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Prevalence of seed mycoflora from different seed of spices under field and storage conditions of Agra region

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ABSTRACT

Coriander, Foeniculum, Cumin and Brassica seeds were evaluated for field and storage fungi from large number of samples from Agra region. The percentage prevalence of these fungi evaluated. More than 53 fungi of different group recorded for toxin production.

Key words: Coriander, Foeniculum, Cumin and Brassica seeds mycoflora, prevalence

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INTRODUCTION

Several fungi found on market seeds used in spices are known to cause considerable damage either directly to those that carry them or to the crops that are raked from such contaminated seed stocks. Generally, we see that the condiments which are used daily in our food material and which are easily stored in our houses get infected by fungi after some time. Also the viability of seed is lost due to the effect of fungi found on the surface of the seeds and loses their viability rapidly during storage. For laying down health standards against seed borne diseases of condiments considerable background information is needed with regard to mycoflora associated with such seeds, their role if any, on the disease outbreaks and the nature and extent of damage they cause. Therefore, due to economic importance in view of Indian scenario the following spices have been selected for the study of seed mycoflora and other investigations:

MATERIALS AND METHODS

The periodic survey for the collection of different seeds of spices was conducted from January 2007 to December 2008. The fresh and stored seeds of *Coriander*, *Foeniculum*, *Cumin* and *Brassica* were collected in and around different rural and city seeds markets of Agra. These samples weighing 15-20 gram each were collected in small paper / polythene packets, properly leveled and bought into the laboratory. A part of the samples were kept and stored under different conditions of temperature and humidity for further investigations. The other part of the samples 5-10 grams each were analyzed for fungal flora. Seeds are highly complex living substance. It contains diverse group of micro-organisms including both parasites and saprophytes. The storage (seeds of spices) was done for a period of one year and the fungi associated with the stored material both externally and internally were detected after a regular interval of three months employing following techniques

The different seed samples collected earlier were examined in dry condition under low power sterio binocular microscope for seed abnormality, spores load on seed surface, fruiting structures of different fungi and removal of

other infected plant parts or soil particles. Two gm. of seeds from each storage condition were taken separately in a sterilized conical flask, having 10 ml of sterilized distilled water. These flasks were then shaken vigorously with the help of electric shaker for one hour and the liquid was centrifuged at 3000 rpm for 30 minutes. The supernatant was decanted off and the sediment was diluted in 5 ml of sterilized distilled water for plating at the rate of 1 ml /Petri plate. The sterilized plates were poured with 18-20 ml of sterilized Czpeck agar containing streptomycin and having 1 ml of the washing onto each plate. The plates were rotated gently to have uniform mixing of the two. Five replicates of each were incubated at 28 degree C for 7-8 days. Total population (Colony Forming Unit CFU) and their percentage abundance for each fungal species calculated / replicate of seeds samples.

Three layers of blotter in double sets (size equivalent to each Petri dish) were soaked in distilled water and kept in each sets of upper and lower lid of the dish. The surplus water was drained out from the plates. Fixed number of seeds was placed in circle at an equal distance in Petri plate. After plating the seeds the Petri plates were incubated for seven days at 20-22 degree centigrade near UV or ordinary tube light with a cycle of 12 hours light and 12 hours darkness. The seeds were examined on the 8^{th} day under stereomicroscope. The identification was done on the basis of sporulation, arrangement of conidia with conidiophores and other fruiting structures. The final Identification was done with help of standard books on fungal taxonomy.

Isolation of internal seed borne pathogen was done by Agar Plate Methods in which Pre sterilized (Hot air) Petri plates were taken and plated either with sterilized Potato Dextrose Agar, Carrot Potato Agar, Malt Extract Agar, Oat Meal agar and Czpeck agar etc. On solidification of agar, pre sterilized seeds (by soaking for two mts. in 1% KoH solution) were selected and plated (10 seed each) aseptically in a circle with equal distance on the agar medium. Such plates were then incubated at 20-25 degree centigrade for 10 days. After the incubation these plates were examined under the dissecting binocular for the colony development of pathogenic fungi coming out from each seeds. A small bit of the mycelium was cut and transferred either on culture tube or on the Petri plates having PDA or CPA media. When these fungi were fully grown were mounted on slide into a drop of stain and glass cover slipped. The excess stain blotted on out, margin sealed. Such slides examined through under research microscope for generic and species level identifications.

