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Fungi associated with Seeds of some economically important plants

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Abstract

Sixteen different species were isolated using Agar Plate method and Blotter method. Isolations were made from the seeds of twelve plants *viz*., *Zea mays* L., *Avena sativa* L., *Nigella sativa* L., *Carum copticum* (L.) Clarke, *Abelmoscus esculentus* L., *Glycine max* (L.) Merrill, *Luffa cylindrica* (L.) Roem. *Pennisetum typhoides* (Burm.) Stapf., *Brassica campestris* (L.) Czern., *Cicer arietinum* L., *Cuminum cyminum* L., and *Hordeum vulgare* L. Genera Isolated from seeds were *Aspergillus, Penicillium, Monilia, Drechslera, Mucor, Alternaria, Cladosporium, Fusarium, Acremonium, Rhizopus, Tubercularia, Phoma,* and *Trichoderma.* Among all the tested plants *Z. mays* seeds were found to be heavily colonized by fungi. , *A. flavus* Link, *A. fumigatus* Fresenius and *A. niger* Van Tieghem were the most prominent fungi isolated in present study. *C. cyminum, H. vulgare* and *C. copticum* were found to be infected only by *Aspergillus.*
Key Words: *Aspergillus*, agar plate, isolation, seeds.

Introduction

Post-harvest spoilage by filamentous fungi is one of the most important threats associated with processed and stored food products worldwide. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production has been linked to moldy contaminated foods (Moreau, 1968, Christensen and Kaufman, 1969). This situation is made worse in the tropics where the warm and humid climates provide these micro organisms with favorable conditions for their spread and subsequent establishment in numerous substrates. Three genera viz. *Fusarium, Penicillium* and *Aspergillus,* all potential mycotoxin producers, could be considered the most significant toxigenic fungi growing in processed and stored foods. Due to their capability to develop in a wide range of environmental conditions, fungi in the genus *Aspergillus* are comparatively more widespread than others (Chelkowski, 1991). Aspergilli are economically, ecologically, and medically important and constitute large genus. Consequently, special care is to be devoted to them, especially as they could play an important role in food decay and mycotoxin formation under certain storage conditions. There are many accounts of *Aspergillus* occurring in processed and stored agricultural commodities (Javaid and Anjum, 2006). Abdel-Gawad and Zohri (1993) & Mazen *et al*., (1990) documented the spectrum and levels of *Aspergillus* growing on nuts and cotton seeds-based products. The presence and incidence of *Aspergillus* spp. in cereal-based foods, milks, oils and peanuts are also well known (Manabe and Tsuruta, 1991; Adebajo, 1993). Carcinogenic aflatoxins produced by *Aspergillus* spp. are common contaminants of seeds (Klich, 2002).

In Pakistan, the phenomenon could be of great concern, especially in areas where food shortages have compelled people to consume low grade food material, even if moulds are visible as contaminants. The present study is aimed at documenting the spectrum of *Aspergillus* spp. growing on seed of selected plants.

Materials and Methods

Seeds of selected plants i.e. *Zea mays*, *Avena sativa, Nigella sativa, Carum copticum, Abelmoscus esculentus, Glycine max, Luffa cylindrica, Pennisetum typhoides, Brassica campestris, Cicer arietinum, Cuminum cyminum,* and *Hordeum vulgare* were collected from Lahore and vicinity. Agar plate and Blotter method were used for isolation of fungi (Mathur *et al*., 1975).

In Agar plate method surface seeds were plated aseptically on PDA (Potato Dextrose Agar) and in blotter method on a layer of three sterilized moisten filter papers @ 10 seeds per plate. These petriplates were incubated at 25 ^{o}C. Each sample was replicated thrice for each method. Fungi growing on different seeds were isolated, purified and identified after reference to Barron (1968), Ellis (1971), Barnett (1972) and Raper and Fennel (1965). Percentage occurrence of each genus was calculated by using the formula.

