Romanian Biotechnological Letters *Vol. 18, No. 5, 2013*
Copyright © 2013 University of Bucharest Printed in Romania. All rights reserved
ORIGINAL PAPER

Differential antagonism of *Bacillus* spp. against isolates of *Macrophomina phaseolina*

Received for publication, June 3, 2013
Accepted, October 2, 2013

M.R.OMAR[1], E.Z.GOMAA[2]*, A.A.ALY[1], AND A.M.A. EL-SAMAWATY [1]
[1]Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[2]Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt
**Corresponding author. E- mail: emann7778@yahoo.com*

Abstract

In view of the ecological hazards of fungicides, a greenhouse study was conducted to determine the efficiency of seven Bacillus isolates against 32 isolates of Macrophomina phaseolina. Bacillus isolates, M. phaseolina isolates, and their interaction were all highly significant sources of variation in infection (damping-off), plant height, and dry weight. The highly significant (p=0.0000) of Bacillus isolates × M. phaseolina isolates interaction implies that a single isolate of the antagonist can be highly effective against an isolate of M. phaseolina, but may have minimal effects on the other isolates of M. phaseolina. Therefore, isolates of the antagonist should be tested against as many isolates of M. phaseolina as possible, as this will improve the chance of identifying antagonist isolates effective against several isolates of M. phaseolina.

Key words: *Macrophomina phaseolina. Bacillus isolates*, cotton, biocontrol

Introduction

Macrophomina phaseolina (Tassi) Goid, the causal agent of charcoal rot on cotton, is a seed-born and soil-born pathogen with a wide distribution and a wide host range [1]. When *M. phaseolina* invades roots or stems of cotton, colonization of internal tissues proceeds rapidly and the plant dies. Examination of affected parts reveals a dry rot, with many tiny black sclerotia distributed throughout the wood and softer tissues [2]. *M. phaseolina* is of a widespread distribution in the Egyptian soil and it is easily and frequently isolated from cotton roots particularly during the late period of the growing season. All commercially grown cotton cultivars in Egypt are susceptible to this pathogen [3]. *M. phaseolina* is difficult to control because of its persistence in the soil and wide host range. Some fungicides are effective in controlling this pathogen; however, these chemicals are expensive and not environmentally safe. Therefore, alternative control methods are needed for controlling this pathogen. Several alternative measures, such as biological control, have been found to be good and safe as an alternative control method to fungicides [4].

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 [5] . *Bacillus* spp. have been identified as potent antagonists against a wide range of pathogens such as *Macrophomina phaseolina* [6, 7], *Fusarium* spp. [8, 9], *Rhizoctonia solani* [10] and *Pythium ultimum* [7].

The purpose of this study was to evaluate the antagonistic effects of seven isolates of *Bacillus* spp. from cotton rhizosphere against 32 isolates of *Macrophomina phaseolina* on cotton seedlings under greenhouse conditions.

Materials and methods

Sources of *Macrophomina phaseolina* isolates

Twenty four isolates of *M. phaseolina* originated from roots of cotton plants were obtained from the fungal collection of the Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt, and eight isolates from other hosts were obtained from other sections in the same institute; three from sunflower, two from sesame and one from each of soybean and cowpea. One isolate was recovered from unidentified host (Table 1).

Table 1. Governorates, regions, and sources of *M. phaseolina* isolates used in the present study**.**

Isolate No.	Governorate	Region	Source
1	Faiyoum	Middle Egypt	Cotton
2	Assiute	Upper Egypt	Cotton
3	Sohag	Upper Egypt	Cotton
4	Minya	Middle Egypt	Cotton
5	Assiute	Upper Egypt	Cotton
6	Minufiya	Middle Delta	Cotton
7	Giza	Middle Egypt	Sesame
8	Minufiya	Middle Delta	Cotton
9	Giza	Middle Egypt	Sunflower
10	Sharqiya	East Delta	Sesame
11	Gharbiya	Middle Delta	Cotton
12	Beheria	West Delta	Cotton
13	Damietta	East Delta	Cotton
14	Daqahilya	East Delta	Cotton
15	Kafr El-Sheikh	North Delta	Cotton
16	Sharqiya	East Delta	Cotton
17	Beheria	West Delta	Cotton
18	Beheria	West Delta	Cotton
19	Kafr El-Sheikh	North Delta	Cotton
20	Sharqiya	East Delta	Sunflower
21	Minya	Middle Egypt	Cotton
22	Giza	Middle Egypt	Sunflower
23	Gharbiya	Middle Delta	Cotton
24	UN	UN	UN
25	Assiute	Upper Egypt	Cotton
26	Sohag	Upper Egypt	Cotton
27	Assiute	Upper Egypt	Cotton
28	Faiyoum	Middle Egypt	Cotton
29	Gharbiya	Middle Delta	Cotton
30	Giza	Middle Egypt	Soybean
31	Assiute	Upper Egypt	Cotton
32	UN	UN	Cowpea

UN means unidentified

Preparation of fungal inoculum

Substrate for fungal growth was prepared in 500-ml glass bottles each contained 50 g of sorghum grains and 40 ml of tap water. Contents of each bottle were autoclaved for 30 minutes. Inoculum, taken from one week old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for three weeks at 26±2°C; during this period the incubated bottles were shacked for 5min every three days to ensure uniform distribution of the fungal growth [11].

