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Fungal flora analysis of stored paddy (*Oryza sativa* L.) seeds at Ghazipur

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Abstract
Paddy is economically important crop that produce rice, ensure food security of billions of people around the world as staple food, and provide livelihoods in rural areas. In the present study, survey the several villages and blocks of Ghazipur district and collected 96 seed samples of paddy and brought to laboratory, and stored at room temperature (20-28°C) for six months. Twenty fungal species were isolated from both external and internal surface by single spore technique. Among them five fungal sp. *viz*. *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium moniliformae* and *Penicillium* sp. were associated both externally and internally on paddy seeds.

Keywords: Paddy seeds, Storage fungi, PDA, Ghazipur district

Introduction
The paddy plant is a member of Poaceae family (Image I & II) and it is nutrient rich and serves as a good source of the vitamins *viz*. thiamine, riboflavin and niacin. Levels of dietary fibre, minerals and vitamins are highest in the bran. It is major crop of Bangladesh, China, India, Korea, Pakistan and Vietnam. It is regularly consumed and forms the main part of a person´s diet in many capitals such as China, Japan, Malaysia, Sri Lanka, the Philippines, Mexico and India. The straw of paddy is used for mushroom cultivation, poultry litter, making mat, and as forage for animal. After processing of rice husk and straw are used as ecofriendly in making paper, cups, plates, packaging of materials etc.
The seed-borne fungi causes the gloss and discoloration of rice, fields is considered a minor disease and is becoming more and more important in almost rice field growing areas in the world. It may lead to a decrease in the weight of grains, seed germination, root length, branch length, and seedling vitality index. Rice cultivation provides employment opportunities for people. In developing nations, it is main source of income. Grain discoloration caused by strains such as oryzae reduces the vitality of seeds, and such seeds usually show death before and after the emergence of seedlings during planting. *Alternaria* and bipolar oryzae mainly occur in the seed coat and endosperm, causing the discoloration of the grains even when the various fungal pathogens alone or in combination.
Seeds are carriers of fungi that spread externally or internally, or both. The intensity of fungal diversity varies from region to region, depending on the climate, storage conditions and seed composition of the seed (Wadsworth, 1994, Shanakht *et al.*, 2014) [30, 27]. The fungus, if not controlled, should cause damage during storage or on site. In addition, they reduce the quality of seeds, that is, the vitality of seeds and the germination rate of seeds.
In the present investigation the fungal flora associated externally and internally of paddy seeds which collected from different blocks of Ghazipur district were analysed.

Materials and Methods
Total 96 seed samples of paddy were collected from 16 blocks *viz*. Barachawar (A), Bhadaura (B),Bhawarkol (C), Birno (D), Devkali (E), Ghazipur Sadar (F), Jakhania (G), Zamania (H), Karanda (I), Kasimabad (J), Manihari (K), Mardah (L), Mohammadabad (M), Revatipur (N), Sadat (O) and Saidpur (P) of Gazipur distrct. The 200g of each seed sample was transfered in tin containers and stored at room temperature (20-28°C) for six months.

Isolation of mycoflora
The fungi of both external and internal surface were analysed by agar plate method of

Muskett (1948) [19]. For external five seeds were transfered in poured Petri plate (PDA) and for internal flora seeds were surface sterilized with 1% $HgCl_2$ of each sample and transfered in Petri plate containing PDA, incubated at room temperature (20-28°C). After six days, all appearing fungi on seeds of both surfaces were isolated and cultured separately by using single spore technique (Table-1&2). Each set having five replicates. The growth of fungi observed after three days and pure culture of each fungus was maintained in PDA slant.

