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Effect of Biocontrol Agents of Bacteria against Seed-borne Pathogens *in vitro* and Under Greenhouse Conditions

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Abstract This study was conducted to check the efficiency of some biocontrol agents on controlling damping-off disease incited by some tested pathogens both in laboratory and under greenhouse conditions. Results showed that all tested BBA's were effective in reducing the radial growth of the tested damping-off pathogens. Moreover *B. subtilis* was more effective (65.55%), whereas *F. semitictum* was the most sensitive to Bacterial biocontrol agents (BBA's) treatments, where reduction rates attained was 70.83%. On the contrary, *M. phaseolina* was the least sensitive to BBA's treatments, where reduction rate was 35.28%**.** Both the tested BBA's treatments significantly reduced TIP incidence caused by the tested cucurbit seed-borne pathogens. Reduction rates attained 46.29% and 42.81% in *B. cereus* and *B. subtili* respectively, compared with control. Moreover, reduction rates were higher in *M. phaseolina* and *F. semitictum* treatments**.** Treatments with the tested BBA's result in significant reductions in PRD values. *B. cereus* realized the highest reduction rates, particularly against *F. moniliforme* (55.95%). *B. subtilis* / *F. semitictum* gave the least effective among the other treatments, were reduction rates in PRD values 47.78%**.***B. cereus* or *B .subtilis* / *F. semitictum* induced the highest reduction rates in PTD values (42.62%), the least reduction rates was obtained in *F. moniliforme* treatments with *B. cereus* (21.75%). Moreover *B. subtilis* was the least effective than *B. cereus*.

Keywords Greenhouse conditions, Biocontrol agents, *In vitro*, *In vivo*, Seed-borne Pathogens

Introduction

Bacterial biocontrol agents BBA's for plant diseases are getting more attention in resent years as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts [1]. Among the BBA commonly used are bacterial agents those related to the genera *Bacillus* spp. More investigations are required to study different aspects concerning application of bacterial biocontrol agents against pathogens attacking cucurbitaceous crops, furthermore evaluation of the efficacy of different bacterial BBA's, behavior of the antagonists against the targeted fungal pathogens and biochemical changes characterizing the antagonist-pathogen interactions, especially production of chitinases and changes host proteins [2, 3]. The objective of this work was to evaluate the efficacy of some bacterial biological control agents against cucurbit seed-borne pathogens both *in vitro* and under greenhouse conditions [4].

Materials and Methods

In vitro study

The bioreaction between the bacterial bioagents and the tested pathogenic fungi [5]

Two bacterial biocontrol agents, i.e. *Bacillus subtilis* and *Bacillus cereus* were kindly obtained from Microbiologics 217 Osseo Avenue North, St. Cloud, MN 56303 USA. to investigate their effect on the growth and development of the tested cucurbits seed-borne pathogens. In order to study the antagonistic effect of the bacterial isolates of *Bacillus subtilis* and *Bacillus cereus* on the growth four tested cucurbits seed-borne pathogens namely *F. solani, F. moniliforme, F. semitectum, F. oxysporium* and *M. phaseolina*, the bacterial bioagent was streaked at two sides on PDA medium plates and incubated for 24 hours at 30 ºC, their one disc (4 mmin diameter) bearing 7-day old growth of one of the tested fungi was placed on the center of plate. Petri dishes were inoculated with each of the pathogenic fungi only to serve as a control. Each treatment was replicated three times and incubated at 25ºC ± 2. Data were calculated according to formula adopted by Sallam et al., [6] as follows:

$$\text{Percentage of reduction (\%)} = \frac{\text{A - B}}{\text{A}} \text{X100}$$

A= diameter of the control hyphal growth
B = diameter of the treated hyphal growth

***In vivo* study**

Autoclaved potted soils were inoculated with the inocula of the tested pathogens as previously mentioned. The bacterial agents isolates, i.e. *B. subtilis* and *B. cereus* were grown in flasks 250 ml containing 50 ml NB medium to obtain suspension of the tested bioagents. After 72 hrs of incubation, bacterial suspensions were adjusted to conc. 10^7/ml. Talc powder formula was prepared by adding 0.5 g carboxymethyl cellulose (CMC) to 50 ml of bacterial suspension, and then mixed with 100 g of talc powder. One ml of this formula was used to treat 2 g seeds. The Seeds treated with the bacterial biocontrol agents were then incubated for one day at 25^{o}C for drying. The all available interactions between cucurbits seed-borne pathogens and bacterial biocontrol agents were investigated:

1. *F. solani* alone
2. *F. solani* and *B. subtilis*
3. *F. solani* and *B. cereus*
4. *F. moniliforme* alone
5. *F. moniliforme* and *B. subtilis*
6. *F. moniliforme* and *B. Cereus*
7. *F. semitectum* alone
8. *F. semitectum* and *B. subtilis*
9. *F. semitectum* and *B. cereus*
10. *M. phaseolina* alone
11. *M. phaseolina* and *B. subtilis*
12. *M. phaseolina* and *B. Cereus*

Ten seeds coated with the tested bacterial biocontrol agents were sown in each potted soil infested with the tested pathogens according to the above mentioned combinations. Four control experiments were designed, in which the first was inoculated with the tested pathogens only, and in others, non-infested soil was used.

Surface sterilized seeds of different cantaloupe cultivars were sawn each in infested plastic pot (12 cm) according to the previous treatments and placed in the greenhouse at approximately 20 ^{o}C. Four replicates (4 pots) of each treatment were used. Pre- and post-emergence damping-off was calculated out 14 days following planting [7, 8].

Results

The aim of this investigation was to check the inhibitory effect of two bacterial bioagents (*Bacillus subtilis* and *B. cereus*) against the five tested cucurbitaceous seed-borne pathogens, i.e. *F. solani, F. moniliforme, F. semitectum, M. phaseolina* and *S. sclerotiorum* both under laboratory and greenhouse conditions.

In vitro study

Bioreaction between the bacterial bioagents and cucurbitaceous seed-borne pathogens *in vitro*

According to the method described in detail in the section of Materials and Methods, Linear growth method was determined for all the tested cucurbitaceous seed-borne pathogens grown in Petri dishes along with the tested bacterial bioagents. Data were then statistically analyzed, presented in table 1. According to the obtained data, generally, it was evident that all the tested bacterial bioagents significantly reduced growth of mycelial growth of

all the tested cucurbitaceous seed-borne pathogens. However, reduction rates differed according to the bioagent or the pathogen tested.

B. subtilis

B. subtilis bioagent proved to be more inhibitory to *F. semitectum* and *F. solani*, however, reduction rate in growth of *F. semitectum* (75.55%) was more than that of *F. solani* (73.33%), then *F. moniliforme* and *S. sclerotiorum* (72.77% and 71.66% less than control). The least inhibitory effect of *B. subtilis* was induced against *M. phaseolina* (34.44%).

B. cereus

F. solani and *F. semitectum* proved to be the most sensitive among the tested pathogens to the inhibitory effect of the bacterial bioagent *B. cereus* where maximum reduction rates were attained (equal rates 66.11%). *M. phaseolina* was the least sensitive to effect of *B. cereus*, compared with control (36.11%), however similar inhibition rates were obtained from *F. semitectum* and *F. moniliforme* (61.66% and 60.00%).

Therefore, from data obtained in table 1the following could be concluded:

- Both the tested bacterial bioagents significantly reduced the mycelial growth of the tested tuber rot pathogens. Reduction rates compared with control were58.00% and 65.55%**.**
- In spite of the pronounced suppression induced by the bacterial bioagent *B. cereus*, however, reduction rates of mycelial growth of the tested pathogens were the least, compared with those produced by the other tested bioagents. Moreover, *M. phaseolina* was less sensitive to the effect of *B. subtilis* than the other tested pathogens.

Table 1: Antagonistic effect of some bacterial bioagents on the mycelial growth of the tested cucurbitaceous seed-borne pathogens

B.C.A	**Reduction (%)**					**Mean**
	The tested pathogen					
	F. solani	***F. moniliforme***	***F. semitectum***	***S. sclerotiorum***	***M. phaseolina***	
B. subtilis	73.33	72.77	75.55	71.66	34.44	65.55
B. cereus	66.11	60.00	66.11	61.66	36.11	58.00
Control	0.00	0.00	0.00	0.00	0.00	0.00
Mean	69.72	66.39	70.83	66.66	35.28	

* Percentage of reduction in the mycelial growth.

