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Pathogenicity of *Bacillus* Strains to Cotton Seedlings and Their Effects on Some Biochemical Components of the Infected Seedlings

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Pathogenicity of eight *Bacillus* strains to seedlings of four cotton cultivars was evaluated under greenhouse conditions. Each of the tested cultivars was individually treated with powdered inoculum of each bacterial strain. Untreated seeds were planted as control treatments in autoclaved soil. Effects of the tested strains on levels and activities of some biochemical components of the infected seedlings were also assayed. The biochemical components included total soluble sugars, total soluble proteins, total free amino acids, peroxidase, polyphenol oxidase, phenols, and lipid peroxidation. ANOVA showed that *Bacillus* strain (B) was a very highly significant source of variation in damping-off and dry weight. Cotton cultivar (V) was a nonsignificant source of variation in damping-off while it was a significant source of variation in dry weight. B × V interaction was a significant source of variation in damping-off and a nonsignificant source of variation in dry weight. *Bacillus* strain was the most important source of variation as it accounted for 59.36 and 64.99% of the explained (model) variation in damping-off and dry weight, respectively. The lack of significant correlation between levels and activities of the assayed biochemical components and incidence of damping-off clearly demonstrated that these biochemical components were not involved in the pathogenicity of the tested strains. Therefore, it was hypothesized that the pathogenicity of the tested strains could be due to the effect of cell wall degrading enzymes of pathogenic toxins. Based on the results of the present study, *Bacillus* strains should be considered in studying the etiology of cotton seedling damping-off.

Keywords **:** amino acid, antioxidant enzymes, damping-off, phenols, sugars

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The genus *Bacillus* represents a large group of Gram-positive bacteria. They are rod-shaped, endospore-forming, catalase-positive bacteria with aerobic or facultatively anaerobic metabolism. *Bacillus* spp. are exceptionally ubiquitous, since they can inhabit a large variety of ecological niches; they are found in soil, water, air, surfaces, and the rhizosphere of plants, as well as many other extreme environments (Fira et al., 2018). In general, rhizobacteria *Bacillus* strains antagonize phytopathogenic fungi through various mechanisms such as competition for niches (e.g., iron through siderophores synthesis) as well as parasitism, which may involve the production of hydrolytic enzymes such as chitinase, β-1,3-glucanase, protease, and cellulase, that lyse fungus cell walls. In addition, *Bacillus* strains

cause inhibition of the pathogen by secreting antimicrobial compounds such as antibiotics, toxins, and biosurfactants, and the induction of systemic resistance in host plants (Kumar et al., 2012; Mohamed and Gomaa, 2012; Sofy et al., 2021).

Contrary to the common belief that *Bacillus* spp. are effective biocontrol agents, among members of this genus, there are numerous species that are pathogenic to plants. For example, *B. macerans* caused a decay of flax and wheat roots (Rempe and Sorokina, 1950). *B. subtilis* and *B. licheniformis* inhibited growth of some Egyptian cotton cultivars (Naim and Hussein, 1956). *B. pumilus*, *B. subtilis*, and *B. polymyxa* were reported to cause postharvest soft rot of vegetables (Chiu et al., 1964). Seedborne and soilborne strains of *Bacillus* sp. increased seed decay, reduced germination, reduced hypocotyl and radicle elongation and stunted seedling plants of soybean under extreme environmental conditions in both greenhouse and field trials (Suslow and Schroth, 1982). *B. megaterium* pv. *cerealis* caused wheat white blotch (Hosford, 1982). Gabr and Gazar (1983) reported that *B. pumilus* and *B. polymyxa* were causal pathogens of head rot of cabbage. *B. polymyxa* is the causal agent of bacterial seedling blight of tomato (Caruso et al., 1984). *B. circulans* caused a disease of date palm seedling in the greenhouse (Leary and Chun, 1989; Leary et al., 1986). *B. pumilus*, *B. subtilis*, *B. coagulans*, and *B. polymyxa* have been reported to be the main causal agents of garlic clove postharvest decay (Galal et al., 2002; Saleh, 1995). *B. pumilus* causes brown spots on fruits and leaves of Balady peach (Saleh et al., 1997). *B. pumilus* causes leaf blight of mango trees in Egypt (Galal et al., 2006). A *Bacillus* sp., identified as a possible *B. pumilus* strain, was associated with leaf and twig dieback of Asian pear trees in China (Li et al., 2009). *B. pumilus* also caused potato soft rot in storage (Bathily et al., 2010) and ginger rhizome rot (Peng et al., 2013). *B. pumilus* has a potential to cause the soft rot disease in pine seedling and symptoms of wetwood disorder in young Scot's pine trees (Kovaleva et al., 2015). *B. amyloliquefaciens* caused bacterial rot of onion (Hwang et al., 2012) and black rot of arrowhead (*Sagittaria sagittifolia* L.) (Zhong et al., 2015). *B. amyloliquefaciens* also caused soft rot of potato tubers in the field (Wang et al., 2017).

