The Pharma Innovation Journal 2023; 12(6): 4860-4867

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 4860-4867

www.thepharmajournal.com
Received: 22-04-2023
Accepted: 28-05-2023

RK Ranjan
Department of Plant Pathology, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Yamleshwar
Young Professional-II under Biotech Kisan Hub ICAR DBT at IGKV Raipur, Chhattisgarh, India

Dinesh Rai
Department of Plant Pathology, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Subhashi Kumar
Technical Assistant, Deptt. of Plant Pathology, Dr. RPCAU, Pusa, Bihar, India

Corresponding Author:
RK Ranjan
Department of Plant Pathology, Dr Rajendra Prasad Central Agricultural University, Pusa Samastipur, Bihar, India

Isolation and identification of seed borne mycoflora associated with wheat seeds

RK Ranjan, Yamleshwar, Dinesh Rai and Subhashi Kumar

Abstract
A total of 70 seed samples collected from 25 locations were tested for associated seed borne mycoflora by using standard blotter method, standard agar plate method and standard paper towel method. In standard blotter paper method, standard agar plate method and standard paper towel method, five mycoflora were identified based on spore morphology namely, *Aspergillus sp., Alternaria triticina, Bipolaris sorokiniana, Fusarium moniliforme* and *Penicillium sp.* In standard blotter paper method, average mycoflora recorded in Samastipur and Muzaffarpur districts seed samples were *Aspergillus* sp. (28.34%), *Alternaria triticina* (15.63%), *B. sorokiniana* (9.29%), *Penicillium* sp. (8.90%) and *Fusarium moniliformae* (6.54%), standard agar plate method, average mycoflora recorded in Samastipur and Muzaffarpur district seed samples were *Aspergillus* sp. (28.46%), *Alternaria triticina* (16.09%), *B. sorokiniana* (9.49%), *Penicillium* sp. (8.63%) and *Fusarium moniliformae* (6.19%) and in standard paper towel method the average mycoflora recorded in Samastipur and Muzaffarpur district seed samples were *Aspergillus* sp. (10.76%), *Alternaria triticina* (4.98%), *B. sorokiniana* (5.99%), *Penicillium* sp. (1.86%) and *Fusarium moniliformae* (2.30%). Fungi *Aspergillus* sp and *Alternaria triticina* was found predominant in standard blotter paper, standard agar plate and standard paper towel methods followed by *Bipolaris sorokiniana, Penicillium* sp and *Fusarium moniliforme*.

Keywords: Standard blotter paper method, standard agar plate method, standard paper towel method, seed health, seed mycoflora

Introduction
India is the second largest wheat producing country in the world after China has the production of about 98.61 million tons with an average productivity of 33.18 q/ha and cultivated over an area of about 29.78 million hectares (ICAR-Indian Institute of Wheat & Barley Research, 2018). Bihar rank 6th in wheat production and cultivated over an area of about 2.04 million ha, production 5.74 million tonnes and productivity 28.16 q/ha (ICAR-Indian Institute of Wheat & Barley Research, 2018). In India, the farmers are growing wheat crop mainly for consumption purpose and they save a portion of produce to use them as a seed for next season. These farmers saved seeds are not tested for their quality. Obviously, the farmers saved seeds are of poor health and quality and often infected by seed borne pathogens. These poor qualities of infected wheat seeds fail to germinate and if germinated the young seedlings from infected seeds die in few days, resulting in germination failure, post emergence damping off and cause seedling blight. The seed borne pathogens present in the seeds or associated with seeds will remain alive in dormant condition with seed lots as long as the seed remains viable.
Wheat crop is affected by approximately 120 different diseases, among them, 42 diseases are seed borne and 35 diseases are caused by fungi (Hasan *et al.*, 2005) [7]. Coincidentally, seed borne diseases of wheat are more destructive in nature and it occurs worldwide. Seeds provide natural substrate for the growth of fungi gets associated with externally or internally or both to the seeds. Fungi associated with seeds as contaminant can cause seed abnormalities, poor germination as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Bateman G. L. and Kwasna 1999; Khanzada, KA *et al.*, 2002) [3, 10]. They not only reduce the quality of seed but also transmitted from one season to other and may introduced new pathogens in a disease-free area, causing quantitative and qualitative crop losses and permanent contamination of the soil (Ora *et al.*, 2011) [12].
Therefore, using a good quality seed and performing seed health test to detect the presence of seed borne fungi becomes paramount to manage the diseases for healthy crop establishment. Considerable work has been done on seed health and detection of seed borne pathogens in wheat seeds from different geographical region of the country (Sharma and Chahal, 1996;

