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SEED BORNE MYCOFLORA OF GROUNDNUT

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Abstract

Using blotter, agar plate and deep freezing method as recommended by ISTA, the seed borne mycoflora of 12 groundnut seed samples collected from different localities of Pakistan was examined. Of the 14 genera and 28 species of fungi isolated, 18 fungal species viz., *Absidia corymbifera* (Cohn) Sacc and Trotter, *Alternaria citri* Ellis and Pierce apud Pierce, *Aspergillus awamori* Nakazawa, *A. candidus* Link ex Link, *A. japonicus* Saito, *A.luchuensis* Inui, *A. panamensis* Raper & Thom, *A. penecilloides* Spegazzini, *A. terricola* Marchal, *A. terreus* Thom, *A. wentii* Wehmer, *Chaetomium globosum* Kunze ex Steud., *C. indicum* Corda, *Cladosporium oxysporum* Berk. & Curt, *Paecilomyces variotii* Bain,*Syncephalastrum racemosum* Cohn ex Schrot, *Trichoderma hamatum* (Bonord) Bain and *Tricothecium roseum* (Pers) Link ex Gray do not appear to have been reported from Pakistan on groundnut seed. *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani, F. oxysporum, Aspergillus flavus* and *A. niger* were found predominant. Higher number of fungi were isolated where blotter method was used as compared to agar plate and deep-freezing method. Surface sterilization of seeds reduced the incidence of *A. flavus* and *A. niger*.

Introduction

Groundnut (*Arachis hypogea* L.) a valuable legume crop, is cultivated over an area of 99.4 hectares in Pakistan with a production of about 1017 Kg/ hectare or 101 tones during 2001-02(Anon., 2002). Groundnut seed contain 50% edible oil. Seeds are rich in fats, protein, vitamin B_1, B_2, B_6, nicotinic acid and other vitamins. It is also a good source of lecithin present to the extent of 0.5-0.7% in decorticated nuts. Peanut butter has become a common edible diet. Groundnut cake has high nutritive value. Peanut flour is suitable for supplementing white flour (Sastri, 1948). Of the various disease causing organisms, *Fusarium solani*, *F.oxysporum* cause damping off of groundnut seedlings (Reddy & Rao, 1980). *Aspergillus flavus* attacks germinating groundnut seed (Clinton, 1960). *A. niger* caused disease of crown rot of peanut (Gibson,1953a, 1953b). Mould fungi are also known to produce mycotoxin (Rodricks, 1976). Many workers have detected different mold fungi and their toxin production ability in stored grains, which deteriorate the stored products (Afzal *et al*., 1979, Vedahayagam *et al*., 1989). Experiments were therefore carried out to determine the composition of the mycoflora of groundnut seeds which is presented herein.

Materials and Methods

Twelve samples of groundnut seed collected from Chakwaal (1), Karachi (8), Lahore (3) were used. Using ISTA technique (Anon., 1976), 400 seeds from each sample were tested. For the standard blotter technique, untreated seed and seed after treatment with 1% Ca $(OCl)_2$ were placed on three layers of moistened blotters, 10 seeds per Petri dish. For the agar plate method, the treated and untreated seeds were plated on potato dextrose agar (PDA), 10 seeds per Petri dish and the dishes were incubated at 24°C in alternating

cycle of 12 hours light and 12 hours darkness for 7 days. In deep freezing method, the treated and untreated seeds were incubated for 1 day each at 20°C and at 0°C in a freezer followed by 5 days incubation at 24°C. Fungi growing on seeds were identified after reference to Ellis (1971), Domsch *et al*., (1980), Nelson *et al*., (1983) and Raper & Fennel (1965).

Data were subjected to Analysis of Variance (ANOVA) or Factorial Analysis of Variance (FANOVA) depending upon the experimental design following the procedure as given by Gomez & Gomez (1984).

Results and Discussion

A total number of 14 genera and 28 species of fungi viz., **Absidia corymbifera* (Cohn) Sacc and Trotter, **Alternaria citri* Ellis and Pierce apud Pierce, **Aspergillus awamori* Nakazawa, **A. candidus* Link ex. Link, *A. flavus* Link ex Gray, **A. japonicus* Saito, **A. luchuensis* Inui, *A. niger* Van Tieghem, **A. panamensis* Raper & Thom, *A. parasiticus* Speare, **A. pencilloides* Spegazzini, **A. terricola* Marchal, **A. terreus* Thom, **A. wentii* Wehmer, **Chaetomium globosum* Kunze ex Steud., **C. indicum* Corda, **Cladosporium oxysporum* Berk and Curt, *Fusarium oxysporum* Schlech, *F. semitectum* Berk & Rav., *F. solani* (Mart.), *Macrophomina phaseolina* (Tassi) Goid, **Paecilomyces variotii* Bain, *Penicillium* sp., *Rhizoctonia solani* Kuhn, *Rhizopus stolonifer* (Ecicuh ex Link) Lind, **Syncephalastrum racemosum* Cohn ex Schrot, **Trichoderma hamatum* (Bonord) Bain and **Tricothecium roseum* (Pers.) Link ex Gray were isolated from 12 samples of groundnut collected from different parts of Pakistan (Table 1). Of these 18 fungal species marked with an asterisk are new report from Pakistan (Ghafoor & Khan, 1976; Ahmad *et al*., 1992).

All the seed samples were found to be infected by *A. flavus* whereas 17% with *R. solani* and *Alternaria citri*, 33% with *M. phaseolina* and 58% with *Fusarium* spp. *Aspergillus* spp., were significantly detected by blotter method ($p< 0.001$). Surface sterilization with 1% Ca $(OCl)_2$ significantly reduced the incidence of *Aspergillus* spp., ($p<0.001$). No significant difference was found in the incidence of *M. phaseolina* and *Fusarium* spp., before or after surface sterilization. The results showed that microbial contamination were eliminated by Chlorine disinfestations as also reported by Limonard (1968). Blotter method showed better results as compared to agar plate and deep-freezing method ($p<0.001$). Jovicevic (1980) also reported that the filter paper method was most practical method for routine analysis of seed health. Such similar results were observed by Khan *et al*., (1988) on rice seed and Dawar & Ghaffar (1991) on sunflower seed. The sample collected from Chakwaal showed the highest incidence of fungi viz., *F. solani, Macrophomina phaseolina, Rhizoctonia solani* and *Trichoderma hamatum*. Present results showed that *A. flavus* and *A. niger* were the predominant fungi of groundnut. Mukherjee *et al*., (1992) also found that *A. flavus* and *A. niger* were the predominant storage fungi of groundnut seed. Similar reports have been made by Dawar & Ghaffar (1991) on sunflower. Species of *Aspergillus, Penicillium* and *Rhizopus* have also been reported on groundnut seed (Lumpungu *et al*., 1989). These species reduced the germination of seeds and damage the seeds in storage (Christensen, 1973). *Fusarium solani* and *F. oxysporum* cause damping off of groundnut seedling (Reddy & Rao, 1980). Of the fungi isolated, *A. flavus* is an important mycotoxin producer and produce four major metabalites of aflatoxin B_1, B_2, G_1 and G_2 which are hepatocarcinogenic (Goldblatt, 1969). There is therefore need for reducing the mold growth and mycotoxin production in groundnut seeds by improving the storage condition.

Table 1. Seed borne mycoflora of groundnut.

Name of fungi	Sterilized seeds						Non-sterilized seeds					
	Agar plate		Blotter method		Deep freezing		Agar plate		Blotter method		Deep-freezing	
	NSI	1% ± SD	NSI	1% ± SD	NSI	1% ± SD	NSI	1% ± SD	NSI	1% ± SD	NSI	1% ± SD
**Absidia corymbifera*	2	0.58±2.73	2	0.45±3.06	1	0.06±0.45	2	0.75±3.50	2	0.68±3.25	2	0.25±1.29
**Alternaria citri*	1	0.58±4.04	1	0.5±1.00	2	0.75±3.50	1	0.72±5.05	1	0.64±4.47	2	1.47±5.65
**Aspergillus awamori*	5	1.64±4.95	9	2.72±3.47	1	0.14±1.01	5	3.06±8.23	9	3.95±6.18	3	0.64±2.51
**A. candidus*	2	0.25±1.24	3	0.56±2.04	0	0	2	0.56±2.62	3	0.85±6.18	1	0.16±2.51
A. flavus	12	8.02±6.71	12	7.16±4.26	12	4.56±1.90	12	11.58±8.80	12	9.75±4.13	12	5.29±2.15
**A. japonicus*	7	1.77±3.31	10	1.68±2.59	2	0.25±1.18	7	2.54±4.76	10	3.56±5.32	1	0.25±1.73
**A. luchuensis*	2	0.58±3.01	9	2.12±3.41	2	0.16±0.77	2	0.87±4.13	9	3.18±5.87	2	0.25±1.24
A. niger	12	7.3±4.22	12	6.79±5.96	11	4.27±3.26	12	10±4.9	12	8.6±7.62	12	4.93±2.50
**A. panamensis*	1	0.22±1.58	7	1.31±2.41	0	0	1	0.27±1.87	6	1.87±4.27	0	0
A. parasiticus	10	1.20±4.01	10	1.35±4.12	8	1.01±3.69	10	1.97±5.18	11	1.75±5.74	8	1.14±4.23
**A. penecilloides*	4	0.93±3.11	3	0.6±2.29	0	0	4	1±3.37	3	0.62±2.77	0	0
**A. terricola*	3	1.04±3.92	3	0.85±3.53	1	0.08±0.57	3	1.72±6.39	3	1.04±4.52	1	0.125±0.866
**A. terreus*	4	1±3.08	5	1.16±3.20	0	0	4	1.39±4.22	5	1.77±5.00	0	0
**A. wentii*	8	1.64±3.60	7	1.62±3.18	2	0.25±1.16	7	2.43±4.86	7	2.29±4.62	2	0.5±2.33
**Chaetomium globosum*	0	0	2	0.72±3.7	2	0.27±1.30	0	0	2	0.75±5.52	2	0.52±2.49
**C. indicum*	1	0.22±1.58	1	0.45±3.17	1	1.66±11.54	1	0.52±3.60	1	0.52±3.60	1	0.31±2.16
**Cladosporium oxysporum*	1	0.6±4.18	1	0.37±2.59	1	0.37±2.59	1	0.66±4.61	1	0.6±4.18	1	0.45±3.17
Fusarium oxysporum	1	0.39±2.74	1	0.41±2.88	1	0.41±2.88	1	0.62±4.33	1	0.72±5.05	1	0.58±4.04
F. semitectum	2	0.33±1.57	2	0.66±3.45	2	0.81±4.51	2	0.83±4.65	2	0.95±4.71	2	0.93±4.91
F. solani	4	1.20±4.01	3	1.35±4.12	3	1.02±3.69	4	1.47±5.18	3	1.75±5.74	3	1.14±4.23
Macrophomina phaseolina	4	1.6±6.42	4	2.39±9.20	4	1.68±5.62	4	1.45±5.10	4	1.97±6.89	4	2.18±7.38
**Paecilomyces variotii*	0	0	1	0.375±2.59	0	0	0	0	1	0.5±3.46	0	0
Penicillium sp.	4	0.14±1.01	4	0.6±1.87	0	0	4	0.25±1.73	4	0.83±3.24	1	0.25±1.73
Rhizoctonia solani	1	0.29±2.02	2	0.62±2.92	1	0.25±1.73	2	0.95±4.65	2	1.37±6.59	1	0.35±2.45
Rhizopus sp.	7	2.41±4.82	7	3.08±7.79	3	0.52±1.94	7	3.91±7.31	7	3.41±7.92	3	0.70±2.58
**Syncephalastrum racemosum*	3	0.93±3.41	3	0.79±3.09	0	0	3	2±8.61	3	1.37±5.08	0	0
**Trichoderma hamatum*	2	0.95±4.65	2	0.64±3.03	1	0.27±1.87	2	1.6±7.51	2	1±4.69	1	0.70±4.90
**Tricothecium roseum*	0	0	1	0.5±2.37	0	0	0	0	1	0.64±3.03	0	0

NSI# = Number of samples infected out of 12 samples tested.

* = New reports on groundnut seeds.

1% ± SD = % of infected seed ± Standard deviation.

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