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Detection and identification of seed borne myco flora of wheat (*Triticum aestivum* L. em. The ll.) Seed samples

Hansraj Dhakar, RS Ratnoo and Anita Jat

Abstract
Seed borne myco flora of Wheat in two district of southern Rajasthan (Chittorgarh and Udaipur) were surveyed. A total of 80 seed samples, 40 of each district, were collected during 2015-16. Data were recorded for seed germination percentage, percent pathogen frequency and major seed borne myco flora, which were identified and quantified using the blotter and agar plate method as recommended by ISTA (International Seed Testing Association).Seed germination percentage were found minimum 82.9% in Chittorgarh district CH-6 sample and maximum 86.10% in CH-14, CH-40 (Chittorgarh), UD-14 and UD-21 (Udaipur). Eight wheat seed borne myco flora *viz., Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme, Rhizopus stolonifer, Mucor* spp. and *Trichoderma viride* were detected and isolated from eighty seed samples by using standard blotter paper and agar plate methods. The maximum infected embryo was observed in Chittorgarh district seed samples while minimum was observed in Udaipur district seed samples.

Keywords: wheat, pathogen frequency, seed borne, myco flora, embryo

1. Introduction
Seed borne diseases have been found to affect the growth and productivity of crop plants. A seed borne pathogen present externally or internally or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection. Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Besides, the mold fungi which grow on the seed substratum produce mycotoxins which are hazardous to humans and animals (Halt, 1994) [6]. Studies were carried out to study the composition of seed borne myco flora occurring in wheat. The significance of sustainable agricultural production is hidden in the use of quality seed. It is the most crucial and vital input for enhancing productivity. Since seed is the custodian of the genetic potential of the cultivars, the quality of the seed determines the limits of productivity to be realized in a given cropping system. Though seeds are of great economic interest and also contribute a major part of diet, they play a vital role in associating microorganism, which prove hazardous for the seed or the new plant created from it, so, any infections agent (bacteria, fungi, nematode, etc.) which is associated with seeds having potential of causing a disease in a seedling or plant, is teemed as seed borne pathogen (Agarwal, 1996) [1].
Hence, the storage fungi are especially insidious because they invade seeds stored at moisture contents that practical grain men consider safe and often cause serious damage before their presence even suspected. Therefore, with few exceptions spoilage of stored fungi, this may be introduced during the post harvest handling process. It is well known fact that several fungi are known to cause considerable damage to seeds in storage and produce various activities. It is in view of this that the current study aimed at detecting seed borne fungal pathogen wheat seed sample of two districts (Chittorgarh and Udaipur) of southern Rajasthan.

2. Materials and Methods
Experimental location
The experiment was conducted at Department of Plant Pathology, Rajasthan College of Agriculture (RCA) MPUAT, Udaipur during 2015-16.

Sources of experimental materials
Eighty seed samples of wheat were collected for the isolation and identification of seed borne fungi from Chittorgarh and Udaipur district of southern Rajasthan.

40 seed sample were collected from each district. All materials except seeds, which used in this experiment, were sterilized using 70% ethyl alcohol. The Petri plates were sterilized at 180 ^{0}C for two hours in hot air oven whereas, Media (Potato Dextrose Agar, Richard's broth), blotter paper and distilled water were sterilized in an autoclave at 1.045 kg/cm^{-2} pressures for 20 min. Seeds are surface sterilized by dipping in 0.1 per cent mercuric chloride ($Hgcl_2$) solution for 2-3 minutes followed by three washing with sterilized distilled water. Fresh polythene bags were sterilized with 5% formalin solution. Cotton blue and lacto phenol were used for staining of the fungal cytoplasm and for providing a light blue background, against which the walls of hyphae can readily be seen (Aneja, 2004) [4]. Plating of the seed component: Standard blotter method and agar plate method described by the International Seed Testing Association (ISTA 1976) [8], was used for the isolation of the seed borne fungi associated with the wheat seed samples.

