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Deciphering of seed Health of common food grains (wheat, rice) of North Eastern UP and Gurgaon Haryana, India

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The stored random samples of food seeds of wheat and rice (60 samples) were purchased from places of Eastern UP and Gurgaon district Haryana. Its moisture contents were estimated. The Mycological investigations of wheat seeds revealed presence of a total number of 16 fungal species viz., *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *A. niger*, *A. ochraceous*, *A. phoenicis*, *A. tamari*, *A. terreus*, *A. sydowi*, *Fusarium moniliforme*, *F. oxysporum F. solani*, *P. glabrum*, *Rhizopus nigricans*, *Trichoderma viride* and *Trichothecium roseum*. While mycological analysis of rice seeds showed presence of 15 fungal species viz., *Alternaria padwickii*, *A. oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, *Aspergillus clavatus*, *A. flavus*, *A. niger*, *Cladosporium* sp., *Nigrospora oryzae*, *Alternaria tenuissima*, *Chaetomium globosum*, *F. solani*, *Microascus cirrosus*, *Helminthosporium oryzae*, *Pyricularia grisea*. It also projected variation in presence of fungal species in blotter and agar plate method of analysis. In wheat Blotter method of analysis showed 16 fungal species while agar plate depicted 13 fungal species. In rice Agar plate method depicted presence of 15 fungal species while blotter method shows presence of 12 fungal species. The insect analysis revealed that wheat samples were infected with *Tribolium castaneum*. While rice seeds sample showed presence of insect *Sitophilus oryzae*. The investigations revealed that *Aspergillus flavus*, *A. niger*, *Sitophilus oryzae* and *Tribolium castaneum* caused reduction in seed weight loss, seed germination, carbohydrate and protein contents of common food grains (wheat, rice). It also revealed that randomly selected A. flavus isolate 1 of wheat showed higher potential of aflatoxin B_1 production (1392.940 µg/l) while rice isolate 2 showed 1231.117 µg/l production.

Losses of food seeds/grains and other stored products of agriculture due to attack of pests are not a new phenomena. They have been observed by farmers since they became food gatherers from food hunters. So priority should be given to post harvest studies particularly in humid tropical climates. At these regions at least half of the food supply gets lost between harvest and consumption. The deterioration in the stored food commodities occurs because of triple agencies viz. fungi, insects and rodents. Infestation of insects occurs in stored grains and grain products to a variable extent depending upon the storage conditions in developing countries[1]. This is because of lack of appropriate storage facilities and pillaging of grains[2].

Globally, postharvest losses account for 24% of the total food produced. It varies from about 9% in developed countries to 20% or more in developing countries[3]. According to Wijayaratne et al.[4], the direct and indirect postharvest losses in humid regions are up to 50%. Losses occurred due to insect infestation in storage are the most serious problem in grain storage, particularly in villages and towns in tropical and subtropical countries, because of humid conditions, poor sanitation and inappropriate storage facilities[5].

Sometimes molds grow in the insect-infested food grains and these molds produce a chemical substance called aflatoxin which is reported to be associated with the lever cancer of human being[6].

Overall food grains production was estimated to be 314.51 million tonnes[7]. Rice is staple food for about 800 million people of India. It plays a major role in diet, economy, employment, culture and history. It is the staple food for more than 65% of Indian population contributing approximately 40% to the total food grain production.

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India grows rice in 43 Mha with production of 112 million tons (Mt) of milled rice and average productivity of 2.6 t −1 ha[8].

The food seeds (wheat, rice) are stored for varying length of time for various purposes. It is estimated that approximately 10–20% of the stored seeds become deteriorated by fungi. Several fungi have been associated with seeds viz., wheat seed[9,10], rice seed[11,12]. The non scientific storage of wheat and rice seeds in rural areas of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur leads to heavy deterioration by fungi and insects. However, detailed studies on such deterioration of stored seeds have not been made so far.

Keeping the above in view, in the present investigation, an extensive survey of stored seeds of wheat and rice were made for fungi and insects in order to find out its role in food seeds deterioration. Aflatoxin B1 production of *Aspergillus flavus* isolate were also investigated.

Materials and methods

Collection of stored seed samples of food. The random stored samples of food seeds (wheat, rice) [3 to 8 months old]were purchased from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur. From this all 10 selected spots from each spot six samples of food seeds (500 g) were purchased and kept separately in pre-sterilized polyethylene bags after labeling the name of district, tahsil and place. Thus all random 60 samples of seeds purchased were brought to Laboratory for analysis.

Estimation of Moisture content. Moisture content have role in seed mycoflora. So moisture content were estimated in all randomly collected 60 samples of food seeds (wheat, rice). The weight of 100 seeds (wheat, rice) were recorded randomly using a electronic balance. The seed moisture content was estimated following oven dry method using three replications each of 20 g (ISTA)[13,14]. After estimating the initial moisture content of seeds, about 200 g seed of each sample was kept in muslin cloth bags, to permit free flow of air, and placed in a seed drying room maintaining a constant temperature of 15 °C and 15% RH. Seed samples were drawn at an interval of seven days to estimate the moisture content. The per cent moisture content was estimated.