In the present study, we evaluated the pathogenicity of eight *Bacillus* strains to seedlings of four cotton cultivars under greenhouse conditions. Effects of the tested strains on levels and activities of some biochemical components of the infected seedlings were also determined.

Materials and Methods

Source of cotton cultivars. Four cultivars of cotton (*Gossypium barbadense* L.) were used in the present study: V1 Giza 86; V2 Giza 87; V3 Giza 88; V4 Giza 90. Seeds of these cultivars were obtained from the Cotton Research Institute, Agricultural Research Center, Giza, Egypt.

Source of bacterial strains. Eight strains of *Bacillus* spp. were used in the present study (Table 1). The *Bacillus* strains originated from roots of cotton plants and were obtained from the bacterial collection of the Cotton Diseases Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Bacterial strains were identified to the species level by standard bacteriological tests (Holt et al., 1994) (Supplementary Table 1).

Preparation of bacterial inoculum. The *Bacillus* strains were grown in nutrient glucose broth at 30ºC for 72 h in a shaker incubator. The growth was adjusted turbidimetrically to 10^8 cfu/ml using Spectro 2000 RSP 220 V, 50 Hz. Bacterial cultures were formulated in a powder form by mixing 400 ml of cell suspension with 1 kg talc as carrier, which was previously autoclaved for 30 min for 2 successive days; 10 grams of carboxy methyl cellulose were added to 1 kg of the carrier and mixed well. The pH of all materials was adjusted to 7.0 by adding calcium carbonate. The bacterial population was assessed as 4×10^7 cfu/g talc.

Pathogenicity of *Bacillus* strains to cotton cultivars. Autoclaved clay soil was dispensed into 15-cm-diameter clay pots. Slightly moist seeds of each of the tested cultivars were individually treated with the powdered inoculum of

Table 1. Classification and geographic origins of *Bacillus* strains used in the present study

Strain no.	Classification	Geographic origin	
		Region	Governorate
B_1	*B. coagulans*	Unknown	Unknown
B_2	*B. globisporus*	East Delta	Daqahliya
B_3	*B. pumilus*	Middle Egypt	Giza
B_4	*B. subtilis*	Upper Egypt	Assiute
B_5	*B. circulans*	West Delta	Beheira
B_6	*B. cereus*	Unknown	Unknown
B_7	*B. coagulans*	Middle Egypt	Minya
B_8	*B. cereus*	East Delta	Sharqiya

each bacterial strain at a rate of 10 g/kg seeds, and thoroughly shaken in plastic bags before being planted in the autoclaved soil at a rate of 10 seeds/pot. Untreated seeds were planted as control treatments in the autoclaved soil. The pots were randomly distributed on a greenhouse bench under a temperature regime that ranged from 25 ± 3 to 32 ± 4°C. The effects of *Bacillus* strains on cotton cultivars were evaluated 45 days after sowing by using the percentage of infection (combined pre-and post-emergence damping-off) and dry weight (mg/plant).

Biochemical studies

Determination of sugar content. Seedlings of treated and untreated cotton plants were pulverized in a mortar and pestle in 5 ml of 80% ethanol (v/v), then heated for 10 min and centrifuged for 10 min at 2,000 rpm. The total soluble sugars were measured using phenol sulfuric acid and the supernatant (Dubois et al., 1956). As a control, pure glucose was employed.

Determination of total soluble protein. The seedlings were powdered in a pH 6.5 sodium phosphate buffer. According to Lowry et al. (1951), the total soluble protein content in the supernatant was measured. The amount of total soluble protein was estimated using the Bovine Serum Albumin standard curve.

Determination of total free amino acids. Moore and Stein (1954) used the ninhydrin method to determine total free amino acid content in cotton seedlings pulverized in a mortar and pestle in 5 ml of 80% ethanol (v/v). The mixture was heated for 10 min and centrifuged at 2,000 rpm for 10 min, and the supernatant was collected.

Determination of enzymatic antioxidant. For peroxidase and polyphenoloxidase, cotton seedlings were crushed in a sodium phosphate buffer at pH 6.5. The activity of the following enzymes was measured in the supernatant. Peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.14.18.1) were measured using Kar and Mishra's technique (Kar and Mishra, 1976).

Determination of total phenols. According to Dihazi et al. (2003), the levels of soluble phenols in cotton seedlings were measured by the absorbance at 725 nm. The amount of soluble phenols was represented as mg gallic acid/g fresh weight, with gallic acid as the reference.

Determination of malondialdehyde. The Heath and Packer (1968) method of calculating malondialdehyde (MDA) content was used to determine lipid peroxidation. At 532 nm and 600 nm, the absorbance of the resultant supernatant was measured. The extinction coefficient of 155/mM/cm was used to calculate the absorbance coefficient of MDA.

