources present. *B. subtilis* can survive in certain dry formulations with effective populations (Turner and Backman, 1991). Also, survived for 120 days in talc powder formulation, and up to one year in pregelatinized corn flour and semolina talc power formulations (Amer and Rania El-Shennawy, 2003; Jayaraj *et.al*,2005).

Table (2): Populations of *P.fluorescens and B.subtilis* in different formulations during 24 months of storage at room temperature.

Formulation type	*Pseudomonas fluorescens* population (x10^{8} CFU/gm^{-1}) at different months of storage								
	0	**1**	**2**	**4**	**8**	**12**	**16**	**20**	**24**
F1	56.66a	55.03b	50.26b	49.36a	40.46b	38.86b	29.36b	16.63b	4.10b
F2	54.33b	50.50d	47.80b	42.46b	33.80c	27.66c	19.63c	10.23d	1.03c
F3	54.33b	51.36c	49.76b	44.13b	33.76c	22.43d	20.20c	12.76c	5.66b
F4	57.1a	56.50a	53.03a	51.13a	48.36a	43.96a	31.40a	19.86a	11.83a
Formulation type	***B.subtilis* population (x10^{8} CFU /gm^{-1}) at different months of storage.**								
	0	**1**	**2**	**4**	**8**	**12**	**16**	**20**	**24**
F1	54.16a	53.56a	54.63a	54.16b	53.8b	53.06b	51.63b	50.70a	47.96a
F2	51.43b	52.50b	52.13b	51.56c	48.66d	45.36d	43.06d	40.16c	39.20c
F3	50.80b	52.06b	55.30a	54.33b	52.70c	51.56c	48.06c	45.06b	41.06b
F4	53.66a	52.66b	54.83a	55.33a	55.90a	54.76a	52.53a	49.70a	47.53a

Means followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05). The formulations were stored up to 24 months, a period adequate for using fresh inoculum of the biocontrol agents. F1: Biogel-vermiculite based powder; F2: Biogel-talc based powder; F3: biogel-charcoal based powder and F4: wettable powder.

Colonization of bean rhizosphere by *P.fluorescens* and *B. subtilis*.

When the biogel-based powder formulations of antibiotic resistant *Ps. fluorescens* and *B. subtilis* were applied as seed coating and soil amendment under green house conditions, the bacteria could be detected in bean rhizosphere (Tables 3 and 4 and Figures 1 and 2), which clear that the bacterial populations were gradually increased by time.

In general, seed coating treatments resulted higher populations in bean rhizosphere than those of soil amendments. The population of *P.fluorescens* applied as seed coating in different formulation was higher than that of soil amendment. The populations in the formulations (F-1) and (F-4) was higher than that of (F-2) and (F-3) after 30 days of sowing. However, after 60 days of sowing, the populations of the bacteria were increased than the population after 30 days. After 30days of sowing, the populations of *P. fluorescens* were 19.8 - 18.3 x10^{6} cfu /gm in F4 and F1 respectively ,and reached to the maximum population after 60 days which recorded 31.5 and 23.4 x10^{6} cfu/gm in F1 and F4 , respectively. With prolonged times, the populations of *Ps.fluorescens* decreased sharply when applied either as seed coating or soil

amendments after 90 days of sowing. These populations were very less compared to other measurements after sowing which declined to 4.1 and 3.5 x10^6 cfu/gm in F4 and F1after 90 days, respectively . In soil application, The rhizosphere population of *P.fluorescens* was 16.7 and 15.4 x10^6 cfu/gm for F1and F4 respectively after 30 days of sowing and increased to 27.1and 21.0 x10^6 cfu/gm in both formulations after 60 days then decreased sharply in after 90 days of sowing which recorded 1.3 and 2.6 x10^6 cfu/gm for both formulations, respectively.

The populations of *B. subtilis* when applied as seed coating were higher during the whole estimation times after sowing. The highest population numbers were recorded after 60 and 90 days of sowing compared to other measurements. After 30 days of sowing , the formulations F-1 and F4 of *B. subtilis* recorded higher populations(13.5 - 14.1x10^6 cfu/gm) followed by F-2 and F-3(10.3 - 11.3x10^6 cfu/gm), respectively. However, after 60 days, the populations in F-1 and F-4 increased gradually which recorded the highest number of viable cells(29.3and 27.5x10^6 cfu/gm)followed by F-2 and F-3respectively. After 90 days of sowing, F-1 and F-4recorded the highest populations(18.7 and 16.5x10^6 cfu/gm) followed by F-2 and F-3. On the other hand, soil application of *B. subtilis* formulations, the rhizosphere bacterial populations were decreased compared to seed coating application in all experimental time. Populations of *B. subtilis* recorded 12.6 and 11.4 x10^6 cfu /gm for F-4and F-1, respectively after 30 days of sowing, increased to be 24.5 and 23.9x10^6 cfu/gm for F-1 and F-4 respectively after 60 days, and remained in sufficient viable cells after 90 days and recorded moderate decreases in numbers. Lugtenberg *et al.*(2001) reported the possibility to predict the rhizosphere competence of *Pseudomonas* spp. and *Bacillus* spp. based on their root colonizing ability , since the root colonizing ability of a bacterium will determine its rhizosphere competence. The most widely studied bacteria by far in relation to biocontrol are *Pseudomonas* spp. and *Bacillus* spp. which are amongst the most effective root colonizing bacteria (Whipps, 2001) .