% occurrence of genus= No of colonies of a genus X 100
Total No of Colonies

Pure cultures of fungi were maintained on PDA and deposited to First Fungal Culture Bank of Pakistan. All data was analyzed by analysis of variance followed by Duncan's Multiple Range Test for mean separation. (Steel and Torrie, 1980)

Results and Discussion

Twenty seven *Aspergillus* isolates belonging to 16 species were isolated from the 12 different plant seeds samples. The *Aspergillus* species isolated from seed samples were *A. flavus, A. fumigatus, A. parasiticus, A. niger, A. sydowi, A. japonicus, A. parasiticus*, *A. alliaceus*, *A. terreus*, *A. versicolor, A. sparsus, A. clavato-flavus, A. carbonarius*, *A. auratus*, *A. pulvinus* and *Emericella nidulans*.

Among tested seeds, maximum infection was recorded in Z*ea mays* seeds as 20 isolates of fungi belonging to 10 different genera *viz*., *Aspergillus Penicillium, Monilia, Drechslera*, *Mucor, Alternaria, Cladosporium, Fusarium, Acremonium* and *Rhizopus* were screened. (Fig. 1). Six different species of Aspergilli i.e., *A. flavus, A. niger, A. alliaceus*, *A terreus, A. versicolor* and *A. glaucus* caused 30% infection in *Z. mays* seeds. In case of seeds of *G. max,* 6 fungal genera namely *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium*, *Tubercularia* and *Phoma* with almost equal percentage frequency of occurrence were recorded (Fig. 2). However only one species of *Aspergillus*, *A. flavus* was recovered in this case. Five fungal genera i. e., *Aspergillus*, *Trichoderma*, *Acremonium*, *Fusarium* and *Monilia* were identified from the seeds of *A. sativa* (Fig. 3). In this case *Aspergillus* and *Trichoderma* were equally distributed with 18% infection in *A sativa* seeds. From the seeds of *L. cylindrica*, 4 different fungal genera i.e., *Aspergillus, Mucor, Monilia* and *Fusarium* were isolated (Fig. 4). In *L. cylindrica*, aspergilli showed 60% infection in seeds. In case of *B. campestris*, (Fig. 5) *Aspergillus*, *Rhizopus*, *Monilia* and *Alternaria* were reported. In this case *Aspergillus* did not appear as the prominent genus which is the only exception in present study. Species of *Aspergillus*, *Acremonium* and *Drechslera* were identified from the seeds of *Pennisetum typhoides* (Fig. 6). Two species of *Aspergillus* i.e. *A. sydowi* and *A. japonicus* constituted 50 % infection of seeds. Seeds of both *Cicer arietinum* (Fig. 7) and *Abelmoscus esculentus* (Fig. 8) were colonized with only with one genus i.e., *Fusarium* and *Monilia*, respectively along with *Aspergillus* which is in higher percentage frequency of occurrence. It was interesting to note that *C. cyminum*, *H. vulgare* and *C. copticum* were found to be 100 percent infected only by *Aspergillus* (Fig. 9).

The 16 different species of *Aspergillus* mentioned in this paper have been reported in other food commodities elsewhere. In general, all the *Aspergillus* spp. referred to in this study are common and distributed in nature worldwide, and have been isolated in a wide array of substrates (Kozakiewicz, 1994). Mazen *et al*. (1990) isolated 12 species of *Aspergillus* from cotton seeds and cotton seeds products in Egypt. Manabe and Tsuruta (1991) isolated 17 *Aspergillus* spp. from stored rice grains. Similarly, in other study**,** Essono *et al*., 2007 reported different species of Aspergilli, some of which are same as reported in present investigation. According to them, stored food products are suitable substrates for the growth and development of *Aspergillus* spp. However, presence of other fungal species in sample analyzed may be due to the differences in methods of isolation which had a greater influence (Essono *et al*., 2007). Presently surface sterilization with NaClO of seeds allowed the colonization of variety of fungal flora, which is in line with Msikita (1995), who attributed this to the lack of a protective shell in foods such as maize, groundnuts and rice. Sauer and Burroughs (1986) argued that such a method (absence of sterilization) is likely to bring about some overestimation in the actual internal composition of the microflora population when portions of studied samples are to be plated onto culture media for analyses.