Statistical analysis. The experimental design of the present study was a randomized complete block with three replicates. Least significant difference (LSD) was used to compare means of *Bacillus* strains within each cotton cultivar. Analysis of variance (ANOVA) and correlation analysis were carried out by the MSTAT-C statistical package. Percentage data were transformed into arc sin angles before carrying out ANOVA to produce approximately constant variance.

Results

ANOVA (Table 2) showed that *Bacillus* strain was a very highly significant source of variation in damping-off and dry weight. Cotton cultivar was a nonsignificant source of variation in damping-off while it was a significant source of variation in dry weight. B × V interaction was a significant source of variation in damping-off and a nonsignificant source of variation in dry weight. *Bacillus* strain was the most important source of variation as it accounted for 59.36 and 64.99% of the explained (model) variation in damping-off and dry weight, respectively. Cultivar was the least important source of variation, as it accounted for 3.36 and 10.51% of the explained (model). B × V interaction was always the second in importance as a source of variation, as it accounted for 37.27 and 24.51% of the explained

Table 2. Analysis of variance of the effects of *Bacillus* strain, cotton cultivar, and their interactions on incidence of damping-off and dry weight of cotton seedlings

Variable and sources of variation	D.F.	M.S.	*F*-value	*P* > *F*
Damping-off				
Replication	2	5.337	0.051	0.950
Bacillus strain (B)	8	1,017.283	9.718	0.000
Cotton cultivar (V)	3	153.735	1.469	0.231
B × V	24	212.907	2.034	0.012
Error	70	104.683		
Dry weight				
Replication	2	5,929.037	0.531	0.590
Bacillus strain (B)	8	104,332.176	9.348	0.000
Cotton cultivar (V)	3	44,981.639	4.030	0.011
B × V	24	13,114.806	1.175	0.295
Error	70	11,161.151		

D.F., degrees of freedom; M.S., mean square.

Table 3. Effect of *Bacillus* strains, cotton cultivars, and their interactions on incidence of damping-off and dry weight (mg/plant) of cotton cultivars

Bacillus strains	V1		V2		V3		V4		Mean	
	%	Trans.	%	Trans.	%	Trans.	%	Trans.	%	Trans.
Incidence of damping-off on cotton cultivars										
B1	33.33	35.25[a]	33.33	35.25	26.67	30.76	60.00	51.15	38.33	38.10
B2	26.67	30.94	26.67	33.03	28.83	32.01	68.33	56.33	37.63	38.08
B3	65.00	55.00	55.00	47.87	90.00	72.15	60.00	50.94	67.50	56.49
B4	61.67	53.29	55.00	47.91	58.33	51.83	65.00	54.67	60.00	51.92
B5	40.00	38.85	43.33	41.07	50.00	45.01	30.00	33.16	40.83	39.52
B6	35.00	36.27	30.00	33.16	33.33	35.25	33.33	35.25	32.92	34.98
B7	38.33	37.90	45.00	42.12	30.00	33.16	18.33	23.36	32.92	34.14
B8	18.33	25.00	30.00	33.16	18.33	25.30	61.67	52.87	32.08	34.08
Control	18.33	25.30	21.67	27.52	23.33	28.54	25.00	27.19	22.08	27.14
Mean	37.41	37.53	37.78	37.90	39.81	39.33	46.85	42.77	40.48	39.38
LSD for B × V (transformed data)	16.54									
Dry weight										
B1	241.67		194.00		310.00		373.00		279.67	
B2	319.33		263.33		477.00		534.33		398.50	
B3	501.67		511.00		668.67		623.67		576.25	
B4	386.00		516.67		413.00		489.00		451.17	
B5	360.33		391.00		415.00		330.00		374.08	
B6	243.67		272.67		265.67		305.67		271.92	
B7	394.33		293.00		295.33		298.00		320.17	
B8	332.00		291.33		389.67		538.67		387.92	
Control	430.00		372.00		395.67		400.00		399.42	
Mean	356.56		345.00		403.33		432.48		384.34	
LSD ($P \leq 0.05$) for *Bacillus*	85.40									
LSD ($P \leq 0.05$) for cultivar	69.73									

LSD, least significant difference.
[a]Percentage data was transformed into arc sine angle before carrying out the ANOVA to produce approximately constant variance.

(model). Due to the significant B × V interaction as a source of variation in damping-off, an interaction LSD was used to evaluate the pathogenicity of *Bacillus* strains on cotton cultivars. The evaluation showed that B_1, B_2, and B_8 were pathogenic only to V_4 and nonpathogenic to the other cultivars. B_3 and B_4 were pathogenic to all the tested cultivars. On the contrary, B_5, B_6, and B_7 were nonpathogenic on all cultivars (Table 3).