Detection of Seed Mycobiota. The mycofloral analysis of all randomly collected 60 samples of food seeds (wheat, rice) were done following techniques (i) Agar plate technique[15] (ii) Standard Blotter Technique[16]. (i) In agar plate technique, Czapek's Dox agar medium with following compositions was used during entire experimentation-Ingredients Gms/Litre (Sucrose 30.00; Sodium nitrate 2.00; Dipotassium phosphate 1.00; Magnesium sulphate 0.50; Potassium chloride 0.50; Ferrous sulphate 0.01; Agar 15.00; Final pH (at 25 °C) 7.3 ± 0.2. The medium was sterilized in an autoclave at 20 lb/square inch pressure for 30 min. After cooling of medium to about 40 °C, 10 mg of Streptopenicillin was thoroughly mixed in them in order to prevent the bacterial contaminations[17]. 10 ml of medium was poured aseptically in each of the pre-sterilized petri plates (80 mm diam) separately and allowed to solidify. Glasswares used were pre-sterilized in an oven at 180 °C overnight. (ii) In standard blotter technique, three pieces of blotting paper were sterilized by dipping in ethyl alcohol. These were allowed to dry and placed inside a pre-sterilized Petri plate (80 mm diam).

Isolation of mycoflora from unsterilized seeds. 5 seeds of each sample of food seeds (wheat, rice) were kept equidistantly in each of the pre-sterilized Petri plates containing moistened blotters and solidified agar media separately.10 such assay plates were prepared comprising 50 seeds of each sample. Petri plates were incubated at 28 ± 2 °C for 7 days. The fungi appearing on the seeds were isolated, purified and their single spore cultures were maintained on Czapek's Dox agar medium in B.O.D. incubator at 10 ± 1 °C.

Isolation of mycoflora from sterilized seeds. All samples of food seeds (wheat, rice) were surface sterilized by dipping them 0.1%Sodium hypochlorite solution. The seeds were then washed thoroughly with sterilized distilled water to remove the traces of disinfectant. The seeds were placed on moistened blotters and solidified Czapek's Dox agar medium. The Petri plates were incubated at 28 ± 2 °C. The fungi appearing on the seeds were isolated on the seventh day.

The fungi were identified by comparing their morphological and cultural characteristics with authentic cultures maintained in Mycology Laboratory, Department of Botany, University of Gorakhpur and Amity Institute of Biotechnology, Amity University Haryana as well as with the help of available literature[18–21].

The per cent frequency of unsterilized and sterilized seeds was calculated by using following formulae- Frequency (%) = No of plates in which individual fungal species occurred × 100/Total no. of plates studied.

Analysis of food seeds (wheat, rice) collected from different places for insects. Stored random samples (60) of food seeds (wheat, rice) [3–8 months old] collected from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur were also observed for their insect infestation. Observations for the occurrence of insects in samples of stored food grains were recorded as presence (+)/absence (−) of the insect in Table 4.

Deterioration caused by fungal species. Freshly harvested sterilized wheat, rice seeds were taken in pre-sterilized polyethylene bags (50 g/bag) and these were inoculated by one disc (5 mm diam) of different fungal species separately. For each fungal species 5 control and 5 treatment sets were made and stored for 20 days under laboratory conditions (28 ± 2 °C). The weight loss, germination percentage, Carbohydrate and protein

content of treated and control sets were observed. For germination seeds were placed on moistened filter paper and germination was recorded at different intervals for treated and control sets.

The per cent germination was calculated by the following formula:

$$\%\text{Seed germination} = \text{No. of seed germinated} \times 100/\text{Total no. of seed kept for germination}$$

Deterioration caused by insect species. The living insects viz., *Tribolium castaneum*, *Sitophilus oryzae* were collected in small glass tube (1′×4′) and plugged with cotton separately. 50 g surface sterilized healthy seeds of grains (wheat, rice) were placed in sterilized jars (in 5 replicate) with tin covers separately for each commodity.2 pairs of insects (2 males and 2 females) collected in glass tube were introduced in each jar having of one seed such as wheat, rice separately. Sterilized white hard paper strip was placed in jar for each movements of insects separately. A small pin size hole was made in each of the tin covers of the glass jar for gaseous exchange. The jars were placed in dark at room temperature (28 ± 2 °C). The observation in control and treatments were made after 2 months in terms of weight loss, germination percentage, Carbohydrate and protein content in each commodity inoculated seeds separately.