Germination Test

Samples were tested in the month of November 2015. The towel paper method (ISTA, 1976) [8] was used for germination test. Two brown colored towel papers of equal size (46 x 27 cm) were jointly soaked in water for 4 hr and placed over a butter paper (39 x 25 cm). Fifty seeds were placed at equal distance over the towel paper, which was subsequently covered by another moist towel paper (46 x 27 cm). Then the towel paper was rolled up, ends of the rolled towel paper tied lightly with rubber bend and placed in germination chamber at 25 ± 2^0 C. fifty seeds were used for each sample. The per cent germination and healthy seedling were recorded on 7th day of incubation. The seedling, which possessed the ability to develop into a fully normal and vigorous seedling, was considered as healthy seedling.

Embryo count method

Rennie and Seaton (1975) [12] developed NaOH soak method for the detection of *Ustilago nuda* in wheat and barley seeds. In the present study, Agarwal *et al*., (1981) [2] modified method was used for detection of seed borne pathogen *in vitro*. The seeds were soaked overnight in 10% NaOH at 22^{o}C and washing with warm water through sieves of decreasing mesh size. Embryos finally cleared in lacto phenol. Per cent diseased stain embryo was counted.

Examination of incubated seeds

Sampling for germination was done at 3 days after incubation, while identification of fungi was done at 7 days. The Petri dishes were brought to the examination area in the laboratory, where each seed was examined under a microscope for growth habits of the various fungi growing in the Petri plates. Slide preparations of the various fruiting structures of the fungi were made and identified under the stereo zoom compound microscope.

Slide preparation and identification

The samples of fungi were taken randomly from each crop. These samples were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi (Aneja, 2004; Rifai, 1969; Barnet and Hunter, 1999) [4, 13, 5]. The binocular compound microscope was used to determine the type of fungus in each plate. The seed borne fungi were identified using identification keys and cross-checked for each seed plates to identify the type of fungus growing on each seed. After seven days of incubation, fungal species found growing on the surface of seeds, were identified.

3. Results

(A) Per cent seed moisture, germination and healthy seedlings of wheat seed samples

All the eighty seed samples collected from two districts of Rajasthan were found viable. The per cent moisture content (mc) ranged from 13.8 (CH-11) to 16.2 (CH-35) in Chittorgarh district seed sample. The per cent germination varied from 82.9% (CH-6) to 86.1% (CH-14 and CH-40). Maximum healthy seedlings were obtained in seed sample CH-14 and CH-40 (76.10%) while, minimum healthy seedling was observed in CH-3, CH-6 and CH-34 (72.2%) (Table 1).

The seed samples collected from Udaipur district showed the per cent moisture content (mc) ranged from 13.9% (UD-5, UD-10, UD-19, UD-22 and UD-28) to 15.8% (UD-3). The per cent germination varied from 83.7% (UD-19) to 86.1% (UD-14 and UD-21). Maximum healthy seedlings were obtained in seed sample UD-14 (75.9%) while, minimum (73.1%) was observed in UD-2, UD-19, UD-20 and UD-39 seed samples. (Table 2).

(B) Embryo count test

Embryo count test was done by using the method of Agarwal *et al.,* (1981) [3]. Maximum infected embryo was observed in Chittorgarh district seed samples (14.0%) and minimum infected embryo was observed in Udaipur (9.09%) district seed samples. Chittorgarh district seed samples maximum (0.6%) infected embryo was found in seed sample CH-9, CH-16 and CH-24 while infected embryo was not found in CH-2, CH-5, CH-6, CH-11, CH-13, CH-17, CH-20, CH-23, CH-27, CH-28, CH-29, CH-30, CH-32 and CH-33 seed samples. In case of Udaipur District seed samples maximum infected embryo was found in UD-13, UD-17, UD-21 and UD-36 while infected embryo was not found in UD-1, UD-8, UD-9, UD-22, UD-29, UD-31 and UD-38 seed samples. (Table 3).

