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Control of peanut root and pod rots diseases using certain bioagents

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Abstract

To evaluate the potential of certain bacterial and fungal bioagents against *Macrophomina phasolina*, *Rhizoctonia solani* and *Fusarium oxysporum* which causing peanut root and pod rots diseases. Thirty three fungal isolates obtained from infected plants showing typical symptoms of peanut root and pod rot, collected from different localities in Assiut, Sohag and Minia governorates, Egypt were identified as *R. solani*, *M. phaseolina*, *F. solani*, *Aspergillus niger*, *A. flavus*, *F. moniliforme*, *F. equesti* and *F. semitectum*. Pathogenicity tests showed that *M. phaseolina* No. 4 recorded was the most pathogenic as incited root-rot and pod rot on the tested peanut cultivars NC followed by *R. solani* No. 6 and *F. solani* No. 7. Under laboratory conditions, twelve isolates of bacterial bioagents *i.e Bacillus subtilis* (BS1 and BS2), *Bacillus megaterium* (BM1 and BM2), *Penibacillus polymyxa* BP, *Pseudomonas fluorescens* (PF1 and PF2) and *Azotobacter* spp. (AZ1, AZ2, AZ3, AZ4 and AZ5), as well as, six isolates of *Trichoderma* spp. (T5, T7, T8, T9, T10 and T34) were tested to study their effects against the mycelial growth of the causal pathogens isolates *i.e. M. phaseolina* No. 4, *R. solani* No. 6 and *F. solani* No. 7. *In vitro*, our results showed that seven bacterial isolates (*B. subtilis* BS1, *B. megaterium* BM2, *B. polymyxa* BP, *P. fluorescense* (PF1 and PF2), *Azotobacter* spp. (AZ2 and AZ5)) and three isolates of *Trichoderma* spp. (T7, T10 and T34) gave the highest significant antagonistic effects against the test pathogens. The ten bioagents isolates which exhibited the best inhibition to the pathogenic fungi *in vitro* were tested *in vivo* during 2019 and 2020 growing seasons and the results revealed that *Trichoderma* spp. T34, *B. subtilis* BS1, *P. polymyxa* BP, *Azotobacter* spp. AZ2, *B. megaterium* BM2 and *Trichoderma* spp. T10, which tested against *M. phaseolina*, *R. solani* and *F. solani*, gave the highest reduction of both peanut root and pod rots diseases.

Keywords: peanut, root rot, pod rot, *Trichoderma* spp., *Pseudomonas fluorescens*, *Bacillus subtilis*.

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1. Introduction

Peanut or groundnut (*Arachis hypogaea* L.) is a leguminous plant belonging to family Fabaceae. It is considered one of the most important crops in Egypt, as well as in many countries of the world, which is due to its seeds high nutritive value for humans, as well as the produced green leafy hay for feeding livestock, in addition to the importance of the seed oil for industrial purposes (Abdalla et al., 2009). Root and pod rots are serious worldwide diseases attacking roots and pods underground (Hilal et al., 1990). Damping–off, root and pod rot diseases caused by *Macrophomina phasolina*, *Rhizoctonia solani* and *Fusarium oxysporum* are the most destructive fungal diseases, which attack peanuts causing quantitative and qualitative losses of yield (Abdel-Elkhair et al. 2016). *Fusarium* spp is known as a pathogen causing different symptoms of infected roots and pods (Hussin Zeinab, 2011; Marei, 2000). Various strategies were developed for controlling such diseases by using plant growth promoting rhizobacteria (PGPR). Different strains of PGPR genera such as *Azoarcus, Pseudomonas, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Clostridium, Burkholdaria, Enterobacter, Gluco-nacetobacter, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavo-bacterium*, are soil inhabitants that are able to colonize plant roots, stimulate plant growth, and increase crop yield, and employ a direct and indirect mechanisms to enhance plant growth and diseases prevention (Mroz et. al. 1994; Kloepper et al., 1989). Fungi of the genus *Trichoderma* are economically important due to their plant growth- and performance-promoting effects, such as improved nutrient supply, myco-parasitism of plant-pathogens and priming of plant defense (Guo et al., 2019). El-Sharkawy (2006) mentioned that *T. harzianum, T. hamatum* and *G. virens* reduced mycelial growth of *R. solani, M. phaseolina, F. oxysporum* and *Verticilium albo-atrum*. The main objective of the present study was to evaluate the efficacy of certain bacterial and fungal bioagents against *Macrophomina phasolina, Rhizoctonia solani* and *Fusarium oxysporum* which causing peanut root and pod rots diseases.