Preparation of bacterial inoculum

Seven *Bacillus* spp. isolates, obtained from Cotton Disease Research Section; Plant Pathology Research Institute; Agricultural Research Centre; Giza, Egypt, were used in this experiment (Table 2). The *Bacillus* spp. isolates were grown in nutrient glucose broth at 30°C for 72 h on a shaker. The growth was adjusted turbidimetrically to10^8 cfu/ml using spectro 2000 RSP 220v. 50Hz. Bacterial culture were formulated in powder form by mixing 400 ml of cell suspension with 1kg talc as carrier which previously autoclaved for 30 min for 2 successive days, ten grams of carboxy methyl cellulose (CMC) was added to 1kg 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.

Table 2. Identification of *Bacillus* spp. isolates used in biological control of *M. phaseolina* isolates and their governorates.

Isolate No.	Identification	Governorate
1	*Bacillus coagulans*	Gharbiya
2	*Bacillus globisporus*	Daqahilya
3	*Bacillus pumilus*	Giza
4	*Bacillus subtilis*	Assiute
5	*Bacillus circulans*	Beheria
6	*Bacillus cereus*	Giza
7	*Bacillus coagulans*	Minya

Biological control of *M. phaseolina* by *Bacillus* spp.

Autoclaved soil infested with each of *M. phaseolina* isolates at a rate of 50 g/kg soil was dispensed into 15-cm-diameter clay pots. Slightly moist seeds of cultivar Giza 89 were treated with the powdered inoculum of each bacterial isolate at a rate of 10g/kg seeds, and thoroughly shaken in plastic bags before being planted in the infested soil at a rate of 10 seeds/pot. Untreated seeds were planted in the control treatments in the infested soil. The pots were randomly distributed on a greenhouse bench under temperature regime ranged from 24±4 and 34±4°C. The effect of *Bacillus* spp. on *M. phaseolina* isolates was evaluated 45 days after sowing by using the percentage of infection (combined pre-and post emergence damping-off), plant height (cm/plant) and dry weight (mg/plant)

Statistical analysis of greenhouse studies

The experimental design was a randomized complete block with three replicates. Least significant difference (LSD) was used to compare means of *M. phaseolina* isolates within each bacterial isolate. Analysis of variance (ANOVA) and correlation analyses were carried out by MSTAT-C statistical package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Percentage data were transformed into arc sine angles before carrying out ANOVA to produce approximately constant variance. Cluster analysis of *M. phaseolina* and *Bacillus* isolates was performed with the software package SPSS 10.0.

Results and discussion

Results illustrated in Table 3 showed that *M. phaseolina* isolates were significant sources of variation in infection and plant height while they were non-significant source of variation in dry weight. *Bacillus* isolates and their interaction with *M. phaseolina* were highly significant sources of variation in all the tested parameters.

Table 3. Analysis of variance of the effects of *Bacillus*, *M. phaseolina* isolates and their interaction on cotton seedling disease variables (cultivar Giza 89) under greenhouse conditions.

Parameter and source of variation[a]	D.F.	M.S.	F. value	P > F
Infection (Damping-off)				
Replication	2	28.786	0.2069	
***Bacillus* isolates (B)**	7	17388.780	124.9728	0.0000
***M. phaseolina* isolates (M)**	31	248.257	1.7842	0.0065
B × M	217	277.963	1.9977	0.0000
Error	510	139.141		
Plant height				
Replication	2	22.746	1.1849	0.3066
***Bacillus* isolates (B)**	7	1200.332	62.5318	0.0000
***M. phaseolina* isolates (M)**	31	35.246	1.8361	0.0044
B × M	217	45.357	2.3629	0.0000
Error	510	19.196		
Dry weight				
Replication	2	62.814	0.0131	
***Bacillus* isolates (B)**	7	241864.750	50.5760	0.0000
***M. phaseolina* isolates (M)**	31	5387.358	1.1265	0.2947
B × M	217	7984.826	1.6697	0.0000
Error	510	4782.199		

[a] Replication is random, while each of *Bacillus* isolate and *M. phaseolina* isolate is fixed.

Bacillus isolates were the most important source of variation in infection, while *Bacillus* × *M. phaseolina* isolates interaction was the most important source of variation in plant height. *Bacillus* isolates and their interaction with *M. phaseolina* were almost equal as a source of variation in dry weight. On the other hand, *M. phaseolina* isolates showed the lowest relative contribution to variation in all the tested parameters (Table 4).

Table 4. Relative contribution of *Bacillus*, *M. phaseolina* isolates and their interaction to variation in cotton seedling disease variables (cultivar Giza 89) under greenhouse conditions.

Source of variation	Relative contribution [a] to variation in		
	Infection	Plant height	Dry weight
***Bacillus* isolates (B)**	64.13	43.35	47.12
***M. phaseolina* isolates (M)**	4.06	5.64	4.65
B × M	31.78	50.78	48.23

[a] Calculated as percentage of sum squares of the explained (model) variation.

