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Evaluation of Seed-borne Mycoflora of Rice (*Oryza sativa* L.) by the Effect of Storage Length on Fungal Invasion under Different Storage Technique

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The incidence of seed-borne mycoflora in sahbhagi rice was screened by Agar plate method and Blotter method. Seed stored in different conditions like Bin and Gunny bag then observation was taken periodically 0, 3, 6, 9, 12 months in each storage condition. Surface sterilization was done by 0.1% mercuric chloride ($HgCl_2$) solution. Both surface sterilized and unsterilized seeds were taken for isolation of fungi. A total number of 16 fungal species including *Rhizopus stolonifer, Mucor hiemalis, Aspergillus flavus, A. niger, A. candidus, A. fumigates, Penicillium rubrum*, *P. citrinum, Alternaria alternata, Drecshlera gramini, Curvularia lunata, Trichoderma harzianum, Microdochium lycopodinum, Fusarium oxysporum,* Dark Sterile Mycelium and White Sterile Mycelium were found to be associated with the Sahabhagirice cultivar. Among them the most predominant seed-borne fungi, associated with seed were *A. niger* (56.67% & 63.33%), *A. flavus* (53.33% & 56.67%), *P. citrinum* (50% & 53.33%) and *M. lycopodinum* (50% & 53.33%) by Agar plate method and *A. niger* (50% & 56.67%), *A. flavus* (46.67% & 53.33%), *P. citrinum* (46.67% & 50%) and *M. lycopodinum* (46.67% & 50%) by Blotter method in Bin and Gunny Bag storage condition, respectively at the end of storage. Visual examination of seed showed that the maximum increase in the number of abnormal seed was recorded in gunny bag than Bin. Highest percent incidence of seed borne fungi was recorded in Gunny bag storage condition than Bin. In both storage procedures control seed yielded more number of seed-borne fungi as compared to sterilized seed during different storage period.

Keywords: Rice, seed pathogens, *A. niger, A. flavus, P. citrinum, M. lycopodinum*.

Seed-borne fungi are one of the most important biotic constrains in seed production worldwide. India is renowned rice (*Oryza sativa* L.) producing country and stands second with an annual production of 155 million tonnes (FAOSTAT, 2011). Rice cultivation takes place in all states of India, but West Bengal, Uttar Pradesh, Madhya Pradesh, Punjab, Orissa and Bihar are the major rice producing states. Sahabhagi Dhan has shown a yield advantage of 0.8 to 1 tonnes per ha. over other varieties under drought conditions (Yamano *et al*., 2013). The tested high yielding variety "Sahabhagi dhan" was released from CRRI in 2009 can be suitably directs sown or transplanted in rainfed upland ecosystem and tolerant to drought and is resistant to leaf blast, moderately resistant to brown spot, sheath rot, stem borer, leaf folder (Samant *et al*., 2015). The crop is affected by as many as 36 seed-borne diseases of which 31 were caused by fungi (Ou, 1985). Seed health testing is one of the conventional methods to detect the presence of seed-borne fungi (ISTA, 1993). The purpose of seed health testing is to assure the safe movement of seed of different crops for research or trade. The aim of this study, isolation of dominant mycoflora of rice at different stages of

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storage by standard technique under different storage. Ahmed *et al*. (2013) in their study on rice seed contamination identified 9 species of seed-born fungi including; *Fusarium oxysporum, F. moniliforme, Bipolaris oryzae, Alternaria padwickii, Curvularia lunata, Aspergillus flavus, A. niger, Penicillium* sp. and *Nigrospora oryzae*. Uma and Wesely (2013) by studying on 5 seed varieties after ending storage period in India, identified seeds contamination to *A. flavus, A. niger, P. citrinum, A. padwickii* and *R. oryzae* in which, *A. flavus* with 18% and *A. niger* with 17.6% had the highest severity. Extensive studies have been carried out on isolation of seed mycoflora from different seeds by several workers from all over the world including India (Christensen, 1952; Cherewick, 1954; Joshi and Gupta, 1980; Gupta *et al.* 1988; Sulaiman and Hussain, 1985; Vijaylaxmi and Rao, 1985; Shah and Jain 1993; Chiejina, 2006; Habib *et al.*, 2007; Jogdand *et al.*, 2010; Panchal and Dhale, 2011; Hajihasani *et al.*, 2012 and Santoshreddy *et al*., 2014)

MATERIALS AND METHODS

The experiment was conducted in the laboratory of department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Seed (fresh & stored) were taken from Banaras Hindu University Agricultural farm through plant breeder. Two storage procedures (Bin and Gunny Bag) of Rice were selected for detailed study.