2. Materials and methods

2.1 Isolation and identification of the causal pathogens of peanut root and pod rots diseases

Peanut samples were collected from different localities in Minia, Assiut and Sohag governorates, Egypt. The infected roots and pods (Shell and kernel), were cut into small pieces, washed thoroughly with tap water, surface sterilized by immersing for 2 minutes in 70% ethayl alcohol. After rinsing several times in sterilized distilled water, samples were dried between two sterilized filter papers, The surface sterilized plant pieces were plated on sterilized Potato Dextrose Agar (PDA) medium in Petri dishes and incubated at 27°C. After 4-5 days of incubation, the developed fungal growth was purified using hyphal tip and single spore techniques. The pure fungal isolates were kept on PDA medium at 27°C and have been used in this study. The isolated fungi were identified based on the morphological characters of mycelium and spores as described by Barnet and Hunter (1977) and Domsch et al. (1980).

2.2 Pathogenicity tests

The pathogenic capabilities of the obtained fungal isolates were carried out on peanut cultivar NC under greenhouse conditions in the Farm of Faculty of Agriculture, Al-Azhar University (Assiut Branch), Egypt during 2017 growing season. Inocula of the tested isolates were prepared by inoculating sterilized conical flasks (250 ml) containing sorghum medium, which contains 75 g sorghum cereal, 25 g clean sand, 2 g sucrose and 50 ml water according to Abdel-Moneem (1996), with equal discs (0.5 cm) taken from 7 days old cultures of the tested fungal isolates. The inoculated flasks were incubated at 27°C for two weeks, then each isolate was mixed with sand clay soil sterilized with formalin 5% at the rate of 2% w/w. Sterilized pots (30 cm in diameter) were filled with infested sand clay soil 7 days before sowing. Uninfested soil pots were used as control. Seeds of peanut cultivar NC were seeded in infested and non-infested sand clay soil (7 seeds /pot). Disease severity of root rot was recorded after 100 days from sowing. The arbitrary (0-5) disease index scale as described by Grunwald et al. (2003) was adopted, where: 0= No visible symptoms, 1= slight hypocotyls lesions, 2= lesions coalescing around epicotyls and hypocotyls, 3= lesions starting to spread into the root system with root tips starting to be infected, 4= epicotyls, hypocotyls and root system almost completely infected and 5= completely infected root. Pod rot severity was recorded by adopting the scale based on area of spots covered on pods and percent disease index (PDI), calculated mean disease index, pods were grouped into six categories described by Wheeler (1969), Where 0=non disease symptoms (disease free), 1=spots cover 1-10%, 2= spots cover 10-30%, 3= spots cover 30-50%, 4= spots cover 50-70% and 5= spots cover >70%. The percentages of disease severity of root rot and pod rots diseases were estimated using the following formula:

$$\text{Disease severity (\%)} = \sum [(n \times V) / 5xN)] \times 100$$

Where; n= number of root or pod within each infection category, V= numerical values of infection categories, N= total number of root or pods examined, 5= constant, the highest numerical value.

2.3 Evaluation of certain antagonistic bacteria against the pathogenic fungi *in vitro*

The antagonistic bacteria were obtained from MIRCEN, Faculty of AgricUlture, Ain Shams University, Cairo, Egypt. The used bacteria in this study were five isolates of *Azotobacter* spp. (AZ1, AZ2, AZ3, AZ4 and AZ5), two isolates of *Bacillus subtilis* (BS1 and BS2), two isolates of *Bacillus megaterium* (BM1 and BM2), one isolate of *Penibacillus polymyxa* as well as two isolates of *Pseudomonas fluorescens* (PF1 and PF2). These isolates were tested against the most aggressive pathogenic fungi *F. solani* No. 7, *R. solani* No. 6 and *M. phaseolina* No.4 *in vitro*. The antagonistic bacteria were grown on nutrient agar medium. Plates were streaked with the bacterial growth which obtained from 2 days old cultures at the periphery using sterilized loop. At the same time, one disc of the pathogen was placed at the center of each plate, then plates incubated at 27°C. Inoculated plates with fungal disc without bacteria

served as control. When growth of the pathogen covered the plate surface (9.0 cm in diameter) of control treatment, antagonistic effect was determined by measuring the free inhibition zone, then percentage of mycelial growth inhibition was calculated according to the formula:

$$\text{Mycelial growth inhibition } \% = [A - B / A] \times 100$$

Where: A = length of hyphal growth of the control. B = length of hyphal growth of the treatment.