Due to the significance of *M. phaseolina* × *Bacillus* isolates interaction as a source of variation in infection, a least significant difference (LSD) was calculated to compare between means of *Bacillus* isolates within each isolate of *M. phaseolina* isolate (Table 5). This comparison showed that the differences in infection between *Bacillus* isolates and the control was not the same for each *M. phaseolina* isolates, that is, *M. phaseolina* isolates responded differently to the application of *Bacillus* isolates. For example, while *Bacillus coagulans* (B7) was effective against isolate no.8 and highly effective against *M. phaseolina* no. 9 , it was ineffective in reducing infection incited by *M. phaseolina* isolates no.1, 11, 16, 23, 26, 27, 29 or 31. *Bacillus cereus* (B6) showed high efficiency against infection by *M. phaseolina* isolates no.6, 11, 12,13,15,17, 23 and 29 tested in this study. *Bacillus globisporus* (B2) showed efficiency equal to that of *Bacillus circulans* (B5) against infection by *M. phaseolina* isolates no. 20, 23 and 32. It is worth mentioning that *M. phaseolina* no.25 caused the lowest damping-off on cotton seedlings; however all *Bacillus* isolates were ineffective against it. This may indicate that the response of *M. phaseolina* isolates to *Bacillus* spp. is not related to their pathogencity.

Table 5. Effect of *Bacillus* isolates , *M. phaseolina* isolates , and their interaction on infection (damping-off) of cotton seedlings (cultivar Giza 89) under greenhouse conditions.

***M. phaseolina* isolates**	***Bacillus* isolates**																	
	B1		**B2**		**B3**		**B4**		**B5**		**B6**		**B7**		**Control**		**Mean**	
	%	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**
1	40.0[a]	(39.15)[b]	16.67	(23.85)	30.00	(33.21)	13.33	(21.15)	30.00	(28.08)	33.33	(35.01)	46.67	(43.08)	73.33	(59.22)	35.42	(35.34)
2	60.0	(50.77)	26.67	(30.99)	36.67	(37.14)	2.333	(28.29)	23.33	(28.78)	26.67	(30.99)	33.33	(35.22)	83.33	(70.08)	39.17	(39.03)
3	53.33	(46.92)	33.33	(35.21)	30.00	(33.21)	2.333	(28.08)	26.67	(30.29)	23.33	(28.78)	33.33	(35.22)	93.33	(81.15)	33.33	(39.83)
4	46.67	(42.99)	43.33	(41.15)	36.67	(37.22)	36.67	(37.22)	40.00	(38.85)	33.33	(35.01)	33.33	(34.92)	66.67	(55.08)	42.08	(40.31)
5	50.00	(45.00)	43.33	(41.07)	16.67	(19.22)	20.00	(22.14)	33.33	(34.92)	33.33	(35.01)	46.67	(43.08)	86.67	(76.92)	41.25	(39.67)
6	30.00	(32.22)	26.67	(30.99)	26.67	(30.29)	23.33	(28.08)	30.00	(33.21)	20.00	(26.07)	53.33	(46.92)	100.0	(90.00)	38.75	(39.72)
7	20.00	(26.07)	16.67	(23.85)	33.33	(34.92)	76.67	(71.07)	26.67	(30.99)	43.33	(40.37)	33.33	(34.22)	100.0	(90.00)	39.32	(43.94)
8	30.00	(33.21)	30.00	(33.21)	26.67	(30.99)	46.67	(47.22)	33.33	(34.14)	30.00	(33.00)	66.67	(56.07)	73.33	(75.00)	42.08	(42.86)
9	46.67	(43.08)	30.00	(33.00)	60.00	(51.93)	26.67	(30.78)	23.33	(28.08)	43.33	(41.07)	10.00	(18.44)	56.67	(49.22)	37.08	(36.95)
10	30.00	(33.00)	40.00	(38.85)	40.00	(39.23)	30.00	(33.00)	23.33	(30.99)	43.33	(41.07)	66.67	(55.78)	86.67	(76.92)	45.00	(43.61)
11	33.33	(35.01)	40.00	(39.15)	43.33	(41.07)	30.00	(33.00)	23.33	(38.85)	16.67	(23.36)	56.67	(50.01)	76.67	(66.93)	40.00	(40.92)

