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Screening of Seed Born Mycoflora of Wheat, Rice, Gram and Mustard

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ABSTRACT

Various food grains are stored for different purposes. During storage these may be contaminated by a number of fungal species. During present study, a survey of fungal flora of food grains like wheat (*Triticum vulgare*), rice (*Oryza sativa*), bengal gram (*Cicer arietinum*) and mustard (*Brassica campestris*) have been investigated. Altogether, 12 species of fungi from wheat, 11 from rice, 10 from gram and 13 species of fungi were isolated from mustard. The fungi were *Aspergillus niger A. flavus, A. terreus, A.sydowi, A. nidulans, Penicillium spp., Alternaria alternata, Curvularia lunata, Fusarium moniliformae, Rhizopus stolonifer, Chaetomium spinossum* spp., *Drechslera hawaiiensis, Helminthosporium* spp., *C. globossum*. The most dominant species on the four food grains were *Aspergillus niger* and *Penicillium* spp. Fungi of the genus *Aspergillus* and *Penicillium* were present on 100% of grain samples tested. The spread and subsequent development of fungi on grains was found to depend on the period of storage and climatic conditions particularly temperature and rain fall. This was evident from the result that after harvest and storage of fresh grains, the number of fungal species and their population gradually increased in succeeding months.

Key words : Food Grain, Storage Fungi, Czapec-dox medium, Seed fungi association, Fungal load.

INTRODUCTION

Chhattisgarh is one of the important food grain producing province of India where paddy or rice (*Oryza sativa*) is the most important crop. However, wheat (*Triticum vulgare*) gram (*Cicer arietinum*) and mustard (*Brassica campestris*) are also grown in this region. In practice, food grains are transferred to various storage systems. These seeds play vital role for the healthy production of crop. They are carriers of some important seed born diseases causing microorganisms which cause considerable losses in the yields (Abou-Heilah, 1984). Several diseases of crops are transmitted through seeds. Association of certain fungi with seeds have been reported by various workers (Christenson and Kaufmann, 1964 ; Flanningan, 1970 ; Roy *et.al*., 1971; Bilgrami *et.al*., 1980). Seed-fungi association has been reported to be a complex phenomenon. Besides deterioration in the physical-chemical quality of seed grains, fungi also affect germinability of seeds. (Gohari *et.al*., 2007; Adisa, 1994; Klyszejko *et. al.,* 2005, Kroiakova *et.al*., 1989). However; stimulatory effect of certain fungal forms have been reported by some workers (Roy and Pandey, 1971). Likewise, seed coat leachates have been reported to cause inhibitory effect on certain seed fungi (Shrivastava and Mishra, 1971). The condition of seed-fungi association becomes complex due to variation in seed and its fungi due to different varients including the seed age and climatic conditions at the site of the activity. The same type of seed may be acted upon by different microorganisms under different climatic conditions

with diverse results. It is therefore, worthwhile to investigate the interrelationship between different seeds collected from various localities and the fungi associated with them.

Studies on the mycoflora of crop seeds from different parts of the world have established that species of *Alternaria, Aspergillus, Penicillium, Aureobasidium, Botrytis, Cephalosporium, Chaetomium, Cladosporium, Curvularia, Drechslera, Epicoccum, Fusarium, Griphosphoeria, Helminthosporium, Leptosphaeria, Nigrospora, Phoma and Rhizopus* are commonly pathogenic to these seeds (Flanningan, 1978 ; Shulten, 1982; Vimla, 1982; Abou-Heilah, 1984). In India also, many workers have worked on the mycoflora of the food grains like rice (Jeswal, 1986), wheat (Sinha and Sinha, 1988), pulses (Sinha *et.al.*, 1981) and oil seeds (Vaidya and Dharmvir, 1989). However, very little information on the fungal association with food grains in Chhattisgarh is available. Therefore, the present piece of work was started with the objective to isolate and identify the fungal species infesting on the four food grains viz., rice, wheat, gram and mustard.

Methodology

Food grain sampling

Two samples of every food grain under study were collected from various shopkeepers of Bilaspur city at 15 days interval. Sample size was 100 gram which were collected in separate polythene packets. The latter were carried to laboratory and refrigerated to check further fungal contamination of grains.

Mycological analysis

Such analysis was made by homogenate culture method. For this purpose 1.00 gm of grains were soaked overnight in pre-sterilized distilled water and homogenised in a mortar pastle. The homogenate was filtered with double layered muslin cloth and supernatant was used for further studies. It was diluted serially and 1.00 ml of the 3rd dilution was plated on petriplates containing culture medium. The plates were incubated at 30°C and the fungal colonies growing after 72 hours of growth were recorded and counted.

Culture medium

Czapec-dox agar culture media was used for isolation of fungi, from storage grains. It has following constitution -

Dextrose	-	10.00 gm
$NaNO_3$	-	6.00 gm
KCl	-	0.52gm
$MgSO_4$	-	0.52gm
$KH_2 PO_4$	-	1.52gm
$CuSO_4$	-	Traces
$ZnSO_4$	-	Traces
$FeSO_4$	-	Traces
Peptone	-	2.0 gm
Yeast Extract	-	1.5 gm
Casein Hydrolysate	-	1.0 gm
Vitamine Mixture	-	1.00 ml

(Containing biotin, pyridoxin nicotinamide, theamine, riboflavin)

Identification of Fungal Species

Isolates were sub-cultured by dilution plating and then identified by using the morphological criteria described by Ellis (1976), Hanlin (1990) and Raper and Fennell (1965).

