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Improving Yield of Cotton Plant by *Bacillus* Species as Biocontrol Agents for Damping-off Disease

Aida H. Afify [1] and A. Z. A. Ashour [2]

[1] Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt
[2] Plant Pathology Research Institute, Agriculture Research Center(ARC), Giza, Egypt

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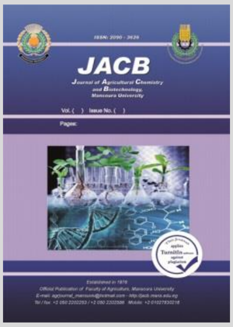

ABSTRACT

Four *Bacillus* species. were isolated from rhizosphere soil of cotton seedlings and identified as *Bacillus subtilis*, *Bacillus* sp., *Bacillus* sp. and *Bacillus amyloliquefaciens*. Bacillus isolates exhibited antagonistic activities against *Fusarium oxysporum* and *Rhizoctonia solani* which were isolated from roots of cotton seedlings showing damping-off symptoms. Greenhouse and field experiments with artificially and naturally infected soils with *F. oxysporum and R. solani* were carried out. Seeds of cotton (Giza 89) treated with the antagonistic bacteria (four *Bacillus* spp.) just before sowing were cultivated. Untreated seeds were involved as a control. In greenhouse experiment, the antagonistic *B. subtilis* and *B. amyloliquefaciens* significantly repressed the disease incidence to the lowest values in both seasons (2022 and 2023) as compared to the untreated control. Also, under field conditions the same *Bacillus* isolates significantly decreased the disease incidence. Finally, the application of *B. subtilis* and *B. amyloliquefaciens* as bio-controllar significantly increased seed cotton yield. Thus, these bacterial bio-controlling agents could be recommended for association in integrated control system of damping-off fungi in cotton and improving quality of cotton yield.

***Keywords*:** biological control, damping-off, *Bacillus* spp., cotton

INTRODUCTION

Cotton (*Gossypium barbadense* L.) is a very important crop and it is widly cultivated in many counteries because of the economic value of its seed for oil and fiber for clothes (Heale and Isaac 1965). The biological control is a safe technique in plant protection to avoid environmental pollution (Zabihulla and Tang, 2020). Cotton is the first crop to be treated with bio-control agents to suppress soil-borne fungi and rhizosphere diseases from microorganisms (Brannen and Kenney 1997). It is well known that *Rhizoctonia solani* and *Fusarium oxysporum* attack a wide range of host plants and destroy the crops. Damping-off is a disease that aims to the prevent germination of plant seeds and suppress growth of young seedlings, which the most important yield constraints represents for farmers in nurseries and fields (Jay, *et al.* 2017). Damping-off disease caused by phytopathogenic fungi. Furthermore, soil –borne fungi specially *Rhizoctonia solani* and *Fusarium oxysporum* being soil inhabitants therefore are difficult to suppress. Also, the chemical fungicides for controlling the plant pathogens not always effective and may cause environmental pollution and occasional phytotoxicity (Lifshitz *et al*. 1984). Antagonistic microorganisms have been used by many workers for controlling soil-borne plant pathogens (Afify and Ashour 1995). *In vitro*, there are several strains of *Bacillus* able to inhibit mycellium fungal growth (Fravel 1988 & Ashour and Afify 2024). Henrique, *et al.* (2020) concluded that the bacteria as *B. velezensis*, *B. amyliquefaciens* and *Paenibacillus* sp. able suppressed *Colletotrichum gossypii* var. *cephalosporioides* (CGC) in both conditions, greenhouse and field experiments. From ANOVA tests Aly *et al.* (2022) reported that species of *Bacillus* exhibited highly significant ability to suppress damping-off disease. This study was carried out in greenhouse and field experiments as an attempt to control damping off disease in cotton using antagonistic bacteria (*Bacillus subtilis*, *Bacillus* spp. and *Bacillus amyliquefaciens*). Moreover, effect of these antagonistic bacteria on cotton yield was also studied

MATERIALS AND METHODS

The used bacteria

The four *Bacillus* isolates (*Bacillus subtilis*, *Bacillus* sp., *Bacillus* sp. and *Bacillus amyloliquefaciens*) were isolated from rhizosphere soil of cotton seedlings. On the basis of cultural, morphological and molecular characteristics these isolates were identified according to Ashour and Afify (2024).

