BIOLOGICAL SEED TREATMENTS FOR CONTROLLING MAJOR PEANUT (Arachis hypogaea L.) SEED-BORNE FUNGI Ghonim, M.I.; I.H. El-Abbasi and A.A. El-Wakil Seed Pathol. Res. Dept., Plant Pathology Res. Inst., ARC, Egypt

ABSTRACT

Seed-borne mycoflora of peanut (Arachis hypogaea L.) cultivar Giza 5 were surveyed in 3 major Egyptian production areas. Seed health test of the collected samples from Sharkyia, Ismailia and North Sinai revealed 18 seed-borne fungi. Major pathogenic seed-borne fungi were tested for their pathogenicity after 15, 30 and 60 days of planting under greenhouse conditions, which proved that Rhizoctonia solani was the most serious pathogen affecting the healthy survival percentage, followed by Macrophomina phaseolina, Fusarium solani and Sclerolium rolfsii. Fusarium oxysporum was the least dangerous fungus in this respect.

In greenhouse experiments, the bio-control agents *Trichoderma harzianum* and *Bacillus subtilis* were experimented to control *S. rolfsii*, *F. oxysporum* and *M. phaseolina* using seed coat treatment combined with soil sterilization. Complete elimination of the three pathogens was achieved when anyone of the two bio-control agents was used as seed treatment under certain condition of soil sterilization; otherwise, different levels of control were recorded.

Examining shells and seeds yield for the presence of the previously concerned pathogens proved that controlling soil-borne inocula was very important base line to accomplish complete control of seed-borne mycoflora using biological seed treatment.

Keywords: Peanut, seed health test, biological seed treatment, Trichoderma harzianum, Bacillus subtilis.

INTRODUCTION

Seeds are the only mean of field crops re-production. This absolute importance of seeds used for cultivation on the purpose of productivity. attracted many plant researchers to do their best efforts studying various topics related to it. Diseases are one of the critical factors influencing viability, re-producibility and seed quality. These critical factors could be reduced or even eliminated by several control means. Biological control is considered as a recent, promising and safe trend in the field of plant disease management. Two bio-control agents; Trichoderma harzianum and Bacillus subtilis are widely used in this respect. Using these two bio-control agents as seed treatments, for controlling peanut seed-borne pathogens was the main goal of the present work, whether under greenhouse or field conditions. Peanut (Arachis hypogaea L.) was chosen because of its importance as an oil crop, which is considered as a main component of many food industries. This valuable crop is a main source of high quality edible oil (36-54%), protein (12-35%) and excellent source of vitamins E, K and B (Savage and Keenan, 1994 and Stalker, 1997). Unfortunately, peanut seeds are suffering from serious diseases, which affecting seed yield quantity and quality (Abd El-Al, 1973; Abu-Arkoub, 1973; Umechuruba et al., 1992; Davis et al., 1996 and El-Wakil et al., 2001).

MATERIALS AND METHODS

A wide range of pathogenic fungi attacks peanut seeds and seedlings. These fungi are seed and/or soil-borne pathogens causing preand post- emergence damping-off. Seed samples of peanut cultivar Giza 5 were collected from different newly reclaimed localities in Sharkiya, Ismailia and North Sinai governorates and surveyed for the presence of seed-borne fungi.

1. Isolation, purification and identification of seed-borne mycoflora:

The selected seed samples were thoroughly washed in running tap water then surface sterilized by immersing in 2% sodium hypochlorite solution for 3 min. Seeds were rinsed in sterilized water and left to dry under laminar flow. Sterilized seed samples were individually transferred to Petri dishes containing PDA medium. One hundred seeds were used from each sample in 10 replicates (10 seeds/dish). Plates were incubated at 20°C ± 2 for 5-7 days under complete darkness, Identification and isolation were carried out whenever fungal cultures were recognizable. All isolated fungi were purified using hyphal tip technique developed by Dhingra and Sinclair (1985). Identification was carried out according to Neergaard (1945), Barnett and Hunter (1972), Ellis (1976 and 1980), Booth (1985) Klitch (1992). Percentages of incidence and frequency of the isolated fungi were calculated.