L.S.D. at 5% for:	Bioagent (B)	Fungi (F)
	1.24	0.862

***In vivo* study**

Effeciency of BCA's in controlling damping-off disease in greenhouse:

This study aimed to check the effect of soil inoculation with different bioagents, namely: *B. cereus*, and *B. subtilis* for controlling pre- and post-emergence damping-off caused by some cucurbitaceous seed-borne pathogens, i.e. *F. solani*, *F. moniliforme*, *F. semitectum* and *M. phaseolina* on cantaloupe (CEREDO F1, ISI 54139 F1 & ANANAS cultivars) under greenhouse conditions. In order to achieve such target, pots were inoculated individually with the four tested pre-emergence damping-off (PRD), post-emergence damping-off (PTD) pathogens under greenhouse conditions. The Two tested bioagents were applied through soil inoculation. Data were calculated as percentages, statistically analyzed, and then presented in Tables (2-5).

F. solani

Soil treatment with *B. subtilis* significantly reduced PRD incidence (40.00 to 66.67% less than control). The highest PRD suppression was detected in *B. subtilis* x ISI 54139 F1 cv. combination. Reduction in PRD disease incidence was the highest in *B. subtilis*, compared with *B. cerues*, while *B. cerues* give reduction range (40-61.11% less than control). PTD values were significantly higher, in general, compared with inoculatedcontrol; however, soil treatment with both BBA's were ineffective in controlling PTD incited by *F. solani* in ISI 54139 F1 cv. (16.67%). The most effective BBA treatment was that of *B. cerues*, since it induced the highest reduction in PTD incidence attaining maximum percentages in tested cantaloupe cultivars (16.67-50.00% less than control). Efficiency of *B. subtilis*

treatment came next to *B. cerues*, particularly on tested cantaloupe cultivars (16.67-33.33% less than control). In general, TIP values in control inoculated with *F. solani*, untreated with *B. cerues* and *B. subtilis*, ranged from (46.98% and 42.43, respectively). Both BBA's treatments significantly reduced TIP of damping-off incited by *F. solani*. The highest reductions in disease incidence (50.00% in ISI 54139 F1 cv., compared with control) was detected by *B. cerues* (Table 2).

Table 2. Efficiency of some Biocontrol agents on controlling damping-off of cantaloupe cvs., incited by *F. solani*.

Treatment	Percentage of seedling infection (PSI)									Mean Value TIP
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
F. solani+ *B. cereus*	15.0	7.5	22.5	17.5	12.5	30.0	20.0	15.0	35.0	29.16
F. solani+ *B. subtilis*	15.0	10.0	25.0	15.0	17.5	32.5	20.0	17.5	37.5	31.66
Control (1) (*F. solani* alone)	25.0	15.0	40.0	45.0	15.0	60.0	40.0	25.0	65.0	55.0
Control (2) (Untreated)	0.0	0.0	0.0	5.0	0.0	5.0	10.0	0.0	10.0	5.0
L.S.D	2.67	2.12	2.51	2.64	2.07	3.81	1.98	1.56	3.92	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

F. moniliforme

Treatment of soil with *B. cereus* was more effective in reducing PRD, incited by *F. moniliforme* in the more resistant, ISI 54139 F1 and ANANAS cvs. (66.67% and 58.33%, respectively) than CEREDO F1 cv. (42.85%). In spite of the significant reduction in PRD by *B. subtilis*, however, reduction rates were lower than those produced by *B. cereus* x ISI 54139 F1. Soil treatment with *B. subtilis* was most effective in controlling PTD incited by *F. moniliforme* in ISI 54139 F1 cv. (33.33%). While too, *B. subtilis* was lower reduction in PTD incited by *F. moniliforme* in ANANAS cv. (14.28%). On the other hand, soil treatment with both BBA's were equal effective in controlling PTD incited by *F. moniliforme* in CEREDO F1 cv.(20.00%). Results of table 3 show that TIP's were higher reductions compared with control in ISI 54139 F1 cv.with *B. cereus* and *B. subtilis* (50.00%), While soil treatment by *B. subtilis* with ANANAS cv. was lower reduction rate in TIP incited (26.67%) compared with control (Table 3).