2.4 Evaluation of some *Trichoderma* isolates against the pathogenic fungi *in vitro*

Petri dish was divided into equal halves. The first half was separately inoculated with standard disc (0.5 cm) of *Trichoderma* spp. isolated from peanut rhizosphere. The second half was inoculated with an equal disc of pathogenic fungi, each treatment was replicated three times, and inoculated plates with the pathogen only were used as control. All Petri dishes were incubated at 27°C for 4 days and data were recorded. The percentage of mycelial growth inhibition was calculated according to the following formula:

$$\text{Mycelial growth inhibition } \% = [A - B / A] \times 100$$

Where: A= the length of mycelial growth in control. B= the length of mycelial growth in treated Petri plates.

2.5 Evaluation of certain bioagents on controlling peanut root and pod rots diseases under greenhouse conditions

Pot experiments were carried out during 2019 and 2020 growing seasons. The inoculum of *Trichoderma harzianum* isolates (T7 and T10) and isolate of *T. asperellum* T34 as antagonistic fungi as well as *F. solani*, *R. solani* and *M. phaseolina* were grown on sorghum medium as mentioned before in pathogenicity tests and mixed with sterilized soil at the rate of 2% (w/w) of soil for each of antagonistic and pathogenic fungi, 15 days before planting. Also, seven antagonistic bacteria *Azotobacter* spp. (AZ2 and AZ5), *Bacillus* (BS1, BM2 and BP) and *Pseudomonas fluorescens* (PF1 and PF2) were applied as soil treatment 15 days before planting by adding 100 ml of bacterial suspensions (10^8 cfu /ml) for each pot, which previously infested with the pathogenic fungi. Seeds of peanut cv. NC were sown (7 seeds /pot) in infested soil with the pathogenic isolates as mentioned before in the pathogenicity tests experiments. After 100 days from sowing, disease severity of peanut root and pod rots diseases were recorded (Hussin Zeinab, 2011).

2.6 Statistical analysis

Comparison of means was performed using Fisher's protected least significant difference (LSD) at $p \leq 0.05$ (Gomez and Gomez 1984) and the standard error was calculated using the statistical analysis software "CoStat 6.4" (CoStat, 2005).

3. Results and Discussion

3.1 Isolation and identification of the causal pathogens of peanut root and pod rots diseases

Thirty three fungal isolates were obtained from the infected roots and pods of peanut plants collected from different localities in Minia, Assiut and Sohag governorates, Egypt were identified as; ten isolates as *Rhizoctonia solani*, seven isolates as *M. phaseolina*, seven isolates as *Fusarium solani*, three isolates as *Aspergillus niger*, three isolates as *Aspergillus flavus* and one isolate as *F. moniliforme, F. equesti* and *F. semitectum*.

3.2 Pathogenicity tests

Thirty three fungal isolates were tested to study their pathogenic capability on peanut cv. NC plants under greenhouse conditions during 2017 growing season. According to data presented in Table (1), all tested fungal isolates proved significantly to be pathogenic and produced typical symptoms of pod and root rots diseases on peanut plants. *Macrophomina phaseolina* No.4, *R. solani* No. 6 and *F. solani* No.7 were the most pathogenic as incited disease severity of root rot 94%, 87%, respectively and 82%, and disease severity of pod rot 74%, 65% and 63%, respectively on the tested peanut cv. NC. On other hand, isolates *R. solani* No. 4 and *M. phaseolina* No. 2 followed by *A. flavus* No. 2 showed the lowest disease severity in peanut root rot disease, which were 14%, 18% and 22% respectively. While, *M. phaseolina* (No. 2 and No. 7), *A. flavus* No. 2 followed by *R. solani* and *F. solani* (No. 4 and 2) gave the lowest disease severity of pod rot, as reached 14% and 19.5%, 20.25 and 21%, respectively.

3.3 Efficacy of certain bacterial bioagents against the causal pathogens of peanut root and pod rots diseases *in vitro*

Data in Table (2) indicated that all test bacterial bioagents were able to inhibit the mycelial growth of all tested pathogenic fungi compared with the control. *Bacillus megaterium* BM2 recorded the highest reduction percentage of mycelial growth of *M .phaseolina,* followed by *Bacillus subtilis* BS1 and *Bacillus megaterium* BM1. While, isolates *Azotobacter* spp. AZ5, *Pseudomonas fluorescens* PF1, *Azotobacter* spp. AZ2 and *Azotobacter* spp. AZ3 recorded the moderate degree of inhibition against the same tested pathogenic fungus. *Bacillus megaterium* BM2, *Pseudomonas fluorescens* PF1, *Bacillus subtilis* BS1 and *Azotobacter* spp. AZ5 showed the highest growth inhibition against *Rhizoctonia solani*, while *Bacillus subtilis* BS2, *Pseudomonas fluorescens* PF2, *Azotobacter* spp. AZ2 and *Penibacillus polymyxa* BP showed moderate effects on the mycelial growth of *R. solani*. On other hand, *Azotobacter* spp. AZ5, *Bacillus megaterium* BM2, *Azotobacter* spp. AZ2, *Bacillus subtilis* BS1 and *Pseudomonas fluorescens* PF1 exhibited the highest growth inhibition of *Fusarium solani*, while *Bacillus megaterium* BM1, (*Bacillus subtilis* BS2, *Azotobacter* spp. AZ1, *Pseudomonas fluorescens* PF2 and *Penibacillus polymyxa* BP showed moderate effects