2. Greenhouse experiments:

2.1. Pathogenicity tests:

From the isolated peanut seed-borne mycoflora, five major fungal isolates namely; Fusarium oxysporum Schlecht, ex Fr., F. solani (Mart.) Sacc. emend. Snyd. & Hans., Macrophomina phaseolina (Tassi) Goid., Rhizoctonia solani Kühn and Sclerotium rolfsii Sacc. were tested for their pathogenicity on peanut seeds cultivar Giza 5 under greenhouse conditions. Plastic pots (25 cm in diam.) were sterilized by immersing in 5% formalin solution for 15 min. and left to dry. Sandy-clay soil (1:1 w/w) was sterilized using 5% formalin solution and left 3 weeks uncovered for complete formalin evaporation. Pots were then filled with the sterilized soil at the rate of 5 kg soil/pot.

Inocula of the five fungal isolates were grown separately on moistened sterilized corn-sand medium for 20 days at 25°C. Soil infestation was carried out by mixing the inoculum of each fungus individually with soil of each individual pot at the rate of 3 gm of well developed pathogen on cornsand medium/kg soil then watered daily for one week before sowing. Pots of control treatment were filled with the same amount of sterilized com-sand medium. Five apparently healthy seeds were surface sterilized using 2 % sodium hypochlorite for 3 min., washed thoroughly in distilled sterilized water, sown in each pot and watered whenever needed. Percentage of pre-, postemergence damping-off and healthy survival plants were recorded after 15, 30 and 60 days, respectively, from planting following the formula used by Emara (1995).

2.2. Biological seed treatments under greenhouse conditions:

Cells or spores suspensions of the biocontrol agents *B. subtilis* or *T. harzianum* were used as peanut seed coat treatment for controlling the seed-bome inocula of *M. phaseolina, F. oxysporum* and *S. rolfsii.*

Sterilized plastic pots (25 cm in diam.) were fuled with 5 kg/pot of sterilized sandy clay soil (1:1 w/w). Artificial infestation with one of the aforementioned pathogenic fungi at the rate of 3 qms, of cornmeal medium per kg soil was carried out. Planting was carried out 15 days after soil infestation and daily mixing and watering. Seeds of cv. Giza 5 were coated with cell suspension of B. subtilis (2.8 x 108 cell/ml.) in 0.1 w/v carboxymethel cellulose as adhesive for one hour (Abd-Allah, 1995), while, other equal amount of seeds were coated with conidial spores suspension of 5-days-old T. harzianum at the rate of 6 x 103 spores/ml. (Abdel-Kader, 1997). Sowing uncoated sterilized seeds in infested soil was done to serve as control. Five seeds in four replicates were planted for each treatment and the usual agricultural practices for peanut crop were followed. Percentages of pre-, post-emergence damping-off and root rot were recorded after 15, 30 and 60 days from planting, respectively. All treatments, including control treatment, received exactly the same agricultural practices till the end of growing season.

2.3. Transmission of seed-borne mycoflora to adult plants:

At harvest time, the resulted pods of each individual treatment were collected. Shells were separated from seeds and received the same following treatments where, thoroughly washed with running tap water and surface sterilized by immersing them in 2% sodium hypochlorite solution for 3 min., then rinsed in sterilized water and dried between two sterilized filter papers. Sterilized shell parts and seeds were separately transferred to sterilized glass Petri dishes containing PDA medium. Plates were immediately incubated at 20°C ±2 and screened daily for fungal development. The isolated fungi were microscopically examined after 5 – 7 days. The percentage of incidence and frequency of seed/plant-transmitted mycoflora were recorded.

