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Survey of seed-borne fungi associated with seeds of rice in Tamil Nadu

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ABSTRACT

Seed health testing to detect seed borne pathogens is an important key step in the management of crop diseases. A total of 30 rice seed samples consisting of 7 cultivars were obtained from different locations of Tamil Nadu, India were used for testing their health status. The pathogens were isolated by using blotter papers and agar plate method. A total of 9 pathogens (Alternaria padwickii, Curvularia lunata, Fusarium moniliforme, Helminthosporium oryzae, Sarocladium oryzae, Pyricularia oryzae, Rhizopus oryzae, Aspergillus niger and Trichoderma species) were identified. Among them the most predominant one was Helminthosporium oryzae which was associated with 62.36 per cent seed samples, followed by Alternaria padwickii (36.63%), Sarocladium oryzae (30.63%), Fusarium moniliforme (28.63%), Curvularia lunata (26.00%). These findings suggest that there is a need for proper storage of rice seed to minimize the fungal infestation and their mycotoxin production.

Key words: *Oryza sativa, seed-borne fungi, blotter method, agar plate method*

Rice is the most widely cultivated food crop in the world. Global rice production was approximately 645 mt in 2007. Rice is being cultivated in 114 countries throughout the world, and more than 50 countries have a minimum annual production of 100,000 t. The majority of the rice (90%) is being produced in Asian countries with China and India being the major producers (IRRI, 2008). The other major rice producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil and Japan.

Frequent heavy rainfall and floods particularly near harvest in the different parts of the country wet the crop and make panicles more prone to invasion by fungal species (Reddy *et al.*, 2004). Fungi are a major cause of reduction in the quality of rice due to high moisture and temperature conditions before its harvest. Microorganisms play an important role in affecting the quality of seed of which fungi are the largest group. These pathogens are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages. Seed borne diseases caused by fungi are relatively difficult to control as the fungal hyphae get established and become dormant.

Apart from being seed borne pathogens, fungi may grow on storage products. These fungi may decrease the seed germinability cause seed discoloration, produce toxins that may be injurious to man and domestic animals and may reduce seed weight also.

Infected seeds germinate poorly and could be a major source of inoculums for new crops raised from them. For example, most pathogens causing abnormal seedling of rice are seed borne (Guerrero *et al*., 1972). There is no accurate estimate about the yield loss of rice due to diseases. However, roughly 10% yield loss of rice may be incurred annually due to seed-borne diseases in the country.

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. Seed health information reveals the organisms carried by the seed and the level of infection or infestation that will be introduced to another region or country. Such information comes from experiments or survey under field conditions where the

seed is grown. The objective of the present study were 1) to assess the prevalence and extent of different seed-borne fungi associated with different varieties of rice 2) to isolate and identify the pathogen associated with seeds of different rice varieties 3) to study the cultural characteristics of pathogen.

Thirty seed samples comprising of seven rice varieties namely ADT 36, ADT 39, ADT 45, ADT 48, White Ponni, Andhra Ponni and BPT 5204 (Table 1) were collected from Rice Research Institutes, Seed Testing Laboratory and farmer's holdings. The seeds were collected in sterilized polythene bags and stored at 4-5°C until used in any treatment. The experiment was conducted in the Department of Plant Pathology, Faculty of Agriculture, Annamalai University during the year 2014.

Two hundred seeds per sample were disinfected in 1% freshly prepared sodium hypochloride solution for 2 min. Seeds were rinsed with sterilized distilled water, air dried and then plated onto 3 layers of moistened filter paper (Whatman no.41) in Petri plates and incubated at 28±2°C under 12h alternating cycles of light and darkness for 7 days (ISTA, 1996). Then the seeds were examined on eighth day under Stereo binocular microscope and the fungi were identified based on the taxonomic characteristics (Booth, 1971; Barnett and Hunter, 1972; Watanabe, 2002). In the agar plate method, two hundred seeds surface sterilized seeds (sodium hypochloride @1%) were plated on the PDA medium (Ainsworth, 1961) and the plated seeds were incubated for 5-7 days at 28±2°C under 12h alternating cycles of light and darkness. Identification was done based on colony characters and morphology of sporulation structures under a Stereo binocular microscope. Semi permanent slides were prepared from the fungal colony and observed under compound microscope. In the agar plate method more than one type of fungal colonies were produced. In this case, identification was done on the most frequently occurring colony present in all the Petri plates and then the second most frequent, the third most frequent and so on. Thereafter, the identification of the different colonies were done visually and then under a stereomicroscope.Pure cultures of isolated fungi were maintained on PDA slants.

