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Diversity, pathogenicity and temporal dynamics of seed-associated mycoflora in major cereal crops

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Abstract

Seed is the most important input for crop production. Pathogen free healthy seed is the urgent need for desired plant populations and good harvest. Wheat, maize and sorghum are the main cereal seeds prone to be attacked by many diseases caused by fungi, bacteria, MLO's etc., which are ectophytic or endophytic in nature and deteriorate the quantity as well as quality of seeds. Seven seed mycoflora were isolated from wheat, maize and sorghum seeds viz., *Alternaria alternata*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Fusarium oxysporum*, *Curvularia lunata* and *Rhizopus sp.* The study was conducted in potato dextrose agar method. The unsterilised seeds yielded the maximum seed mycoflora as compared to sterilised seeds in potato dextrose agar plate method. The pathogenicity test of seed mycoflora of wheat, maize and sorghum were confirmed by seed inoculation technique. Among the seven seed mycoflora, *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata* and *Fusarium oxysporum* found the most predominant on all the three cereal crops.

For calculating the abundance level of seed mycoflora at different time period, isolation of seed mycoflora of different cereals was carried out by potato dextrose agar plate method. Maximum abundant mycoflora in sterilized wheat seed was *Alternaria alternata* in 180 days after storage followed by *Aspergillus niger* in 300 days after storage. *Aspergillus niger* was also found to be maximum in 300 DAS on unsterilized seeds. *Rhizopus sp.* was absent on all sterilized cereal seeds for all the time period, but it was found on unsterilized seeds.

Keyword: Seed mycoflora, Cereal crops, Pathogenicity, Fungal diversity, Seed storage

Introduction

Cereals are rich sources of carbohydrates and play an important role in nutrition for majority of vegetarian population in India. India ranks second in the world in terms of cereal production (10% of total world production). Seed is the most important input for crop production. Pathogen free healthy seed is the need for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops.

Among various seed-borne agents, the fungi form, a major group of pathogens that can be seedborne or transmitted through seeds therefore, pathogen free and healthy seeds are the basic requirement for growing a disease-free crop. Only seed borne agents capable of initiating disease in plants or seeds are considered to be as a seed-borne pathogen. Seed-borne pathogens have a greater capacity for spreading the disease in growing crops than pathogens which are not seedborne. If effective steps are not taken immediately to check the introduction of foreign pathogens through seeds, the situation might become difficult when the pathogen gets established in our soils.

The present investigation was undertaken to find out the seed borne fungi associated with the seeds of selected cereals. Considering the nutritional, agronomical and industrial value of cereals and its grain yield, the present study aims to investigate seed-borne fungi associated with wheat (*Triticum aestivum L.*), maize (*Zea mays L.*) and sorghum (*Sorghum bicolor L.*) from Banaskantha district (Gujarat, India). Wheat (*Triticum aestivum L.*) is a staple food globally ranks third in terms of production after maize and rice. Maize (*Zea mays L.*) is important as a source of energy and protein in the human diet throughout the world. *Sorghum* (*Sorghum bicolor L.*) is a hardy crop compared to corn, requires less water than corn, and ranks 5th in cereals for global production.

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Material and Methods

The present investigation entitled, “Seed mycoflora of cereal crops and their management” was carried out in the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The materials and methodology adopted in present investigation for various aspects are as under.

Glasswares

Different corning brand glasswares viz., Petri plates, test tubes, conical flasks, measuring cylinders, glass rods, slides, cover slips etc. were used throughout the study and cleaned by overnight soaking in six per cent chromic acid solution (potassium dichromate 80 g, sulphuric acid 400 ml and water 300 ml). The glasswares were immersed in water for 24 hours then rinsed with tap water and finally washed with distilled water, air dried and then sterilised at 180 °C temperatures for one hour in hot air oven for three consecutive days before use.

Equipments

The laboratory equipments used were autoclave, oven, incubator, refrigerator, physical balance, electronic balance,

compound microscope, stage and ocular micrometer, camera lucida etc.

Culture media

The culture media in the conical flasks were sterilised in the autoclave at 121.6 °C and 15 lbs psi steam pressure for 20 minutes.

Collection of seeds from different sources and isolation of seed mycoflora

Collection of seed samples

Seed samples of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) were collected from Agriculture Produce Marketing Committee, Palanpur and Deesa of Banaskantha district of Gujarat state for seed mycoflora study. For investigating the range of mycoflora associated with seeds, the seed samples of each crop, obtained from above mentioned sources were assayed separately, while in the study of management aspects, the equal quantities of seed samples of each crop obtained from different sources were mixed thoroughly and used.



Fig 1: Pure cultures of major seed mycoflora

Isolation of seed mycoflora

External and internal mycoflora of wheat, sorghum and maize seeds were isolated by using Agar plate method (ISTA, 1976) [1].

Agar plate method

One hundred seeds from each crop variety were used as sterilised and unsterilised sets separately distributed 10 each in 10 separate Petri plates of each sample. To sterilise seed, it was treated with 2.5 to 3 per cent NaOCl (Sodium hypochlorite) for one minute followed by three washings with sterilised distilled water. For unsterilised seed sets, they were kept as such without

any treatment. Ten seeds were placed equidistance aseptically per Petri plate (9cm diameter) containing twenty ml of PDA. Ten plates per sample were incubated at 27±1 °C for 12 hours of light alternating with 12 hours dark period. The fungal growth on the seeds was examined regularly up to 10 days of incubation. Then the individual fungal growth emerged from seeds was aseptically transferred on slant of test tubes by using sterilised needle or wire loop to allow pure culture. The pure culture of individual isolate observed by preparing slides in binocular microscope.

Identification of seed mycoflora

The preliminary identification of the seed mycoflora in agar plate method was done on the basis of the colony characters developed on the agar medium and the morphology of the individual fungal species on the basis of sporulation, conidial characters, either sexual or asexual spore or fruiting structure under stereo binocular microscope. The isolated seed mycoflora were sent to National Center of Fungal Taxonomy, New Delhi for detailed identification.

