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Antagonistic Activity of Selected Strains of Rhizobacteria Against *Macrophomina phaseolina* of Soybean Plants

[1]Ebtehag El-Barougy, [2]Nemat M Awad, [2]Azza Sh. Turky and [3]Hoda A Hamed

[1]Plant Pathology Res. Inst., Agric. Res. Center, Giza, Egypt
[2]Agric. Microbiology Dept. National Research Center, Cairo, Egypt
[3]Microbial Chemical Dept. National Research Center, Cairo, Egypt

Abstract: Root diseases caused by soil-borne pathogens are often main constraints in legume crop production. Chemicals are effective in controlling these pathogens but, these chemicals are expensive and not environmental friendly.Pots and field experiments were conducted in Ismailia, Agric. Res. Station to evaluate the impact of some strains of plant growth promoting (PGPR), plant growth promoting rhizobacteria (PGRP) (*Bradyrhizobium japonicum* strain USDA 110, *Azotobacter chroococcum*, *Azospirillum brasilense*, phosphate solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum*), potassium solubilizing bacteria (*B. cereus*) and *Pseudomonas fluorescens*) for their efficacy against *Macrophomina phaseolina* on soybean plants and to study their effect on percentage of healthy plant and growth of soybean. In pots, all tested PGPR were significantly decreased damping-off, rotted and wilted plants and increased healthy plants compared with the control (infested soil). *B. megaterium* showed the highest percentage of healthy plants. Data suggest the positive impact of PGPR in improving the stand and vigour of soybean plants in *Macrophomina* infested soil. In the field trial, results indicated that, all tested PGPR significantly decreased root rot and wilt disease incidence compared with the control (untreated seeds). *B. megaterium* treated plots was the most effective treatment followed by the combination of *Azotobacter chroococcum* and *Azospirillum brasilense* and *B. megaterium*. The reduction in disease incidence reflected on plant growth. Shoot weight and shoot height of plants grown in treated with plant growth promoting bacteria plot were greater compared with non treated ones.In view of the apparent bacterial plant growth promoting and bacterial biocontrol agents could provide a mean for reducing the incidence of root rot and wilt diseases complex of soybean in addition to avoiding the use of fungicides. Such biocontrol approach should be employed as a part of integrated pest management (IPM) system.

Key words: *Bradyrhizobium japonicum* · *Macrophomina phaseolina* · Plant growth promoting rhizobacteria (PGPR) · Soybean plants

INTRODUCTION

Leguminous crops have been cultivated since ancient time. They are a source of food for humans and feed for domestic animals as well as provide nitrogen for subsequent crops in the crop rotations. Nutritionally the legumes are complementary to cereals as a source of the amino acid lysine, which is limited in cereals, whilst cereals supply the amino acids methionine and cysteine, which are limited in legumes [1]. On the other hands, soybean *(Glycine max* (L.) Merr) has many benefits for human and animal nutrition. It can be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming systems [2,3].

Soil-borne fungal diseases are among the most important factors limiting the yield production of soybean in many countries, resulting in serious economic losses. Several soil-pathogens attack the roots and crowns of soybean plants [4-8]. *Macrophomina phaseolina* (Tassi) is also pathogenic to soybean roots causing damping off, charcoal rot [9-12]. These pathogens are difficult to control because of their persistence in the soil and wide host range. Some chemicals are effective in controlling these diseases but, these chemicals are expensive and not

Corresponding Author: Ebtehag El-Barougy, Plant Pathology Res. Inst., Agric. Res. Center, Giza, Egypt

environmental friendly. Therefore, alternative control methods are needed for managing these pathogens. Several alternative measures are being tested, natural resources such as biological control, found to be good and safe mean of diseases control especially the soilborne diseases. Many researchers have used bacterial biological control as a means of protection against soil-borne plant diseases as an alternative control method to fungicides [13]. From the plant growth promoting rhizobacteria *Bacillus* have shown promising results for the biological control of various plant pathogens as well as growth promoters of some crops [14,15].