RESULTS AND DISCUSSION

1. Peanut seed-borne mycoflora:

Data presented in Table (1) show the percentage of incidence and frequency of fungal species and genera isolated from peanut seed samples in the three surveyed governorates.

Data in Table (1) show 18 fungal isolates belong to 12 species and 15 genera. Among the recorded fungal isolates, there were 7 fungal species presented in high frequency percentages ranged between 20.0 to 36.7% as total frequency; namely *Rhizopus nigricans* (36.7%), *Fusarium solani* (34.3%), *Macrophomina phaseolina* (32.5%), *Aspergillus flavus* (24.9%), *A. niger* (23.4%), *F. oxysporum* (20.9%) and *Sclerotium rolfsii* (20.0%). Concerning the frequency percentage of the 7 species in each individual governorate, *Rhizopus nigricans* (8.3, 11.8 and 16.6), *Fusarium solani* (14.5,

9.6 and 10.2%), Macrophomina phaseolina (9.4, 11.5 and 11.6%), Aspergillus flavus (7.9, 10.5 and 6.5%), A. niger (6.6, 8.3 and 8.5%), F. oxysporum (7.9, 5.8 and 7.2%) and Sclerotium rolfsii (9.3, 5.4 and 5.1) as recorded from Sharkyia, Ismailia and North Sinai governorates, respectively. Pathogenic fungi such as M. phaseolina, F. oxysporum and S. rolfsii used to attack peanut seeds and pods causing serious disease problems. In similar study, El-Wakii and Ghonim (2000) showed that the pathogenic fungal species include R. solani, S. rolfsii, M. phaseolina, F. solani and F. oxysporum were the most frequently isolated in Sharkia governorate. On the other hand, Abd El-Al (1973) reported differences in disease incidence and distribution from one location to another.

Table (1): Incidence percentage and frequency of seed mycoflora isolated from peanut seed samples collected from three Egyptian governorates.

Sharkyia

Ismailia

North Sinai

Fungal isolates	% of incidence	Frequency %	% of incidence	Frequency %	% of Incidence	Frequency %	frequency %
Alternaria alternata	3.7	5.1	2.7	3.5	2.7	4.6	13.2
Aspergillus flavus	5.7	7.9	8.2	10.5	3.8	6.5	24.9
Aspergillus niger	4.8	6.6	6.5	8.3	5.0	8.5	23.4
Botrydiplodia thiobromae	1.3	1.8	3.0	3.8	2.2	3.8	9.4
Cladosporium sp.	1.7	2.3	2.5	3.2	1.8	3,1	8.6
Curvularia spp.	1.7	2.3	2.2	2.8	0.0	0.0	5.1
Drechslera spp.	0.8	1,1	1.7	2.2	0.0	0.0	3.3
Epicoccum sp.	0.8	1.2	1.2	1.5	1.2	2.0	4.7
Fusarium moniliforme	4.8	6.6	5.5	7.2	3.0	5.1	18.9
F. oxysporum	5.7	7.9	4.5	5.8	4.2	7.2	20.9
F. solani	10.5	14.5	7.5	9.6	6.0	10.2	34.3
Macrophomina phaseolina	6.8	9.4	9.0	11.5	6.8	11.6	32.5
Myrothecium sp.	1.8	2.5	1.3	1.7	2.2	3.8	8.0
Nigrospora oryzea	1.5	2.1	1.8	2.2	0.0	0.0	4.3

2. Greenhouse experiments:

3.8

4.3

6.0

6.7

5.2

5.9

8.3

9.3

100.0

2.1. Pathogenicity tests:

Penicillium spp.

Rhizoctonia solani

Rhizopus nigricans

Scierotium rolfsil

Total

Data presented in Table (2) indicate that among the five fungi tested for their pathogenic potentiality; *R. solani, S. rolfsii* and *M. phaseolina* were the most pathogenic ones, which caused the highest percentages of preemergence damping-off after 15 days, being 51.7, 40.7 and 40.3%, on cv.