on the same pathogen. Generally, the seven bacterial isolates (*Bacillus subtilis* BS1, *Bacillus megaterium* BM2, *P. polymyxa* BP, *Pseudomonas fluorescens* PF1, *Azotobacter* spp. AZ2, *Azotobacter* spp. AZ5 and *Pseudomonas fluorescens* PF2) achieved the highest records in reducing mycelial growth of the three tested pathogenic fungi. These results are similar to those obtained by El-Mougy et al. (2011) who examined the influence of the antagonistic isolates *B. subtilis* and *P. fluorescens* and their culture filtrates against soil-borne root rot pathogens *R. solani* and *F. solani in vitro*. The tested antagonists reduced the linear growth of the fungal pathogens. Mahmoud et al. (2006) tested seventeen bacterial isolates *in vitro* for their antagonistic effect against *F. solani* and *M. phaseolina* and they recorded that the most effective isolates in reducing the mycelium growth of pathogenic fungi were *P. fluorescens* followed by *B. subtills* and *Bacillus* sp.

Table 1: Pathogenicity tests of 33 fungal isolates on peanut cv. NC under greenhouse conditions during 2017 growing season.

Fungal isolate	Isolate No.	Disease severity (%)	
		Root rot	Pod rot
Fusarium solani	1	58	42.5
	2	23	21
	3	64	25.75
	4	75.5	55
	5	50	34
	6	52	32
	7	82	63
Fusarium moniliforme	1	27	25
Fusarium semitectum	2	29	24
Fusarium equesti	3	43	25
Aspergillus niger	1	33	27.5
	2	45	26.5
	3	33.5	25.75
Aspergillus flavus	1	32	31
	2	22	20.25
	3	41	25.75
Rhizoctonia solani	1	36	22.25
	2	35	27.25
	3	57	32.5
	4	14	21
	5	59	40.5
	6	87	65
	7	44	25
	8	44.5	30
	9	25	34
	10	42	41
Macrophomina phaseolina	1	29	26
	2	18	14
	3	27	24
	4	94	74
	5	24	28
	6	39	24.25
	7	37	19.5
Control		0	0
L.S.D. at 5%		4.5	4.94

Table 2: Antagonistic effects of various bacterial bioagents isolates against different pathogens of peanut pod and root rots diseases.

Bacterial isolate	Mycelial growth inhibition (%)		
	Macrophomina phaseolina	*Rhizoctonia solani*	*Fusarium solani*
Bacillus subtilis BS1	75.2	81.5	74
Bacillus subtilis BS2	9.2	74.8	59.2
Penibacillus polymyxa BP	8.5	66.6	52.6
Bacillus megaterium BM1	64	63	61.8
Bacillus megaterium BM2	78	89.6	79.3
Pseudomonas fluorescens PF1	56	86.6	64
Pseudomonas fluorescens PF2	14	69	57.4
Azotobacter spp. AZ1	10.5	53	58
Azotobacter spp. AZ2	40	68	78
Azotobacter spp. AZ3	30	66.5	41
Azotobacter spp. AZ4	19.5	62.5	52.5
Azotobacter spp. AZ5	59.5	80	83
Control	0	0	0
L.S.D at 5%	4.34	4.19	4.34

3.4 Efficacy of dual culture of various *Trichoderma* isolates against the causal pathogens of peanut root and pod rots diseases *in vitro*