Beneficial bacteria can be a significant component in the management of the soil environment so as to achieve attainable crop yield [16]. These beneficial bacteria live in the rhizosphere, the region around the root, which is rich in nutrients due to the exudation of 0.40 of plant nutrients from the roots [17]. By benefiting from the nutrients secreted by plant roots within the rhizosphere, the bacteria influence the plants in a direct or indirect way. One influence may be stimulation of plant growth [18]. Bacteria inhabiting the rhizosphere and positively influencing plant growth are referred to as plant growth promoting rhizobacteria (PGPR) [19]. Significant yield increases have been achieved in crops such as maize, rice, potato, wheat and canola after inoculation with PGPR [20-23] which resulted in increased interest in PGPRs [23,24]. There are several hypotheses about the mechanisms by which rhizobacteria enhance growth. One direct mechanism is production of the auxin indoleacetic acid (IAA) [25]. Another direct mechanism may be increased availability of nutrients in the rhizosphere by means of solubilization of unavailable forms of nutrients and/or production of siderophores [26,27]. Free living diazotrophic bacteria such as *Azosprillum* also are involved in promoting the growth of many tropical grasses, by fixing nitrogen asymbiotically and transferring it to the plant [28]. Biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzyme, hydrogen cyanide and siderophore or through competition for nutrient and space can significantly improve plant health and promote growth by increasing of seedling emergence, vigor and yield. Antibiotics produced by *Pseudomonas* species [29], *Bacillus cereus* [30] play an important role in the biological control of plant diseases. Seed and soil treatments with biocontrol agents *Bacillus megaterium*, *B. subtilis* and *Pseudomonas fluorescens* significantly reduced chickpea *Fusarium* wilt disease intensity and increased chickpea seed yield [31].

The objectives of the present study are therefore to evaluate some strains of PGPR for growth promotion of soybean plants under greenhouse and field conditions and to investigate their effect on percentage of healthy plant and growth of soybean plant in M. *phaseolina*-infested soil.Also, to study their effect on root rot and wilt disease complex of soybean in field under natural conditions.

MATERIALS AND METHODS

Microorganisms Used and Method of Inoculated *Macrophomina Phaseolina* Inoculum and Pathogenicity Test

Isolation of *M.phaeolinai* from Soybean: *M. phaseolina* was originally isolated on potato dextrose agar (PDA) medium from diseased soybean plants collected from different localities of Ismailia Governorate. Purification of the isolated fungus was carried out using hyphal tip techniques as described by Toussoun and Nelson [32]. Isolated fungus were identified according to their morphological characters according Nelson *et. al.*[33] and Barnett and Hunter [34].

Pathogenicity Test: The pathogenicity test of *M. phaseolina* was carried out at Ismailia Agric. Res. Station., on four cultivars of soybean, namely, Giza35, Giza111, Giza 22 and Kilarce. in pot infested soil using the homogenized culture technique

Preparation of Fungal Inoculum: The inoculum of *M. phaseolina* was prepared from one week old culture grown on 100 ml Potato Dextrose (PD) broth medium in flask (500 ml) and incubated at 25°C. The content of the flask were homogenized in a blender for one min. Pots (30cm in diameter) were filled with un-sterilized soil. On the other hand, soil infestation was performed by mixing the prepared inoculum of *M. phaseolina* with the upper layer of potted soil at the rate of 100 ml homogenized culture per pot, one day before planting. Four pots were used as replicates for each cultivar and another 4 pots with equal amount of sterile PD liquid medium without fungal inoculation were served as control. Ten seeds were sown in each pot. Percentages of pre and post emergence damping off were recorded 15 and 30 days respectively after planting. Survival of survived plants (healthy and infested) were calculated 3 months after seeding. Infested survival plants were evaluated three months after seeding any discoloration of internal tissue was recorded. Healthy survival plants = no visual evidence of disease. Disease severity indexing (DSI) was determined with scale proposed by Haware and Nene [35] based on 0-4 scale according percentage of foliage yellowing or necrosis (0=0%, 1=1-33%, 2=34-66%,3=67-l00%, 4= dead plant).

