Mycopath (2019) 17(2): 93-96 **Research Article**

Mycofloral study of wheat seeds grown in district Poonch, Azad Jammu and Kashmir

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Abstract

The study was carried out on prevalence of mycoflora associated with wheat (*Triticum aestivum* L.) seed using blotter paper method. Wheat seed samples were collected from six different locations of district Poonch, Azad Jammu and Kashmir. A total of seven species of fungi belonging to species of *Penicillium*, *Fusarium*, *Aspergillus* and *Pythium* were identified. *Aspergillus flavus* and *Aspergillus niger* were predominant species in all locations. It is suggested to maintain temperature and relative humidity during storage to avoid fungal associated diseases on wheat seeds.

Keywords: Blotter test, Mycoflora, Pathogenic fungi, Wheat.

Introduction

Wheat is a major cereal crop which occupies central position in the diverse farming system of Pakistan. It is major staple food worldwide, contributing 70% of the daily diet of people. In Pakistan, wheat is grown approximately on an area 9.05 million hectors with production 25.750 million tons (GOP, 2017). Wheat seed quality is questioned by number of seed associated mycoflora, which results in reduced quality and plant vigor (Barabara *et al*., 2004; Klyszejko *et al*., 2005). These fungi are responsible for both pre and post-emergence seedling death results in reduced germination and low planting density (Rajput *et al*., 2005; Niaz and Dawar, 2009). Early identification of seed-borne pathogens allows timely management of diseases and helps to avoid epidemics (Nafula, 1997). District Poonch fall in temperate region where prevalence of pathogenic and saprophytic fungi is very high. For the purpose of seed health, present study was conducted to evaluate the presence of seed associated mycoflora in natural storage conditions around the district Poonch.

Materials and Methods

Seed sampling was done from the various locations of district Poonch, Azad Jammu and Kashmir (Fig. 1). The seeds were collected from household seed stores to ensure the natural existence of the associated flora. Samples were carried to the faculty of agriculture department of Plant Pathology, University of the Poonch, Rawalakot druing May-September 2017. Isolation of associated mycoflora was done by socking of seeds using blotter paper for primary mycelial growth of fungi. Three layers of blotting paper were placed in the Petri-dishes and moistened with distilled water. Twenty-five seeds per petri-dish were placed on equal distances (Fig. 2). The plates were incubated at 26±2 ºC for a period of eight days. After eight days the infected seeds with fungal growth were counted and shifted to separate plate containing PDA (potato dextrose agar) medium incubated at 26±2 ºC for 72 hours for colony development. The percentage frequency of occurrence was calculated by using following formula:

$$\text{Frequency } (\%) = \frac{\text{No. of infected seeds}}{\text{Total no. of seeds}} \times 100$$

Purification of fungal culture was done by repeated isolation of fungus for colonies of pure culture. Colony color was observed and studied under stereomicroscope. Detail morphological studies were carried out by preparation of mounts and identification through literature. Fungi isolated from each sample were documented as wheat associated mycoflora.

Results and Discussion

Identification of fungi

The isolated fungi were recognized according to there colony characteristics and morphology of their fruiting bodies and spores.

Fusarium oxysporum

White colonies, cottonlike aerial mycelium with pink pigmentation on the underside. Abundant cylindrical (occasionally oval) microconidia (8.9 × 3.6 µm), sparse macroconidia (15.2 × 4.1 µm), slightly curved and slender, 3 septate with hooked basal cell and pointed apical cell.

***Fusarium* spp.**

White colonies, cotton like aerial mycelium with orange/red pigmentation on the underside, abundant cylindrical microconidia (8.2 × 3.3 µm), macroconidia extremely sparse (15.4 × 3.9 µm),

slightly curved and slender, 3 to 4 septate with hooked basal cell and pointed apical cell.

***Penicillium* sp.**

The first initially produced white mycelium that later developed greyish green sporulation with white margins; colonies were sulcate, velutinous, with clear exudate, turning yellow on the reverse side of the plate. The second initially appeared white but later developed bluish green sporulation lacking white margins; colonies were circular with dense velvety texture and produced a brown Color on the reverse side of plates, developing a dry, powdery appearance after 10 to 15 days.

