

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Seed-Borne Fungi of Peanut in Egypt: Pathogenicity and Transmission

Mohamed A. Elwakil and Mohamed A. El-Metwally
Plant Pathology Department, Faculty of Agriculture,
Mansoura University, El-Mansoura, Egypt, 35516

Abstract: Fifty seed samples of peanut seed collected from the commercial markets of Egypt were tested for the presence of seed-borne fungi. Twenty-seven fungal species were isolated and identified. Use of the blotter seed health testing method resulted in the isolation of a larger number of seed-borne fungi than the deep freezing method. The deep freezing method was more effective for the isolation of *Aspergillus nidulans*, *A. versicolor* and *A. carneus*, while the blotter method was more effective for the isolation of *Alternaria alternate*, *Mucor* sp., *Chaetomium* sp. and *Stemphylium* sp. Isolation of *Cephalosporium* sp. was the first record of the fungus on peanut seeds in Egypt. No previous reports of this fungus on peanut were found.

Pathogenicity tests showed that *Sclerotium bataticola* had a significant effect on seed emergence, followed by *Fusarium solani*, in seed inoculation experiments. *Rhizoctonia solani* showed a highly significant effect, followed by *Sclerotium bataticola*, in soil infestation treatments of Giza 4 and Giza 5 cultivars.

Transmission of *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Cephalosporium* sp. and *Verticillium* sp. from seed to mature plant of peanut showed that their translocation gradually decreased from the terminal part of the root towards the upper portions of the plants. The five tested fungi were reisolated from all plant portions in Giza 4 and Giza 5 cultivars, except *Verticillium* sp. while several attempts failed to reisolate it from the middle and upper parts of the stem or shoot tips.

The present investigation studied the detect, identify and survey the seed-borne fungi of local and introduced seeds in Egypt, study the nature of isolated fungi and their effect on peanut plants and elucidate the transmission of some pathogen fungi from seed to mature plant.

Key words: Peanut, seed-borne fungi, pathogenicity, transmission

Introduction

Peanut seeds and seedlings are highly susceptible to several pathogens. Peanut pod rot is a worldwide soil-borne disease and a limiting factor in peanut production. Infected pods show various degrees of discoloration, from superficial russeting to complete blackening of the hulls, plus various stages of hull and kernel decay. Pegs can be infected and the junction between peg and pod is weakened to an extent that substantial loss of pods occurs at digging. Several authors isolated the following fungi from peanut pods, shells and seeds: *Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp., *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp., *Trichothecium*, *Macrophomina phaseolina*, *Alternaria* spp., *Botrytis cinerea*, *Helminthosporium* spp., *Mucor* spp., *Curvularia* sp., *Cladosporium* sp., *Botryodiplodia theobromae*, *Chaetomium* sp. (Abou-Talib, 1970; El-Khadem *et al.*, 1974; El-Akkad, 1982; Porter *et al.*, 1990; Richardson, 1990; Baird *et al.*, 1993; Dharmaputra and Retnowati, 1996; Shim *et al.*, 1996).

Numerous *Sclerotium* species attack peanut plants, causing a considerable loss in yield. *S. rolfsii* and *S. bataticola* have been isolated from peanut in Egypt (El-Khadem *et al.*, 1974; El-Akkad, 1982; Porter *et al.*, 1990; Hollowell *et al.*, 1998). The floury rot disease, caused by *Verticillium* sp., attacks peanut pods at a later stage of maturity and has been isolated from seeds (Frezzi, 1965; Mathur *et al.*, 1975; Melouk *et al.*, 1983). *Rhizoctonia solani* is the most prevalent fungus associated with mature sound or rotted pods, and is the major causal organism of seedling damping off (Abou-Talib, 1970; Tawfik 1975; El-Khadem *et al.*, 1974; El-Akkad, 1982; Porter *et al.*, 1990; Richardson, 1990; Baird *et al.*, 1993; Hollowell *et al.*, 1998). *Fusarium* spp. are predominant in the soil, rhizosphere, and fresh harvested kernels and are also common in stored kernels (Abou-Talib, 1970; Tawfik, 1975; El-Khadem *et al.*, 1974; El-Akkad, 1982; Porter *et al.*, 1990; Richardson,

1990; Baird *et al.*, 1993; Dharmaputra and Retnowati, 1996).

Comparative seed health testing methods for the detection of seed-borne pathogens were studied by several investigators. Mathur *et al.* (1975) found that the deep freezing method was more suitable for the detection of *Fusarium* spp. in sorghum seeds. Khan *et al.* (1988) found that blotter and agar plate methods were more suitable for the detection of *Fusarium* spp. in rice seeds. Similar results have been observed by Dawar (1994) who reported that the blotter technique yielded significantly higher numbers of fungi than the agar plate and deep freezing methods with sunflower seeds. Elwakil and Ghoneem (1999a,b) reported that the blotter seed health testing method proved to be more effective than the deep freezing method in detecting large numbers of seed-borne fungi from both blonde psyllium and black cumin seeds.