Source of pathogens isolates

Fusarium oxysporum Schlech and *Rhizoctonia solani* kuhn as fungal pathogens were isolated from infected cotton seedlings. At plant pathology Research Institute, ARC, Giza, Egypt these fungi were purified and identified. These fungi were maintained on potato-dextrose agar medium (PDA) and kept at 4ºC for further study.

The antagonistic effects of *Bacillus* spp.

Antagonism between the bacterial isolales (*Bacillus subtilis*, *Bacillus* sp., *Bacillus* sp. and *Bacillus amyloliquefaciens*) and fungal isolates (*F. oxysporum* and *R. solani*) were previously recorded by Ashour and Afify (2024). Data recorded in Table (1) represent the details of the antagonistic activities of the four bacilli isolates used against both fungal isolates.

* Corresponding author.
E-mail address: aidaafify@yahoo.com
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Table 1. Inhibition zones (mm) of mycelial growth of both fungal isolates by four bacilli isolates as previously determined by Ashour and Afify (2024).

Isolates of *Bacillus* spp.		Inhibition zone (mm) *F. oxysporum*	*R. solani*	Means (mm)
B. subtilis	*B*.sp.- 1	11.8	13.8	12.8
Bacillus sp.	*B*.sp.- 4	10.4	12.4	11.4
Bacillus sp.	*B*.sp.- 12	11.6	9.6	10.6
B.amyloliquefaciens	*B*.sp.14	13.0	11.6	12.3

Several microorganisms able to antagonize different *Fusarium* species (Sundaramoorthy and Balabaska, 2013). Also, strain of *B. subtilis* is a potential alternative for the inhibation of *Fusarium* species in the soil (Abdelmoteleb, *et al.,* 2017). In addition, rhizobacteria *Bacillus* strains reduce phytopathogenic fungi by different mechanisms and cause suppression of these fungal pathogen by producing antimicrobial compounds (Sofy *et al.* 2021 & Ashour and Afify 2024).

Experimental conditions:

1.Treatment of cotton seed with the antagonistic bacteria

Cotton seeds were surface sterilized by gentle agitation for 3 min. in 2.5% calcium hypochlorite solution. After thorough washing in six changes of sterile distilled water, the seeds were aseptically air dried, placed in flasks containing 150 ml bacterial suspension (10^9 cfu/ml) for 24 hr and sown in greenhouse potted soil and/or field experiment (Mew and Rosales 1986).

2.Preparation of fungal inocula

Substrate for growth of each fungus was prepared in 500 ml glass bottles, each bottle contained 100 g of sorghum grains and 80 ml of water. Contents of bottles were autoclaved for 30 min at 121ºC. Fungal inoclum taken from one-week-old culture on PDA, was aseptically introduced into the bottles and allowed to colonize sorghum for 3 weeks. The fungus-sorghum mixture was air-dried to a powder in a blender and was stored in plastic bags at 5ºC until use. In the present study batches of soil were placed on greenhouse and infested separately with inoculum of each fungus at the rates of 0, 5, 10 and 50g/kg soil of *F. oxysporum* and *R. solani* respectively. In 20 cm diameter clay pots infested soils were planted with 10 cotton seeds per pot (cultivar Giza 89). In the control treatments, no fungi were added to soil. The experiments were carried out during two successive seasons (2022 and 2023).

3.Variable of the tested plants

After one week of soils infection, soils were planted with the antagonistic coated seeds and kept under the decided conditions for 5-6 weeks before recording the percentage of diseased seedlings with *F. oxysporum* and *R. solani*. Each treatment of each tested bio-controller was replicated 5 times among twice attempts in each season. Finally the produced cotton yield (kentar/fed) was recorded at the end of growth season (Henis *et al.* 1978).

Statistical analysis

Before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Percentage data of greenhouse and field experiments were transformed into arc sine angles. After that, ANOVA was performed by the software A Microcomputer Program for the Design (MSTAT-C, Michigan State Univ., USA).

