FIELD APPLICATION OF POWDER FORMULATION OF Bacillus subtilis FOR CONTROLLING DAMPING-OFF AND ROOT ROT OF SOYBEAN

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ABSTRACT: Three isolates of Bacillus subtilis were developed as a talc powder formulations, viable counts (cfu) in the formulations were decreased with time and remain in sufficient populations for up to 120 days at refrigerator with percent viability 77, 71 and 74% for Bs-1, 4 and 6 respectively, and at room temperature with 64, 65 and 65% viability, respectively.

Powder formulations were used for seed coating and soil application and proved effective in reducing soybean damping-off and root rot under field conditions.

Seed coating application was more effective in reducing damping-off than soil application (76 – 77% and 67 – 68% surviving plants respectively). Seed coating combined with Topsin-M or Rhizolex-T was the most effective treatment (81.86% surviving plants) compared to control treatment (44% surviving plants).

Application of B. subtilis formulations to the soil was less effective treatment than seed coating (11.9 – 16.4% and 8 – 9.8% root rot incidence respectively). Addition of B. subtilis formulations as seed coating combined with Tospin-M or Rhizolex-T were more effective in reducing soybean root rot incidence (6 – 11.1%) as compared to control (22.7%).

Application of the B. subtilis formulations as seed coating or soil application increased the yield.

Key words: Soybean, Root rot, Damping-off, Biological control, Bacillus subtilis.

INTRODUCTION

Soybean (Glyclne max L.) is an important crop, and a major source for high quality feed for lifestock. It is subjected to the attack by several destructive pathogens belongs to the genera Pythlum, Scierotium, Macrophomina, Fusarium and Rhizoctonia, cause damping-off of seedlings and root rot under field conditions (Fadl and Hussien, 1978 and Mahrous and Ibrahim, 1984). These pathogens are unspecialized and can't be prevented by

CFOP rotation or the development of resistant crop varieties. Control of these pathogens depends mainly on fungicides (Waraitch et al., 1986 and Vyas, 1994). Chemical fungicides pollute the environment and disturb the ecological balances for all living microorganisms and cause harmful effect for beneficial microorganisms (Hooda and Grover, 1983).

Biological control is becoming an important component of plant disease management and offers solutions to many of the persistent problems in agriculture (Cook and Baker, 1983).

Bacillus species as a group, offer several advantages as seed inoculants for protection against root pathogens, including longer shelf life, because of their ability to form endospores and the broad spectrum activity of their antibiotics (Turner and Backman, 1991).

Many reports of rhizosphere colonization and root disease control with *Bacillus* spp. introduced as seed inoculants, including *B. cereus* UW85 for control of damping-off of alfalfa (Handelsman et al., 1990), *B. megaterium* B 153-2-2 for control of Rhizoctonia root rot of soybean (Liu and Sinclair, 1991, 1992, 1993), *B. subtilis* GB03 for control of damping-off of cotton (Mahafee and Backman, 1993), *B. subtilis* has been reported as an effective antagonist for the control of damping-off and root rot fungl in legumes like soybean (Jharia and Khare, 1986; Liu and Sinclair, 1991).

One of the major problems in the exploitation of biocontrol agents for disease control in field is the lack of suitable growth and delivery systems.

The purpose of this investigation was to:

- 1. Formulate B. subtilis isolates with talc powder for easily field delivery system and application.
- 2. Study the long term surviving ability of *B. subtllis* in the powder formulation.
- 3. Assess their efficacy in controlling damping-off and root rot of soybean under field conditions.

MATERIALS AND METHODS

The antagonist:

Ba*cillus subtilis isolates 1, 4 and 6 were obtained from Department of Agricultural Botany Collection, Faculty of Agriculture, Minufiya University.

Talc powder Formulation of Bacillus subtilis:

The formulation was developed as described by Vidhyasekaran and Muthamilan (1995) using a mixture of 10 gm of carboxylmethyl-cellulose and 1 kg of talc powder. The pH was adjusted to 7.0 by adding calcium carbonate and the mixture was autoclaved for 30 mln on each of 2 successive days.

Each strain was grown in two litre liquid potato dextrose broth medium for 72 hrs as a shake culture at 150 rpm at room temperature (25 ± 2°C).

