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Pathogenic and/or Saprophytic Fungi of Wilted Peanut Seedling and Their Role in Pathogenicity of Peanut Seeds

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Abstract: The gross fungal counts of peanut seedling (20 samples) on glucose- (264 colonies) was higher than on cellulose- (214 colonies) Czapek's agar. The average number of fungal colonies per sample was higher on glucose (5-23 colonies) compared with cellulose (4-17 colonies) medium with 93% and 84.5% infected seedlings by pathogenic and/or saprophytic fungi listed on the two isolation media, respectively. A total of 11 species of 6 genera were isolated and identified from the wilted seedling samples on glucose- and cellulose- (6 & 10 species of 4 & 6 genera, respectively) Czapek' s agar at 18 ± 1 ^{0}C. Three genera (*Aspergillus*, *Fusarium* and *Gibberella*) had the highest counts on glucose (52.2%, 29.2% & 10.2% of gross fungal count, respectively) and cellulose (59.8%, 18.6% & 6%, respectively) media and frequencies (50-100% and 30-95% of the samples, respectively). *A. flavus* had the highest counts and frequencies followed by *F. oxysporum* and *G. fujikuroi*. Two species (*A. niger* and *Penicillium funiculosum*) of two genera were detected on the two media in less counts (collectively, 13.6% and 9.8%) and frequencies (25-30%, respectively). Six species of 4 genera were isolated and identified on only one medium, where *P. chrysogenum* appeared only on glucose whereas, *A. fumigatus*, *A. terreus*, *P. coryophilum*, *Nectria haematococca* and *Emericella nidulans* were only detected on cellulose medium in low counts (collectively, 22.9% of fungal count). The most dominant species (*A. flavus*, *F. oxysporum* and *G. fujikuroi*) were tested for their pathogenicity against peanut seed (healthy and surface disinfected) germination on three soil types (sandy, loam and clay). *G. fujikyroi* and *F. oxysporum* were highly pathogens on germination of peanut seeds (average: 28.6% and 34.6%, respectively) whereas, *A. flavus* was poorly effect (72% seed germination) compared with control (73.6%). Also, sandy soil had the best in rate of germination compared with loam and clay soils. It is worthy to mention that, the germination of peanut seeds in treated seeds with the spore suspensions of the most dominant isolates tested consumed time compared with untreated (control) seeds.
Key words: peanut, seedling, fungi, pathogens, saprophytes, diseases, germination

Introduction

Peanut (*Arachis hypogaea* L.) is a major species of family Leguminosae, considered to be the crop of poor people that contains oils, proteins, minerals, vitamins, compounds of medicinal importance, and also serves as good cattle feed. Peanut productions and yields are compromised by various biotic threats, such as viruses, bacteria, fungi, and aphides as well as abiotic factors like drought, salinity and temperature. Fungi are superior of peanut diseases (more than 40 diseases) caused by several fungal species including leaf, stem, root and pod (IPI, 2019).

Seed rot, pre- and post-emergence death of seedling, dark brown inadequate sunken lesions on stems, seedling are stunted or die at a later date stand may result. The species, *Rhizoctonia solani*, *Pythium* spp. and *Fusarium* spp. were known as the most common species infected peanut seedlings (Porter *et al.* 1990, Bird *et al.* 1993 and Shim-Hyoenghwon *et al*. 1996).

Pathogenicity tests have been shown a significant effects on the emergence of seeds. Of peanut seeds, *Rhizoctonia solani* showed highly effects followed by *Scloretium batacola* and *Fusarium solani* in inoculation of seeds or soil treatments (Hashimi, 1988) and peanut pod rot lead to damage of peanut kernels by *Pythium* spp. and *Rhizoctonia solani* (Terry *et al*. 2016). Of lentil, *Rhizoctonia solani* and *F. solani* showed damping off and root rot (Montaser & Abo-Elyouser, 2012). Of asparagus, *F. oxysporum*, F. sp. *asparagi*, *F. proliferatum*, *F. redolens*, and *F. solani* had crown and root rot of the plant (Wadi, 2015). Of melon, *Macrophomina phaseolins* caused damping of melon "*Cucumis melon*" (Saleh *et al*., 2009).

Therefore, The present manuscript aimed for isolation and identification of pathogenic and/or saprophytic fungi associated with peanut seedling wilting off and pathogenicity of the dominant fungi against peanut seed germination was lso assessed.