(C) Detection of seed myco flora by standard techniques

(a) Standard blotter paper method (SBPM)

Incubation of all the wheat seed samples by standard blotter paper method revealed the presence of seven different fungi *viz., Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium moniliforme, Rhizopus stolonifer* and *Mucor* spp. (Table 4).

The unsterilized and sterilized wheat seeds were placed over moist blotter paper in sterilized Petri plates for detection of external seed borne myco flora. In unsterilized wheat seed samples, the total incidence of myco flora recorded were (33.89%) and (36.0%) respectively in Chittorgarh and Udaipur districts unsterilized seed samples while Sterilized seeds showed maximum percentage (12.09%) of myco flora in Chittorgarh district seed samples and minimum (10.68%) in Udaipur district seed samples.

Alternaria alternata was observed maximum (2.63%) in Chittorgarh unsterilized seed samples and minimum in Udaipur (0.44%) sterilized seed samples. However, *Aspergillus flavus* was found maximum (9.61%) in unsterilized and minimum (3.94%) in sterilized seed samples of Udaipur district. In Udaipur district unsterilized seed samples *Curvularia lunata* was found maximum (5.15%) and minimum in Udaipur (0.64%) sterilized seed samples. while *Fusarium moniliforme* was found higher (7.4%) in

unsterilized seed samples of Udaipur and lower in (1.54%) sterilized seed samples of Udaipur district. *Rhizopus stolonifer* was observed maximum in Chittorgarh (3.60%) sterilized seed samples and minimum in Udaipur (0.43%) sterilized seed samples. while *Mucor* spp. was found maximum (4.0%) and minimum (1.24%) in Chittorgarh unsterilized and Udaipur sterilized seed samples respectively.

(b) Agar plate method

The unsterilized and sterilized seeds were placed over PDA medium in sterilized Petri plates. All the fungi detected with seeds of wheat by using standard blotter method were also detected by the agar plate method. *Alternaria alternata* was found maximum (1.96%) and minimum (0.44%) in Udaipur unsterilized and sterilized seed samples respectively. *Aspergillus flavus* was observed maximum (8.90%) and minimum (1.55%) in Chittorgarh unsterilized and sterilized seed samples. In Udaipur district unsterilized seed samples *Aspergillus niger* was found maximum (5.2%) and minimum (0.27%) in Chittorgarh district sterilized seed samples. *Curvularia lunata* was observed maximum (5.3%) and minimum (0.96%) in Udaipur district unsterilized and sterilized seed samples respectively. In Chittorgarh district seed samples *Fusarium moniliforme* was found maximum (5.06%) in unsterilized and minimum (2.37%) in sterilized seed samples. *Rhizopus stolonifer* was found maximum (3.7%) and minimum (0.025%) in Chittorgarh unsterilized and sterilized seed samples. Maximum (3.06%) incidence of *Mucor* spp. was found in Chittorgarh unsterilized seed samples while minimum (0.25%) in Udaipur unsterilized seed samples. *Trichoderma viride* was observed only in Udaipur district seed samples with frequency of maximum (2.08%) in unsterilized and minimum (0.25%) in sterilized seed samples\ (4.6.2).

(D) Isolation, purification and identification of the seed borne myco flora

The seed borne myco flora detected by standard incubation technique were isolated by transferring on potato dextrose agar medium in Petri plates. The plates were incubated at 25±2 ^{0}C for the fungal growth.

The seed borne myco flora was identified on the basis of cultural, morphological characters using selective standard methods and literature (Holliday, 1980; Rifai, 1969; Rape and Fennell, 1965; Nargale *et al*., 2013; Mordue, 1988) [7, 13, 11, 10, 9] as mention below and the wheat seed borne myco flora identity was confirmed *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium moniliforme*, *Rhizopus stolonifer*, *Mucor* spp. and *Trichoderma viride*.