Gopalakrishnan and Valluvaparidasan, 2009; Gopalakrishnan *et al*., 2010; Archana and Prakash, 2013; Sharma and Kapoor, 2016; Singh *et al*., 2018) [14, 15, 5, 6, 1, 16]. But, the information on seed health of wheat varieties collected from farmers of different location in Bihar is scanty. Thus, farmers saved wheat seeds can be infected by many seed borne pathogens. Seed associated mycoflora and seed borne diseases are mainly responsible for low yield and production of poor quality wheat seeds in Bihar.

In view of the above facts and importance of seed associated mycoflora, the present study was taken up to determine the prevalence and extent of different seed borne mycoflora associated with different popular wheat varieties collected from farmers of different location.

Materials and methods

Collection of wheat seed samples

Total 70 seed samples of different variety were collected from 25 different locations of the farmers of Samastipur and Muzaffarpur district of Bihar state (Table 1). The collected seed samples were kept in cloth bag and stored in a well-ventilated room of the laboratory in Department of Plant Pathology, RPCAU, Pusa, Bihar (India).

Table 1: Detailed information of wheat seed samples collected from different villages of farmers:

1. District: Samastipur

Sample No.	Farmers Name	Village	Variety
S-1	Vinod Shah	Harpur pusa	HD-3470
S-2	Vijay Shankar	Harpur pusa	UP-262
S-3	Md. Rizwan	Harpur pusa	HD-3171
S-4	Rajkumar Jha	Harpur pusa	Unknown
S-5	Ashutosh Kumar	Harpur pusa	HD-2733
S-6	Ramsevak Mahto	Karua	PBW-373
S-7	Prabhulal Mahto	Karua	HD-2733
S-8	Subalal Sahu	Hazipur	Unknown
S-9	Ramchandra Das	Hazipur	Unknown
S-10	Shreenivas Sharma	Malinagar	HD-2967
S-11	Bablu Sahney	Malinagar	HD-2967
S-12	Shiv kumar	Saidpur	PBW-644
S-13	Mithlesh Kumar Pandey	Saidpur	HD-2967
S-14	Gunu Mallik	Sahoori	HD-2967
S-15	Niranjan Kumar	Bakhtiyarpur	HD-2967
S-16	Arjun Paswan	Matiyara	HD-2733
S-17	Arisum Mahto	Harpur pusa	Unknown
S-18	Maheshwar	Harpur pusa	Unknown
S-19	Gessri Mahto	Bhuskaul	Unknown
S-20	Mahesh Mahto	Bhuskaul	Unknown
S-21	Vijay Shah	Gaddopur	HI-1544
S-22	Ramanand Kumar	Gaddopur	Unknown
S-23	Ramanand Kumar	Gaddopur	UP-262