All the experiments were of completely randomized design (CRD) and repeated twice. Data were subjected to analyses of variance and treatment means were compared by an appropriate Duncan's multiple range test ($P<0.05$). The IRRISTAT package version 92-1, developed by the International Rice Research Institute Biometrics Unit, Philippines, was used for analysis (Gomez and Gomez, 1984).

A total of 9 genera of fungi namely *Alternaria padwickii, Curvularia lunata, Fusarium moniliforme, Helminthosporium oryzae, Sarocladium oryzae, Pyricularia oryzae, Rhizopus oryzae, Aspergillus niger*and *Trichoderma* species were found to be associated with the seed samples (Table 2). Among them, the most predominant one was *Helminthosporium oryzae* which was associated with 62.36 per cent seed samples, followed by *Alternaria padwickii* (36.63%), *Sarocladium oryzae*(30.63%), *Fusarium moniliforme* (28.63%), *Curvularia lunata* (26.00%). The following fungi *viz*., *Pyricularia oryzae, Aspergillus niger, Rhizopus oryzae* and *Trichoderma* species observed to an extent of 10.3, 5.3, 3.3 and 3.0 per cent in the seed samples, respectively.

Mian and Fakir (1989) reported that the most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata,*

Table 1. List of rice seed samples collected from different rice growing areas of Tamil Nadu

Place of collection	Source	Variety	No. of samples
Aduthurai	Tamil Nadu Rice Research Station	ADT 36	4
		ADT 39	5
		ADT 45	4
		ADT 48	2
Chidambaram	Farmer's holding	White ponni	7
Madurai	Seed Testing Laboratory	Andhra ponni	4
Pudukkottai	Farmer's holding	BPT 5204	4
		Total	30

Table 2. Occurrence of seed-borne fungi in rice seeds by standard blotter paper method

Fungus detected	Seed lot infected (%)	Range of infection percentage in infected seed samples**									
		1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Alternaria padwickii	36.63 b*	100	23	12	2	6	12	-	-	-	-
Curvularia lunata	26.00 e	116	8	-	-	-	-	-	-	-	-
Fusarium moniliforme	28.63 d	67	12	-	-	-	-	-	-	-	-
Helminthosporium oryzae	62.36 a	124	7	14	8	6	10	-	-	-	-
Sarocladium oryzae	30.63 c	40	21	8	6	2	3	-	-	-	-
Pyricularia oryzae	8 f	16	4	-	-	-	-	-	-	-	-
Rhizopus oryzae	3.3 h	7	-	-	-	-	-	-	-	-	-
Aspergillus niger	5.3 g	11	-	-	-	-	-	-	-	-	-
Trichoderma species	3.0 h	8	-	-	-	-	-	-	-	-	-

**Means of four replications; - means no infection

*Values with different letters within a column differ significantly at 5% level of significance as per DMRT

Aspergillus spp. And *Trichoconis padwickii*. Gopalakrishnan *et al.* (2010) reported that 8 genera comprising twelve species were found to be associated with the seed samples. Among them, the most predominant one was *Bipolaris oryzae* which was associated with 58.89 per cent seed samples, followed by *Alternaria padwickii* (52.96%). In blotter method, (Ora *et al*. 2011) identified a total of 12 seed borne pathogens, of which, *Bipolaris oryzae* and *Fusarium moniliforme* were more predominant one on the most of the rice varieties Recently, Archana and Prakash (2013) reported that 69 rice seed samples were collected from different states of India reported that the most predominant one was *H. oryzae* which was associated with 82.08% followed by *A. padwickii* (63.36%).

Out of 200 seeds tested, seeds carried *Helminthosporium oryzae*. Among the 169 seeds, 124 carried 1-10 per cent, 7 carried 11-20 per cent, 14 carried 21-30 per cent, 8 carried 31-40 per cent, 6 carried 41-50 per cent and 10 carried 51-60 per cent seed infection (Table 2). This reveals the presence of diverse mycoflora both pathogenic and non-pathogenic in rice seeds in ruling varieties in Tamil Nadu. The plants developed from discoloured seeds might serve as a source of inoculums for many serious problems of the crop (Gopalakrishnan and Valluvaparidasan, 2007). Since rice is a stable food, better seed health management is a prerequisite for successful rice cultivation.