Hashmi (1988) studied the transmission of *Fusarium moniliforme* and *F. oxysporum* in capsicum and *F. solani* in coriander from seed to plant. He was able to isolate *F. moniliforme* from the root, the lower and the upper hypocotyl, the cotyledonary leaf and the seed coat. He showed necrotic lesions on the lower stem and roots and deformation of primary and secondary roots, which confirmed the pathogenic nature of *F. oxysporum*. He also reported that *F. solani* was seed transmitted. Elwakil *et al.* (1989) reported that *Phialophora gregata* (*Cephalosporium gregata*) can be seed-transmitted in soybean. Khan and Fakir (1992) reported that the occurrence of *Fusarium* spp. infections in seeds at different stages of development increased with increasing seed age in jute seeds. Heppner and Heitefuss (1995) reported that *Verticillium dahliae* could be isolated only from the pericarp and not from the embryo in rape. Santos *et al.* (1996) reported that *F. oxysporum* were transmitted by seed in bean. Elwakil and Ghoneem (1999a, b) reported that the translocation of all

El-Wakil and El-Metwally: Seed-Borne fungi of peanut in Egypt

the tested fungi gradually decreased from the root towards the upper portions of the plants. *F. moniliforme*, *F. oxysporum* and *F. solani* were isolated from the root, hypocotyl and lower stem of blonde psyllium, while *F. moniliforme*, *F. oxysporum* and *Verticillium* sp. were restricted to the root and lower portion of the stem. *F. solani* was the only fungus isolated from the root of black cumin.

Materials and Methods

Sources of seed samples: Fifty seed samples of mixed cultivars of peanut were collected from commercial markets in different parts of Egypt, including Ismailia, Sharkia and Nobarria, during 1996, 1997 and 1998.

Seed Health Testing: Seed health testing (SHT) for seed-borne fungi was carried out following the Rules of International Seed Testing Association (ISTA, 1993). Standard blotter and deep freezing methods were selected for this study. Aliquots of 200 seeds were randomly taken from each sample for SHT.

Blotter method: Replicates of ten seeds were plated in 11 cm diameter Petri-dishes containing three layers of water-soaked blotters. The plates were incubated at $20 \pm 2^\circ\text{C}$ for 7 days under 12 hour alternating cycles of cool white fluorescent light and darkness.

Deep freezing method: Plated seeds (as described above) were incubated at 20°C for 24 hours and transferred to a freezer at -20°C for 24 hours. This was followed by 7 days incubation at $20 \pm 2^\circ\text{C}$ under 12 hour alternating cycles of cool white fluorescent light and darkness. Seeds were then ready for examination under a stereoscopic binocular microscope (6-50X) for the presence and identification of seed-borne fungi. A compound microscope was used as necessary to confirm fungal identity by examining the morphology of the conidia and conidiophores.

In addition, fungi present on infested seeds were identified using the fungus description sheets of the Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications and information from Booth (1985), Burrges *et al.* (1988), Chidambaram *et al.* (1973), Ellis (1971), Raper and Fennell (1965) and Singh *et al.* (1991).

Maintaining pure cultures of the isolated seed-borne fungi: Hyphal tips from the fungus colonies present on the seeds were transferred to plates containing potato dextrose agar (PDA). Heat-stretched capillary tubes were used to pick the hyphal tips. Pure cultures of the fungi were obtained and all isolates were maintained on PDA slants.

Pathogenicity tests of important seed-borne fungi on Peanut

Seed borne fungi used in the pathogenicity tests: Seven fungal species, *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Rhizoctonia solani*, *Verticillium* sp., *Cephalosporium* sp. and *Sclerotium bataticola*, were tested for their pathogenic effects on peanut seeds and seedlings.

Preparation of inocula: The previously isolated pure cultures were used to prepare inocula for seeds. Sub-cultures of the fungi were transferred to FDA media and incubated for several days in the dark at $24 \pm 2^\circ\text{C}$. When hyphal growth reached 3 cm diameter, a 0.5 cm disk was transferred to a 100 ml flask containing 50 ml potato dextrose broth media (PD) and incubated in the dark for several days at $24 \pm 2^\circ\text{C}$

until the hyphal mat covered the surface of the medium. The hyphal mats were harvested and washed with sterile distilled water. Fifty grams of fresh mat of each fungus was blended in 500 ml of sterile distilled water to produce a sticky suspension. These suspensions were then used to inoculate the seeds.

Seed inoculation: Peanut seeds from pathogen-free seed lots were disinfected in 1% sodium hypochlorite solution for five minutes, rinsed in tap water three times, and placed on sterilized tissue paper at room temperature until dry. The sterile seeds were then soaked in the fungal suspensions for 12 hours and left to dry at room temperature before sowing. The inoculated seeds were planted in plastic bags containing sterilized soil.