RESLTUS AND DISCUSSION

In greenhouse experiment data of Table (2) indicated that, antagonistic bacteria reduced the disease incidence of two pathogenic fungi in both seasons as compared with the untreated control. Among the single biocontrol agents, *B. subtilis* in 2022 and *B. amyloliquefaciens* in 2023 reduced the disease incidence to the lowest values, *i.e*., 47.33 and 50.00% while untreated treatment exhibited the maximum values viz, 72.33 and 72.00%. Each of *B. subtilis* and *B. amyloliquefaciens* was found to gave good disease controlling comparing with untreated treatment (Table 2). These results are in agreement with the eariler reports (Xu *et al.* 1993) and also, with recantly reports Henrique, *et al.* (2020) who concluded that the strains of *B. velezensis*, *B. amyliquefaciens* and *Paenibacillus* sp. controlled *Colletotrichum gossypii* var. *cephalosporioides* (CGC) in both conditions greenhouse and field experiments. By ANOVA Aly *et al.* (2022) recorded that strain of *Bacillus* showed highly significant activity in suppressing damping off disease

Table 2. The effects of biocontrol agents on the incidence of cotton seedling damping off under greenhouse condition.

Season	Biocontrol agents	Diseased-Seedling%[b] Pathogenic fungi: None	F. oxysporum	R. solani	Mean
2022	None	97.0 (81.00)	60.0 (50.82)	60.0 (50.82)	72.33 (60.88)
	B. subtilis	54.0 (47.31)	44.0 (41.54)	44.0 (41.54)	47.33 (43.46)
	Bacillus sp.	64.0 (53.18)	52.0 (46.15)	48.0 (43.85)	54.67 (47.73)
	Bacillus sp.	54.0 (47.31)	48.0 (43.85)	44.0 (41.54)	48.67 (44.23)
	B. amyloliquefaciens	56.0 (48.46)	46.0 (42.69)	46.0 (42.69)	49.00 (44.62)
	Mean	65.0 (55.45)	50.0 (45.01)	48.4 (44.08)	
LSD for biocontrl agents (3.294 P< 0.05)					
2023	None	80.0 (63.73)	68.0 (55.71)	68.0 (55.71)	72.00 (58.39)
	B. subtilis	64.0 (53.14)	56.0 (48.46)	58.0 (49.62)	59.33 (50.41)
	Bacillus sp.	58.0 (49.65)	56.0 (48.46)	52.0 (46.15)	55.33 (48.09)
	Bacillus sp.	62.0 (51.96)	52.0 (46.15)	50.0 (45.00)	54.67 (47.70)
	B. amyloliquefaciens	58.0 (49.65)	46.0 (42.69)	46.0 (42.69)	50.00 (45.00)
	Mean	64.4 (53.63)	55.6 (48.29)	54.8 (47.83)	
LSD for biocontrl agents (2.596 P <0.05)					

b Percentage data were tranformed into arc-sine angles .

Data in Table (3) indicated that the biocontrol agents as a whole decreased the incidence of disease as compared to the untreated control which gave 74.69 and 63.36% in 2022 and 2023, respectively. Although *B. subtilis* was effective in decreasing the incidence of damping-off disease in cotton along two successive seasons 2022 and 2023giving the values

61.16% and 51.64% comparing with 74.69% and 63.36% for untreated treatment. Hence, these data are in full agreement with those recorded by Kaur and Mukhopadhyay (1992). In addition, Henrique, *et al.* (2020) reported that *Bacillus* spp. are importantl tools to produce high cotton yield and fiber of good quality

Table 3. The effect of biocontrol agents on the incidence of cotton seedling disease under field conditions

Season	Biocontrol agents	Diseased-Seedling%[b] Pathogenic fungi: None	F. oxysporum	R. solani	Mean
2022	None	80.30 (63.71)	71.43 (57.79)	72.33 (58.34)	74.69 (59.94)
	B. subtilis	67.88 (55.51)	57.53 (49.34)	58.08 (49.65)	61.16 (51.50)
	Bacillus sp.	63.68 (52.96)	50.43 (45.24)	55.70 (48.28)	56.60 (48.83)
	Bacillus sp.	65.60 (54.11)	49.90 (44.94)	51.13 (45.64)	55.54 (48.23)
	B. amyloliquefaciens	61.53 (51.67)	54.65 (47.68)	57.65 (49.43)	57.94 (49.60)
	Mean	67.80 (55.59)	56.79 (48.99)	58.98 (50.27)	
LSD for biocontrl agents (2.26 P< 0.05)					
2023	None	81.63 (64.60)	56.08 (45.80)	52.38 (46.36)	63.36 (53.15)
	B. subtilis	56.20 (48.57)	52.60 (46.49)	46.13 (42.78)	51.64 (45.94)
	Bacillus sp.	54.00 (47.30)	53.33 (46.91)	40.00 (39.20)	49.11 (44.47)
	Bacillus sp.	45.30 (42.28)	44.15 (41.60)	38.73 (38.48)	42.73 (40.79)
	B. amyloliquefaciens	45.50 (42.41)	50.93 (45.53)	40.00 (39.20)	45.48 (42.38)
	Mean	56.53 (49.03)	51.42 (45.27)	43.45 (41.20)	
LSD for biocontrl agents (3.234 P <0.05)					

b Percentage data were tranformed into arc-sine angles.