In agar plate method, 8 seed borne pathogens were identified associated with rice seeds (Fig. 1, 2). These were *Alternaria padwickii, Curvularia lunata, Fusarium moniliforme, Helminthosporium oryzae, Sarocladium oryzae, Pyricularia oryzae, Rhizopus oryzae* and *Aspergillus niger* (Table 3). InADT 36, highest percentage (17.66 %) of *Alternaria padwickii* association was observed. *Fusarium moniliforme* showed 17.00 per cent occurrence in ADT 36 followed by 12.33 per cent in ADT 39. Uma and Wesely (2013) reported that in Karnataka ponni, highest percentage (18%) of *A. flavus* association was observed. *Aspergillus niger* showed 17.6% of occurrence in ADT 39 followed by 17.5% in Karnataka ponni. The frequency of fungal association was varied in different varieties of rice seeds. Earlier observations reported the occurrence of *Pyricularia oryzae, Alternaria alternata, A. padwickii, A. longissima, Curvularia oryzae, C. lunata, Drechslera oryzae, A. niger, A. flavus, Fusarium moniliforme, F. semitectum, F. oxysporum, Penicillium citrinum, Rhizopus oryzae, F. solani*and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Penicillium, Myrothecium*and *Colletotrichum* from seeds of different varieties of rice (Nguefack *et al.*, 2007; Utobo *et al*., 2011; Islam *et al*., 2012).

The present study reveals the presence of diverse micro flora of both pathogenic and non-pathogenic in rice seeds in ruling varieties in Tamil Nadu. The plants developed from discoloured seeds might serve as a source of inoculums for many serious problems of the crop. Since rice is a staple food, better seed health management is a prerequisite for successful rice cultivation.

Fig.1. Axenic cultures of seed borne pathogens. *1. Curvularia lunata 2. Fusarium moniliforme 3. Sarocladium oryzae 4. Helminthosporium oryzae 5. Alternaria padwickii 6. Trichoderma* species *7. Rhizopus oryzae 8. Aspergillus niger 9. Pyricularia oryzae*

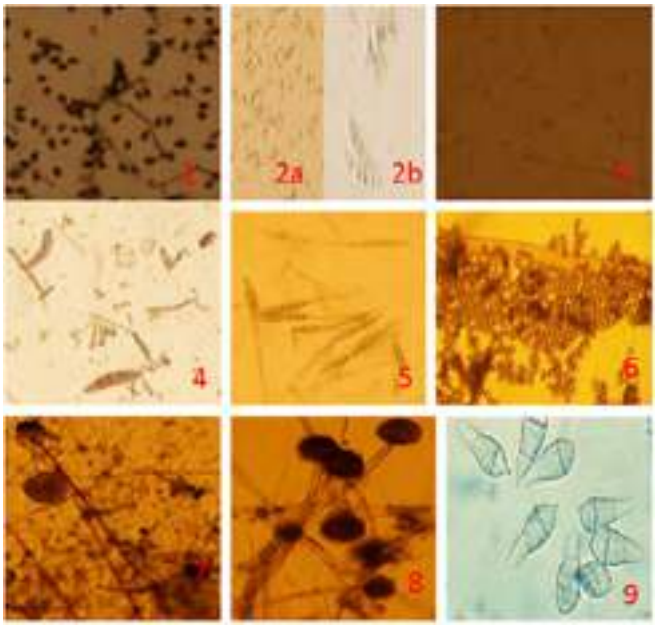

Fig.2. Conidia of rice seed borne pathogens**.** *1. Curvularia lunata 2. Fusarium moniliforme a. Micro conidia, b. Macro conidia 3. Sarocladium oryzae 4. Helminthosporium oryzae 5. Alternaria padwickii 6. Trichoderma* species *7. Rhizopus oryzae 8. Aspergillus niger 9. Pyricularia oryzae*

Table 3. Incidence of different seed-borne fungi associated with rice seeds by agar plate method

Variety	Percentage of association**							
	A. padwickii	*C. lunata*	*F. moniliforme*	*H. oryzae*	*S. oryzae*	*P. oryzae*	*R. oryzae*	*A. niger*
ADT 36	17.66 a *	10.00 a*	17.00 a*	15.33 a*	14.66 a*	10.66 a*	11.33 a*	5.66 a*
ADT 39	16.00 a	8.33 b	12.33 b	10.00 c	12.00 b	10.00 a	8.66 b	5.00 a
ADT 45	17.00 a	8.00 b	12.00 b	12.66 b	12.00 b	8.33 b	7.00 b	3.33 b
ADT 48	14.66 b	5.00 c	8.66 c	10.00 c	10.00 c	8.00 b	7.33 b	6.33 a
White ponni	5.00e	2.33 d	5.00 d	6.33 d	7.66 d	9.66 a	4.00 c	5.00 a
Andhra ponni	10.00 c	4.00 c	2.00 e	4.00 e	4.33 e	6.33 c	2.00 d	3.00 b
BPT 5204	8.66 d	2.00 d	1.66 e	6.00 d	2.66 f	2.00 d	2.33 d	1.00 c

**Means of four replications

*Values with different letters within a column differ significantly at 5% level of significance as per DMRT

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