Table (3): Survival of *Pseudomonas fluorescens* antibiotics marked isolate in bean rhizosphere following seed and soil treatment with different formulations.

Formulation	Seed coating treatment with *P. fluorescens* (x10^6 cfu /gm) after/days of sowing			Soil treatment with *P . fluorescens* (x10^6 cfu /gm) After/days of sowing		
	30	60	90	30	60	90
F1	18.3 b	31.5 a	3.5 b	16.7 a	27.1 a	1.3 c
F2	14.5 c	19.7 c	1.5 d	11.3 d	17.4 d	0.7 d
F3	13.3 d	16.6 d	2.3 c	12.1 c	17.7 c	1.9 b
F4	19.8 a	23.4 b	4.1 a	15.4 b	21.0 b	2.6 a
Control	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e

Means followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05).

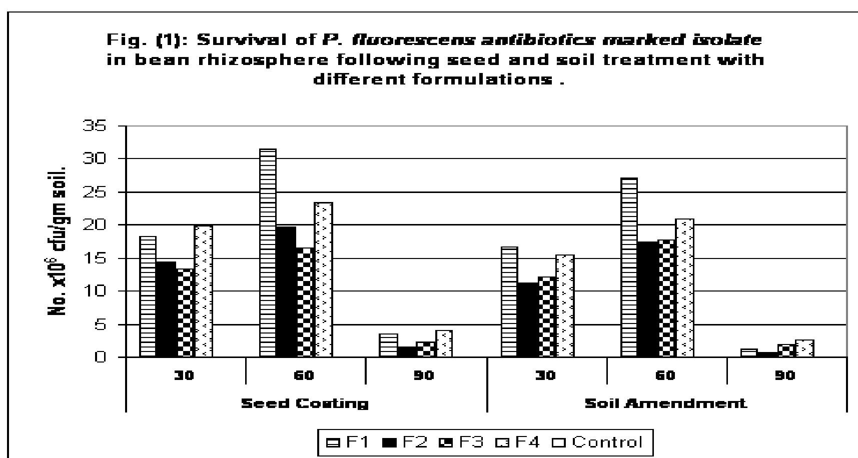
F1:Biogel-vermiculite based powder; F2:Biogel-talc based powder; F3:biogel-charcoal based powder and F4:wettable powder.

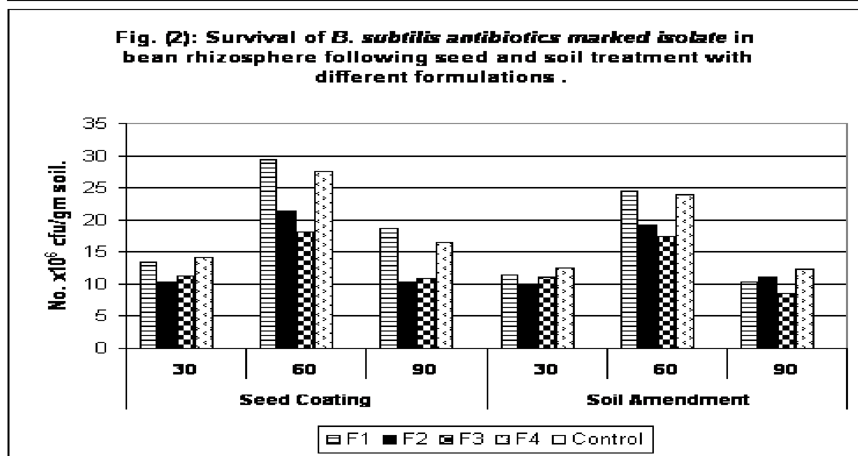
Table (4): Survival of *Bacillus subtilis* antibiotics marked isolate in bean rhizosphere following seed and soil treatment with different formulations

Formulation	Seed coating treatment with *Bacillus subtilis* (x10^6 cfu /gm) After/days of sowing			Soil treatment with *Bacillus subtilis* (x10^6 cfu /gm) After/days of sowing		
	30	60	90	30	60	90
F1	13.5 b	29.3 a	18.7 a	11.4 b	24.5 a	10.3 c
F2	10.3 d	21.4 c	10.4 d	10.0 d	19.3 c	11.1 b
F3	11.3 c	18.1 d	10.9 c	11.1 c	17.4 d	8.6 d
F4	14.1 a	27.5 b	16.5 b	12.6 a	23.9 b	12.3 a
Control	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e

Means followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05).

