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Detection of Seed Borne Mycoflora Associated with Paddy Varieties of Eastern Vidarbha

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ABSTRACT: Paddy grain discolouration is an important disease and it has led to huge losses when the favourable conditions are available. It is caused by a complex of mycoflora. Paddy seeds of 7 varieties viz. PKV Kisan, D100, Sindewahi, RPN 7, RPM, MTU 1001 and 1010 were collected from different regions of Eastern Vidarbha. The maximum discoloured grade of each variety were tested for seed mycoflora by Standard blotter paper method and agar plate method. From the two methods, a total of 20 fungal species belonging to 16 fungal genera viz., Fusarium moniliforme, Fusarium solani, Fusarium semitectum, Fusarium equiseti, Curvularia lunata, Chaetomium sp., Alternaria padwickii, Tilletia sp., Rhizoctonia solani, Taeniolina sp., Aspergillus flavus, Sarocladium oryzae, Aspergillus niger, Bipolaris oryzae, Acremoniella sp., Pyricularia oryzae, Nigrospora sp., Pestalotia sp., Cladosporium sp. and Rhizopus sp. were detected. Pre-treatment was given to all the seven varieties of paddy and reduction of superficial and saprophytic fungi growth along with pathogenic fungi was recorded in pre-treated seeds. Thus, the study has proved that paddy seeds are associated with different mycoflora. Grain discolouration should be the current concern in order to halt the disease from affecting paddy production in the country. Therefore, production and post production activities of crop should be done carefully for disease free, quality seeds and to minimize crop failure.

Keywords: Paddy, blotter paper method, seed mycoflora, agar plate method, pre-treatment.

INTRODUCTION

Paddy (*Oryza sativa* L.) is the staple food for about four billion people. It plays a vital role in the culture and heritage of many Asian countries. It is the staple food for more than 65 per cent of the country's population, playing an important role in livelihood and food security of people and directly contributes to the attainment of Sustainable Development Goal (SDG). Globally, paddy production amounts to approximately 508.7 million tonnes (Anonymous, 2020a). India grows paddy in 43 Mha which is 22 per cent of crop land with production of 112 million tons (Mt) of milled paddy and average productivity of 2.6 t/ha (Anonymous, 2020b). It is cultivated in both large scale and small scale by many farmers around the world, except in Antartica (Muthayya *et al.*, 2014).

Of all biotic stresses that affects paddy, discolouration of paddy grains is an important disease that damages paddy seeds, thus limiting paddy production in many countries (Rajappan *et al.*, 2001; Pizzatti and Cortesi, 2008). Paddy is exposed to attack of 50 diseases that includes 21 mycoflora (Ilyas *et al.*, 2012). The symptoms of glume discolouration maybe exhibited internally or externally on kernels. The favourable conditions for disease development includes higher temperature, higher humidity, maturity stage with rainfall, pollination during high pressure wind and

periods of low light (Ashfaq et al., 2017; Dirchwolf et al., 2018). Mycoflora such as Alternaria spp., Fusarium spp., Pyricularia oryzae, Sarocladium oryzae, Sclerotium spp., Drechslera oryzae, Penicillium spp., Aspergillus spp., Phoma spp. and Cercospora spp. are reported to be associated with paddy grain discolouration (Islam and Ahmed 2017; Arshad et al., 2009; Rehman et al., 2013; Javaid and Anjum 2006; Misra and Vir 1990; Rajappan *et al.*, 2001; Ou, 1985). In India, the paddy crop succumbs to various diseases. Grain discolouration of paddy involves many mycoflora such as Alternaria padwickii, Alternaria longissima, Curvularia oryzae, C. lunata, Fusarium moniliforme, F. semitectum, F. oxysporum, F. solani, Pyricularia oryzae, Aspergillus niger and species of Phoma, Cercospora, Chaetomium, Penicillium, Myrothecium and Colletotrichum. Pre and post emergence death of seedlings caused due to the reduction of seed viability is due to grain discolouration caused by Bipolaris oryzae, M. grisea, Fusarium moniliforme, F. graminearum, S. oryzae, C. oryzae and Alternaria padwickii (Duraiswamy, 1982). Alternaria alternata and Curvularia lunata which causes ashy grey discolouration and black discolouration, with dark brown spots on seeds respectively are found mostly on coat of seed and endosperm region of seed (Sachan and Agrawal, 1995).