The culture was centrifuged at 5000 rpm for 10 min, and the supernatent discarded. The resulting cell pellet was resuspended in 400 ml of phosphate buffer as a final volume.

Bacterial suspension (400 ml) was added to the carrier (1 kg for each isolate) and mixed well under sterile condition to form a pesta. The pesta was air dried in laminar flow for 24 hrs. After drying it was powdered using a mixer and sleved.

The final powder was packed in polyethelene bags, sealed and sample were stored at refrigerator or at room temperature for studying its shelf life.

Survival of B. subtilis in the formulation:

Samples were drawn at 30 days periodical intervals and the colony forming unit (cfu) of bacterial populations were assessed using potato dextrose agar medium by dilution plate technique. Three independent samples were analysed with three replicates of each.

Field treatments:

The efficacy of the powder formulations in controlling damping-off and root rot of soybean was assessed under field conditions. Experiment was carried out at Gemmiza Research Station, Agricultural Research Center.

a. Seed treatment:

Soybean seeds (cv. Giza21) were treated with the powder formulation at 10 gm / kg seeds, according to the method of Weller and Cook (1983). Seeds were surface sterilized with 2.4% sodium hypochlorite solution for 2 – 3 min, rinsed in sterile distilled water and dried overnight under sterile air stream. Seeds were treated with the powder formulation at the rate of 10 gm / kg seed as slurry treatment. The slurry was prepared by mixing 10 gm powder in 40 ml of sterilized water.

The following treatments were carried out to seeds: (1) 25 gm seeds for each replicate plots were dipped in the slurry of the powder formulation or (2) the slurry along with the recommended dose of the fungicides Topsin-M or Rhizolex-T (2.5 gm / kg seeds), (3) seeds were treated with the fungicides alone, and (4) untreated seeds were served as control.

Soil treatment:

The powder formulation was mixed with sieved sand for easy of handling and distribution at the rate of 25 gm powder / 500 gm sand. The mixture was applied to the base of sowing side of the row and mixed with surface soil using a wooden stick.

The soil application of the fungicides used was carried out as in case of powder formulation of *B. subtilis* isolates, mixed with sand at the rate of 2.5 gm / kg sand and applied to soil as mentioned earlier with powder formulations, untreated seeds and soil served as controls treatments.

Pre and post-emergence damping-off were recorded after 30 and 60 days of sowing respectively. Percentage of root rot incidence was recorded after 90 days of sowing. There were three replicated plots of each treatment, with three rows for each replicate, each raw was 3 m. long.

RESULTS AND DISCUSSION

Survivability of B. subtilis in powder formulation:

Survival of B. subtilis isolates (Bs-1, 4 and 6) in the powder formulations.

Results obtained revealed that, *B. subtilis* isolates population were recorded a gradual reduction in the number of colony forming unit (cfu). After 120 days of storage, sufficient number of viable colonies of *B. subtilis* was obtained during this period (Fig. 1 and 2).

Survival (Log colony forming units / gm of formulations) of *B. subtilis* isolates stored in refrigerator (about 0°C) was 8.4 log cfu at zero time and 6.5 log cfu after 120 days for *B. subtilis* (Bs-1), 9.0 to 6.7 for *B. subtilis* (Bs-4) and 8.7 to 6.2 for *B. subtilis* (Bs-6) (Fig. 1) with percent viability 77, 74% and 71% for Bs-1, Bs-4 and Bs-6, respectively.

Under room temperature (22 \pm 2°C) (Fig. 2) the survival of *B. subtilis* isolate (Bs-1) was 8.4 at zero time to 5.4 log cfu with 64% survival, for Bs-4 from 9.0 to 5.9 log cfu with 65% survival and for Bs-6 from 8.7 to 5.4 log cfu with 65% survival.

Carriers could improve product stability, shelf life, and also protect the bacteria against environmental extremes in soil. Powder formulations of the biocontrol plant growth promoting rhizobacteria (PGPR) for large scale field use may be ideal. This study shown that *B. subtilis* can survive in powder formulations for up to 120 days under refrigeration or at room temperature. PGPR have been reported to survive in certain dry formulations with effective populations (Suslaw and Schroth, 1982; Turner and Backman, 1991 and Amer and Utkhede, 2000).

