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Antagonistic Activity of *Bacillus* and *Pseudomonas* Isolates Alone or in Combination with Fungicides Against Some Soil Borne Plant Pathogens under Laboratory and Greenhouse Conditions

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Abstract: Nine bacterial isolates belongs to *Bacillus* and *Pseudomonas* genera were tested for their antagonistic activity alone or in combination with fungicides against some soil borne plant pathogens under laboratory and greenhouse conditions. Laboratory studies reveal that all bacterial isolates were highly tolerance to all fungicides at 25, 50 and 100 ppm and they were slightly tolerance at 200 and 400 ppm. According to turbidity values estimated as optical density (OD), the reduction in the growth of bacterial isolates caused by each of mancozeb, metalaxyl M + mancozeb, pencycuron, thiophanate-methyl and thiram + tolclofos-methyl, at 25 ppm, were in the range of 5.1 to 35.9, 0.1 to 12.2, 1.6 to 21.1, 4.3 to 34.5 and 1.0 to 51.8%, respectively. *Bacillus pumilus* (Bp_2, Bp_2 and Bp_3), *B. subtilis* (Bs_3), *P. fluorescens* (Pf_2), Pf_1, *B. megaterium* (Bm), Bs_2 and Bs_1 isolates was highly tolerance to pencycuron at 25 ppm, which recorded reduction in (OD) estimated as 1.6, 2.4, 4.1, 4.4, 5.2, 7.1, 8.5, 20.5 and 21.1%, respectively. Under laboratory conditions, the tested bacterial isolates in combination with the fungicide pencycuron at 25 ppm were highly reduced the growth of soil borne pathogens than bacterial isolates alone. Isolates of Bs_3, Bp_3 and Pf_2 reduced the growth of *F. solani* by 30.0, 54.7 and 75.6%, when used alone, while the reduction attained to 55.6, 61.1 and 88.9%, when used in combination with pencycuron at 25 ppm, respectively. Greenhouse studies evaluated the effect of soil infestation with each of Bs_3, Bp_3 and Pf_2 isolates inocula alone or in combination with the fungicide pencycuron as seed dressing on the incidence of bean damping-off and root rot diseases caused by *F. solani*. Obtained results reveal that application of each of Bs_3, Bp_3 and Pf_2 isolates in combination with pencycuron is a promising approach for the improvement of bean root rot control, bean growth parameters in terms of shoot and root length (cm), leaves number per plant, fresh and dry weight (g) of plant, as well as reduction of the dose of pencycuron.

Key words: *Bacillus* and *Pseudomonas* Isolates · Antagonistic Activity · Combination with Fungicides and Soil Borne Plant Pathogens

INTRODUCTION

Control of the plant diseases using chemicals sometimes cause serious ecological problems. In recent years, the increasing use of potentially hazardous pesticides and fungicides in agriculture has been the result of growing concern of both environmentalists and public health authorities. Biocontrol agents can be used for controlling several diseases such as damping-off, root-rot, seed decay, collar rot, crown rot and wilt as well as foliar diseases. Biological control methods are safe and environmental friendly pesticides alternatives in agriculture application [1]. But biological control alone sometimes un-sufficient to eradicate or reduced the propagules of the pathogens. Therefore integration methods between biocontrol agents and the fungicides could be effective. The tolerance of bacterial biocontrol agents to fungicides was studied by many workers over the world. Malathi *et al*. [2] tested the systemic fungicides *viz*., thiophanate methyl and carbendazim combined with *Pseudomonas fluorescens* Migula for managing sugarcane red rot disease caused by *Colletotrichum*

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falcatum Went. They found that the growth of *P. fluorescens* (about 11 strains) was not affected up to 500 ppm of those systemic fungicides *viz*., thiophanate methyl and carbendazim, according to turbidity values of bacterial growth *in vitro* tests. They added that in pots experiment the treatment receiving thiophanate methyl and *P. fluorescens* recorded the maximum plant survival of 81.67%. *Bacillus subtilis* Chon and *P. fluorescens* also were tolerance to azoxystrobin 23 SC *in vitro* at high concentration of 300 ppm [3]. Also, Devi and Prakasam [4] showed that *B. subtilis* and *P. fluorescens* were tolerance to azoxystrobin 25 SC at the concentrations of 5, 10, 50, 100 and 250 ppm. The tolerance of three isolates of each *Bacillus* and *Pseudomonas* with common used fungicides in tea plantation such as hexaconazole, carbendazim, mancozeb and copper oxychloride were tested *in vitro* by Pallavi *et al*. [5]. They reported that all the six selected biocontrol isolates showed growth tolerance in carbendazim, followed by hexaconazole. Valarmathi *et al*. [6] reported that *B. subtilis* and *P. fluorescens* were also tolerance to copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm. The tolerance of *B. subtilis* and *P. fluorescens* to carbendazim, mancozeb, metalaxyl, captan, thiram and nemacur, commonly used for the control of soil borne plant pathogens, was studied by Mohiddin and Khan [7]. They found that *P. fluorescens* was more tolerance to fungicides than *B. subtilis* and the maximum tolerance concentration for the former being 2500 µg/ml of thiram, 1600 µg/ml of mancozeb and 50,000 µg/ml for captan and carbendazim.

Bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for human consumption in the world. Several soil borne fungi attacked bean plants causing damping-off and root rot diseases. Thes diseases were commonly attributed to *Fusarium solani* (Mart.) Sacc. in complex with *Rhizoctonia solani* and *F. oxysporum* [8-10]. The disease incidence of damping-off and bean root rot has risen with the increased acreage of this crop, the shortening of crop rotation intervals and the use of highly susceptible cultivars. Yield losses in several infested areas approach 50% [11, 10].

The research was conducted (i) to study the inhibitor effect of five commercial fungicides against the growth of both some soil borne fungal pathogens and the nine bacterial isolates belongs to *Bacillus* and *Pseudomonas* genera *in vitro*; (ii) to evaluate the antagonistic activity of the nine bacterial isolates alone or in combination with fungicides against some soil borne plant pathogens *in vitro* and (iii) to determine the effect of bacterial isolates alone or in combination with the fungicide pencycuron on the incidence of bean damping-off and root rot caused by *F. solani* under greenhouse conditions.