Conventional growing-on test: Five seed replicates of both inoculated and non-inoculated seeds were planted in plastic bags (20 cm diam.) containing autoclaved sandy soil, and allowed to grow in a greenhouse under ambient conditions during the summer season. Daily records of germination, symptoms of pre- and post-emergence damping off and stunted seedlings were made for a one month period.

Soil inoculation: Fungal inocula were prepared by growing each fungus on media consisting of ground peanut shells at $24 \pm 2^\circ\text{C}$ for 14 days. Soil in pots was infested with the fungal preparations at a rate of 1% (w/w). Pots containing soil mixed with equivalent amounts of non-infested media served as checks. Ten replicates were used per treatment. The pots were kept in a greenhouse for seven days with a daytime temperature range of 30°C to 34°C to allow the fungi to adapt before sowing seeds. The soil was moistened as necessary.

Seeds were first germinated in sandy soil and five germinated seeds of both cultivars Giza 4 and Giza 5 were sown in 20 cm plastic pots containing steamed sterilized sandy soil. Ten additional pots, prepared in the same manner but without adding the fungus (non-inoculated medium was used), served as checks.

Disease-free seeds selected from previously tested lots were surface sterilized and planted (5 seeds/pot) in both infested and non-infested soil. The pots were kept in a greenhouse under ambient conditions during the summer season. Daily observations for germination, symptoms of pre- and post-emergence damping off, and stunted seedlings were recorded. Re-isolations of the tested fungi were made from seeds and seedlings manifesting disease symptoms.

Transmission of certain pathogenic fungi from seed to mature plants: Peanut seeds were inoculated with *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Verticillium* sp. and *Cephalosporium* sp. The methods of inoculum preparation, seed inoculation, soil sterilization and planting in plastic bags were the same as those used for the pathogenicity tests. Ten replicates of plastic bags containing seeds inoculated with each fungus were prepared, with 5 seeds planted in each bag.

Seedlings that emerged from the inoculated seeds were rated and left to grow. Recovery rates of the fungi from different plant parts at intervals of 90 days were determined. At the end of the 90 days, five plants were removed from the bags, washed, disinfected and dissected under sterile conditions. The various plant parts (terminated part of the root, basal part of the root, basal part of the

EI-Wakil and EI-Metwally: Seed-Borne fungi of peanut in Egypt

stem, middle part of the stem, upper part of the stem, shoot tip and peg or gynophore) were plated on sterile moist blotters and incubated for 7-10 days at 24°C. Fungi recovered for each treatment were identified, and the transmission percentage was recorded.

Statistical analysis: Data obtained from this study were analyzed with the CoStat program (1990). One way ANOVA was carried out to test the significance between the treatments. Duncan's multiple range test was used to determine the differences among the means (Duncan, 1955).

Results

Seed Health Testing: A total of 27 fungal species belonging to 17 genera were isolated and identified. They were *Alternaria alternata* (Fr.) Keissler, *Aspergillus carneus* Blochwitz, *Aspergillus flavus* Link ex. Gray, *Aspergillus niger* Van Tieghem, *Aspergillus nidulans* (Eidam) Wint., *Aspergillus ochraceus* Wilhelm, *Aspergillus oryzae* (Ahlburg) Cohn, *Aspergillus tamarii* Kite, *Aspergillus versicolor* (Vuill) Tiraboschi, *Botryodiplodia theobromae* Pat., *Botrytis cinerea* pers.ex. pers., *Cephalosporium* sp., *Chaetomium* sp., *Cladosporium* sp., *Drechslera* sp., *Fusarium moniliforme* Sheild, *Fusarium oxysporum* Schlecht, *Fusarium semitectum* Berk & Rav, *Fusarium solani* (Mart.) Sacc., *Mucor* sp., *Penicillium* sp., *Rhizoctonia solani* Kuhn, *Rhizopus* sp., *Sclerotium bataticola* Taub., *Stemphylium* sp., *Trichothecium* sp. and *Verticillium* sp. The occurrence of each fungus was recorded in terms of percentage and range from the lowest to highest as shown in Table 1.

In a comparison of the two tested methods, the blotter method yielded a greater number of fungi than the deep-freezing method. In the blotter method, the predominant detected fungi were *Aspergillus niger*, maximum incidence of 94% and percentage ranging from 2-85% *A. flavus*, *Penicillium* sp., *Fusarium solani* and *Rhizopus* sp., maximum incidence of 92% and percentage ranging from 1-96, 1-30, 1-51 and 2-40%, respectively, *Fusarium oxysporum*, maximum incidence of 82% and percentage ranging from 1-76% and *Rhizoctonia solani*, maximum incidence of 54% and percentage ranging from 1 to 20%.

The deep-freezing method detected the following fungi: *Fusarium solani*, maximum incidence of 96% and percentage ranging from 1-45%, *Aspergillus flavus*, maximum incidence of 88% and percentage ranging from (2-75%), *Aspergillus niger*, maximum incidence of 82% and percentage ranging from 6-90%, *Penicillium* sp., maximum incidence of 78% and percentage ranging from 1-79%, *Fusarium oxysporum*, maximum incidence of 68% and percentage ranging from 1-40% and *Fusarium moniliforme*, maximum incidence of 58% and percentage ranging from 1-32%.