33

3.7

9.2

4.2

4.2

4.7

11.8

5.5

100.0

3.0

4.0

9.7

3.0

5.1

6.8

16.6

5.2

100.0

14.6

17,1

36.7

20.0

Giza 5 peanut plants, respectively under greenhouse conditions. It could be easily recognized that there were significant differences between the effect of the Fusaria group and other pathogens with no significance between themselves, or between M. phaseolina and S. rolfsii. While, after 30 days from planting, M. phaseolina recorded the highest percentage of postemergence damping-off (50.7%), followed by R. solani (43.3%), F. solani (42.3%), F. oxysporum (31.7%) and S. rolfsii was the least effective when recorded 29.0%. All obtained data show significant differences between treatments and when comparing any treatment with the control. Results of the same table also show that after 60 days from planting, pathogenic fungi caused wilt were F. oxysporum (41.3%) followed by F. solani and S. rolfsii (21.0%, for each), with clear significant difference between the first pathogen and both of the second and the third ones. Also, results of all treatments significantly differed from the control treatment. On the other hand, R. solani and M. phaseolina gave similar level of root rot symptoms (66.6 and 64.3%, respectively) with significant differences with the following fungus S. rolfsii (38.3%) or with the control treatment (0.33%).

Regarding the results of survived plants, pathogens could be classified to three categories according to their dangerous effects, include *R. solani* and *M. phaseolina*, which significantly decreased healthy survival percentage from 98.3% for the control treatment to 10.0 and 11.3%, respectively. *Fusarium solani* and *S. rolfsii* could be considered as the second category (medium dangerous effect), which resulted 28.3 and 29.3% of healthy survival plants with insignificant difference. Although, *F. oxysporum* is usually considered as a destructive pathogen, but the obtained results in the present study categorized this pathogen as the least effective one since there was a percent of 48.3 of sown peanut seeds were able to escape from its pathogenic potentiality.

Table (2): Pathogenicity test of 5 major pathogenic fungl isolated from

	Days from planting								
	15	30	60						
Fungi tested	% Pre- emergence damping-off	% Post- emergence damping-off	% of wilt	% of root rot	% of healthy survivals				
Fusarium oxysporum	27.7	31.7	41.3	0.0	48.3				
F. solani	30.0	42.3	21.0	0.0	28.3				
Macrophomina phaseolina	40.3	50.7	0.0	64.3	11.3				
Rhizoctonia solani	51.7	43 3	0.0	66.6	10.0				
Sclerotium rolfsii	40.7	29.0	21.0	38.3	29.3				
Control	0.0	0.33	0.0	0.33	98.3				
L.S.D. at 5%	4.1	5.81	3.54	6.28	6.45				

2.2. Effect of biological seed treatments under greenhouse conditions:

Results obtained from the greenhouse experiments – presented in Table (3) – indicate that the tested bio-control agents; *B. subtilis* (B.) and *T. harzianum* (T.) proved to be successful mean of biological control against wilt and root rot causal pathogens, when used as peanut seed treatment. Percentages of pre-, post-emergence damping-off and root rot obtained from the biologically treated seeds - using the bacterial or the fungal agent - were significantly reduced when compared with the untreated seeds (control treatment). Peanut seeds which were coated with any of cell or spore suspensions of *B. subtilis* or *T. harzianum* and sown in soil infested with one of the seed-borne pathogens; *S. rolfsii* (S.r.), *F. oxysporum* (F.o.) or *M. phaseolina* (M.p.). Data in Table (3) indicate that, the two biological agents (B. & T.) significantly decreased the percentage of pre- and post-emergence damping-off and percentage of wilt and/or root rot. Also, significant increases in the percentages of the survived plants were noticed when compared with plants grown in infested soils with one of the three seed-borne pathogens.