The deterioration caused by fungi/insect in terms of carbohydrate content in wheat and rice seed were studied following Anthrone method[22]. The Carbohydrates were dehydrated through Conc. H2SO4 for forming furfural. Furfural then condenses with anthrone (10-Keto-9, 10 dihydro anthracene) to form a blue-green coloured complex. This was measured through calorimeter at 630 nm. The protein content estimation was done following Lowry et al.[23] by taking bovine serum albumin as standard. The optical density of each chickpea seed sample was taken at 650 nm.

Detection of aflatoxigenic isolates of *Aspergillus* species. Four isolates of A. flavus were randomly selected from each food seeds samples of wheat, rice separately to determine their Aflatoxin B_1 producing potential by Thin Layer Chromatography (TLC)[24]. Fifty µl conidial suspension (≈10^6 conidia/ml) of selected A. flavus isolates were separately inoculated in 49.5 ml SMKY (Sucrose, 200 g; $MgSO_4.7H_2O$, 0.5 g; KNO_3, 0.3 g; Yeast extract, 7.0 g; Distilled water, 1000 ml) broth medium in 150 ml Erlenmeyer flask and mixed properly followed by incubation at 27 ± 2 °C for 10 days. Content of each flask was filtered after incubation and filtrate was extracted with chloroform (40 ml) in a separating funnel. The separated chloroform extract was dried on water bath at 60–70 °C. The residue left after evaporation was re-dissolved in 1 ml chloroform and 50 µl of it was spotted on TLC plate (20 × 20 cm^2 of silica gel). The plate was developed in toluene: isoamyl alcohol: methanol (90:32:2; v/v/v) solvent system and intensity of AFB_1 was observed under ultra violate fluorescence analysis cabinet at an excitation wavelength of 360 nm[25]. The fluorescent blue spots on TLC plate containing AFB_1 were scraped in 5 ml cold methanol and centrifuged at 3000 rpm for 5 min. Absorbance of supernatant was recorded at 360 nm and AFB_1 content was quantified[26].

Results and discussion

It is evident from Table 1, that the 100 seed weight of wheat and rice seeds were in 4.60 ± 0.23, 2.68 ± 0.13(g) respectively which indicates the seed size diversity. After seven days of incubation grains showed 6.71 ± 0.53, 7.32 ± 0.43, per cent moisture content respectively.

A total number of 16 fungal species viz., *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus*, *A. niger*, *A. ochraceous*, *A. phoenicis*, *A. tamari*, *A. terreus*, *A. sydowi*, *Fusarium moniliforme*, *F. oxysporum F. solani*, *P. glabrum*, *Rhizopus nigricans*, *Trichoderma viride* and *Trichothecium roseum* were isolated by both agar plate as well as blotter paper methods from 60 random samples of places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur stored seeds of Wheat (*Triticum aestivum L.*) (Tables 2, 3). In which *Aspergillus flavus*, *A. niger*, *A. ochraceus* and *A. terreus* were dominant showing 29.0, 27.0, 23.0, 21.0 in blotter and 29.0, 22.0, 22.0, 20.0% in agar plate method of study (Table 4). In wheat seeds the Blotter method of analysis showed 16 fungal species while agar plate depicted 13 fungal species (Table 4). Time to time researchers have isolated fungi the difference may be due to different climatic conditions. 25 genera and 59 species of seed-borne fungi from Egypt with the highest dominance of the genus *Aspergillus* (18 species + 2 varieties), followed by *Penicillium* (12 species + 1 variety, *Fusarium* on third in this regard (5 species + 1 variety), followed by *Rhizopus* spp., *Mucor* spp., *Alternaria* spp., and *Curvularia* spp.[27]; A total of 28 genera and 72 species of seed-borne fungi, most common species viz., *A. niger*, *A. flavus*, *A. terreus*, *A. nidulans*, *A. alternata*, *Cladosporium herbarum*, and *F. oxysporum*[28]; *A. tenuis*[29]; *Chaetomium globosum*, *Drechslera hawaiiensis*, *Fusarium subglutinens* and *Rhizoctonia solani* by using blotter paper method[30]; *Fusarium* spp., *Bipolaris* spp., *Alternaria* spp., *Curvularia* spp., *Aspergillus* spp., and *Penicillium*

Weight of seeds of wheat and rice (100 seeds) [g]		Moisture content in per centage	
		Moisture content on 0 days%	Moisture content on 7th day%
Wheat	4.60 ± 0.23	9.01 ± 0.46	6.71 ± 0.53
Rice	2.68 ± 0.13	9.27 ± 0.33	7.32 ± 0.43

Table 1. Weight of seeds of wheat and rice and its moisture content under constant seed drying environment. *Values given are mean of three replicates; SD = Standard Deviation.