Among all tested seeds, *Z. mays* showed maximum colonization and *B. campestris* least colonized by Aspergilli members, which support earlier research showing the role of the seed coat as a barrier for invasion (Carter, 1973). Tannins, waxes, amino compounds and structural features in seed coat have been implicated in resistance to invasion by *Aspergillus* species (Horn *et al*., 2005). In contrast to mycoflora examined on other plant seeds *viz*., *C. copticum*, *C. cyminum* and *H. vulgare* were found to be 100 percent infected only by *Aspergillus* attributed to the fact the source of infection by other fungi in inoculated seeds and grain was primarily soil. Moreover, competitive ability in fungi is complex and depends on environmental variables such as substrate composition, water activity, temperature and inoculum density (Rayner and Webber, 1984). *Aspergillus* species have been reported from diverse substrates but are found most frequently in oil-rich seeds and grains (Payne, 1998) where they often have a competitive advantage over other

fungi at water activities of <0.96 and a temperature of approximately 30 °C (Marín *et al.*,1998). In addition to environmental parameters, physiological characters such as rate of spore germination, rate of mycelial growth and enzymatic capability greatly influence competitive saprophytic ability in fungi (Garrett 1970).

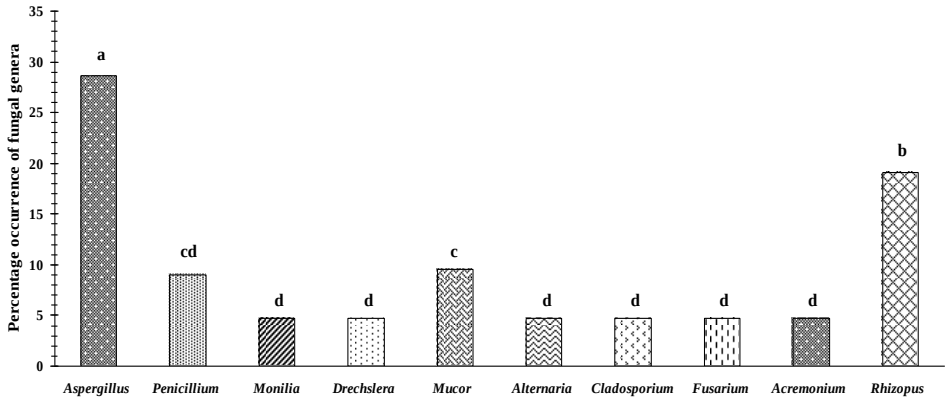

Fig 1: Fungal genera isolated from *Zea mays* seeds.

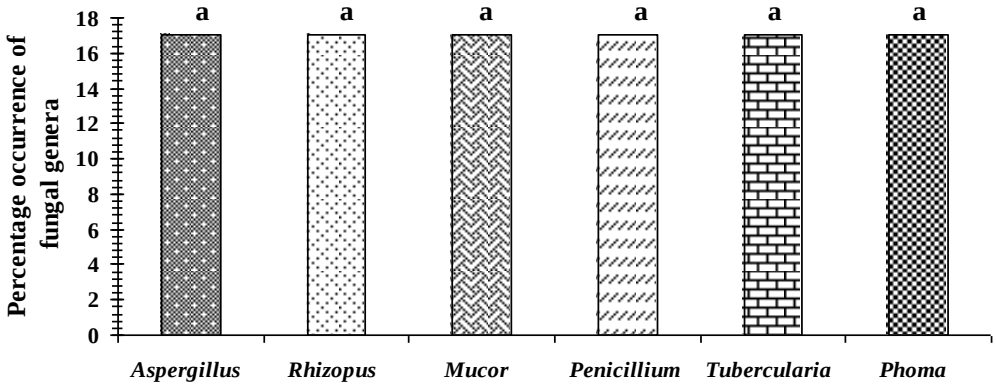

Fig 2: Fungal genera isolated from *Glycine max* seeds.