Pathogenicity test of seed mycoflora

Mass cultures of all these mycoflora isolated from seeds of respective crops were prepared and used in pathogenicity test. The pathogenicity tests were carried out by employing seed inoculation technique on wheat, sorghum and maize seeds.

Seed inoculation technique

Sixty apparently healthy seeds were taken. The seeds were then rolled, separately on seven days old sporulating culture of each fungus thriving on PDA in Petri plates. The inoculated seeds were air dried and sown separately in seedling trays filled with sterilised soil. Each seeds were sown at equal distance in each tray. After sowing trays were kept in a mist house and watered as and when required. Observations on per cent pre and post emergence mortality were recorded and calculated mathematically while seedling vigour and per cent seedling showing disease symptoms were recorded from 20th to 25th days after sowing.

Abundance of seed mycoflora on different cereals at different time interval

The experiment was conducted in the laboratory of Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Seeds of maize, sorghum and wheat collected from selected APMCs of Banaskantha district were washed thoroughly with distilled water. The seeds were then dried back again to its original weight and used for further studies. Three replications of each treatment were taken. The seeds were preserved for one year and seed mycoflora recorded six times at two months interval. Isolation of seed mycoflora from these cereal seeds was followed by agar plate method.

The Petri plates were incubated at 25 ± 2 °C under 12 hrs alternating cycles of light and darkness for seven days. Seed samples were analysed by 'Agar plate method' following International Rules for Seed Health Testing Association (ISTA, 1976) [1]. After seven days of incubation, the germination and the prevalence of the pathogens with seeds were recorded. The pathogens were detected on the basis of their growth character on the incubated seeds in agar plate under stereobinocular

microscope and confirmed after preparing slides by examining under compound microscope.

Results and Discussion

The present study entitled, "Seed mycoflora of cereal crops and their management" was carried out in *in vitro* conditions. The results of different aspects of investigation obtained are presented and discussed as follows.

Isolation of seed mycoflora

Isolation of seed mycoflora was carried out from wheat, maize and sorghum seed samples, collected from APMC, Palanpur and Deesa of Gujarat state. Six seed mycoflora viz., *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Curvularia lunata* were isolated from wheat, maize and sorghum seed samples by employing the effective Potato Dextrose Agar (PDA) method. The isolated seed mycoflora cultures were identified by National Center of Fungal Taxonomy, New Delhi and assigned the authentic identification ITCC No. NCFT 8120.16.- 8126.16.

Seed mycoflora of wheat

Higher percentage of seed mycoflora was detected on PDA method in both sterilised and unsterilised seeds. The results presented in Table 3.1 and Plate I indicated that the seeds collected from APMC, Deesa showed the maximum per cent mycoflora i.e., 27.0 per cent in unsterilised and 15.0 per cent in sterilised seeds. While, the less per cent seed mycoflora were recorded in seeds collected from APMC, Palanpur i.e., 24.0 per cent in unsterilised and 12.0 per cent in sterilised seeds. The maximum seeds without mycoflora were observed in seeds collected from APMC, Palanpur 76.0 and 88.0 per cent in unsterilised and sterilised seeds, respectively where APMC, Deesa recorded 73.0 and 85.0 per cent in unsterilised and sterilised seeds, respectively. In all the seed samples studied *Alternaria alternata* found predominant with high frequency followed by *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Curvularia lunata*. The maximum frequency of *Alternaria alternata* was observed in seeds collected from APMC, Deesa i.e., 7.0 per cent in unsterilised and 4.0 per cent in sterilised seeds, while, *Aspergillus niger* 6.0 per cent in unsterilised and 4.0 per cent in sterilised seeds, respectively.

These results are in accordance with the results obtained by Jogdand and Talekar (2010) [2]. They observed the highest population of seed mycoflora like *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium oxysporum* and *Alternaria alternata* from groundnut seeds isolated by potato dextrose agar plate method.

Table 3.1: Number of isolated seed mycoflora of wheat by potato dextrose agar method

Sr. No	Isolated seed mycoflora	APMC, Deesa		APMC, Palanpur	
		US	S	US	S
1.	<i>Aspergillus niger</i>	6	4	5	3
2.	<i>Alternaria alternata</i>	7	4	6	4
3.	<i>Aspergillus fumigatus</i>	3	2	3	1
4.	<i>Fusarium oxysporum</i>	4	3	4	2
5.	<i>Penicillium chrysogenum</i>	3	1	3	1
6.	<i>Curvularia lunata</i>	2	1	2	1
7.	<i>Rhizopus</i> sp.	2	0	1	0
Per cent seed mycoflora		27	15	24	12

US = Unsterilised seeds; S = Sterilised seeds.

Seed mycoflora of maize

The seeds collected from APMC, Deesa showed the maximum per cent mycoflora i.e., 31.0 per cent in unsterilised and 18.0 per cent in sterilised seeds. While a bit less per cent seed mycoflora were observed in seeds collected from APMC, Palanpur i.e., 28.0 per cent in unsterilised and 16.0 per cent in sterilised seeds. In all the seed samples studied *Aspergillus niger* was predominant with high frequency followed by *Aspergillus fumigatus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Curvularia lunata* and *Alternaria alternata*. The maximum frequency of *Aspergillus niger* was observed in seeds

collected from APMC, Deesa i.e., 8.0 per cent in unsterilised and 6.0 per cent in sterilised seeds, while *Aspergillus fumigatus*, 6.0 per cent in unsterilised and 4.0 per cent in sterilised seeds, respectively. These results are in accordance with the results obtained by [Ishrat and Shahnaz \(2009\)](#) [3]. They recorded the highest number of seed mycoflora like *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus wentii* and *Penicillium* species from maize samples isolated by potato dextrose agar plate method. The present results of the fungal predominance are in accordance with the previous findings.