Table (3): Effect of seed treatments with the biocontrol agents Bacillus subtilis (B.) and Trichoderma harzlanum (T.) on controlling damping-off and will caused by some seed-borne pathogens

Treatments	% pre- emergence damping-off			% post- emergence damping-off			% of wilt or root rot symptoms			% of healthy survivals		
	S.r.	F.o.	M.p.	S.r.	F.o.	M.p.	S.r.	F.o.	M.p.	S.r.	F.o.	M.p.
Seeds sown in infested soil with the pathogen	60.3	45.0	32.3	30.0	20.7	25.7	64.3	65,7	75.0	9.7	34.3	42.0
Seeds treated with (B.) and sown In infested soil		17.7	16.3	15.3	9.3	10.7	12.3	16.0	15.7	53.7	71.0	73.0
Seeds treated with (T.) and sown in infested soll		20.7	20.7	19.3	16.0	12.3	20.7	22.3	22.3	55.7	63.3	67.0
Seeds treated with (B.) and sown in sterillzed soil		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
Seeds treated with (T.) and sown in sterilized soil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
Untreated seeds sown In sterilized soll		0.0	0.0	1.0	0.7	1.0	0.0	1.0	1.0	97.3	99.3	99.0
L.S.D. at 5%	8.28	4.38	1.29	9.82	1.40	3.91	3.62	3.90	4.54	4.90	6.37	2.01

Data in the same table show that, seed treatment with any of the biocontrol agent and sown in sterilized soil proved to be quite effective in pre- and post-emergence damping-off, while the percentage of healthy survived plants was 100%. No sign of infection was seen on plants grown from seeds pretreated with any of the bio-control agents and sown in sterilized soil. These obtained data proved that biological seed treatments with one of the tested biocontrol agents would be practically enough for controlling peanut seed-borne pathogens, if seeds were the only source of inoculum. This was also confirmed when untreated seeds (naturally infected) were sown in sterilized soil, which gave very low percentage of pre-, post-emergence damping-off or even wilt or root rot symptoms (< 2.0%). On the same time, the healthy survival percent of peanut plants resulted from this treatment was excellent (> 97.0%) with no significant differences compared to both treatments of biologically treated seeds and sown in sterilized soils which gave 100.0% of healthy survivals. Similar results were obtained by Saleh (1997) when greenhouse experiment was conducted, where *B. subtilis* significantly decreased the incidence of root rot and wilt symptoms of groundnuts caused by *M. phaseolina, R. solani* and *F. compactum,* when it was applied on the day of sowing to soil infested one week earlier with the mentioned pathogenic fungi.

2.3. Transmission of pathogenic fungi from seed to adult plant:

Data presented in Table (4) indicate that no seed infection with any of the three concerned pathogens were recorded when one of the tested biocontrol agents (B. or T.) was used side by side with soil sterilization. Such results would strongly recommend sterilizing soil, which could be achieved by getting the benefits of the cheap and safe solar energy, especially under Egyptian sunny weather. Similar recommendations were reported by Katan (1980); Ei-Shami et al. (1990); Kalomoira and Tjamos (1992) and Hilal et al. (2002).

Table (4): Incidence percentages and frequencies of major seed-borne pathogens isolated from yield of seed and shell parts resulted from different treatments grown under greenhouse conditions.