4. Discussion

Seeds of many crops are subjected to invasion by pathogen during development and before or after harvest by a great variety of myco flora. Seed carrying a pathogen serves as the primary source of infection and has very important role in the epidemiology of disease. Myco flora present on or in the seed may result in prolonged dormancy, reduce germination and seedling survival (Christensen and Lopezf, 1963).

In dates back to 1729, Michelli first demonstrated seed transmission of a pathogen while, Tillet (1755) confirmed and established that *Tilletia caries* Tull, the fungus responsible for bunt disease of wheat is seed borne. Since then the knowledge of seed borne disease of crop has been greatly increased and now there is hardly any cultivated crop where at least one seed borne fungal parasite is not known. Consequently, it has become clear in Plant Pathology that seed borne myco flora is largely associated with the occurrence of number of diseases.

In present study wheat seed sample were collected from two districts of Rajasthan namely Udaipur and Chittorgarh for seed myco flora studies. Forty seed samples were collected from each district. Seeds of these samples were tested for germination, moisture per cent and seedling vigour.

The variation in moisture per cent, seed germination and per cent seedling vigour amongst all eighty seed samples were observed. Since all seed samples were collected and stored uniformly, such variation could have been due to (1) Physiochemical nature of the seeds (2) Agriculture operations (3) Climatologically condition of locality under sampling (4) Infected with pathogenic and non pathogenic seed myco flora.

Apart from testing the samples for moisture per cent, germination and seedling vigour, they were subjected to seed health tests *viz.*, inspection of dry seeds, blotter paper test and agar plate test. If the results of per cent moisture, seed germination and per cent normal seedlings obtained are compared with results of dry seed inspection, then it will become clear that Chittorgarh districts sample CH-3, CH-6 and CH-34 have maximum shriveled seeds and gave rise to minimum seedlings. The seed sample UD-14 from Udaipur district has minimum shriveled and gave rise to showed maximum germination of seeds as well as maximum seedlings. Relationship between seed discoloration and germination were also observed in UD-2, UD-19, UD-20 and UD-39 seed sample where minimum germination was with maximum seed discoloration. Whereas, minimum discoloration exhibited in CH-14 seed sample in which germination and seedling were maximum. These investigations proved the usefulness of dry inspection in testing seed health of wheat.

Dry seed inspection of wheat seed samples revealed the presence of deformed, discoloured, damaged seeds and other inert material together with healthy seeds. It is likely that development of different types of seed myco flora during storage may lead to such deformation of seed. Presence of such kind of seeds and other impurities in "*Moth"* has also been reported by Sharma (1986).

In present investigation, methods *viz.*, blotter paper method, agar plate method were used for detection of seed myco flora of wheat. *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium moniliforme*, *Rhizopus stolonifer*, *Mucor* spp. and *Trichoderma viride* were isolated from seeds. Similarly, Singh *et al*., (2011) found many fungal species associated with wheat seeds and their effect on germination. On examination of seed myco flora by agar plate method and blotter method. Total sixteen fungal species were isolated from test cultivars by the standard techniques. Fungi isolated and identified were *Alternaria alternata, A. solani, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. terreus, Curvularia lunata, Fusarium roseum, F semitectum, Penicillium citrinum, P. rubrum, Rhizopus stolonifer* and *Trichoderma harzianum*. However, Bashir *et al.,* (2012) also reported five fungal species namely *Rhizopus nigricans, Mucor* spp., *Penillium jenseni, Aspergillus niger,* and *Fusarium moniliforme*. Similarly, Zrari (2013) observed ten seed borne fungi in wheat (*Alternaria* spp., *Aspergillus* spp., *Aureobasidium* spp., *Cladosporium* spp., *Dreschslera spp., Penicillium* spp., *Rhizoctonia* spp., *Stemphylium* spp., *Mucor* spp. *and Rhizopus* spp.).