Data presented in Table (1) show that, seed coating was more effective in reducing damping-off than direct soil application.

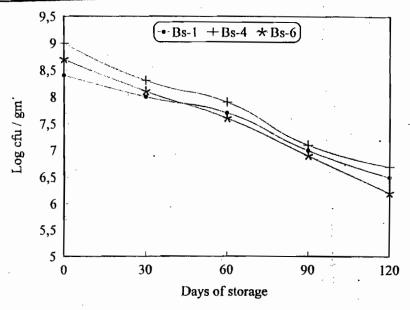


Fig. 1. Viability of *B. subtilis* isolates in the powder formulations during storage under refrigerator (about 0°C).

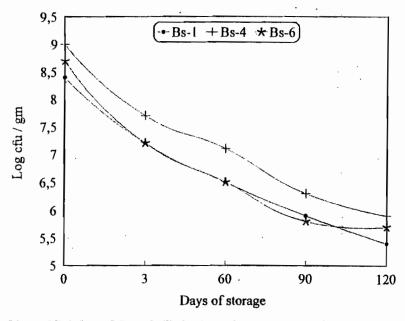


Fig. 2. Viability of *B. subtilis* isolates in the powder formulations during storage under room temperature (about 22°C).

Table (1): Effect of seed and soil application with powder formulations of *B. subtilis* isolates alone or combined with the fungicides Topsin-M or Rizolex on the percentages of damping-off on soybean under field conditions.

	% Damping-off					
	Seed coating			Soil application		
Treatments	Pre-emergence	Post-	Survival	Pre-	Post-	Surviving
	%	emergence	%	emergence	emergence	plants %
		%		%	%	
B. subtilis (Bs)1	21.6 ^b	2.4°	76.3°	26.3 ^b	6.3 ^{de}	67.3°
Bs-4	20.0 ^b	2.1°	77.0°	27.0 ^b	6.7 ^{de}	67.0°
Bs-6	22.0 ^b	2.7°	76.0°	26.0 ^b	5.3 ^{de}	68.7°
Topsin-M (T)	14.7°	11.3 ^b	74.0°	19.0°	11.0 ^{bc}	70.0 ^{bc}
Rizolex (R)	13.3 ^c	12.3 ^b	74.3 ^c	17.7°	11.7 ^b	70.3 ^{bc}
Bs.1 + T	15.7°	3.3°	81.0 ^b	17.3°	4.7 ^{de}	78.0 ^a
Bs.4 + T	14.6°	1.5°	84.0 ^{ab}	16.0°	7.3 ^{cd}	76.7ª
Bs.6 + T	14.0°	1.3°	84.7 ^{ab}	16.7°	8.3 ^{bcd}	75.0 ^{ab}
Bs.1 + R	14.0°	0.6 ^c	85.3ª	19.3°	5.3 ^{de}	75.3 ^{ab}
Bs.4 + R	13.0°	1.0°	86.0ª	18.0°	3.3°	78.7ª
Bs-6 + R	14.7°	1.7°	83.7 ^{ab}	18.7°	4.3 ^{de}	77.0 ^a
Control	37.0ª	19.0°	44.0 ^d	38.3°	18.3°	43.3 ^d
LSD _{0.05}	3.7	2.7	3.7	4.0	3.9	5.1

Values in each column followed by the same letter are not significantly different (p = 0.05) according to Duncan's multiple range test.

Pre- and post emergence damping-off were recorded after 15 and 30 days after sowing respectively.

Seed coating:

Seed coating with B. subtilis isolates combined with the fungicides was the most effective treatment in reducing damping-off. It increased the percentages of surviving plants to the range of 81 – 86% compared to 44% in control. This was followed by B. subtilis isolates alone which recorded 76 – 77% of surviving plants. The seed treatment with the fungicides Topsin-M or Rizolex-T showed to be less effective and recorded 74.0 – 74.3% survivals respectively.