Fungal Species Recorded	Wheat (*Triticum aestivum* L.)	Rice (*Oryza sativa* L.)
Alternaria alternata (Fr.) Keissler	+	–
Alternaria padwickii (Ganguly) M.B. Ellis,	–	+
Alternaria tenuissima Samuel Paul Wiltshire	–	+
Aspergillus candidus Pers ex	+	–
Aspergillus clavatus Desm	–	+
Aspergillus flavus Link	+	+
Aspergillus niger van Tieghem	+	+
Aspergillus ochraceous Wilhelm	+	–
Aspergillus oryzae E. Cohn	–	+
Aspergillus sydowi (Bainier and Sartory) Thom and Church	+	–
Aspergillus tamarii Kita	+	–
Aspergillus terreus Thom	+	–
Chaetomium globosum Kunze	–	+
Cladosporium herbarum (Pers.) Link	–	+
Curvularia lunata (Wakker) Boedijn	–	+
Fusarium moniliforme Sheldon	+	+
Fusarium oxysporum von Schlechtendal	+	–
Fusarium solani (Mart.) Sacc	+	+
Helminthosporium oryzae Breda de Haan	–	+
Microascus cirrosus Zukal	–	+
Nigrospora oryzae (Berk. & Broome) Petch	–	+
Penicillium glabrum (Wehmer) Westling	+	–
Pyricularia grisea Sacc	–	+
Rhizopus nigricans Ehr	+	–
Syncephalastrum racemosum Cohn	+	–
Trichoderma viride Pers.ex.Fr	+	–
Trichothecium roseum (Persoon)	+	–
Total	16	15

Table 2. Number of Fungal species recorded from Wheat (*Triticum aestivum* L.) and Rice (*Oryza sativa* L.).

spp.[31]; *A. flavus*, *A. niger*, *A. alternata,* and *F. verticillioides*[32]*;* A total of 14 genera and 22 species, among which *Drechslera sorokiniana* with maximum mean frequency (18.1%), other pathogenic fungi include *D. tetramera* (15.66), *D. teres* (12.5), *Alternaria alternata* (9.75), *A. tritici* (4.33), *A. triticola* (6.41), *Fusarium semitectum* (10.58), *Cercospora* spp. (2.75) *F. solani* (1.08), *F. oxysporum* (1.66), *Stemphylium solani* (5.66), *S. botryosum* (2.55), *Cladosporium herbarium* (3.41), *Phoma* spp. (6.5) and *Sclerotinia sclerotiorum* (3.25)[33]; *Alternaria alternata* (55.10%), *Bipolaris sorokiniana* (34.69%) and *Cladosporium herbarum* (7.19%)[34], *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium moniliforme*, *Rhizopus stolonifer*, *Mucor* spp. and *Trichoderma viride* from eighty seed samples by using standard blotter paper and agar plate methods[35]; *Alternaria alternata* (infection rate 6.8–19.5%), *Tilletia caries* (1–2%), *Fusarium* spp. (0.5–3.5%), *Cladosporium herbarum* (1.5–3.5%), *Bipolaris sorokiniana* (1.0–4.8%), *Mucor* spp. (1.5–12%), *Penicillium* spp. (0.5– 1.5%), and *Aspergillus* spp. (1–1.5%) on the seeds[36]; Aspergillus, Penicillium, Fusarium, and Alternaria[9] and Forty-four fungal species belonging to 20 genera, Two prevalent pathogens (average incidence > 40%) Alternaria alternata and Cladosporium spp. Ustilago tritici was present in only seven of the 25 governorates, and less abundant than Tilletia tritici[10]. It is established fact that fungal contamination reduces the viability and ultimately affects the germination of the wheat seeds[37].

The fungal investigations on 60 samples of different stored food seeds of rice (*Oryza sativa* L.) from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur showed presence of 15 fungal species viz., *Alternaria padwickii*, *A. oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, *Aspergillus clavatus*, *A. flavus*, *A. niger*, *Cladosporium* sp., *Nigrospora oryzae*, *Alternaria tenuissima*, *Chaetomium globosum*, *F. solani*, *Microascus cirrosus*, *Helminthosporium oryzae*, *Pyricularia grisea*. Out of these fungal species *Aspergillus flavus*, *A. niger* was found to be dominant on the basis of per cent frequency. Agar plate method depicted presence of 15 fungal species while blotter method shows presence of 12 fungal species. Agar plate method showed higher per cent frequency while blotter method showed lower frequency of fungal species (Table 5). Time to time researchers have isolated fungi the difference may be due to different climatic conditions viz., *Curvularia*[38]; *Alternaria alternata*, *A. tenuissima*, *Aspergillus niger*, *A. flavus*, *A. terreus*, *Chaetomium globosum* and *Curvularia lunata*[39]; *Drechslera oryzae*, *Alternaria padwickii.*[40]; *Gibberella zeae* (anamorph, *Fusarium graminearum*) and *Fusarium semitectum*, *with F. acuminatum*, *F. anguioides*, *F. avenaceum*, *F. chlamydosporum*, *F. equiseti*, and *F.* oxysporum[41]; *A. padwickii*, *A. longissima*, *Curvularia oryzae*, *C. lunata*, *Drechslera oryzae*, *A. niger*, *Fusarium moniliforme*, *F. semitectum*, *F. oxysporum*, *F. solani* and species of *Phoma*, *Cercospora*, *Chaetomium*, *Sclerotium*, *Penicillium*, *Myrothecium* and *Colletotrichum* from seeds of rice varieties[42]; *Bipolaris oryzae*, *Fusarium moniliforme*, *Pyricularia oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae*,