Rolled Towel Paper method was adopted by using **t**he Kraft's paper or filter paper having the capillary movement of water at vertical direction (30 mm rise/ min.). The paper used was free from any toxic materials, fungi, bacteria and had sufficient water holing capacity during the process of experiments. The Texture was as such that the roots of germinating seedlings grew on and not into the paper. The seeds were placed between the paper had been, rolled in to the towels. The rolled towel was placed in an incubator (20-25 degree C) in an upright position for 10 days. These seeds were then examined for seed mycoflora as well germinability and root infection by any pathogenic fungi.

RESULTS AND DISCUSSION

The results obtained on prevalence, isolations and identification of seed mycoflora of different spices conducted on Storage fungi parasitizing *Brassica juncea, Coriandrum sativum, Cumin cyminum* and *Foeniculum vulgare* are presented for Agra region from 2006 to 2009. These seeds were brought into the laboratory and analyzed .Two hundred seeds of each sample were tested through Blotter test. The unsterilized seeds were plated on presoaked (in water) blotter and placed on lower lid on which 20-25 seeds were placed. The plates were covered with upper lid which also was soaked in water in double layers.

For Agar test again 200 infected seeds were surface sterilized with sodium hypochlorite (3%) for 3 min. and washed several times with sterilized water, then transferred to plates containing Czapek's/Potato Dextrose medium. Plates were incubated at 25°C for the growth of storage fungi. Fungi isolated were purified and identified according to Raper and Fennell (1965), Gilman (1957) and Barnett and Hunter (1972). Fungal cultures were maintained on PDA slants until needed.

Seeds were surface sterilized with sodium hypochlorite (3%) for 3 min. and washed several times with sterilized water, then transferred to plates containing Czapek's/Potato Dextrose Agar medium. Plates were incubated at 25°C for the growth of storage fungi

Prevalance of storage fungi from seeds of different spices

The study on prevalence of different group of fungi on seeds of Brassica *juncea* (Table1), *Coriandrum sativum* (Table2), *Cumin cyminum* (Table3) and *Foeniculum vulgare* (Table 4) have been carried out through Blotter test and Agar plating methods. The percentage infection by fungi from each sample was calculated and depicted in the following tables.

Table 1: Prevalance of seed mycoflora on *brassica juncea*

S.NO.	FUNGI ASSOCIATED	NO.SEEDS INFECTED	% SEEDS INFECTED
1	*Absidia glauca*	23	05.75 %
2	*Aspergillus flavus*	35	08.75 %
3	*Aspergillus niger*	30	07.50 %
4	*Aspergillus fumigatus*	65	16.25 %
5	*Aspergillus oryzae*	40	10.00 %
6	*Cladosporium cladosporio*	25	06.25 %
7	*Chaetomium indicum*	22	05.50 %
8	*Fusarium chlamydosporu*	25	06.25 %
9	*Phoma exigua*	32	08.00 %
10	*Rhizoctonia solani*	15	03.75 %
11	*Sclerotium rolfsii*	05	01.25 %
12	*Trichoderma viride*	28	07.00%
13	*Emericella nidulanse*	23	05.75 %
14	*Epicoccum purpurascence*	19	04.75 %
15	*Glomerella cingulata*	13	03.25 %
	Total seeds examined	400	