Agar plate method gave higher number of fungi as compared to blotter test. It may be possible that variation may be due to

reason that week and slow growing fungi could not grown well on blotter paper as compared to agar plate tests (Neergaard and Saad, 1962). The blotter paper and agar plate test revealed that per cent incidence of all species isolated from sterilized seeds were low as compared to unsterilized ones and in some sterilized seed samples. Pathak and Razia (2013) also found that *Fusarium moniliforme, Rhizopus* spp., *Mucor* spp., *Alternaria alternata, Aspergillus niger, A. flavus, Curvularia lunata, Drechslera* spp., *Alternaria* spp. *and Penicillium* spp., were most frequently isolated from the wheat seeds, which is similar to result, observed in present investigation. Similar results with agar plate test also have been reported by Bashir *et al.*, (2012) and Gohari *et al.*, (2007).

Table 1: per cent seed moisture, germination and healthy seedlings of wheat seed samples collected from Chittorgarh district of Rajasthan

S. No.	Seed sample	Moisture content (%)	Germination (%)	Healthy seedlings (%)
1	CH-1	15.2	84.4	73.2
2	CH-2	14.9	85.2	72.6
3	CH-3	15.1	83.1	72.2
4	CH-4	15.6	85.3	74.6
5	CH-5	13.9	85.7	74.3
6	CH-6	14.2	82.9	72.2
7	CH-7	14	84.9	73.9
8	CH-8	15.1	83.9	73.3
9	CH-9	15.3	84.2	74.8
10	CH-10	14.9	85.3	75.2
11	CH-11	13.8	85.8	75.3
12	CH-12	15.2	86	75.9
13	CH-13	15.3	85	74.8
14	CH-14	15	86.1	76.10
15	CH-15	14.7	84.2	74.7
16	CH-16	14.6	85.1	75.0
17	CH-17	13.9	84.2	75.1
18	CH-18	15.8	85.1	75.3
19	CH-19	15.2	86	76.1
20	CH-20	14	83.9	73.2
21	CH-21	14.9	84.8	73.5
22	CH-22	14.8	84.7	73.6
23	CH-23	15	85.2	74.8
24	CH-24	15.2	85.7	74.9
25	CH-25	15.3	85.3	74.9
26	CH-26	15.5	85	75.0
27	CH-27	15.7	86	75.8
28	CH-28	14.7	84.9	74.9
29	CH-29	14.2	84.3	74.5
30	CH-30	15.2	84.5	75
31	CH-31	14.3	85.2	75.3
32	CH-32	15.3	84.6	75.1
33	CH-33	14.9	85.9	75.4
34	CH-34	15.1	83.2	72.2
35	CH-35	16.2	84.7	74.3
36	CH-36	15.3	83.9	73.8
37	CH-37	14.7	84.2	73.7
38	CH-38	14.8	85.3	74.8
39	CH-39	14.2	85.4	75.2
40	CH-40	14.8	86.1	76.1
Mean		14.89	85	74.64

Table 2: Per cent seed moisture, germination and healthy seedlings of wheat seed samples collected from Udaipur district of Rajasthan

S. No.	Seed sample	Moisture content (%)	Germination (%)	Healthy seedlings (%)
1	UD-1	14.6	85.2	73.2
2	UD-2	15.2	83.8	73.1
3	UD-3	15.8	84.8	75.1
4	UD-4	14.3	84.3	74.8
5	UD-5	13.9	83.9	73.3
6	UD-6	14.7	85.3	75.2
7	UD-7	14.2	84.9	74.7
8	UD-8	15.1	83.8	73.6
9	UD-9	15.6	85.2	75.1
10	UD-10	13.9	85.7	75.3
11	UD-11	14.4	86.2	75.8
12	UD-12	14.7	86.0	75.6
13	UD-13	14.9	85.3	75.2