Fungal species recorded	Basti, U.P	Deoria, U.P	Gorakhpur, U.P	Maharajganj, U.P	Siddhartha Nagar, U.P	Gurgaon, U.P	Farrukhnagar, Haryana	Manesar, Haryana	Pataudi, Haryana	Sohna, Haryana	Bilaspur, Haryana
Alternaria alternata (Fr.) Keissler	−	+	+	+	−	+	+	+	−	+	−
Alternaria padwickii (Ganguly) M.B. Ellis,	+	+	+	−	+	+	−	+	+	−	+
Alternaria tenuissima Samuel Paul Wiltshire	+	−	−	+	+	+	+	+	+	−	+
Aspergillus candidus Pers ex	+	+	+	+	−	+	+	+	+	+	−
Aspergillus clavatus Desm	−	+	+	+	+	−	+	−	+	+	−
Aspergillus flavus Link	−	+	+	−	+	+	+	−	−	+	+
Aspergillus niger van Tieghem	−	+	+	−	+	+	−	+	+	+	−
Aspergillus ochraceous Wilhelm	+	−	−	+	+	+	+	−	−	+	+
Aspergillus oryzae E. Cohn	−	+	+	−	−	+	+	−	+	−	+
Aspergillus sydowi (Bainier and Sartory) Thom and Church	+	−	+	+	−	+	+	+	+	−	+
Aspergillus tamarii Kita	+	−	+	+	−	+	−	+	+	+	−
Aspergillus terreus Thom	−	+	+	+	+	−	+	+	−	+	+
Chaetomium globosum Kunze	+	−	+	+	+	+	−	+	+	−	+
Cladosporium herbarum (Pers.) Link	+	+	−	−	+	+	+	−	+	+	−
Curvularia lunata (Wakker) Boedijn	+	−	+	+	+	−	+	+	+	−	+
Fusarium moniliforme Sheldon	−	+	+	−	+	+	−	+	+	−	+
Fusarium oxysporum von Schlechtendal	+	+	−	+	+	−	+	−	+	−	+
Fusarium solani (Mart.) Sacc	+	−	+	−	+	+	−	+	−	+	−
Helminthosporium oryzae Breda de Haan	−	+	+	+	−	+	+	−	+	+	−
Microascus cirrosus Zukal	+	−	+	−	+	+	+	+	+	−	+
Nigrospora oryzae (Berk. & Broome) Petch	−	+	+	+	+	+	−	+	+	+	−
Penicillium glabrum (Wehmer) Westling	+	+	−	+	+	+	+	+	+	−	+
Pyricularia grisea Sacc	+	+	−	+	+	−	−	+	+	+	−
Rhizopus nigricans Ehr	+	+	+	−	−	+	+	+	−	+	+
Syncephalastrum racemosum Cohn	−	+	+	+	+	−	−	+	+	+	+
Trichoderma viride Pers. ex.Fr	+	−	+	+	+	−	+	+	+	+	−

Continued

Fungal species recorded	Basti, U.P	Deoria, U.P	Gorakhpur, U.P	Maharajganj, U.P	Siddhartha Nagar, U.P	Gurgaon, U.P	Farrukhnagar, Haryana	Manesar, Haryana	Pataudi, Haryana	Sohna, Haryana	Bilaspur, Haryana
Trichothecium roseum (Persoon)	+	+	–	+	+	–	–	+	+	+	+
Total	17	18	20	18	20	19	17	20	21	17	16

Table 3. Number of Fungal species recorded from different places of Eastern Uttar Pradesh and Haryana. +: Presence of fungal species; –: Absence of fungal species.

