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BIOLOGICAL SEED TREATMENTS FOR CONTROLLING MAJOR PEANUT (*Arachis hypogaea* L.) SEED-BORNE FUNGI

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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 *Sclerotium 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 pre- and 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 corn-sand medium/kg soil then watered daily for one week before sowing. Pots of control treatment were filled with the same amount of sterilized corn-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-, post-emergence 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-borne inocula of *M. phaseolina, F. oxysporum* and *S. rolfsii.*

Sterilized plastic pots (25 cm in diam.) were filled 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 gms. 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* (2.8×10^8 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 6×10^3 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-Wakil 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.

Fungal isolates	Sharkyia		Ismailia		North Sinai		Total
	% 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
Penicillium spp.	3.8	5.2	3.3	4.2	3.0	5.1	14.6
Rhizoctonia solani	4.3	5.9	3.7	4.7	4.0	6.8	17.1
Rhizopus nigricans	6.0	8.3	9.2	11.8	9.7	16.6	36.7
Sclerotium rolfsii	6.7	9.3	4.2	5.5	3.0	5.2	20.0
Total		100.0		100.0		100.0	

2. Greenhouse experiments:

2.1. Pathogenicity tests:

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 pre-emergence damping-off after 15 days, being 51.7, 40.7 and 40.3%, on cv.

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 post-emergence 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 fungi isolated from peanut seeds grown under greenhouse conditions.

Fungi tested	Days from planting				
	15	30	60		
	% 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 harzianum* (T.) on controlling damping-off and wilt caused by some seed-borne pathogens on peanut plants under greenhouse conditions.

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	31.0	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 soil	25.6	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 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
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 soil	1.7	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 bio-control 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 pre-treated 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 bio-control 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); El-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.

Treatments	Yield part	*Sclerotium rolfsii*		*Fusarium oxysporum*		*Macrophomina phaseolina*	
		% incidence	% frequency	% incidence	% frequency	% incidence	% frequency
Untreated seeds sown in infested soil with the pathogen	Seeds	10.0	43.5	25.0	78.9	15.0	39.5
	Shells	35.0	43.8	40.0	42.4	30.0	46.2
Seeds treated with (B.) and sown in infested soil	Seeds	5.0	21.7	0.0	0.0	8.0	21.1
	Shells	20.0	25.0	16.0	17.0	10.0	15.4
Seeds treated with (T.) and sown in infested soil	Seeds	8.0	34.8	0.0	0.0	12.0	31.6
	Shells	25.0	31.3	13.3	14.1	20.0	30.8
Seeds treated with (B.) and sown in sterilized soil	Seeds	0.0	0.0	0.0	0.0	0.0	0.0
	Shells	0.0	0.0	0.0	0.0	0.0	0.0
Seeds treated with (T.) and sown in sterilized soil	Seeds	0.0	0.0	0.0	0.0	0.0	0.0
	Shells	0.0	0.0	0.0	0.0	0.0	0.0
Untreated seeds sown in sterilized soil	Seeds	0.0	0.0	6.7	21.1	3.0	7.9
	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 علي بذور الفول السوداني باستخدام المعاملات لبيولوجية للتقاوى

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قسم بحوث أمراض البذور- معهد بحوث أمراض النباتات- مركز البحوث الزراعية- الجيزة- مصر

تم حصر الفطريات المحمولة على تقاوى الفول السوداني صنف جيزة ٥ فى ثلاث مناطق إنتاج رئيسية فى مصر. حيث تم تعريف ١٨ فطرا بإجراء اختبارات سلامة البذور على عينات البذور التى تم جمعها من محافظات الشرقية ، الإسماعيلية وشمال سيناء. وباختبار القدرة المرضية للفطريات الهامة المعزولة من البذور بعد ١٥ ، ٣٠ ، ٦٠ يوم من الزراعة تحت ظروف الصوبة ، وجد أن الفطر ريزوكتونيا سولانى كان أخطرهم تأثيرا على نسبة النباتات الباقية ثم تبعه كل من الفطريات ماكروفومينا فاسيولينا ، فيوزاريوم سولانى ثم الفطر سكليروشيم رولفساى. وكان الفطر فيوزاريوم أوكسيسبورم أقلهم خطورة فى هذا المجال.

فى تجارب الصوبة ، تم إختبار كلا من الفطر تريكودرما هارزيانم و البكتيريا باسلس سبتلس لمكافحة كل من الفطريات سكليروشيم رولفساى ، فيوزاريوم أوكسيسبورم و ماكروفومينا فاسيولينا كمعاملة بذور 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 العشرة القرون والبذور الناتجة من المعاملات المختلفة لتسجيل نسب تواجد الفطريات الثلاثة الممرضة تحت الدراسة ، وجد أن القضاء على اللقاحات الفطرية فى التربة عامل هام جدا لتحقيق مكافحة تامة لكافة اللقاحات المحمولة عن طريق البذرة باستخدام معاملة البذور بالعوامل الحيوية الأمنة.