Visual examination of deterioration of seed by different storage procedure

Seed stored in different condition like Bin and Gunny bag then observation was taken periodically 0, 3, 6, 9 and 12 months in each storage condition. It is very common method for identifying cultivars in the laboratory by examining the seed by naked eyes as well as under stereoscopic binocular microscope for the presence of abnormal seeds i.e., fungal infested seeds, insect eaten seeds, inert matter, plant debris, seeds of other crops, discolouration, mouldy growth, formation of lumps, etc. in the seeds. Seeds were also observed for musty odour and smells of decay.

Isolation of seed mycoflora

The isolation of fungi from seeds was done by the standard technique i.e. Agar plate method and Blotter method (ISTA, 1966) at different period of storage. The micro fungi associated with fresh and stored seed of Rice were isolated. One lot of seed is treated with $HgCl_2$ and one is controlled. These seeds were dried back again to its original weight and used for further study and three replication of each treatment were prepared. The experimental data were recorded from fresh as well as stored, after every three months (0, 3, 6, 9 and 12 months) of storage period.

Agar plate method (Muskett, 1948)

The nutrient medium used for isolation and observation of fungi was Potato Dextrose Agar (PDA) medium. Sterilized (15 psi for 20 min.) melted medium was poured aseptically into sterilized Petri-dishes were allowed to cool and settle down. Ten seeds were placed in each Petri-plate containing solidify PDA medium with flame sterilized forceps under aseptic conditions. Both surface sterilized and control (unsterilized) seeds were taken for isolation of fungi. Surface sterilization was done by 0.1% mercuric chloride ($HgCl_2$) solution. All the Petri-plates containing seeds were incubated at 25± 1°C for a week under 12 hours alternating cycles of light and darkness. Fungi growing on seeds were isolated and identified under microscope.

Blotter method (de Tempe, 1953)

The blotting paper was sterilized and then three pieces of sterilized blotting papers in folds moistened with sterilized distilled water were placed in each sterilized Petri dish of 9 cm diameter. Ten seeds were placed equal distance on blotter in each Petri dish. Both surface sterilized and unsterilized seeds were taken for isolation of fungi. The Petri-plates were incubated at 25 ± 1°C under 12 hours alternating cycle of light and darkness. Plated seeds were periodically observed for the presence and growth of fungal species on the seeds.

Incidence of different fungal pathogen was recorded as under

$$\text{Incidence (\%)} = \frac{\text{No. of infected seeds}}{\text{Total no of seeds assessed}} \times 100$$

RESULTS AND DISCUSSIONS

In the present work the seed mycoflora of fresh and stored seeds of rice was studied it was found that Agar plate method of fungal isolation are effective, consistently and routinely applicable and provide reliable results than Blotter

method. The occurrence of fungi most frequently encountered is recorded. The seed mycoflora were isolated periodically 0, 3, 6, 9 and 12 months by Agar plate method and Blotter method from treated seed with $HgCl_2$ and control in each storage condition (Bin and Gunny bag).The finding of the experiment conducted during the present investigation and presented by using following methods.

Visual examination

It is most common method for identifying cultivar in the laboratory examining through naked eyes as well as binocular microscope for abnormalities i.e. discoloration. Seed abnormalities were observed to be increasing with the length of storage. It was observed to increase in both storage techniques with the advancement of storage period. At the end of period, maximum increase in the number of abnormal seeds was recorded in gunny bag (20) followed by bin (17) out of 100.(Table-1).The discoloration of grains by fungal infection was reported by several workers (Godika *et al.,* 2000; Srivastava and Jaiswal, 2000, Singh *et al*, 2013).

