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Seed mycoflora of paddy varieties in Tamil Nadu and its effect on germination and seedling vigour

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Abstract
Seed health testing to detect seed borne pathogens is important for diagnosis and proper management of crop diseases. A survey has been conducted in major paddy growing areas of Tamil Nadu during 2015-2018 and a total of 28 popularly grown paddy varieties including two traditional varieties were collected from farmers' holdings, Research stations and KVKs. The moisture per cent ranged from 10.4 to 13.4 and the germination per cent ranged from 32 (TRY 3) to 94 (CO 51). A total of seven pathogens viz., *Drechslera oryzae*, *Fusarium moniliforme*, *Aspergillus flavus*, *A. fumigatus*, *Curvularia* sp., *Penicillium* sp., and *Alternaria padwickii* were detected by blotter method. Among these pathogens, the most predominant was *D. oryzae* which is associated with 86 % of the collected seed samples followed by *Fusarium moniliforme* (57%) and *Aspergillus flavus* (46%). The abnormal seedlings and ungerminated seeds in rolled paper towel method were mostly associated with *D. oryzae*, *F. moniliforme* and *A. flavus*.

Keywords: Blotter method, rolled paper towel, *Drechslera oryzae*, *Aspergillus flavus*, seed borne pathogens, abnormal seedlings

Introduction
Seed is the primary basis of crop production and is the most important available input for small holding farmers. In most parts of the world, small farmers use their produced seeds for next year planting, and they attempt to stock their own produced seeds for several months to several years. These seeds are often of poor quality, impure and contaminated with pathogens (Fujisaka *et al*. 1993) [10]. Seed contamination of pathogens during storage could reduce seed vigour, germination and cause negative effect on appearance and chemical composition of seeds. In addition to seed deterioration, it can also inhibit germination and transmission of the pathogen from seed to seedling or main plant leading to reduction in crop yield and threatened food security.

India is renowned rice (*Oryza sativa*) producing country and stands second with an annual production of 121.46MT (Ministry of Agriculture and Farmers' Welfare, 2020-21). Tamil Nadu is one of the leading rice growing state in India with an area of 1.86 M ha, production of 7.28 MT and productivity 3923 kg/ha (Agricultural Statistics at a Glance 2018) [1]. There are several constraints in the production of rice, of which, diseases caused by fungi, bacteria, viruses and nematodes are responsible for major economic losses in India (Mew and Gonzales 2002) [20]. The crop is affected by as many as 36 seed borne diseases of which 31 were caused by fungi (Ou 1985) [25]. These pathogens are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages.

Paddy seed plays an important role in carrying pathogens. Farmers generally use different rice varieties and face difficulties to many diseases. As most of the pathogens are seed borne, there is a chance to transmit new race of the pathogen. Due to increasing seed demand and subsequent increase of international import, endemic plant pathogens continue to be a challenge in safeguarding plant health. Therefore early and accurate diagnoses and pathogen surveillance will help in development and application of mitigation strategies. So assessment of the seed health standard of paddy varieties is important for farmer and food security.

Seed health testing is one of the conventional methods to detect the presence of seed borne fungi (ISTA 1993) [15]. The purpose of seed health testing is to assure the safe movement of seed of different crops for research and 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.

2. Materials and Methods

2.1. Collection of paddy seed samples

A survey has been conducted in the major paddy growing areas of Tamil Nadu during 2015-18 to collect stored paddy seeds. The paddy seeds comprising twenty eight varieties were collected from Agricultural Research Stations, KVKs and farmers holdings. The samples were collected in sterilized polythene bags and kept under cold storage for further study. The moisture per cent was recorded immediately after the collection of samples.

2.2 Estimation of seed moisture content

The stored paddy seeds collected were subjected to estimation of seed moisture content. The seed moisture content was estimated by oven drying method approved by ISTA.