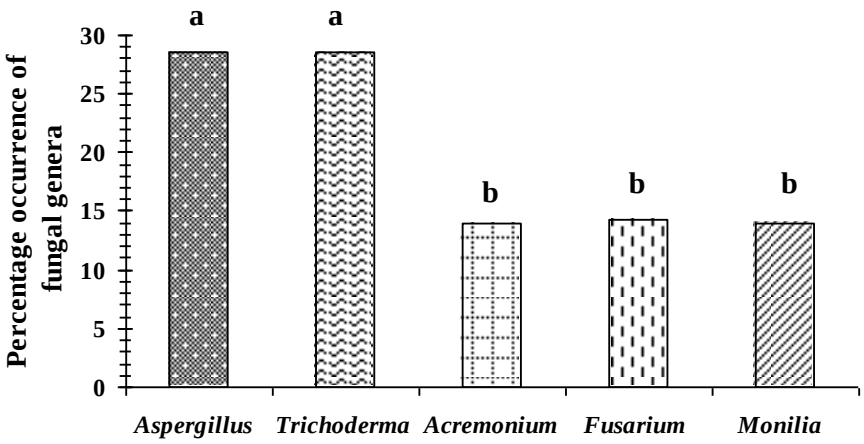

Fig 3: Fungal genera isolated from *Avena sativa* seeds.

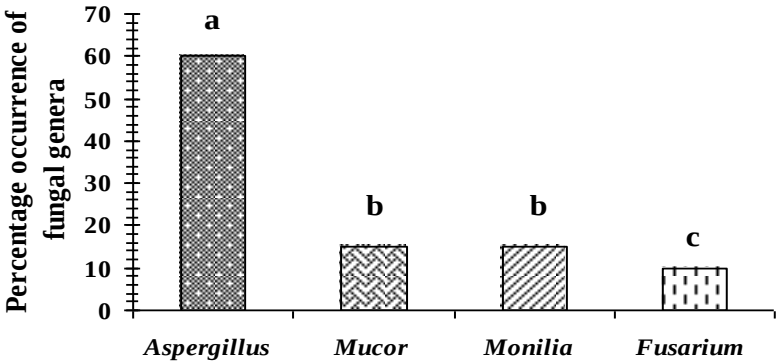

Fig 4: Fungal genera isolated from *Luffa cylindrica* seeds.

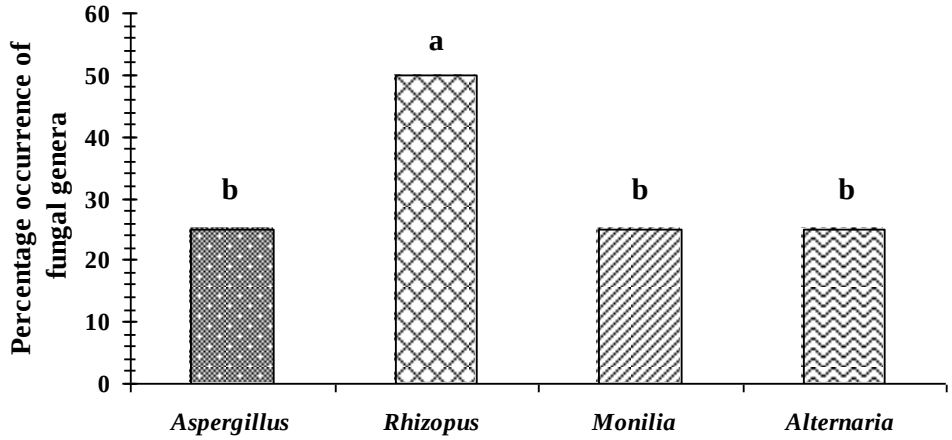

Fig 5: Fungal genera isolated from *Brassica campestris* seeds.

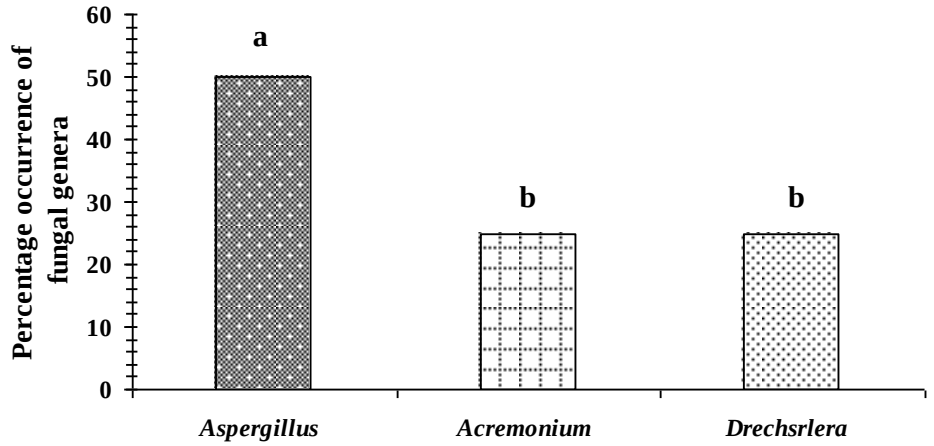

Fig 6: Fungal genera isolated from *Pennisetum typhoides* seeds.