MATERIALS AND METHODS

***Bacillus* and *Pseudomonas* Isolates:** Nine bacterial isolates *viz*., three isolates of *B. subtilis* (Bs_1, Bs_2 and Bs_3), three isolates of *B. pumilus* Meyer and Gottheil (Bp_1, Bp_2 and Bp_3), one isolates of *B. megaterium* de Bary (Bm) and two isolates of *P. fluorescens* (Pf_1 and Pf_2) used in this study, were isolated from Egyptian soil using serial dilution method [12]. Bacterial isolates were identified in Plant Pathology Department, National Research Centre (NRC) according to their morphological and cultural characterestics as described by Dowson [13] and Lelliott and Stead [14]. Bacterial isolates were stained by Gram staining. Finally, several physiological and biochemical tests were carried out according to Bergey's Manual of Systematic Bacteriology [15].

Soil Borne Plant Pathogens: Five soil borne plant pathogens, *i.e. Fusarium oxysporum* Schlecht. Emend. Snyd. Et Hans, *F. solani, Rhizoctonia solani* Kühn, *Macrophomina phaseolina* (Tassi.) Goid and *Sclerotinia sclerotiorum* (Lib.) de Bary, obtained from Plant Pathology Department, National Research Centre (NRC), were used in this study.

Fungicides: Five commercial fungicides *viz*., mancozeb (Tridex 80%), metalaxyl M + mancozeb (Ridomil Glod 68%), pencycuron (Monceren 25% WP), thiophanate-methyl (Topsin-M 70% WG) and thiram + tolclofos-methyl (Rizolex T 50% WP), were obtained from the Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt.

Laboratory Experiments

Inhibitor Effect of Tested Fungicides Against Some Soil Borne Fungal Pathogens: Effect of all tested fungicides at the concentrations of 0, 100, 200, 400 and 600 ppm against the radial mycelial growth of *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. sclerotiorum* were evaluated *via* the poisoned food technique described by Schmitz [16] and Borum and Sinclair [17]. The experiment was conducted in Completely Randomized Design. The autoclaved potato dextrose agar (PDA) medium was used. Fungicides concentrations were prepared based on their active ingredient and then was added to PDA medium before its solidification to obtain the proposed concentrations of 0, 25, 50, 100, 200 and 400 ppm. About 0.1ml of Tween 80 (Sigma) was mixed gently with medium to enhance solubility. Then, 15 ml of fungicide amended PDA medium was poured in sterilized Petri plates (9 cm-diameter). The amended medium was allowed to solidify. Tween 80 amended PDA medium without fungicide was kept as

control. Then, 0.5 cm fungal mycelial disc of each *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. sclerotiorum* was picked from 7-days-old purified culture with the help of a sterilized cork borer and then the disc was inoculated in the center of each plate. Four Petri plates were used as replicates for each treatment as well as untreated control. The diameter (cm) of each soil borne fungi growth was measured when the fungal growth of the control plates reached full growth of the Petri plate. The percent inhibition in radial mycelial growth of the pathogenic fungi caused by fungicides over the control was calculated using the formula given by Vincent [18] as follow:

Pathogen growth inhibition (%) = [(dc-dt)/dc] × 100

where
dc = Average diameter of pathogen growth in control. dt = Average diameter of pathogen growth in fungicide treatment.

Inhibitor Effect of Tested Fungicides Against the Growth of Bacterial Isolates: The tolerance of Bs_1, Bs_2, Bs_3, Bp_1, Bp_2, Bp_3, Bm, Pf_1 and Pf_2 isolates to the tested fungicides was evaluated *via* the turbidometric method [19]. The Nutrient Broth medium NB (g/l): peptone 5 g, beef extract 3 g, sodium chloride 5 g, glucose 20 g, pH 7; was dispersed in 50 ml quantities into 250 ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min. Fungicides concentrations were prepared based on their active ingredients and then added to NB medium to obtain the proposed concentrations of 0, 25, 50, 100, 200 and 400 ppm and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Flasks containing NB medium with 0.1% Tween 80 only was kept as control. Then, one ml of the bacterial suspension (10^7-10^9 colony forming units (CFU)/ml of bacterial antagonist) grown on NB medium for 48 h was transferred to each flask. Five flasks were used as replicates for each treatment as well as the control. The inoculated flasks were incubated at 28±2°C in a shaker incubator for 48h. At the end of incubation period the optical density values of the culture broth were determined using Unic UV-2000 Spectrophotometer at 610 nm [4]. The percent reduction in bacterial growth of *Bacillus* and *Pseudomonas* isolates in fungicides treatments was calculated based on the optical density value in the control using the following formula:

Bacterial growth reduction (%) = [(A-B)/A] × 100

where,
A = Optical density (OD) value in control and B = OD value in fungicide treatment.

Antagonistic Activity of *Bacillus* and *Pseudomonas* Isolates Against Some Soil Borne Fungal Pathogens: The antagonistic effect of Bs_1, Bs_2,Bs_3, Bp_1, Bp_2, Bp_3, Bm, Pf_1 and Pf_2 isolates against some soil borne plant pathogens were tested *via* the dual culture technique using the method described by Estrella *et al*. [20]. Each bacterial isolate was cultured (by streaking) at 1cm from the edge of a 9-cm diameter Petri plate containing freshly sterilized PDA medium. On the opposed position 1cm away from the other set of the plate a 5-mm plug taken from the leading edge of a 5-days old culture of *Fusarium solani*, *F.oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotinia sclerotiorum*, was cultured individually. For untreated plates, an agar disc (5-mm in diameter) of pathogenic fungi only was placed at 1cm from the edge of a 9-cm diameter Petri plate containing PDA medium. Five plates were used as replicates for each treatment as well as the control. Inoculated plates were incubated at 25 ± 1°C except for *Sclerotinia sclerotiorum* which was incubated at 20±2°C until the fungal growth of the control plates reached full growth of the plate. The reduction in mycelial growth of the pathogenic fungi was calculated using the formula suggested by Pandy *et al*. [21] as follows: R = C – T / C × 100, whereas: R = Mycelial growth reduction (%) of the pathogen, C = Radial growth of the pathogen in control plates (cm) and T = Radial growth of the pathogen in dual culture plate (cm).