Among the single biocontrol agents *B. subtilis* increased seed cotton yield significantly up to the highest values *i.e.*, 5.883 and 5.886 kentar/fed for 2022 and 2023 seasons (Table 4). The similar application of bacterial strains for controlling cotton seedling damping-off under field conditions, as demonstrated here is in agreement with Aly *et al.* (2021) who reported that strains of *Bacillus* spp. significantly developed yield of cotton. In addition, the same authors stated that *Bacillus* strains treatments significantly increased yield at different locations in Egypt.

Table 4. Effect of biocontrol agents on seed cotton yield (kentar*/feddan) under field conditions

Season	Biocontrol agents	Pathogenic fungi: None	F. oxysporum	R. solani	Mean
2022	None	3.990	4.972	5.060	4.674
	B. subtilis	5.665	5.920	6.063	5.883
	Bacillus sp.	4.842	5.600	5.822	5.421
	Bacillus sp.	5.087	5.438	5.772	5.432
	B. amyloliquefaciens	4.610	5.397	5.428	5.145
	Mean	4.839	5.465	5.629	
LSD for biocontrol agents (0.4556 P< 0.05)					
2023	None	4.900	5.188	5.330	5.139
	B. subtilis	5.505	5.942	5.210	5.886
	Bacillus sp.	4.980	5.515	5.530	5.612
	Bacillus sp.	5.355	5.628	5.768	5.584
	B. amyloliquefaciens	5.145	5.512	5.612	5.423
	Mean	5.177	5.557	5.49	
LSD for biocontrol agents (0.3101 P <0.05)					

***Kentar = 157.5 kg**

CONCLUSIONS

The strains of *Bacillus* species controlled damping-off fungi on cotton plants in both conditions greenhouse and field experiments. Therefore, *B. subtilis* and *B. amyloliquefaciens* produced the best results in the incidence of cotton seedlings disease and cotton yield, performing even better than the untreated cotton plants. These *Bacillus* species could be a potential candidate as a bioagents for soil-borne fungi and could be used for commercial exploration.

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تحسين محصول نبات القطن بواسطة أنواع الباسيلس كعوامل مقاومة حيوية لمرض موت البادرات

عايده حافظ عفيفى[1] و عبد الودود زكى عاشور[2]

[1]قسم الميكروبيولوجى – كلية الزراعه – جامعة المنصوره – المنصوره- مصر
[2] معهد أمراض النباتات - مركز البحوث الزراعيه – الجيزه – مصر

الملخص

قد تم إختبار قدرة أربعة سلالات من بكتيريا جنس الباسيلس على تثبيط نمو فطرين من فطريات مرض موت بادرات القطن هما : فيوزاريوم أوكسيسبورم وريزوكتونيا سولانى والتى تم عزلها من جذور بادرات القطن المصابة .وفى هذه الدراسة أقيمت تجارب الزراعة فى الصوبة وكذلك فى الحقل فى موسمين 2023/2022 حيث تم زراعة بذور نبات القطن المعاملة بسلالات بكتيريا الباسيلس الأربعة منفردة وأخرى غير معاملة مقارنة (كنترول) كما تم معاملة التربة بالمسببات المرضية الفطرية . وقد سجلت تجربة الزراعة فى الصوبة أن عوامل المقاومة الحيوية من بكتيريا الباسيلس قللت معنويا نسبة الإصابة بالمرض وذلك فى النباتات المعاملة عن غير المعاملة (الكنترول) وسجلت السلالاتين باسيلس ستلس وباسيلس أميلوفاكشن أقل قيمة فى نسبة الإصابة بالمرض كما أكدت تجارب الحقل أن المعاملة بالسلالات البكتيرية أدت إلى زيادة محصول القطن . ومن نتائج هذه التجارب يمكن أن نوصى بإستخدام تلك السلالات من بكتيريا الباسيلس كعوامل مقاومة حيوية لفطرى فيوزاريوم أوكسيسبورم وريزوكتونيا سولانى حيث أنها تقلل إصابة البادرات وبالتالى تحسن وتزيد محصول القطن .