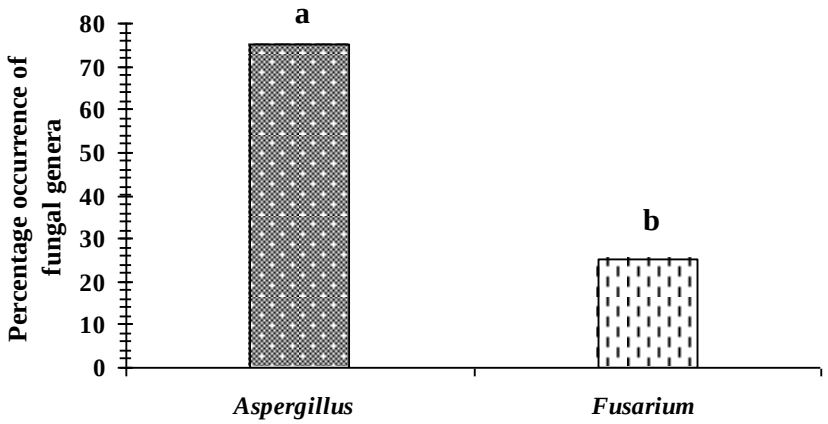

Fig 7: Fungal genera isolated from *Cicer arietinum* seeds.

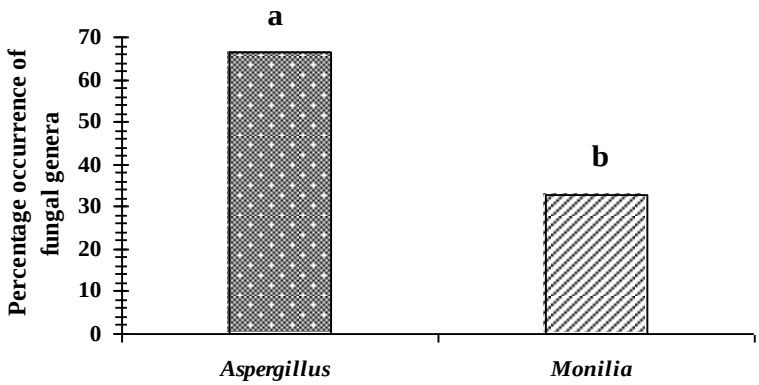

Fig 8: Fungal genera isolated from *Abelmoscus esculentus* seeds.

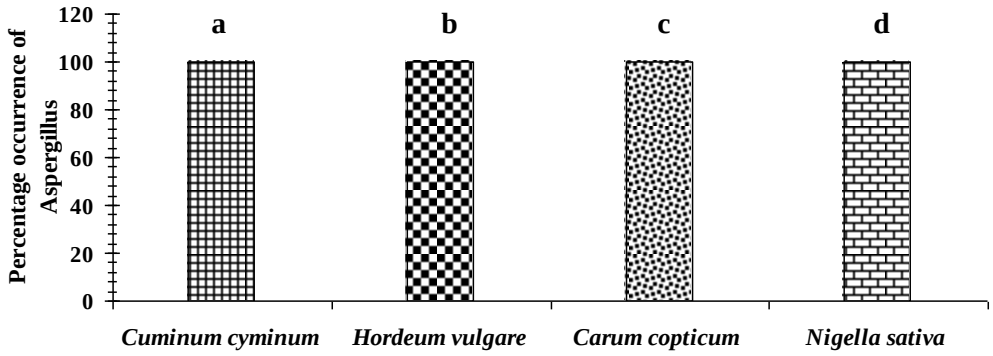

Fig 9: Aspergilli isolated from seeds.

Note: Values with different letter show significant difference (P≤ 0.05) as determined by Duncan's Multiple Range Test.

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