Identification of Mycoflora

Fungi were identified with the help of standard mycology and plant pathology literature (Gilman, 1967, Booth, 1971, Alexopoulos *et al*., 1979, Domsch *et al*., 1980, Barnett and Hunter, 1998, Mehrotra and Aggarwal, 2007, Webster and Weber, 2007, Singh, 2009) [7, 4, 1, 6, 2, 18, 32, 29] and by comparing their morphological and cultural characteristics

Results

20 fungal species were isolated from 96 stored samples of paddy seeds of 12 genera *viz*. *Alternaria alternata*, *A. padwickii*, *Aspergillus flavus*, *A. fumigatus*, *A. japonicas*, *A. niger*, *Bipolaris oryzae*, *Cercospora* sp., *Chaetomium* sp., *Colletotrichum* sp. *Curvularia lunata*, *C. Oryzae*, *Fusarium chlamydosporum*, *F. Moniliformae*, *F. oxysporum*, *F. Solani*, *Myrothecium* sp., *Penicillium* sp., *Rhizopus arrhizus* and *Sclerotium* sp. 5 fungal species were associatedon both external and internal surfaces of paddy seeds (*Oryza sativa*) *viz. Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *F. Moniliformae*, *Penicillium* sp. (Table-3).

Discussion

It is well known that several fungi are associated with stored paddy seeds on both external and internal suefaces and cause diseases. Richardson (1990) [26], Khan *et al*. (2000) [13], and But *et al*. (2011) [5] in their research found that some species like *Fusarium moniliforme*, *F. semitectum*, *Alternaria alternata*, *A. padwickii*, *Curvularia oryzae*, *Drechlera oryzae* were pathogenic in nature with 46.79% to 16.77% external and internal infestation. Some fungi such as *Alternaria padwickii*, *Curvularia lunata*, *C. oryzae*, *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, *Pyricularia oryzae* and *Phoma* species have been isolated from seeds of different locations (Neergaard, 1979, Richardson, 1979, Leeper, 1984, Ou, 1985, Wahid *et al*., 2001, Javaid *et al*.,2002) [20, 25, 17, 22, 31, 10]. The researchers from all around the world reported *Trichconus padwickii* and several other fungi (Imolehin, 1987, Khan *et al*, 1988, Kim and Lim, 1989, Odebunmi, 1989, Khan *et al*., 2000, Wahid *et al*., 2001, Javaid *et al*., 2002, Johnson *et al*., 2003, Ibiam *et al*., 2006) [9, 12, 15, 21, 13, 31, 10, 11, 8].

In 2014, Aurangzeb *et al*. were studied for the presence of seed-borne fungal flora and isolated 18 species of fungi from 8 genera *viz*. *Alternaria*, *Bipolaris*, *Cuirvularia*, *Fusarium*, *Nigrospora*, *Pyricularia*, *Phoma* and *Tellitia* which associated with paddy seeds. *Aspergillus flavus*, *A. niger*, *Pencillium* sp. and *Fusarium* sp. were isolated by Kumar *et al*. (2020) [16] from paddy seeds. Many researchers reported that fungal infection reduce the seed germination, increase seedling abnormalities and seed discoloration (Singh *et al*., 2005, Khatun *et al*., 2009, Patil *et al*.,2012, Pathak and Zaidi, 2013) [29, 14, 24, 23].

Conclusion

The present findings reveals that fungi are associated with paddy seeds externally as well as internally. Mostly fungi cause seed deterioration, establishment of inoculums in disease free soil and also contribute towards the lesser seed germination, seed discoloration, reduce seed weight and harmful to humans and animals. The testing against storage fungi of paddy is under process in laboratory.

Fig 1: Paddy crop

Fig 2: Paddy crop

Plate-I: Paddy seeds

Plate-II: Paddy seeds

Table 1: Fungi associated externally with paddy (*Oryza sativa* L.) seeds after six month storage