Table 3.2: Number of isolated seed mycoflora of maize by potato dextrose agar method

Sr. No.	Isolated seed mycoflora	APMC, Deesa		APMC, Palanpur	
		US	S	US	S
1.	<i>Aspergillus niger</i>	8	6	7	6
2.	<i>Alternaria alternata</i>	2	1	2	0
3.	<i>Aspergillus fumigatus</i>	6	4	6	3
4.	<i>Fusarium oxysporum</i>	5	3	5	4
5.	<i>Penicillium chrysogenum</i>	4	3	4	2
6.	<i>Curvularia lunata</i>	3	1	3	1
7.	<i>Rhizopus</i> sp.	3	0	1	0
Per cent seed mycoflora		31	18	28	16

US = Unsterilised seeds; S = Sterilised seeds.

Seed mycoflora of sorghum

Higher percentage of seed mycoflora was detected on PDA method in both sterilised and unsterilised seeds. The result presented in Table 3.3 and Plate III showed that the seeds collected from APMC, Deesa recorded the maximum per cent seed mycoflora i.e., 28.0 per cent in unsterilised and 15.0 per cent in sterilised seeds. While, somewhat less the per cent seed mycoflora were observed in seeds collected from APMC, Palanpur i.e., 25.0 per cent in unsterilised and 13.0 per cent in sterilised seeds. In all the seed samples studied *Aspergillus fumigatus* was predominant with high frequency followed by *Aspergillus niger*, *Alternaria alternata*, *Penicillium*

chrysogenum, *Fusarium oxysporum* and *Curvularia lunata*. The maximum frequency of *Aspergillus fumigatus* was in seeds collected from APMC, Deesa i.e., 8.0 per cent in unsterilised and 5.0 per cent in sterilised seeds, while, *Aspergillus niger*, 5.0 per cent in unsterilised and 3.0 per cent in sterilised seeds, respectively. These results are in accordance with the research findings obtained by [Jogdand and Talekar \(2010\)](#) [2]. They observed the highest population of seed mycoflora like *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium oxysporum* and *Alternaria alternata* from groundnut seeds isolated by potato dextrose agar plate method.

Table 3.3: Number of isolated seed mycoflora of sorghum by potato dextrose agar method

Sr. No.	Isolated seed mycoflora	APMC, Deesa		APMC, Palanpur	
		US	S	US	S
1.	<i>Aspergillus niger</i>	5	3	4	3
2.	<i>Alternaria alternata</i>	4	2	4	2
3.	<i>Aspergillus fumigatus</i>	8	5	7	4
4.	<i>Fusarium oxysporum</i> <i>Penicillium</i>	3	1	3	1
5.	<i>chrysogenum</i>	4	3	4	2
6.	<i>Curvularia lunata</i>	2	1	2	1
7.	<i>Rhizopus</i> sp.	2	0	1	0
Per cent seed mycoflora		28	15	25	13

US = Unsterilised seeds; S = Sterilised seeds.

Purification and identification of cereal seed mycoflora

The seed mycoflora associated with different cereal crops seed were purified by single spore isolation technique and maintained at 4°C in refrigerator for further study.

The pure cultures of seed mycoflora (Plate IV) were identified as *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Curvularia lunata* by National Center of Fungal Taxonomy, New Delhi and assigned the authentic identification with Reference ID. No. NCFT 8120.16-8126.16.

Pathogenicity test

All the fungal cultures isolated from seeds of respective crops

were tested for their pathogenicity, under the mist house condition. Mass cultures of all isolated fungi were prepared and used in pathogenicity test.

Pathogenicity of seed mycoflora of wheat

The pathogenicity of seed mycoflora of wheat was tested by seed inoculation technique. It is apparent from the data presented in the Table 3.4 and Plate V that the minimum seed germination was observed in *Aspergillus niger* inoculated seeds (40.00%) over rest of the treatments. It was having at par result with *Aspergillus fumigatus* inoculated seeds (41.07%). While the maximum seed germination was recorded in *Fusarium oxysporum* inoculated seeds (52.38%). Significantly higher pre-

emergence mortality was observed in *A. niger* (49.00%) over rest of the treatments. This was followed by *A. fumigatus* (42.00%), *Fusarium oxysporum* (40.83%) and *Alternaria alternata* (39.40%) which were found at par with each other. The minimum pre-emergence mortality was recorded in *Penicillium chrysogenum* (32.67%). Seed inoculated with *Fusarium oxysporum* resulted the maximum post-emergence seedling mortality (33.17%) over rest of the treatments followed

by *Alternaria alternata* (33.00%). The minimum post-emergence seedling mortality was recorded in *Penicillium chrysogenum* (27.00%). Hussain *et al.* (2013) [4] also stated the presence of *Aspergillus niger*, *Fusarium oxysporum* and other *Aspergillus sp.* in all the varieties of wheat caused detrimental reduction in seed germination and vigour index. Present results are in confirmity with the above findings.

Table 3.4: Pathogenicity of wheat seed mycoflora by seed inoculation technique

Sr. No.	Seed mycoflora	Seed germination (%)	Seedling mortality (%)	
			Pre-emergence	Post-emergence
1.	<i>Alternaria alternata</i>	43.77 ^d	39.50 ^c	33.00 ^a
2.	<i>Aspergillus niger</i>	40.00 ^e	49.00 ^a	31.00 ^{ab}
3.	<i>Aspergillus fumigatus</i>	41.17 ^e	42.00 ^b	30.67 ^b
4.	<i>Fusarium oxysporum</i>	52.38 ^b	40.83 ^{bc}	33.17 ^a
	<i>Penicillium chrysogenum</i>	50.25 ^b	32.67 ^e	27.00 ^c
6.	<i>Curvularia lunata</i>	47.00 ^c	37.50 ^d	30.00 ^b
	Control(without inoculation)	79.67 ^a	23.00 ^f	17.67 ^d
7.				
S.Em.±		0.64	0.57	0.66
C.V.%		2.19	2.63	3.97

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Pathogenicity of seed mycoflora of maize

The pathogenicity of seed mycoflora of maize was tested by seed inoculation technique. The data presented in the Table 3.5 and Plate VI revealed that the minimum seed germination was observed in *Aspergillus niger* inoculated seeds (38.00%). This was followed by *Aspergillus fumigatus* (40.00%) inoculated seeds. While, the maximum seed germination was recorded in *Curvularia lunata* (54.00%) inoculated seeds. Significantly higher pre-emergence mortality was observed in *A. niger*

(52.67%) which was at par with *A. fumigatus* (51.83%) over rest of the treatments. The minimum pre-emergence mortality was recorded in *Curvularia lunata* (31.22%). With respect to post-emergence seedling mortality *Penicillium chrysogenum* (33.39%) and *Fusarium oxysporum* (33.00%) yielded the maximum seedling mortality and found at par with each other. The minimum post-emergence seedling mortality was recorded in *Curvularia lunata* (24.39%).