Antagonistic Activity of *Bacillus* and *Pseudomonas* Isolates in Combination with the Fungicide Pencycuron Against Some Soil Borne Fungal Pathogens: The antagonistic effect of Bs_1, Bs_2,Bs_3, Bp_1, Bp_2, Bp_3, Bm, Pf_1 and Pf_2 isolates in combination with the fungicide pencycuron at 25 ppm (the low inhibitor concentration against bacterial isolates), against soil borne plant pathogens, were assessed by poisoned food technique and dual culture technique using PDA medium according to the method described by Bardia and Rai [22]. The prepared PDA medium was dispersed in 200 ml quantities into 250 ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min. Pencycuron concentration were prepared based on its active ingredients and then added

to PDA medium before its solidification to obtain the concentration of 25 ppm and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Then 15 ml of fungicide amended PDA medium was poured in sterilized 9 cm Petri plates. The amended medium was allowed to solidify. Each bacterial isolate was cultured (by streaking) at 1cm from the edge of fungicide amended Petri plate. On the opposed position 1cm away from the other set of the plate a 5-mm plug from the leading edge of a 5-days old culture of *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. sclerotiorum*, cultured on PDA medium were inoculated individually. Fungicide amended PDA medium inoculated with the pathogen only served as control. Five plates were used as replicates for each treatment as well as the control. Inoculated plates were incubated at 25 ± 1°C except for *Sclerotinia sclerotiorum* which was incubated at 20±2°C until the fungal growth of the control plates reached full growth. Inhibition of the pathogenic fungal growth (%) was calculated as mentioned before.

Greenhouse Experiment: In this experiment the promissing treatments of bacterial isolates, fungicide and combination between them in controlling Fusarium damping-off and root rot diseases of bean plants were selected as a model for semi applied test.

Plant Material: White bean seeds cv. Giza 4 was obtained from Vegetable Crops Research Department, Agric. Res. Centre, Giza, Egypt.

Damping-off and Root Rot Pathogen: Pathogenic isolate of *F. solani*, the causal organism of damping-off and root rot diseases in bean, was obtained from Department of Plant Pathology, NRC. Isolation, purification, identification and pathogenicity test were confirmed in previous study [23]. Fungal mass production used for soil infestation in pots was obtained by growing the tested isolate on sand-barley medium. This natural medium was prepared by mixing sand and barley (100 g ground barley grains mixed with 40 g washed sand and 60 ml distilled water); then the mixture placed in 500-ml glass bottles sealed with cotton plugs were sterilized three times (1 hr each time) at 121°C. The autoclaved medium was then inoculated with a 5-mm disk obtained from 5-day old culture of *F. solani* and then incubated at 25 ± 1°C for two weeks [24].

Bacterial Isolates: The bioagent isolates of *B. subtilis* (Bs_2), *B. pumilus* (Bp_3) and *P. fluorescens* (Pf_2) used in the greenhouse experiment were selected based on their inhibitor effect against soil borne fungal pathogens as well as their high tolerance to the tested fungicides *in vitro*.

Fungicide: The fungicide pencycuron (Monceren 25% WP), used in the greenhouse experiment were selected based on its inhibitor effect against soil borne fungal pathogens as well as its low inhibitor effect against bacterial isolates *in vitro*.

Experimental Trails: Pots experiment was carried out in the greenhouse of Pest Rearing Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Dokki, Giza, Egypt. The experiment was conducted to evaluate the role of soil application with bacterial biocontrol agents of Bs_3, Bp_3 and Pf_2, used alone or in combination with seed dressing with pencycuron, in control *F. solani*, the causal organism of damping-off and root rot diseases of bean. For soil infestation, 120g of *F. solani* inoculum were added to 30cm diameter pots, filled with steam sterilized sandy loamy soil 15 days before cultivation to ensure the distribution of the inocula [11].

For soil treatments, inocula of bacterial isolates were prepared by inoculated one loopful of each of *B. subtilis* (Bs_2), *B. pumilus* (Bp) and *P. fluorescens* (Pf), into Nutrient Broth medium and incubated on a shaker incubator (125 rpm) at 28 ± 1°C. Antagonistic bacterial cells were then harvested after 48 hours of growth in culture medium by centrifugation at 6,000 rpm for 15 min and re-suspended in a phosphate buffer (0.01 M, pH 7.0). The cell suspension was adjusted by plate count technique to an approximately 10^8 colony forming unit CFU / ml as mentioned by Mosa *et al*. [25]. Infested pots were treated with the bacterial suspension at a dose of 500ml / pot one week before seed sowing [26]. White bean seeds cultivar Giza 4 was surface sterilized by immersion in a solution of 2.5% NaOCl for 3 min, then rinsed three times in sterile water. Surface sterilized seeds were sown in the pots by the rate of ten seeds per pot.

For seed treatments, the fungicide pencycuron at the rate of 1.0g/kg seeds (= 250 ppm, ten folds of the tested laboratory concentration) were applied to surface sterilized bean seeds moistened with 1% carboxymethyle cellulose in polyethylene bag and then were shaken well. A set of four pots was used for every treatment *viz*., soil treatments, seed treatments and combined treatments as well as the control (infested pots with *F. solani* only). Treatments included:

T_1= *F. solani* only.
T_2= *F. solani* + *B. subtilis* (Bs_3).
T_3= *F. solani* + *B. pumilus* (Bp_3).
T_4= *F. solani* + *P. fluorescens* ($Pf_{2)}$.
T_5=*F. solani* + Pencycuron (Pe).
T_6=*F. solani* + Bs_3 + Pe.
T_7=*F. solani* + Bp_3 + Pe.
T_8=*F. solani* + Pf_2 + Pe.

Disease Assessment: Percentages of bean damping-off incidence at pre- and post-emergence stages were calculated after 15 and 30 days, respectively. Thereafter, the percentages of dead plants due to infection by root rot was recorded up to 60 days after sowing [27].

Plant growth parameters: Effect of different treatments on the growth parameters of bean plants in terms of shoot and root length (cm), leaves number per plant, fresh and dry weight (g) of plant were recorded after 60 days of seed sowing [23].

Statistical Analysis: All experiments were arranged in Complete Randomized Design. All experiment was also repeated twice and the obtained data were analyzed by using least squares analysis of variance (ANOVA), Least Significant Difference (L. S. D.) test was used at the 0.05% level of significance [28].

RESULTS

Laboratory Experiments

Inhibitor Effect of Tested Fungicides Against Some Soil Borne Fungal Pathogens: The inhibitor effect of tested fungicides against the growth of some soil borne plant pathogens at the concentrations of 0.0, 25, 50, 100, 200 and 400 ppm are listed in Table 1. There was a significant differences among pathogenic fungi in their response to the tested fungicides, where the inhibitor efficacy was increased with the increase of fungicide concentrations. The fungicide mancozeb was completely inhibited the growth of *S. scleratiorum*, while it was not showing any

Table 1: Inhibitor effect of tested fungicides against the growth of some soil borne plant pathogens *in vitro*.