Six isolates of *Trichoderma* spp. were tested to study their effect against *M. phaseolina, R. solani* and *F. solani*, under laboratory conditions. Data in Table (3) indicated that all the tested isolates of *Trichoderma* spp. exhibited different degrees of reaction against *M. phaseolina, R. solani* and *F. solani*. The mycelial growth of *Trichoderma* spp. T34 gave the high significant antagonistic effect against the three tested pathogenic fungi, followed by *Trichoderma* spp. T7 and *Trichoderma* spp. T10. While, *Trichoderma* spp. T9 exhibited the lowest antagonistic effect on the fungal growth of tested pathogens. The other isolates, *Trichoderma* spp. T5 and *Trichoderma* spp. T8 showed moderate antagonistic effects on the fungal growth of pathogens. *Trichoderma* spp. T7 and *Trichoderma* spp. T10 were identified as *Trichoderma harzianum*. While, *Trichoderma* spp. T34 identified as *Trichoderma asperillum*. Generally, *T. asperillum* T34 achieved the highest records in reducing the mycelial growth of the tested pathogenic fungi followed by *T. harzianum* T7, respectively. These results are similar to those obtained by Ha (2010) who found that antagonistic effect might be due to direct influence of the tested fungi against pathogens through coiling their hyphae around the hyphae of the pathogens to prevent their continued growth (Adekunle et al., 2006; Chu & Wu, 1981). The antagonistic properties of *Trichoderma* are based on the activation of multiple mechanisms. *Trichoderma* strains act as biocontrol agents against fungal phytopathogens either indirectly or directly. Indirect mechanism comprises competition for nutrients and space, modification of the environmental conditions, antibiosis and induction of plant defensive mechanisms, however direct mechanism encompasses mycoparasitism (Harsukh et al., 2013).

3.5 Efficacy of certain bioagents on controlling of peanut root rot disease under greenhouse conditions

Data presented in Table (4) showed that

tested bioagents significantly reduced the disease severity of peanut root rot disease compared with control. *Trichoderma asperillum* T34, *Penibacillus polymyxa* BP, *Bacillus megaterium* BM2, *Azotobacter* spp. AZ2 and *B. subtilis* BS1 exhibited the highest reduction of disease severity of peanut root rot disease caused by *M. phaeolina* recording 21.4, 24.38, 24.7, 24.8 and 28.5% respectively, followed by *T. harzianum* T10 and *Pseudomonas fluorescens* PF2 with 37.2 and 38%, respectively. While *P. fluorescens* PF1 recorded the lowest reduction of disease severity, followed by *Azotobacter* spp. AZ5 and *T. harzianum* T7 (49, 45.22 and 43%, respectively). *Azotobacter* spp. AZ2, *T. asperillum* T34 and *T. harzianum* T10 exhibited the highest reduction of disease severity of peanut root rot disease caused by *R. solani* (21.48, 21.57 and 25.7%, respectively). In this respect, *B. subtilis* BS1, *P. polymyxa* BP, *B. megaterium* BM2 and *P. fluorescens* PF2 showed moderate effects of the disease severity reduction. While, *P. fluorescens* PF1 exhibited the lowest reduction of disease severity of peanut root rot disease caused by *R. solani* followed by *T. harzianum* T7 and *Azotobacter* spp. AZ5 with 43.8, 42.79 and 40%, respectively. *Bacillus subtilis* BS1, *Azotobacter* spp. AZ2 and *T. asperillum* T34 isolates showed the greatest decrease in disease severity caused with the pathogen *F. solani*, as reached 19, 19, and 23.3% respectively. While, isolate *P. fluorescens* PF2 recorded the lowest reduction of the disease severity followed by *Azotobacter* spp. AZ5 (40.94 and 35.7% respectively). Such results are in line with those reported by Gowily Ahlam et al. (1993), who found that inoculation with *A. chroococcum*, *A. brasilense* and *B. japonicum* reduced soybean root-rot disease caused by *R. solani* and *F. solani*. El-Habbaa et al. (2002) suggested that addition of *Rhizobium* and *B. subtilis* to soil before sowing peanut seeds significantly reduced root-rot disease of peanut caused by *S. rolfsii*, *R. solani*, *M. phaseolina* and *F. solani*. Mahmoud et al. (2006) and Ibrahim et al. (2008) studied the effects of certain bacterial isolates against *R. solani*, *S. rolfsii*, *F. solani* and *M. phaseolina* causing root rot disease of peanut in greenhouse experiments and they were found that the most effective isolates in reducing the diseases of peanut were *P. fluorescens* followed by *B. subtills* and *Bacillus* sp. Vargas et al. (2008) mentioned that inoculation with *Trichoderma* spp. recorded the lowest incidence of peanut root rot disease caused by *F. solani*.

Table 3: Antagonistic effects of dual culture of various *Trichoderma* spp. isolates against different pathogens of peanut pod and root rots diseases.

Fungal isolate	Mycelial growth inhibition (%)		
	Macrophomina phaseolina	*Rhizoctonia solani*	*Fusarium solani*
Trichoderma spp. T5	69	77.8	77
Trichoderma spp. T7	77	89	83.3
Trichoderma spp. T8	72.2	80	73.5
Trichoderma spp. T9	66.7	77	72
Trichoderma spp. T10	83.3	83.3	77
Trichoderma spp. T34	80	92	83.3
Control	0	0	0
L.S.D at 5%	5.9	5.12	5.63

Table 4: Effect of certain bioagents on disease severity percentages of peanut root rot disease under greenhouse conditions during 2019 and 2020 growing seasons.