Bacterial Inoculums, Preparation and Inoculation Techniques: Highly efficient strains of plant growth promoting rhizobacteria (PGPR) (*Bradyrhizobium japonicum* strain USDA 110, *Azotobacter chroococcum, Azospirillum brasilense*, phosphate solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum*), potassium solubilizing bacteria (*B. cereus*) and *Pseudomonas fluorescens* were obtained from cultural collocation of Agric. Microbiology Dep. National Research Centre. The growth promoting rhizobacteria were independently grown in nutrient broth for 48 hours at 30°C in a rotary shaking incubator. The density of each bacterial culture in the broth was counted using a haemocytometer. Liquid broth cultures initially containing $8x10^7$, $7x10^8$, $5x10^7$ and $3x10^7$ viable cell/ml respectively. In PGPR treatments, 10 ml of either tested microorganisms suspension were added to the soil in each pot just after sowing. In field inoculation, 100 ml of each microorganism was just to the pot after planting.

Effect of some strains of plant growth promoting rhizobacteria (PGPR) on controlling damping off and survived soybean seedlings grown in soil infested with *M. phaseolina*

Pots experiment: In this experiment, PGPR were used to evaluate their efficiency in controlling root rot disease (*M. phaseolina*) of soybean. Soybean seeds Giza 35 (10 seeds/pot) were sown in 30-cm pots filled with unsterilized *M. phaseolina* infested soil at the rate of 100 ml homogenized culture per pot as previously mentioned, one day before planting. All plants were inoculated with *Bradyrhizobium japonicum* strain USDA 110. *Rhizobium* inoculation was done by 10 ml per pot. The treatments were as follows: plant inoculated with *Azotobacter chroococcum* and *Azospirillum brasilense* and/or *B. megaterium* and/or *B. cereus* and/or *Pseudomonas fluorescens*. The control treatments were soil infested with *M. phaseolina* was used as control 1 and uninfected soil as control 2. A set of 4 pots for each treatment were used. Each pot received equal amounts of water. Other agricultural processers were performed according to normal practice. Percentages of pre and post emergence damping off were recorded 15 and 30 days after planting, respectively. Healthy and infected survival plants were evaluated 3 months after seeding. Plant growth parameters (shoot weight, plant height, number of pods and seed weight/plant (aver. of 5 plants) were also recorded three months after planting.

Disease Severity of Root Rot: Disease severity of root rot and any discoloration of internal tissue were recorded. Severity of inside browning of internal tissue was recorded and conducted with scale proposed by Haware and Nene[35] based on 0-4 scale according percentage of foliage yellowing or necrosis (0=0%, 1=1-33%, 2=34-66%,3=67-loo%, 4= dead plant).

Field Experiment: An experiment was conducted in a field at Ismailia Agric. Res. Station for controlling root rot and wilt diseases complex of soybean in naturally infested field by soil treatment with some bacterial plant growth promoting and bacterial biological agents. The same treatments in pot experiment were used arranged in a complete randomized block designed with four replicates. The field plot was 3x3 m with 5 rows, 200 seeds were sown in each plot (40 seed/row). Plant samples were taken at 15 and 30 days after planting, respectively to observations the percentages of pre and post emergence damping off. Healthy and infected survival plants were evaluated 3 months after seeding. Plant growth parameters (Shoot weight, plant height, number of pods and seed weight /plant (aver. of 5 plants) were also recorded three months after planting.

Disease Severity of Root Rots and Wilt Disease Complex: Incidence (%) of diseased plants (Total number of dead plants/Total number of plants at plots (%) was calculated 2 and 3 months after planting. Disease severity was also recorded on a random sample of plants of the plots (20 plants) four months after planting. Disease severity indexing (DSI) of root rot and any discoloration of tissue were recorded according to based on 0-4 scale according percentage of foliage yellowing or necrosis Haware and Nene [35] based on (0=0%, 1=1-33%, 2=34-66%,3=67-loo%, 4= dead plant).

Statistical Analysis: All the data were statistically processed by the analysis of variance and by determining the significance threshold using Duncan's test [36].