Estimation of seed borne fungi at different period of storage by different storage technique

Agar plate method and Blotter method was employed for this study and two sets of seeds were analyzed i.e., treated and control seeds during 0, 3, 6, 9, 12 months of storage under different storage condition(Bin and Gunny bag) are presented in Table 2, 3, 4 and 5. All fungi were identified on the basis of their cultural and morphological characteristics. In the present study it was found that the Agar plate method of fungal isolation is effectively applicable and provides reliable result than Blotter method. The occurrence of fungi most frequently encountered is recorded. A total of 16 different fungi were isolated and the prominent seed associated mycoflora of rice are the *Rhizopus stolonifer, Mucor hiemalis, A. flavus, A. niger, A. candidus, A. fumigates, Penicillium rubrum, P. citrinum, Alternaria alternata, Drecshlera gramini, Curvularia lunata, Trichoderma harzianum, Microdochium lycopodinum, Fusarium oxysporum,* Dark Sterile Mycelium and White Sterile Mycelium.The fungal species that were observed only in fresh seeds are *R. stolonifer, A. niger, A. flavus, P. citrinum, M. lycopodinum*.The fungal species that were observed after 12 months of storage are *R. stolonifer, M. hiemalis, A. flavus, A. niger, A. candidus, A. fumigates,P. rubrumP. citrinum, A. alternata, C. lunata, T. harzianum, M. lycopodinum, D. gramini, F. oxysporum,* White Sterile Mycelium and Dark Sterile Mycelium are presented in Table 2-5.

Comparison between Agar plate method and Blotter method

Table 2, 3, 4 and 5 related to isolation of seed mycoflora reported that more fungi were isolated by Agar plate method than the Blotter method. Slow growing fungi could not grow in successfully in culture plates in competition with fast growing fungi (Agarwal *et al., 1972;* Singh *et* al., 2005; Dawar *et al.,* 2007; Jogdand *et al.,* 2010 and Panchal and Dhale, 2011)

Comparison between Bin and Gunny bag storage technique

The maximum number of seed borne fungi recorded in Gunny bag storage condition than Bin in both treated and control seed by both Agar plate method and Blotter method and the percent incidence of seed-borne mycoflora of rice also highest in gunny bag storage condition in comparison to bin storage condition.

Table 1. Visual examination of seed by different storage techniques

Storage methods	Storage period (in months)									
	Fresh		3		6		9		12	
	N	A	N	A	N	A	N	A	N	A
Bin	95	5	93	7	90	10	87	13	83	17
Gunny bag	95	5	92	8	88	12	85	15	80	20

N=Normal per cent A=Abnormal per cent

Table 2. Percent incidence of seed borne fungi on rice seeds stored in Bins at different period of storage by Agar plate method