Fungi recorded	Unsterilized seed		P-value	Sterilized seed		P-value
	Blotter method	Agar plate method		Blotter method	Agar plate method	
Alternaria alternata (Fr.) Keissler	3.1 ± 0.16	5.1 ± 0.08	0.000059	2.1 ± 0.10	–	0.0000097
Aspergillus candidus Pers ex	2.1 ± 0.04	4.1 ± 0.09	0.0000099	–	–	–
A. flavus Link	29.0 ± 0.13	29.0 ± 0.14	1.0	4.6 ± 0.15	9.7 ± 0.11	0.00000012
A. niger van Tieghem	27.0 ± 0.12	22.0 ± 0.15	**0.000997**	3.7 ± 0.13	7.7 ± 0.19	0.0000004
A. ochraceous Wilhelm	23.0 ± 0.10	22.0 ± 0.07	**0.159902**	4.6 ± 0.13	7.7 ± 0.09	0.0000028
A. tamarii Kita	3.0 ± 0.12	3.2 ± 0.17	**0.070484**	–	–	–
A. terreus Thom	21.0 ± 0.45	30.6 ± 0.33	**0.000225**	4.5 ± 0.40	6.7 ± 0.11	0.000069
A. sydowi (Bainier and Sartory) Thom and Church	5.3 ± 0.16	5.0 ± 0.18	0.06532	2.1 ± 0.19	1.0 ± 0.18	**0.001041**
Fusarium moniliforme Sheldon	3.1 ± 0.11	5.1 ± 0.17	0.0000064	2.0 ± 0.13	–	0.00000414
F. oxysporum von Schlechtendal	3.1 ± 0.21	3.1 ± 0.37	1	2.1 ± 0.43	1.4 ± 0.44	**0.000193**
F. solani (Mart.) Sacc	3.1 ± 0.05	3.0 ± 0.44	**0.327928**	2.1 ± 0.26	1.2 ± 0.29	**0.000571**
Penicillium glabrum (Wehmer) Westling	5.1 ± 0.22	2.3 ± 0.24	0.0000048	–	–	–
Rhizopus nigricans Ehr	3.1 ± 0.29	0.3 ± 0.28	0.0000003	–	–	–
Syncephalastrum racemosum Cohn	4.1 ± 0.41	–	0.0000006	–	–	
Trichoderma viride Pers. ex.Fr	3.0 ± 0.35	–	0.0000001	–	–	–
Trichothecium roseum (Persoon)	3.4 ± 0.25	–	0.000000004	–	–	–

Table 4. Percent frequency of isolated mycobiota from of stored seeds of Wheat (*Triticum aestivum* L.) from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur. –: Absence of fungal species; US: Unsterilized seeds; SS: Sterilized seeds. *Values given are mean of three replicates; SD = Standard Deviation. Significant values are in bold.

Sclerotium oryzae, *Microdochium oryzae*, *Curvularia lunata* are associated with seed infection of rice causes yield reduction, quality deterioration and germination failure[43]; Totally 8 genera of fungi viz., *Alternaria*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Curvularia*, *Fusarium*, *Sarocladium* and *Trichoderma* comprising twelve species[44]; *Helminthosporium oryzae*[45]; *Penicillium globosum*, *Rhizoctonia* sp., *Phoma* sp. were isolated in higher frequency from blotter paper method and *Curvularia lunata* and *Drechslera* sp. from agar plates[46]; *Alternaria padwickii*, followed *by Curvularia lunata*, (5.9–14%) *Fusarium oxysporium* (9.9–13.5%) and *Verticillium* sp (2–9.5%)[47]; six genera viz. *Bipolaris oryzae* (2.5 to 8.53%), *Alternaria padwickii* (5.3 to 13.35%), *Fusarium moniliforme* (11.66 to 21.67%), *Fusarium oxysporum* (1.25 to 4.35%), *Curvularia lunata* (1.95 to 7.5%) and *Aspergillus* sp.(1.75 to 6.54%)[48]; *Alternaria padwickii*, *Curvularia lunata*, *Fusarium moniliforme*, *Helminthosporium oryzae*, *Sarocladium oryzae*, *Pyricularia oryzae*, *Rhizopus oryzae*, *A. niger* and *Trichoderma* sp.[49]; *Penicillium* sp. and *Aspergillus* sp.[50]; *Aspergillus flavus*, *A. niger*, *Penicillium* sp. and *Fusarium* sp.[11]; *Aspergillus* sp., *Fusarium* sp., *Rhizopus* sp., *Gibberella* sp., *Tilletia* sp. and *Penicillium* sp.[12]. As evident from Table 4, the wheat samples collected from Basti, Deoria and Maharajganj, Farrukhnagar, Manesar, Pataudi consisted of *Tribolium castaneum*. Sohna and Bilaspur rice sample showed absence of insect *Sitophilus oryzae*. It is interesting to note that sample of food seeds of Basti was badly infested from where maximum insect population was recorded (Table 6).

Time to time previous investigators have reported observations on presence of storage insects on common seeds/grains. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the major insect pests of stored seeds[51,52]. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is the most important stored-product insect pest infesting rice (*Oryza sativa* L.)[53].

Fungi recorded	Unsterilized seed		P-value	Sterilized seed		P-value
	Blotter method	Agar plate method		Blotter method	Agar plate method	
Alternaria padwickii (Ganguly) M.B. Ellis,	1.0 ± 0.16	3.1 ± 0.24	0.000018	1.1 ± 0.14	–	**0.000126**
Alternaria tenuissima Samuel Paul Wiltshire	–	2.2 ± 0.06	0.000000045	–	–	–
Aspergillus clavatus Desm	1.0 ± 0.09	1.1 ± 0.08	**0.481817**	–	–	–
A. flavus Link	27.0 ± 0.24	37.1 ± 0.17	0.00000057	13.9 ± 0.22	9.3 ± 0.16	0.00000048
A. niger van Tieghem	24.0 ± 0.22	29.1 ± 0.23	0.00000039	14.9 ± 0.24	9.1 ± 0.24	0.00000012
A. oryzae E. Cohn	5.2 ± 0.16	11.1 ± 0.24	0.000000053	4.7 ± 0.14	4.7 ± 0.28	1
Chaetomium globosum Kunze	–	2.3 ± 0.17	0.000000019	–	–	–
Cladosporium herbarum (Pers.) Link	–	9.9 ± 0.14	0.00000011	–	6.1 ± 0.16	0.000000001
Curvularia lunata (Wakker) Boedijn	2.7 ± 0.11	5.1 ± 0.35	0.0000014	1.3 ± 0.16	2.1 ± 0.25	0.0000269
Fusarium moniliforme Sheldon	0.5 ± 0.21	1.9 ± 0.22	0.000073	–	1.1 ± 0.26	0.00000116
F.solani (Mart.) Sacc	1.2 ± 0.15	3.1 ± 0.12	0.0000021	0.3 ± 0.16	0.7 ± 0.13	0.000043
Helminthosporium oryzae Breda de Haan	5.2 ± 0.06	11.1 ± 0.25	0.0000023	4.7 ± 0.09	4.7 ± 0.35	1
Microascus cirrosus Zukal	1.3 ± 0.23	2.3 ± 0.22	0.000042	–	–	–
Nigrospora oryzae (Berk. & Broome) Petch	4.1 ± 0.13	9.9 ± 0.18	0.000000016	1.3 ± 0.15	6.1 ± 0.11	0.000000025
Pyricularia grisea Sacc	2.7 ± 0.25	5.1 ± 0.23	0.00000075	1.3 ± 0.24	2.1 ± 0.12	0.0000845

Table 5. Percent frequency of isolated mycobiota from of stored seeds of rice (*Oryza sativa* L.) from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj and Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur. –: Absence of fungal species; US: Unsterilized seeds; SS: Sterilized seeds. *Values given are mean of three replicates; SD = Standard Deviation. Significant values are in bold.

Name of places from which food grains samples were collected	Presence/ absence of insects in stored food grains and pulses	
	Wheat	Rice
Basti	++	++
Deoria	+	+
Gorakhpur	–	+
Maharajganj	+	+
Siddhartha Nagar	–	+
Farrukhnagar	+	+
Manesar	+	+
Pataudi	+	+
Sohna	–	–
Bilaspur	–	–

Table 6. Intensity of insect in stored food seeds (wheat, rice) from places of Eastern UP viz., Basti, Deoria, Gorakhpur, Maharajganj, Siddhartha Nagar and Gurgaon district Haryana viz., Farrukhnagar, Manesar, Pataudi, Sohna, Bilaspur. – Nil.

As evident from Table 7, *Aspergillus flavus*, *A. niger, Tribolium castaneum* L. played important role in wheat seed weight loss and seed germination. The *Aspergillus flavus* inoculated wheat seeds showed 42.15 ± 0.19, *A. niger* 39.12 ± 0.14, *Tribolium castaneum* L 37.13 ± 0.16% Carbohydrate. content respectively. The *Aspergillus flavus* inoculated seeds showed 6.7 ± 0.14, *A. niger* 5.9 ± 0.15 while *Tribolium castaneum* L. inoculated wheat seeds showed 4.7 ± 0.19% protein content respectively (Table 7). Results reported that *A. niger* filtrate has a adverse effect on the germination rate of wheat seeds and the development of their seedlings. It could be due to the ability of the fungus to produce Aflatoxins. These findings are consistent with previous findings. Ijaz et al.[54] reported

Fungal species/insect	Weight loss (in/g)		Germination%		Carbohydrates%		Protein%	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Aspergillus flavus	Nil	0.179 ± 0.21	90.43 ± 0.11	50.57 ± 0.12	70.78 ± 0.10	42.15 ± 0.19	12.0 ± 0.10	6.7 ± 0.14
A. niger	–	0.157 ± 0.12	89.43 ± 0.11	47.30 ± 0.15	–	39.12 ± 0.14	–	5.9 ± 0.15
Tribolium castaneum L.	–	0.78 ± 0.14	92.00 ± 0.18	49.24 ± 0.126	–	37.13 ± 0.16	–	4.7 ± 0.19

Table 7. Fungal species/insect species vis-à-vis weight loss, germination and Nutritional composition of mature wheat seeds. – Nil; *Values given are mean of five replicates; SD = Standard Deviation.