2. District: Muzaffarpur

Sample No.	Farmers Name	Village	Variety
M-1	Kuldeep Mahto	Salah	Shriram 272
M-2	Alok Kumar	Salah	HD-2967
M-3	Janardhan Mishra	Prakhand Salah	Unknown
M-4	Garimnath Sharma	Gangapur	PBW-343
M-5	Shyamnandan Singh	Gangapur	Unknown
M-6	Turantlal Rai	Pilkhi	PBW-343
M-7	Harishankar Prasad	Pilkhi	PBW-343
M-8	Rajesh Kumar Rai	Pilkhi	HD-2733
M-9	Avadh KumarJha	Pilkhi	PBW-550
M-10	Pankaj Kumar	Manikamanohar, Narouli	HD-2733
M-11	Lakshman Rai	Manikamanohar, Narouli	HD-2733
M-12	Chandeshwar Rai	Manikamanohar, Narouli	HD-2967
M-13	Ramesh Rai	Manikamanohar, Narouli	HD-2733
M-14	Shri Lakshman Ram	Meerapur	Unknown
M-15	Chandeshwar Ram	Meerapur	PBW-343
M-16	Umesh Rai	Muroul, Dholi	HD-2964
M-17	Ranjeet Singh	Muroul, Dholi	HD-2733
M-18	Ramchandra Rai	MuroulTola, Jahangirpur	HD-2733
M-19	Saroj Kumar	Muroul, Dholi	HD-2733
M-20	Devendra Rai	Muroul	PBW-343
M-21	Satyadev Kumar	Muroul	HD-3086
M-22	Ramkumar Rai	Bishanpur, Bakhari	HD-2733
M-23	Devlal Rai	Rampur, Bakhari	HD-2967
M-24	Shri Ramshobhit Mahto	Basantpur, Bakhari	Unknown

M-25	Jagan Rai	Mohammadpur, Damodar	HI-8381
M-26	LaluPandit	Mohammadpur, Damodar	HD-2733
M-27	Shri Vishwanath Choudhary	Mohammadpur, Damodar	HD-2733
M-28	Ramchandra Rai	Mohammadpur, Damodar	PBW-343
M-29	Vishvajeet Kumar	Mohammadpur, Damodar	PBW-343
M-30	Ramnath Pandit	Mohammadpur, Damodar	PBW-550
M-31	Feku Ram	Meerapur	PBW-343
M-32	Satyadev Rai	Meerapur	PBW-343
M-33	Ramanand Paswan	Belamode	PBW-343
M-34	Ramnaresh Mishi	Dwarikanagar	HD-2733
M-35	Krishna Kumar Choudhary	Dholi	HD-2733
M-36	Sudheer Kumar	Mushouri, Gangapur	PBW-343
M-37	Shri Ramanand Shahi	Goraiya	PBW-343
M-38	Shri Premsagar Shahi	Goraiya	PBW-357
M-39	Shri Niteshwarprasad Shahi,	Goraiya	UP-262
M-40	Shri Ramanand Shahi	Goraiya	HD-2967
M-41	Badari Shahi	Goraiya	PBW-343
M-42	Premsagar Shahi	Goraiya	PBW-343
M-43	Devanand Shahi	Goraiya	PBW-343
M-44	Shri Pappu Shahi	Goraiya	UP- 262
M-45	Premsagar Shahi	Goraiya	HD-2967
M-46	Ram Iqbal Rai	Rampurbakhri, Dholi	HD-2733
M-47	Ram Iqbal Rai	Rampur Bakhri, Dholi	Unknown

Isolation and identification of seed mycoflora associated with wheat seed samples

Wheat seed samples collected from different locations of farmers were subjected to seed health testing by using (i) Standard blotter paper method (ii) Standard agar plate method and (iii) Standard paper towel method (ISTA, 2001) [9].

Standard blotter paper method

Two sheets of blotting papers were placed at the bottom of sterilized Petri plate aseptically and moistened by sterilized distilled water. 20 seeds from each collected seed samples were placed at an equal distance in each Petri plate 100 seeds were tested from each samples). The Petri plates were incubated at 28±1 °C for12 h of light alternating with 12 hour of dark period. The seeds were examined alternatively on 3rd to 7th day of incubation. The fungal hyphae/spore was observed under compound microscope. Observation were recorded for individual seed mycoflora present on each seed samples of wheat.

Standard agar-plate method

Sterilized Petri plates each containing 20 ml potato dextrose agar media were used for incubation of seeds. 20 seeds per Petri plate were equispaced kept aseptically and incubated at 28±1 °C. The fungal colonies emanating from seeds were examined alternately from 3rd to 7th day of incubation. The fungal hyphae/spore was observed under compound microscope. Observations were recorded for individual seed mycoflora present on each seed samples of wheat.