RESULTS

Isolation of *M. Phaseolina* from Soybean and Pathogenicity: Isolation trails from rotted plants collected from different localities of Ismailia Governorate Fig. (1) yielded a fungus which was

(A) (B) (A) (B)

Fig. 1: Typical symptoms of root rot (natural infection)
A: Healthy soybean plant, B: Rotted soybean plant

(A) (B)

Fig. 2: Artificial inoculation in soil infested with *M. phaseolinai* one weak, before sowing soybean seeds of Giza 35 cultivar.
A= Non-infested soil (Control), B =Soil infested with *M. phaseolina*

identified as *Macrophomina phaseolina*. In seedling stage, infested plants may be seen with damping off symptoms. In adult stage the plant rotted. Disease symptoms attributed to *M. phaseolinai* were observed on soybean plants grown in soil artificially infested with the tested fungus in pots experiment (Fig.2). Symptoms were almost similar to those noticed under the field conditions.

Pathogenicity Test: *M. phaseolina* isolated from the roots of soybean plants was tested for the pathogenicity on seedling of Giza35, Giza22, Giza 111 and Kilarce soybean cultivars. Data were recorded as percentage of pre- and post-emergence damping-off and survival plants at 15, 30, 90 days after planting, respectively. With respect to the percentage of pre-emergence damping-off the cultivar Giza 111exhibited the least infection level which reached 20% followed by Kilarce, Giza 35 which recorded 26.7% and 23.3% while, Giza 22 cultivar suffered 53.3% pre-emergence infection. Post-emergence infection ranged between 3.3 recorded for Giza 22 and 20% recorded for Giza 111. Surviving plants of each cultivar were examined to identify those which were infected and continued to grow (infected survivals), thus were categorized into healthy and infected. All cultivars were almost similar in the percentage of infected survivals ranging from 6.7 to 16.7%. Data in Table 1 revealed that Giza 111 showed

Table 1: Pathogenicity with *M phaseolini* isolated from soybean roots on four cultivars

	Macrophomina phaseolina				
	% Damping off		% Survival plants		
Cultivars	Pre-emergence	Post- emergence	Infested survival	Healthy survival	Disease severity Score
Giza 35	233.0b	20.0a	10.0a	46.7ab	2.5
Giza 111	20.0b	13.3a	6.7a	60.0a	2.2
Giza 22	53.3a	3.3a	16.7a	26.7b	3.7
Kilarce	26.7b	13.3a	16.7a	43.3a	2.3
L.S.D	28.7	26.6	16.3	17.2	

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

Table 2: Effect of some bacterial plant growth promoting (PGPR) on damping off and survival plants of soybean seedling infected with *Macrophomina phaseolina*

	Macrophomina phaseolina				
	% Damping off		% Survival plants		
Treatments	Pre-emergence	Post- emergence	Infested survival	Healthy survival	Disease severity Score
Azc+Asb	12.5bc	5.0bc	15.0a	67.0ba	1.6c
BC	12.5bc	12.5ab	2.5b	72.5ab	1.8bc
BM	20.0ab	2.5bc	2.5b	75.0ab	1.3cd
PF	12.5ab	2.5bc	0.0b	62.5bc	2.3b
Con No.1	25.0a	20.0a	17.5a	37.5c	3.6a
Con No.2	5.0c	0.0c	0.0b	95.0a	0.7d
LSD	11.1	10.6	8.02	20.06	0.6

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

the highest percentage of healthy plants (60) followed by Giza 35 (46.7). On the other hand, Cv.22 was more susceptible to *M. phaseolina* showing only 6.7% healthy plants. Disease severity was also recorded in Table 1, the lowest degree was obtained from Giza 111 cultivar (2.2) but the highest one (3.7) was observed on Giza22.