Due to the nonsignificant B × V interaction as a source of variation in dry weight, the general means were used to compare among *Bacillus* strains and among cultivars. These comparisons showed that B_1 and B_6 significantly reduced dry weight, B_3 significantly increased dry weight, and the other strains did not significantly affect dry weight. The only significant difference among cultivars was observed between V_2 and V_4 (Table 3). A positive significant correlation was observed between damping-off and dry weight of all cultivars except V_1 (Table 4).

Table 4. Correlation between damping-off (%) and dry weight (mg/plant) of four cotton cultivars infected with *Bacillus* strains

Cotton cultivar	Correlation
V1	0.444[a] (0.231)[b]
V2	0.713 (0.031)
V3	0.748 (0.020)
V4	0.781 (0.013)

[a]Linear correlation coefficient.
[b]Probability level and $n = 9$.

Bacillus strain was a very highly significant source of variation in sugars, proteins, and amino acids, as it accounted for 48.95, 40.01, and 22.8%, respectively of the explained (model). Cultivar was a nonsignificant source

Table 5. Analysis of variance of the effects of *Bacillus* strains, cotton cultivars, and their interactions on some biochemical components of cotton seedlings

Biochemical component and sources of variation	D.F.	M.S.	*F*-value	*P* > *F*
Sugars				
Replication	2	4.751	0.247	0.782
Bacillus strain (B)	8	265.175	13.800	0.000
Cotton cultivar (V)	3	45.295	2.357	0.079
B × V	24	86.526	4.503	0.000
Error	70	19.215		
Proteins				
Replication	2	0.715	0.340	0.713
Bacillus strain (B)	8	21.280	10.128	0.000
Cotton cultivar (V)	3	9.218	4.387	0.007
B × V	24	9.485	4.514	0.000
Error	70	2.101		
Amino acids				
Replication	2	9.846	0.754	0.474
Bacillus strain (B)	8	142.874	10.936	0.000
Cotton cultivar (V)	3	174.010	13.320	0.000
B × V	24	139.502	10.678	0.000
Error	70	13.064		

D.F., degrees of freedom; M.S., mean square.

of variation in sugars while it was very highly significant source of variation in proteins and amino acids as it accounted for 3.14, 6.50, and 10.41%, respectively of the explained (model). B × V interaction was always a very highly significant source of variation in all biochemical components as it accounted for 47.92, 53.50, and 66.78%, respectively of the explained (model). *Bacillus* strains and B × V interaction were almost equally important as sources of variation in sugars. B × V interaction was the most important source of variation in proteins and amino acids. Cultivar was the least important source of variation in all biochemical components (Table 5).

Due to the significant effect of V × B interaction as a source of variation in biochemical components, an interaction LSD was used to compare the effects of *Bacillus* strain on levels of biochemical components within each cultivar. This comparison showed that the differences in biochemical components and the control were not the same for each cultivar. Cultivars responded differently to the application of *Bacillus* strains. For example, B_4 significantly increased sugar content of V_1 while it did not affect sugar content of V_3. The significant B × V interaction also indicated that the differences between strains may vary depending on the tested cultivar. For instance, the difference between B_3 and B_4 in sugar content was significant in V_1 and nonsignificant in V_2 (Table 6).

Table 6. Effect of *Bacillus* strains, cotton cultivars, and their interactions on content of total soluble sugars (mg glucose/g), total soluble proteins (mg/g), and total free amino acids (mg/g) of cotton seedlings

Bacillus strains	Cotton cultivars				
	V1	V2	V3	V4	Mean
Total soluble sugars					
B1	16.68	15.29	12.69	11.23	13.97
B2	11.32	17.63	14.24	9.80	13.25
B3	22.96	12.85	13.65	13.81	15.82
B4	35.76	16.93	21.81	22.60	24.28
B5	10.21	7.68	14.41	14.13	11.61
B6	26.79	22.99	19.77	22.17	22.93
B7	19.40	19.67	25.77	23.87	22.18
B8	28.29	13.64	16.21	27.39	21.38
Control	7.83	24.53	21.97	19.31	18.41
Mean	19.92	16.80	17.84	18.26	18.21
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	7.09				
Total soluble proteins					
B1	7.33	6.71	7.13	5.83	6.75
B2	12.40	7.34	5.35	5.04	7.53
B3	8.60	7.47	6.80	5.90	7.20
B4	11.45	7.07	7.42	12.06	9.50
B5	6.95	7.67	9.29	6.77	7.67
B6	7.81	10.58	8.66	11.38	9.61
B7	9.22	7.07	8.84	9.34	8.62
B8	11.20	8.26	9.40	11.65	10.13
Control	5.51	6.66	7.24	6.70	6.53
Mean	8.94	7.65	7.79	8.30	8.17
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	2.34				
Total free amino acids					
B1	28.77	10.07	11.28	7.60	14.43
B2	6.20	8.78	13.83	8.47	9.32
B3	29.12	11.18	10.15	8.75	14.80
B4	8.10	2.40	3.72	15.53	7.44
B5	21.82	8.60	9.03	27.72	16.79
B6	14.93	9.93	6.80	13.55	11.30
B7	13.12	13.43	6.20	14.12	11.72
B8	3.82	9.00	7.72	5.52	6.51
Control	2.77	8.52	20.95	19.25	12.87
Mean	14.29	9.10	9.97	13.39	11.69
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	5.84				

LSD, least significant difference.