Soil application:

The addition of B. subtilis formulation combined with the fungicides (Topsin-M or Rizolex-T) was more effective in reducing damping-off and increasing the percentages of surviving plants (75.0 - 78.7%) compared to control (43.3%), followed by the application of fungicides alone to soil which resulted 70.0 - 70.3% of surviving plants. The application of B. subtilis formulation isolates to soil was the least effective treatment (67 - 68%) compared to other treatments.

Biological control may be achieved by the inactivation of the pathogen by another microbe as a result of antibiosis, parasitism or competition for nutrients (Lewis, 1991 and deFreitas and Germida, 1991).

Seed pelleting is the cheapest delivery system for the antagonistic organisms to the rhizosphere of crop plants to be protected from soil-borne diseases. Biological protection of the seeds along with the emerging roots has been achieved using antagonists like *B. subtilis* in potato (Thirumalachar and Obrien, 1977), chickpea (Singh and Mehrothra, 1980) and aginst a number of soil borne fungi (Fiddaman and Rossal, 1993 and Etheridge and Parry, 1993).

The results obtained revealed that, seed treatment with *Bacillus subtilis* isolates alone or combined with the fungicides (Topsin-M or Rizolex-T) was more effective than soil application in reducing damping-off. These revealed that the biocontrol against applied to seeds were able to grow along with germinating seeds and elongating roots, and protect the roots against infection with pathogens (Klopper and Schroth, 1981). At the same time the application of biocontrol agents to soil are less effective than seed treatment and this because the failure of the inoculent to become established and / or to express antagonism in soil (Powell, 1992 and Amer and Utkhede, 2000).

The effect of seed coating and soil application with *B. subtilis* isolates alone or combined with the fungicides on root rot incidence was shown in Table (2). Data presented indicate that, seed treatment was more effective than soil application. The combination between *B. subtilis* isolates and the fungicides gave the lowest percentage of root rot incidence (6.4%) as

compared with control 22.7% followed by seed treatment with B. subtilis isolates alone (9.0 – 9.8%) or the fungicides (16.1 and 17.5%).

Table (2): Effect of seed and soil application with powder formulation of *B. subtilis* isolates alone or combined with the fungicides Topsin-M or Rizolex on percentage of root rot incidence of soybean under field conditions.

Treatments	Root rot incidence (%)			
reaunencs	Seed coating	Soil application		
B. subtilis (Bs)1	9.5 ^{ef}	15.1 ^{efg}		
Bs-4	8.0 ^{gh}	11.9 ^h		
Bs-6	9.8°	16.4 ^{cde}		
Topsin-M (T)	16.1°	22.0 ^b		
Rizolex (R)	17.5 ^b	22.8 ^b		
Bs-1 + T	7.6 ^h	17.3 ^{cd}		
Bs-4 + T	6.4	14.1 ^{fg}		
Bs-6 + T	6.5 ⁱ	16.7 ^{cde}		
Bs-1 + R	8.7 ^{fg}	15.6 ^{def}		
Bs-4 + R	6.7 ⁱ	13.8 ^g		
Bs-6 + R	11.1 ^d	17.5°		
Control	22.7 ^a	35.7ª		
LSD _{0.05}	0.9	1.5		

Values in each column followed by the same letter are not significantly different (p = 0.05) according to Duncan's multiple range test.

Data were recorded after 90 days of sowing.

Seed treatment with *Bacillus* spp. has been tested on several plants to control root rot diseases they are appealing candidates for biocontrol because they produce endospores that are tolerant to heat, desiccation and drought conditions (Broadbent et al., 1971), also *B. subtilis* applied as seed coat decreased pigeon pea wilt and this may be due to the production of the antibiotic bulbiformin around seed coat and rhizosphere which causes lysis of mycelium of *Rhizoctonia solani* (Weller, 1988).

Soil application of B. subtilis alone or combined with the fungicides were

most effective (11 - 17%) than the fungicides alone (22.0 - 22.8%) as compared to control (35.7%). Lewis and Papavizas (1984) also reported similar phenomenon that in the approach of an integrated the pathogen was first weakened by the chemical subsequently effectively controlled for longer periods by the biocontrol agents.

The yield of soybean plants increased as a result of seed coating and soil application of *B. subtilis* isolates alone or in combination with the fungicides. Data presented in Table (3) show that, the seed coating and soil application increased the yield of soybean as dry seeds. Seed coating was more effective in increasing the yield than soil application.