Effect of Some Strains of Bacterial Plant Growth Promoting (PGPR) on Controlling Damping off and Survived Soybean Seedlings Grown in Soil Infested with *M. Phaseolina*

Pots Experiment: In this experiment, PGPR (*Azotobacter chroococcum, Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum*, *B. cereus* and *Pseudomonas fluorescens*) were used to evaluate their efficiency in controlling root rot disease (*M. phaseolina*) of soybean. Data in Table 2 show that all bacterial plant growth promoting decreased damping off and percentage of infested plants and increased healthy plants compared with the control treatment. Data indicate that soil infested with *M. phaseolina* used as a control show the highest percentage of pre emergence damping off (25%) compared with the lowest percentage (5%) recorded from non-infested soil (control No. 2) followed by soil artificially infested and treated with *B. megaterium* (20%) while, 12.5% obtained from all of *A. chroococcum* + *A. brasilense, B. cereus*, *Pseudomonas fluorescens* treatments. For post emergence damping off, Control No.1 recorded the highest percentage of infected plants (20) but the lowest percentage which reached 2.5% was obtained from both of *B. cereus* and *Pseudomonas fluorescens* treatments. Concerning the percentage of healthy plants, the most effective treatment was *B. megaterium* at (75%), however *B. cereus* showed 72.5% followed by *A. chroococcum* + *A. brasilense* (67.5%) while *Pseudomonas fluorescens* showed 62.5 % healthy survival compared with the lowest percentage (37.5%) recorded from infested soil (control No. 1). Root-rot severity was 3.6 when *M. phaseolina* was applied alone (control1) and severity was reduced to 2.3, 1.8, 1.6 and 1.3 in plants grown from seed treated with *Pseudomonas fluorescens*, *Bacillus cereus*. *A. chroococcum* + *A. brasilense* and *Bacillus megaterium*

Table 3: Effect of some bacterial plant growth promoting on shoot height, shoot weight, No. of pods, Seed weight of soybean plants as a mean of 5 plants

	Parameters growth			
Treatments	Shoot height (cm/plant)	Shoot weight (g/plant)	No. of pods/plant	weight of 100 Seed
Azc+Asb	35.0bc	30.3bc	18.75bc	8.2bc
BC	31.3cd	27.0cd	22.5b	9.5abc
BM	36.7b	34.2ab	28.8a	10.3ab
PF	33.3cd	25.0d	28.3a	10.7ab
Con No.1	16.3d	13.2c	12.0c	4.6c
Con No.2	40.0a	35.6a	32.0a	14.1a
LSD	2.6	4.8	6.8	4.7

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

Table 4: Effect of bacterial plant growth promoting and bacterial biocontrol agents alone or in combination on root rot and wilt diseases complex of soybean plant under field condition

	Incidence (%) of diseased plants					
	One months after sowing		Two months after owing		Three months after owing	
Treatments	Disease Incidence %	Reduction over control (%)	Disease Incidence %	Reduction over control(%)	Disease Incidence %	Reduction over control(%)
Azc+Asb	7.0d	89.20	25.0e	70.5	30.0c	66.6
Bc	39.0b	40.00	46.7cd	45.5	57.5b	35.5
Bm	12.3cd	81.07	15.0f	82.3	11.3d	87.5
Pf	33.2bc	48.90	40.0d	52.9	37.5c	58.3
Azc+Asb + Bc	16.0cd	75.30	67.5b	26.4	62.5b	30.5
Azc+Asb+ Bm	24.0bcd	63.07	20.0ef	76.5	31.2c	63.3
Azc+Asb+Pf	27.3b	58.00	55.0c	35.3	37.5c	57.3
Azc+Asb+ Bc+Bm+Pf	43.0 b	33.80	50.0c	41.2	52.5b	41.6
Control	65.0a	-	85.0a	-	90.0a	-
LSD	15.8		9.02		10.11	

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

respectively, when sown in soil infested with *M. phaseolina* comparing with the value of 0.7 which recorded from plants grown in non- infested soil (Control No.2).