12	40.00	(38.85)	30.00	(32.30)	43.33	(41.07)	23.33	(28.07)	23.33	(30.29)	16.67	(23.36)	43.33	(40.86)	100.0	(90.00)	40.00	(40.60)
13	46.67	(43.08)	33.33	(33.00)	33.33	(35.01)	30.00	(32.22)	13.33	(17.71)	10.00	(15.00)	83.33	(61.22)	100.0	(90.00)	43.75	(40.90)
14	43.33	(41.07)	30.00	(33.00)	40.00	(39.15)	16.67	(19.92)	16.67	(23.36)	30.00	(33.00)	100.0	(90.00)	90.00	(78.93)	45.83	(44.80)
15	30.00	(33.91)	26.67	(30.78)	40.00	(39.15)	20.00	(25.35)	33.33	(34.92)	40.00	(28.85)	70.00	(57.70)	63.33	(53.07)	40.42	(39.13)
16	53.33	(46.92)	30.00	(33.00)	20.00	(26.07)	26.67	(30.99)	40.00	(39.06)	33.33	(35.01)	70.00	(66.15)	80.00	(68.85)	44.17	(43.26)
17	33.33	(34.22)	26.67	(30.99)	33.33	(35.01)	26.67	(30.99)	33.33	(34.92)	23.33	(28.78)	36.67	(36.93)	90.00	(78.93)	37.92	(38.85)
18	33.33	(35.22)	16.67	(23.36)	40.00	(39.23)	50.00	(45.00)	23.33	(28.78)	36.67	(36.85)	90.00	(78.93)	80.00	(68.07)	46.25	(44.43)
19	13.33	(17.22)	33.33	(35.22)	36.67	(37.14)	36.67	(36.93)	36.67	(36.15)	26.67	(30.99)	60.00	(51.15)	80.00	(73.08)	40.42	(39.73)
20	23.33	(28.08)	20.00	(26.07)	46.67	(41.15)	26.67	(30.78)	20.00	(26.07)	33.33	(35.22)	46.67	(43.08)	100.0	(90.00)	39.58	(40.06)
21	36.67	(37.22)	40.00	(39.23)	26.67	(30.99)	30.00	(33.00)	33.33	(34.22)	30.00	(32.71)	43.33	(40.37)	96.67	(83.85)	42.08	(41.45)
22	13.33	(21.15)	33.33	(35.22)	36.67	(37.22)	30.00	(32.71)	26.67	(30.78)	43.33	(41.07)	36.67	(36.93)	93.33	(77.71)	39.17	(39.10)
23	36.67	(37.22)	20.00	(26.07)	26.67	(30.99)	16.67	(19.92)	20.00	(26.07)	16.67	(19.22)	50.00	(44.92)	60.00	(51.85)	30.84	(32.03)
24	33.33	(34.92)	26.67	(30.78)	46.67	(34.08)	16.67	(23.85)	36.67	(36.15)	26.67	(30.99)	30.00	(32.22)	93.33	(81.15)	38.75	(39.14)
25	20.00	(26.07)	23.33	(28.78)	26.67	(30.78)	26.67	(30.99)	33.33	(35.01)	43.33	(40.08)	43.33	(41.15)	26.67	(30.78)	30.42	(32.96)
26	33.33	(35.01)	30.00	(32.30)	16.67	(23.36)	20.00	(26.07)	36.67	(37.22)	36.67	(37.22)	40.00	(38.85)	56.67	(49.92)	33.75	(34.99)
27	33.33	(35.22)	26.67	(30.78)	40.00	(39.15)	43.33	(41.07)	50.33	(46.71)	56.67	(48.93)	64.33	(52.86)	80.00	(67.86)	49.33	(45.32)
28	56.67	(48.85)	43.33	(39.99)	30.00	(33.00)	26.67	(30.99)	26.67	(30.00)	33.33	(34.92)	46.67	(42.70)	80.00	(64.93)	42.92	(40.67)
29	40.00	(39.15)	46.67	(43.08)	33.33	(35.22)	43.33	(41.07)	13.33	(17.71)	10.00	(15.00)	60.00	(50.85)	60.00	(56.15)	38.33	(37.27)
30	36.67	(37.14)	10.00	(15.00)	23.33	(28.08)	26.67	(26.07)	30.00	(32.71)	43.33	(41.15)	46.67	(43.08)	86.67	(76.92)	37.92	(37.52)
31	53.33	(47.22)	20.00	(26.07)	20.00	(26.07)	33.33	(35.01)	23.33	(28.78)	23.33	(28.78)	66.67	(55.78)	83.33	(70.08)	40.42	(39.72)
32	36.67	(36.93)	30.00	(33.00)	36.67	(37.14)	46.67	(42.78)	30.00	(33.00)	40.00	(38.85)	60.00	(51.15)	86.67	(72.78)	45.84	(43.21)
Mean	37.08	(36.92)	29.48	(32.17)	34.77	(34.89)	30.21	(32.62)	28.55	(31.78)	31.53	(31.78)	52.11	(47.18)	80.63	(70.79)	41.67	(39.77)

[a] Mean of three replicates.

[b] Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

LSD (transformed data) for *Bacillus* isolates × *M. phaseolina* isolates interaction = 18.92 ($P \leq 0.05$) or 24.90 ($P \leq 0.01$).

The interaction between *Bacillus* isolates and *M. phaseolina* isolates was also a highly significant source of variation in plant height (cm / plant) and dry weight (mg / plant). The significance of this interaction implies that a single isolate of the antagonist can be highly effective in increasing plant height and dry weight in soil infested with an isolate of *M. phaseolina*, but may be ineffective in increasing these parameters in soil infested with the other isolates of *M. phaseolina*. All *Bacillus* isolates failed in improving plant height and dry weight significantly when the soil was infested with *M. phaseolina* isolates no.1,4,9,15,23,25,26,or 28 (Tables 6 &7) . On the contrary, all the isolates significantly improved plant height and dry weight of the surviving seedlings, with different levels of efficiency, when the soil was infested with *M. phaseolina* isolates no. 3, 5, 8 and 24.

Table 6. Effect of *Bacillus* isolates , *M. phaseolina* isolates , and their interaction on plant height (cm/plant) of cotton seedlings (cultivar Giza 89) under greenhouse conditions.