Table 2: Prevalance of seed mycoflora on coriandrum sativum

S.NO.	FUNGI ASSOCIATED	NO.SEEDS INFECTED	% SEEDS INFECTED
1	*Aspergillus flavus*	35	08.75 %
2	*Aspergillus niger*	22	05.50%
3	*Aspergillus parasiticus*	31	07.75%
4	*Chaetomium globosum*	15	03.70%
5	*Curvularia pallescence*	23	05.75%
6	*Cladosporium oxysporum*	15	03.75%
7	*Colletotrichum gloeosporioides*	10	02.50%
8	*Cunninghamella echinulata*	25	04.00%
9	*Drechslera specifer*	17	04.25%
10	*Fusarium moniliforme*	40	10.00%
11	*Memnoniella echinulata*	45	11.25%
12	*Microascus cinereous*	21	05.25%
13.	*Myrothecium roridrum*	12	03.00%
14	*Nigrospora oryzae*	15	03.75%
15	*Penicillium citrinum*	21	05.25%
16	*Phoma glomerata*	20	08.00%
17	*Rhizoctonia solani*	05	03.70%
18	*Rhizopus stolonifer*	16	04.00%
19	*Trichothecium roseum*	12	03.00%
	Total Seeds tested	400	

Table 3: Prevalance of seed mycoflora on *cumin cyminum*

S.NO.	FUNGI ASSOCIATED	NO.SEEDS INFECTED	% SEEDS INFECTED
1	*Absidia corymbifera*	17	04.25%
2	*Acremonium kiliense*	23	08.25%
3	*Alternaria alternate*	35	08.75%
4	*Aspergillus clavatus*	20	05.00%
5	*Aspergillus flvus*	30	07.50%
6	*Aspergillus terreus*	35	08.75%
7	*Botryodiplodia theobrome*	25	06.25%
8	*Cunninghamella echinulata*	12	03.00%
9	*Fusarium oxysporum*	34	04.25%
10	*Fusarium solani*	27	06.75%
11	*Neosartoria fischeri*	22	05.50%
12	*Peniciliium citrinum*	32	07.10%
13.	*Peniciliium italicum*	27	06.75%
14	*Trichoderma virense*	22	05.50%
15	*Trichoderma viride*	38	09.50%
	Total seeds examined	400	

Table 4: Prevalance of seed mycoflora on *foeniculum vulgare*

S.NO.	FUNGI ASSOCIATED	NO.SEEDS INFECTED	% SEEDS INFECTED
1	*Acremonium strictum*	18	04.50%
2	*Aspergillus flavus*	35	08.75%
3	*Aspergillus oryzae*	26	06.50%
4	*Chaetomium bostrychoides*	27	06.75%
5	*Curvularia lunata*	15	03.75%
6	*Drechslera rostrata*	20	05.00%
7	*Fusarium oxysporum*	30	07.50%
8	*Fusarium pallidoroseum*	19	04.75%
9	*Microascus cinereous*	28	07.00%
10	*Mucor circinelloides*	17	04.25%
11	*Paecilomyces variotii*	22	05.50%
12	*Penicillium chrysogenum*	32	08.00%
13.	*Periconia byssoides*	10	02.50%
14	*Phoma glomerata*	17	04.25%
15	*Rhizopus stolonifer*	14	03.50%
16	*Nigrospora oryzae*	12	03.00%
17	*Trichoderma harzianum*	25	06.25%
18	*Verticillium alboatrum*	33	08.25%
	Total Seeds tested	400	

TABLE 5: Total number of storage fungi isolated from four different samples collected around markets of Agra

S.NO.	FUNGI ASSOCIATED	NO.SEEDS INFECTED	% SEEDS INFECTED
1	*Acremonium kiliense*	23	01.43%
2	*Acremonium strictum*	18	01.25%
3	*Absidia corymbifera*	17	01.06%
4	*Absidia glauca*	23	01.43%
5	*Alternaria alternate*	35	02.18%
6	*Aspergillus clavatus*	20	01.25%
7	*Aspergillus flavus*	135	08.43%
8	*Aspergillus fumigatus*	65	04.00%
9	*Aspergillus niger*	52	03.25%
10	*Aspergillus oryzae*	66	04.12%
11	*Aspergillus parasiticus*	31	01.97%
12	*Aspergillus terreus*	35	02.18%
13.	*Botryodiplodia theobrome*	25	01.56%
14	*Chaetomium bostrychoides*	27	01.68%
15	*Chaetomium globosum*	15	00.93%
16	*Chaetomium indicum*	22	01.37%
17	*Cladosporium cladosporioides*	25	01.56%
18	*Cladosporium oxysporum*	15	00.93%
19	*Curvularia lunata*	15	00.93%
20	*Curvularia pallescence*	23	01.43%
21	*Colletotrichum gloeosporioides*	10	00.63%