Effect of Some Strains of Bacterial Plant Growth Promoting (PGPR) on Parameters Growth of Soybean Plants Grown in Soil Infested with *M. Phaseolina*: Data presented in Table 3 revealed that the application of *Azotobacter chroococcum, Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum*, *B. cereus* and *Pseudomonas fluorescens* with the pathogen caused a significant increase in shoot height. Shoot weight, no. of pods and seed weight over the control with pathogen without any antagonists. The data showed that the lowest values of plant height, shoot weight, no. of pods and seed weight (16.3 cm, 13.2 g, 12 pods and 4.6g / plant respectively, as a mean of 5 plants) were observed with the control No. 1, where soil infested with *M. phaseoli* without any treatments, while the greatest plant height, shoot weight, no. of pods and Seed weight of soybean plants grown in *M. phaseoli* - infested soil (36.7cm, 34.2g, 28.8 pods and 10.4g) was recorded from *B. megaterium* treatment compared with 40cm, 35.6g, 32 pods and 14.1g/plant obtained from soybean plant, growing in non infested soil (control No. 2).

Field Experiment: The effect of PGPR (*Azotobacter chroococcum*(Azc), *Azospirillum brasilense* (Asb), *Bacillus megaterium* var. *phosphaticum* (Bm), *B. cereus* (Bc) and *Pseudomonas fluorescens* (Pf)) alone and in combination on incidence of root rot and wilt diseases complex of soybean under field is shown in Table 4.

The incidence of diseased soybean plants (7%) recorded from plants grown in plots treated with *Azotobacter chroococcum*+ *Azospirillum brasilense* followed by *B. megaterium* (12.3%), *Pseudomonas fluorescens* (33.2%) and *B. cereus* (39 %) compared with

Table 5: Severity of root rot and wilt diseases complex on soybean plant under field condition

	Diseases severity Index**		
Treatments	One months after sowing	Two months after sowing	Three months after sowing
Azc+Asb	1.5b	2.0ef	2.6c
Bc	2.3ab	2.8cd	3.5ab
Bm	1.3b	1.8c	1.4d
Pf	1.5b	2.5cde	2.8bc
Azc+Asb + Bc	2.0ab	3.3ab	3.5ab
Azc+Asb+Bm	1.5b	3.0abc	2.3c
Azc+Asb+ Pf	1.5b	2.3def	2.5c
Azc+Asb+Bc+Bm+Pf	1.5b	2.7def	3.0abc
Control	3.0a	3.6a	3.8a
LSD	1.13	0.66	0.37

**Disease severity of root rot and any discoloration of tissue were recorded according to Haware and Nene (1980) based on 0-4 scale according percentage of foliage yellowing or necrosis (0=0%, 1=1-33%, 2=34-66%,3=67-loo%, 4= dead plant)

Table 6: Effect of bacterial plant growth promoting alone or in combination on plant growth parameters of soybean plant grown in natural infested soil

	Plant growth parameters			
	Shoot height (cm/plant)		Shoot weight (g/plant)	
	Shoot height	Increasing over control(%)	Shoot weight	Increasing over control(%)
Azc+Asb	47.8b	58.3	15.5b	307.8
BC	37.2c	24.0	5.9c	55.2
BM	65.4a	108.0	17.4ab	355.2
PF	62.6a	108.6	15.6b	310.5
Azc+Asb+ BC	39.8bc	32.6	4.8c	26.3
Azc+Asb+ BM	78.6a	162.0	18.7a	392.1
Azc+Asb+ PF	47.0bc	56.6	5.8c	52.6
Azc+Asb+BC+BM+PF	48.4bc	60.0	4.1c	7.8
Control	30.0c		3.8c	
LSD	9.16		4.27	

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test

the highest percentage (65%) in the untreated plots (control) one month after sowing. It is also obvious that the lowest incidence (15% and 11.3 %) of diseased soybean plants recorded 2 and 3 months after sowing respectively from plots treated with *B. megaterium* followed by *Azotobacter chroococcum, Azospirillum brasilense* (25 % and 30%), *Pseudomonas fluorescens* (40% and 37.5%) and *B. cereus* (46.7% and 57.5%) compared (85% and 90%) in the untreated plots (control). Data in Table (4) also show the effect of PGPR in combination on incidence of root rot and wilt diseases complex of soybean. It is noticed that protection against infection occurred, where The incidence of diseased soybean plants with both of (Azc+Asb) and (Bm) in combination decreased from 65 to 24%, from 85 to 20% and from 90 to 31.2% one, two and three months after sowing respectively (Table 4).Significantly differences were realized between the control and other treatments.