Pathogenicity Test of Important Seed-Borne Fungi of Peanut: Results obtained from pathogenicity tests of *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Rhizoctonia solani*, *Sclerotium bataticola*, *Cephalosporium* sp. and *Verticillium* sp. are presented in Table 2 and 3. Observations of symptoms on seeds and seedlings were made 30 days after sowing.

Symptoms recorded were pre- and post-emergence damping off and stunted seedlings. Pre-emergence damping off was observed as rotted seeds covered by mycelium and spores of the tested pathogens. Post-emergence damping off infections showed lesions on lower stems near the soil

surface and thread-like roots. Wilt was accompanied by general yellowing, discoloration of internal tissues, stunting, and shoot dryness.

Table 1: Incidence of seed-borne fungi in 50 mixed samples of peanut seeds, using blotter and deep-freezing methods

Fungus	Blotter		Deep Freezing	
	NSI*	% of detected fungi**	NSI*	% of detected fungi**
<i>Alternaria alternata</i>	9	18 (1-61)	0	0
<i>Aspergillus carneus</i>	0		1	2 (4)
<i>Aspergillus flavus</i>	46	92 (1-96)	44	88 (2-75)
<i>Aspergillus niger</i>	47	94 (2-85)	41	82 (6-90)
<i>Aspergillus nidulans</i>		0	10	20 (1-4)
<i>Aspergillus ochraceus</i>	18	36 (1-12)	23	46 (1-29)
<i>Aspergillus oryzae</i>	5	10 (1-61)	11	22 (1-13)
<i>Aspergillus tamarii</i>	1	2 (1)	14	28 (2-12)
<i>Aspergillus versicolor</i>	0		9	18 (1-5)
<i>Botryodiplodia theobromae</i>	23	46 (1-121)	18	38 (1-12)
<i>Botrytis cinerea</i>	10	20 (2-39)	1	(1-5)
<i>Cephalosporium</i> sp.	7	14 (1-41)	23	46 (1-9)
<i>Cheetomium</i> sp.	6	12 (4-61)	0	0
<i>Cladosporium</i> sp.	1	2(1)	20	40 (1-25)
<i>Drechslera</i> sp.	9	18 (1-4y)	3	6 (1-2)
<i>Fusarium moniliforme</i>	23	46 (2-131)	29	58 (1-32)
<i>Fusarium oxysporum</i>	41	82 (1-761)	34	68 (1-40)
<i>Fusarium semitectum</i>	10	20 (1-71)	5	10 (1-4)
<i>Fusarium solani</i>	46	92 (1-51y)	48	96 (1-45)
<i>Mucor</i> sp.	10	20 (1-61)	0	
<i>Penicillium</i> sp.	46	92 (1-301)	39	78 (1-79)
<i>Rhizoctonia solani</i>	27	5 (2-201)	2	4 (3-6)
<i>Rhizopus</i> sp.	46	92 (2-401)	10	20 (1-5)
<i>Scierotium bataticola</i>	23	46 (1-81)	21	42 (1-6)
<i>Stemphylium</i> sp.	1	2111	0	0
<i>Trichothecium</i> sp.	9	18 (1-61)	15	30 (4-35)
<i>Verticillium</i> sp.	8	16 (1-4)	18	36 (1-12)

*NSI: No. of samples infected. Numbers in parentheses indicate infection range. **% of detected fungi: (NSI/Total No. of samples (50)) × 100

Seed inoculation treatments: Data in Table 2 illustrate the percentages of infection expressed as symptoms of pre- and post-emergence damping off and stunted seedlings.

Syndrome of *Fusarium moniliforme*: *Fusarium moniliforme* had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 8, 8 and 48%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 4, 4 and 40%, respectively, in the Giza 5 cultivar. The percentage of normal seedlings was 36% in Giza 4 and 52% in Giza 5.

Syndrome of *Fusarium oxysporum*: *Fusarium oxysporum* had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 8, 8 and 56%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 4, 8 and 72%, respectively, in the Giza 5 cultivar. The percentage of normal seedlings was 28% in Giza 4 and 16% in Giza 5.

Syndrome of *Fusarium solani*: *Fusarium solani* had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 28, 12 and 28%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 24, 8 and 16%, respectively, in the Giza 5 cultivar. The percentage of normal seedlings was 32% in Giza 4 and 52% in Giza 5.