Aspergillus niger

The fungal colonies were colourless to pale on the reverse side and covered with a dense layer of dark brown-to-black conidial heads. Conidia were globose to sub globose (3.5 to 5.0 µm in diameter), dark brown to black, and rough walled.

Aspergillus flavus

The single-conidia isolates developed into yellow-green colonies with white mycelia at the edges, 65 to 70 mm in diameter. The biseriate conidial heads ranged in size from 400 to 800 µm and were finely rough walled. Conidia were globose with relatively thin, finely or moderately roughened walls. Colonies developed orange Color on the reverse of the plate.

***Pythium* sp.**

Rapidly growing colony, coenocytic mycelia typical of a *Pythium* species grew out from all roots. Older cultures showed globose sporangia and ornamented oogonia. Oogonia had an average diameter of 23 µm, and projections were conical, measuring an average length of 3 µm.

Alternaria alternata

Colonies were black, conidia were club-shaped, in branched acropetal chains, 12.1 to 29.0 (18.0) µm long and 7.5 to 12.6 (10.6) µm wide, with transverse and longitudinal septa and the morphology matched the description of *A. Alternata.*

Seven fungal species belong to 5 genera were isolated from wheat seeds collected from 6 different location of district Poonch. *Aspergillus* was most dominant genus in all six sides, followed by *Fusarium*, *Penicillium*, *Alternaria* and *Pythium*. Where *Alternaria* and *Pythium* are worldwide distributed fugus responsible for pre- and post-emergence plant death and dumping off, while *Aspergillus*, *Penicillium* and *Fusarium* are commonly found mould responsible for natural decomposing (Table 1). Seven genera of fungi both saprophytic as well as pathogenic were obtained from samples of wheat seeds. Fungi isolated were *Alternaria alternata*, *Pythium* sp., *Peniciilum* sp. *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* sp. Percentage infection ranges from 10 to 65% in samples in all tested locations. Seeds collected from Mendhole showed 64.5% infection followed by showing 64% and 60% from seeds collected from Devigali and Paniola, respectively. Two locations *i.e.* seeds collected from Abbaspur and Hajira showed 52% infection, while seeds from Jandali showed 40% infection. These results are in close conformity with Hussain *et al*. (2013), who isolated eleven fungi from wheat seed by using agar plate and blotter paper technique. The most frequent isolated fungi were *Alternaria alternata* (7.15%) and *Aspergillus niger* (6.22%). It is found that all tested commercial wheat verities were contaminated by seed borne fungi. These seed associated fungi cause dire disease in wheat which reduce the germination capacity. These results are also similar to with Fakhrunnisa *et al*. (2006), who isolated and identified 12 genera and 21 species of fungi *viz. Alternaria alternata, A. niger, A. flavus, A. sulphureus, A. candidus, Absidia* spp., *Curvularia lunata*, *Cephalosprium* spp., *Cheatomium globosum, Drechslera halodes, D. hawaiiensis, D. tetramera, F. oxtsporum, F. moniliforme, F. pallidoroseum, Penicillium* sp. and *Rhizopus* sp. from wheat seeds and *Penicillium* spp., *A. alternata*, *A. flavus* and *A. niger* were found to be promintant.

Conclusions

Wheat seeds of district Poonch found to be associated with many fungi, but their presence varied with locations. All local wheat variety tested have various percentage frequency of the fungal pathogen. Variety with low frequency of seed infection may be grown in disease prone area and wheat seeds should be produced in areas with relatively lower temperature and low relative humidity.

Table 1: Percentage frequency of fungi in seeds of wheat from six different locations.