Materials and Methods

Collection of samples:-

Twenty infected peanut seedling samples from farmer's fields of 5 governorates (El-Giga, Assiut, Sohag, Qena and Aswan) in Upper Egypt were collected. The samples were separately placed in sample bags, sealed and placed in another bags

which were also sealed. The samples were kept cool during transferring (3-5^{0}C).

Isolation and identification of fungi:-

The roots and cotyledons were cut with removing soil particles, surface sterilized by sequential immersion in 75% ethyl alcohol for 1 min. (act as surfactant), 0.93-1.3 M solution of sodium hypo-chloride (3- 5% available chlorine) for 3 min (actual-sterilizing agent), ethyl alcohol for 0.5 min, and rinsed in sterile distilled water for 1 min, then dried between sterile filter paper (Lodge *et al*. 1996 and Bayman *et al*. 1997).

The treated segments (10 segments) of each samples (root and cotyledon) were put with fixing on surface of two isolation media and these were: 1% glucose- (glucose, 10 g; $NaNO_3$, 2 g; KH_2PO_4, 1g; KCl, 0.5 g; $MgSO_4$. $7H_2O$, 0.5 g; agar agar, 15 g per liter H_2O) and cellulose- (cellulose , 10 g instead of glucose) Czapek,s media. Rose bengal (1/30000 g) and chloramphenicol (1/15000 g) were used as antibacterial agents (Smith & Dawson, 1944; Al-Doory, 1980 and Redlin & Carris, 1996).

The plates of the two isolation media (10 Petri dishes) containing the segments (each, 2 segments) were incubated at 18 ± 1 ^{0}C for 2 weeks. The growing colonies were examined, counted (per segment), isolated and identified based on morphological features according the keys of Raper and Thom, 1949; Raper and Fennell, 1965; Booth, 1971; Christensen and Raper, 1978; Moubasher, 1993, and Nelson, *et al*., 1983;

Pathogenicity test of peanut seeds:-

A healthy peanut seeds (1200 seeds) were surface disinfected, washed by sterilized distilled water, and dried under sterilized conditions. The seeds were classified into 4 groups (each, 300 seeds), 3 groups were treated by 5 ml spore suspensions (spore +mycelia) of the three most dominant isolated fungi (*Aspergillus flavus*, *Fusarium oxysporum* and *Gibberella fujikuroi*) that grown on PDA to have mass production of growth and the fourth acts as control (dist. H_2O). The treated and untreated seeds (M.C. 30% based on dry weight) were re-classified into 12 groups (each, 100 seeds) which were grown on large pots (1.2x1 meter) contained three types of soils (sandy, loam and clay). The soils were irrigated several times by tap water and the rate of germinations were estimated every 7 days (3 times; 7, 15 and 21days) and the summations were calculated as pathogenicity indicator.

Results

A- Pathogenic and/or saprophytic fungi of peanut infected seedling:-

Glucophilic fungi:

Based on the results obtained, the gross total fungal count on 1% glucose-Czapek's agar of 20 samples (200 segments) was 264 colonies. Sixteen samples were highly infected (10-23 colonies per sample), whereas only 4 samples had less infections (5-9 colonies) after surface disinfection. A total of 6 species belonging to 4 genera were isolated and identified from wilted peanut seedling at 18±1 ^{0}C to isolate the causing pathogens (Table, 1).

Aspergillus was the dominant genus based on number of cases of isolation (100% of the samples) and total count (52.2% of gross count). It was represented by two species and these were *A. flavus* and *A. niger*. *A. flavus* was superior in count (84.7% of total *Aspergillus* and 44.3% of gross count) and frequency (100% of the samples), whereas *A. niger* was less in count (15.2% and 7.9%) and frequency (30%).

Fusarium oxysporum occupied the second place based on occurrence and count (75% of the samples and 29.2% of gross count) and *Gibberella fujikuroi* had the third place (50% and 10.2%, respectively). *Penicillium* was less in frequency and count (50% and 8.3%, respectively) and represented by two species: *P. funiculosum* and *P. chrysogenum* (30% & 20% of samples and 5.6% & 2.7% of count).

Cellulose–decomposing fungi

Of cellulose-decomposing fungi, the gross total fungal count on 1% cellulose-Czapek's agar of the 20 samples was 214 colonies. Fourteen samples were highly infected (10-17 colonies per sample), whereas 6 samples had low infections (4-6 colonies). Ten species of 6 genera were identified (6 of 4 on glucose medium) from infected peanut seedlings (Table, 1).