Standard paper towel method

This method generally used for measuring seed germination, seed borne fungi that cause seed decay, seedling blight, or seedling abnormalities can also be detected by using the same method. Two sheets of square brown blotter paper (23 x 26.5 cm size) paper towels were moistened in distilled water, and then placed 100 seeds from each seed samples in equal distance on one of the wet paper towel, used another wet paper towel to cover the seed. Rolled the towels, covered with a butter paper sheet and closed the ends with rubber band to maintain humidity. Whole setups were incubated for 7 days at 28±1 ºC under a 12-hour light regime. After 7 days unrolled the towels, and examined seeds carefully for growth of mycoflora. Mycoflora were identified and isolated from diseased parts of the seeds on PDA plates under aseptic condition.

Observations on percent incidence of individual seed mycoflora present on each seed samples of wheat were recorded in all three incubation methods. After 24 - 48 hours, fragments of hyphal tip from the growing point were transferred to fresh PDA slants for pure culture preparation. Pure culture was maintained on media slants by sub culturing it at 30 days intervals.

Result & Discussion

Collection of wheat seed samples

Total 70 seed samples of wheat collected from 25 different locations of farmers of different villages of Samastipur (23 samples from 10 villages) and Muzaffarpur (47 samples from 15 villages) districts of Bihar were analysed for seed health and quality. The list of collected seeds samples with their code number were presented in table 1.

Isolation and identification of seed mycoflora associated with wheat seed samples.

Total seventy seed samples of wheat collected from farmers were used for the isolation and identification of mycoflora associated with seeds. Three methods i.e., (i) standard blotter paper method (ii) standard agar Plate method and (iii) standard paper towel method were used for this purpose.

Standard blotter paper method

Standard blotter paper methods were used for isolation and identification of mycoflora associated with collected seed samples. The results revealed that the presence of five different fungi *viz.*, *Aspergillus sp.*, *Alternaria triticina*, *Bipolaris sorokiniana*, *Fusarium moniliforme* and *Penicillium sp.* The results were presented in table 2. In Samastipur and Muzaffarpur seed samples range of percent incidence of fungi recorded were *Aspergillus sp.* (11 to 37%), *Alternaria*

triticina (5 to 31%), *Bipolaris sorokiniana* (0 to 17%), *Fusarium moniliforme* (0 to 20%) and *Penicillium* sp. (0 to 25%). The average mycoflora recorded in Samastipur and Muzaffarpur districts seed samples were *Aspergillus* sp. (28.34%), *Alternaria triticina* (15.63%), *B. sorokiniana* (9.29%), *Penicillium* sp. (8.90%) and *Fusarium moniliformae* (6.54%).

Standard agar-plate method

Standard agar plate method were used for isolation and identification of mycoflora associated with seed samples. The results were presented in table 3. In Samastipur and Muzaffarpur seed samples range of percent incidence of fungi recorded were *Aspergillus* sp. (11 to 41%), *Alternaria triticina* (8 to 35%), *Bipolaris sorokiniana* (0 to 20%), *Fusarium moniliforme* (0 to 15%) and *Penicillium* sp. (0 to 27%). The average mycoflora recorded in Samastipur and Muzaffarpur district seed samples were *Aspergillus* sp. (28.46%), *Alternaria triticina* (16.09%), *B. sorokiniana* (9.49%), *Penicillium* sp. (8.63%) and *Fusarium moniliformae* (6.19%).

Standard paper towel method

Standard paper towel method were used for isolation and identification of mycoflora associated with seed samples. The results revealed that the presence of five different fungi *viz.*, *Aspergillus* sp., *Alternaria triticina*, *Bipolaris sorokiniana*, *Fusarium moniliforme* and *Penicillium* sp. The results were presented in table 4. In Samastipur and Muzaffarpur seed samples range of percent incidence of fungi recorded were *Aspergillus* sp. (2 to 25%), *Alternaria triticina* (1 to 11%), *Bipolaris sorokiniana* (2 to 12%), *Fusarium moniliforme* (0 to 6%) and *Penicillium* sp. (0 to 8%). The average mycoflora recorded in Samastipur and Muzaffarpur district seed samples were *Aspergillus* sp. (10.76%), *Alternaria triticina* (4.98%), *B. sorokiniana* (5.99%), *Penicillium* sp. (1.86%) and *Fusarium moniliformae* (2.30%).