***M. phaseolina* isolates**	***Bacillus* isolates**								
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**Control**	**Mean**
1	19.15	19.72	20.52	21.09	21.99	22.11	24.36	21.73	21.33
2	20.00	22.72	22.56	21.21	22.87	25.21	23.97	14.78	21.67
3	20.14	23.25	22.96	22.15	22.47	24.32	25.88	9.07	21.28
4	20.04	23.19	21.90	21.98	20.99	24.50	25.40	23.79	22.72
5	19.87	25.86	21.08	25.54	26.78	26.27	25.27	8.00	22.33
6	17.96	25.27	22.42	26.20	23.50	24.83	26.19	0.00	20.80
7	16.95	23.09	24.00	24.57	23.37	23.46	25.23	0.00	20.09
8	21.40	23.31	23.28	23.80	24.99	24.09	25.31	10.00	22.02
9	22.79	24.31	22.26	25.86	23.43	25.44	25.11	22.66	23.98
10	20.51	23.82	22.36	24.28	28.59	26.18	23.45	8.75	22.24
11	20.77	24.12	22.13	26.17	23.29	26.66	23.61	15.87	22.83
12	22.38	23.00	22.22	25.59	24.90	24.06	25.13	0.00	20.91
13	22.99	24.54	22.13	24.60	23.44	25.10	23.64	0.00	20.81
14	25.04	24.52	22.50	24.11	23.16	22.65	0.00	7.78	18.72
15	24.95	24.10	22.52	25.96	25.04	22.41	25.50	22.95	24.18
16	25.73	22.95	21.71	23.96	24.52	23.02	7.11	25.34	21.79
17	25.44	22.82	21.79	25.88	24.15	23.35	24.86	9.78	22.26
18	24.29	24.63	21.56	25.49	22.57	23.61	9.67	16.08	20.99
19	22.89	23.17	23.22	23.25	24.33	22.77	24.21	10.50	21.79
20	23.56	22.81	21.98	22.77	23.56	23.69	24.74	0.00	20.39
21	21.84	22.29	22.52	25.42	25.03	23.23	25.43	10.00	21.97
22	22.89	20.57	22.90	23.86	23.48	22.67	24.19	16.58	22.14
23	21.40	22.76	23.26	24.58	22.26	23.19	24.49	22.89	23.10
24	22.77	21.62	22.46	22.50	22.34	22.19	26.53	7.48	20.99
25	21.89	21.33	22.16	22.56	23.28	23.57	24.99	24.72	23.06
26	22.34	21.87	21.29	22.58	26.21	22.78	24.19	23.88	23.14
27	22.85	24.67	22.53	25.16	24.71	22.15	25.53	15.78	22.92
28	21.28	21.91	22.53	22.79	26.23	24.60	25.72	24.89	23.74
29	23.29	23.68	22.28	22.94	24.01	24.41	26.35	18.94	23.24
30	23.92	24.85	22.12	24.17	25.18	23.83	25.39	7.92	22.18
31	23.10	22.12	23.55	23.00	23.79	24.80	27.18	15.67	22.90
32	23.11	24.30	22.94	22.44	25.99	25.25	25.03	15.83	23.11
Mean	22.11	23.26	22.36	23.95	24.08	23.95	23.24	13.49	22.06

LSD for *Bacillus* isolates × *M. phaseolina* isolates interaction = 7.03 ($p \leq 0.05$) or 9.25 ($p \leq 0.01$).

Table 7. Effect of *Bacillus* isolates , *M. phaseolina* isolates and their interaction on dry weight (mg/plant) of cotton seedlings (cultivar Giza 89) under greenhouse conditions**.**

***M. phaseolina* isolates**	***Bacillus* isolates**								
	B1	B2	B3	B4	B5	B6	B7	Control	Mean
1	334.33	345.33	298.00	325.33	299.00	341.67	342.67	284.67	321.38
2	347.67	335.33	328.33	342.33	289.67	331.33	318.33	229.67	318.71
3	434.67	350.33	347.00	347.67	333.67	336.33	364.67	141.67	332.00
4	337.33	329.33	328.00	339.33	345.00	350.67	332.67	305.67	333.50
5	320.33	372.67	307.67	333.00	337.67	341.33	387.33	146.00	318.25
6	323.67	342.00	321.33	334.67	347.33	317.00	355.00	0.00	292.63
7	348.00	313.00	436.67	342.33	352.00	330.00	385.00	0.00	303.13
8	331.33	298.67	344.00	332.67	329.67	333.67	367.00	116.33	306.67
9	363.67	316.67	436.67	332.33	321.67	331.67	364.67	311.67	336.13
10	328.33	316.67	340.33	365.00	343.67	349.33	358.00	152.67	319.25
11	336.00	342.00	330.67	347.67	329.67	349.67	324.67	263.67	328.00
12	358.00	306.00	352.00	354.67	333.67	352.67	391.00	0.00	306.00
13	371.67	327.33	341.00	332.67	347.33	342.67	370.67	0.00	304.17
14	381.67	333.00	336.00	333.67	340.33	362.00	0.00	145.67	279.04
15	272.00	353.00	357.00	332.67	335.33	327.33	335.67	313.33	328.29
16	324.67	335.33	326.33	333.00	345.00	336.00	140.00	288.00	303.54
17	334.33	338.00	317.00	345.00	331.67	327.00	382.67	143.00	314.83
18	319.67	343.00	340.00	329.33	347.67	320.33	141.67	273.67	302.17
19	316.33	341.00	322.00	328.67	343.33	372.00	326.67	140.33	311.29
20	327.00	333.00	321.33	347.00	344.67	341.00	363.00	0.00	297.13
21	319.33	369.00	340.67	329.67	329.00	327.33	353.00	139.67	313.46
22	326.33	336.00	322.00	336.67	317.00	329.67	317.33	247.33	316.54
23	341.33	337.67	332.33	328.33	323.33	316.67	367.33	307.67	331.83
24	356.33	337.00	316.67	331.00	346.33	358.67	360.00	141.67	318.55
25	340.33	327.67	339.33	353.00	334.67	327.67	318.67	283.00	328.04
26	324.00	333.67	346.00	342.00	333.00	344.33	371.00	323.33	339.67
27	333.333	322.33	339.00	324.33	329.33	347.00	371.00	242.67	326.25
28	329.67	329.33	324.00	353.33	308.67	353.67	398.33	313.33	338.79
29	371.00	331.00	330.00	329.33	320.67	340.67	336.33	239.00	324.75
30	328.67	331.00	312.33	319.33	333.67	321.67	373.67	148.33	308.67
31	344.33	332.67	336.00	358.00	324.67	344.00	350.67	298.00	336.04
32	305.67	348.00	344.67	353.00	342.33	359.33	383.67	268.00	338.08
Mean	339.31	334.62	332.32	338.66	332.58	339.51	332.92	194.00	317.99

LSD for *Bacillus* isolates × *M. phaseolina* isolates interation = 110.9 ($p \leq 0.05$) or 146.0 ($p \leq 0.01$).