S. No.	Fungal Species	Fresh		3 months		6 months		9 months		12 months	
		T	C	T	C	T	C	T	C	T	C
1	*Rhizopus stolonifer*	0 (0.00)*	3.33 -6.14	3.33 -6.14	6.67 -12.28	10 -18.42	13.33 -21.13	16.67 -23.84	20 -26.55	23.33 -28.76	26.67 -30.98
2	*Mucor hiemalis*	0 0	0 0	0 0	0 0	3.33 -6.14	10 -18.42	13.33 (21.13)	16.67 -23.84	20 -26.55	26.67 -30.98
3	*Aspergillus flavus*	6.67 -12.28	10 -18.42	16.67 -23.84	23.33 -28.76	26.67 (30.98)	30 -33.19	33.33 (35.20)	40 -39.21	46.67 -43.05	53.33 -46.9
4	*Aspergillus niger*	13.33 -21.13	16.67 -23.84	20 -26.55	26.67 -30.98	30 -33.19	33.33 -35.2	36.67 -37.2	46.67 -43.05	50 -44.98	56.67 -48.92
5	*Aspergillus candidus*	0 0	0 0	0 0	0 0	3.33 -6.14	10 -18.42	6.67 -12.28	13.33 -21.13	10 -18.42	16.67 -23.84
6	*Aspergillus fumigatus*	0 0	0 0	0 0	3.33 -6.14	6.67 -12.28	10 -18.42	10 -18.42	13.33 -21.13	16.67 -23.84	20 -26.55
7	*Penicillium rubrum*	0 0	0 0	0 0	0 0	0 0	00.00 (0.00)	00.00 (0.00)	3.33 -6.14	3.33 -6.14	6.67 -12.28
8	*Penicillium citrinum*	10 -18.42	13.33 -21.13	16.67 -23.84	20 -26.55	26.67 -30.98	30 -33.19	33.33 -35.2	40 -39.21	43.33 -41.13	50 -44.98
9	*Alternaria alternata*	0 0	0 0	3.33 -6.14	6.67 -12.28	10 -18.42	13.33 -21.13	16.67 -23.84	20 -26.55	20 -26.55	23.33 -28.76
10	*Drecshlera gramini*	0 0	0 0	0 0	0 0	0 0	00.00 (0.00)	00.00 (0.00)	3.33 -6.14	00.00 (0.00)	6.67 -12.28
11	*Curvularia lunata*	0 0	0 0	3.33 -6.14	6.67 -12.28	0 0	3.33 -6.14	6.67 -12.28	10 -18.42	13.33 -21.13	16.67 -23.84
12	*Trichoderma harzianum*	0 0	0 0	0 0	0 0	0 0	3.33 -6.14	0 0	6.67 -12.28	3.33 -6.14	10 -18.42
13	*Microdochium lycopodinum*	10 -18.42	13.33 -21.13	16.67 -23.84	20 -26.55	23.33 -28.76	30 -33.19	33.33 -35.2	36.67 -37.2	46.67 -43.05	50 -44.98
14	*Fusarium oxysporum*	0 0	0 0	0 0	0 0	0 0	00.00 (0.00)	00.00 (0.00)	3.33 -6.14	6.67 -12.28	10 -18.42
15	Dark Sterile Mycelium	0 0	0 0	0 0	0 0	0 0	3.33 -6.14	3.33 -6.14	6.67 -12.28	10 -18.42	13.33 -21.13
16	White Sterile Mycelium	00.00 (0.00)	0 0	0 0	0 0	0 0	00.00 (0.00)	00.00 (0.00)	3.33 -6.14	3.33 -6.14	6.67 -12.28
	CD(0.05)	4.83	5.56	8.37	9.13	8.14	8.27	8.85	11.52	9.7	9.03

*Note:Figures within Brackets are Transformed values

Table 3. Percent incidence of seed borne fungi on rice seeds stored in Gunny bags at different period of storage by Agar plate method