14	UD-14	14.3	86.1	75.9
15	UD-15	15.2	85.3	75.1
16	UD-16	15.1	84.8	74.2
17	UD-17	14.3	83.9	74
18	UD-18	14.6	84.7	74.1
19	UD-19	13.9	83.7	73.1
20	UD-20	15.1	85.2	73.1
21	UD-21	15.3	86.1	75.3
22	UD-22	13.9	83.9	73.2
23	UD-23	14.8	84.3	74.6
24	UD-24	14.6	84.8	73.3
25	UD-25	14.2	84.3	73.9
26	UD-26	15.1	84.9	73.5
27	UD-27	15	84.7	73.3
28	UD-28	13.9	85.1	74.5
29	UD-29	15.1	85.2	74.3
30	UD-30	14.9	85.3	74.6
31	UD-31	14.7	85.8	74.9
32	UD-32	14.2	84.7	73.8
33	UD-33	14.8	84.8	74.6
34	UD-34	15.1	84.3	74.3
35	UD-35	15.2	85.1	75.2
36	UD-36	14.8	85.6	75.3
37	UD-37	14.7	84.8	74.9
38	UD-38	15.3	84.9	74.8
39	UD-39	15.2	83.7	73.1
40	UD-40	14.9	85	75.2
Mean		14.73	84.97	74.5

Table 3: Per cent infected embryo in seed samples of wheat

Per cent infected embryo				
S. No.	**Sample No.**	**Chittorgarh seed samples**	**Udaipur seed samples**	**Total**
1	S.1	0.2 (2.56)	0.0 (0.0)	0.2
2	S.2	0.0 (0.0)	0.4 (3.63)	0.4
3	S.3	0.5 (4.05)	0.2 (2.56)	0.7
4	S.4	0.4 (3.63)	0.4 (3.63)	0.8
5	S.5	0.0 (0.0)	0.3 (3.14)	0.3
6	S.6	0.0 (0.0)	0.1 (1.81)	0.1
7	S.7	0.2 (2.56)	0.2 (2.56)	0.4
8	S.8	0.4 (3.63)	0.0 (0.0)	0.4
9	S.9	0.6 (4.44)	0.0 (0.0)	0.6
10	S.10	0.5 (4.05)	0.1 (1.81)	0.6
11	S.11	0.0 (0.0)	0.3 (3.14)	0.3
12	S.12	0.2 (2.56)	0.2 (2.56)	0.4
13	S.13	0.0 (0.0)	0.5 (4.05)	0.5
14	S.14	0.2 (2.56)	0.1 (1.81)	0.3
15	S.15	0.4 (3.63)	0.4 (3.63)	0.8
16	S.16	0.6 (4.44)	0.2 (2.56)	0.8
17	S.17	0.0 (0.0)	0.5 (4.05)	0.5
18	S.18	0.2 (2.56)	0.3 (3.14)	0.5
19	S.19	0.1 (1.81)	0.4 (3.63)	0.5
20	S.20	0.0 (0.0)	0.3 (3.14)	0.3
21	S.21	0.4 (3.63)	0.5 (4.05)	0.9
22	S.22	0.2 (2.56)	0.0 (0.0)	0.2
23	S.23	0.0 (0.0)	0.2 (2.56)	0.2
24	S.24	0.6 (4.44)	0.2 (2.56)	0.8
25	S.25	0.5 (4.05)	0.3 (3.14)	0.8
26	S.26	0.2 (2.56)	0.4 (3.63)	0.6
27	S.27	0.0 (0.0)	0.2 (2.56)	0.2
28	S.28	0.0 (0.0)	0.2 (2.56)	0.2
29	S.29	0.0 (0.0)	0.0 (0.0)	0
30	S.30	0.0 (0.0)	0.3 (3.14)	0.3
31	S.31	0.2 (2.56)	0.0 (0.0)	0.2
32	S.32	0.0 (0.0)	0.3 (3.14)	0.3
33	S.33	0.0 (0.0)	0.4 (3.63)	0.4
34	S.34	0.2 (2.56)	0.1 (1.81)	0.3
35	S.35	0.2 (2.56)	0.3 (3.14)	0.5
36	S.36	0.2 (2.56)	0.5 (4.050	0.7