B_2 significantly increased protein content of V_1 while it did not affect protein content of V_2. The difference between

Table 7. Analysis of variance of the effects of *Bacillus* strains, cotton cultivars, and their interactions on some biochemical components of cotton seedlings

Biochemical component and sources of variation	D.F.	M.S.	*F*-value	*P* > *F*
Peroxidase				
Replication	2	9.944	1.255	0.291
Bacillus strain (B)	8	86.495	10.914	0.000
Cotton cultivar (V)	3	33.116	4.179	0.009
B × V	24	33.242	4.195	0.000
Error	70	7.925		
Polyphenol oxidase				
Replication	2	2.455	0.509	0.603
Bacillus strain (B)	8	92.196	19.129	0.000
Cotton cultivar (V)	3	15.368	3.188	0.029
B × V	24	21.994	4.563	0.000
Error	70	4.820		

D.F., degrees of freedom; M.S., mean square.

B_2 and B_3 was significant in V_1 and nonsignificant in V_2 (Table 6). B_3 significantly increased amino acid content of V_1 while it did not affect amino acid content of V_2. The difference between B_1 and B_2 was significant in V_1 and nonsignificant in V_2 (Table 6). Each *Bacillus* strain and the B × V interaction were very highly significant sources of variation in activities of peroxidase and polyphenol oxidase. Cultivar was a highly significant and/or a significant source of variation in activities of peroxidase and polyphenol oxidase, respectively (Table 7).

Each *Bacillus* strain and the B × V interaction were very highly significant sources of variation in contents of phenols and lipid peroxidation. Cultivar was a nonsignificant source of variation in phenol content while it was a very highly significant source of variation in lipid peroxidation content (Table 8). *Bacillus* strain was the first in importance as a source of variation in polyphenol oxidase activity, phenol content, and lipid peroxidation content as it accounted for 56.24, 61.22, and 50.10%, respectively of the explained (model). B × V interaction was the first in importance as a source of variation in peroxidase activity as it accounted for 50.20% of the explained (model), while it was the second in importance as a source of variation in the other variables. Cultivar was always the least important source of variation in peroxidase, polyphenol oxidase, phe-

Table 8. Analysis of variance of the effects of *Bacillus* strains, cotton cultivars, and their interactions on some biochemical components of cotton seedlings

Biochemical component and sources of variation	D.F.	M.S.	*F*-value	*P* > *F*
Phenols				
Replication	2	0.059	0.337	0.715
Bacillus strain (B)	8	2.663	15.091	0.000
Cotton cultivar (V)	3	0.353	2.002	0.122
B × V	24	0.518	2.937	0.000
Error	70	0.176		
Lipid peroxidation				
Replication	2	8,867.370	2.140	0.125
Bacillus strain (B)	8	55,470.850	13.389	0.000
Cotton cultivar (V)	3	23,031.441	5.559	0.002
B × V	24	15,540.226	3.751	0.000
Error	70	4,143.066		

D.F., degrees of freedom; M.S., mean square.

Table 9. Effect of *Bacillus* strains, cotton cultivars, and their interactions on peroxidase enzyme and polyphenol oxidase activity/g FW/h of cotton seedlings

Bacillus strains	Cotton cultivar				
	V1	V2	V3	V4	Mean
Peroxidase enzyme activity					
B1	15.51	14.47	12.52	11.42	13.48
B2	11.49	16.22	13.68	10.35	12.94
B3	19.22	12.64	13.24	13.36	14.62
B4	26.82	16.70	19.36	19.95	20.71
B5	10.66	9.66	13.81	12.60	11.68
B6	20.09	17.24	14.83	16.63	17.20
B7	14.55	14.75	19.33	17.90	16.63
B8	21.22	10.23	12.16	20.54	16.04
Control	10.87	15.40	16.48	20.48	15.81
Mean	16.71	14.15	15.05	15.91	15.46
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	4.55				
Polyphenol oxidase activity					
B1	21.27	15.88	15.79	12.00	16.24
B2	18.76	15.23	13.40	16.25	15.91
B3	13.10	17.17	15.51	17.53	15.83
B4	18.78	17.74	18.49	17.27	18.07
B5	16.05	16.57	8.83	9.56	12.75
B6	17.52	15.07	13.46	14.31	15.09
B7	14.10	18.11	17.57	19.31	17.27
B8	18.65	22.95	22.21	24.72	22.13
Control	9.46	16.15	13.13	14.50	13.31
Mean	16.41	17.21	15.38	16.16	16.29
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	3.55				

LSD, least significant difference.

Table 10. Effect of *Bacillus* strains, cotton cultivars, and their interactions on content of total phenols (mg gallic acid/g) and lipid peroxidation (nmol/cm) of cotton seedlings

Bacillus strains	Cotton cultivars				
	V1	V2	V3	V4	Mean
Total phenols					
B1	3.46	2.58	2.57	1.95	2.64
B2	2.72	2.48	2.18	2.79	2.54
B3	2.13	2.79	2.52	2.85	2.58
B4	3.06	2.89	3.01	2.81	2.94
B5	2.45	2.53	1.43	1.55	1.99
B6	2.85	2.45	2.19	2.33	2.45
B7	2.29	2.95	2.86	3.14	2.81
B8	3.04	3.73	3.61	4.02	3.60
Control	1.54	2.63	2.14	2.20	2.13
Mean	2.62	2.78	2.50	2.63	2.63
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	0.68				
Lipid peroxidation					
B1	249.67	167.33	177.00	103.00	174.25
B2	473.00	164.33	86.67	153.00	219.25
B3	187.00	164.67	108.00	88.33	137.00
B4	326.33	145.33	201.67	307.00	245.08
B5	231.00	222.33	267.67	194.00	228.75
B6	253.67	320.00	276.33	339.00	297.25
B7	279.00	244.33	272.00	264.67	265.00
B8	365.33	395.00	296.00	388.00	361.08
Control	132.67	187.67	223.33	191.33	183.75
Mean	277.52	223.44	212.07	225.37	234.6
LSD ($P \leq 0.05$) for *Bacillus* × cultivar	104.06				

LSD, least significant difference.

nol and lipid peroxidation, as it accounted for 6.25, 3.52, 3.04, and 7.80%, respectively of the explained (model).

B_4 significantly increased peroxidase activity in V_1 while it did not significantly affect its activity in V_2. The difference between B_5 and B_8 in peroxidase activity was significant in V_1 and nonsignificant in V_2 (Table 9). B_1 significantly increased polyphenol oxidase activity in V_1 while it did not affect its activity in V_2. The difference between B_4 and B_8 in polyphenol oxidase activity was nonsignificant in V_1 and significant in V_4 (Table 9). B_1 significantly increased phenol content of V_1 while it did not affect phenol content of V_2. The difference between B_4 and B_8 in phenol content was nonsignificant in V_1 and significant in V_4 (Table 10). B_2 significantly increased lipid peroxidation content in V_1 while it did not affect its content in V_2. The difference between B_1 and B_2 in lipid peroxidation content was significant in V_1 and nonsignificant in V_2 (Table 10). Data shown in Table 11 indicated the lack of significant correlation between damping-off incidence and any of the tested components.

Discussion

Plant bacterial diseases are generally characterized by plant morphological aberrations such as leaf and fruit spots, cankers, blights, vascular wilts, rots, and tumors. Phytopathogenic bacteria provoke diseases in plants by penetrating into host tissues (Buonaurio, 2008). Microbial pathogenicity has often been defined as the biochemical mechanisms whereby pathogenic microorganisms cause disease in a host organism (Fuchs, 1998). Microbial virulence is defined as the degree or measure of pathogenicity shown by one or more plants. Pathogenicity and/or virulence of Gram-negative plant pathogenic bacteria are strictly de-

Table 11. Correlation between contents and activities of some biochemical components in infected cotton seedlings of four cultivars and damping-off incidence rating under the effects of *Bacillus* strains

Component	Cotton cultivar			
	V1	V2	V3	V4
Total soluble sugars	0.482[a] (0.189[b])	–0.481 (0.189)	–0.221 (0.567)	–0.291 (0.448)
Total soluble proteins	0.133 (0.734)	–0.179 (0.645)	–0.188 (0.629)	–0.060 (0.878)
Total free amino acids	0.531 (0.141)	–0.166 (0.670)	–0.247 (0.522)	–0.650 (0.058)
Peroxidase	0.509 (0.162)	–0.152 (0.697)	0.047 (0.905)	–0.282 (0.462)
Polyphenol oxidase	0.011 (0.978)	0.121 (0.757)	–0.149 (0.701)	0.282 (0.462)
phenols	0.051 (0.896)	0.095 (0.807)	–0.151 (0.698)	0.339 (0.372)
Lipid peroxidation	–0.170 (0.661)	–0.342 (0.368)	–0.431 (0.247)	–0.148 (0.704)

[a]Pearson's correlation coefficient, which measures the degree of association between the designated component and damping-off incidence rating.
[b]Probability level and $n = 9$.

pendent on the production of secretion apparatuses in their cells, through which they secrete proteins or nucleoproteins involved in their virulence within the apoplast or inject these substances into host cells (Buonaurio, 2008). Bacteria colonize a host by growing between the cells and absorbing the cells nutrients that leak into intercellular space or grow within the vascular tissue of the plant. Depending on the species of bacteria and the tissue infected they can produce enzymes that degrade cell walls, growth regulators that alter the plants normal growth, toxins that degrade cell membranes and complex sugars that plug water conducting tissue (Agrios, 2005).