		Linear mycelial growth (cm) and growth reduction (%) of pathogenic fungi									
		F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. sclerotiorum*	
Fungicide	Conc. (ppm)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
Mancozeb	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	25	9.00	0.0	8.90	1.1	5.32	40.9	9.00	0.0	4.70	47.8
	50	9.00	0.0	8.30	7.8	4.67	48.1	9.00	0.0	2.30	74.4
	100	8.90	1.1	7.67	14.8	3.60	60.0	9.00	0.0	0.00	100.0
	200	7.40	17.8	6.73	25.2	2.97	66.7	9.00	0.0	0.00	100.0
	400	7.20	20.2	4.10	54.4	2.30	74.4	9.00	0.0	0.00	100.0
Metalaxyl M - Mancozeb	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	25	9.00	0.0	9.00	0.0	5.65	37.2	9.00	0.0	4.88	45.8
	50	9.00	0.0	8.80	2.2	3.50	61.1	9.00	0.0	2.44	72.9
	100	8.60	4.4	8.30	7.8	2.63	70.7	9.00	0.0	0.00	100.0
	200	6.27	30.4	6.17	31.5	1.43	83.3	9.00	0.0	0.00	100.0
	400	4.47	41.2	3.50	61.1	0.00	100.0	9.00	0.0	0.00	100.0
Pencycuren	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	25	9.00	0.0	9.00	0.0	8.80	2.2	9.00	0.0	9.00	0.0
	50	9.00	0.0	8.90	1.1	8.12	9.8	9.00	0.0	8.50	5.6
	100	8.60	4.4	8.67	3.7	7.90	12.2	9.00	0.0	7.30	18.9
	200	6.40	28.9	5.90	34.4	3.83	55.6	9.00	0.0	4.30	52.2
	400	5.17	32.0	5.70	36.7	3.57	60.4	9.00	0.0	2.70	70.0
Thiophanate - methyl	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	25	9.00	0.0	8.50	5.6	8.48	5.7	9.00	0.0	9.00	0.0
	50	8.00	11.1	8.00	11.1	7.98	11.9	8.70	3.3	8.50	5.6
	100	7.47	17.1	7.37	18.2	7.30	18.9	7.50	16.7	7.60	15.6
	200	5.00	44.4	4.97	44.8	1.47	83.3	2.60	71.1	4.77	46.7
	400	3.03	60.1	3.50	61.1	1.00	88.9	0.00	100.0	0.00	100.0
Thiram +Tolelofos-methyl	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	25	7.00	22.2	8.48	5.8	5.30	41.1	4.00	55.6	4.46	50.4
	50	5.30	41.1	8.10	10.0	4.40	51.1	2.30	75.4	3.30	63.3
	100	4.20	53.3	7.27	19.2	0.00	100.0	1.27	85.6	1.00	88.9
	200	0.80	91.1	1.97	78.2	0.00	100.0	0.00	100.0	0.00	100.0
	400	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0
L.S.D. $_{0.05}$		Fungicide (F)		Pathogen (P)		F x P		Concentration (C)		F x C	P x C	F x P x C
Mycelial growth (cm)		0.04		0.04		0.08		0.05		0.09	0.09	0.18
Mycelial reduction (%)		1.28		1.28		2.56		1.43		2.86	2.86	5.71

inhibitor effect against *M. phaseolina* at all tested concentrations. The growth inhibition of *F. solani*, *F. oxysporum* and *R. solani* caused by mancozeb was in the ranges of 0.0-20.2%, 1.1-54.4% and 40.9-74.4%, respectively. Metalaxyl M + mancozeb was completely inhibited the growth of *S. scleratiorun* at the hight concentrations, *viz*., 100, 200 and 400ppm, while it was completely inhibited the growth of *M. phaseolina* at all the tested concentrations.

Metalaxyl M + mancozeb was inhibited the growth of *F. solani*, *F. oxysporum* and *R. solani* in the ranges 0.0-41.2%, 0.0-61.1 % and 37.2-100.0%, respectively (Table 1). Pencycuren was completely inhibited the growth of *M. phaseolina* at all tested concentrations. The reduction caused by pencycuren against the growth of *F. solani*, *F. oxysporum*, *R. solani* and *S. scleratiorun* was in the range of 0.0-32.0%, 0.0 -36.7%, 2.2-60.4% and 0.0-70.0%, at all tested concentrations, respectively. Thiophanate-methyl inhibited the growth of *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. scleratiorun* by the range of 0.0-60.1%, 5.6-61.1%, 11.9-88.9%, 0.0-100% and 0.0-100%, at all tested concentrations, respectively. Thiram + tolelofos-methyl was completely inhibited the growth of *F. solani*, *F. oxysporum* at 400 ppm, while the fungicide was completely inhibited the growth of *R. solani*, *M. phaseolina* and *S. scleratiorun* at 200 and 400 ppm (Table 1).

Inhibitor Effect of Tested Fungicides Against the Growth of Bacterial Isolates

Inhibitor Effect of Mancozeb: The reduction in the growth of the tested bacterial isolates caused by the fungicide mancozeb was in the range of 5.1-77.5% at all tested concentrations. Among the tested bacterial isolates, Bp_3 was highly tolerance to mancozeb at 25 and 50 ppm, which recorded reduction in OD estimated as 5.1and 6.9 %, respectively. *Bacillus megaterium* (Bm) isolate was also highly tolerance to mancozeb at 25 ppm only, which recorded reduction in OD estimated as 6.9% (Table, 2). Other bacterial isolates were highly tolerance to mancozeb at 25, 50 and 100 ppm and they were slightly tolerance at 200 and 400 ppm.

Inhibitor Effect of Metalaxyl M + Mancozeb: The reduction in the growth of the tested bacterial isolates caused by the fungicide metalaxyl M + mancozeb was in the range of 0.1 - 64.0% at all tested concentrations. *Bacillus megaterium* (Bm) isolate was highly tolerance to metalaxyl M + mancozeb at 25, 50 and 100 ppm, which recorded reduction in OD estimated as 1.1, 4.5 and 8.2 %, respectively. Meanwhile, Bs_1 and Bs_3 isolates was highly tolerance to metalaxyl M + mancozeb at 25 and 50 ppm, which recorded reduction in OD estimated as 2.3 and 9.2 and 4.9 and 7.8%, respectively. Isolates of BP_3, Pf_1, Bs_2, Bp_2, Bp_1 and Pf_2 were highly tolerance to metalaxyl M + mancozeb at 25 ppm, which recorded reduction in OD estimated as 2.1, 4.4, 5.8, 9.0, 9.5 and 12.5, respectively (Table, 3). Other bacterial isolates were highly tolerance to metalaxyl M + mancozeb at 25, 50 and 100 ppm and they were slightly tolerance at 200 and 400 ppm.