Plate 1: Pathogenicity of seed mycoflora of wheat

Control

- T₁: *Alternaria alternata*
- T₂: *Aspergillus niger*
- T₃: *Aspergillus fumigatus*

- T₄: *Fusarium oxysporum*
- T₅: *Penicillium chrysogenum*
- T₆: *Curvularia lunata*

Table 3.5: Pathogenicity of maize seed mycoflora by seed inoculation technique

Sr.No.	Seed mycoflora	Seed germination (%)	Seedling mortality (%)	
			Pre-emergence	Post-emergence
1.	<i>Alternaria alternata</i>	51.00 ^{^c}	37.00 ^{^d}	26.17 ^{^c}
2.	<i>Aspergillus niger</i>	38.00 ^{^e}	52.67 ^{^a}	29.00 ^{^b}
3.	<i>Aspergillus fumigatus</i>	40.00 ^{^d}	51.83 ^{^a}	28.50 ^{^b}
4.	<i>Fusarium oxysporum</i>	50.00 ^{^c}	41.17 ^{^c}	33.00 ^{^a}
5.	<i>Penicillium chrysogenum</i>	51.00 ^{^c}	48.17 ^{^b}	33.39 ^{^a}
6.	<i>Curvularia lunata</i>	54.00 ^{^b}	31.22 ^{^e}	24.39 ^{^d}
7.	Control(without inoculation)	79.67 ^{^a}	23.33 ^{^f}	17.00 ^{^e}
S.E.m.±		0.63	0.85	0.53
C.V.%		2.10	3.63	3.36

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance. [Schenik and Kennedy \(1995\)](#) ^[5] also reported the

detrimental reduction in seed germination and vigour index due to depletion of reserve food in seeds due to fungal invasion. Present results are in accordance with their research findings.

**Plate 2:** Pathogenicity of seed mycoflora of maize

Control

- T₁: *Alternaria alternata*
- T₂: *Aspergillus niger*
- T₃: *Aspergillus fumigatus*
- T₄: *Fusarium oxysporum*
- T₅: *Penicillium chrysogenum*
- T₆: *Curvularia lunata*

Pathogenicity of seed mycoflora of sorghum

The pathogenicity of seed mycoflora of sorghum was tested by seed inoculation technique. It is evident from the data presented in Table 3.6 and Plate VII that the minimum seed germination

was recorded in *Aspergillus fumigatus* inoculated seeds (41.33%) over rest of the treatments. This was followed by *Aspergillus niger* (44.22%) inoculated seeds. While the maximum seed germination was recorded in *Curvularia lunata* (59.00%) inoculated seeds. Significantly higher pre-emergence mortality was observed in *A. niger* (53.50%) and *A. fumigatus* (48.00%) over rest of the treatments. The minimum pre-emergence mortality was recorded in *Curvularia lunata* (33.00%). Considering the post-emergence seedling mortality *Fusarium oxysporum* resulted the maximum mortality (31.11%) followed by *Aspergillus niger* (27.83%). The minimum post-emergence seedling mortality was recorded in *Penicillium chrysogenum* (22.00%).

Table 3.6: Pathogenicity of sorghum seed mycoflora by seed inoculation technique

Sr. No.	Seed mycoflora	Seed germination (%)	Seedling mortality (%)	
			Pre-emergence	Post-emergence
1.	<i>Alternaria alternata</i>	47.00 ^{^e}	33.33 ^{^e}	26.17 ^{^bc}
2.	<i>Aspergillus niger</i>	44.22 ^{^f}	53.50 ^{^a}	27.83 ^{^b}
3.	<i>Aspergillus fumigatus</i>	41.33 ^{^g}	48.00 ^{^b}	26.00 ^{^bc}
4.	<i>Fusarium oxysporum</i>	50.00 ^{^d}	42.10 ^{^c}	31.11 ^{^a}
5.	<i>Penicillium chrysogenum</i>	53.00 ^{^c}	39.50 ^{^d}	22.00 ^{^d}
6.	<i>Curvularia lunata</i>	59.00 ^{^b}	33.00 ^{^e}	25.17 ^{^c}
7.	Control(without inoculation)	78.33 ^{^a}	19.50 ^{^f}	16.00 ^{^e}
S.E.m.±		0.73	0.39	0.68
C.V.%		2.37	1.76	4.50

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Hussain *et al.* (2013) ^[4] also stated the presence of *Aspergillus niger*, *Fusarium oxysporum* and other *Aspergillus sp.* in major cereals caused detrimental reduction in seed germination and

vigour index. Present results are in accordance with the findings.