37	S.37	0.4 (3.63)	0.3 (3.14)	0.7
38	S.38	0.2 (2.56)	0.0 (0.0)	0.2
39	S.39	0.5(4.05)	0.4 (3.63)	0.9
40	S.40	0.2 (2.56)	0.4 (3.63)	0.6
Total		14.0	9.09	
		SEm±	CD at 5%	CV %
Districts (D)		0.0056	0.0157	2.53
Infected Embryo (IE)		0.0251	0.0701	
D x DE		0.0355	0.0992	

* The value in parentheses is angular transformed

Table 4: Per cent incidence of seed myco flora associated with seed samples of wheat tested by agar plate method

S. No.	Seed myco flora	Chittorgarh seed sample		Udaipur seed sample	
		Unsterilized	Sterilized	Unsterilized	sterilized
1	*Alternaria alternata*	1.93 (7.93)	0.61 (4.43)	1.96 (8.03)	0.44 (3.79)
2	*Aspergillus flavus*	8.9 (17.34)	1.55 (7.14)	8.16 (16.59)	2.13 (8.38)
3	*Aspergillus niger*	1.36 (6.69)	0.27 (2.97)	5.2 (13.17)	1.96 (8.04)
4	*Curvularia lunata*	4.2 (11.82)	1.43 (6.77)	5.3 (13.30)	0.96 (5.61)
5	*Fusarium moniliforme*	5.06 (12.99)	2.37 (8.78)	4.9 (12.78)	3.2 (10.30)
6	*Rhizopus stolonifer*	3.7 (11.08)	0.025 (0.90)	3.63 (10.97)	1.0 (5.73)
7	*Mucor* Spp.	3.06 (10.07)	2.32 (8.68)	0.25 (2.86)	0.86 (5.31)
8	*Trichoderma viride*	0.0 (9.27)	0.0 (2.86)	2.08 (9.62)	0.25 (2.86)
Seed without myco flora		69.19	91.17	67.8	89.2
Seed with myco flora		30.81	8.83	32.2	10.8
SEm±		0.139	0.21	0.149	0.074
CD at 5%		0.417	0.632	0.448	0.223
CV%		2.21	6.86	2.38	2.07

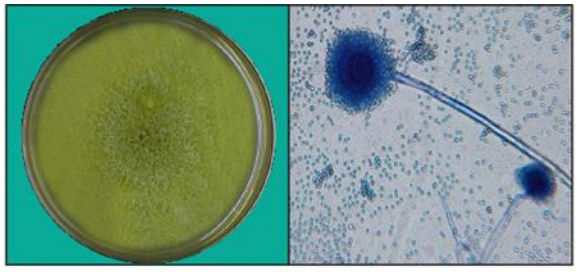
* The value in parentheses is angular transformed

Table 5: Per cent incidence of seed myco flora associated with seed samples of wheat tested by standard blotter paper method