Bioagents isolate	Disease severity (%)								
	Macrophomina phaseolina			*Rhizoctonia solani*			*Fusarium solani*		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis BS1	24.7	31.4	28.05	23.2	31.42	27.31	20	18	19
Bacillus megaterium BM2	28.55	20.9	24.7	21.87	36.18	29	23.76	31.41	27.6
Pseudomonas fluorescens PF1	45.7	52.36	49	37.14	50.45	43.8	27.6	24.73	26.2
Pseudomonas flurescens PF2	35.23	40.95	38	36.2	40.9	38.55	34.27	47.61	40.94
Azotobacter spp. AZ2	20.18	29.5	24.84	18.26	24.7	21.48	15.23	22.83	19
Azotobacter spp.AZ5	41.9	48.55	45.22	33.32	46.65	40	28.55	42.85	35.7
Penibacillus polymyxa BP	25	23.67	24.38	32.36	22.8	27.58	30.45	39	34.7
Trichoderma harzianum T7	40.9	45.2	43	41.9	43.68	42.79	33.32	35.23	34.3
Trichoderma harzianum T10	39	35.2	37.2	23.8	27.6	25.7	29.5	40.95	35.22
Trichoderma asperillum T34	25.69	17.14	21.4	26	17.14	21.57	24.73	21.86	23.3
Control	87.61	90.33	88.97	80	85.6	82.8	72.36	76	74.2
L.S.D at 5%	4.72	4.48		3.28	5.92		4.04	3.62	

3.3 Efficacy of certain bioagents on controlling of peanut pod rot disease under greenhouse conditions

Data presented in Table (5) showed that treated soil with all bacterial and fungal bioagents significantly reduced the disease severity of peanut pod rot disease compared with the control. *Trichoderma asperillum* T34, *Azotobacter* spp. AZ2, *Penibacillus polymyxa* BP and *Bacillus subtilis* BS1 recorded the highest reduction of disease severity of peanut pod rot disease caused by *M. phaeolina* (32.48, 34.48, 36.83 and 37.5% respectively), while BM2, *T. harzianum* T7 and *T. harzianum* T10 gave moderate effects of the reduction of the disease severity (39.15, 40 and 40.5%, respectively). *Trichoderma asperillum* T34, *Azotobacter* spp. AZ2 and *B. subtilis* BS1 achieved the highest reduction of disease severity of peanut pod rot disease caused by *R. solani* (30.33, 30.65 and 30.98%, respectively).

Table 5: Effect of certain bioagents on disease severity percentages of peanut pod rot disease under greenhouse conditions during 2019 and 2020 growing seasons.

Bioagents isolate	Disease severity (%)								
	Macrophomina phaseolina			*Rhizoctonia solani*			*Fusarium solani*		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Bacillus subtilis BS1	35	40	37.5	31.67	30.3	30.98	30.3	21.67	25.98
Bacillus megaterium BM2	42	36.3	39.15	38.3	29	33.65	31.67	18.67	25.17
Pseudomonas fluorescens PF1	50.67	45.67	48.17	45.67	47.67	46.67	40.3	38.67	39.33
Pseudomonas flurescens PF2	47.67	41.67	44.67	43.67	37.67	40.67	41.67	37	39.48
Azotobacter spp. AZ2	36.67	32.3	34.48	35	26.3	30.65	35	17.3	26.15
Azotobacter spp.AZ5	49	50.3	49.65	42.67	46.3	44.48	45.3	40.67	42.98
Penibacillus polymyxa BP	43.67	30	36.83	36.67	33.67	35.17	43.67	44.3	43.98
Trichoderma harzianum T7	44	36	40	40	40.3	40.15	36.67	30	33.33
Trichoderma harzianum T10	48	33	40.5	41.67	32.3	36.98	37.3	35	36.15
Trichoderma asperillum T34	38.67	26.3	32.48	39	21.67	30.33	28	25.67	26.83
Control	87.3	77	82.15	80.67	72	76.33	77	71	74
L.S.D at 5%	3.3	2.55		2.88	3.06		3.17	2.9	

Bacillus megaterium BM2, *P. polymyxa* BP, *T. harzianum* T10 and *T. harzianum* T7 showed moderate reduction of the disease severity (33.65, 35.17, 36.98 and