Table (3): Effect of seed and soil application with powder formulation of *B. subtilis* isolates alone or combined with the fungicides Topsin-M or Rizolex on the yield (dry seeds gm / plot) of soybean under field conditions.

Treatments	Yield (gm / plot)		
Treatments	Seed coating	Soil application	
B. subtilis (Bs)1	847 ^{ab}	663ª	
Bs-4	763 ^{bc}	670ª	
Bs-6	657 ^{cd}	617 ^{ab}	
Topsin-M (T)	573 ^{cd}	540 ^b	
Rizolex (R)	613 ^{cd}	518 ^{bc}	
Bs-1 + T	990°	718ª	
Bs-4 + T	960 ^{ab}	667 ^a	
Bs-6 + T	882 ^{ab}	663ª	
Bs-1 + R	908 ^{ab}	677 ^a	
Bs-4 + R	· 878 ^{ab}	674 ^a	
Bs-6 + R	859 ^{ab}	687ª	
Control	548 ^d	417°	
LSD _{0.05}	177	109	

Values in each column followed by the same letter are not significantly different (p = 0.05) according to Duncan's multiple range test.

Seed coating with *B. subtilis* isolates in combination with the fungicides gaves the maximum increase in the yield (859 – 990 gm / plot) as compared to control (549 gm / plot) followed by *B. subtilis* isolates alone (657 – 847 gm / plot), the fungicides were more or less are similar to control treatment.

Soil application at all treatments increased the yield (617 - 718 gm / plot) as compared to control (417 gm / plot).

Results clearly indicated that the addition of biocontrol agent through seeds or soil increased the yield and this was reported in many research investigations and findings of several workers (Chao et al., 1986). The results suggest that the organism B. subtills may be act as a plant growth-promoting rhizobacterium which has potential for use in seed bacterization for higher growth and yield. Similar results were obtained by Singh and Mehrotra (1980), Kumar and Dube (1992), Kim et al. (1997), Liu and Sinclair (1991), Reddy and Rahe (1989).

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التطبيق الحقلى للباسياس سبتاس في صورة بودرة لمقاومة موت بادرات وعفن جذور فول الصويا

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الملخص العربي:

تسم إسستخدام بسودرة التلك في عمل مركب بودرة لثلاث عزلات من بكتريا الباسيلس سبتلس وتم حدوث نقص تدريجي في تعداد الخلايا الحية في المركب بمرور الوقت ولكنها كانت في أعداد كافية حتى ١٢٠ يوم من التخزين تجت ظروف الثلاجة حيث أعطت ١٢٠ ، ١٧ ، ٧٤ % حسيوية للسثلاث عزلات Bs-1, 4 and 6 وتحت ظروف الغرفة أعطت ٢٤ ، ٦٥ ، ٦٠ حيوية لنفس العزلات على التوالى .

تسم استخدام مركبات البودرة في تغليف البذور ومعاملة التربة والإثنين معاً ، وأظهروا فعالية في تقليل موت البادرات وعفن جذور فول الضويا تحت ظروف الحقل .

تغليف البذور كانت أكثر فعالية من معاملة التربة في تقليل موت البادرات وأعطت نسبة من ٧٦ إلى ٧٧% و ٦٧ إلى ٦٨% نباتات حية على التوالي .

تغليف البذور بالبودرة والمبيدات (توبسن – م أو ريزوكلس – ت كانت أكثر المعاملات فعالية وأعطت من ٨١ إلى ٨٦% نباتات حية مقارنة بالكنترول ٤٤%.

إضافة الباسيلس سبتلس في صورة بودرة إلى التربة كان أقل فعالية من تغليف البذرة في تقليل نسبة الإصابة بعض الجذور حيث أعطت من 11,9 إلى 11,8 ومن 11,9 على التوالى .

معاملة تغليف البذور بالبودرة والمبيدات توبسن - م أو ريزوكلس - ت كانت أكثر فعالية في تقليل عفن الجذور وأعطت من ٦ إلى ١١,١% عفن جذور مقارنة بالكنترول ٢٠٧٠.