Thus, reduction in yield of paddy as much as 75 per cent is due to reduction in grain weight, floret sterility, germination inhibition, reduction of stand as well as due to year to year transmission due to seed borne nature of pathogen (Trung et al., 1993). The paddy seeds which are infected poses a serious problem due to reduced quality of the seed, hence seed certification and marketing is hindered (Pham et al., 2001). Losses due to the detrimental effect of seed mycoflora is approximately 2.5 million tons annually (Alam et al., 2014). Among the limiting factors of successful growing of paddy, seed mycoflora is one of the most important factors other than climate and crop management. Thus, the present study was therefore undertaken to investigate on the seed mycoflora of paddy.

MATERIAL AND METHODS

Collection of paddy seed samples

From farmers field of different regions of Eastern Vidarbha during 2020 (*Kharif* season), the different varieties of paddy viz. 1010, PKV Kishan, RPM, RPN 7, D100, MTU 1001 and Sindewahi were collected from.

Detection and identification of seed pathogen

Seed borne mycoflora from paddy seed was detected by standard blotter paper method and agar plate method.

A. Standard blotter paper method (ISTA, 1976)

Moistened with sterile distilled water, three layers of sterilized blotter paper (90mm diameter) were placed in Petri plates (90mm diameter). The excess water was drained out from the plates. The paddy seeds were transferred to the plates containing three-layer moist blotter papers. In each plate, twenty five seeds were placed at equal distance and they were incubated at 2° C under alternate cycles of 12 hrs. of light and darkness for 7 days. Hundred such seeds from each grade of each cultivar were tested in each replication and they were maintained in four replications. The seeds were examined under stereoscopic binocular microscope for associated fungi and identification was done based on habit and colony characters after seventh day of incubation.

Seed borne mycoflora from paddy seed was detected by standard blotter paper method and agar plate method.

a. Untreated seed

b. Seed treatment with NaOCl (0.1%) solution.

To detect seed borne fungi, seeds were surface disinfected by dipping in sodium hypochlorite (NaOCl) solution at 0.1 per cent concentration for one minute and washed subsequently in sterile distilled water with 3 changes in order to remove traces of sodium hypochlorite. The seeds were dried and plated. Seeds without surface disinfection were also used for detection of seed borne mycoflora.

B. Agar plate method

Northern Ireland first used this method for seed health management. Pre sterilized Petri plates were poured with 18-20 ml of autoclaved potato dextrose agar media. On cooling the medium, the seeds were equidistantly placed aseptically with ten seeds per Petri plate were then placed. Forty seeds were used per test. The plates were incubated at 28 ± 2^{0} C under diurnal conditions. The seeds were examined under stereoscopic binocular microscope for associated fungi and identification was done based on habit and colony characters after seventh day of incubation

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RESULTS AND DISCUSSIONS

A. Detection using Standard Blotter Method

Results obtained in table number 1 and figure 1 showed that in blotter paper method in maximum discoloured grade of seeds, the highest mycoflora per cent was found in variety Sindewahi i.e. 84 per cent and it was followed by varieties D100 and PKV Kisan with per cent mycoflora association of 63 and 38 per cent respectively. Variety RPN 7 showed mycoflora association of 36 per cent, RPM showed 32 per cent, varieties 1010 and MTU 1001 showed 32 and 20 per cent association respectively. Among the mycoflora, both pathogenic and saprophytic ones were found. Fusarium solani (79 per cent), Curvularia lunata (77 per cent), Tilletia sp.(41 per cent), Fusarium moniliforme (30 per cent), Alternaria padwickii (21 per cent), Aspergillus flavus (21 per cent), Sarocladium oryzae (9 per cent), Bipolaris oryzae (8 per cent), Fusarium semitectum (3 per cent), Aspergillus niger (3 per cent), Pestalotia sp. (3 per cent), Fusarium equiseti (2 per cent), Rhizoctonia solani (2 per cent), Acremoniella sp. (2 per cent), Nigrospora sp. (2 per cent), Pyricularia oryzae (1 per cent) and Taeniolina sp. (1 per cent) were the fungi detected.