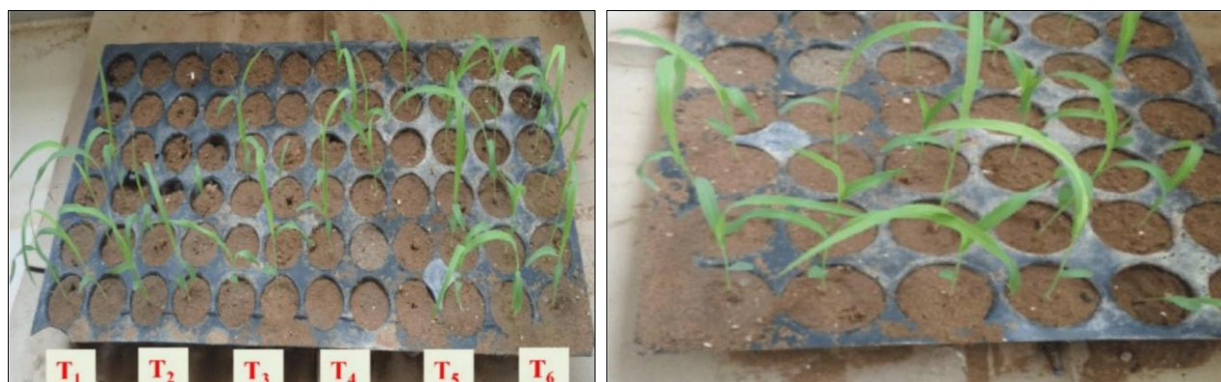


Plate 3: Pathogenicity of seed mycoflora of sorghum

Control

- T1 - *Alternaria alternata*
- T4 - *Fusarium oxysporum*
- T2 - *Aspergillus niger*
- T5 - *Penicillium chrysogenum*
- T3 - *Aspergillus fumigatus*
- T6 - *Curvularia lunata*

Abundance of seed mycoflora on different cereals at different time intervals

Depending upon the various seasons, the abundance of seed mycoflora varies on different cereals. Some mycoflora won't be present on particular months in cereal crops. For determining the abundance level of mycoflora at sterilised and unsterilised conditions, isolation of seed mycoflora from these cereal seeds were done by agar plate method.

Abundance of seed mycoflora on sterilised wheat seeds at different time interval

It is revealed from the data presented in the Table 3.7 that the

maximum abundance of *Alternaria alternata* was recorded on 180 DAS (43.33%) followed by 240 DAS (35.67%). The second abundant mycoflora on wheat was *Aspergillus niger* which was found to be at its maximum number on 300 DAS (41.00%) followed by 120 DAS (24.00%) duration. The third abundant mycoflora on wheat was *Aspergillus fumigatus* which was found to be at its maximum number on 300 DAS (15.67%) followed by 360 DAS (14.33%). *Fusarium oxysporum* was abundant in more number on 120 DAS (22.67%). *Penicillium chrysogenum* was recorded in abundant form on 360 DAS (10.67%).

Maximum incidence of *Curvularia lunata* was recorded from March to June time period (7.67%). Singh *et al.* (2010) ^[6] investigated per cent abundance of seed-borne microbiota on different varieties of mungbean and reported *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Alternaria sp.*, *Curvularia sp.*, *Fusarium sp.*, *Macrophomina phaseolina* and *Penicillium sp.* as predominant mycoflora. Their results on the abundance level of various mycoflora on different storage periods are in accordance with the present findings.

Table 3.7: Abundance of seed mycoflora on sterilised wheat seeds at different time interval

Sr.No.	Name of isolated seed mycoflora	Time interval					
		Frequency in sterilised seeds (%)					
		60 DAS	120 DAS	180 DAS	240 DAS	300 DAS	360 DAS
1.	<i>Alternaria alternata</i>	17.00 ^{gh}	18.33 ^{^g}	43.33 ^{^a}	35.67 ^{^c}	5.67 ^{^p}	15.67 ^{^hi}
2.	<i>Aspergillus niger</i>	20.00 ^{^f}	24.00 ^{^d}	12.67 ^{^kl}	14.00 ^{^jk}	41.00 ^{^b}	21.67 ^{^e}
3.	<i>Aspergillus fumigatus</i>	12.33 ^{^l}	6.00 ^{^p}	12.00 ^{^lm}	7.67 ^{^o}	15.67 ^{^hi}	14.33 ^{^j}
4.	<i>Fusarium oxysporum</i>	15.33 ^{^j}	22.67 ^{^de}	3.67 ^{^qr}	2.00 ^{^st}	2.67 ^{^rs}	5.33 ^{^p}
5.	<i>Penicillium chrysogenum</i>	9.33 ^{^n}	5.67 ^{^p}	2.67 ^{^rs}	1.00 ^{^tu}	5.00 ^{^pq}	10.67 ^{^mn}
6.	<i>Curvularia lunata</i>	7.67 ^{^o}	1.00 ^{^tu}	5.00 ^{^pq}	4.67 ^{^pq}	1.67 ^{^st}	7.67 ^{^o}
7.	<i>Rhizopus sp.</i>	0.00 ^{^u}	0.00 ^{^u}	0.00 ^{^u}	0.00 ^{^u}	0.00 ^{^u}	0.00 ^{^u}
Mycoflora x Time interval							
S. Em. ±				0.44			
C.V.%				7.04			

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance

Results and Discussion

Abundance of seed mycoflora on unsterilised wheat seeds at different time interval

The data revealed from the Table 3.8 that the maximum abundance of *Aspergillus niger* was recorded on 300 DAS (49.00%) followed by 120 DAS (43.33%) on unsterilised wheat seeds. The second abundant mycoflora on unsterilised wheat seed was *Alternaria alternata* which was found to be at its

maximum number on 180 DAS (45.67%) followed by 120 DAS (40.00%) duration. The third abundant mycoflora on wheat was *Fusarium oxysporum* which was found to be at its maximum number on 120 DAS (30.00%) followed by 60 DAS (21.00%). *Aspergillus fumigatus* was abundant in more number on 180 DAS (22.00%). *Penicillium chrysogenum* was recorded in abundant form on 60 DAS (17.67%). Maximum incidence of *Curvularia lunata* was recorded on 60 DAS (12.00%) which

was followed by 360 DAS (10.67%). *Rhizopus sp.* was maximum abundant on 120 DAS (12.00%). Considering the results of both the sterilised and unsterilised methods employed, the period from July to December is more conducive for seed mycoflora of wheat. Parvathi *et al.* (2014)^[7] conducted a study on the incidence of various seed mycoflora like *A. flavus*, *A.*

fumigatus, *A. niger*, *Fusarium sp.* and *Penicillium sp.* on different seasons and the mycotoxins they produce. The present findings are in accordance with their study on abundance level of mycoflora on unsterilised cereal seeds at different time interval.