Table 2: Result of isolation and identification of mycoflora associated with wheat seed samples by standard blotter paper method.

Samples	% of mycoflora associated with wheat seed samples				
	***Aspergillus* sp.**	***Alternaria triticina***	***Bipolaris sorokiniana***	***Penicillium* sp.**	***Fusarium moniliforme***
S-1	18	12	6	6	6
S-2	23	13	13	16	0
S-3	33	15	12	12	6
S-4	20	13	6	6	20
S-5	26	15	10	15	0
S-6	22	11	0	0	11
S-7	16	11	5	5	0
S-8	23	11	5	11	11
S-9	25	25	12	0	15
S-10	31	18	12	6	0
S-11	18	12	6	6	0
S-12	20	20	6	20	6
S-13	37	17	11	5	0
S-14	25	12	6	6	6
S-15	11	5	5	0	0
S-16	20	13	6	13	6
S-17	17	19	11	11	11
S-18	16	16	11	0	5
S-19	25	6	12	0	12
S-20	33	16	5	0	0
S-21	23	15	17	5	12
S-22	30	25	6	6	6
S-23	20	13	13	6	0
M-1	25	18	12	18	6
M-2	23	13	6	20	13
M-3	28	31	12	0	6
M-4	30	16	15	15	0
M-5	32	16	12	11	8
M-6	28	12	5	22	5
M-7	36	24	10	13	0
M-8	35	22	11	0	11
M-9	36	25	6	12	0
M-10	37	18	6	12	6
M-11	32	12	15	6	12
M-12	36	26	12	6	0
M-13	17	19	13	0	13
M-14	33	18	12	0	12
M-15	27	22	11	5	16
M-16	17	11	17	23	11
M-17	35	11	5	25	0
M-18	31	12	8	12	11
M-19	31	18	12	0	0

M-20	34	17	11	11	6
M-21	32	21	13	11	0
M-22	30	15	9	18	8
M-23	23	20	13	0	13
M-24	25	15	10	18	9
M-25	30	16	13	20	6
M-26	32	16	9	0	12
M-27	26	16	7	22	10
M-28	26	10	10	15	0
M-29	31	22	9	11	11
M-30	20	13	13	6	0
M-31	23	11	17	0	13
M-32	27	18	8	15	8
M-33	23	15	11	0	12
M-34	23	15	12	0	15
M-35	30	13	13	13	10
M-36	23	17	10	13	7
M-37	25	15	12	12	0
M-38	27	13	6	11	11
M-39	20	16	13	16	13
M-40	23	11	7	11	5
M-41	27	16	6	16	0
M-42	36	18	5	6	12
M-43	22	16	5	16	11
M-44	31	13	11	17	0
M-45	30	17	8	18	8
M-46	33	23	5	13	6
M-47	31	17	7	11	7
Average	25.74	15.63	9.29	8.89	6.54

Table 3: Result of isolation and identification of mycoflora associated with wheat seed samples by standard agar plate method.

Samples	**% of mycoflora associated with wheat seed samples**				
	***Aspergillus* spp.**	***Alternaria triticina***	***Bipolaris sorokiniana***	***Penicillium* spp.**	***Fusarium moniliforme***
S-1	34	16	16	0	6
S-2	38	35	0	0	0
S-3	30	18	11	16	11
S-4	20	20	6	13	8
S-5	41	11	12	0	0
S-6	38	11	8	11	4
S-7	20	13	6	6	11
S-8	16	16	5	27	5
S-9	25	25	12	0	15
S-10	30	15	12	12	6
S-11	31	11	9	25	0
S-12	38	17	11	0	5
S-13	38	12	12	5	11
S-14	17	13	11	0	9
S-15	31	18	12	6	0
S-16	31	12	6	18	12
S-17	28	8	12	12	10
S-18	34	18	11	0	6
S-19	18	16	6	18	6
S-20	32	12	10	0	11
S-21	33	16	11	11	4
S-22	27	13	7	6	9
S-23	20	13	13	6	6
M-1	31	13	7	11	11
M-2	11	17	11	16	6
M-3	25	17	10	5	5
M-4	31	12	12	18	6
M-5	31	13	5	19	11
M-6	18	18	11	12	0
M-7	26	17	6	12	11
M-8	27	16	11	16	10
M-9	22	20	8	12	0
M-10	33	20	7	0	13