The results of the present study confirmed the results of Pleban et al. [12], who found that inoculating cotton seedlings with *B. cereus* reduced disease incidence caused by *R. solani* in the greenhouse by 51%, and the results of Asran [13] who reported that *B. coagulans* and *B. cereus* showed significant increases of survival and reduced pre-emergence damping-off. Akhtar and Siddiqui [14] mentioned that *Bacillus pumilus* decreased disease incidence caused by *M. phaseolina* in chickpea plants. In general, species of *Bacillus* have been shown to be effective and are commercially applied as biological control agents against fungal pathogens [15,16].

The significant interaction between *Bacillus* isolates and *M. phaseolina* isolates indicates that apparently many genes from both organisms interact to regulate the amount of antagonism between *Bacillus* and *M. phaseolina* isolates [17]. The interaction also has an important bearing on antagonism testing methods. Isolates of antagonists should be tested against as many isolates of *M. phaseolina* as possible, as this will improve the chance of identifying antagonist isolates effective against several isolates of *M. phaseolina*. The interaction also suggests that it may be more prudent to evaluate blends of antagonist isolates for wider application against more isolates of *M. phaseolina.* In this investigation, the interaction between *M. phaseolina* isolates and the antagonist isolates was evaluated under greenhouse conditions in a soil and at temperature favorable for the growth of both *M. phaseolina* and the antagonist. Under field conditions, soil nutrients and temperature during the different periods of cotton growing season may be more favorable for *M. phaseolina* isolates or the antagonist isolates. Thus, the results of this work are not expected to be necessarily related to the degree of biological control that may be observed in the field, but should reflect the capacities and genetic variability of the antagonist isolates and of the various *M. phaseolina* to resist antagonism [18].

Correlations among variables used for evaluating pathogenicity of *M. phaseolina* isolates when they were subjected to the effects of *Bacillus* isolates are shown in Table 8. In case of *Bacillus coagulans* (B.7), a highly significant positive correlation ($P < 0.01$) was found between plant height and dry weight; however, a significant negative correlation was found between damping-off and dry weight. On the contrary, a significant positive correlation was found between damping-off and dry weight in case of *Bacillus coagulans* (B.1). Correlations among the tested variables were nonsignificant in case of the remaining isolates.

Table 8. Correlation among variables used for evaluating pathogenicity of *M. phaseolina* isolates on seedlings of cotton (cultivar Giza 89) under the effect of isolates of *Bacillus* spp.

***Bacillus* isolates**	**Variable**	**Variable**	
		2	**3**
Bacillus coagulans	1- Damping-off (%)	- 0.005[a]	0.413*
(B1)	2- Plant height (cm/plant)		-0.214
	3- Dry weight (mg/plant)		
Bacillus globisprus	1- Damping-off (%)	0.130	-0.016
(B2)	2- Plant height (cm/plant)		0.148
	3- Dry weight (mg/plant)		
Bacillus pumilus	1- Damping-off (%)	0.090	0.257
(B3)	2- Plant height (cm/plant)		0.037
	3- Dry weight (mg/plant)		
Bacillus subtilis	1- Damping-off (%)	0.094	-0.051
(B4)	2- Plant height (cm/plant)		0.024
	3- Dry weight (mg/plant)		
Bacillus circulans	1- Damping-off (%)	0.115	-0.141

(B5)	2- Plant height (cm/plant)		-0.071
	3- Dry weight (mg/plant)		
Bacillus cereus	1- Damping-off (%)	-0.253	0.111
(B6)	2- Plant height (cm/plant)		0.297
	3- Dry weight (mg/plant)		
Bacillus coagulans	1- Damping-off (%)	-0.647	-0.610*
(B7)	2- Plant height (cm/plant)		0.961**
	3- Dry weight (mg/plant)		

[a] Linear correlation coefficient (r) is significant at P <0.05 (*) or P < 0.01 (**).

The application of cluster analysis has been suggested for assessing similarity and/or dissimilarity in gene for gene host-parasite relationships [19]. The method was also used to express exactly the genetic similarity among 20 isolates of *Macrophomina phaseolina* and 52 isolates of *Rhizoctonia solani* [20].

Three groups of isolates were identified by cluster analysis (Fig.1). The first group (isolates no.6,8,10,11,12,13,14,15,16,17,18,19,20,23,29,and32) included all the isolates collected from Lower Egypt (Nile Delta) governorates. Middle and Upper Egypt isolates (isolates no.1,2,3,4,5,21,25,26,27,28,30,and 31) were placed in three remotely related sub-clusters. Isolates no.7, 9, 22, and 24 were quite different from the others in their pathogenicity patterns. This grouping of isolates indicates that the Nile Delta isolates were less diverse in their pathogenicity patterns compared with Middle and Upper Egypt isolates.