2.3. Detection of seed borne pathogens in seeds

Blotter method

The collected paddy samples were analyzed for the presence of major seed borne fungal pathogens by blotter method following the international rules for seed testing (ISTA 1996) [16]. Three layers of well moistened blotter paper were placed at the bottom of 9cm plastic Petri dish. Four hundred seeds of each sample were counted at random and placed on the moist filter paper at the rate of 25 seeds per plate. Four replications were maintained with 100seeds per replication. The plated seeds were incubated at 25 ± 2°C under 12hours cycle of alternate near ultra-violet (NUV) and darkness for 7 days. Each seed was observed under stereomicroscope to record the presence of fungal colony after incubation based on the growth habit. Also, temporary slides were prepared from fungal colony and observed under compound microscope for proper identification of the fungus. The fungus was identified as per the keys suggested by Malone and Musketee 1964 [19]; Booth 1971 [5]; Ellis 1971 [9]; Chidambaram and Mathur 1975 [7] and Neergaardand Saad, 1962.

2.4. Detection of seed borne pathogens in seedlings

To determine the effect of seed infection on germination, rolled paper towel method developed by Warham (1990) [30] was followed. One hundred seeds were randomly taken from each variety and 25 seeds were placed between a pair of moist paper towels. Four replications for each variety were maintained. The towels were rolled and the ends were closed by threads and covered by polythene paper to prevent drying and incubated for a period of 14 days. After incubation, the observations pertaining to germination per cent, ungerminated seed (hard seed and rotten seed), shoot length, root length, vigour index and incidence of seed borne pathogens were recorded.

For determination of the seed borne pathogens the fungal growth on the infected seedlings were taken with the needle and observed under compound microscope. For determination of seedling vigour 10 seedlings (normal) were randomly selected from each variety and their individual shoot and root length was measured. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of the root to the largest available lateral root apex. Vigour of the seedling was determined by the following formula (Baki and Anderson 1972) [3].

Vigour Index = (Mean of root length + Mean of shoot length) × Germination per cent

3. Results and Discussion

A total of 28 varieties of paddy samples were collected from the Cauvery delta zone of Tamil Nadu. All the paddy varieties were grown in low land condition. After harvest, the farmers store the seeds in gunny bags stacked over the wooden plank. The per cent moisture content of the paddy samples ranged from 10.4 to 13.2. The seed samples subjected to seed health test by blotter method revealed that a total of seven pathogens *Drechslera oryzae, Fusarium moniliforme, Aspergillus flavus, Curvularia* sp., *A. fumigatus, Trichoconis padwickii,* and *Penicillium* sp. were found associated with the seeds of different varieties of rice. The frequency of fungal association and occurrence varied in different varieties of paddy seed. Among the pathogens detected, *Drechslera oryzae* is prevalent in most of the varieties except ADT 38, ADT 39, IW Ponni, AD 07367. The highest incidence of *D. oryzae* (20%) and *F. moniliforme* (18%) was recorded in the variety TRY 3 and ADT 46 respectively. The overall pathogen infection was maximum (33.00 %) in the paddy variety (TRY 3) followed by ADT 46 (30.00%) and minimum (2.00%) in TKM 9 and IW Ponni (Table 1). The result was in accordance with the findings of earlier observations that the occurrence of *Pyricularia oryzae, Alternaria alternata, A. padwickii, A. cougissima, Curvularia oryzae, C. lunata, Drechslera oryzae, A. niger, Fusarium moniliforme, F. semitectum, F. oxysporum, F. solani* and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Penicillium, Myrothecium and Colletotrichum* from seeds of different varieties of rice (Khan 2000; Wahid *et al*. 2001; Javaid *et al*. 2002; Nguefack *et al*. 2007) [18, 29, 17, 24]. Five pathogenic fungi viz., *Aspergillus flavus, A. niger, Penicillium citrinum, Alternaria padwickii and Rhizopus oryzae* were found to be associated with different varieties of rice seeds from South Tamil Nadu (Uma and Wesely, 2013) [27]. Imolehin (1983) [13] reported that *H. oryzae, Fusarium moniliforme, Penicillium* sp., *Curvularia lunata, Aspergillus* sp., *Rhizopus arrhizus, Geotrichum* sp. *and Alternaria* sp. were detected from the upland and lowland rice cultivars from Nigeria. He also observed that the most frequently isolated seed borne fungus was *Helminthosporium oryzae* followed by *Pyricularia oryzae* and *Fusarium moniliforme.* Mian and Fakir (1989) [21] reported that the most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus* sp. And *Trichomonas padwickii*. Four fungal species viz., *Fusarium moniliforme, Alternaria* sp., *Helminthosporium* sp. and *Curvularia* sp. were found associated with the seeds of different varieties of rice was reported (Butt *et al*. 2011) [6]. Smilarly, Gopalakrishnan *et al*. (2010) [11] reported that eight genera of fungi viz., *Trichoconis, Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma* were associated with the rice seed samples among which the most predominant one was *Bipolaris oryzae* followed by *Trichomycosis padwickii*.