M-11	11	23	5	17	0
M-12	34	16	12	22	6
M-13	31	19	11	6	0
M-14	30	11	9	0	11
M-15	20	13	10	7	0
M-16	35	18	0	12	6
M-17	29	12	6	6	13
M-18	20	16	15	0	0
M-19	31	11	11	0	11
M-20	25	19	11	13	0
M-21	38	16	13	0	8
M-22	29	18	12	12	0
M-23	31	18	6	18	6
M-24	32	17	11	0	0
M-25	35	17	6	17	0
M-26	35	13	12	0	0
M-27	22	28	11	6	11
M-28	27	17	20	0	7
M-29	31	18	10	18	6
M-30	29	13	14	0	6
M-31	36	13	9	0	0
M-32	28	17	5	22	10
M-33	25	18	13	0	13
M-34	19	19	8	13	0
M-35	24	16	12	6	6
M-36	34	12	9	12	6
M-37	28	17	6	0	0
M-38	39	22	11	6	0
M-39	32	12	6	0	13
M-40	36	16	12	17	11
M-41	27	17	6	0	13
M-42	18	16	12	12	0
M-43	29	12	12	6	10
M-44	23	16	7	13	13
M-45	34	23	8	0	0
M-46	19	13	10	19	0
M-47	25	24	6	18	7
Average	28.46	16.09	9.49	8.63	6.19

Table 4: Result of isolation and identification of mycoflora associated with wheat seed samples by standard towel paper method.

Samples	**% of mycoflora associated with wheat seed samples**				
	***Aspergillum* sp.**	***Alternaria triticina***	***Bipolaris sorokiniana***	***Penicillium* sp.**	***Fusarium moniliforme***
S-1	7	4	3	3	2
S-2	8	7	6	1	1
S-3	8	8	7	0	3
S-4	7	3	8	0	0
S-5	5	3	10	2	5
S-6	8	7	5	0	2
S-7	4	5	7	3	4
S-8	8	4	9	2	6
S-9	5	7	4	0	0
S-10	11	3	5	1	3
S-11	3	7	9	2	1
S-12	9	4	8	0	0
S-13	15	7	4	5	2
S-14	5	3	8	0	5
S-15	10	5	8	4	2
S-16	14	7	4	0	0
S-17	16	5	7	2	3
S-18	20	5	5	0	6
S-19	23	5	7	4	1
S-20	19	7	4	0	0
S-21	8	3	6	0	3
S-22	6	5	9	5	2
S-23	11	4	5	2	4
M-1	16	3	7	0	2

M-2	21	4	4	5	1
M-3	7	11	2	0	0
M-4	8	7	3	1	4
M-5	15	6	5	0	3
M-6	11	6	3	1	4
M-7	8	3	5	0	3
M-8	24	6	9	0	0
M-9	5	1	7	3	3
M-10	6	8	6	0	0
M-11	15	3	4	2	0
M-12	13	7	8	3	4
M-13	14	4	6	0	0
M-14	11	3	5	1	4
M-15	11	4	7	0	1
M-16	5	2	3	3	2
M-17	4	6	3	4	0
M-18	7	2	3	0	0
M-19	3	9	4	3	3
M-20	2	4	4	1	0
M-21	5	6	9	0	3
M-22	16	3	2	5	0
M-23	2	1	5	2	2
M-24	20	8	4	2	4
M-25	7	4	2	6	3
M-26	16	2	3	4	0
M-27	7	5	4	2	2
M-28	13	2	3	1	0
M-29	4	3	4	2	0
M-30	22	3	3	2	3
M-31	11	4	6	0	2
M-32	17	7	11	1	2
M-33	7	5	4	2	3
M-34	25	3	7	0	0
M-35	11	5	4	2	3
M-36	13	3	7	2	3
M-37	19	7	8	3	5
M-38	17	5	11	4	3
M-39	16	4	8	0	2
M-40	9	8	5	5	2
M-41	7	8	6	2	4
M-42	17	8	8	8	6
M-43	10	9	4	5	3
M-44	16	3	6	3	3
M-45	13	3	9	3	6
M-46	6	4	8	4	3
M-47	9	5	12	4	3
Average	10.76	4.98	5.99	1.86	2.30