The above method is commonly used for identifying seed borne fungi and the findings were similar with Naveenkumar et al., (2016), Singh et al., (2018) and Sultana et al., (2020). Singh et al., (2018) carried out study to assess the mycoflora of paddy and a total of 21 fungi including like Acremoniella sp., Alternaria sp., Curvularia lunata, C. oryzae, Bipolaris oryzae, Fusarium moniliforme, F. semitectum, Pyricularia grisea, F. oxysporum, Rhizoctonia sp., Sarocladium oryzae, Tilletia sp., Aspergillus flavus, A. niger, Penicillium sp. and Rhizopus stolonifer were recorded. Chaudhary et al., (2021) recorded twenty-five species of fungi belonging to 15 genera associated with the paddy varieties which included A. niger, A. flavus, Curvularia lunata, Bipolaris oryzae, Fusarium moniliforme, Nigrospora oryzae, F. oxysporum, F. solani, Penicillium sp. and Sarocladium oryzae.

Table 1: Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Blotter paper method.

Variety	Per cent association of seed mycoflora							Total	i
Seed mycoflora	PKV KISAN	MTU 1001	D100	RPM	RPN 7	SINDEWAHI	1010	fungal load	Mean
Fusarium moniliforme	5			5	2	16	2	30	4.28
Fusarium solani	7	8	16	7	13	22	6	79	11.28
Fusarium semitectum	3						-	3	0.42
Fusarium equiseti		2						2	0.28
Curvularia lunata	11	6	12	4	8	26	10	77	11
Alternaria padwickii	7		5	2	6		1	21	3
Tilletia sp.			14	14	5		8	41	5.85
Rhizoctonia solani							2	2	0.28
Taeniolina sp.	1							1	0.14
Aspergillus flavus			14			7		21	3
Sarocladium oryzae	4					5		9	1.28
Aspergillus niger						3		3	0.42
Bipolaris oryzae		-	2		2	3	1	8	1.14
Acremoniella sp.						2		2	0.28
Pyricularia oryzae		1						1	0.14
Nigrospora sp.							2	2	0.28
Pestalotia sp.		3					-	3	0.42
Total	38	20	63	32	36	84	32		

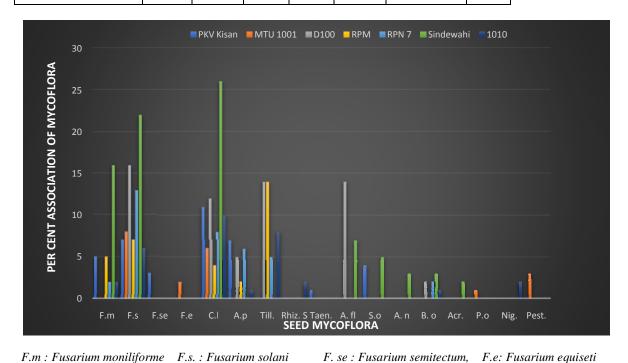


Fig. 1. Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Blotter paper method.

A.p : Alternaria padwickii

B.o: Bipolaris oryzae

S.o: Sarocladium oryzae

Taen.: Taeniolina sp.

Acr.: Acremoniella sp.

Till.: Tilletia sp.

A. fl.: Aspergillus flavus

P.o: Pyricularia oryzae

Pest.: Pestalotia sp.

C.l: Curvularia lunata

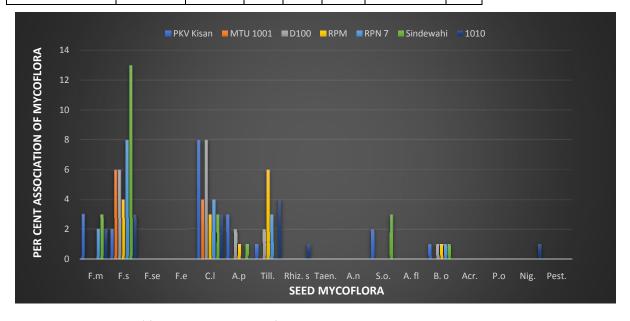
A. n: Aspergillus niger

Rhiz. s.: Rhizoctonia solani

Nig.: Nigrospora sp.

Table 2: Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Blotter paper method (Pre-treatment).