F1:Biogel-vermiculite based powder; F2:Biogel-talc based powder; F3:biogel-charcoal based powder and F4:wettable powder.





F1:Biogel-vermiculite based powder; F2: Biogel-talc based powder; F3: biogel- charcoal based powder and F4: wettable powder.

Effect of formulated biological control agents on root rot of bean.

Efficacy of *P. fluorescens* and *B. subtilis* formulations on root rot incidence in bean under greenhouse conditions is listed in Table (5). Generally, root rot on bean plants caused by *S. rolfsii* was significantly reduced by all formulated biocontrol agents applied as seed coating or soil amendment as compared with the control. Seed coating was effective than soil amendment and *B.subtilis* was more effective in reducing root rot disease incidence than *P.fluorescens*. The formulation (F1)was most effective than other formula used. Data presented in Table (5) indicate that, after 30 days of sowing, the formulation(F1) of *B. subtilis* applied as seed coating recorded the lowest root rot incidence (2.71%) compared to other formulations. The fungicide Rovral was the most effective treatment (1.30% root rot incidence) as compared to control. After 60 days of sowing, The formulation (F1) of *B.subtilis* applied as seed coating was the most effective one and recorded 5.70 % disease incidence the fungicide Rovral recorded the lowest percentage of disease incidence(4.2%). After 90 days of sowing *B. sutilis* was the most effective one and recorded 0.93for F3 and 1.30% for F4 of root rot incidence compared to control (4.1%).The applied formulations of both biocontrol agents indicated that, the formulations F1 and F4 of *B. subtilis* gave the best results in reducing the root rot disease incidence than *P. fluorescens*.

At the present time, there is considerable interest in the biological control of *S. rolfsii* through the introduction of biological control agents.
There are three strategies in considering biological control with introduced biocontrol agents: (a) to reduce the population of the pathogen; (b) to prevent the pathogen to infect the plant; and (c) to limit the disease development after infection (Cook, 1993).

Seed coating with the power formulated biological control agents reduced the incidence of root rots on bean more than soil amendment. This demonstrates the potential of seed coating to control the disease during the early plant growth stages. This revealed that the biocontrol agents applied to seeds were able to grow along with germinating seeds and elongating roots, and protect the roots against infection with pathogens (Kloepper and Schroth, 1981), this also my be due to the production of the antibiotics around the seed coat and rhizosphere which causes lysis of mycelium of *S. rolfsii* (Weller,1988). The obtained results revealed that, soil application of powder formulated biocontrol agents was effective in reducing root rot incidence and this explained by the fact that biogel based powder formulation in the soil acts as food base for biocontrol agents for period of time in the soil and lead to increase its population, therefore the population of the pathogen was decreased. These results are correlated with the ability of biocontrol agents to inhibit the hyphe of *S. rolfsii* through antibiosis potential (Elad *et al.,* 1983; Henis *et al.,* 1984; Freitas *et al.*, 1997; Keel *et al.,* 1992).

Effect of seed and soil treatment with formulations of *Ps. fluorescens* and *B.subtilis* on the number of sclerotia formed .

Pseudomonas fluorescens and *B.subtilis* treatments significantly reduced the number of sclerotia formed by *S. rolfsii* compared with the control (Table 6). Such reduction was obvious in the case of *B. subtilis* than *Ps.*

fluorescens. Moreover, in both bioagents, soil treatment was more effective than seed treatment in reducing number of the fungal sclerotia.It was found that, soil treatment with formulation type(F1) of *B. subtilis* was the most effective one in reducing sclerotia number(50.33 sclerotia/gm soil) followed by (F4),(F3) and (F2) which recorded (64.66 ; 69 ; 72 sclerotia/gm soil) respectively. Nevertheless, fungicide treatment (Rovral) gave the lowest number of *S. rolfsii* sclerotia (60 and 56.6 sclerotia/gm soil) as compared with the control.

A successful biological control agents should be most effective against the survival structures of the pathogen (Cook and Baker,1983 and Cook,1993).

Table (5): Effect of *P.fluorescens* and *B.subtilis* in different formulations on bean root rot incidence under green house conditions.