Inhibitor Effect of Pencycuron: The tested bacterial isolates were highly tolerance to pencycuron at 25, 50 and 100 ppm, while it was slightly tolerance at 200 and 400 ppm. The reduction in OD caused by pencycuron was in the range of 1.6 - 63.3 % at all tested concentrations. Isolates of Bp_3, Bs_3, Pf_2, Pf_1, Bp_2, Bp_1, Bm, Bs_2 and Bs_1 were highly tolerance at 25 ppm, which recorded reduction in OD estimated as 1.6, 2.4, 4.1, 4.4, 5.2, 7.1, 8.5, 20.5 and 21.1 %, respectively. Meanwhile, isolates of Bp_3, Pf_2 and Bs_3 isolates were highly tolerance at 50 ppm, while Bp_3 and Pf_2 isolates were highly tolerance at 100 ppm, which recorded reduction in OD estimated as 3.7, 7.4, 7.4, 7.0 and 9.0%, respectively (Table, 4).

Inhibitor Effect of Thiophanate-Methyl: At the concentration of 25 ppm, Bs_3, Bs_2, Pf_1, Bp_2 and Bp_1 isolates was highly tolerance to thiophanate-methyl, where the recorded reduction in OD were 4.3, 6.3, 6.3, 6.7 and 9.4%, respectively. While at 50 ppm, Bp_3 isolate only showed high tolerance, where the reduction in OD were 9.6%. Other bacterial isolates were high tolerance to thiophanate-methyl at 25, 50 and 100 ppm and slightly tolerance at 200 and 400 ppm (Table, 5).

Inhibitor Effect of Thiram + Tolclofos-Methyl: The tested bacterial isolates were highly tolerance to thiram + tolclofos-methyl at 25, 50 and 100 ppm and they were slightly tolerance at 200 and 400 ppm. The reduction in OD caused by thiram + tolclofos-methyl was in the range of 1.0-98.5 % at all tested concentrations. At the concentration of 25 ppm, Bs_3, Pf_2, Bp_3 and Bp_1 isolates was highly tolerance to, where the reduction in OD were 1.0, 2.0, 5.8 and 6.5%, respectively. Isolates of Bs_3 and Pf_2 was highly tolerance at 50 ppm, which recorded reduction in OD estimated as 2.2 and 6.1%, respectively. Isolate of Pf_1 was highly tolerance to thiram + tolclofos-methyl at 100 ppm, where the reduction in OD was 9.3% (Table, 6).

Table 2: Inhibitor effect of mancozeb against *Bacillus* and *Pseudomonas* isolates *in vitro*.

	Bacterial growth as optical density (OD) and reduction (%)										
	Mancozeb concentrations (ppm)										
	0.0	25		50		100		200		400	
Bacterial isolate	OD	OD	Red.	OD	Red.	OD	Red.	OD	Red.	OD	Red.
B. subtilis (Bs_1)	1.640	1.051	35.9	0.900	45.0	0.813	50.4	0.715	56.4	0.493	70.0
B. subtilis (Bs_2)	1.773	1.481	16.5	1.071	39.6	0.831	53.1	0.805	54.5	0.705	60.3
B. subtilis (Bs_3)	1.156	0.933	21.1	0.980	22.9	0.716	38.0	0.607	46.1	0.539	53.5
B. pumilus (Bp_1)	1.347	1.198	10.3	0.976	26.0	0.879	33.3	0.824	37.5	0.763	42.2
B. pumilus (Bp_2)	1.732	0.949	45.2	0.773	55.3	0.768	55.6	0.762	56.0	0.680	60.7
B. pumilus (Bp_3)	1.744	1.655	5.1	1.623	6.9	1.359	21.9	1.115	35.2	0.866	49.9
B. megaterium (Bm)	1.558	1.455	6.9	1.117	28.3	0.591	61.0	0.261	71.2	0.248	77.5
P. fluorescens (Pf_1)	1.133	0.936	17.3	0.887	21.8	0.809	28.6	0.421	41.3	0.376	66.8
P. fluorescens (Pf_2)	1.415	1.043	25.3	0.785	43.8	0.758	45.4	0.725	47.8	0.366	73.9
$L.S.D._{0.05}$	Isolates (I)				Concentrations (C)				I x C		
	0.052				0.042				0.126		

Table 3: Inhibitor effect of metalaxyl M + mancozeb against *Bacillus* and *Pseudomonas* isolates *in vitro*.

	Bacterial growth as optical density(OD) and reduction (%)										
	Metalaxyl M + mancozeb concentrations (ppm)										
	0.0	25		50		100		200		400	
Bacterial isolate	OD	OD	Red.	OD	Red.	OD	Red.	OD	Red.	OD	Red.
B. subtilis (Bs_1)	1.640	1.602	2.3	1.489	9.2	1.210	26.3	1.176	28.2	0.590	64.0
B. subtilis (Bs_2)	1.773	1.670	5.8	1.346	13.0	1.503	17.8	1.180	33.4	1.110	37.3
B. subtilis (Bs_3)	1.156	1.132	4.9	1.069	7.8	0.947	17.9	0.916	19.6	0.827	28.4
B. pumilus (Bp_1)	1.347	1.205	9.5	1.147	13.4	1.035	23.5	0.849	35.3	0.747	42.9
B. pumilus (Bp_2)	1.732	1.576	9.0	1.118	35.9	0.971	44.0	0.830	52.1	0.749	56.8
B. pumilus (Bp_3)	1.744	1.741	0.1	1.707	2.1	1.475	14.7	1.368	21.3	1.026	40.2
B. megaterium (Bm)	1.558	1.540	1.1	1.489	4.5	1.433	8.2	0.761	51.2	0.582	62.6
P. fluorescens (Pf_1)	1.133	1.083	4.4	0.914	19.3	0.868	23.5	0.799	29.5	0.791	30.2
P. fluorescens (Pf_2)	1.415	1.242	12.2	0.935	32.5	0.905	34.7	0.836	39.8	0.775	44.5
$L.S.D._{0.05}$	Isolates (I)				Concentrations (C)				I x C		
	0.053				0.043				0.129		

Table 4: Inhibitor effect of pencycuron against *Bacillus* and *Pseudomonas* isolates *in vitro*.