S. No.	Fungal Species	Fresh		3 months		6 months		9 months		12 months	
		T	C	T	C	T	C	T	C	T	C
1	*Rhizopus stolonifer*	0 (0.00)*	3.33 -6.14	6.67 -12.28	10 -18.42	13.33 -21.13	16.67 -23.84	23.33 -28.76	26.67 -30.98	26.67 -30.98	30 -33.19
2	*Mucor hiemalis*	0 0	0 0	3.33 -6.14	6.67 -12.28	6.67 -12.28	10 -18.42	16.67 -23.84	20 -26.55	23.33 -28.76	30 -33.19
3	*Aspergillus flavus*	6.67 -12.28	10 -18.42	23.33 -28.76	30 -33.19	33.33 -35.2	36.67 -37.2	36.67 -37.2	43.33 -41.13	50 -44.98	56.67 -48.82
4	*Aspergillus niger*	13.33 -21.13	16.67 -23.84	23.33 -28.76	30 -33.19	33.33 -35.2	36.67 -37.2	43.33 -41.13	53.33 -46.9	56.67 -48.82	63.33 -52.75
5	*Aspergillus candidus*	0 0	0 0	6.67 -12.28	10 -18.42	6.67 -12.28	13.33 -21.13	10 -18.42	16.67 -23.84	13.33 -21.13	20 -26.55
6	*Aspergillus fumigatus*	0 0	0 0	3.33 -6.14	6.67 -12.28	10 -18.42	13.33 -21.13	13.33 -21.13	16.67 -23.84	20 -26.55	23.33 -28.76
7	*Penicillium rubrum*	0 0	0 0	0 0	0 0	0 0	0 0	0 0	3.33 -6.14	3.33 -6.14	6.67 -12.28
8	*Penicillium citrinum*	10 -18.42	13.33 -21.13	23.33 -28.76	26.67 -30.98	30 -33.19	33.33 -35.2	40 -39.21	46.67 -43.05	46.67 -43.05	53.33 -46.9
9	*Alternaria alternata*	0 0	0 0	6.67 -12.28	10 -18.42	10 -18.42	13.33 -21.13	20 -26.55	23.33 -28.76	23.33 -28.76	26.67 -30.98
10	*Drecshlera gramini*	0 0	0 0	3.33 -6.14	6.67 -12.28	6.67 -12.28	10 -18.42	10 -18.42	13.33 -21.13	10 -18.42	16.67 -23.84
11	*Curvularia lunata*	0 0	0 0	3.33 -6.14	6.67 -12.28	6.67 -12.28	10 -18.42	6.67 -12.28	13.33 -21.13	13.33 -21.13	16.67 -23.84
12	*Trichoderma harzianum*	0 0	0 0	0 0	3.33 -6.14	3.33 -6.14	6.67 -12.28	3.33 -6.14	10 -18.42	6.67 -12.28	13.33 -21.13
13	*Microdochium lycopodinum*	10 -18.42	13.33 -21.13	20 -26.55	23.33 -28.76	26.67 -30.98	33.33 -35.2	36.67 -37.2	43.33 -41.13	46.67 -43.05	53.33 -46.9
14	*Fusarium oxysporum*	0 0	0 0	0 0	0 0	3.33 -6.14	3.33 -6.14	3.33 -6.14	6.67 -12.28	6.67 -12.28	10 -18.42
15	Dark Sterile Mycelium	0 0	0 0	0 0	6.67 -12.28	6.67 -12.28	10 -18.42	3.33 -6.14	10 -18.42	13.33 -21.13	16.67 -23.84
16	White Sterile Mycelium	0 0	0 0	0 0	0 0	0 0	0 0	3.33 -6.14	6.67 -12.28	6.67 -12.28	10 -18.42
	CD(0.05)	4.83	5.56	12.02	11.06	12.14	7.91	10.68	9.31	10.15	6.9

***Note:** Figures within Brackets are Transformed values

Table 4. Percent incidence of seed borne fungi on rice seeds stored in Bins at different period of storage by Blotter method