40.15%, respectively). *Bacillus megaterium* BM2, *B. subtilis* BS1 and *Azotobacter* spp. AZ2 achieved the highest reduction of disease severity of peanut pod rot disease caused by *F. solani* (25.17, 25.98 and 26.15%, respectively). Generally, *P. fluorescens* PF1, *Azotobacter* spp. AZ5 and PF2 gave the lowest reduction of disease severity of pod peanut rot disease caused by the three fungal pathogens *M. phaseolina, R. solani* and *F. solani*. The results obtained are in agreement with both Mahmoud (2004) and Ziedan (2000) who found that *B. subtilis* and *P. fluorescens* significantly reduced incidence of all types of pod rots caused by *Fusarium* spp**.** Ahmed (2006) revealed the superiority of *T. harzianum* followed by commercial products Rhizo-N and Plant-guard which showed high reduction of pod rot disease of peanut caused by the *R. solani, M. phaseolina, S. rolfsii* and *F. moniliforme*. Plant growth promoting rhizobacteria are applied as active ingredients in several commercial bio-inoculants. Each product has special set of mechanisms for plant growth promotion and biocontrol of plant pathogens. In the same way, *Trichoderma* spp. parasitize other fungi and induce systemic resistance in host plants (Harman, 2011). As for, bacteria produce siderophore and provide N, P and Fe for plant growth, release several antibiotics and volatiles for suppression of plant pathogens (Sharifi and Ryu, 2016; Sharifi et al., 2010), as well as improve plant health and compete with plant pathogens by colonizing root tissues (Ghanbarzadeh et al., 2016). The efficiency of *T. harzianium* to inhibit fungal growth may be through competition for space and nutrients, mycoparasitism and production of antibiotic compounds. It was found that the hyphae of *T. harzianium* coil around the hyphae of the pathogen and penetrate the host mycelium through degrading cell wall by secretion hydrolytic enzymes followed by assimilation of cell contents (Siameto et al., 2011; Howell, 2006).

References

Abdalla AA, El-Howeity MA, Desoky AH, 2009. Response of peanut crop cultivated in newly reclaimed soil to inoculation with plant growth-promoting rhizobacteria. Menoufia Journal of Agricultural Research **34**(6): 2281–2304.

Abd-El-Khair H, Hagag KHE, Elshahawy IE, 2016. Soil application of *Bacillus pumilus* and *Bacillus subtilis* for suppression of *Macrophomina phaseolina* and *Rhizoctonia solani* and yield enhancement in peanut. International Journal of ChemTech Research **9**(6): 1.

Abd-Elmoneem HMK, 1996. Effect of micronutrients on incidence of sesame charcoal rot, root rot and wilt diseases complex. Assiut Journal of Agricultural Sciences **27**(3): 181–195.

Adekunle AT, Ikotun T, Fiorini DA, Cardwell KF, 2006. Field evaluation of selected formulations of *Trichoderma* species as seed treatment to control damping-off of cowpea caused by *Macrophomina phaseolina.* African Journal of Biotechnology **5**(5): 419–424.

Ahmed KA, 2006. Integrated control of peanut fruit rot diseases and their side effects on the biological effects on soil. Ph.D. Thesis, Faculty of Agriculture, Moshtohor, Benha University, Egypt, 140 pp.

Barnett HL, Hunter BB, 1977. Illustrated genera of imperfect fungi. 3rd ed., Burgess Publishing Company, Minneapolis, Minnesota, USA, 421 pp.

Chu FF, Wu WS, 1981. Antagonistic action of *Trichoderma* spp. and *Penicillium* spp. on *Rhizoctonia solani*. Memories of the College of Agriculture, National Tiwan University **21**(2): 4–18.

CoStat 2005. CoStat program, Version 6.4., CoHort Software, Monterey, CA., USA.

Domsch KH, Gams W, Anderson TH, 1980. Compendium of soil fungi. Academic Press, A subsidiary of Harcout Brace Jovanovich, Publishers, London, United Kingdom, **1**: 859.

El-Habbaa GM, Felaifel MS, Zahra AM, Abdel-Ghany RE, 2002. *In vitro* evaluation of some fungicides, commercial bio-control formulations and natural plant extracts on peanut root-rot pathogens. Egyptian Journal of Agriculture Research **80**(3): 1017–1030.

El-Mougy NS, Abdel-Kader MM, Alhabeb RS, 2011. *In vitro* antifungal activity of chitinolytic enzymes produced by bio-agents against root rot pathogenic fungi. Archives of Phytopathology and Plant Protection **44**(7): 613–622.

El-Sharkawy RMI, 2006. Studies on root-rot and wilt diseases of strawberry with special reference to their control in Egypt. Ph.D. Thesis, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, 138 pp.