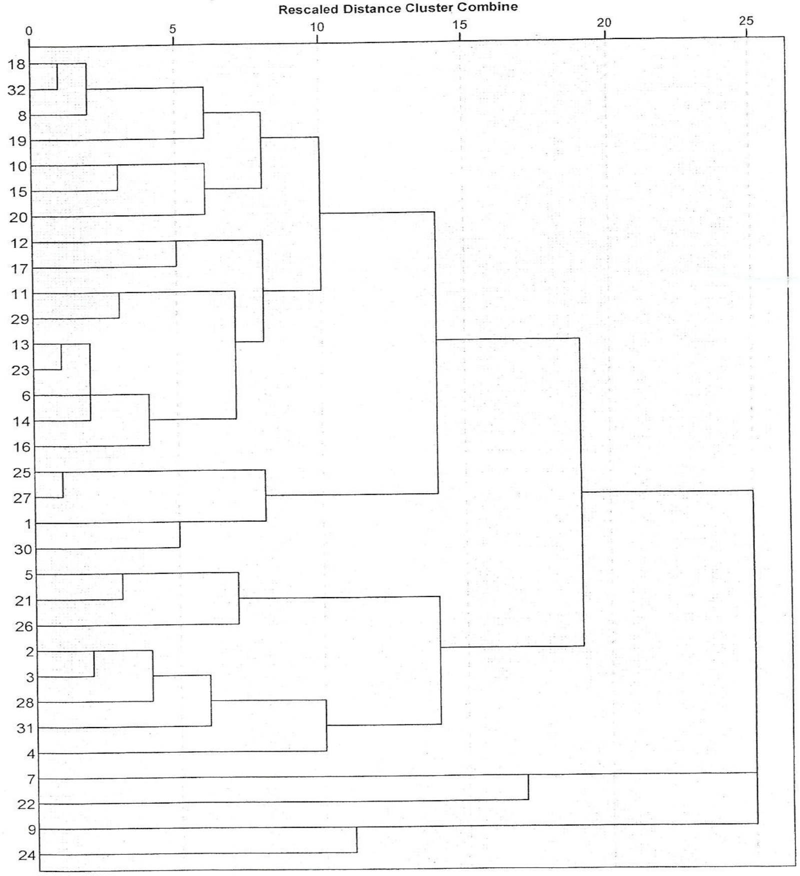

Figure 1. Phenogram based on average linkage cluster analysis of pathogenicity of 32 *Macrophomina phaseolina* isolates on cotton cultivar Giza 89 under the effects of *Bacillus* spp. Geographic origins and sources of isolates are shown in Table1.

The results of cluster analysis of *Bacillus* spp. isolates based on their antagonistic efficiency parameters are shown in Fig. 2. There were two main subclusters. The first one included isolates B. 3, B.4, B.5 and B.6. The second cluster included isolates B.1, B.2, and B.7. Grouping the isolates based on their antagonistic efficiency parameters was not related to their geographic origins. Similar results were found by El-samawaty et al.[20] . For example, isolates B.5 and B.6 showed a very high similarity although they were isolated from Behera (West Delta) and Giza (Middle Egypt), respectively. Grouping the isolates was also unrelated to their taxonomic position, thus , isolates B.1 and B.7 were remotely related although they belong to *Bacillus coagulans.*