Syndrome of *Rhizoctonia solani*: *Rhizoctonia solani* had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 4, 4 and 36%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 32, 0 and 16%, respectively, in

El-Wakil and El-Metwally: Seed-Borne fungi of peanut in Egypt

Table 2: Symptoms of tested fungi on inoculated peanut seeds

Pathogen	Pre-emergence damping-off		Post-emergence damping-off		Stunted seedling		Normal seedling	
	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5
Check	4d	4d	0b	0a	0e	0e	96a	96a
<i>Cephalosporium</i> sp.	2013-d	10a-c	12a	8a	40bc	44bc	28b-d	20cd
<i>Fusarium moniliforme</i>	8cd	4d	8a	4a	48bc	40bc	36b-d	52b
<i>Fusarium oxysporum</i>	8cd	4d	8a	8a	56ab	72a	28b-d	16cd
<i>Fusarium solani</i>	28a-c	24b-d	12a	8a	28cd	1fide	32b-d	52b
<i>Rhizoctonia solani</i>	4d	32ab	4a	0a	36b-d	16de	56b	52b
<i>Sclerotium bataticola</i>	32ab	44a	8a	4a	44bc	40bc	16cd	12d
<i>Verticillium</i> sp.	4d	8cd	8a	4a	48bc	44bc	40cd	44bc

Table 3: Symptoms of tested fungi on peanut seeds and seedlings grown in infested soil

Pathogen	Pre-emergence damping-off		Post-emergence damping-off		Stunted seedling		Normal seedling	
	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5
Check	0f	4ef	0b	0b	0g	0g	100a	96a
<i>Cephalosporium</i> sp.	24c-f	28c-f	16ab	28a	28a-d	24b-e	32d-f	20d-g
<i>Fusarium moniliforme</i>	28c-f	24c-f	1 2ab	4b	44a	36a-c	16d-g	36de
<i>Fusarium oxysporum</i>	16d-f	3fcd	0b	8b	44a	36a-c	40od	20d-g
<i>Fusarium solani</i>	16d-f	12d-f	8b	16ab	16d-g	8e-g	60bc	64b
<i>Rhizoctonia solani</i>	92a	76ab	8b	0b	4fg	12d-g	4g	12e-g
<i>Sclerotium bataticola</i>	52bc	64b	4b	16ab	36a-c	20c-f	8fg	0g
<i>Verticillium</i> sp.	32c-e	28c-f	4b	8h	28a-d	40ab	36de	24d-g

Table 4: Recovery of fungi from plant parts 90 days after sowing artificially inoculated peanut seeds

Portions of plant	Pathogen									
	<i>Fusarium solani</i>		<i>Fusarium oxysporum</i>		<i>Fusarium moniliforme</i>		<i>Cephalosporium</i> sp.		<i>Verticillium</i> sp.	
	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5	Giza 4	Giza 5
Terminal part of the root	100	100	80	80	100	100	100	100	100	80
Basal part of the root	100	100	80	80	100	100	100	100	100	80
Basal part of the stem	100	100	60	80	100	100	60	80	40	20
Middle part of the stem	100	80	60	40	80	60	40	20	0	0
Upper part of the stem	100	80	20	40	80	60	40	20	0	0
Shoot tip	80	80	20	40	80	60	40	20	0	0
Peg (Gynophore)	100	80	40	60	80	60	40	20	20	0

the Giza 5 cultivar. The percentage of normal seedlings was 56% in Giza 4 and 52% in Giza 5.

Syndrome of *Sclerotium bataticola*: *Sclerotium bataticola* had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 32, 8 and 44%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 44, 4 and 40%, respectively, in the Giza 5 cultivar. The percentage of normal seedlings was 16% in Giza 4 and 12% in Giza 5.

Syndrome of *Cephalosporium* sp.: *Cephalosporium* sp. had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 20, 12 and 40%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 10, 8 and 44%, respectively, in the Giza 5 cultivar. The percentage of normal seedlings was 28% in Giza 4 and 20% in Giza 5.

Syndrome of *Verticillium* sp.: *Verticillium* sp. had symptoms of pre- and post-emergence damping off and stunted seedlings at percentages of 4, 8 and 48%, respectively, on seeds and seedlings of the Giza 4 cultivar. These percentages were 8, 4 and 44%, respectively, in the Giza 5 cultivar. The percentage of normal seedlings was 40% in Giza 4 and 44% in Giza 5.

Data presented in Table 2 show that *Sclerotium bataticola* had a highly significant effect with respect to pre-emergence damping off, followed by *F. solani* and *Cephalosporium* sp., a low significant effect was observed with

F. oxysporum and *F. moniliforme* and no significant effect with *R. solani* and *Verticillium* sp. in the Giza 4 cultivar. *Sclerotium bataticola* had a highly significant effect with respect to pre-emergence damping off, followed by *R. solani* and *F. solani*, a low significant effect was observed with *Cephalosporium* sp. and *Verticillium* sp. and no significant effect with *F. moniliforme* and *F. oxysporum* in the Giza 5 cultivar. Post-emergence damping off was non-significant in both cultivars compared to the check. The percentage of stunted seedlings was significant with all pathogenic fungi in the Giza 4 cultivar. The highest significant effect was due to *F. oxysporum*, followed by *Cephalosporium* sp., *Verticillium* sp., *F. moniliforme*, and *Sclerotium bataticola*, while the lowest significance was due to *F. solani* and *R. solani* in the Giza 5 cultivar. Percentages of normal seedlings were higher with *R. solani*, followed by *Verticillium* sp., *F. moniliforme*, *F. solani*, *F. oxysporum* and *Cephalosporium* sp. The percentages were lower with *Sclerotium bataticola* in the Giza 4 cultivar. The lowest percentage was observed with *Cephalosporium* sp., *F. oxysporum* and *Sclerotium bataticola* in Giza 5 compared to the check.