S. No.	Fungal Species	Fresh		3 months		6 months		9 months		12 months	
		T	C	T	C	T	C	T	C	T	C
1	*Rhizopus stolonifer*	0	3.33	10	13.33	16.67	20	20	23.33	20	26.67
		0	-6.14	-18.42	-21.13	-23.84	-26.55	-26.55	-28.76	-26.55	-30.98
2	*Mucor hiemalis*	0	0	0	6.67	10	13.33	16.67	20	20	23.33
		0	0	0	-12.28	-18.42	-21.13	-23.84	-26.55	-26.55	-28.76
3	*Aspergillus flavus*	6.67	10	13.33	20	23.33	26.67	30	36.67	40	46.67
		-12.28	-18.42	-21.13	-26.55	-28.76	-30.98	-33.19	-37.2	-39.21	-43.05
4	*Aspergillus niger*	13.33	16.67	16.67	23.33	26.67	30	33.33	43.33	46.67	50
		-21.13	-23.84	-23.84	-28.76	-30.98	-33.19	-35.2	-41.13	-43.05	-44.98
5	*Aspergillus candidus*	0	0	0	0	3.33	6.67	3.33	10	6.67	13.33
		0	0	0	0	-6.14	-12.28	-6.14	-18.42	-12.28	-21.13
6	*Aspergillus fumigatus*	0	0	0	3.33	3.33	6.67	6.67	10	13.33	16.67
		0	0	0	-6.14	-6.14	-12.28	-12.28	-18.42	-21.13	-23.84
7	*Penicillium rubrum*	0	0	0	0	0	0	0	0	0	3.33
		0	0	0	0	0	0	0	0	0	-6.14
8	*Penicillium citrinum*	10	13.33	13.33	16.67	23.33	26.67	30	36.67	40	46.67
		-18.42	-21.13	-21.13	-23.84	-28.76	-30.98	-33.19	-37.2	-39.21	-43.05
9	*Alternaria alternata*	0	0	0	3.33	10	10	13.33	16.67	16.67	20
		0	0	0	-6.14	-18.42	-18.42	-21.13	-23.84	-23.84	-26.55
10	*Drecshlera gramini*	0	0	0	0	0	0	0	0	0	3.33
		0	0	0	0	0	0	0	0	0	-6.14
11	*Curvularia lunata*	0	0	0	3.33	0	0	3.33	6.67	10	13.33
		0	0	0	-6.14	0	0	-6.14	-12.28	-18.42	-21.13
12	*Trichoderma harzianum*	0	0	0	0	0	0	0	3.33	3.33	10
		0	0	0	0	0	0	0	-6.14	-6.14	-18.42
13	*Microdochium lycopodinum*	10	13.33	13.33	16.67	20	26.67	30	33.33	43.33	46.67
		-18.42	-21.13	-21.13	-23.84	-26.55	-30.98	-33.19	-35.2	-41.13	-43.05
14	*Fusarium oxysporum*	0	0	0	0	0	0	0	0	3.33	6.67
		0	0	0	0	0	0	0	0	-6.14	-12.28
15	*Dark Sterile Mycelium*	0	0	0	0	0	0	0	3.33	6.67	10
		0	0	0	0	0	0	0	-6.14	-12.28	-18.42
16	*White Sterile Mycelium*	0	0	3.33	6.67	10	13.33	16.67	20	23.33	26.67
		0	0	-6.14	-12.28	-18.42	-21.13	-23.84	-26.55	-28.76	-30.98
	CD(0.05)	4.39	5.56	5.89	10.57	7.11	7.37	8.49	8.55	9.6	9.13

***Note:** Figures within Brackets are Transformed values

Table 5. Percent incidence of seed borne fungi on rice seeds stored in Gunny bags at different period of storage by Blotter method