Table 3: 8 Abundance of seed mycoflora on unsterilised wheat seeds at different time interval

Sr. No.	Name of isolated seed mycoflora	Time intervalFrequency in unsterilised seeds (%)					
		60 DAS	120 DAS	180 DAS	240 DAS	300 DAS	360 DAS
1.	<i>Alternaria alternata</i>	30.67 ^{^e}	40.00 ^{^d}	45.67 ^{^b}	38.67 ^{^d}	7.33 ^{rs}	21.33 ^{gh}
2.	<i>Aspergillus niger</i>	27.67 ^{^f}	43.33 ^{^c}	15.67 ^{kl}	27.33 ^{^f}	49.00 ^{^a}	31.33 ^{^e}
3.	<i>Aspergillus fumigatus</i>	15.67 ^{kl}	9.33 ^{opqr}	22.00 ^{^g}	15.00 ^{^l}	19.33 ^{hij}	19.00 ^{ij}
4.	<i>Fusarium oxysporum</i>	21.00 ^{ghi}	30.00 ^{^e}	7.67 ^{qrs}	6.33 st	7.33 ^{rs}	9.67 ^{nopqr}
5.	<i>Penicillium chrysogenum</i>	17.67 ^{jk}	14.67 ^{^l}	11.67 ^{no}	9.33 ^{opqr}	10.67 ^{nop}	14.00 ^{lm}
6.	<i>Curvularia lunata</i>	12.00 ^{mn}	10.00 ^{nopq}	6.67 ^{^s}	6.33 st	4.33 ^{^t}	10.67 ^{nop}
7.	<i>Rhizopus sp.</i>	9.00 ^{pqr}	12.00 ^{mn}	8.33 ^{pqrs}	10.00 ^{nopq}	11.67 ^{no}	8.00 ^{qrs}
Mycoflora x Time interval							
	S. Em. ±			0.71			
	C.V.%			6.90			

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Abundance of seed mycoflora on sterilised maize seeds at different time interval

It is revealed from the data presented in the Table 3.9 that the maximum abundance of *Aspergillus niger* was recorded on 180 DAS (50.33%) which was at par with the abundance recorded on 240 DAS (49.33%). The second abundant mycoflora on maize was *Aspergillus fumigatus* which was found to be at its maximum number on 300 DAS (38.33%) followed by 180 DAS (34.00%) duration. The third abundant mycoflora on maize was *Fusarium oxysporum* which was found to be at its maximum number on 60 DAS (20.67%) followed by 360 DAS (17.67%).

Penicillium chrysogenum was abundant in more number on 60 DAS (18.00%). *Alternaria alternata* was recorded in abundant form on 60 DAS (15.33%). Maximum incidence of *Curvularia lunata* was recorded on 60 DAS (15.00%) which was at par with its incidence on 360 DAS (14.00%). Ahmad *et al.* (2014)^[8] studied the effect of different storage periods from June to May on the abundance of seed mycoflora. They recorded *Aspergillus fumigatus*, *Aspergillus niger*, *Alternaria alternata* and *Fusarium oxysporum* on maize seeds. The results on the abundance level of various mycoflora on different storage periods in maize are in accordance with the present findings.

Table 3: 9 Abundance of seed mycoflora on sterilised maize seeds at different time interval

Sr.No.	Name of isolated seed mycoflora	Time intervalFrequency in sterilised seeds (%)					
		60 DAS	120 DAS	180 DAS	240 DAS	300 DAS	360 DAS
1.	<i>Alternaria alternata</i>	15.33 ^{^j}	4.67 ^{mn}	1.00 ^{qr}	0.00 ^{^r}	4.00 ^{mno}	11.00 ^{^k}
2.	<i>Aspergillus niger</i>	31.33 ^{^e}	37.00 ^{^c}	50.33 ^{^a}	49.33 ^{^a}	43.00 ^{^b}	26.67 ^{^f}
3.	<i>Aspergillus fumigatus</i>	23.33 ^{^g}	26.00 ^{^f}	34.00 ^{^d}	32.00 ^{^e}	38.33 ^{^c}	23.00 ^{^g}
4.	<i>Fusarium oxysporum</i>	20.67 ^{^h}	2.67 ^{opq}	4.33 ^{mno}	0.33 ^{^r}	7.33 ^{^l}	17.67 ^{^i}
5.	<i>Penicillium chrysogenum</i>	18.00 ^{^i}	5.67 ^{lm}	7.33 ^{^l}	4.33 ^{mno}	3.00 ^{nop}	15.33 ^{^j}
6.	<i>Curvularia lunata</i>	15.00 ^{^j}	0.33 ^{^r}	1.00 ^{^r}	0.00 ^{^r}	2.33 ^{pq}	14.00 ^{^j}
7.	<i>Rhizopus sp.</i>	0.00 ^{^r}	0.00 ^{^r}	0.00 ^{^r}	0.00 ^{^r}	0.00 ^{^r}	0.00 ^{^r}
Mycoflora x Time interval							
	S. Em. ±				0.57		
	C.V.%				7.01		

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Abundance of seed mycoflora on unsterilised maize seeds at different time interval

It is revealed from the data presented in the Table 3.10 that the maximum abundance of *Aspergillus niger* was recorded on 240 DAS (58.33%) which was recorded to be at par with the abundance obtained on 180 DAS (57.33%) followed by 120 DAS (52.00%) on unsterilised maize seeds. The second abundant mycoflora on unsterilised maize seed was *Aspergillus fumigatus* which was found to be at its maximum number on 300 DAS (42.67%). The third abundant mycoflora on maize was *Fusarium oxysporum* which was found to be at its maximum number on May-June (25.67%) followed by 360 DAS (22.33%). *Penicillium chrysogenum* was abundant in

more number on May-June (20.00%). *Alternaria alternata* was recorded in abundant form on May-June (18.67%). Maximum incidence of *Curvularia lunata* was recorded on 360 DAS (18.33%) which was at par with May-June (17.67%). *Rhizopus sp.* was maximum abundant on 300 DAS (18.00%). It can be concluded that majority of the maize seed mycoflora needs somewhat high RH while the fungus like *Fusarium oxysporum* found activated more in hot and low humidity which prevails usually before monsoon during the year i.e. March to June. Moreover, maize is grown in monsoon as well as winter season also. Hence, this time period also suitable for certain seed mycoflora like *Aspergillus fumigatus*, *Aspergillus niger* and *Rhizopus sp.*...