Ghanbarzadeh B, Safaie N, Mohammadi Goltapeh E, Rezaee Danesh Y, Khelghatibana F, 2016. Biological control of Fusarium basal rot of onion using *Trichoderma harzianum* and *Glomus mosseae*. Journal of Crop Protection **5**: 359–368.

Gomez KA, Gomez AA, 1984. Statistical Procedures for Agriculture Research, Second ed., Wiley, New York, USA, 680 pp.

Gowily Ahlam, Bouthina E, Abdel-Ghany F, Omar SA, 1993. Effect of biofertilizer application on controlling soybean root-rot disease. Egyptian Journal of Applied Sciences **8**(9): 20–31.

Grunwald NJ, Coffman VA, Kraft JM, 2003. Source of partial resistance to fusarium root rot in the *pisum* core collection. Plant Disease **87**: 1197–1200.

Guo Y, Ghirardo A, Weber B, Schnitzler JP, Benz JP, Rosenkranz M, 2019. *Trichoderma* species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor*. Frontiers in Microbiology **10**: 891.

Ha TN, 2010. Using *Trichoderma* species for biological control of plant pathogens in Viet Nam. Journal of International Society for Southeast Asian Agriculture Science **16**(1): 17–21.

Harman GE, 2011. *Trichoderma*-not just for biocontrol anymore. Phytoparasitica 39: 103–108.

Harsukh G, Rinkal D, Sunil P, Mansukh K, Balubhai G, 2013. Molecular mechanism of *Trichoderma* as bio-control agents against phytopathogen system – a review. Current Research in Microbiology and Biotechnology **1**(4):

133–142

Hilal AA, Metwally AH, El-Deeb AA, 1990. Peanut diseases in Egypt: II- Factors affecting healthy survival plants, pod rots and yield. Annals of Agricultural science, Moshtohor, **28**: 1557–1567.

Howell CR, 2006. Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. Phytopathology **96**: 178–180.

Hussin Zeinab N, 2011. New approaches for controlling peanut root and pod rots caused by *Rhizoctonia solani* in Egypt and Nigeria. Ph.D. Thesis, Institute of African Research and Studies, Cairo University, Cairo, Egypt, 156 pp.

Ibrahim MM, Mahmoud EY, Wagida AM, 2008. The ability of some antagonistic bacteria on control of peanut root rots diseases compared to fungicides efficiency. Minufiya Journal of Agricultural Research **33**(5): 1107–1125.

Kloepper JW, Lifshiftz R, Zablotowicz RM, 1989. Free living bacterial inoculation for enhancing crop productivity. Trends in Biotechnology 7: 39–44

Mahmoud E, Shokry Y, Samia YM, Hussin Zeinab N, 2006. Induction of resistance in peanut plants against root rot disease under greenhouse conditions by some chemical inducers. Mansoura University Journal of Agriculture science **31**(6): 3511–3524.

Mahmoud EY, 2004. Integrated control of pod rot disease of peanut. Ph.D. Thesis, Faculty of Agriculture, Ain-Shams University, Egypt, 154 pp.

Marei TA, 2000. Studies on pod rots of peanut. Ph.D. Thesis, Faculty of Agriculture, Moshtohor, Zagazig University, Egypt, 143 pp.

Mroz A, Martiniuk S, Kus J, 1994. Response of winter wheat to seed applied microorganisms. Phytopathology **19**: 15–20.

Sharifi R, Ahmadzadeh M, Sharifi-Tehrani A, Talebi-Jahromi K, 2010. Pyoverdine production in *Pseudomonas fluorescens* UTPF5 and its association with suppression of common bean damping off caused by *Rhizoctonia solani* (Kuhn). Journal of Plant Protection Research **50**: 72–78.

Sharifi R, Ryu CM, 2016. Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, alarm signals to *Arabidopsis* seedlings for eliciting induced resistance, or both?. Frontiers in Microbiology **7:** 196.

Siameto EN, Okoth S, Amugune NO, Chege NC, 2011. Molecular characterization and identification of biocontrol isolates of *Trichoderma harzianum* from Embu District, Kenya. Tropical and Subtropical Agroecosystems **13**: 81–90.

Vargas GS, Meriles JM, Haro R, Casini C, March GJ, 2008. Crop rotation and tillage systems as a proactive strategy in the control of peanut fungal soilborne diseases. BioControl **53**(4): 685–698.

Wheeler BEJ, 1969. An Introduction to Plant Diseases, John Wiley and sons Ltd., London, England, 374 pp.

Ziedan EH, 2000. Soil treatment with biofertilizers for controlling peanut root and pod rot diseases in Nobaria Province. Egyptian Journal of Phytopathology **28** (1-2): 17–26.