Table 3: Efficiency of some Biocontrol agents on controlling damping-off of cantaloupe cvs., incited by *F. moniliforme*

Treatment	Percentage of seedling infection (PSI)									Mean Value TIP
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
F. moniliforme + *B. cereus*	10.0	10.0	20.0	10.0	12.5	22.5	12.5	12.5	25.0	22.50
F.moniliforme+ *B. subtilis*	10.0	10.0	20.0	12.5	10.0	22.5	12.5	15.0	27.5	23.33
Control (1) (*F.moniliforme*alone)	17.5	12.5	30.0	30.0	15.0	45.0	30.0	17.5	47.5	40.83
Control (2)(Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	5.0	1.66
L.S.D	2.41	2.67	2.83	2.54	2.62	3.44	2.88	3.11	3.97	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

F. semitectum

Both the tested BBA's against PRD in cantaloupe cvs., caused by *F. semitectum*, significantly reduced PRD incidence. Reduction rates differed depending upon the tested BBA's and the cultivar. In ISI 54139 F1 cv., the highest reduction rate was realized with *B. cereus* (62.50% less than control). In ANANAS cv., the highest reduction rate was realized with *B. subtilis* (60.00%less than control). While, in CEREDO F1 cv., the lowest reduction rate was realized with *B. subtilis* (33.33% compared with control). All PTD values resulted from soil treatment of cantaloupe cvs. with the tested BBA's were significant compared with control. The highest reduction rates were obtained by *B. cereus* or *B. subtilis* with CEREDO F1 cv. (60.00%), followed by, *B. cereus* or *B. subtilis* with ANANAS cv. (42.86%), while, the lowest reduction rates were obtained by *B. cereus* or *B. subtilis* with ISI 54139 F1 cv. equal rates (25.00% less than control). TIP values in both tested BBA's /cultivar combinations were

significantly reduced in treatments. The highest reductions in disease incidence was induced by *B. cereus*, Significantly reduced TIP in CEREDO F1, ISI 54139 F1 and ANANAS cvs. (54.54%, 50.00%, and 41.18%, respectively), compared with control, whereas *B. subtilis* reductions were (45.45%, 41.67% and 52.94, respectively). In generally, rates reduction TIP obtained by *B. cereus* and *B. subtilis* (48.57%and 46.68%, less than control, respectively) (Table 4).

Table 4: Efficiency of some Biocontrol agents on controlling damping-off of cantaloupe cvs., incited by *F. Semitectum*

Treatment	Percentage of seedling infection (PSI)									Mean Value TIP
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
F. semitectum + *B. cereus*	7.5	5.0	12.5	7.5	7.5	15.0	15.0	10.0	25.0	17.50
F. semitectum + *B. subtilis*	10.0	5.0	15.0	10.0	7.5	17.5	10.0	10.0	20.0	17.50
Control (1) (*F. semitectum*alone)	15.0	12.5	27.5	20.0	10.0	30.0	25.0	17.5	42.5	33.33
Control (2) (Untreated)	5.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	1.66
L.S.D	1.92	2.14	2.61	2.30	2.46	2.72	2.22	2.63	2.96	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

M. phaseolina

All the tested PRD values in both the tested BBA's /cultivars combinations were significant, compared with control inoculated alone with *M. phaseolina*. Moreover, *B. subtilis* with ISI 54139 F1 cv. was the most effective in reducing PRD values (66.67%) than any of the other tested PRD, followed by *B. cerues* with ISI 54139 F1 cv., was reduction rate (60.00%, less than control). While, the lowest reduction rate in treatment PRD was by *B. subtilis* with CEREDO F1 cv. (37.50%, compared with control). All the tested PTD values in both the tested BBA's /cultivars combinations were significant, compared with control inoculated alon with *M. phaseolina* except, *B. subtilis* x ISI 54139 F1 cv.. Significant reduction in PTD values were obtained as a result of soil inoculation with *B. cerues*x all cultivars (50.00%, 20.00% and 44.44% in CEREDO F1, ISI 54139 F1 and ANANAS cvs., respectively),and *B. subtilis* x CEREDO F1, ISI 54139 F1 and ANANAS cvs. (50%, 0.0% and 33.33%, respectively). Soil treatment with *B. cerues* gave the most significant reduction TIP in ANANAS cvs. (52.17.% less than control), followed by, *B. cerues* with CEREDO F1 cv. or ISI 54139 F1 cv., or *B. subtilis* with ISI 54139 F1 cv. were equal rate(50.00%, less than control). On the other hand, soil treatment by *B. subtilis* with CEREDO F1 cv., was the lowest reduction rate in TIP (42.86 % less than control) (Table 5).