Application method	Formulation type	Root rot disease incidence % /days after sowing					
		P. fluorescens			*B. subtilis*		
		30	60	90	30	60	90
Seed coating	F1	12.34e	14.86d	2.78e	2.71g	5.70f	2.00d
	F2	14.83d	17.20bc	3.60d	3.90e	6.40de	1.90d
	F3	13.30e	14.68d	3.20de	3.40f	6.13ef	0.93f
	F4	15.30cd	16.60c	1.64g	4.30d	6.60de	1.36e
Soil amendment	F1	15.86cd	15.33d	2.77e	4.40d	7.50b	2.60c
	F2	17.56b	18.60b	4.70c	5.10c	6.86cd	2.20d
	F3	16.66bc	17.30bc	3.30de	3.90e	6.30de	2.06d
	F4	16.03cd	18.40b	2.13f	6.13b	7.30bc	2.10d
Rovral		2.70f	10.40e	6.26b	1.30h	4.20g	6.50b
Control		40.56a	20.20a	9.30a	49.50a	16.13a	7.10a

Means followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05). F1:Biogel-vermiculite based powder; F2:Biogel-talc based powder; F3:biogel-charcoal based powder and F4:wettable powder.

Table (6): Effect of seed and soil treatment with different formulations of *P.fluorescens* and *B.subtilis* on sclerotial number formed by *S. rolfsii* .

Application method.	Formulation type	Number of sclerotia formed/gm soil	
		Ps. fluorescens	*B.subtilis*
Seed coating	F1	125.76b	112.66a
	F2	129.33b	102.66a
	F3	99.00d	106.00a
	F4	116.00c	82.00b
Soil amendment	F1	68.33f	50.33c
	F2	82.00e	72.00bc
	F3	67.33f	69.00bc
	F4	77.33e	64.66bc
Rovral		60.00g	56.66c
Control		167.00a	120.66a

Means followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05). F1:Biogel-vermiculite based powder; F2:Biogel-talc based powder; F3:biogel-charcoal based powder and F4:wettable powder

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كفاءة تحميل بكتريا المقاومة الحيوية على بودرة البوليميرات الطبيعية في مقاومة مرض عفن الجذور في الفاصوليا المتسبب عن الفطر سكليروشيم رولفسياى

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أجريت هذه الدراسة بغرض تحميل اثنان من البكتريا المضادة حيويا على بودرة البوليمرات الطبيعية وهما باسيلس ستلس *Bacillus subtilis* و سيدوموناس فلوريسنس *Pseudomonas fluorescens* لاستخدامها في أربع تراكيب مختلفة وهى بودرة الفيرميكولايت biogel-vermiculite ، وبودرةالتلك biogel-talc powder – بودرة الشاركول biogel-charcoal - بودرة قابلة للبلل bioge- wettable powder – . تم اختبار قدرة هذه المركبات على حيوية البكتريا المضادة عند تخزينها لمدة عامين على درجة حرارة الغرفة وكذلك قدرتها على مقاومة مرض عفن الجذور في الفاصوليا المتسبب عن الفطر سكليروشيم رولفسياى. وقد وجد أن حيوية بكتريا سيدوموناس فلوريسنس المحملة على بودرة الفيرميكولايت و البودرة القابلة للبلل كانت ثابتة لمدة 16 شهر من التخزين ثم تدهورت بشدة بعد ذلك حتى 24 شهر بينما قل معدل حيوية البكتريا المحملة على بودرة التلك – بودرة الشاركول خلال سنة من التخزين. على العكس من ذلك استمرت حيوية خلايا البكتريا المضادة باسيلس ستلس ثابتة حتى عامين من التخزين مع بعض النقص الغير معنوي في كل من التراكيب المحملة عليها. وكان عدد الخلايا البكتيرية في منطقة الريزوسفير نشطا بكل من البكتريا المضادة باسيلس ستلس و سيدوموناس فلوريسنس سواء باستخدامها بطريقة تغليف البذور أو معاملة التربة وكان أفضلها معاملة تغليف البذور. وكان أفضل المواد المحمل عليها بودرة الفيرميكولايت حيث أعطت أعلى مستوى من البكتريا في منطقة الريزوسفير يليها البودرة القابلة للبلل لكل من نوعى البكتريا المضادة. وبصفة عامة كانت معاملة تغليف البذور أفضل من معاملة التربة في تقليل شدة الإصابة بمرض عفن الجذور وكان معاملة البذرة ببكتريا باسيلس ستلس المحملة على بودرة الفيرميكولايت الأفضل في تقليل شدة الإصابة بالمرض بينما كانت معاملة التربة افضل من معاملة البذرة في تقليل الاجسام الحجرية للفطر الممرض ، و كانت معاملة التربة ببكتريا باسيلس ستلس المحملة على بودرة الفيرميكولايت الأفضل في تقليل الاجسام الحجرية للفطر الممرض.

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