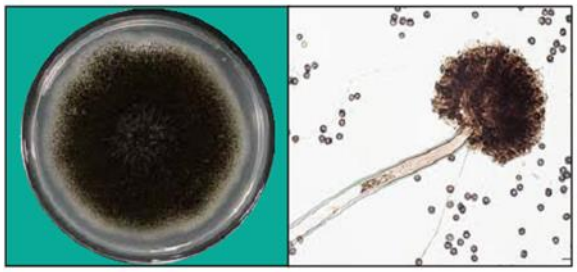
S. No.	Seed myco flora	Chittorgarh seed sample		Udaipur seed sample	
		Unsterilized	Sterilized	Unsterilized	Sterilized
1	*Alternaria alternata*	2.63 (9.32)	0.65 (4.62)	2.60 (9.27)	0.44 (3.73)
2	*Aspergillus flavus*	9.6 (18.09)	4.0 (11.53)	9.61 (18.05)	3.94 (11.44)
3	*Aspergillus niger*	3.1 (10.29)	2.18 (8.48)	4.75 (12.58)	2.45 (9.0)
4	*Curvularia lunata*	4.93 (12.82)	1.06 (5.90)	5.15 (13.06)	0.64 (4.59)
5	*Fusarium moniliforme*	6.03 (14.58)	1.99 (8.09)	7.4 (15.77)	1.54 (7.12)
6	*Rhizopus stolonifer*	3.6 (10.92)	0.61 (4.46)	2.63 (9.32)	0.43 (3.75)
7	*Mucor* spp.	4.0 (11.52)	1.60 (7.26)	3.86 (11.32)	1.24 (6.38)
Seed without myco flora		66.11	87.91	64	89.32
Seed with myco flora		33.89	12.09	36	10.68
SEm±		0.298	0.147	0.160	0.206
CD at 5%		0.904	0.447	0.488	0.626
CV%		4.13	3.55	2.18	5.44

* The value in parentheses is angular transformed

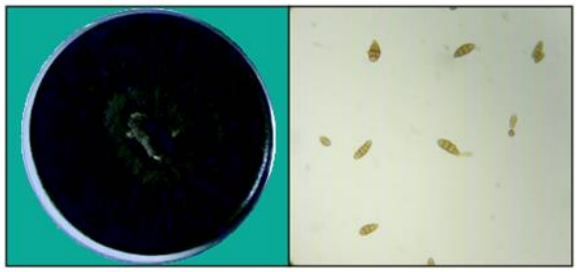
Alternaria alternata



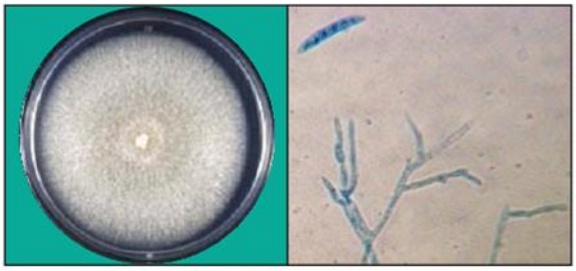
Aspergillus flavus



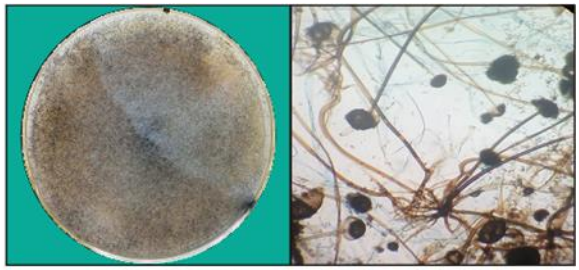
Aspergillus niger



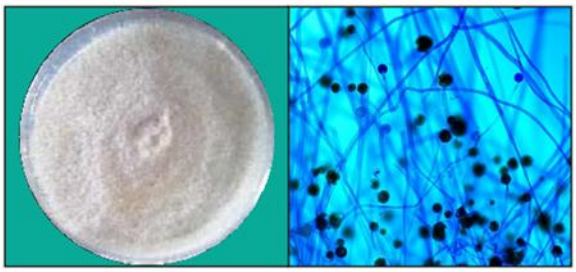
Curvularia lunata



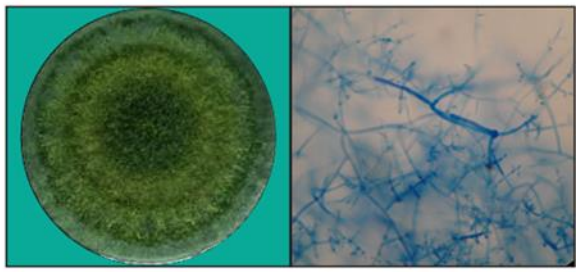
Fusarium moniliforme



Rhizopus stolonifer



Mucor spp



Trichoderma viride

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