22	*Cunninghamella echinulata*	37	02.31%
23	*Drechslera specifer*	17	01.06%
24	*Drechslera rostrata*	20	01.25%
25	*Emericella nidulanse*	23	01.43%
26	*Epicoccum purpurascence*	19	01.18%
27	*Fusarium chlamydosporum*	25	01.56%
28	*Fusarium moniliforme*	40	02.50%
29	*Fusarium oxysporum*	64	04.00%
30	*Fusarium pallidoroseum*	19	01.18%
31	*Fusarium solani*	27	01.68%
32	*Glomerella cingulata*	13	00.81%
33	*Memnoniella echinulata*	45	02.81%
34	*Microascus cinereous*	48	03.00%
35	*Mucor circinelloides*	17	01.06%
36	*Myrothecium roridrum*	12	00.75%
37	*Mucor circinelloides*	22	01.37%
38	*Nigrospora oryzae*	27	01.68%
39	*Periconia byssoides*	10	00.62%
40	*Paecilomyces variotii*	22	01.37%
41	*Penicillium citrinum*	53	03.31%
42	*Penicillium chrysogenum*	32	02.00%
43	*Peniciliium italicum*	27	01.68%
44	*Phoma glomerata*	37	02.31%
45	*Phoma exigua*	32	02.00%
46	*Rhizoctonia solani*	05	00.31%
47	*Rhizopus stolonifer*	30	01.87%
48	*Sclerotium rolfsii*	05	00.31%
49	*Trichoderma harzianum*	25	01.56%
50	*Trichoderma virense*	22	01.37%
51	*Trichoderma viride*	66	04.12%
52	*Trichothecium roseum*	12	00.75%
53	*Verticillium alboatrum*	30	01.87%
	Total Seeds tested	1600	

The following fungi isolated from infected seed of *Brassica juncea, Coriandrum sativum, Cumin cyminum and Foeniculum vulgare* have been listed against their four range of availability in percentage

(1) Range from 00.00 to 00.09 % (*Chaetomium globosum, Cladosporium oxysporum, Curvularia lunata, Myrothecium roridrum, Rhizoctonia solani, Sclerotium rolfsii, Trichothecium roseum*) 2). Range from 01.00 to 01.99 % *(Acremonium kiliense, Acremonium strictum , Absidia corymbifera, Aspergillus clavatus, Aspergillus parasiticus , Botryodiplodia theobrome., Cladosporium cladosporioides, , Curvularia pallescence,, Drechslera specifer, Drechslera rostrata, Emericella nidulanse, Epicoccum purpurascence, Fusarium chlamydosporum, Fusarium pallidoroseum,, Fusarium solani, Mucor circinelloides, Mucor circinelloides, Nigrospora oryzae, Paecilomyces variotii, Peniciliium italicum, Trichoderma harzianum, Trichoderma virense, Trichoderma viride, Verticillium alboatrum.*) 3). Range from 02.00 to 02.99 % (*Alternaria alternate, Aspergillus terreus, Cunninghamella echinulata, Fusarium moniliforme, Memnoniella echinulata, Penicillium chrysogenum, Phoma glomerata* and *Phoma exigua*) 4).Range from 03.00 to 03.99 % (*Microascus cinereous, Penicillium citrinum).* 5) Range from 04.00 to 04.99 % (*Aspergillus fumigatus, Aspergillus oryzae, Fusarium oxysporum, Trichoderma viride)* 6).Range from 05.00 to 08.99 % (*Aspergillus flavus),*