Discussion

In the present investigations, total 70 wheat seed samples of different variety were collected from the farmers of 25 different villages of Samastipur and Muzaffarpur districts of Bihar state to study the quality and health status of wheat seeds. Also to isolate and identify the seed mycoflora associated with seeds by different methods.

In the present study, three detection methods *i.e.*, standard blotter paper, standard agar plate and standard paper towel methods used for detection of seed associated in seeds collected from farmers. In all the three detection methods five fungus were recorded, which is found to associated with seeds *i.e.*, *Aspergillus* sp., *Alternaria triticina*, *Bipolaris sorokiniana*, *Fusarium moniliforme* and *Penicillium* sp. These recorded pathogens were varying in range in different methods. However, maximum mycoflora were recorded in standard blotter paper, standard agar plate methods and standard paper towel method recorded less mycoflora.

Singh *et al*., (2011) also confirm the findings, they isolated 16 sixteen fungal species by the standard agar plate and standard blotter paper method. The isolated fungi were identified as *Alternaria solani*, *A. alternata*, *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*.

Similarly, Zrari (2013) [19] in their study observed 10 mycoflora found to associated in wheat *i.e.*, *Alternaria* spp., *Aspergillus* spp., *Aureobasidium* spp., *Cladosporium* spp., *Drechslera* spp., *Penicillium* spp., *Rhizoctonia* spp., *Stemphylium* spp., *Mucor* spp. and *Rhizopus* spp. by the agar and blotter paper mathods. The higher number of fungi detected in agar plate method as compare to blotter paper method. Variation in detections, may be due to reason that week and slow growing fungi unable to grow well on blotter paper method as compared to agar plate method (Neergaard and Saad, 1962) [11]. Which is similar to our findings isolated

and identified the mycolfora from seeds by different detection methods and vary in different detection method.

Pathak and Razia (2013) [13] in their study recorded *Fusarium moniliforme, Rhizopus* spp., *Mucor* spp., *A. alternata, A. niger, A. flavus, C. lunata, Drechslera spp.* and *Penicillium* spp. in wheat seed samples. It also confirms our findings the seed mycoflora present in wheat seeds. The same observations were also recorded in agar plate method stated by Bashir *et al.,* (2012) [2] and Gohari *et al*., (2007) [4].

Tonu *et al.,* (2017) [18] in studied the health and quality of farmer saved wheat seeds. They recorded thirteen fungi in farmers' saved seed sample. The 5 major pathogenic fungi identified were *B. sorokiniana, A. tenuis, C. lunata, F. oxysporum* and *A. flavus.* This is also confirming to our findings.

In the present investigations, all the samples subjected to standard blotter paper, standard agar plate and standard paper towel methods of detection were found to be associated with either one or more fungi and none of the sample was free from the fungal infection. In standard blotter paper and standard agar plate, highest number of mycoflora was detected than standard paper towel method. Fungi *Aspergillus* sp and *Alternaria triticina* was found predominant in standard blotter paper, standard agar plate and standard paper towel methods followed by *Bipolaris sorokiniana*, *Penicillium* sp and *Fusarium moniliforme*. All the wheat seed samples collected from 25 different locations of Samastipur and Muzaffarpur districts of Bihar showed varying percent of occurrence of seed borne fungi. Since, wheat is the main crop of this region use of pathogen free seed and seed health test to detect and identify the associated mycoflora becomes vital in order to take up appropriate management strategies for successful wheat cultivation.