Table 5: Efficiency of some Biocontrol agents on controlling damping-off of cantaloupe cvs., incited by *M. phaseolina*

Treatment	Percentage of seedling infection (PSI)									Mean Value TIP
	CREDO F1			ISI 54139 F1			ANANAS			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	
M. phaseolina+ *B. cereus*	10.0	7.5	17.5	15.0	10.0	25.0	15.0	12.5	27.5	23.33
M. phaseolina + *B. subtilis*	12.5	7.5	20.0	12.5	12.5	25.0	17.5	15.0	32.5	25.83
Control (1) (*M. phaseolina* alone)	20.0	15.0	35.0	37.5	12.5	50.0	35.0	22.5	57.5	47.5
Control (2) (Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	5.0	1.66
L.S.D	1.93	1.85	2.38	2.18	2.36	2.54	2.95	3.26	3.53	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection

Discussion

In vitro results of the present studies suggested that biocontrol agents (BBA's) *viz*., *B. cereus* and *B. subtilis*, have significantly reduced linear growth of all tested fungi compared to control. In the present work, *B. subtilis* proved to be

more effective in reducing growth of the tested damping-off pathogens *in vitro* than *B. cereus*. Suppression in growth of *Fusarium solani* by *B. cereus* and *B. subtilis* during the present studies supports the findings of Amalraj *et al.,* [9]. *Bacillus* species have a number of characteristics useful for biocontrol of plant diseases. They form endospores, which can withstand ecological stresses like high temperature and moisture stress for long periods [10]. The varying level of growth inhibition of *F. solani* by the antagonist might indicate the existence of the different mechanisms of antibiosis exhibited by different antagonistic. Moreover, the *Bacillus* antagonists kept the growth of *F. solani* more 62%. Similar results were reported on direct relationships between the population density of *Bacillus* and yeast antagonists on the spore germination and hyphal growth of anthracnose (*Colletotrichum gloeosporioides*) on mango [11, 12].

Results of experiments were conducted to evaluate the role of bacterial bioagent which proved a good antagonistic activates against *F. moniliforme* under laboratory, *B. subtilis* was more effect then *B. cereus*. Similar results were obtained [13]. Antagonistic activity of *B. subtilis* was also confirmed by Chet *etal*., 1990 [14] and Basha and Ulaganathan (2014) [15]. Similarly, in this study, *Bacillus subtilis* strains isolated from cow dung, rhizosphere soil and soil compost inhibited *Fusarium moniliforme* growth *in vitro* up to a maximum of about 41.1%, 61.2% and16.3% respectively. *Bacillus subtilis* from soil compost was earlier found to inhibit the growth of *Fusarium oxysporum* to the extent of 62%. *F. semitectum* was the most sensitive among all the pathogens tested to BBA's. Antagonistic capability of *B. cereus* and *B. subtilis* were tested *in vitro* against *F. semitectum*. The percentage of linear growth of the pathogen was recorded when its growth covered the plate surface in control treatment. Results of this study revealed that all tested BBA's inhibited growth of *F. semitectum*. *B. subtilis* gave the greatest percentage of growth inhibition. Such results are in agreement with those reported by Ramzan *et al*., [16] *Sclerotinia sclerotiorum* was sensitive to tested BBA's, while decrease growth and not produce scloricia. BBA's were inhibited *in vitro* the mycelial growth of *S. sclerotiorum*. Similar results *in vitro* have been reported by Levy *et al*., [17].