Effect of Bacterial Plant Growth Promoting Alone or in Combination on Severity of Root Rot and Wilt Diseases Complex: In this experiment, the effect of PGPR on severity of root rot and wilt diseases complex of soybean plants under field is shown in Table 5 disease severity was recorded on a random sample of some plants of each plot, 0ne, two and three months after sowing (Table 5). Results show that, *B. megaterium* treatment gave the lowest value of degree (1.3, 1.8 and 1.4 one, two and three months after sowing respectively) comparing with the control treatment which revealed the highest value of degree (3.0, 3.6 and 3.8) from the sample were noticed one, two and three months after sowing, respectively. Generally, data in Table (5) showed that the most effective treatment was *B. megaterium* followed by the combination of *Azotobacter chroococcum, Azospirillum brasilense* and *B. megaterium*.

(C) (A1) (C) (A2) (C) (A3)

Fig. 3: Effect of inoculation with plant growth promoting bacteria on plant height of soybeanin in field experiments
C=Uninoculated plants, A1=Inoculated plants with *Azotobacter chroococcum+Azospirillum brasilense*
A2=Inoculated plants. with *Bacillus megaterium,* A3=Inoculated plants with *Azotobacter chroococcum +Azospirillum brasilense* in combination with. *Bacillus megaterium*

Effect of Bacterial Plant Growth Promoting Alone or in Combination on Plant Growth Parameters of Soybean Plant Grown in Natural Infested Soil: Data presented in Table 6 and illustrated in Fig. 3 indicate that the highest value of plant high (78.6cm) was obtained from *Azotobacter chroococcum, Azospirillum brasilense* and *B. megaterium* in combination followed by 65.4cm which recorded from treatment of B. *megaterium* alone. Meanwhile, control recorded the lowest value of plant high (30cm) as compared with other treatments. The same trained was noticed with shoot weight. The most effective treatment was also the combination of *Azotobacter chroococcum, Azospirillum brasilense* and *B. megaterium* treatment (18.7g) compared with 3.8g/plant which obtained from the control treatment.

DISCUSSION

Isolation trails from rotted soybean plants yielded *Macrophomina phaseolina* conforming to other reports [5,11,12,37,38]. They reported that *M. phaseolina* is one of the most important pathogens of soybean. Pathogenicity test was conducted and led to symptoms which were almost similar to those noticed under field conditions. The present investigation demonstrated that the isolated fungus from naturally infested field could reduce seedling emergence and healthy plants and could directly affect yield.

The use of pesticides to control soil-borne diseases and pests of economically important crops has been used in agriculture for many years. However, recently the use of chemical has been reduced for several reasons, including pollution of environment, particularly ground water and food supplies. Recently, an increasing desire to reduce the use of pesticides is seen through the attempts to develop integrated pest management approaches, where natural resources are put to maximum use. Biological control is high on the list of potential alternative tactics. However, the use of pesticides will continue but at lower rate, wherever it is necessary. Therefore, it was thought to be of value to evaluate a series of bacterial biocontrol agents to be included in the protection of the crop. The effect of some bacterial plant growth promoting [*Azotobacter chroococcum* (Azc), *Azospirillum brasilense* (Asb), *B. cereus* (Bc), *Bacillus megaterium* (Bm) and *Pseudomonas fluorescens* (Pf)] were evaluated in pots against *M. phaseolina* infecting soybean plants. Bacterial plant growth promoting (PGPR) can promote plant growth directly or indirectly. Indirect effects are related to production of metabolites, such as antibiotics, that decrease the growth of phytopathogens and other deleterious microorganisms [30,39, 40]. Direct effects are dependent on production of plant growth regulators. Some rhizobacterial strains promote legume nodulation and nitrogen fixation by producing flavonoid-like compounds and/or stimulating the host legume to produce more flavonoid signal molecules [41]. Nodulation and subsequent nitrogen fixation by soybean plants are inhibited by low root zone temperatures (RZTs). Plant growth promoting bacteria can help overcome these deleterious effects. Yuming *et al.*, [42] reported that three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 were shown to have plant growth promoting activity on pouch-grown soybean plants under greenhouse conditions at low root zone temperatures (RZTs) and under field conditions in a short growing season area. And provided increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain yield of soybean plants.