Table 3: 10 Abundance of seed mycoflora on unsterilised maize seeds at different time interval

Sr.No.	Name of isolated seed mycoflora	Time intervalFrequency in unsterilised seeds (%)					
		60 DAS	120 DAS	180 DAS	240 DAS	300 DAS	360 DAS
1.	<i>Alternaria alternata</i>	18.67 ^{^j}	6.67 ^{opqr}	4.00 ^{stuv}	0.00 ^{^w}	7.00 ^{nopq}	14.00 ^{^k}
2.	<i>Aspergillus niger</i>	42.33 ^{^d}	52.00 ^{^b}	57.33 ^{^a}	58.33 ^{^a}	47.00 ^{^c}	34.00 ^{^f}
3.	<i>Aspergillus fumigatus</i>	28.00 ^{gh}	37.00 ^{^e}	40.67 ^{^d}	40.00 ^{^d}	42.67 ^{^d}	28.67 ^{^g}
4.	<i>Fusarium oxysporum</i>	25.67 ^{^h}	9.67 ^{lmn}	6.00 ^{pqrst}	3.33 ^{uv}	8.67 ^{mnop}	22.33 ^{^i}
5.	<i>Penicillium chrysogenum</i>	20.00 ^{ij}	9.33 ^{lmno}	10.00 ^{lm}	9.33 ^{lmno}	5.67 ^{qrstu}	18.33 ^{^j}
6.	<i>Curvularia lunata</i>	17.67 ^{^j}	1.67 ^{vw}	2.33 ^{vw}	0.00 ^{^w}	4.00 ^{stuv}	18.33 ^{^j}
7.	<i>Rhizopus sp.</i>	12.00 ^{kl}	12.00 ^{kl}	13.67 ^{^k}	6.00 ^{pqrst}	18.00 ^{^j}	8.33 ^{mnopq}
Mycoflora x Time interval							
S. Em. ±				0.81			
C.V.%				7.18			

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Parvathi *et al.* (2014) ^[7] conducted a study on the incidence of maize seed mycoflora and recorded *A. flavus*, *A. fumigatus*, *A. niger*, *Fusarium sp.* and *Penicillium sp.* on different seasons and the mycotoxins they produce. The present findings are in accordance with their study on abundance level of mycoflora on unsterilised maize seeds at different time interval.

Abundance of seed mycoflora on sterilised sorghum seeds at different time interval

The data presented in Table 3.11 indicated that the maximum abundance of *Aspergillus niger* was recorded on 300 DAS (54.67%) which was followed by 240 DAS (36.67%). The second abundant mycoflora on sorghum was *Aspergillus fumigatus* which was found to be at its maximum number on 180 DAS (22.00%) followed by 300 DAS (21.00%) and 240 DAS (20.67%). The third abundant mycoflora on sorghum was

Penicillium chrysogenum which was found maximum number on 60 DAS (13.67%). *Fusarium oxysporum* was abundant in more number during 60 DAS (10.67%). *Alternaria alternata* was recorded in abundant form on 60 DAS (10.67%). Maximum incidence of *Curvularia lunata* was recorded on 120 DAS (8.00%) which was at par with its incidence on 60 DAS (7.67%). *Rhizopus sp.* was absent on sterilised sorghum seeds for all the time period. Ahmad *et al.* (2014) ^[8] studied on the effect of different storage periods from June to May on the abundance of seed mycoflora and reported *Aspergillus fumigatus*, *Aspergillus niger*, *Alternaria alternata* and *Fusarium oxysporum* on maize seeds. Their results on the abundance level of various mycoflora on different storage periods are in accordance with the present findings.

Table 3: 11 Abundance of seed mycoflora on sterilised sorghum seeds at different time interval

Sr.No.	Name of isolated seed mycoflora	Time intervalFrequency in sterilised seeds (%)					
		60 DAS	120 DAS	180 DAS	240 DAS	300 DAS	360 DAS
1.	<i>Alternaria alternata</i>	10.67 ^{^j}	9.67 ^{jk}	4.00 ^{no}	8.33 ^{kl}	4.00 ^{no}	8.33 ^{kl}
2.	<i>Aspergillus niger</i>	16.00 ^{gh}	14.67 ^{hi}	19.00 ^{def}	36.67 ^{^b}	54.67 ^{^a}	16.00 ^{gh}
3.	<i>Aspergillus fumigatus</i>	19.67 ^{de}	17.00 ^{fg}	22.00 ^{^c}	20.67 ^{cd}	21.00 ^{cd}	18.33 ^{ef}
4.	<i>Fusarium oxysporum</i>	10.67 ^{^j}	8.67 ^{kl}	8.33 ^{kl}	1.00 ^{pq}	0.00 ^{^q}	9.00 ^{ijkl}
5.	<i>Penicillium chrysogenum</i>	13.67 ^{^i}	13.00 ^{^i}	3.67 ^{no}	4.33 ^{no}	7.33 ^{lm}	9.67 ^{jk}
6.	<i>Curvularia lunata</i>	7.67 ^{klm}	8.00 ^{kl}	0.67 ^{pq}	2.33 ^{op}	0.00 ^{^q}	5.67 ^{mn}
7.	<i>Rhizopus sp.</i>	0.00 ^{^q}	0.00 ^{^q}	0.00 ^{^q}	0.00 ^{^q}	0.00 ^{^q}	0.00 ^{^q}
Mycoflora x Time interval							
S. Em. ±				0.64			
C.V.%				10.79			

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Abundance of seed mycoflora on unsterilised sorghum seeds at different time interval