Variety	Per cent association of seed mycoflora								
Seed mycoflora	PKV KISAN	MTU 1001	D100	RPM	RPN 7	SINDEWAHI	1010	Total fungal load	Mean
Fusarium moniliforme	3				2	3	2	10	1.42
Fusarium solani	2	6	6	4	8	13	3	42	6
Fusarium semitectum									
Fusarium equiseti									
Curvularia lunata	8	4	8	3	4	3	3	33	4.71
Alternaria padwickii	3		2	1		1		7	1
Tilletia sp.	1		2	6	3		4	16	2.28
Rhizoctonia solani							1	1	0.14
Taeniolina sp.									
Aspergillus flavus									
Sarocladium oryzae	2					3		5	0.71
Aspergillus niger									
Bipolaris oryzae	1		1	1	1	1		5	0.71
Acremoniella sp.									
Pyricularia oryzae									
Nigrospora sp.							1	1	0.14
Total	20	10	19	15	18	24	14		



F.m: Fusarium moniliforme F.s.: Fusarium solani C.l : Curvularia lunata A.p : Alternaria padwickii A. n: Aspergillus niger B.o: Bipolaris oryzae Nig.: Nigrospora sp. S.o: Sarocladium oryzae Rhiz. s.: Rhizoctonia solani

Taen.: Taeniolina sp. Acr.: Acremoniella sp. Till.: Tilletia sp.

F. se: Fusarium semitectum, F.e: Fusarium equiseti A.fl: Aspergillus flavus P.o: Pyricularia oryzae Pest.: Pestalotia sp.

Fig. 2. Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Blotter paper method (Pre-treatment).

B. Detection with Agar Plate Method

Same 7 varieties which were tested in blotter paper method, were tested through agar plate method for detection of mycoflora on untreated paddy seeds as presented in Table 3 and Fig. 3.

Totally 15 fungal species belonging to 13 different genera were found. Among them, *Curvularia lunata* with 135 per cent association of mycoflora was the highest followed by Fusarium solani (132.5 per cent), Fusarium moniliforme (67.5 per cent), Aspergillus flavus (55 per cent), Aspergillus niger (55 per cent), Rhizoctonia solani (41.5 per cent), Cladosporium sp. (40 per cent), Alternaria padwickii (32.5 per cent), Pyricularia oryzae (24.5 per cent), Rhizopus sp. (15 per cent), Bipolaris oryzae (15 per cent), Acremoniella sp. (15 per cent), Pestalotia sp. (12.5 per cent) and Nigrospora sp. (2.5 per cent). Both pathogenic and saprophytic fungi were found in this method.

The above results were similar with the research findings of Ora et al., (2011), Signaboubo et al., (2016) and Singh et al., (2018). Ora et al., (2011) carried out experiment on seed borne mycoflora of paddy and a total of 12 pathogens (Rhizopus stolonifer, Aspergillus spp., Phoma sp., Bipolaris oryzae, Fusarium moniliforme, Curvularia lunata, Penicillium sp., Nigrospora oryzae, Alternaria tenuissima, Chaetomium globosum and Tilletia barclayana) were identified. It was also corroborated with the works of Singh et al., (2018) who carried out study to assess the seed borne mycoflora of paddy and a total of 21 fungi were recorded including Acremoniella sp., Alternaria sp., Curvularia lunata, C. oryzae, Bipolaris oryzae, F. oxysporum, Fusarium moniliforme, F. semitectum,

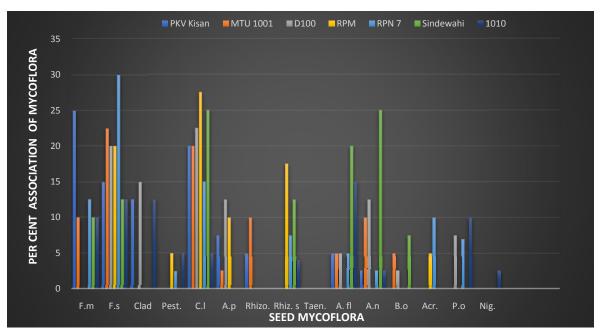
Rhizoctonia sp., Pyricularia grisea., Sarocladium oryzae, Tilletia sp., Stilaginoidea virens, Aspergillus flavus. Penicillium sp., A. niger, and Rhizopus sp.

Effect of Pre-Treatment: To study the effect of pre-treatment, the maximum discoloured grades of each variety were selected and selected samples were given pre-treatment. The observation is presented in Tables 2 and 4, plates 2 and 3, depicted graphically in Figs. 2 and 4.

