Content list available at http://epubs.icar.org.in, www.kiran.nic.in; ISSN: 0970-6429

Indian Journal of Hill Farming

December 2019, Volume 32, Issue 2, Page 316-321

Fungal Infestation Level of Farmers' Rice Seed in Hill Districts of Manipur

Th. Tilakraj Singh

Department of Botany, Ideal Girls' College, Akampat, Imphal East-795001, Manipur

ARTICLE INFO

Article history:
Received 4 May 2019
Revision Received 22 August 2019
Accepted 26 September 2019

Key words:
Farmer saved seed, Infestation level, Pathogenic, Mycoflora

ABSTRACT

The study analyzes the fungal infestation level in farmer saved seed in hill districts of Manipur. 63 fungal species belonging to 22 fungal genera and two sterile forms could be identified during the isolation of microfungi from 250 seed samples collected from the five hill districts of Manipur. Highest number of fungal species (59 species out of 65 total fungal species) was Isolated from rice seed samples collected from Tamenglong districts. A number of fungal species which were known to be pathogenic to rice such as *Alternaria padwickii*, *Aspergillus oryzae*, *Cercospora oryzae*, *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforme*, *Rhizoctonia solani*, *etc.* were consistently isolated from all the five hill districts. Two important rice pathogens *Pyricularia solani* and *Rhizoctonia solani* were also isolated from Senapati districts at very low percent frequencies. The infestation levels for *A. padwickii*, *D. oryzae* and *Fusarium moniliforme* were comparatively higher. Whereas the infestation levels of *Cercospora oryzae*, *Curvularia lunata*, *Pyricularia solani*, *Rhizoctonia solani* were low. Farmers' save hill rice seeds are found to be more associated with seed borne mycoflora and study suggest that rice seed should be properly detected to check contamination by pathogens before showing for effective and healthy crop production.

1. Introduction

Rice (*Oryza sativa* L.) is the major crop of the people of Manipur in term of area, production and consumption in the state. The state comprises of both hill and valley areas. Hill area constitutes 90 per cent of the total geographic area of the state. Agriculture in this area is in the most primitive form. Most of the operation are labor intensive performed mainly with the uses of small hand tools and are completely ignorant of the modern concept of scientific farming system.

In the hill districts shifting cultivation (jhuming), terrace cultivation and contour cropping are practiced according to the nature of soil and physiographic (slope and gradient) of the farming area. According to Prasad (1987) shifting cultivation is a primitive form of farming system of North Eastern hills leading to heavy soil erosion, declining fertility, loss of flora and fauna and eventually disastrous effects on restoring capacity of the land. The south districts of Manipur hill areas the duration of Jhum cycle is about is about 3-5 years only. About 3 lakhs tribal population is engaged in the age-old practice of Jhuming. For every 5 members tribal family about one hectare of Jhum land is required. Thus, they are extending their Jhum land in the new virgin land. About 900 sq.km. of virgin forest is burnt down every year for the extension of new Jhum land (Laiba,1992). The hill farmers in this region traditionally store the seeds in kotlus, gunny bags, bamboo mats, mud pastured storage structure and even in tins, drums and cemented bins. Fungi alone cause 55 rice diseases of which 43 are seed borne or seed transmissible (Mew and Misra, 1994). The seed mycoflora grow and thrive best under favorable conditions during storage (Main and Fakir, 1989). Fungal population varied with changed in seed moisture and length of storage having significant on seed health (Deka *et al.,* 1995). Rice seeds are infected by fungi and perpetuated from one season to another through infected seeds (Zope and Thrimuty 2004).

Corresponding author: tilakigc2016@gmail.com

In India, only 20% is meet by certified seed and remaining 80% from farmers saved seed (Raj *et al.,* 2007; Atwal 2013). The total area under rice crop of hill during 2000-2001 is 67,960 ha. with an annual production of 1,31,960 tones. On the other hand, valley districts products 2,49,730 tones from an acreage of 89,000 ha., which is much below the national average of 2.08 t/ha. One of the reasons for low production of rice in this farming system may be inferior quality of rice cultivars as well as rice seed health used by the hill farmers. Considering the above facts, the present study was planned to study different qualitative and quantitative aspects of fungal infestation of rice seeds in the five hill districts of Manipur.