Growth of *Macrophomina phaseolina* was found to be inhibited by *B. cereus* and *B. subtilis*. Similar results have been reported by Weller 2002, [18, 6, 16] reported that, growth of *M. phaseolina* was inhibited by 11 fungi *viz., A. fusispora, Aspergillus flavus A. fumigatus, A. niger, Drechslera hawaiiensis, Emericella nidulans, Penicillium chrysogenum, P. citrinum, S. atra, T. harzianum* and *T. virens,* and seven bacteria *viz*., *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, M. varians* and *P. fluorescens*. However, the genus *Bacillus* is one of the most important biocontrol agents used against insect plant pathogens. *Bacillus* has been shown to be effective and was commercially applied against plant pathogenic fungi. *B. subtilis* produces five antibiotics *viz.,* bacillin, bacitracin, bacilomycin, subtilin and subtenolin. Moreover, produced chitinase enzyme [15]. Several species of *Bacillus* have been reported as antifungal agents. *B. subtilis* strain GBO3 was shown to possess strong antifungal activity by producing a special class of pore-forming lipopeptides [11], *B. brevis* decreased the fusarial wilt of pigeon peas by producing several antibiotics [19]. *B. subtilis* AF1 has been shown to produce N-acetyl glucosaminidase enzymes that suppress the growth of *Aspergillus niger* [20] However, the chitinolytic mechanism proved to be a major mechanism in the control of fungal pathogens [14] Herein, we report a chitinolytic soil bacterium *B. subtilis* BC121 that has broad spectrum of antifungal activity against various fungal pathogens tested. Further, the purification and characterization of the chitinase enzyme, Chi25 from *B. subtilis* BC121 was carried out. The purified enzyme, Chi25 was shown to inhibit the growth of fungi tested. This may be due to the hydrolytic action of Chi25 on fungal cell wall component, chitin that is made up of with the units of N-acetyl-D-glucosamine along with sugars, proteins, lipids and polysaccharides [21]. In vivo results of the present studies suggested that bacterial biocontrol agents (BBA's) including *B. cereus* and *B. subtilis,* proved to be antagonistic to all the tested pathogens, both *in vitro* and *in vivo*. Many researches confirmed the significant role of BBA's in controlling damping-off diseases, particularly Most of these researches dealt with controlling damping-off diseases incited by *R. solani, F. solani, M. phaseolina, P. ultimum, A. alternata, S. sclerotiorum, F. oxysporum, Sclerotium rolfsii, Cladosporium* sp., *F. moniliforme* and *F. semitectum*. To improve biological control of the disease, antagonistic bacterial isolates of *B. cereus* and *B. subtilis* with different carriers (talc based powder and wheat bran) were tested on incidence of cantaloupe damping-off caused by of the tested pathogens in greenhouse and field on conditions. Under greenhouse conditions, application of antagonistic BBA's one week before planting showed higher percentage of survival plant of pre and post emergence damping-off compared control. In pre emergence damping-off formulation of isolate *B. cereus* gave the highest number of survival plants percentage followed by *B. subtilis*. The two primary mechanism of action associated with nonpathogenic *Fusarium* spp. are induced systemic

resistance and competition for nutrients in the soil and parasitic competition for infection sites on the roots [13]. The distinguished antagonistic effect of *B. subtilis* both *in vitro* or in greenhouse was confirmed against damping-off and root rots incited by *F. solani* and *M. Phaseolina* [22, 23]. *Bacillus* is one of the first successful biocontrol agents used against insects and plant pathogens. *Bacillus* spp. rapidly and aggressively colonize the rhizosphere of various crops and have a broad spectrum of antagonistic activity against many pathogens. *Bacillus* spp. have been identified as potent antagonists against a wide range of pathogens such as *Macrophomina phaseolina* [18], *Fusarium* spp., *Rhizoctonia solani* and *Pythiumultimum.* According to the available literature, the suppressive effect of *B. subtilis* was attributed to competition with different phytopathogenic fungi on seed or root surface for nutrients, iron or infection or attachment sites or regions into which plant exuadates emerge [12, 24]. Similar results were observed by Nalisha *et al*., 2006 [8]. However, we believed that this suggested mechanism could be effective in controlling damping-off pathogens only under conditions of low nutrient availability. Production of antibiotics by *B. subtilis*, i.e. bacilysin and iturin-like lipopeptides was found to be possible effective mechanism explaining the suppression effect of this bacilysin against various phytopathogenic fungi**.**It is believed that, the modes of action of antibiotics and enzymes against pathogens are scarcely clarified. A better understanding of the mode of action is essential to allow for the prediction of the likelihood of resistance of the target pathogens to the antibiotics [25]. In addition, a better understanding of the mode of action might allow for the development of more effective synthetic antibiotic analogues. Many objectives were realized throughout our study on the application of BBA's in controlling damping-off and root rot pathogens. Some of the tested untraditional BBA's such, *B. cereus* proved to be more effective than *B. subtilis*.

Conflict of Interest

It is assured that there is no conflict of interest of any kind in any spheres.

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