In Blotter method, pre-treatment reduces mean incidence of pathogenic fungi like Fusarium solani from 11.28 per cent to 6 per cent, Curvularia lunata from 11 to 4.71 per cent, *Tilletia* sp. from 5.85 to 2.28 per cent. Fusarium moniliforme from 4.28 to 1.42 per cent, Alternaria padwickii from 3 to 1 per cent, Sarocladium oryzae from 1.28 to 0.71 per cent, Bipolaris oryzae from 1.14 to 0.71 per cent, Rhizoctonia solani from 0.28 to 0.14 per cent. Saprophytic fungi like Aspergillus niger and Aspergillus flavus were completely eliminated. Similarly, in agar plate method, the same 7 varieties were tested and growth of saprophytic fungi was completely eliminated. Pre-treatment reduces incidence of pathogenic fungi like Fusarium solani from 18.85 per cent to 12.50 per cent, Curvularia lunata from 19.28 to 10 per cent, Fusarium moniliforme from 9.64 to 6.07 per cent, Rhizoctonia solani from 5.92 to 2.50 per cent, Alternaria padwickii from 4.64 to 2.50 per cent, Pyricularia oryzae from 3.5 to 1.42 per cent. Saprophytic and superficial fungi like Aspergillus flavus, Aspergillus niger and Rhizopus sp. were completely eliminated.

Table 3: Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Agar plate method.

Variety	Per cent association of seed mycoflora								
Seed mycoflora	PKV KISAN	MTU 1001	D100	RPM	RPN 7	SINDEWAHI	1010	Total fungal load	Mean
Fusarium moniliforme	25	10			12.5	10	10	67.5	9.64
Fusarium solani	15	22.5	20	20	30	12.5	12.5	132.5	18.85
Cladosporium sp.	12.5		15				12.5	40	5.71
Pestalotia sp.				5	2.5		5	12.5	1.78
Curvularia lunata	20	20	22.5	27.5	15	25	5	135	19.28
Alternaria padwickii	7.5	2.5	12.5	10				32.5	4.64
Rhizopus sp.	5	10						15	2.14
Rhizoctonia solani				17.5	7.5	12.5	4	41.5	5.92
Taeniolina sp.									
Aspergillus flavus	5	5	5		5	20	15	55	7.85
Aspergillus niger	2.5	10	12.5		2.5	25	2.5	55	7.85
Bipolaris oryzae		5	2.5			7.5		15	2.14
Acremoniella sp.				5	10			15	2.14
Pyricularia oryzae			7.5		7		10	24.5	3.5
Nigrospora sp.							2.5	2.5	0.35
Total	92.5	85	97.5	85	92	112.5	79		



F.m: Fusarium moniliforme C.l: Curvularia lunata A. fl: Aspergillus flavus A

P.o : Pyricularia oryzae

ne F.s : Fusarium solani A.p : Alternaria padwickii A. n: Aspergillus niger Nig. : Nigrospora sp. Clad.: Cladosporium sp. Rhizo.: Rhizopus sp. B.o: Bipolaris oryzae Rhiz. s: Rhizoctonia solani Pest.: Pestalotia sp. Taen.: Taeniolina sp. Acr.: Acremoniella sp.

Fig. 3. Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Agar plate method.

Table 4 : Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Agar plate method (Pre-treatment).

Variety	Per cent association of seed mycoflora								
Seed mycoflora	PKV KISAN	MTU 1001	D100	RPM	RPN 7	SINDEWAHI	1010	Total fungal load	Mean
Fusarium moniliforme	20	5			5	7.5	5	42.5	6.07
Fusarium solani	10	15	12.5	10	20	10	10	87.5	12.5
Cladosporium sp.	7.5							7.5	1.04
Pestalotia sp.									
Curvularia lunata	5	10	20	10	5	17.5	2.5	70	10
Alternaria padwickii	2.5	2.5	5	5			2.5	17.5	2.5
Rhizopus sp.									
Rhizoctonia solani				5		10	2.5	17.5	2.5
Taeniolina sp.									
Aspergillus flavus									
Aspergillus niger									
Bipolaris oryzae		5	2.5		2	7.5		17	2.42
Acremoniella sp.					5			5	0.71
Pyricularia oryzae			2.5				7.5	10	1.42
Nigrospora sp.							2.5	2.5	0.35
Total	45	37.5	42.5	30	37	52.5	32.5		