S. No.	Fungal Species	Fresh		3 months		6 months		9 months		12 months	
		T	C	T	C	T	C	T	C	T	C
1	*Rhizopus stolonifer*	0	3.33	13.33	16.67	20	23.33	23.33	26.67	23.33	30
		0	-6.14	-21.13	-23.84	-26.55	-28.76	-28.76	-30.98	-28.76	-33.19
2	*Mucor hiemalis*	0	0	3.33	10	13.33	16.67	20	23.33	23.33	26.67
		0	0	-6.14	-18.42	-21.13	-23.84	-26.55	-28.76	-28.76	-30.98
3	*Aspergillus flavus*	6.67	10	20	23.33	26.67	30	33.33	40	46.67	53.33
		-12.28	-18.42	-26.55	-28.76	-30.98	-33.19	-35.2	-39.21	-43.05	-46.9
4	*Aspergillus niger*	13.33	16.67	20	26.67	30	33.33	36.67	46.67	50	56.67
		-21.13	-23.84	-26.55	-30.98	-33.19	-35.2	-37.2	-43.05	-44.98	-48.92
5	*Aspergillus candidus*	0	0	3.33	6.67	6.67	10	6.67	13.33	10	16.67
		0	0	-6.14	-12.28	-12.28	-18.42	-12.28	-21.13	-18.42	-23.84
6	*Aspergillus fumigatus*	0	0	0	3.33	6.67	10	10	13.33	16.67	20
		0	0	0	-6.14	-12.28	-18.42	-18.42	-21.13	-23.84	-26.55
7	*Penicillium rubrum*	0	0	0	0	0	0	0	3.33	3.33	6.67
		0	0	0	0	0	0	0	-6.14	-6.14	-12.28
8	*Penicillium citrinum*	10	13.33	16.67	20	26.67	30	33.33	40	43.33	50
		-18.42	-21.13	-23.84	-26.55	-30.98	-33.19	-35.2	-39.21	-41.13	-44.98
9	*Alternaria alternata*	0	0	6.67	10	10	13.33	16.67	20	20	23.33
		0	0	-12.28	-18.42	-18.42	-21.13	-23.84	-26.55	-26.55	-28.76
10	*Drecshlera gramini*	0	0	0	3.33	3.33	6.67	3.33	6.67	3.33	6.67
		0	0	0	-6.14	-6.14	-12.28	-6.14	-12.28	-6.14	-12.28
11	*Curvularia lunata*	0	0	0	0	0	3.33	6.67	10	13.33	16.67
		0	0	0	0	0	-6.14	-12.28	-18.42	-21.13	-23.84
12	*Trichoderma harzianum*	0	0	0	3.33	0	0	3.33	6.67	6.67	13.33
		0	0	0	-6.14	0	0	-6.14	-12.28	-12.28	-21.13
13	*Microdochium lycopodinum*	10	13.33	16.67	20	23.33	30	33.33	36.67	46.67	50
		-18.42	-21.13	-23.84	-26.55	-28.76	-33.19	-35.2	-37.2	-43.05	-44.98
14	*Fusarium oxysporum*	0	0	0	3.33	0	3.33	3.33	6.67	6.67	10
		0	0	0	-6.14	0	-6.14	-6.14	-12.28	-12.28	-18.42
15	*Dark Sterile Mycelium*	0	0	0	3.33	0	3.33	3.33	6.67	10	13.33
		0	0	0	-6.14	0	-6.14	-6.14	-12.28	-18.42	-21.13
16	*White Sterile Mycelium*	0	0	3.33	6.67	13.33	16.67	20	23.33	26.67	30
		0	0	-6.14	-12.28	-21.13	-23.84	-26.55	-28.76	-30.98	-33.19
	CD(0.05)	4.83	5.56	9.47	12.07	8.6	9.71	10.28	9.52	9.96	7.95

***Note:** Figures within Brackets are Transformed values

Comparison between treated ($HgCl_2$) and control seeds

Seed associated mycoflora in control seeds were more than treated seeds in both storage condition (Jogdand *et al.*, 2010; Panchal and Dhale, 2011; Singh *et al.* 2011; Singh *et al*., 2013 and Bhoyar *et al.*, 2014). Among all seed-borne fungi, the percent incidence was more in control than treated seed by Agar plate method and Blotter method.

CONCLUSION

The fungi associated with seed samples were *R. stolonifer, M. hiemalis, A. flavus, A. niger, A. candidus, A. fumigates, P. rubrum, P. citrinum, A. alternata, D. gramini, C. lunata, T. harzianum, M. lycopodinum, F. oxysporum*, Dark Sterile Mycelium and White Sterile Mycelium were found to be associated with the Sahbhagi rice cultivar detected by Agar plate method and Blotter method. Among them the most pre-dominant seed-borne fungi, associated with seed were *A. niger*, *A. flavus*, *P. citrinum* and *M. lycopodinum* in Bin and Gunny bag storage condition, respectively at the end of storage by Agar plate method and Blotter method. Among all seed borne fungi *A. niger* showed maximum incidence in under bin as well as gunny bag storage condition. Visual examination of seed showed that the maximum increase in the number of abnormal seed was recorded in gunny bag than Bin. Highest percent incidence of seed borne fungi was recorded in Gunny bag storage condition than Bin by both Agar plate method and Blotter method. Maximum number of fungi recorded in Agar plate method than Blotter method. In both storage procedure unsterilized (control) seed yielded more number of seed borne fungi as compared to sterilized seed during different storage period.

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