	Bacterial growth as optical density(OD) and reduction (%)										
	Pencycuron concentrations (ppm)										
	0.0	25		50		100		200		400	
Bacterial isolate	OD	OD	Red.	OD	Red.	OD	Red.	OD	Red.	OD	Red.
B. subtilis (Bs_1)	1.640	1.293	21.1	1.088	33.6	0.983	40.0	0.898	45.2	0.668	59.3
B. subtilis (Bs_2)	1.773	1.409	20.5	0.959	46.0	0.860	51.4	0.756	57.4	0.675	62.0
B. subtilis (Bs_3)	1.156	1.128	2.4	1.055	8.9	1.000	13.3	0.674	41.5	0.423	63.3
B. pumilus (Bp_1)	1.347	1.239	7.1	0.912	30.8	0.858	35.1	0.738	44.0	0.667	49.2
B. pumilus (Bp_2)	1.732	1.642	5.2	1.128	34.9	1.021	41.0	0.568	67.2	0.533	59.2
B. pumilus (Bp_3)	1.744	1.717	1.6	1.679	3.7	1.613	7.0	1.287	26.8	0.941	45.5
B. megaterium (Bm)	1.558	1.425	8.5	1.175	24.6	0.960	36.5	0.861	44.8	0.672	57.0
P. fluorescens (Pf_1)	1.133	1.083	4.4	0.956	15.7	0.901	23.2	0.750	33.8	0631	44.8
P. fluorescens (Pf_2)	1.415	1.369	4.1	1.319	7.4	1.288	9.0	1.044	24.9	0.861	38.3
$L.S.D._{0.05}$	Isolates (I)				Concentrations (C)				I x C		
	0.074				0.060				0.180		

Table 5: Inhibitor effect of thiophanate-methyl against *Bacillus* and *Pseudomonas* isolates *in vitro*.

	Bacterial growth as optical density (OD) and reduction (%)										
	Thiophanate-methyl concentrations (ppm)										
	0.0	25		50		100		200		400	
Bacterial isolate	OD	OD	Red.	OD	Red.	OD	Red.	OD	Red.	OD	Red.
B. subtilis (Bs_1)	1.640	1.084	33.8	1.049	36.0	0.858	47.5	0.655	60.1	0.570	65.2
B. subtilis (Bs_2)	1.773	1.661	6.3	1.237	34.1	0.972	41.2	0.823	53.6	0.721	59.3
B. subtilis (Bs_3)	1.156	1.100	4.3	0.957	15.7	0956	17.3	0.761	42.6	0.628	45.6
B. pumilus (Bp_1)	1.347	1.211	9.4	1.117	15.0	0.913	31.0	0.766	42.1	0.653	50.0
B. pumilus (Bp_2)	1.732	1.109	36.0	0.895	48.3	0.861	50.3	0.826	52.3	0.730	57.9
B. pumilus (Bp_3)	1.744	1.623	6.7	1.568	9.6	1.329	23.3	1.021	41.1	1.005	41.9
B. megaterium (Bm)	1.558	1.083	30.5	0.997	36.0	0.730	53.1	0.711	54.3	0.445	71.4
P. fluorescens (Pf_1)	1.133	1.061	6.3	0.973	14.1	0.876	22.7	0.792	28.8	0.712	37.2
P. fluorescens (Pf_2)	1.415	0.916	34.5	0.849	38.3	0.716	48.3	0.710	49.1	0.427	69.4
$L.S.D._{0.05}$	Isolates (I)				Concentrations (C)				I x C		
	0.078				0.064				0.191		

Table 6: Inhibitor effect of thiram + tolclofos-methyl against *Bacillus* and *Pseudomonas* isolates *in vitro*.

	Bacterial growth as optical density (OD) and reduction (%)										
	Thiram + tolclofos-methyl concentrations (ppm)										
	0.0	25		50		100		200		400	
Bacterial isolate	OD	OD	Red.	OD	Red.	OD	Red.	OD	Red.	OD	Red.
B. subtilis (Bs_1)	1.640	1.330	18.9	0.646	60.6	0.439	73.2	0.200	89.6	0.162	90.0
B. subtilis (Bs_2)	1.773	1.370	22.8	1.098	38.1	1.075	39.4	0.982	44.6	0.885	50.1
B. subtilis (Bs_3)	1.156	1.144	1.0	1.130	2.2	1.024	11.2	0.907	21.4	0.821	28.9
B. pumilus (Bp_1)	1.347	1.269	6.3	0.872	33.5	0.548	58.6	0.529	60.0	0.456	65.3
B. pumilus (Bp_2)	1.732	1.115	35.6	1.022	41.0	0.685	60.4	0.542	68.7	0.445	74.3
B. pumilus (Bp_3)	1.744	1.641	5.8	0.914	47.1	0.189	89.1	0.161	90.6	0.079	95.3
B. megaterium (Bm)	1.558	1.260	19.3	1.133	27.3	0.914	41.3	0.672	56.7	0.671	63.3
P. fluorescens (Pf_1)	1.133	1.111	2.0	1.064	6.1	1.028	9.3	0.899	19.3	0.798	29.5
P. fluorescens (Pf_2)	1.415	0.674	51.8	0.231	86.5	0.066	95.3	0.030	97.9	0.020	98.5
$L.S.D._{0.05}$	Isolates (I)				Concentrations (C)				I x C		
	0.050				0.041				0.123		

Table 7: Antagonistic activity of *Bacillus* and *Pseudomonas* isolates against the growth of some soil borne pathogens *in vitro*.

	Mycelial growth (cm) and reduction (%)									
	F.solani		*F. oxysporum*		*R. solani*		*M.phaseolina*		*S. sclerotiorum*	
Bacterial isolate	Growth	Red.	Growth	Red.	Growth	Red.	Growth	Red.	Growth	Red.
B. subtilis (Bs_1)	3.9	56.7	1.8	80.0	6.2	30.0	2.0	65.6	2.7	70.0
B. subtilis (Bs_2)	6.6	26.7	6.5	27.8	7.0	22.2	3.1	65.6	3.0	66.7
B. subtilis (Bs_3)	6.3	30.0	4.2	53.3	7.6	15.6	4.1	54.4	2.4	73.3
B. pumilus (Bp_1)	6.6	26.7	7.1	21.1	7.9	12.2	2.8	68.9	2.6	71.1
B. pumilus (Bp_2)	4.6	48.9	3.0	66.7	2.5	72.2	3.6	60.0	2.1	76.7
B. pumilus (Bp_3)	4.1	54.4	7.3	18.9	8.0	11.1	3.8	57.9	3.1	65.6
B. megaterium (Bm)	7.8	13.3	4.9	45.6	7.8	13.3	4.2	53.3	2.5	72.2
P. fluorescens (Pf_1)	7.1	21.1	6.9	23.3	7.5	16.7	5.0	44.4	1.1	87.8
P. fluorescens (Pf_2)	2.2	75.6	1.4	84.4	2.3	74.4	3.7	58.9	3.0	66.7
Control	9.0	-	9.0	-	9.0	-	9.0	-	9.0	-
$L.S.D._{0.05}$	Isolates (I)			Pathogens (P)				I x P		
	0.1			0.03				0.1		

Table 8: Antagonistic activity of *Bacillus* and *Pseudomonas* isolates in combination with pencycuron against the growth of some soil borne pathogens *in vitro*.