Soil infestation treatments: Data presented in Table 3 illustrate the percentages of infection due to soil infestation. The pre- and post-emergence damping off and stunted seedlings incited by *Fusarium moniliforme* reached percentages of 28, 12 and 44%, respectively, on seeds and seedlings of the Giza 4 cultivar, while it was 24, 4 and 36%, respectively, on seeds and seedlings of the Giza 5

El-Wakil and El-Metwally: Seed-Borne fungi of peanut in Egypt

cultivar. The percentage of normal seedlings was 16% in Giza 4 and 36% in Giza 5 compared with the check (100% in Giza 4 and 96% in Giza 5).

The pre- and post-emergence damping off and stunted seedlings due to soil infestation with *Fusarium oxysporum* was 16, 0 and 44%, respectively, on seeds and seedlings of the Giza 4 cultivar and was 36, 8 and 36%, respectively, on seeds and seedlings of the Giza 5 cultivar. The percentage of normal seedlings was 40% in Giza 4 and 20% in Giza 5.

Fusarium solani produced symptoms of pre- and post-emergence damping off and stunting seedlings in 16, 8 and 16%, respectively, of seeds and seedlings of the Giza 4 cultivar and 12, 16 and 8%, respectively, of seeds and seedlings of the Giza 5 cultivar. The percentage of normal seedlings was 60% in Giza 4 and 64% in Giza 5.

Rhizoctonia solani produced symptoms of pre- and post-emergence damping off and stunted seedlings in 92, 8 and 4%, respectively, of seeds and seedlings of the Giza 4 cultivar and 76, 0 and 12%, respectively, of seeds and seedlings of the Giza 5 cultivar. The percentage of normal seedlings was 4% in Giza 4 and 12% in Giza 5.

Sclerotium bataticola produced symptoms of pre- and post-emergence damping off and stunted seedlings in 52, 4, and 36%, respectively, of seeds and seedlings of the Giza 4 cultivar, and 64, 16 and 20%, respectively, of seeds and seedlings of the Giza 5 cultivar. The percentage of normal seedlings was 8% in Giza 4 and 0% in Giza 5.

Cephalosporium sp. produced symptoms of pre- and post-emergence damping off and stunted seedlings in 24, 16 and 28%, respectively, of seeds and seedlings of the Giza 4 cultivar, and 28, 28 and 24%, respectively, of seeds and seedlings of the Giza 5 cultivar. The percentage of normal seedlings was 32% in Giza 4 and 20% in Giza 5.

Verticillium sp. produced symptoms of pre- and post-emergence damping off and stunted seedlings in 32, 4 and 28%, respectively, of seeds and seedlings of the Giza 4 cultivar and 28, 8 and 40%, respectively, of seeds and seedlings of the Giza 5 cultivar. The percentage of normal seedlings was 36% in Giza 4 and 24% in Giza 5.

In conclusion, data in Table 3 show that *R. solani* had the highest percentage of infection expressed as pre-emergence damping off, followed by *Sclerotium bataticola*. The percentage of normal seedlings was the highest with *F. solani* infection, followed by *F. oxysporum*, *Verticillium* sp. and *Cephalosporium* sp. It was lowest with *F. moniliforme*, followed by *Sclerotium bataticola* and *R. solani*, in the Giza 4 cultivar. No normal seedlings were seen in the Giza 5 cultivar following soil infestation with *Sclerotium bataticola*.

Fungus transmission from seed to mature plants: Among the fungi associated with peanut seeds, five species with a long history of seed transmission, *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Cephalosporium* sp. and *Verticillium* sp. were selected for study using the growing-on test technique.

Data presented in Table 4 illustrate the degree of movement of each fungus within the plant 90 days after seed inoculation and sowing. The tested fungi were isolated from all portions of the roots and stems in both cultivars, except *Verticillium* sp., which was isolated only from terminal and basal parts of the root and stem and from pegs (gynophores), but not from middle and upper parts of the stem or the shoot tip in Giza 4. It was isolated from the terminal and basal parts of the root and basal part of the

stem, but not from the middle and upper parts of the stem, shoot tips, or pegs (gynophores) in Giza 5. The degree of translocation gradually decreased from the terminal parts of the root towards the upper portions of the plant.