Location	Fungal species							
	Alternaria alternata	*Aspergillus flavus*	*A. niger*	*Fusarium* spp.	*F. oxysporum*	*Penicilli-um* spp.	*Pythium* spp.	Frequency (%)
Mandhole	2	4	5	0	2	3	0	64.5
Abbaspur	0	0	6	4	0	0	3	52
Hajira	3	2	0	0	0	4	0	52
Devigali	0	6	5	3	2	0	0	64
Jandali	0	3	0	3	0	4	0	40
Paniola	3	5	0	0	3	0	4	60

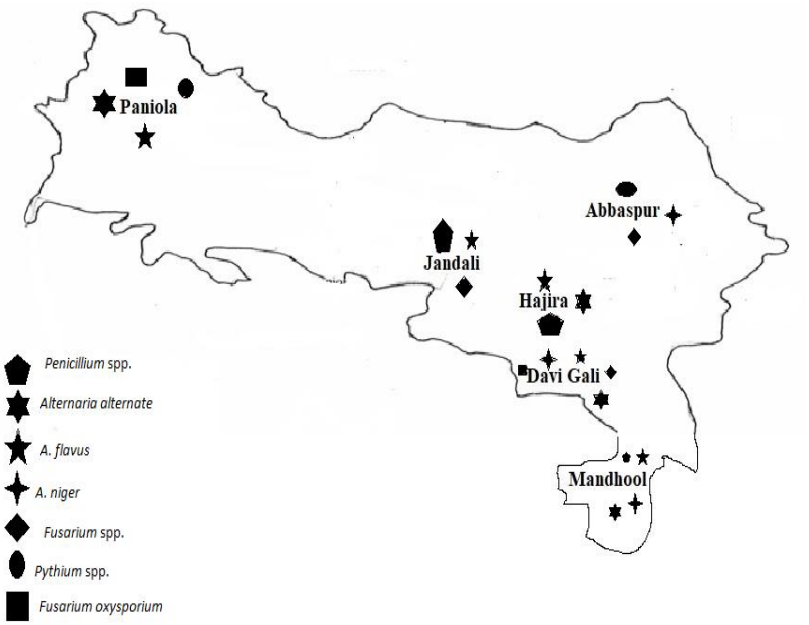

Fig. 1: Sampling sites for the collection of mycoflora from district Poonch AJK.

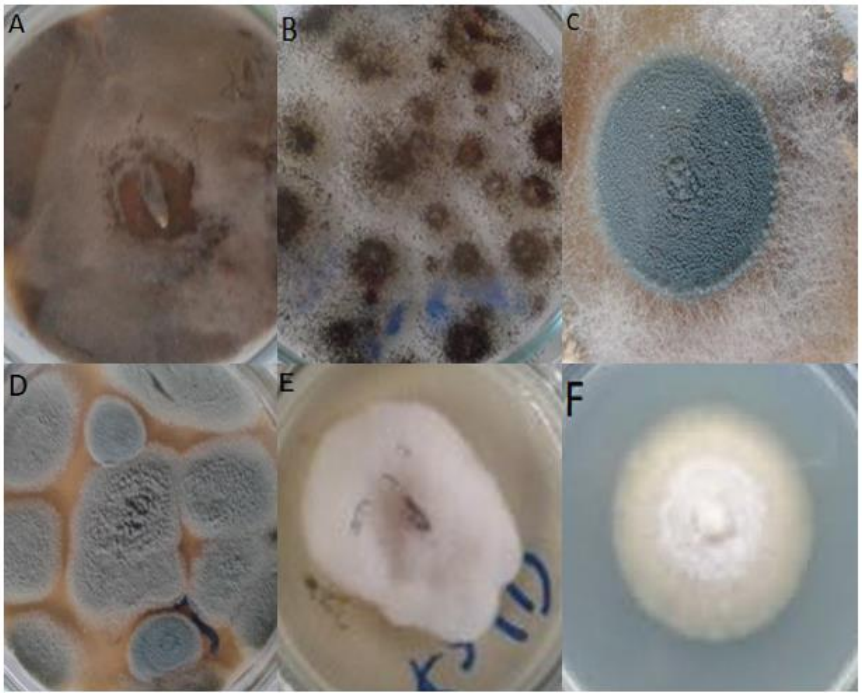

Fig. 2: Colonies of different fungal isolates.

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