	Mycelial growth (cm) and reduction (%)									
	F.solani		*F. oxysporum*		*R. solani*		*M.phaseolina*		*S. sclerotiorum*	
Treatment	Growth	Red.	Growth	Red.	Growth	Red.	Growth	Red.	Growth	Red.
B. subtilis (Bs_1) + Pe	3.7	58.9	1.5	83.3	0.6	93.3	1.0	88.9	1.0	88.9
B. subtilis (Bs_2) + Pe	5.2	42.2	3.7	58.9	0.0	100	2.6	71.1	1.8	80.0
B. subtilis (Bs_3) + Pe	4.0	55.6	2.3	74.4	1.0	88.9	3.0	66.7	1.0	88.9
B. pumilus (Bp_1) + Pe	3.2	64.4	2.5	72.2	0.0	100	2.6	71.1	1.3	85.6
B. pumilus (Bp_2) + Pe	4.0	55.6	3.0	68.7	0.0	100	1.9	78.9	1.0	88.9
B. pumilus (Bp_3) + Pe	3.5	61.1	5.0	44.4	1.4	84.4	2.0	77.8	1.0	88.9
B. megaterium (Bm) + Pe	5.5	38.9	2.3	74.4	0.0	100	3.5	61.1	1.0	88.9
P. fluorescens (Pf_1) + Pe	5.5	38.9	3.5	61.1	0.0	100	2.7	70.0	0.0	100
P. fluorescens (Pf_2) + Pe	1.0	88.9	1.0	88.9	0.0	100	1.7	81.1	1.0	88.9
Pencycuron (Pe) at 25 ppm	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0
$L.S.D._{0.05}$	Treatments (T)				Pathogens (P)			T x P		
	0.5				0.3			0.1		

Table 9: Effect of *Bacillus* and *Pseudomonas* isolates alone or in combination with pencycuron on the incidence of damping-off and root rot of bean plants grown under soil infestation with *F. solani* in pots.

	Pre- and post- emergence damping-off (%)			
Treatment	Pre-	Post-	Root rot (%)	Survived plants (%)
F. solani + *B. subtilis* (Bs_3)	13.3	9.1	6.7	70.9
F. solani + *B. pumilus* (Bp_3)	13.3	11.7	7.1	67.9
F. solani + *P. fluorescens* (Pf_2)	0.0	6.7	7.7	85.6
F. solani + Bs_3 + Pe	6.7	7.7	4.7	80.9
F. solani + Bp_3 + Pe	6.7	7.1	3.6	82.6
F. solani + Pf_2 + Pe	0.0	5.7	0.0	94.3
F. solani + Pencycuron (Pe)	13.3	15.7	7.7	63.3
F. solani only	40.0	22.2	27.8	10.0
$L.S.D._{0.05}$	0.6	0.1	0.1	0.6

Table 10: Effect of *Bacillus* and *Pseudomonas* isolates alone or in combination with pencycuron on vegetative growth parameters of bean plants grown under soil infestation with *F. solani* in pots.

	Vegetative growth parameters				
Treatment	Shoot length (cm)	Root length (cm)	Leaves No./plant	Plant fresh weight (g)	Plant dry weight (g)
F. solani + *B. subtilis* (Bs_3)	46.5	17.5	6.5	12.8	2.3
F. solani + *B. pumilus* (Bp_3)	46.8	19.8	5.3	12.2	2.2
F. solani + *P. fluorescens* (Pf_2)	52.0	21.8	5.8	17.1	3.2
F. solani + Bs_3 + Pe	51.0	22.5	6.8	13.4	3.4
F. solani + Bp_3 + Pe	46.8	22.5	7.8	13.6	3.7
F. solani + Pf_2 + Pe	52.2	23.5	6.5	20.8	4.0
F. solani + Pencycuron (Pe)	44.3	19.0	5.8	12.2	3.2
F. solani only	32.8	16.0	5.0	9.5	2.1
$L.S.D._{0.05}$	6.8	6.9	1.7	7.8	0.1

Antagonistic Activity of Bacterial Isolates Alone or in Combination with Pencycuron at 25 ppm Against Some Soilborne Fungal Pathogens: The tested bacterial isolates reduced the radial growth of *F. solani, F. oxysporum*, *R. solani, M. phaseolina* and *S. sclerotiorum* by the ranges of 13.3 -75.6; 18.9-88.9; 11.1-74.4; 44.4-88.9 and 65.6-87.8 %, respectively, when used alone. While the bacterial isolates reduced the same pathogens by the ranges of 38.9-88.9; 44.4-88.9; 84.4-100.0; 61.1-88.9 and 80.0-100.0%, respectively, when used in combination with pencycuron at 25 ppm (Tables, 7 and 8). Although pencycuron at 25 ppm only did not showing reduction in linear growth of the tested fungi, but the growth appeared more weak than the growth in un-amended medium. Isolates of Pf_2, Bs_1, Bp_3, Bp_2, Bs_3, Bs_2, Bp_1, Pf_1 and Bm, reduced the growth of *F. solani* by 75.6, 56.7, 54.4, 48.9, 30.0, 26.7, 26.7, 21.1 and 13.3%, when used alone, while the reduction attained to 88.9, 58.9, 61.1, 55.6, 42.2, 64.4, 38.9 and 38.9%, when used in combination with pencycuron,

respectively. Furthermore, isolates of Pf_2, Bs_1, Bp_2, Bs_3, Bm, Bs_2, Pf_1, Bp_1 and Bp_3, reduced the growth of *F. oxysporum* by 84.4, 80.0, 66.7, 53.3, 45.6, 27.8, 23.3, 21.1 and 18.9%, when used alone, while the reduction reached to 88.9, 83.3, 68.7, 74.4, 74.4, 58.9,61.1, 72.2 and 44.4%, when used in combination with pencycuron, respectively (Tables, 7 and 8).