The percentage of recovery of *F. solani* in Giza 4 was 100% in all portions of the plant except shoot tips, which were 80%. It was 100% in the terminal and basal parts of the root, basal part of the stem and 80% in the middle and upper parts of the stem, shoot tips and pegs (gynophores) in Giza 5.

The percentage of recovery of *F. oxysporum* was 80% in the terminal and basal parts of the root, 60% in the basal and middle parts of the stem, 40% in the pegs (gynophores) and 20% in the upper part of the stem and shoot tips in Giza 4. It was 80% in the terminal and basal parts of the root and stem, 60% in the pegs and 40% in the middle and upper parts of the stem and shoot tips in Giza 5.

Recovery of *F. moniliforme* was 100% in the terminal and basal parts of the root and stem in both cultivars. The recovery percentage of *Cephalosporium* sp. was 100% in the terminal and basal parts of the root in both cultivars, 60% in the middle part of the stem in Giza 4, 80% in Giza 5, and 40% in the other portions of Giza 4, 20% in Giza 5. The recovery percentage of *Verticillium* sp. was 100% in the terminal and basal parts of the root, 40% in the lower parts of the stem, and 20% in the pegs (gynophores). The fungus was not recovered from other plant parts in Giza 4. It was isolated at a percentage of 80% from the terminal and basal parts of the roots and 20% of the lower parts of the stem, while there was no recovery from other plant parts in Giza 5.

Discussion

Intensive seed health testing showed that the type and number of fungi recovered from peanut seed varied depending on the method used. A larger number of seed-borne fungi were recovered with the blotter method than with the deep freezing method. It may be more advantageous to use the blotter method for routine seed-health testing of peanut.

Cephalosporium sp. was recovered for the first time from peanut seeds. No other record of this fungus on peanut was found in an extensive literature search.

Sclerotium bataticola had a severe effect on pre-emergence damping off with soil infestation and less effect when seeds were inoculated (52 and 32% respectively in Giza 4 and 64 and 44% in Giza 5). The same trend was found with the post-emergence damping off. *Rhizoctonia solani* caused extensive damage with soil infestation and low damage on seedlings raised from inoculated seed. However, variation might be due to different fungal requirements for nutrients/growth factors found in soil. The different soil compounds might enhance fungal activity, leading to variable effects on the infection process. These observations help to identify conditions under which each fungus could potentially be most infective on peanut plants. A new study to investigate this aspect will be undertaken. One of the objectives of this study was to determine the ability of some important seed-borne fungi of peanut to move from seed to different plant parts. In common with earlier work on other crops, it was observed that the recovery of the five tested fungi, *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Cephalosporium* sp. and *Verticillium* sp., gradually decreased from the terminal parts of the root toward the above ground portions of the plant.

El-Wakil and El-Metwally: Seed-Borne fungi of peanut in Egypt

Despite the lower level of recovery of the fungi from the upper portions of the plant, it is still evidence of the mechanism whereby the tested fungi are transmitted from seed to seed. It also leaves no doubt that one of the most important aspects of seed quality is freedom from seed-borne pathogens.

An analogous situation occurred when soybean was raised from *Cephalosporium gregata* (*Phialophora gregata*) infected seeds (El-Wakil *et al.*, 1989).

The results of this study may provide valuable information for growers and extension specialists to use in establishing programs to control seed-borne diseases of peanut. They also substantiate the need for a management program to control seed-borne diseases and reduce their impact on peanut production in Egypt. It has also been noted that the isolated seed-borne fungi did not significantly affect seed germination, resulting in the production of seeds of poor quality, including seed contaminated with mycotoxin-producing fungi.

Acknowledgments

The authors thank Dr. Conrad J. Krass, Primary State Plant Pathologist, California Department of Food and Agriculture, Sacramento, CA, USA, for critical review of the manuscript.