S.N.	Fungal sp.	*Blocks of Ghazipur district															
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Alternaria alternata*	+	-	+	+	-	-	+	+	+	-	+	+	-	-	+	+
2	*A. Padwickii*	+	+	+	-	+	-	+	-	+	-	+	+	-	+		+
3	*Aspergillus flavus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	*A. fumigatus*	+	+	+	+	-	-	+	-	+	-	+	+	-	+	+	+
5	*A. japonicas*	+	+	+	-	+	-	+	-	+	+	+	+	-	+	+	+
6	*A. niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	*Bipolaris oryzae*	+	+	+	+	+	-	+	-	+	+	-	+	+	+	-	-
8	*Cercospora* sp.	-	+	+	+	-	+	+	-	+	-	+	+	-	+	+	+
9	*Chaetomium* sp.	-	+	+	-	-	+	-	+	-	+	+	-	+	+	+	-
10	*Colletotrichum* sp.	+	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-
11	*Curvularia lunata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	*C. oryzae*	-	+	+	-	-	+	-	+	-	+	+	+	+	+	+	-
13	*Fusarium chlamydosporum*	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	-
14	*F. moniliformae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	*F. pxysporum*	-	-	+	+	-	+	-	+	+	-	+	-	+	-	+	-
16	*F. solani*	+	-	+	+	+	+	-		+	-	-	+	-	-	+	-
17	*Myrothecium* sp.	+	-	+	+	-	+	+	-	+	-	+	-	+	-	+	-
18	*Penicillium* sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	*Rhizopus arrhizus*	-	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-
20	*Sclerotium* sp.	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-

*A= Barachawar; B= Bhadaura; C= Bhanwarkol; D= Birno; E= Devkali; F= Ghazipur Sadar; G= Jakhania; H= Zamania; I= Karanda; J= Kasimabad; K=Manihari; L= Mardah, M= Mohammadabad; N= Revatipur; O= Sadat and P= Saidpur

Table 2: Fungi associated internally with paddy (*Oryza sativa* L.) seeds after six month storage

S.N.	Fungal sp.	*Blocks of Ghazipur district															
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Alternaria alternata*	+	-	+	-	-	-	+	-	+	-	+	+	-	-	-	+
2	*A. Padwickii*	+	-	+	-	-	-	+	-	+	-	+	+	-	+	-	+
3	*Aspergillus flavus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	*A. fumigatus*	+	+	-	+	-	-	+	-	+	-	+	+	-	+	-	+
5	*A. japonicas*	-	+	+	-	+	-	+	-	+	+	-	+	-	+	-	+
6	*A. niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	*Bipolaris oryzae*	+	+	-	-	+	-	+	-	+	+	-	+	+	+	-	-
8	*Cercospora* sp.	-	+	+	+	-	-	+	-	+	-	+	+	-	-	+	+
9	*Chaetomium* sp.	-	+	+	-	-	+	-	+	-		+	-	+	+	+	-
10	*Colletotrichum* sp.	+	+	-	-	+	-	+	-	+	+	-	+	+	+	+	-
11	*Curvularia lunata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	*C. oryzae*	-	-	+	-	-	+	-	-	-	+	+	-	+	+	+	-
13	*Fusarium chlamydosporum*	+	+	-	+	-	+	+	+	+	-	-	+	+	-	+	-
14	*F. moniliformae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	*F. pxysporum*	-	-	-	+	-	+	-	+	+	-	+	-	+	-	+	-
16	*F. solani*	+	-	+	+	-	+	-	-	+	-	-	+	-	-	+	-
17	*Myrothecium* sp.	+	-	+	+	-	+	-	-	+	-	+	-	+	-	+	-
18	*Penicillium* sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	*Rhizopus arrhizus*	-	+	-	+	-	+	-	+	+	-	+	-	+	-	+	-
20	*Sclerotium* sp.	+	-	+	+	-	+	+	-	+	-	+	-	+	-	+	-

*A= Barachawar; B= Bhadaura; C= Bhanwarkol; D= Birno; E= Devkali; F= Ghazipur Sadar; G= Jakhania; H= Zamania; I= Karanda; J= Kasimabad; K=Manihari; L= Mardah, M= Mohammadabad; N= Revatipur; O= Sadat and P= Saidpur

Table 3: Fungi associated externally and internally both surface with paddy (*Oryza sativa* L.) seeds after six month storage

S.N.	Name of fungal sp.	*Blocks of Ghazipur district															
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Aspergillus flavus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	*A. niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	*Curvularia lunata*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	*F. moniliforme*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	*Penicillium* sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*A= Barachawar; B= Bhadaura; C= Bhanwarkol; D= Birno; E= Devkali; F= Ghazipur Sadar; G= Jakhania; H= Zamania; I= Karanda; J= Kasimabad; K=Manihari; L= Mardah, M= Mohammadabad; N= Revatipur; O= Sadat and P= Saidpur

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