In this study, *Drechslera oryzae* was the most frequently isolated fungus followed by *Fusarium moniliforme* which reduces the germination per centin the rice cultivars. This result is in accordance with the findings that rice seeds infected with *D. oryzae* has been reported to reduce seed germination (Misra & Singh 1969; Rath 1974) [22, 26] and also cause seedling blight (Guerrero *et al*. 1972) [12]. The high frequency of *D. oryzae* from the rice cultivars collected in the study indicates the high incidence of seed borne Drechslera in most of rice fields in the Cauvery delta zone of Tamil Nadu.

The next most frequently isolated fungus *F. moniliforme* was associated with poor seed germination confirming the earlier reports on this pathogen (Bedi and Dhaliwal, 1971) [4].

Aspergillus sp and *Penicillium* sp. which is regarded as surface contaminant were also isolated from the seeds of rice cultivars. Besides the production of aflatoxins by *Aspergillus* sp., they also deteriorate the stored grains (Vidhyasekaran *et al*. 1970) [28]. The other seed borne fungi like *Curvularia* and *Alternaria padwickii* were rarely isolated from the seeds of rice cultivars *Curvularia lunata* was reported to cause glume and kernel discolouration on rice.

The detection of seed mycoflora in seedlings in rolled paper towel method, revealed that the maximum seed germination (96%) was observed in the variety CO 51, and the lowest seed germination (32%) with maximum ungermianted seeds (42%) was observed in TRY 3. The highest vigour index (2930.4) was recorded in the cultivar Kattuyanam, the traditional paddy variety collected from farmers holdings (Table 2).

These findings indicate that the decreased germination percent was due to infected normal seeds and ungerminated seeds. The results are in accordance with the findings of Islam *et al*. (2000) [14] that significant variation in respect of germination percent and pathogenic infection. Also, the seed germination decreased with increased seed infection (Islam *et al*. 2012) [14].

Spoilage of stored rice is due to storage fungi which were introduced during the post- harvest handling process. (Javid *et al*. 2002). Uma & Wesely (2013) [27] reported that the fungus *A. niger* is widely associated with unmilled rice grain and in storage which result in deterioration in the form of discolouration and bad odours. The presence of *Aspergillus* sp especially

A. niger and *A. flavus* on seeds of rice in higher frequencies and its association with ungerminated seeds of rice confirmed the cause of low germination in seeds.

Table 1: Seed borne mycoflora of paddy varieties in Tamil Nadu (Blotter method)