2. Materials and Methods

The rice seed samples were collected using area sampling technique (Kothari, 1990) from the five hill districts of Manipur during the sowing season of (April – June) of 2012. From each district 5 villages and from each village 10 farmers were randomly selected for seed sample collection. Thus, the total number of seed samples collected was 250. The moist blotter technique as recommended by International Seed testing Association (ISTA, 1985) was employed for isolation of seed mycoflora. Plastic Petri dishes (dia.9 cm.) with circular moist blotter paper was sterilized by autoclaving. Twenty-five seeds were aseptically placed, with appropriate spacing, in each sterile Petri Dish. For each seed sample eight such Petri dish were prepared, thus, the total number of seeds examined for each seed sample was 200. The Petri dishes loaded with seeds were incubated at 27 ± 1^{o} C under alternate cycles of 12 hours of darkness and artificial daylight using white fluorescent tubes arranged at 40cm. above this plates. Observations of fungal growth on the seeds were made at 24 hours intervals upto 8 days after the initial incubation. Effort was made to identify the mycoflora upto species level with the help of literatures (Barnett and Hunter 1972; Mathur and Kongsdal 2003; Mew and Gonzals 2003). The result was expressed in terms of percent frequency of each fungal species from the following formula.

$$\text{Percent frequency} = \frac{\text{No. of seeds on which growth of the particular fungal species is detected} \times 100}{\text{Total number of seeds examined}}$$

Number of smut ball (sclerotia of *Ustilaginoidea virens*) was also counted from contaminated seed samples.

3. Result and Discussion

During isolation of micro fungi from the 250 seed samples 63 fungal species belonging 22 fungal genera and two sterile forms could be identified from the five hill districts of Manipur (Table 1). As seen from the table, district wise fungal infestation levels (% frequency) were 22.46% in Churachandpur, 27.08% in Chandel, 31.80% in Senapati, 29.46% in Ukhrul and 30.30% in Tamenglong district. A number of fungal species which are known to be pathogenic to rice such as *Alternaria padwickii, Aspergillus oryzae, Cercospora oryzae, Curvularia lunata, Fusarium moniliforme, Rhizoctonia solani*, *etc.* were consistently isolated from all the five districts. A total of two pathogenic fungi *A. padwickii and D. oryzae* were most frequently isolated from all the districts. Their percent frequencies were comparatively higher than the other fungal species. Two important rice pathogens *Pyricularia oryzae* and *Rhizoctonia solani* were also isolated from the Senapati district at very low percent frequencies. Table 2 shows the percent frequency of seed infestation levels of important rice pathogens.

The infestation levels for *A. padwickii, D. oryzae and F. moniliforme* were comparatively higher. Whereas, the infestation levels of *Cercospora oryzae, Curvularia lunata, Pyricularia oryzae, Rhizoctonia solani* were low. Table 3 shows the distribution of seed samples in different ranges of smut ball (*Ustilaginoidea virens*) contamination in the five districts. Low contamination by smut balls was observed in all the five hill districts. Earlier workers have also reported various seed borne pathogens, *Alternaria padwickii, Curvularia oryzae, C. lunata, Bipolaris oryzae, Aspergillus niger, Fusarium moniliforme, F. semitectum, Fusarium solani* and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Penicillium and Myrothecium* from seeds of different varieties of rice in many parts of the world (Wahid *et al.,* 2001; Khan *et al.,* 2000; Javaid *et al.,* 2002; Nguefack *et al.,* 2007; Utobo *et al.,* 2011). A total of 69 rice seed samples from different state of India were tested their health status and sixteen genera of fungi *viz. Acremonium, Alternaria, Aspergillus, Bipolaris, Chaetomium, Cladosporium, Curvularia, Exserohilum, Fusarium, Micridochium, Nigrospora, Phoma, Pyricularia, Rhizoctonia, Rhizopus, Verticillum* comprising 27 species were found to be associated with the rice seed samples (Archana and Prakash 2013). Different pathogenic fungi like B. oryzae, Pyricularia oryzae, Rhizoctonia oryzae, Alternaria alternate, *Alternaria padwickii, Alternaria longissima, Curvularia oryzae, C. lunata, B. oryzae, Aspergillus niger*, Aspergillus flavus and species of *Penicillium, Fusarium,*

Table 1. Percent frequency of different fungal species on rice seeds.

