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DETECTION OF SEED BORNE MYCOFLORA ASSOCIATED WITH SOME RICE VARIETIES GROWN IN HIMACHAL PRADESH

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ABSTRACT
A total of 12 seed samples collected from two agroclimatic zones (I and II) of Himachal Pradesh were tested for associated mycoflora based on four categories viz., original seeds, apparently healthy, partially discoloured and discoloured seeds. Significant variation was observed in different varieties and treatments. Total 12 genera of fungi *viz., Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Epicoccum, Fusarium, Mucor, Penicillium, Phoma, Rhizopus, Rhizoctonian* comprising of sixteen species were found associated with rice seeds by blotter and agar-plate methods. Among them, *Curvularia lunata* and *Bipolaris oryzae* were predominant pathogens whereas, *Fusarium solani* was detected from all the varieties. The agar plate method was found more efficient than blotter method for the detection of associated fungi. Pusa-1121, Jhumka and HKR-126 showed lowest pathogenic incidence in all the seed treatments. Among treatments, maximum number of mycoflora was found to be associated with discoloured seeds. The number and per cent incidence of associated mycoflora with seeds was varied from location to location and this might be due to the variation in weather parameters of different agroclimatic zones.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food grain for nearly half of the world's population. "Rice is life", the theme of International Year of Rice, 2004 reflects the importance of rice which holds the key to our country's ability to produce enough food for our people. India is the second largest producer and consumer of rice in the world (Shivalingaiah and Umesha, 2011; Sharma *et al*., 2013). In Himachal Pradesh, there is a great diversity of agro-climatic conditions under which rice is cultivated and its cultivation extends from foot-hills (350m) to high hills (upto 2300m) (http://www.rkmp.co.in/).

Rice is affected by as many as 36 seed borne diseases of which 31 were caused by fungi which are mostly *viz., Pyricularia oryzae*, *Alternaria padwickii*, *Helminthosporium* sp., *Gibberella fujikuroi*, *G. rosa*, *Fusarium cereali*, *Nigrospora* sp., *Epicoccum* sp., *Phyllosticta glumarum*, *Alternaria* sp. and *Helicoceras oryzae*. The most common storage fungi are *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus* sp., *Chaetomium*, *Dematium*, *Monilia*, *Oidium*, *Streptomyces*, *Syncephalastrum* and *Verticillium* (Ou 1972; Ou 1985; Dwivedi and Mehrotra 1984; Jha and Prasad, 1984; Basak and Mridha 1985; Mishra and Dharamvir, 1988; Ali and Deka1996;Khan *et al.*,1999; Gopalkrishanan and Valluvaparidasan, 2009). About 20 species of fungal pathogens were detected from rice seed at any one time (Mew and Gonzales, 2002). Due to associated mycoflora with seeds various harmful effects recorded in seeds are; loss of germination capacity, seed discolouration, decay, increase in fatty acids and utilization of carbohydrates for the synthesis of protein and toxin production (Christensen, 1955; Krogh *et al.,* 1966; Christensen and Kaufmann, 1969; Misra *et al.,* 1994). They not only reduce the quality of seed but are also transmitted from one season to other. Seed health testing is important to assure the safe movement of seed of different crops for research or trade (Archana and Prakash, 2013). Infected seeds helps in the perpetuation of many diseases as well as source inoculum and a medium for survival of pathogens, their transmission to distant places, distribution in field as well as infect the uninfected soils (Singh *et al*., 2014).

Several studies have been carried out to study the seed health status of rice throughout the country (Sharma and Chahal, 1996; Gopalakrishnan and Valluvaparidasan, 2009; Gopalakrishnan *et al.,* 2010; Archana and Prakash, 2013). But, the information on seed health status of rice varieties under different agroclimatic conditions of Himachal Pradesh is scanty. In Himachal Pradesh, rice is mainly cultivated in Zone I and II and the seed health is influenced by climatic conditions of the area especially during harvesting time. Therefore, the present study was conducted to determine the prevalence and extent of different seed borne fungi associated with seeds in the varieties grown in different agroclimatic conditions in Himachal Pradesh.

MATERIALS AND METHODS

Collection of samples

Working seed samples of rice varieties were collected from different localities of Himachal Pradesh, where rice is commercially cultivated (Table 1). Samples of seed of three varieties of rice were collected from farmers of each agroclimatic zones *viz*., I (V_1 = Kasturi Basmati, V_2 = Hybrid-

6444 and V_3 = Pusa-1121) and II (V_4 = Jhumka, V_5 = Parmal and V_6 = Jattoo). Simultaneously samples of two recommended varieties were collected from HAREC, Dhaulakuan (V_7 = PAU-201 and V_8 = HKR-126), Bajaura (V_9 = Nagardhan and V_{10} = Yunlen 18 (s)) and RWRS, Malan (V_{11} = HPR-2143 and V_{12} = HPR-1068). Seeds of each variety were grouped into four categories: i) Original seeds (T_1), ii) Apparently healthy seeds (T_2), iii) Partially discoloured seeds (T_3), iv) Discoloured seeds (T_4).

Studies of associated seed mycoflora

All the varieties were tested for associated mycoflora based on four treatments using standard procedures described by International Seed Testing Association (ISTA, 1985) with slight modifications. Since the seeds are known to serve as a source of carrying wide variety of mycoflora, attempts were, therefore, made to isolate both ectophytic and endophytic mycoflora of the seeds with the help of following methods:

Blotter method

One hundred seeds were placed in a plastic Petri plates (9 cm dia.) lined with two layers of blotting papers moistened with distilled water to study the association of different mycoflora with rice seeds following the International rules for seed testing (ISTA, 1985; Neergaard, 1977). Twenty five seeds were placed in each Petri plates equidistantly. The Petri plates were incubated at 25 ± 1ºC for seven days and the seeds were examined regularly for the presence of different fungi. Incubated seeds were examined visually and under stereo binocular microscope for the associated mycoflora. Associated fungi which could not be identified were isolated in Potato Dextrose Agar (PDA) for further identification.

Agar plate method

Sterilized glass Petri plates (10 cm dia.) containing PDA were used to detect the associated mycoflora with rice seeds by agar plate method following the International rules for seed testing (ISTA, 1985). The seeds were treated with 0.1 per cent mercuric chloride for three minutes, washed three times in sterilized water, and then dried over sterilized blotting papers. Twenty seeds per Petri plate were placed equidistantly aseptically with the help of a sterilized pair of forceps. Total of 100 seeds of each treatment of 12 varieties were tested. The plates were incubated under similar conditions as described under the blotter method. The seeds were examined on 3rd, 5th and 8th day of incubation under stereobinocular microscope for associated mycoflora.

Identification

Isolated pathogens were identified on the basis of sporulation, conidial characters and fruiting structures using compound microscope and stereo binocular following the appropriate keys (Barnet, 1962; Booth, 1971; Mew and Misra, 1994; Mew and Gonzales, 2002).

RESULTS

Studies on seedborne mycoflora

The fungi associated with the seed samples of rice were detected by different methods:

Blotter method

The blotter method test of each rice variety based on four treatments indicated the association of in total 15 fungi *viz., Alternaria alternata* (3-49%), *A. padwickii* (7-13%), *Aspergillus* sp. (1-3%), *Bipolaris oryzae* (1-71%), *Chaetomium* sp. (5-15%), *Curvularia lunata* (2-54%), *Epicoccum purpurascens* (2-28%), *Fusarium moniliforme* (1-23%), *Fusarium solani* (3-25%), *Fusarium* sp. (2-25%), *Mucor* sp. (1-2%), *Penicillium* sp. (1-2%), *Phoma sorghina* (1-71%), *Pyricularia oryzae* (5-15%), *Rhizopus stolonifer* (2-9%) (Table 1).

Significant variation with respect to associated mycoflora was observed. The maximum mycoflora was detected from variety V_1 and V_9. *Curvularia lunata* was found to be predominant on variety V_1 with a frequency ranging from 41-54 per cent whereas, *Bipolaris oryzae* was predominant on seed samples of variety V_9 with a frequency ranging between 30-45 per cent. The predominant pathogens were *Fusarium moniliforme* and *F. solani* found to be present with all varieties. The highest incidence of *Curvularia lunata* and *Bipolaris oryzae* was recorded in each treatments. The maximum incidence of *B. oryzae* ranging from 40-71 per cent was detected on variety V_6. The occurrence of *Pyricularia oryzae* ranging from 5-15 per cent was observed in only one variety V_9 in all treatments. *Chaetomium* sp., *Mucor* sp. and *Rhizopus stolonifer* were also detected from one variety. *Alternaria padwickii* was detected from two varieties V_5 and V_9 collected from zone II of Himachal Pradesh. *Aspergillus* sp. and *Penicillium* sp. were observed from variety V_1 in all treatments wheras in only T_1 and T_2 of variety V_9. The highest per cent infection of *Phoma sorghina* was observed in variety V_4 followed by V_9. Overall the low per cent incidence of seed borne pathogens was detected from varieties V_3 and V_8 in each treatment compare to other varieties.

Agar plate method

The study of per cent incidence of mycoflora associated with four treatments of each rice variety by agar plate method revealed the presence of 16 fungi *viz., Alternaria alternata* (2-27%), *Alternaria padwickii* (1-11%), *Aspergillus* sp. (1-52%), *Bipolaris oryzae* (2-60%), *Chaetomium olivaceum* (16-29%), *Chaetomium* sp. (3-35%), *Curvularia lunata* (3-54%), *Epicoccum purpurascens* (2-11%), *Fusarium moniliforme* (3-25%), *Fusarium solani* (5-30%), *Fusarium* sp. (2-12%), *Mucor* sp. (2-5%), *Penicillium* sp. (1-6%), *Phoma sorghina* (2-23%), *Rhizopus stolonifer* (15-37%) and *Rhizoctonia* sp. (2-3%) (Table 2).

Among the varieties the maximum mycoflora was detected from variety V_1 followed by V_{10}. *Curvularia lunata* was predominant fungal species detected from variety V_1 and *Bipolaris oryzae* from variety V_{10}. Minimum mycoflora was detected from variety V_4 in all the treatments. *Curvularia lunata* and *Bipolaris oryzae* were found in highest frequency in each category and *Fusarium solani* was detected from all the varieties. *Alternaria padwickii* and *Rhizoctonia* sp. were found to be present in only one variety. *Chaetomium olivacearum* was detected from two varieties (V_1 and V_{10}) and *Epicoccum purpurascens* was from varieties (V_1 and V_7). In both of the methods, the per cent incidence was higher in T_4 and lowest in T_2 and microscopic examination of associated mycoflora detected by two methods exhibited different microscopic structures (Plate 1).

Table 1: Seed-borne mycoflora associated with rice varieties collected from different locations of Himachal Pradesh by blotter method

Treatment	*Alternaria alternata*	*Alternaria padwickii*	*Aspergillus* sp.	*Bipolaris oryzae*	*Chaeto mium* sp.	*Curvularia lunata*	*Epicoccum purpur ascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusa. rium* sp	*Mucor* sp.	*Peni cillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*
V1T1	10	0	2	0	0	53	26	14	10	10	2	2	0	0	9
V1T2	4	0	1	0	0	41	9	5	12	8	1	0	0	0	2
V1T3	7	0	3	0	0	53	10	5	10	15	1	1	0	0	3
V1T4	9	0	2	0	0	54	28	5	15	20	1	1	0	0	0
V2T1	11	0	0	14	0	24	2	0	15	12	0	0	5	0	0
V2T2	8	0	0	10	0	17	0	0	6	5	0	0	3	0	0
V2T3	13	0	0	17	0	34	2	0	25	5	0	0	10	0	0
V2T4	16	0	0	24	0	34	3	0	25	15	0	0	8	0	0
V3T1	0	0	0	0	0	0	0	8	9	2	0	0	0	0	0
V3T2	0	0	0	0	0	0	0	5	10	2	0	0	0	0	0
V3T3	0	0	0	0	0	0	0	2	10	3	0	0	0	0	0
V3T4	0	0	0	0	0	2	0	2	15	3	0	0	5	0	0
V4T1	0	0	0	3	5	0	0	0	20	4	0	0	71	0	0
V4T2	0	0	0	1	5	0	0	0	25	12	0	0	37	0	0
V4T3	0	0	0	1	8	0	0	0	25	5	0	0	65	0	0
V4T4	0	0	0	3	15	0	0	0	25	10	0	0	69	0	0
V5T1	8	10	0	25	0	12	0	8	15	0	0	0	0	0	0
V5T2	3	7	0	15	0	6	0	10	10	5	0	0	0	0	0
V5T3	4	8	0	30	0	14	0	10	10	0	0	0	0	0	0
V5T4	10	11	0	35	0	15	0	3	18	5	0	0	0	0	0
V6T1	47	0	0	71	0	9	0	6	10	11	0	0	0	0	0
V6T2	20	0	0	40	0	4	0	10	15	7	0	0	0	0	0
V6T3	41	0	0	60	0	10	0	9	10	4	0	0	0	0	0
V6T4	49	0	0	71	0	14	0	6	11	11	0	0	0	0	0
V7T1	0	0	0	0	0	0	10	15	10	7	0	0	1	0	0
V7T2	0	0	0	0	0	0	5	15	15	10	0	0	0	0	0
V7T3	0	0	0	0	0	0	5	12	15	3	0	0	0	0	0
V7T4	0	0	0	0	0	0	14	12	15	5	0	0	1	0	0
V8T1	0	0	0	0	0	0	0	2	5	0	0	0	5	0	0
V8T2	0	0	0	0	0	0	0	1	3	0	0	0	3	0	0
V8T3	0	0	0	0	0	0	0	1	3	0	0	0	3	0	0
V8T4	0	0	0	0	0	0	0	2	3	0	0	0	7	0	0
V9T1	35	13	3	45	0	30	0	16	9	25	0	1	40	8	0
V9T2	20	10	0	30	0	18	0	9	11	20	0	1	28	5	0
V9T3	40	7	0	40	0	35	0	15	14	24	0	0	45	7	0
V9T4	40	13	0	40	0	35	0	15	14	25	0	0	35	10	0
V10T1	12	0	0	40	0	46	20	18	8	20	0	0	0	0	0
V10T2	7	0	0	25	0	30	10	15	14	18	0	0	0	0	0
V10T3	15	0	0	35	0	45	25	10	12	22	0	0	0	0	0
V10T4	18	0	0	40	0	50	25	12	15	20	0	0	0	0	0
V11T1	20	0	0	26	0	29	0	19	10	8	0	0	22	15	0
V11T2	12	0	0	17	0	19	0	9	11	5	0	0	23	5	0
V11T3	26	0	0	25	0	32	0	6	10	7	0	0	25	13	0
V11T4	33	0	0	31	0	40	0	16	15	10	0	0	33	13	0
V12T1	12	0	0	22	0	42	0	23	9	20	0	0	5	0	0
V12T2	10	0	0	10	0	28	0	15	17	15	0	0	0	0	0
V12T3	15	0	0	18	0	45	0	18	9	20	0	0	5	0	0
V12T4	15	0	0	23	0	50	0	13	12	10	0	0	5	0	0

On the basis of 100 seeds tested of each cultivar

DISCUSSION

In the present study, the results on detection of seed borne pathogens of rice revealed that seeds were infected with various pathogens including those which lead to devastating diseases such as stack burn disease caused by *Alternaria padwickii*, brown spot caused by *Bipolaris oryzae*, rice blast caused by *Pyricularia oryzae*, bakanae disease caused by *Fusarium moniliforme*, sheath blight caused by *Rhizoctonia solani*, black kernel caused by *Curvularia lunata*. The storage fungi *Aspergillus*, *Penicillium, Mucor, Rhizopus stolonifer* and *Chaetomium* were also present. The similar pathogens have also been found associated with rice seeds by various workers (Ou, 1972; Basak and Mridha, 1985; Riaz *et al*., 1995; Sharma and Chahal, 1996; Khan *et al*., 1999; Islam *et al*., 2000; Haque *et al*., 2007; Mandhare *et al*., 2008; Gopalakrishnan *et al*., 2010; Ora *et al*., 2011; Archana and Prakash, 2013).

It is evident from the present study that both the methods of seed health testing are suitable for isolating large number of mycoflora. Khan *et al*. (1988) on rice, Dawar and Ghaffar (1991) on sunflower and Tariq *et al*. (2005) on soybean also found blotter and agar plate methods suitable for the detection of seed borne fungi. But, among the blotter and agar methods, the later had been found to be superior in isolating a large number of fungi. Total numbers of 15 fungal species were detected by blotter method whereas, 16 fungal species were isolated by agar plate in present study. The agar plate method was found to be superior in isolating *Curvularia* spp., *Bipolaris* spp. and *Rhizopus stolonifer*. Khan *et al*. (1988) also found agar plate method as a more effective method for the isolation of *Curvularia* spp. and *Bipolaris* spp. from disinfected seeds of rice. However, in present study frequency of all *Fusarium* spp. was found to be prominent on blotter method than the agar plate method. These findings were in accordance to the findings of Menten (1978) who also reported that agar plate method could detect more micro-organisms than blotter method and association of *Alternaria*, *Rhizoctonia* and *Macrophomina* was more on agar and *Fusarium* on blotter method in beans. Apart from those, *Alternaria padwickii* was also predominant on blotter method than agar method. These observations corraborate with the findings of Agarwal *et al*. (1972) they found blotter technique better for the isolation of *Alternaria padwickii* and *Bipolaris oryzae* than agar plate method. Similarly, Khan *et al*. (1988) and Farias *et al*. (2007)

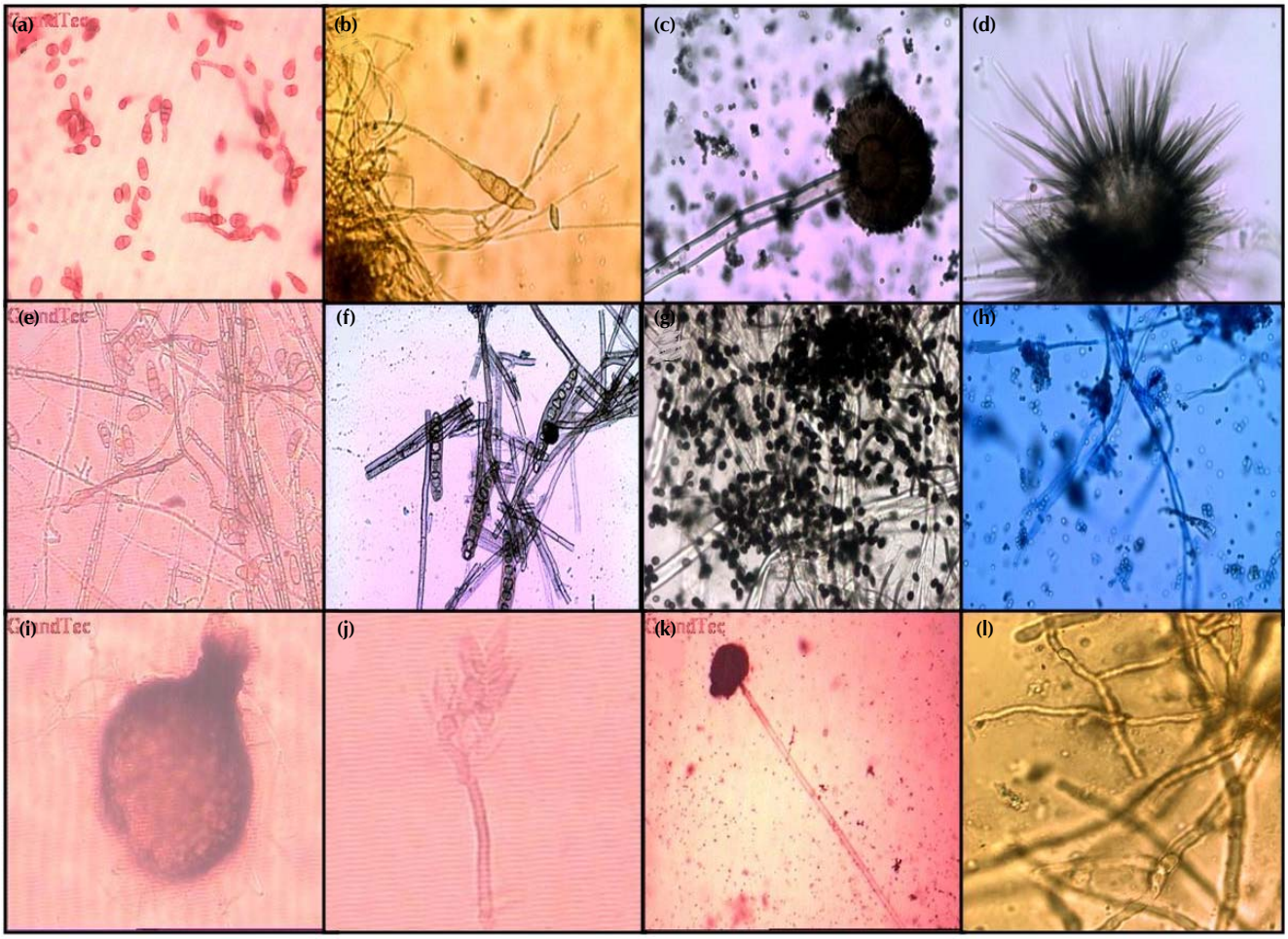

Plate 1: Microscopic observations of associated seedborne mycoflora of rice; (a) *Alternaria alternata*, (b) *Alternaria padwickii*, (c) *Aspergillus* sp., (d) *Chaetomium olivaceum*, (e) *Curvularia lunata*, (f) *Bipolaris oryzae*, (g) *Epicoccum purpurascens*, (h) *Penicillium* sp., (i) *Phoma sorghina*, (j) *Pyricularia oryzae*, (k) *Rhizopus stolonifer*, (l) *Rhizoctonia* sp.

Table 2: Seed-borne mycoflora associated with rice varieties collected from different locations of Himachal Pradesh by agar plate method

Treatment	*Alternaria alternata*	*Alternaria padwickii*	*Aspergillus* sp	*Bipolaris oryzae*	*Chaetomium* olivaceum	*Chaetomium* sp.	*Curvularia lunata*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Rhizopus stolonifer* `	*Rhizoctonia* sp.
V1T1	5.0	0.0	2.0	0.0	25.0	28.0	54.0	5.0	5.0	9.0	3.0	2.0	1.0	0.0	15.0	3.0
V1T2	2.0	0.0	0.0	0.0	16.0	23.0	37.0	5.0	5.0	9.0	3.0	4.0	2.0	0.0	0.0	3.0
V1T3	5.0	0.0	2.0	0.0	29.0	35.0	51.0	3.0	6.0	10.0	5.0	4.0	4.0	0.0	0.0	2.0
V1T4	6.0	0.0	3.0	0.0	22.0	25.0	46.0	7.0	5.0	5.0	2.0	3.0	5.0	0.0	37.0	3.0
V2T1	10.0	0.0	6.0	16.0	0.0	0.0	48.0	0.0	0.0	10.0	5.0	2.0	3.0	0.0	0.0	0.0
V2T2	11.0	0.0	0.0	14.0	0.0	0.0	25.0	0.0	0.0	18.0	5.0	0.0	1.0	0.0	0.0	0.0
V2T3	17.0	0.0	6.0	19.0	0.0	0.0	52.0	0.0	0.0	20.0	8.0	4.0	5.0	0.0	0.0	0.0
V2T4	15.0	0.0	4.0	28.0	0.0	0.0	34.0	0.0	0.0	30.0	11.0	2.0	3.0	0.0	0.0	0.0
V3T1	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	5.0	7.0	2.0	0.0	0.0	0.0	0.0	0.0
V3T2	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	8.0	9.0	2.0	0.0	0.0	0.0	0.0	0.0
V3T3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	3.0	10.0	2.0	0.0	0.0	0.0	0.0	0.0
V3T4	0.0	0.0	1.0	0.0	0.0	0.0	2.0	0.0	5.0	10.0	2.0	0.0	0.0	5.0	0.0	0.0
V4T1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.0	2.0	0.0	0.0	20.0	0.0	0.0
V4T2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	15.0	0.0	0.0
V4T3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0	6.0	0.0	0.0	23.0	0.0	0.0
V4T4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	22.0	0.0	0.0
V5T1	10.0	11.0	0.0	25.0	0.0	5.0	15.0	0.0	3.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0
V5T2	2.0	1.0	0.0	15.0	0.0	0.0	3.0	0.0	9.0	10.0	3.0	0.0	0.0	0.0	0.0	0.0
V5T3	5.0	3.0	0.0	17.0	0.0	6.0	14.0	0.0	9.0	12.0	7.0	0.0	0.0	0.0	0.0	0.0
V5T4	7.0	3.0	0.0	20.0	0.0	8.0	10.0	0.0	10.0	12.0	3.0	0.0	0.0	0.0	0.0	0.0
V6T1	0.0	0.0	0.0	52.0	0.0	0.0	6.0	0.0	4.0	10.0	4.0	0.0	0.0	0.0	0.0	0.0
V6T2	0.0	0.0	0.0	29.0	0.0	0.0	3.0	0.0	7.0	12.0	2.0	0.0	0.0	0.0	0.0	0.0
V6T3	0.0	0.0	0.0	60.0	0.0	0.0	5.0	0.0	2.0	13.0	0.0	0.0	0.0	0.0	0.0	0.0
V6T4	2.0	0.0	0.0	43.0	0.0	0.0	7.0	0.0	5.0	10.0	10.0	0.0	2.0	0.0	0.0	0.0
V7T1	6.0	0.0	0.0	7.0	0.0	0.0	18.0	2.0	12.0	9.0	5.0	0.0	3.0	0.0	25.0	0.0
V7T2	3.0	0.0	0.0	3.0	0.0	0.0	10.0	3.0	10.0	15.0	5.0	0.0	1.0	0.0	0.0	0.0
V7T3	5.0	0.0	0.0	4.0	0.0	0.0	3.0	10.0	10.0	13.0	2.0	0.0	5.0	0.0	15.0	0.0
V7T4	6.0	0.0	0.0	2.0	0.0	0.0	15.0	11.0	14.0	16.0	2.0	0.0	6.0	0.0	25.0	0.0
V8T1	8.0	0.0	0.0	12.0	0.0	0.0	20.0	0.0	5.0	30.0	6.0	0.0	0.0	0.0	0.0	0.0
V8T2	5.0	0.0	0.0	10.0	0.0	0.0	15.0	0.0	8.0	20.0	7.0	0.0	0.0	0.0	0.0	0.0
V8T3	10.0	0.0	0.0	14.0	0.0	0.0	15.0	0.0	14.0	27.0	3.0	0.0	0.0	0.0	0.0	0.0
V8T4	12.0	0.0	0.0	15.0	0.0	0.0	25.0	0.0	6.0	29.0	4.0	0.0	0.0	0.0	0.0	0.0
V9T1	0.0	0.0	35.0	14.0	0.0	0.0	14.0	0.0	18.0	12.0	6.0	5.0	0.0	2.0	0.0	0.0
V9T2	0.0	0.0	25.0	10.0	0.0	0.0	16.0	0.0	12.0	15.0	6.0	2.0	0.0	0.0	0.0	0.0
V9T3	0.0	0.0	40.0	15.0	0.0	0.0	21.0	10.0	10.0	14.0	2.0	2.0	4.0	0.0	0.0	0.0
V9T4	0.0	0.0	52.0	17.0	0.0	0.0	20.0	0.0	16.0	15.0	5.0	2.0	3.0	0.0	0.0	0.0
V10T1	12.0	0.0	6.0	40.0	16.0	20.0	35.0	0.0	20.0	12.0	11.0	2.0	3.0	0.0	0.0	0.0
V10T2	8.0	0.0	5.0	29.0	9.0	15.0	28.0	0.0	17.0	19.0	11.0	1.0	1.0	0.0	0.0	0.0
V10T3	10.0	0.0	4.0	42.0	20.0	22.0	37.0	0.0	15.0	15.0	12.0	4.0	3.0	0.0	0.0	0.0
V10T4	12.0	0.0	6.0	42.0	20.0	22.0	39.0	0.0	18.0	20.0	10.0	4.0	6.0	0.0	0.0	0.0
V11T1	20.0	0.0	0.0	30.0	0.0	0.0	35.0	0.0	25.0	14.0	8.0	0.0	0.0	0.0	0.0	0.0
V11T2	15.0	0.0	0.0	25.0	0.0	0.0	20.0	0.0	5.0	16.0	10.0	0.0	0.0	0.0	0.0	0.0
V11T3	25.0	0.0	0.0	29.0	0.0	0.0	35.0	0.0	14.0	16.0	10.0	0.0	0.0	0.0	0.0	0.0
V11T4	12.0	0.0	6.0	42.0	20.0	22.0	39.0	0.0	18.0	20.0	10.0	4.0	6.0	0.0	0.0	0.0
V12T1	5.0	0.0	1.0	10.0	0.0	10.0	25.0	0.0	15.0	10.0	12.0	0.0	0.0	0.0	0.0	0.0
V12T2	3.0	0.0	3.0	2.0	0.0	3.0	15.0	0.0	6.0	19.0	5.0	0.0	0.0	0.0	0.0	0.0

*On the basis of 100 seeds tested of each cultivar

also found *A. padwickii* as a predominant pathogen on blotter than agar method.

The results in the present study showed that maximum mycoflora was detected from variety Kasturi Basmati by both the methods with higher frequency of *Curvularia lunata* followed by varieties Nagardhan and Yunlen 18 (s) where, *Bipolaris oryzae* was a predominant pathogen. In support of present research Mishra and Dharamvir (1988) also detected *Bipolaris oryzae* followed by *Curvularia lunata* as predominant pathogen in Bihar from discoloured seeds of rice cultivars. Pham Van Du *et al*. (2001) reported *Curvularia* spp. (13.4%) as a dominant pathogen through blotter method. In both the methods, maximum infection was recorded from discoloured seed category and minimum on apparently healthy seed category. This is because of the fact that fungal infections are mainly responsible for grain discolouration (Misra *et al.,* 1994).

The extent of seed infection was varied from variety to variety and location to location. One of the reasons of variation in number of mycoflora and their frequency among different samples could be the variation in the weather condition of agroclimatic zones, especially during harvesting period. These factors may be responsible for differences in discolouration and per cent frequency of mycoflora in different locations. Roy (1983), Ou (1985) and Sunder *et al*. (1989) reported that the extent of discolouration varied with season, locality and variety.

The presence of diverse mycoflora was found to be associated with rice seeds from different zones of Himachal Pradesh. Since pathogen free seed is a vital input in agriculture, proper seed health testing and management of associated mycoflora is a prerequisite for successful rice cultivation.

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