In general antagonistic isolates of Pf_2, Bp_2, Bs_1, Bs_2, Pf_1, Bs_3, Bm, Bp_1 and Bp_3, reduced the growth of *R. solani* by 74.4, 72.2, 30.0, 22.2, 16.7, 15.6, 13.3, 12.2 and 11.1%, when used alone, while the reduction attained to 100, 100, 93.3, 100, 100, 88.9, 100, 100 and 100%, when used in combination with pencycuron, respectively. Isolates of Bp_1, Bs_1, Bs_2, Bp_2, Pf_2, Bp_3, Bs_3, Bm and Pf_1, reduced the growth of *M. phaseolina* by 68.9, 65.6, 65.6, 60.0, 58.9, 57.9, 54.4, 53.3 and 44.4%, when used alone, while the reduction attained to71.1, 88.9, 71.1, 78.9, 81.1, 77.8, 66.7, 61.1 and 70.0%, when used in combination with pencycuron, respectively. Isolates of Pf_1, Bp_2, Bs_3, Bm, Bp_1, Bs_1, Bs_2, Pf_2 and Bp_3, reduced the growth of *S. sclerotiorum* by 87.8, 76.7, 73.3, 72.2, 71.1, 70.0, 66.7, 66.7 and 65.6%, when used alone, while the reduction reached to 100, 88.9, 88.9, 88.9, 85.6, 88.9, 80.0, 88.9 and 88.9%, when used in combination with pencycuron, respectively (Tables, 7 and 8).

Greenhouse Experiment: Soil application with each of Bs_3, Bp_3 and Pf_2 isolates, reduced the incidence of pre- and post-emergence damping-off incited by *F. solani* by 66.75 and 59.0; 66.75 and 47.3% and 100 and 69.8%, when used alone, while the reduction reached to 83.25 and 65.3; 83.25 and 68.0 and 100 and 74.3 %, when combined with pencycuron seeds dressing at 1.0g/kg, respectively (Table, 9). The root rot incidence was also highly restricted in combined treatments than individual one. *Pseudomans fluorescens* (Pf_2) proved to be the most effective isolate followed by *B. subtilis* (Bs_3) and *B. pumilus* (Bp_3) isolates. The treatment receiving Pf_2 isolates (soil application) plus pencycuron (seeds dressing) recorded the maximum plant survival of 94.3 %, while (Bs_3) + pencycuron and (Bp_3) + pencycuron recorded plant survival of 80.9 and 82.6, respectively, compared to 10.0% survived plants in the control (Table, 9). Other treatments showed moderate effect.

On the other hand, data presented in Table (10) show the effect of soil application with each of Bs_3, Bp_3 and Pf_2 isolates alone or in combination with pencycuron seeds dressing on bean vegetative growth. Results reveal that the shoots length (cm), root length (cm), number of leaves per plant, fresh and dry weight (g) / plant of bean plants were in the range of 46.5 to 52.0 cm, 17.5 to 23.5 cm, 5.3 to 7.8, 12.2 to 20.0 g and 2.2 to 4.0g in biological treatments alone or in combination with pencycuron, compared to 44.3 and 32.8 cm, 19.9 and 16.0 cm, 5.8 to 5.0, 12.2 and 9.5 g and 3.2 and 2.1 g in the treatments of pencycuron alone and control (infested soil with *F.solani* only), respectively.

DISCUSSION

In the present study, according to OD values, bacterial isolates belongs to *Bacillus* and *Pseudomonas* genera *viz*., Bs_1, Bs_2, Bs_3, Bp_1, Bp_2, Bp_3, Bm, Pf_1 and Pf_2, were highly tolerance to the five tested fungicides *viz*., mancozeb, metalaxyl M + mancozeb, pencycuron, thiophanate-methyl and thiram + tolclofos-methyl, at 25, 50 and 100 ppm and they were slightly tolerance at 200 and 400 ppm. This may be due to the reason that, some bacteria can use pesticides as nutrients and hence can tolerate chemicals [29, 30, 4]. The isolates Bs_3, Bp_3 and Pf_2 found to be more tolerance than other isolates. This is may be due to the genetic differences between isolates to their response to the fungicides. The obtained data are in agreement with those obtained by Mohiddin and Khan [7]. They reported that *P. fluorescens* was found to be more tolerance to fungicides than *B. subtilis* and the maximum tolerance concentration for the former being 2500 µg/ml of thiram,1600 µg/ml of mancozeb and 50,000 µg/ml for captan and carbendazim. The present study also reveal that the bacterial isolates in combination with pencycuron highly reduced the growth of *F. solani, F. oxysporum*, *R. solani*, *M. phaseolina* and *S. sclerotium* than bacterial isolates alone *in vitro* tests. This is may be due to the synergistic effects between the bacterial isolates and the fungicide pencycuron at 25 ppm. The fungicide pencycuron suppress the growth of the pathogens and make the hyphae more weak to be easily attacked with the bacterial isolates. The obtained results are in accordance with several workers [31-33].

Under greenhouse conditions, chemical, biological and integrated approaches to damping off and root rot control of bean were compared. The values of each Bs_3, Bp_3 and Pf_2 isolates for damping-off and root rot incidence control was evident under artificial soil infestation with *F. solani*. However, it is clearly demonstrated that, *F. solani* pathogen can be successfully managed by combined application of bacterial biocontrol agents with a fungicide pencycuron used at low dose more than those obtained by a single application. The Pf_2 proved to be the most effective isolate in controlling the tested diseases

followed by Bs_3 and Bp_3 isolates. The synergistic effects between the bacterial isolates and the fungicide pencycuron at the low dose are involved. Soil infestation with bacterial isolates before sowing reduced the inoculum potential of *F. solani* pathogen. Although pencycuron seed dressing protect bean seeds from infection during emerge stage. The tolerance of bacterial isolates to the fungicide allow them to grow well and protect the tap and the lateral roots from further infection at any stage of plant development. The obtained results are in harmony with those recorded by [31,32,34,35,33,7]. The potentiality of *B. subtilis*; *B. pumilus* and *P. fluorescens* as biocontrol agents of phytopathogenic fungi in several crops is well known especially to *Fusarium* spp. [36-39]. There are many mechanisms suggested to clarify the role of antagonistic organisms in suppression of the growth pathogens and thus to control diseases. Their action could be through (i) mycoparasitism, (ii) production of antibiotic-type secondary metabolites and (iii) competition for nutrients and space. However, the bacterial isolates only showed the (ii) and (iii) mechanisms that can inhibit the growth of pathogens [38]. The increase of bean vegetative growth obtained in this study could be related to the effect of *B. subtilis*; *B. pumilus* and *P. fluorescens* as plant growth promoters [40]. The present results lead to strategy for the improvement of bean damping-off and root rot control. It also could be an important means to maintain sustainable bean productivity as well as reduced the amount of chemical fungicide need for seed treatment. Further studies to explore the interaction between bacterial biocontrol agents and fungicides in field experiment are desirable.

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