Sl. No.	Fungal Species	Churach-Candpur	Chan-del	Sena-pati	Ukural	Tameng-long	Average	Total
1.	*Alternaria padwickki*	4.22	4.32	5.42	5.46	5.60	5.004	25.2
2.	*Alternaria sp.*	-	-	0.04	-	0.12	0.032	0.16
3.	*Arthrobotrys javanica*	0.10	0.30	0.28	0.38	0.42	0.296	1.48
4.	*Aspergillus candidus*	0.10	0.38	0.22	0.30	0.72	0.344	1.72
5.	*A. clavatus*	0.20	0.12	0.28	0.18	0.14	0.184	0.92
6.	*A. flavipes*	-	0.60	-	-	0.04	0.128	0.64
7.	*A. flavus*	0.48	1.00	1.26	0.98	0.48	0.912	4.56
8.	*A. fumigates*	0.28	0.52	0.74	0.72	0.30	0.512	2.56
9.	*A. funiculosus*	0.04	-	0.06	-	0.30	0.800	0.40
10.	*A. gloucus*	0.20	-	-	-	-	0.040	0.20
11.	*A. niger*	0.84	1.04	1.10	0.98	1.22	1.036	5.18
12.	*A. wenti*	0.14	-	0.06	0.44	0.50	0.228	1.14
13.	*A. oryzae*	-	0.44	-	0.16	0.14	0.148	0.74
14.	*Aspergillus. sp.*	-	0.16	0.44	0.36	0.32	0.256	1.28
15.	*A. sydowi*	0.26	0.44	0.16	0.38	0.28	0.304	1.52
16.	*A. tereus*	-	-	-	0.86	0.60	0.292	1.46
17.	*A. versicolor*	0.10	0.08	0.20	0.12	0.06	0.112	0.56
18.	*Cephalosporium sp.*	0.28	0.54	0.44	0.14	0.34	0.340	1.70
19.	*Cercospora oryzae*	-	-	0.36	-	0.16	0.120	0.60
20.	*Chaetomium botrychodes*	0.54	0.26	0.38	0.24	0.26	0.316	1.58
21.	*C. cochloides*	0.18	0.68	0.58	0.16	0.16	0.372	1.86
22.	*C. globosum*	0.42	0.24	0.30	0.18	0.12	0.260	1.30
23.	*C. homopilatum*	0.04	0.58	0.42	0.10	0.24	0.252	1.26
24.	*C. indicum*	0.22	0.66	0.12	0.24	0.26	0.300	1.50
25.	*C. olivaceum*	0.70	0.98	0.72	1.10	0.38	0.776	3.88
26.	*Cladosporium herbarum*	0.22	0.08	0.20	0.34	0.34	0.236	1.18
27.	*Curvularia lunata*	0.18	0.30	0.72	0.46	0.80	0.492	2.46
28.	*C. pallescens*	-	0.24	0.32	0.36	0.28	0.240	1.20
29.	*Dark sterile mycelium*	-	-	-	0.04	-	0.008	0.04
30.	*Diplosporium flavum*	0.03	-	-	-	0.48	0.156	0.78
31.	*Drechslera oryzae*	3.88	4.14	5.18	5.68	6.22	5.020	25.10
32.	*Drechslera sp.*	-	0.10	0.38	-	0.48	0.192	0.96
33.	*Fusarium diamerum*	0.16	0.04	0.20	0.14	-	0.108	0.54
34.	*Fusarium equiseti*	0.16	0.04	0.20	0.14	-	0.108	0.54
35.	*F. graminearum*	0.16	0.10	0.38	0.32	0.14	0.220	1.10
36.	*F. moniliforme*	2.16	2.78	2.24	1.04	1.34	1.912	9.56
37.	*F. oryzae*	0.28	0.06	-	0.14	0.42	0.180	0.90
38.	*F. oxysporium*	0.42	0.22	0.94	0.72	0.22	0.504	2.52
39.	*F. semitectum*	-	0.04	0.22	0.22	0.08	0.112	0.56
40.	*Fusarum sp.*	0.22	-	0.12	0.34	-	0.136	0.68
41.	*Gleocladum roseum*	0.34	0.18	0.50	0.48	0.24	0.348	1.74
42.	*Memnoniella echinata*	0.12	0.60	-	-	0.08	0.160	0.80
43.	*Mucor sp.*	0.36	0.4	0.28	0.54	0.4	0.396	1.98
44.	*Penicillium frequetans*	-	0.22	0.24	0.04	0.14	0.128	0.64
45.	*P. granulatum*	0.06	0.08	0.26	0.22	0.16	0.156	0.78
46.	*P. herqui*	0.08	0.16	0.28	-	0.06	0.116	0.58
47.	*P. implicatum*	-	-	0.06	-	0.12	0.036	0.18
48.	*P. islandicum*	0.22	-	0.16	0.04	0.26	0.136	0.68