The microbiological quality of spice samples had been demonstrated (Table 1, 2, 3, 4, 5) and that microbial counts vary according to individual spice, the harvest and storage conditions prior to drying. So, the observed counts are thus a reflection of the original fungal load, of growth, as well as of die-off which are probably enhanced by oxidation and the presence of active compounds in spices. The total percent fungal count on four spices varies from 05.00 to 08.99 % to *Aspergillus flavus* infestation, followed to 04.00 to 04.99 % for Aspergillus fumigatus,Aspergillus oryzae, Fusarium oxysporum, Trichoderma viride infection, 03.00 to 03.99 % to Microascus cinereous, Penicillium citrinum infection. 02.00 to 02.99 % to Alternaria alternate, Aspergillus terreus, Cunninghamella echinulata, Fusarium moniliforme, Memnoniella echinulata, Penicillium chrysogenum, Phoma glomerata and Phoma exigua infection. 01.00 To 01.99 % to Acremonium kiliense, Acremonium strictum, Absidia corymbifera, Aspergillus clavatus, Aspergillus parasiticus, Botryodiplodia theobrome., Cladosporium cladosporioides, , Curvularia pallescence,, Drechslera specifer, , Drechslera rostrata, Emericella nidulanse,,

Epicoccum purpurascence, Fusarium chlamydosporum,, Fusarium pallidoroseum,, Fusarium solani, Mucor circinelloides, Nigrospora oryzae, Paecilomyces variotii, Peniciliium italicum, Trichoderma harzianum, Trichoderma virense, Trichoderma viride, Verticillium alboatrum. Infection and least 00.00 to 00.09 % to Chaetomium globosum, Cladosporium oxysporum, Curvularia lunata, Myrothecium roridrum, Rhizoctonia solani, Scleroderma rolfsii, and Trichothecium roseum infection. Similar results were obtained by Athar et al (1988), while studying mycoflora of Indian spices, Aziz et al (1998), on contamination of some spices by fungi, Chahal (1981) on seed borne infection of Alternaria brassicae in Indian Mustered and its elimination during storage. Chand, , et al. (2000) on Alternaria spp associated with cumin (Cuminum cyminum L.) seeds, Christenson and Kaufmann(1965), on deterioration of stored grains by fungi, Deena and Basuchaudhury (1984)on Seed mycoflora of Chilies., Dwivedi et al (1982) fungi isolated from seed of spiced stored at different length of time, Elwakil and Ghoneem (1999) on detection and location of seed-borne fungi of Black *Cumin* and their transmission in seedlings, Geetha and Reddy(1980) on *Aspergillus flavus* and its occurrence in relation to other mycoflora on stored spices. Hashmi, and Ghaffar (1991) on seed-borne mycoflora of Coriandrum sativum L from Pakistan, Mandeel, (2005) on fungal contamination of some imported spices, Mousuymi and Sarkat (2003) for microbiological quality of some retail spices in India. Purohit and Bohra (1999) on seed mycoflora associated with some important spice seeds. Rani and Aggarwal (1995) on qualitative and quantitative estimation of seed mycoflora of some spices. Regina and Tulsi (1988) on fungi associated with fenugrass seed and their role in seed deterioration during storage. Srivastava and Jain (1992) on seed mycoflora of some spices. Srivastava and Chandra (1985) on qualitative and quantitative estimations on seed mycoflora of some spices in India.

Without having knowledge of correct identity of a fungal organism, all studies concerning the seed deterioration during storage by fungi are misleading. Hence identification and taxonomic characterization of all 54 different storage fungi from four different spices were conducted through the development of axenic cultures. These fungi were made for micro slides and Camera Lucida drawings, microphotographs and identified as per literature of Booth, C. (1971) for genus *Fusarium*, Ellis (1976) for Dematiaceous Hyphomyctes. , Kozakiewicz (1989) for Aspergillus species on stored products; Pitt (1985) a laboratory guide to common *Penicillium* species, Raper and Fennell, (1977) for genus *Aspergillus*, Samson, *et al*. (1988) for food borne fungi, Samson, *et.al* (1995) for food-borne Fungi (fourth edition), Christensen and Kaufmann (1965) for deterioration of stored grains by fungi.

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