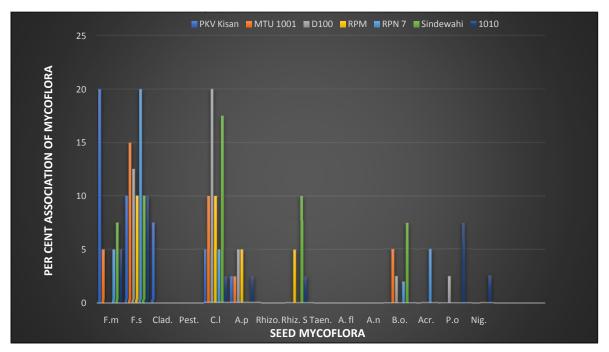


Fig. 4. Per cent association of mycoflora in maximum discoloured grade of different varieties of paddy under Agar plate method (Pre-treatment).

F.m: Fusarium moniliform C.l : Curvularia lunata A.fl: Aspergillus flavus P.o : Pyricularia oryzae

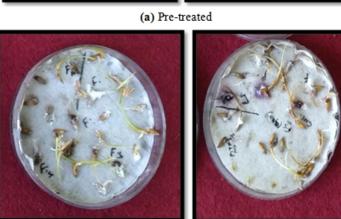
 $F.s: Fusarium\ solani$ A.p : Alternaria padwickii A.n: Aspergillus niger

Nig.: Nigrospora sp.

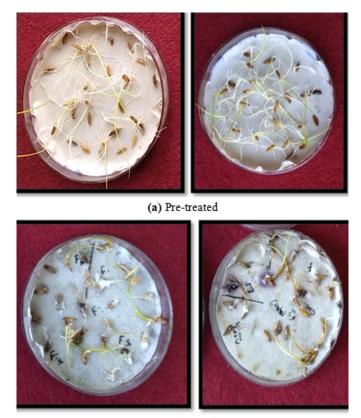
Clad.: Cladosporium sp. Rhizo.: Rhizopus sp. B.o : Bipolaris oryzae Rhiz. s : Rhizoctonia solani

Pest.: Pestalotia sp. Taen. : Taeniolina sp. Acr.: Acremoniella sp.





(b) Untreated Plate 1: Growth of mycoflora on paddy seed in (a) Blotter paper method (b) Agar plate method.



(b) Untreated

Plate 2: Comparison of mycoflora growth between pre treated and untreated paddy seeds in Blotter paper method.

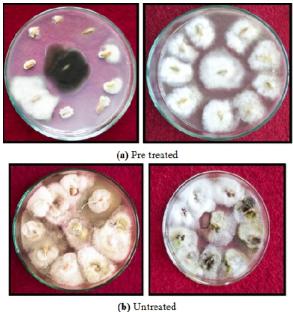


Plate 3: Comparison of mycoflora growth between pretreated and untreated paddy seeds in agar plate method.

The results were similar to the works of Niaz and Dawar, (2009); Mounisha, (2015). Niaz and Dawar (2009) carried out surface disinfection of seed with NaOCl and their was reduction in the incidence of Aspergillus sp., and other superficial fungi. However, slow growing deep seated seed borne fungi like Curvularia sp., Bipolaris Fusarium sp., sp., Macrophomina phaseolina and Botryodiplodia theobromae were still observed. The above results were also corroborated by the findings of Mounisha, (2015) who tested 3 varieties collected from eastern Vidarbha region by applying the same process.

CONCLUSION

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All the seed samples were tested for seed mycoflora by blotter paper method and agar plate method. From the

two methods, a total of 20 fungal species belonging to 16 fungal genera viz., Fusarium moniliforme, Fusarium solani, Fusarium semitectum, Fusarium equiseti, Curvularia lunata, Chaetomium sp., Alternaria padwickii, Tilletia sp., Rhizoctonia solani, Taeniolina sp., Aspergillus flavus, Sarocladium oryzae, Aspergillus niger, Bipolaris oryzae, Acremoniella sp., Pyricularia oryzae, Nigrospora sp., Pestalotia sp., Cladosporium sp. and Rhizopus sp. were detected. After pre-treatment, there was reduction of superficial and saprophytic fungi growth along with pathogenic fungi in all the seven varieties. Thus, the study has shown that mycoflora associated with seed discolouration are major constraints in production of quality seeds of paddy. Therefore, the seed mycoflora are economically important since they affect the attributes of paddy seed and hence, research for the better seed health management of paddy seed is recommended.

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