A. niger as the most damageable storage fungi among fungal pathogens which leads to lower quality and seed germination. Culture filtrates of *Aspergillus* sp. have reported in causing a reduction in seed germination and root-shoot elongation[55]. The germination rate of wheat grains irrigated with the filtrate of *A. niger* and *Rhizopus* sp. was 20% and 80% respectively, compared with 100% of the control grains, which were irrigated with water. The culture filtrates of both *A. niger* and *Rhizopus* sp. affect not only percentage of grains germination but also the morphology of wheat seedlings[56].

As evident from Table 8, *Aspergillus flavus*, *A. niger, Sitophilus oryzae* L. played important role in seed rice weight loss and seed germination. The *Aspergillus flavus* inoculated rice seeds showed 41.17 ± 0.11, *A. niger* 37.11 ± 0.12, *Sitophilus oryzae* 33.13 ± 0.13% Carbohydrate. content respectively. The *Aspergillus flavus* inoculated rice seeds showed 4.7 ± 0.11, *A. niger* 4.1 ± 0.10 while *Sitophilus oryzae* L. inoculated rice seeds showed 4.9 ± 0.10% protein content respectively (Table 8). It is evident from investigations that *Aspergillus flavus*, *A. niger* were dominant fungi causing harm to seeds of wheat and rice (Fig. 1). A study reported that there was high negative significant correlation between seed infestation by microflora and seed germination[57]. Jalander and Gachande[55] by study on the effect of different fungal species of seed-borne fungi of *Aspergillus* on germination and seedling growth of Bean and Cereals reported that *A. niger* caused reduction in germination percentage, growth of the plumule and radicle. As a negative impact, *A. niger* fungi affects on all rice seed germination characteristic more than all other fungi. In accordance with the results of the present study, Islam et al.[58] stated that there is negative and significant correlation [R = −97%] between rate of fungal contaminations and germination percentage in different rice cultivars. Among the studied factors, *A. niger* had high negative impact compared to other factors on all rice seed germination characteristics[59].

As evident from Table 9, the randomly selected A. flavus isolate 1 of wheat showed higher potential of aflatoxin B_1 production (1392.940 µg/l) while rice isolate 2 showed 1231.117 µg/l production. While for both some isolates were non toxigenic. Other A. flavus isolates isolated from wheat and rice showed lower level of aflatoxin production.

Fungal species/insect	Weight loss (in/g)		Germination%		Carbohydrates%		Protein%	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Aspergillus flavus	Nil	0.189 ± 0.21	86.43 ± 0.11	44.57 ± 0.13	79.78 ± 0.10	41.17 ± 0.11	8.0 ± 0.10	4.7 ± 0.11
A. niger	–	0.177 ± 0.12	87.43 ± 0.11	49.30 ± 0.12	–	37.11 ± 0.12	–	4.1 ± 0.10
Sitophilus oryzae L.	–	0.06 ± 0.14	90.00 ± 0.18	48.24 ± 0.12	–	33.13 ± 0.13	–	4.9 ± 0.10

Table 8. Fungal species/insect species vis-à-vis weight loss, germination and Nutritional composition of mature rice seeds. – Nil; *Values given are mean of five replicates; SD = Standard Deviation.

Figure 1. A look on dominant fungi appearing on seeds of wheat and rice.

Fungal isolate	Food grains/pulses	Toxigenicity	AFB_1 Content µg/l
A. flavus 1	Wheat	toxigenic	1392.940 ± 0.20
A. flavus 2	Wheat	non toxigenic	–
A. flavus 3	Wheat	toxigenic	1112.230 ± 0.23
A. flavus 4	Wheat	non toxigenic	–
A. flavus 1	Rice	toxigenic	1013.239 ± 0.22
A. flavus 2	Rice	toxigenic	1231.117 ± 0.27
A. flavus 3	Rice	non toxigenic	–
A. flavus 4	Rice	non toxigenic	–

Table 9. Aflatoxigenic potential of *A. flavus* isolates of common food grains (wheat, rice). – Nil.

Aflatoxins (AFs) are a group of mycotoxins produced as secondary metabolites by the spoilage of Aspergillus fungi, particularly Aspergillus flavus and Aspergillus parasiticus[60]. The most important members are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). They are highly toxic and carcinogenic compounds that cause disease in livestock and humans[61]. In recent years, numerous studies have revealed high levels of aflatoxins and fungal contamination in rice in many countries[62]. The maximum AFB1 concentration of 606 microg kg(−1) was observed in a wheat sample from the state of Uttar Pradesh[63]. AFB1 contamination in rice ranged from 0.014 to 0.123 µg/kg[64]. Out of 1200 rice samples, 67.8% showed AFB1 ranging from 0.1 to 308.0 microg/kg. All the paddy samples from Chattishgarh, Meghalaya and Tamil Nadu showed AFB1 contamination. Milled rice grains from different states showed below the permissible levels of AFB1 (average 0.5–3.5 micro g/kg)[65].

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

N.K.-did experiment compiled dataS.M.P.K.-guided for experimentV.N.P.-guided for experiment.

Competing interests
The authors declare no competing interests.

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