The infected seedlings in this study showed growth retardation, root discoloration, wilting, necrotic reactions, distortions of leaves and roots, or stunting of plants as reported by Schippers et al. (1987). High pathogenicity of *Bacillus* strains reduced the number of surviving seedlings in pots, which was accompanied with a high dry weight due to the low level of competition among seedlings in these pots. Vascular wilts caused by bacteria primarily affect herbaceous plants such as vegetables, field crops, ornamentals, and some tropical plants. The causal pathogen enters, multiplies in, and moves through the xylem vessels of the host plant and interferes with the translocation of nutrients and water by producing gum. The pathogen will often destroy parts of the cell wall of the xylem vessels resulting in pockets of bacteria, gums, and cellular debris. The symptoms of bacterial wilt disease include wilting and death of the aboveground parts of the plant. In some cases, bacterial ooze seeps out through stomata or cracks onto the surface of infected leaves. Usually, this ooze does not occur until the infected plant tissue is dead (Agrios, 2005).

Hence, the observed positive correlation between damping-off and dry weight of all cultivars except V_1. However, this interpretation does not rule out the possibility that the increases in dry weight of cotton seedling could be attributed to the biomass of the *Bacillus* cells especially if one takes into account that *Bacillus* strains accounted for 64.99% of the total variation in dry weight. It has been suggested that a variety of substances contained in plant cells are involved in resistance or susceptibility to infection by pathogens. Among these are sugars (Bezrutezyk et al., 2018), proteins (Strange, 2003), amino acids (Aly et al., 2008), peroxidase (Agrios, 2005), polyphenol oxidase (Agrios, 2005), phenols (Agrios, 2005), and MDA as indicated for lipid peroxidation (Göbel et al., 2003).

As far as we know, the effects of pathogenic *Bacillus* strains on these components in infected cotton seedlings have never been reported. Therefore, in the present study, we evaluated levels and activities of these components in infected cotton seedlings as well as their potential roles in susceptibility of cotton seedlings to pathogenic strains of *Bacillus*. Sugars are the primary photosynthetic products forming the building blocks for all other chemical constituents of plants. The reduction in total soluble sugars in cotton seedlings treated with some *Bacillus* strains may be due to a reduction in net Co_2 assimilation due to the changes in stomatal conductance and intercellular Co_2 concentration. On the other hand, the increase in soluble sugars may be due to the increase in photosynthetic activity in cotton seedlings (Mohamed and Akladious, 2017).

Currently, there are two hypothetical models for how sugars influence plant resistance to pathogens. The first hypothesis (pathogen starvation) is the simplest: that pathogens infect plants with the primary goal of gaining access to resources (sucrose) needed for reproduction, a process that proceeds from a few cells at the time of infection to billions of bacteria when symptoms become apparent. There is no doubt that host-derived sugars are transferred to the pathogen, at least in the case of fungi. This hypothesis depends on the assumption that apoplasmic sugar pools are low, thereby limiting pathogen growth (Sutton et al., 1999). An alternative is the sugar signaling hypothesis, in which altered levels of sugar in the infection site trigger signaling cascades that result in salicylic acid pathway activation and defense gene upregulation, ultimately generating physiological changes that repel pathogens (Gebauer et al., 2017). On the other hand, sugars themselves can act as signals that induce defense genes (Gebauer et al., 2017), supporting a sugar signaling hypothesis.

In the present study, we assessed proteins in infected cotton seedlings after the appearance of early disease symptoms. It seems likely that the assessed proteins included both constitutive proteins and pathogenesis-related proteins, which are induced in response to pathogen attack. Constitutive protein has a role to play in plant defense through a variety of mechanisms. For instance, it may affect the structural components of the pathogen wall or interferes with the synthesis of pathogen wall. It may also destabilize the pathogen membrane (Strange, 2003). On the other hand, pathogenesis-related proteins show strong antifungal activity. For example, some of them inhibit spore release and germination whereas others are associated with strengthening of the host cell wall and its outgrowth and papillae (Agrios, 2005). Proteins secreted by bacteria are transported via molecular systems out of bacterial cells; unrelated virulence factors often share the same secretion mechanism (Fuchs, 1998). Bacterial pathogenicity depends upon bacterial secretion systems (types i-iv), quorum sensing, plant cell wall degrading enzymes, toxins, hormones,

polysaccharides, proteinases, siderophores, and melanin. All of these systems and substances, which are essential for pathogenic infection and virulence, are produced by pathogens during bacterial pathogen–plant interactions (Agrios, 2005). They are all regulated by proteins through signal transduction systems.