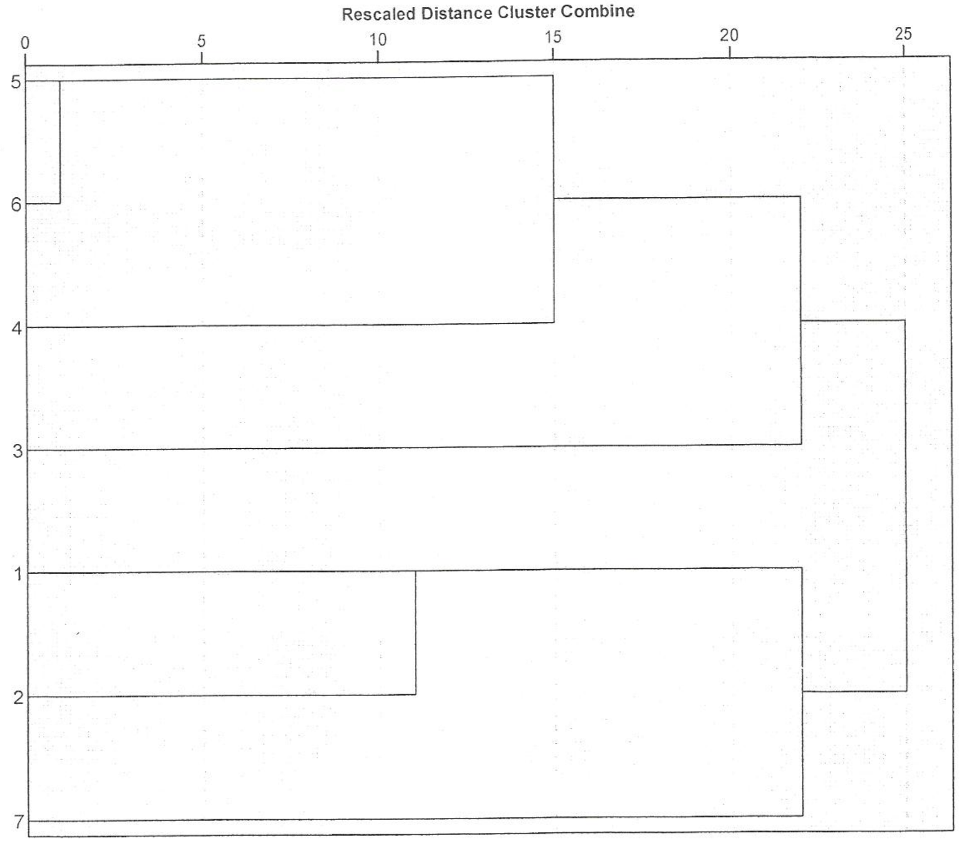

Figure 2. Phenogram based on average linkage cluster analysis of biocontrol capacity of 7 isolates of *Bacillus* spp. against 32 isolates of *Macrophomina phaseolina*. The tested *Bacillus* spp. were *Bacillus coagulans* (1), *Bacillus globisporus* (2), *Bacillus pumilus* (3), *Bacillus subtilis* (4), *Bacillus circulans* (5), *Bacillus cereus* (6), and *Bacillus coagulans* (7)

References

1.O.D. DHINGRA, J.B. SINCLAIR, Biology and pathology of *Macrophomina phaseolina* . Imprensia Universidade Federal de Viscosa, Brazil p.166 (1978).

2.G.M. WATKINS, Compendium of cotton diseases. The American Phytopathological Society. St. Paul, Minnesota, p. 87(1981).

3.M.R., OMAR, Pathological and biochemical studies on *Macrophomina phaseolina* Pathogenic on cotton. Ph.D. Thesis, Seuz Canal Univ., Egypt, p. 178(2005).

4.E. ZAVALETA, Valuing ecosystem services lost to Tamarix invasion in the United States. In H.A. Mooney , R.J. Hobbs ed., Invasive species in a changing world. Island Press, Washington, D.C P. 261-300 (2000).

5.D.M. WELLER, J.M. RAAIJIMAKERS, B.B.M. GARDNERS, L.S. THOMASHOW, Microbial population responsible for specific soil suppressiveness to plant pathogens. *Ann. Rev. Phytopathol*., **40**, 309, 348(2002).

6.K.K. PAL, K.V.B. TILAK, A.K SAXENA, R. DEY, S. SINGH, Suppression of maize root disease caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graninearum* by plant growth promoting rhizobacteria. *Microbiol. Res*., **156**, 209, 223(2001).

7.S. MUHAMMAD, N.A. AMUSA, *In vitro* inhibition of growth of some seedling blight including pathogens by compost inhabiting microbes. *Afr. J. Biotechnol*., **2**,161,164(2003).

8.S. BAPAT, A.K. SHAH, Biological control of fusarial wilt of pigeon pea by *Bacillus brevis*. *Can. J. Microbiol*., **46**,125,131(2000).

9.Y.K.CHAN, A.M. WAYNE, K.A. SEIFERT, Characterization of antifungal soil bacterium and its antagonistic activities against *Fusarium* species. *Can. J. Microbiol*., **49**, 253, 262(2003).

10.C.E. JENSEN, J.A. PERCICH, P.H.GRAHAM, Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. *Field Crop Res*., **74**,107,115(2002).

11.A.A. ALY, Incidence of fusarium wilt of flax as influenced by biotic and abiotic factors. Ph.D. Thesis, South Dakota State Univ., Brookings, p. 173(1988).

12.S. PLEBAN, F. INGEL, I.CHET, Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp. European *J. Plant Pathology*, **101**, 665, 672(1995).

13.A.A. ASRAN , Studies on the cotton rhizosphere microorganisms and their role in biocontrol of root infecting fungi, Ph.D. Thesis, Fac. Agric., Cairo Univ., p.155(2001).

14.S.M. AKHTAR, Z.A. SIDDIQUI, Glomus intraradices, Pseudomonas alcaligenes and *Bacillus pumilus*, effective agents for the control of root-rot disease complex of chickpea *Cicer arietinum* L. *J. Gen. Plant Pathol*., **74**, 53,60(2008).

15.Z.Q. JIANG, Y.H. GUO, S.M. LI , Evaluation of biocontrol efficiency of different *Bacillus* preparation and field application methods against Phytophthora blight of bell pepper. *Biocontrol*., **36**, 216,223(2006).

16.G.L. JIAN, Z.Q. JIANG, L.P XU, F.F. SUN, J.H. GUO, Characterization of chitinase secreted by *Bacillus cereus* strain CH2 and evaluation of its efficacy against *Verticillium* wilt of eggplant. *Biocontrol*., **53**, 931, 944 (2008).

17.H.D.WELLS, D.K.BELL, Antagonism *In vitro* between isolates of *Trichoderma harzianum* and *Rhizoctonia solani* AG4. *Phytopathology*, **73**, 507-512 (1983).

18.D.K. BELL, H.D.WELLS, C.R. MARKHAM, *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology*, **72**, 379–382 (1982).

i.LEBEDA, T. JENDRULEK, Application of cluster analysis for establishment of genetic similarity in gene for gene host parasite relationships. *J. Phytopathol*., **119**,131,141(1987).

19.A.M.A.EL-SAMAWATY, A.A. ASRAN, M.R. OMAR, K.A. ABD-ELSALAM, Anastomosis groups, pathogenicity and cellulase production of *Rhizoctonia solani* from cotton. *Pest Technology*, **1**, 117,124(2008).