The obtained results revealed that percentage of healthy plants remaining in the pots, depending on the treatments. In the infested pots, non-treated with Bacterial plant growth promoting (control No.1), only about third of the plants were still alive at the end of season. However, it has been found that sufficient control of damping off was obtained by using bacterial agents. All pots treated with *Azotobacter chroococcum* (Azc), *Azospirillum brasilens* (Asb), *B. cereus* (Bc), *Bacillus megaterium* (Bm) and *Pseudomonas fluorescens* (Pf) decreased significantly damping-off as well as infested survival plants caused by *M. phaseolina* and increased healthy survival plants, Plant height and shoot weight over the control, these findings are in agreement with those recorded by many researcher such as Zheng and Sinclair [43] showed that there was a significant positive correlation (r2 = 0.78) between root colonization by *Bacillus megaterium* strain B153-2-2 or its mutants and suppression of Rhizoctonia root rot of soybean plants. Whips [44] reported that many bacterial genera are being used and tested in bacterization including, *Azospirillum, Azotobacter, Bacillus, Bradyrhizobium* and *Pseudomonas* to enhancement symbiotic or associative nitrogen fixation, degradation of xenobiotic compounds, plant growth promotion and biological control of plant pathogenic [31] proved that seed and soil treatments with biocontrol agents *Bacillus megaterium, B. subtilis* and *Pseudomonas fluorescens* significantly reduced chickpea *Fusarium* wilt disease intensity and increased chickpea seed yield. Akhtar and Siddiqui [45] they mentioned that *Pseudomonas alcaligenes* and *Bacillus pumilus* decreased disease incidence caused by *M. phaseolina* in chickpea plants. The mechanism by which bacterial biocontrol agents affecting fungal growth may be attributed to the presence of some effective substance such as antibiotics which play an important role in the biological control of plant diseases. Many investigators confirmed these results such as Liu and Sinclair [46,47] and Handelsman *et al.*, [48].

Effect of bacterial plant growth promoting on root rot and wilt disease complex of soybean plant under field condition was also studied. Data showed that soil treated with *Azotobacter chroococcum*(Azc)+*Azospirillum brasilens* (Asb) or *Bacillus cereus* (Bc) or *Bacillus megaterium* (Bm) or *Pseudomonas fluorescens* (Pf) alone significantly reduced diseased plants comparing with the control (untreated). The reduction reached 66.6, 35.5, 87.5 and 58.3% respectively, when recorded three months after sowing. But reached 30.5% from combined treatments of Azc+ Asb plus Bc and reached 63.3% from Azc+Asb plus Bm while, 57.3% from Azc+Asb plus (Pf) and 41.6% from the combined of all treatments (Azc+Asb plus Bc plus Bm plus Pf). The application of *Azotobacter chroococcum, Azospirillum brasilense* or *Bacillus megaterium* var. *phosphaticum* or *B. cereus* or *Pseudomonas fluorescens* treatments alone resulted in higher survival plants than did these treatments in combination. Generally, data showed that Bc was the most effective treatments followed by the combination of Azc+Asb and Bc.

The reduction in disease incidence reflected on plant growth. Shoot weight and shoot height of plants grown in treated with bacterial biocontrol plot were greater compared with non treated ones. These results are relatively similar to those obtained by Yuming *et al.* [42] they reported that three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 provided increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain yield of soybean plants.

In view of the apparent bacterial plant growth promoting and bacterial biocontrol agents could provide a mean for reducing the incidence of root rot and wilt diseases complex of soybean in addition to avoiding the use of fungicides. Such biocontrol approach should be employed as a part of integrated pest management (IPM) system.

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