Aspergillus was superior in count (59.8% of total fungal count) and frequency (95% of the samples tested). The genus was represented by 4 species (2 on glucose) of which *A. flavus* was the dominant (42.5% of fungal count, 71.1% of *Aspergillus* and 95% of samples), whereas, the other 3 species (*A. niger*, *A. fumigatus* and *A. terreus*) were less in count (collectively, 28.9% of *Aspergillus* and 17.3% of isolated fungi) and occurrence (10-30% of samples). Also. *Fusarium (F. oxysporum*)occupied the second place based on count (18.9% of fungal count) and frequency (65% of samples).

Two genera were isolated in moderate frequencies (30%-40% of the samples) and these were *Penicillium* and *Gibberella*. The first genus was represented by *P. funiculosum* and *P.*

corylophilum with low frequencies (each, 25% of samples) and low counts (collectively, 7.9% of gross count). , *G. fujikuroi* had also low count (6% of count). Finally two species, *Nectria haematococca* and *Emericella nidulans* were isolated in low frequencies (25% % 20% of the samples) and low counts (collectively, 7.5% of total fungal count).

It is worthy to mention that one species (*P. chrysogenum*) appeared on glucose- and disappeared on cellulose-Czapek's agar, whereas five species (*A. fumigatus, A. terreus, P. coryophilum, N. haematococca,* and *E. nidulans*) were only detected on cellulose medium .

pathogenicity test:-

Of pathogenicity test, health peanut seeds were surface disinfected, dried, treated (soaked) by spore suspensions of the dominant fungal species (*Aspergillus flavus, Fusarium oxysporum* and *Gibberella fujikuroi*) isolated from infected seedlings, and cultivated in three types of soils (sandy, loam and clay). The rate of germination was estimated 3 times (7, 15 & 21 days) as indication of organism-causing pathogenicity (Table, 2).

According to the results of this experiment, *G. fujikuroi* and *F. oxysporum* proved to be highly pathogens (average, 28.6% & 34.6% seed germination, respectively), whereas *A. flavus* had 72% germination of peanut seeds which was more or less parallel to control (73.6%) that un-treated and irrigated by tap water. Of the three types of soils (germination average of the three fungal species tested), sandy had the best rate of germination (average, 50.3%), followed by loam and clay (45% & 40%) compared with control (82%, 76% and 63%, respectively). Regarding to germination of peanut seeds on the soil types, the appearance of green parts over the surface of soil was faster in control compared with the treated seeds by fungal isolates, Also, the infected seeds did not germinate or germinated for short period, wilted and died.

Discussion

Mycological survey of wilted peanut seedling on two isolation media (1% glucose and 1% cellulose-Czapek's agar) revealed that, the gross fungal count on glucose was more than on cellulose. Also, the number of colonies per segment using glucose was higher than cellulose. In this respect, glucose is easy and quickly utilizing mono-saccharide in glycolysis than other carbohydrates (Sequeira *et al*. 2019). In the other hand , the number of isolated fungal genera and species was higher on cellulose compared with glucose medium. In this respect, cellulose especially with pH 8 as carbon source restricted the growth of heavy sporulating filamentous fungi which gives good chance for growth of other fungal species (El-Maghraby & El-Maraghy, 1988), also the species isolated amongst moderate or high cellulose decomposing fungi (Moubasher & Mazen, 1991)

Of isolated fungal species, *Aspergillus flavus* and *Fusarium oxysporum* were quite the dominant species on the two isolation media followed by *Gibberella fujikuroi* (*F. moniliforme*). Concerning the previous 3 species, *A. flavus* amongst toxigenic storage fungi (Horn, 2003), widely detected in storage peanut seeds in Egypt (El-Maghraby & El-Maraghy, 1987, 1988; Youssef, 2009). *F. oxysporum* and *G. fujikuroi* occupied the second place of wilted and/or damping off of peanut seeds in addition to *Nectria haematococca* (*F. solani*) on cellulose medium only. Concerning to *Fusarium* species, fusaria amongst toxigenic field fungi (Horn, 2003), *F. oxysporum* and *F. moniliforme* are heavy sporulating *Fusarium* species (Nelson *et al*. 1983) and widely detected in Egyptian soils as reported by by Moubasher and his collaborates.