It is revealed from the data presented in the Table 3.12 that the maximum abundance of *Aspergillus niger* was recorded on 300 DAS (61.67%) followed by the abundance obtained on 240 DAS (51.67%) on unsterilised sorghum seeds. The second abundant mycoflora on unsterilised sorghum seed was *Aspergillus fumigatus* which was found to be at its maximum number on 180 DAS (30.00%). The third abundant mycoflora on sorghum was *Penicillium chrysogenum* with its maximum number on 60 DAS (18.00%) followed by 360 DAS (15.33%). *Fusarium oxysporum* was abundant in more number during 120

DAS (17.00%). *Alternaria alternata* was recorded in abundant form on 60 DAS (12.00%) which stood at par with the abundance found on 360 DAS (12.00%). Maximum incidence of *Curvularia lunata* was recorded on 120 DAS (12.33%). *Rhizopus sp.* was maximum during July to October (12.00%). Parvathi *et al.* (2014) ^[7] conducted a study on the incidence of maize seed mycoflora. They recorded *A. flavus*, *A. fumigatus*, *A. niger*, *Fusarium sp.* and *Penicillium sp.* on different seasons in abundance and studied the mycotoxins they produce. The present findings are in accordance with their study on abundance level of mycoflora on unsterilised maize seeds at different time interval.

Table 3.12: Abundance of seed mycoflora on unsterilised sorghum seeds at different time interval

Sr. No.	Name of isolated seed mycoflora	Time interval Frequency in unsterilised seeds (%)					
		60 DAS	120 DAS	180 DAS	240 DAS	300 DAS	360 DAS
1.	<i>Alternaria alternata</i>	12.00 ^{kl}	11.67 ^{lm}	7.33 ^{pqr}	11.67 ^{lm}	5.33 ^{rs}	12.00 ^{kl}
2.	<i>Aspergillus niger</i>	23.67 ^{af}	20.67 ^{ag}	23.67 ^{af}	51.67 ^{ab}	61.67 ^a	31.33 ^c
3.	<i>Aspergillus fumigatus</i>	29.00 ^{cde}	29.33 ^{cde}	30.00 ^{cd}	27.33 ^{ae}	28.33 ^{de}	28.00 ^{de}
4.	<i>Fusarium oxysporum</i>	12.00 ^{kl}	17.00 ^{hi}	11.00 ^{lmn}	5.33 ^{rs}	0.00 ^t	14.33 ^k
		18.00 ^h	14.33 ^{jk}	7.00 ^{pqr}	10.00 ^{lmno}	9.33 ^{mnp}	15.33 ^j
5.	<i>Penicillium chrysogenum</i>	9.33 ^{mnp}	12.33 ^{kl}	0.00 ^t	6.33 ^{qrs}	6.33 ^{qrs}	8.67 ^{nop}
		8.67 ^{nop}	12.00 ^{kl}	12.00 ^{kl}	7.67 ^{opq}	4.67 ^s	7.00 ^{pqr}
6.	<i>Curvularia lunata</i>						
7.	<i>Rhizopus sp.</i>						
Mycoflora x Time interval							
S.E.m. $\pm$				0.69			
C.V.%				7.43			

Treatments with the letter(s) in common are not significant by Duncan's New Multiple Range Test at 5 per cent level of significance.

Conclusion

Cereals are rich sources of carbohydrates and play an important role in nutrition for majority of vegetarian population in India. India is the world's second largest producer of rice, wheat and other cereals occupying 14 per cent of the world's area and 10 per cent of the production. Cereals are the most important source of food for human being and animals.

Seed is the most important input for crop production. Pathogen free healthy seed is the urgent need for desired plant population and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops. Seed health test is the most important for seed pathology to screen out the major seed mycoflora which show a drastic effect on storage viability, quality deterioration, seed germination, plant stand and yield.

Seed samples of wheat, maize and sorghum were collected from APMC, Deesa and Palanpur in Banaskantha district of Gujarat state. The seed mycoflora were isolated from the cereals seed by potato dextrose agar method. Seven seed mycoflora were isolated from wheat, maize and sorghum seeds viz., *Alternaria alternata*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Fusarium oxysporum*, *Curvularia lunata* and *Rhizopus sp.* The unsterilised seeds yielded the maximum seed mycoflora as compared to sterilised seeds. The maximum seed mycoflora were isolated from the seed samples collected from APMC, Deesa as compared to seeds collected from APMC, Palanpur.

The pathogenicity tests of seed mycoflora of wheat, maize and sorghum were confirmed by seed inoculation technique. Among seven seed mycoflora, *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata* and *Fusarium oxysporum* found the most predominant on all the three cereal crops. The isolated seed mycoflora were identified by National Center of Fungal Taxonomy, New Delhi for authentic identification and given the ID.No. NCFT 8120.16- 8126.16.

For calculating the abundance level of seed mycoflora at different time period, isolation of seed mycoflora of three cereal crops was carried out by employing potato dextrose agar plate method. The results indicated that more number of mycoflora registered in unsterilised seeds than sterilised seeds. Maximum abundant mycoflora in wheat seed was *Alternaria alternata* in 180DAS (45.67%) followed by *Aspergillus niger* in 300DAS (49.00%) from unsterilised seeds. In the sterilised set *A. alternata* and *A. niger* recorded 43.33 and 41.00 per cent during the same period.

In case of maize, *A. niger* and *A. fumigatus* found predominant during 180DAS period of the year in unsterilised seeds. In sterilised seeds of maize, *A. niger* registered the higher abundance during the same period, but *A. fumigatus* recorded the maximum abundance in 300DAS (38.33%) in sterilised seeds.

In unsterilised sorghum seeds, *A. niger* recorded the maximum abundance (61.67%) during 300DAS followed by 240DAS (51.67%) while *A. fumigatus* the second highest predominant fungi recorded almost similar high abundance round the year in unsterilised seeds. In sterilised seeds the same fungi recorded the same tune for period with lower the fungi abundance as compared to unsterilised seeds. *Rhizopus sp.* was absent on all sterilised cereals seed for all the time periods, but it was recorded on unsterilised seeds.

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