S. No.	Paddy Varieties	Moisture (%)	Seed Mycoflora (%)							Total Infection (%)
			D.o	*F.m*	*A.fl*	*Cur*	*A.fu*	*A.p*	*Pen*	
1.	ADT 37	11.9	9.00	0.00	0.00	4.00	0.00	0.00	0.00	13.00
2.	ADT 38	13.2	0.00	7.00	0.00	4.00	0.00	0.00	0.00	11.00
3.	ADT 39	12.6	0.00	5.00	0.00	0.00	0.00	1.00	0.00	5.00
4.	ADT 42	10.4	14.00	0.00	0.00	0.00	0.00	0.00	0.00	14.00
5.	ADT 43	11.1	15.00	3.00	0.00	0.00	0.00	0.00	0.00	18.00
6.	ADT 45	11.5	10.00	8.00	0.00	4.00	0.00	0.00	0.00	22.00
7.	ADT 46	12.2	10.00	18.00	0.00	2.00	4.00	0.00	0.00	30.00
8.	ADT 48	11.1	1.00	2.00	0.00	1.00	0.00	0.00	0.00	4.00
9.	ADT 49	11.3	4.00	0.00	0.00	0.00	0.00	0.00	0.00	4.00
10.	ADT 50	11.7	2.00	0.00	0.00	2.00	0.00	0.00	1.00	5.00
11.	TKM 9	10.6	1.00	2.00	1.00	0.00	0.00	0.00	0.00	2.00
12.	TKM 13	11.1	2.00	2.00	0.00	0.00	0.00	0.00	0.00	4.00
13.	TRY 3	12.4	20.00	5.00	8.00	0.00	0.00	0.00	0.00	33.00
14.	TRY 4	12.9	4.00	5.00	3.00	0.00	0.00	0.00	0.00	12.00
15.	CO 50	12.6	4.00	4.00	0.00	0.00	0.00	0.00	0.00	11.20
16.	CO 51	11.1	2.00	1.00	0.00	1.00	0.00	0.00	0.00	4.00
17.	IW Ponni	11.6	0.00	0.00	1.00	2.00	0.00	0.00	0.00	2.00
18.	CR 1009	13.4	2.00	3.00	0.00	1.00	0.00	0.00	0.00	7.00
19.	CR 1009 Sub 1	11.8	4.00	0.00	5.00	3.00	0.00	0.00	3.00	10.00
20.	BPT 5204	11.3	3.00	0.00	5.00	0.00	0.00	1.00	0.00	8.00
21.	Swarna Sub 1	12.6	8.00	5.00	5.00	0.00	0.00	0.00	0.00	18.00
22.	Seeragasamba	11.4	1.00	2.00	0.00	1.00	0.00	0.00	0.00	4.00
23.	Andhra culture	12.2	15.00	0.00	13.00	0.00	3.00	0.00	0.00	28.00
24.	MDU 6	11.2	4.00	3.00	0.00	1.00	0.00	0.00	0.00	10.00
25.	Mapillai samba	11.9	2.00	0.00	2.00	0.00	0.00	0.00	0.00	4.00
26.	Kattuyanam	11.5	3.00	0.00	0.00	0.00	0.00	0.00	0.00	4.00
27.	ADO 7367	11.0	0.00	0.00	8.00	0.00	0.00	0.00	0.00	8.00
28.	ADT 250	11.2	3.00	0.00	5.00	2.00	0.00	0.00	0.00	10.00

D.o- Dreschleraoryzae; F.m- Fusarium moniliforme; A.fl - Aspergillus flavus; Cur-Curvularia sp.; A.fu-Aspergillusfumigatus; A.p- Alternaria padwickii; A.n -Aspergillusniger; Pen–Penicillium sp.

Table 2: Influence of seed borne mycoflora on germination and seedling vigour

S. No	Paddy Varieties	Germination (%)	Seedling Length (cm)	Seedling Vigour
1.	ADT 37	54	28.20	1522.8
2.	ADT 38	54	27.30	1474.2
3.	ADT 39	74	26.30	1946.2
4.	ADT 42	46	14.11	649.1
5.	ADT 43	72	22.79	1640.8
6.	ADT 45	60	20.30	1218.0
7.	ADT 46	52	20.00	1040.0
8.	ADT 48	84	25.30	2125.2
9.	ADT 49	64	19.60	1254.4
10.	ADT 50	76	23.30	1770.8
11.	TKM 9	92	26.69	2455.4

12.	TKM 13	74	21.90	1620.6
13.	TRY 3	32	34.39	1100.4
14.	TRY 4	68	26.10	1774.4
15.	CO 50	52	29.10	1513.2
16.	CO 51	94	28.70	2697.8
17.	IW Ponni	90	22.00	1980.0
18.	CR 1009	72	29.50	2124.0
19.	CR1009Sub 1	58	28.50	1653.0
20.	BPT 5204	80	24.19	1935.2
21.	Swarnasub 1	52	26.50	1378.0
22.	Seeragasamba	82	26.21	2149.2
23.	Andhraculture	36	24.00	864.0
24.	MDU 6	66	25.20	1663.2
25.	Mapillaisamba	80	34.50	2760.0
26.	Kattuyanam	88	33.30	2930.4
27.	AD 07367	78	25.00	1950.0
28.	AD 7250	76	27.00	2052.0

4. Conclusion

This study reveals the presence of diverse mycoflora of both pathogenic and non-pathogenic fungi in paddy seeds of ruling varieties in Tamil Nadu. Since rice is a staple food, better seed health management is a prerequisite for successful rice cultivation. In developing countries where farmers have to save their own seeds for planting, knowledge of seed health is very important to crop and pest management. Seed health testing can also be a means of quality control to improve seed stocks for crop production by farmers. It is also useful for seed certification by seed growers and public seed suppliers to farmers. Also the findings suggest that there is a need for proper storage of rice seed to minimize the fungal infestation in near future.

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