Of pathogenicity test, the three dominant species on the two isolation media were tested against healthy peanut seeds. Two species caused disease (damping off) and these were *G. fujikuroi* and *F. oxysporum* (only, 28.6% & 34.6% germinated seeds) whereas, there were no clear effect of *A. flavus* (72 %) compared with control (73.6%). In this respect concerning the previous studies, *Scloretium batacola* had a significant effect on seeds (peanut) emergence, followed by *F. solani* in seeds treatment, whereas *Rhizoctonia solani* had significant effect followed by *S. batacola* in soil treatment of two peanut cultivars in Egypt (El-Wakil & El-Matwally, 2001). Also, the infection may transmitted in form of spores on seed surfaces as downy mildew of peanut (Hashim, 1988). Also, the treated (spore suspensions of 3 fungal isolates) of peanut seeds consumed time to germinate compared with untreated one. In this respect, the treated peanut seeds with mycotoxins (aflatoxins or trictothecenes and zearalenone) consumed more time to germinate compared with untreated seeds (Ibrahim, 2007; El-Maghraby *et al*., 2009)., Also, section Flavi as well as *A. niger* over broad ranges of deleterious effect on peanut plants and plant productivity (Horn & Dorner, 1998).

In conclusion, mono-saccharides (e.g. glucose) stimulate heavy sporulating fungi to grow, whereas polysaccharides (e.g. cellulose) have some depression of heavy sporulating which are a good chance for non-heavy sporulating to grow. Also, *Aspergillus* (e.g. *A. flavus*) amongst saprophytic, whereas *Fusarium*

(e.g. *F. oxysporum* and *F. monliforme*) are pathogenic fungi.

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Table (1): Total count (TC) calculated per 20 peanut seeding samples (each, 10 segments), total count percentage per gross count (TC%), number of cases of isolation (NCI) and occurrence remark (OR) of fungal genera and species isolated on glucose- and cellulose Czapek's agar at 18 ±1 ^{0}C

Genera and species	Glucose-Czapek's agar			Cellulose Czapek's agar		
	TC	TC%	NCI & OR	TC	TC%	NCI & OR
Aspergillus	**138**	**52.2%**	**H 20**	**128**	**%59.8**	**19 H**
A. flavus Link	**117**	**44.3%**	**H 20**	**91**	**%42.5**	**H19**
A. niger Van Teighem	**21**	**7.9%**	**M 6**	**14**	**%6.5**	**5 L**
***A. fumigatus* Fresenius**	-	-	-	**16**	**%7.4**	**6 M**
***A. terreus* Thom**	-	-	-	**7**	**%3.2**	**3 L**
***Fusarium oxysporum* Schlecht ex Fr.**	**77**	**%29.2**	**H 15**	**40**	**%18.6**	**H 13**
***Gibberella fujikuroi* (Swada) Ito**	**2.7**	**%10.2**	**10 M**	**13**	**%6**	**M6**
Penicillium	**22**	**%8.3**	**M 10**	**17**	**%7.9**	**M 8**
***P. Funiculosum* Thom**	**15**	**5.6%**	**6 M**	**7**	**%3.2**	**5 L**
***P. chrysogenum* Thom**	**7**	**2.7%**	**4 L**	-	-	-
***P. corylophilum* Direckx**	-	-	-	**10**	**%4.6**	**5 L**
***Nectria haematococca* Berkely & Brown**	-	-	-	**7**	**%3.2**	**L5**
***Emericella nidulans* Eidam Vuillemin**	-	-	-	**9**	**4.2%**	**4 L**
Gross total count	**264**			**214**		
Number of fungal species and genera	**6 species/4 genera**			**10 species/6genera**		
Number of colonies (per 10 segments)	**5-23 colonies**			**4-17 colonies**		
Percentage of infected segments	93%			84.5%		

Occurrence Remark:
H: High occurrence, more than 50% of the samples (20 samples)
M: moderate occurrence, between 30-50% of the samples
L: Low occurrence, 25% of the samples or less

Table (2): Percentage of peanut seed germination treated with spore suspensions of three fungal isolates cultivated in three soil types.

Soil type / **Fungal genera and species**	**Sandy soil (% of germination)**	**Loam soil (% of germination)**	**Clay soil (%of germination)**	**Average of the 3 soil types**
Gibberella fujikuroi	**%34**	**%29**	**%23**	**%28.6**
Fusarium oxysporum	**%39**	**%33**	**%32**	**%34.6**
Aspergillus flavus	**%78**	**%73**	**%65**	**%72**
Average of the 3 isolates tested	**50.3%**	**45%**	**40%**	**%**
Control (Dist.water)	**%82**	**%76**	**%63**	**%73.6**