References

1. Archana B, Prakash HS. Survey of seedborne fungi associated with rice seeds in India. International Journal of Research in Applied Microbiology. 2013;3(1):25-29.
2. Bashir M, Mani MA, Kutama AS. Seed-borne mycoflora of local and improved wheat (*Triticum aestivum* L.) cultivars in Kano, Nigeria. Bayero Journal of Pure and Applied Science. 2012;5:101-103.
3. Bateman GL, Kwasna. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Applied Soil Ecology. 1999;13(3):271-282.
4. Gohari AM, Sedaghat N, Nikkhah MJ, Riseh RS. Mycoflora of wheat grains in the main production area in kerman province, Iran. International Journal of Agriculture and Botany. 2007;9:635-637.
5. Gopalakrishnan C, Valluvaparidasan V. Seed borne microflora associated with rice seeds in Tamil Nadu, India. Crop Research (Hisar). 2009;37:207-209.
6. Gopalakrishnan C, Kamalakannan A, Valluvaparidasan V. Survey of seed-borne fungi associated with rice seeds in Tamil Nadu, India. Libyan Agriculture Research Center Journal International. 2010;1(5):307-309.
7. Hasan MM, Chowdhury SP, Alam S, Hossain B, Alam MS. Antifungal Effects of Plant Extracts on Seed-Borne Fungi of Wheat Seed Regarding Seed Germination, Seedling Healthy and Vigor Index. Pak. J. Biol. Sci. 2005;8(9):1284-1289.
8. ICAR - Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India. Annual Report. 2018.
9. ISTA (International Rules for Seed Testing Association), International Rules for Seed Testing. Rules Amendments. Seed Science and Technology. 2001;29:1-127.
10. Khanzada KA, *et al*. Effects of seed dressing fungicide for the control of seed borne mycoflora of wheat. Asian Journal of Plant Science. 2002;1(4):441-444.
11. Neergaard P, Saad A. Seed health testing of rice: A contribution to development of laboratory routine test methods. Indian Phytopathology. 1962;15:85-111.
12. Ora N, NAN Faruq, MT Islam, N Akhtar, Rahman MM. Detection and identification of seed borne pathogens from some cultivated hybrid rice varieties in Bangladesh. Middle-East Journal of Scientific Research. 2011;10:482–488.
13. Pathak N, Razia K Zaidi. Fungi Associated with Wheat Seed Discolouration and Abnormalities in *In vitro* Study. J. Agril. Sci. 2013;4(9):516-520.
14. Sharma RC, Chahal HS. Multilocational evaluation of seed health of parental lines of hybrid rice. Plant Disease Research. 1996;11(2):169-172.
15. Sharma A, Kapoor AS. Detection of seed borne mycoflora associated with some rice varieties grown in Himachal Pradesh. The Bioscan. 2016;11(4):2733-2739.
16. Singh AR, Boopathi T, Singh SB, Dutta SK, Singh LS, Saha SL, *et al*. Seed Borne Mycoflora of Tribal farmers' Saved Hill Rice, Oryza sativa in Northeast of India. Indian Journal of Hill Farming, Special issue. 2018. p. 58-65.
17. Singh J, Srivastava S, Shikha Sinha A, Bose B. "Studies on seed mycoflora of wheat (*Triticum aestivum* L.) treated with potassium nitrate and its effect on germination during storage". Research Journal of Seed Science. 2011;4:148- 156.
18. Tonu NN, Islam MR, Karim MM, Chowdhury MSM, Mia MAT. Quality and Health Status of Farmer Saved Wheat Seed in Bangladesh. International Journal of Research Science & Management. 2017;4(9):10-24.
19. Zrari TJO. Detection of Aflatoxin from Some Aspergillus sp. Isolated from Wheat Seeds. Journal of Life Sciences, USA. 2017;7(10):1041-1047.