References

- Abou-Talib, E.M.E., 1970. Studies on groundnut diseases in UAR. M.Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.
- Baird, R.E., T.B. Breneman, B.G. Mullinix, D.K. Bell, A.K. Culbreath and J.D. Moore, 1993. The effects of chemical treatment, harvest date and specific isolation media on the peanut shell mycobiota of two peanut cultivars. *Plant Dis.*, 77: 736-741.
- Booth, C., 1985. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK., pp: 237.
- Burrages, L.W., C.M. Liddell and B.A. Summerell, 1988. Laboratory Manual for *Fusarium* Research: Incorporating a Key and Descriptions of Common Species Found in Australia. 2nd Edn., *Fusarium* Research Laboratory, Department of Plant Pathology and Agricultural Entomology, University of Sydney, Sydney, Australia, ISBN-13: 9780949269560, Pages: 156.
- Chidambaram, P., S.B. Mathur and P. Neergaard, 1973. Identification of seed-borne *Drehslera* species. *Friesia*, 10: 165-207.
- Dawar, S., 1994. Studies of some seed-borne fungi associated with sunflower. Ph.D. Thesis, Department of Botany, University of Karachi, Pakistan, pp: 213.
- Dharmaputra, O.S. and I. Retnowati, 1996. Fungi isolated from groundnuts in some locations of West Java. *Biotropia*, 9: 15-25.
- Duncan, D.B., 1955. Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- El-Akkad, S.A.F., 1982. Pathological studies on pod rot diseases of peanut. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt.
- El-Khadem, M., K.H. Naguib and A. Ghani, 1974. Aflatoxins in food-stuff in Egypt: I. Peanut microflora and toxicity, *Mikrobiologica* II. Ph.D. Thesis, Tawfik,
- Ellis, M.B., 1971. *Dematiaceous Hyphomycetes*. 1st Edn., Commonwealth Mycological Institute, Kew, Surrey, UK., ISBN-13: 978-0851986180, Pages: 608.
- Elwakil, M.A. and K.M. Ghoneem, 1999a. Detection and location of seed-borne fungi of black cumin and their transmission in seedlings. *Pak. J. Biol. Sci.*, 2: 559-564.
- Elwakil, M.A. and K.M. Ghoneem, 1999b. Detection and location of seed-borne fungi of blonde psyllium and their transmission in seedlings. *Pak. J. Biol. Sci.*, 2: 38-44.
- Elwakil, M.A., S.B. Mathur and E.A. Fayzalla, 1989. World wide distribution of *phialophora gregata* of soybean with special reference to perpetuation and seed transmission ability. Proceedings of the 22nd International Seed Testing Congress, June 21-30, 1989, Edinburgh, Scotland.
- Frezzi, M.J., 1965. Floury rot of groundnut pods at Manfredi (Cordoba) caused by *Verticillium* sp. *Revta. Investens Agrop. Ser.*, 5: 5-12.
- Hashmi, M.H., 1988. Seed-borne mycoflora of some spices detection techniques and pathogenicity. Ph.D. Thesis, Department of Botany, University of Karachi, Pakistan, pp: 164. <http://eprints.hec.gov.pk/1232/1/951.html.htm>.
- Hepner, C. and R. Heitefuss, 1995. Investigation on the occurrence of *Verticillium* sp. Kelb and other fungi on seeds of oilseed rape (*Brassica napus* L. var. *Oleifera metzgar*). *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, 47: 57-61.
- Hollowell, J.E., B.B. Shew, M.K. Beute and Z.G. Abad, 1998. Occurrence of pod rot pathogens in peanuts grown in North Carolina. *Plant Dis.*, 82: 1345-1349.
- ISTA., 1993. International rules for seed testing. *Seed Sci. Technol.*, 21: 1-288.
- Khan, A.A. and G.A. Fakir, 1992. Occurrence of seed-borne fungal pathogens during development and maturation of jute seed. *Ann. Bangladesh Agric.*, 2: 83-86.
- Khan, S.A.J., A.K. Khanzada, N. Sultana and M. Aslam, 1988. Evaluation of seed health testing techniques for the assessment of seed borne mycoflora of rice. *Pak. J. Agric. Res.*, 9: 502-505.
- Mathur, S.K., S.B. Mathur and P. Neergaard, 1975. Detection of seed-borne fungi in sorghum and location of *Fusarium moniliforme* in seed. *Seed Sci. Technol.*, 3: 683-690.
- Melouk, H.A., D.F. Wadsworth and J.L. Sherwood, 1983. Effect of *Verticillium wilt* on root and tap weight of peanut cultivar Tamnut 74. *Plant Dis.*, 67: 1349-1350.
- Porter, D.M., D.H. Smith and R. Rodriguez-Kabana, 1990. Compendium of Peanut Diseases. American Phytopathological Society (APS), St. Paul, MN., USA.
- Raper, K.B. and D.I. Fennell, 1965. The Genus *Aspergillus*. Williams and Wilkins Co., Baltimore, Maryland, Pages: 686.
- Richardson, M.J., 1990. An Annotated List of Seed-Borne Diseases. 4th Edn., The International Seed Testing Association, Switzerland.
- Santos, G.R.D., H. Costa, J.M. Peluzio and G.V. Miranda, 1996. Transport, transmission and pathogenicity of bean (*Phaseolus vulgaris* L.) seed-borne fungi. *R. Ceres*, 48: 621-627.
- Shim, H., D. Lee, G. Lee, H. Yu-Seung and H. Shim *et al.*, 1996. Fungi associated with seed and seedling diseases of peanut. *RDA J. Agric. Sci. Crop Prot.*, 38: 507-515.
- Singh, K., J.C. Frisvad, U. Thrane and S.B. Mathur, 1991. An Illustrated Manual on Identification of Some Seed-Borne *Aspergilli*, *Fusaria*, *Penicillia* and their Mycotoxins. Danish Government Institute for Seed Pathology for Developing Countries, Hellerup, Denmark, ISBN-13: 9788770263177, Pages: 133.
- Tawfik, F.H., 1975. Studies on fungi associated with groundnut (*Arachis hypogea* L.) and rice (*Oryza sativa* L.) seeds. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt