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IDENTIFICATION OF SEED-BORNE MYCOFLORA ASSOCIATED WITH PEANUT (*ARACHIS HYPOGEA* L.) IN POTHWAR, PAKISTAN

Muhammad Farooq Aslam[1], Gulshan Irshad[1], Hafiza Madeeha Khan[2], Salman Ghuffar[1], Farooq Azam[1]
[1] *Department of Plant Pathology, Faculty of Crop and Food Sciences, PMAS-Arid Agriculture University Rawalpindi, Pakistan.*
[2] *Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan.*

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ABSTRACT

Peanut (*Arachis hypogaea* L.) occupies the second largest area among the oilseed crops after mustard and rapeseed in Pakistan. It is the main legume crop and primarily grown as oilseed crop. Due to the presence of high percentage of fatty acids in its oil, its nutritional properties are considered as favorable for human and animals. In the present study, seed-borne fungi were recovered from different varieties of peanut with their incidence percentage. Data revealed that all the varieties were affected by the fungal species however, 68 % of *Aspergillus niger* on agar plate method and 64 % on standard blotter paper while *Rhizopus nigricans* had the least percent incidence of upto 22 % and 6% on agar plate method and standard blotter paper respectively. It is concluded from the present study that due to potato dextrose agar content, agar plate method found to be suitable for germination seed borne fungi are known to affect adversely seed germination and fatty acid composition due to the production of toxic metabolites in the peanut seeds.

Corresponding Author: Muhammad Farooq Aslam
Email: farooqch.96@gmail.com

INTRODUCTION

Peanut (*Arachis hypogea* L.) which belongs to the family Leguminosae and also known as ground nut, monkey nut, manila nut, earth nut and ground bean. In the genus, only *A. hypogaea* L. is significantly important economically. Botanical name of groundnut is derived from the Greek word "arachis" meaning "legume" and hypogaea meaning "below ground", referring to the formation of pods in soil (Pattee and Stalker, 1995). Peanut (*Arachis hypogea* L.) is known as very important legume crop also considered as oilseed crop which is cultivated on an area of 99.4 hectares in Pakistan with average production of approximately 1017KG/Hectare (Khan and Uddin, 2009). Major part of peanut world production is used in human consumption, for processed food as a raw ingredient, such as sacks and peanut butter and also produce oil and flour. (Kaya et al., 2009; Nakai et al., 2008; Passone et al., 2008). Seeds of peanut consists high nutritional and commercial value due to presence of adequate amount of protein, carbohydrates, fatty acids, fiber contents, beside vitamins, calcium and phosphorus (Nakai et al., 2008). It contains 44-56% oils and 22-30% proteins, therefore being considered as an excellent source of energy (564 kcal/100 g) (Andersen et al., 1998).

It is grown mainly in rainfed areas of Pakistan and among total peanut production area, about 84% lies in Punjab 13 % in KPK province and 3 % in Sindh (GOP, 2008). Peanut

crop is commonly grown in southern drier parts of the Pothowar region (Ali et al., 2002). Among total cultivated area, approximately 80% lies in pothowar tract contributing 92% of the overall production of the country (GOP, 2011).

Peanut is attacked by 50 genera of fungal, 1 bacterial, 16 nematodes, 15 viruses and 2 phanerogamic parasites (Naikoo et al., 2013). Aspergillus spp. is the most important fungal pathogen of tropical as well as temperate countries (Elwakil and El-Metwally, 2001). Most of the diseases of the peanut crop is caused by seed borne fungi that can easily survive in infected peanut seeds (Magnoli et al., 2006). One of the main reason of reduce seed viability of the peanut seeds in storage condition (Olkowski et al., 1995).

MATERIALS AND METHODS

Samples collection: Peanut seed samples were collected from22 different locations of pothowar region of Pakistan. These seed samples were taken Mycology Laboratory PMAS Arid Agriculture University Rawalpindi for further studies.

Detection of seed mycoflora: Peanut seed samples were analyzed for their association of seed mycoflora by using standard blotter paper method and Agar plate method (ISTA, 1985). Percent incidence of seed borne mycoflora recorded in percent incidence of seed borne fungi associated with unsterilized seeds. The fungal pathogen associated with peanut seeds were isolated using two different techniques.

Standard blotter paper method: Isolation of seed borne mycoflora was isolated by placing 4-5 seeds in a petri plate with soaked blotter paper as described by Doyer (1938) and De Temp (1953) Plates were incubated at 25°C±2 for 7-8 days. Obtained fungal counts were then confirmed with microscopic studies.

Agar plate method: Seed borne fungal pathogens were isolated by directly plating 4-5 seeds in each petri plate on PDA medium as described by Muskett (1948) and incubation was done at 25ºC±2for 7 days. Recovered fungal counts on PDA medium were purifies by using hyphal tip methods and were subjected to microscopic studies.

RESULTS AND DISCUSSION

Total 25 different fungal isolates were recovered and were examined under microscope for their spore size, shape, colony color, hyphal characters and presence or absence of nucleus. On the basis of microscopic identification of all recovered isolates, 7 isolates were identified as *Aspergillus niger,* 3 isolates were *Fusarium oxysporium,* 4 isolates were *Penicillium expansum,* 3 isolates of *Mucor* spp., while 4 isolates were identified as *Rhizopus nigricans* and *Alternaria* spp. respectively.

Six major fungal pathogens were found associated with peanut seeds such as *Aspergillus niger, Fusarium oxysporum, Penicillium expansum, Mucar* spp.*, Rhizopus nigrican and Alternaria* spp. In case of standard blotter paper, the percent incidence of *Aspergillus niger* (64%) was highest followed by *Fusarium oxysporum* (42%) and *Penicillium expansum* (36%). Alternaria spp., (32%), Mucor spp., (18%) and *Rhizopus nigricans* (6%) were intermediates within the range of 6-32%. *Mucor* spp.*, and Rhizopus nigricans found to be least*.

In agar plate, *Aspergillus niger* (68%) gave highest percent incidence followed by *Fusarium oxysporium* (45%). *Penicillium expansum*, (44%), *Alternaria* spp.*,* (42%), *Mucor* spp. (24%) and *Rhizopus nigricans* (22%) which found to be least on agar plate method (Table 1).

Table 1. Percent Incidence of major mycoflora associated with peanut seeds.

Fungi	% Incidence of Mycoflora	
	Blotter Paper Method	Agar Plate Method
Aspergillus niger	64	68
Fusarium oxysporum	42	45
Penicillium expansum	36	44
Mucor spp.	18	24
Rhizopus nigricans	6	22
Alternaria spp.	32	42

Agar plate method showed better results as compared to standard blotter paper for germination of seed borne mycoflora of peanut seeds due to presence of potato dextrose agar contents.

Aspergillus niger: Septatic and hyaline hyphae (Figure 1A). At apex, conidiophores were terminated in vesicle. Round conidia having radial chains over the phialides (Figure 1B). Conidial heads of Specie have conidial head and loosely columnar. Conidiophores of the pathogen's dimensions 800 x 15-20, 20-45 µm. Morgan (1998) and De Hoog et al. (2000) also observed similar characteristics thus confirming these isolates as *A. niger*.

Fusarium oxysporum: Colony contain woolen to cottony, flat and spreading having whitish, creamy, yellowish, and surface is pinkish, colorless to purple and brown on reverse (Figure 2A). Colonies of *Fusarium oxysporum* are whitish to ting with the salmon on the surface and purple on reverse. Micro and macro conidia were seen under microscope. Microconidia are smaller in size having 1-2 cells while macroconidia were 3-5 celled that are slightly curved and pointed at tips (Figure 2B). Similar findings were recorded by Hennequin et al. (1999).

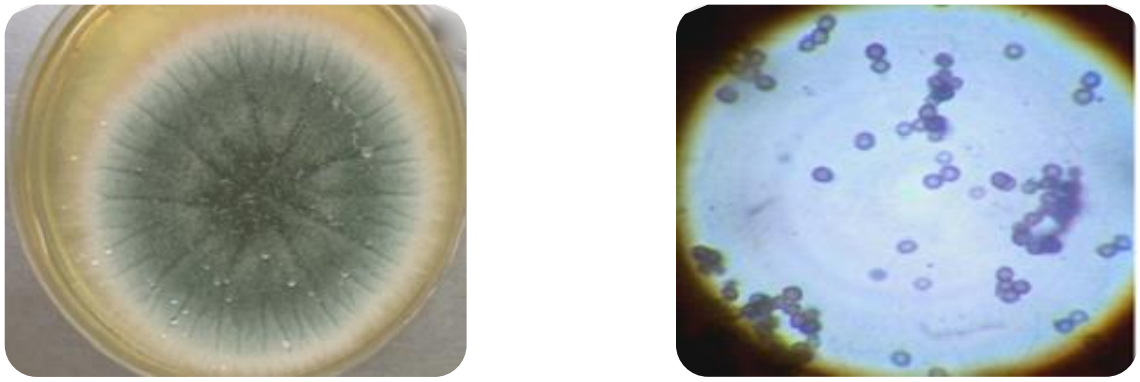

Figure 1A. Culture of *A. niger,* B. Round conidia having radial chains of *A. niger.*

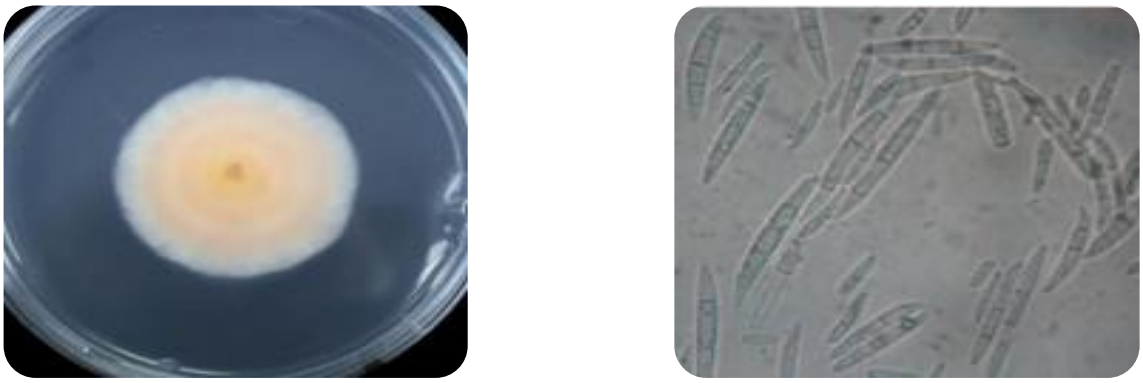

Figure 2A. Wooly to cottony having pinkish shade colony of *F. oxysporum,* B. Spores of *F. oxysporum*.

Penicillium expansum: Colony showed the growth rapidly and is flat and filamentous, have cottony textured. Colonies are blue green from white, turning from pinkish to yellowish with age (Figure 3A). Pale colored from back. The conidia round, single celled, un branched at apix of the Phialides (Figure 3B). Morgan (1998) described the similar features.

***Mucor* spp.:** *Mucor's* colonies were found fast growing at temperature of 25 - 30 °C and covered the growing media's surface rapidly. Appeared fluffy having color initially white, then become grayish brown (Figure 4A). Hyphae 6-15 µm, broad. Rhizoid, stolon both were not present. Sporangiophores were small and erected (Figure 4B).

Rhizopus nigricans: The texture of the colonies of *Rhizopus* spp. was cottony candy like and it was white initially from the front and then it turned from grey to brown color with age (Figure 5A). Firstly, whitish and then pale from back (Morgan, 1998). Broad hyphae with the diameter 6-15 µm, non-septate (Figure 5B). Similar findings were recorded by Morgan (1998).

***Alternaria* spp.:** Rapid growth was shown by the colonies of the pathogen like flat and downy to cottony (Figure 6A). The surface was grey having brown to black reverse. These characteristics were also described by Guarro and de Hoog (2005). Hyphae is septate whereas conidiospores were also Septatic and

dark, large having 8-16 x 23-50 µm, singled or in acropetal chain with both, transverse and longitudinal septations (Figure 6B). Similar characters were described by Guarro and de Hoog (2005).

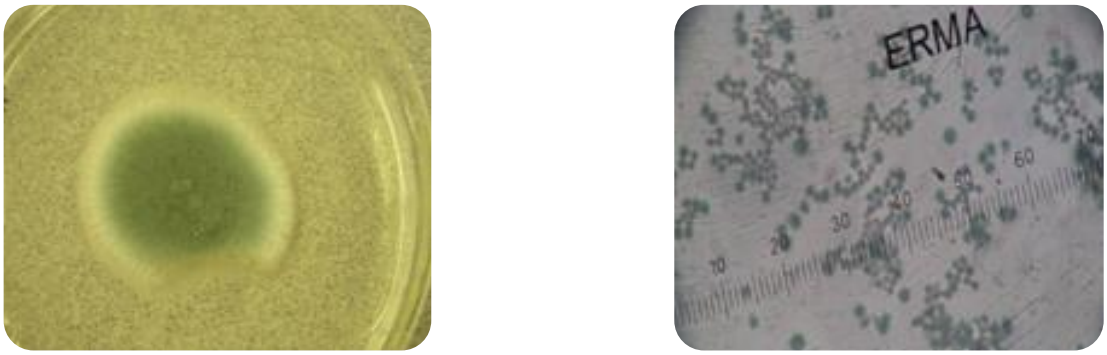

Figure 3A. Flat and cottony colony of *P. expansum,* B. Round single celled conidia.

Figure 4A. White, fluffy colonies of *Mucor* spp. B. Erect and small sporangiophore.

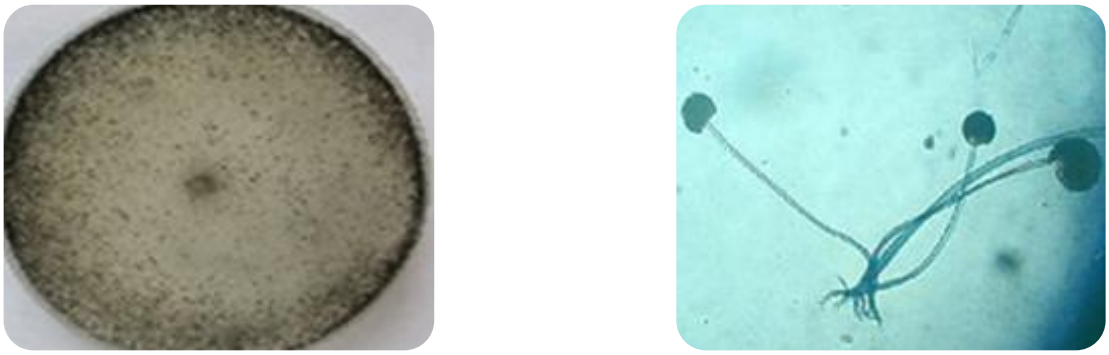

Figure 5A. Cottony, candy like colony of *R. nigricans.* B. Broad, non-septate hyphae.

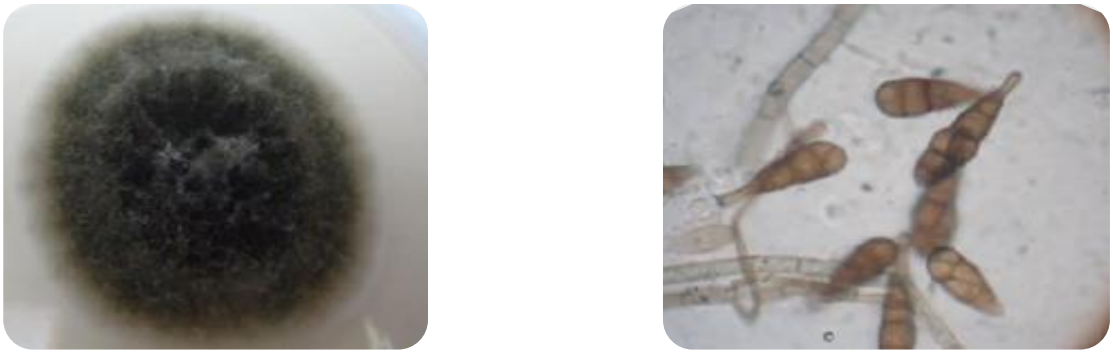

Figure 6A. Brown to black reverse colony of *Alternaria* spp. B. Septate conidiospores with transverse and longitudinal septations

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