Purification and Maintenance of Fungal Cultures

All the Fungi isolated from food grain samples were purified by the method of single colony isolation. Pure cultures were maintained at lower temperature (< 5°C) on slants containing supplemented czapec-dox culture media.

Investigation of Fungal Load on the four food grains

Total fungal load on the four food grains was also studied during present investigation. This has been represented in the form of fungal colony forming units(C.F.Us.). For this purpose, 2.0 grams of each sample was mixed with 100 ml of normal saline containing 0.05% tween-80. This was shaken on a horizontal shaker for 30 minutes at normal speed. The suspension now contained conidia/ spores or segments of hyphae emerging out from the seed surface. 1.00 ml of 3rd dilution of this filtered suspension was plated on supplemented czapec dox medium plates and the fungal growth was counted after 72 hrs of growth at 30°C. The fungal load on the four crop seeds have been presented in Table-2.

RESULTS AND DISCUSSION

The outcome of mycological studies carried out during January to June, 2009 on food grains collected from different shop-keepers of Bilaspur revealed that all the samples of rice, wheat, gram and mustard are highly contaminated with fungi. This is evident from data presented in Table-1 that altogether 11 fungal species were present in rice and 12 in wheat. Likewise, samples of gram or bengal gram exhibited contamination of 10 fungal species. From mustard 13 fungal species could be isolated. Among these, the fungal species were *Aspergillus niger, A. flavus, A. terreus, A. sydowi, Penicillium* spp., *Alternaria alternata, Curvularia lunata, Fusarium moniliformae, Rhizopus stolinifer, Chaetomium spinossum, C.globossum, Cladosporium* spp., *Drechslera hawaiiensis, Helminthosporium* spp., *Aspergillus nidulan* and *Trichoderma* spp. *Aspergillus* spp. and *Penicillium* spp. were specific to wheat. Drechslera hawaiensis was prevalent only on rice and gram. *Aspergillus nidulans* and *Trichoderma* sp. were infesting only on mustard. *C. spinosum* was totally absent in gram.

Table 1: List of fungi isolated from the four food grain under study

S. No.	Fungal Species	Food grains			
		Wheat	Rice	Gram	Mustard
1	*Aspergillus niger*	+	+	+	+
2	*A. flavus*	+	+	+	+
3	*A. terreus*	+	-	-	+
4	*A. sydowi*	+	-	+	+
5	*Aspergillus nidulans*	-	-	-	+
6	*Penicillium* spp.	+	+	+	+
7	*Alternaria alternata*	+	+	+	+
8	*Curvularia lunata*	+	+	+	+
9	*Fusarium moniliformae*	+	+	-	+
10	*Rhizopus stolonifer*	+	+	+	+
11	*Chaetomium spinossum*	+	+	-	+
12	*C. globossum*	+	+	+	+
13	*Cladosporium* spp.	+	-	-	-
14	*Drechslera hawaiiensis*	-	+	+	-
15	*Helminthosporium* spp.	-	+	+	+
16	*Trichoderma* spp.	-	-	-	+
Total	12	11	10	13	

Table 2: Year round (2009) fungal load on rice, wheat, gram and mustard.

S. No.	Month	Fungal load (CFUs/2.0g of grains)			
		Rice	Wheat	Gram	Mustard
1	January	3.2×10^4	7.1×10^5	6.3×10^5	2.0×10^5
2	February	4.1×10^4	6.5×10^5	5.8×10^5	1.6×10^5
3	March	8.0×10^5	8.1×10^5	2.9×10^5	1.1×10^4
4	April	8.1×10^5	8.8×10^4	2.6×10^4	6.3×10^4
5	May	8.0×10^5	8.1×10^4	3.6×10^4	9.3×10^5
6.	June	1.0×10^6	3.0×10^6	1.1×10^6	1.0×10^6

Fusarium moniliformae did not show its appearance in any of the samples of gram. *Cladosporium* appeared only in the samples of wheat while it never showed the contamination of *Helminthosporium* sp.

Total fungal load on the four food grains was also studied during present investigation which was represented in the form of colony forming units (CFUs). Such studies were made from January 2009 to June 2009. The results thus obtained revealed that in wheat the fungal CFUs varied from 8.1×10^4 to 3.0×10^6. In rice samples it ranged between 3.2×10^4 - 1.0×10^6. Likewise, in gram and mustard the range of fungal contamination were 2.6×10^4 - 1.3×10^6 and 1.1×10^4 - 2.6×10^6. It should be considered worthy of attention that the high fungal contamination load on all the four food grains was in the month of June. In contrast, the lowest contamination was in the month of harvesting of the crop. That means it was in April for wheat, January for rice, April for gram and March for mustard. (Table - 2). In this way, the present findings clearly illustrate that there is a gradual contamination of food grains during storage and as a suggestion it is needed to ensure strict safety measures for their safe storage.

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