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7	Yleld		otium fsii		rlum orum	Macrophomina phaseolina		
Treatments	part	%	%	%	%	%	%	
		Incidence	frequency	Incidence	frequency	incidence	frequency	
Untreated seeds sown	Seeds	10.0	43.5	25,0	78.9	15.0	39.5	
in infested soll with the pathogen	Shells	35.0	43.8	40.0	42.4	30.0	46.2	
Seeds treated with (B.)	Seeds	5.0	21.7	0.0	0.0	8.0	21.1	
and sown in infested soli	Shells	20.0	25.0	16.0	17.0	10.0	15.4	
Seeds treated with (T.)	Seeds	8.0	34.8	0.0	0.0	12.0	31.6	
and sown iл Infested soil	Shells	25.0	31.3	13 3	14.1	20.0	30.8	
Seeds treated with (B.)	Seeds	0.0	0.0	0.0	0.0	0.0	0,0	
and sown in sterilized soil	Shells	0,0	0.0	0.0	0.0	0.0	0.0	
Seeds treated with (T.)	Seeds	0.0	0.0	0.0	0.0	0.0	0.0	
and sown in sterilized soil	Shells	0.0	0.0	0.0	0.0	0.0	0.0	
Untreated seeds sown	Seeds	0.0	0.0	6.7	21.1	3.0	7.9	
in sterilized soll	Shells	0,0	0.0	25.0	26.5	5.0	7.7	

Data in Table (4) also reveal that incidence percentages of shell infections were always higher than seed infections. Fusarium oxysporum recorded the highest infection percentage when untreated seeds sown in infested soil whether on shells (40.0%) or on seeds (25.0%). On the other hand, the same pathogen recorded also the highest infection percentage on both shells and seeds when untreated seeds sown in sterilized soil being 25.0 and 6.7%, respectively.

It could be concluded from the data in Table (4) that sowing untreated seeds in infested soil would result – with no doubt – in high seed infection, levels, while sowing untreated seeds in sterilized soil may positively reduce the presence of seed-borne mycoflora. This led us to throw the light on the necessity of applying the integrated pest management since no single control measurement will be sufficient for controlling plant pathogens in soil or on seeds.

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مقاومة الفطريات الهامة المحمولة على بذور الفول السوداني باستخدام المعلملات لبيولوجية للتقاوي

مجدى إبراهيم غنيم ، إبراهيم حافظ العباسى وعبد الفتاح عبد الحميد الوكيل قسم بحوث أمراض البذور – معهد بحوث أمراض النباتات– مركز البحوث الزراعية– الجيزة– مصر

تم حصر الفطريات المحمولة على تقاوى الفول السوداني صنف جيزة ٥ في شــلاث منساطق البتاج رئيسية في مصر . حيث تم تعريف ١٨ فطرا باجراء اختبارات سلامة البنور على عينات البـــنور الني تم جمعها من محافظات الشرقية ، الإسماعيلية وشمال سيناء. وباختبار الفئرة المرضية الفطريـــات اللهامة المعزولة من البنور بعد ٢٠ ، ٢٠ ، ٢٠ يوم من الزراعة تحت ظروف الصوبة ، وجد أن الفطــو ريز وكتونيا سولاني كان اخطرهم تأثيرا على نسبة النياتات الباقية ثم تبعه كل من الفطريات ماكروفومينا فاسيولينا ، فيوز اريوم الوكسيسبورم القلــه فلميولينا ، فيوز اريوم الوكسيسبورم القلــه خطورة في هذا المجال.

فى تجارب الصوبة ، تم اختبار كلا من الفطر تريكودرما هارزياتم و البكتيريا باسلس سسلتلس لمكافحة كل من الفطريات سكليروشيم روافساى ، فيوز اريوم لوكسيسببورم و ماكروفومينا فاسبولينا كمعاملة بنور فى وجود تربة معقمة ، حيث تم القضاء نهائيا على كل من الفطريات الثلاثة الممرضة ، بينما فى المعاملات الأخرى (بدون تربة معقمة) فقد تم تسجيل مستويات مختلفة من التأثير .

عند فحص محصول قَشْرة القرون والبنور النائجة من المعاملات المختلف التسجيل نسبب تولجد الفطريات الثلاثة الممرضة تحت الدراسة ، وجد أن القضاء على اللقاحات الفطريسة فسى التربسة عامل هام جدا لتحقيق مكافحة تامة لكافة اللقاحات المحمولة عن طريق البنرة باستخدام معاملسة البنور بالعوامل الحيوية الأمنة.