The relationship between amino acids and resistance to plant diseases is well documented in a large body of literature. For example, El-Hamalawy and Menge (1995) found that the total free amino acid content of the avocado bark tissue was highly correlated with canker size on stem (r = 0.89) caused by *Phytophthora citricola*. Through correlations and path coefficient analysis of a field trial with peas cv. Rachana (resistant) and T163 susceptible, Bhattcharya and Shukla (1996) concluded that severity of powdery mildew (*Erysiphe polygoni*) on field pea is substantially increased by the accumulation of free amino acids. Omokolo et al. (2002) found a significant negative correlation (r = –0.65, $P \leq 0.05$) between the level of amino acids in cacao (*Theobroma cacao* L.) pods and the lesion size caused by *P. megakarya*.

The importance of peroxidase in disease resistance stems from its property to oxidize phenolic compounds to quinones and semiquinones, which are often more toxic to pathogens than the original phenols. Peroxidase not only oxidizes phenolics but also increases the rate of polymerization of such compounds into lignin-like substances, which are deposited in cell walls and papillae and interfere with the growth and development of the pathogen (Agrios, 2005; Mohamed and Abd-El Hameed, 2014; Mohamed et al., 2016; Hussain et al., 2022). Direct antifungal effects of peroxidase on spore germination and mycelial growth have also been demonstrated (Joseph et al., 1998). Thus, it was reasonable to find a significant and negative correlation between peroxidase activity and severity of flax powdery mildew (Mohamed et al., 2012). Polyphenoloxidase is an enzyme of broad distribution among plants. Most of the reports on polyphenoloxidase indicated a function to defence plants against pathogens. The mode of action proposed for polyphenoloxidase is based on its capacity to catalyze the hydroxylation of monophenols to diphenols and their oxidation to diquinones (Aly et al., 2012a, 2012b, 2013; Helmi and Mohamed, 2016; Mansour et al., 2020). The quinones formed may act in several ways leading to protection of plants (El-Beltagi et al., 2019; Melo et al., 2006). Thus, polyphenoloxidase showed a significant and negative correlation with disease severity (Avtar et al., 2003, Gawande et al., 2002; Mohamed et al., 2012; Rao et al., 2007).

It is well known that the toxic effect of most phenolics is attributed mainly to their interaction with lipids or phospholipids, causing an increase in pathogen membrane permeability, leakage of cell contents, and cytoplasm aggregation (Dallagnol et al., 2011). Thus, an increase in the levels of post-infection phenolic compounds in host tissues may enhance resistance against infection by pathogen (Ashry et al., 2018; Avtar et al., 2003; Mohamed et al., 2012; Rao et al., 2007; Satisha et al., 2008). The MDA produced during lipid peroxidation is an indicator of cellular damage. It is well known that lipid peroxidation in the plasma membrane of cell wall by reactive oxygen species is caused by the occurrence of any type of stress to the cells (Abd El-Rahman and Mohamed, 2014; Abd El-Rahman et al., 2012; Dallagnol et al., 2011). MDA showed significant positive correlation with severity of flax powdery mildew (Mohamed et al., 2012).

In the present study, the lack of significant correlation between levels and activities of the measured biochemical components and incidence of damping-off, regardless of cultivar, after the application of *Bacillus* strains clearly demonstrated that these components were not involved in the pathogenicity of the tested strains to cotton seedlings. Therefore, other mechanisms for pathogenicity should be considered. A large number of enzymes with the potential to harm plant cell walls have been identified in the genus *Bacillus* like xylanase, pectate lyase, β-1,4-endoglucanase, and other pectinolytic and cellulolytic enzymes (Peng et al., 2013). Therefore, it seems reasonable to hypothesize that pathogenicity of the tested *Bacillus* strains might be due to the production of certain cell wall degrading enzymes.

Alternatively, the pathogenicity of *Bacillus* strains could be attributed to the production of pathogenic toxins. Some specific pathogenicity factors enable these bacteria to spread disease in the plants. Five major types of factors are well known in this regard. These factors are effector proteins, cell wall degrading enzymes, exopolysaccharides, phytohormones, and toxins (Abu-Shahba et al., 2021; Gagne-Bourgue et al., 2013). Deleterious rhizobacteria (DRB) are rhizosphere bacteria that affect plants by their metabolites without parasitizing plant tissue. *Bacillus* spp. are among rhizobacteria that conform to this definition. DRB induce growth retardation, root discoloration, wilting, necrotic reactions, distortions of leaves and roots, or stunting of plants (Schippers et al., 1987).

The incidence of damping-off of cotton seedlings traditionally has been attributed to fungal infections, and pathogenicity of several fungi has been demonstrated (Watkins, 1981). To our knowledge, no attempts have been made to identify organisms other than fungi, and usually bacteria that are detected are treated as contaminants.

Based on the results of the present study, we believe that

rhizobacteria in particular *Bacillus* spp. can play an important role in the incidence of cotton damping-off disease, and it is likely that, with more comprehensive isolation, a greater involvement of rhizobacteria in the etiology of cotton damping-off will be demonstrated.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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