Sl. No.	Fungal Species	Churach-Candpur	Chan-del	Sena-pati	Ukural	Tameng-long	Average	Total
49.	*P. lanosum*	-	-	0.06	0.12	-	0.036	0.18
50.	*P.pallidum*	0.1	0.46	-	0.08	0.10	0.148	0.74
51.	*P. purpurogenum*	0.88	0.48	0.86	0.68	0.82	0.744	3.72
52.	*Penicillium sp.*	0.22	0.24	0.16	0.18	0.26	0.212	1.06
53.	*P. terrestre*	0.08	0.16	-	0.22	-	0.092	0.46
54.	*P. turbatum*	0.12	0.04	0.36	0.34	0.18	0.208	1.04
55.	*Phoma sp.*	0.24	-	0.28	0.18	0.38	0.216	1.08
56.	*Pyricularia oryzae*	-	-	0.1	-	0.06	0.032	0.16
57.	*Rhizoctonia solani*	-	-	0.6	0.10	0.46	0.232	1.16
58.	*Rhizopus cohini*	0.4	0.44	0.52	0.76	0.28	0.480	2.40
59.	*Rhizopus nigricans*	0.08	0.18	0.1	-	0.22	0.116	0.58
60.	*Stachybotrys altra*	0.62	0.58	0.84	0.26	0.36	0.532	2.66
61.	*Trichoderma sp.*	0.24	0.32	0.14	0.28	0.22	0.240	1.20
62.	*Trichoderma viride*	0.14	0.24	0.04	0.22	0.10	0.148	0.74
63.	*Verticillium terrestre*	0.38	0.14	0.44	0.42	0.18	0.312	1.56
64.	*Verticillium sp.*	-	0.1	0.2	0.28	0.16	0.148	0.74
65.	*White sterile mycelium*	0.4	0.5	0.56	0.38	0.60	0.488	2.44
Total No. of fungal species		22.4649	27.0850	31.8055	29.4653	30.3059	28.22	141.0

Sclerotium, Colleotrichum were found to be associated with seeds (Uma and Wesely, 2013; Dash, 2013). We found similar fungal diversity associated with rice seed in all five districts. Among them, species of *Aspergillus* and *Penicillium* were most frequent. Islam *et al.* (1994) found that *Drechslera oryzae* and *Trichoconis padwickii* was much higher in the north of the country compared to south of Bangladesh. Soave *et al.* (1997) also concluded that *D. oryzae* is the most important pathogen causing upland rice seed spots in the Brazilian central western region. Similar observation was also made by Singh, (2000). Infestation of both apparently healthy and discolored seeds was highest with *Alternaria padwickii* followed by *Curvularia sp.* in the seed samples collected from 7 different regions of the Philippines (Mishra *et al.,* 1995).

4. Conclusion

The result of present study revealed that rice seeds used by the hill farmers are infested by a number of pathogenic fungi and storage fungi. We also found the fungal diversity in our study. It also shows that a number of important rice diseases prevalent in the hill are seed transmitted. This will result in lower viability of the seeds low performance of the seedling raised from these seed. Finding of the present study may be useful to design effective management of seed borne fungal disease to check the transmission of fungal pathogen from seed to field.

5. References

Archana B. and HS Prakash (2013). Survey of seed borne fungi associated with rice seeds in India. *International Journal of Research in Pure and Applied Microbiology* 3: 25-29.

Atwal SS (2013). Successful entrepreneurship through seed production. In: Kumar a, Kumar R, Gupta A, Atwal SS (eds.) Entrepreneurship development through seed production, Indian Agriculture Research Institute, Regional Station, Karnal, Haryana, India pp:1.

Barneet HL, and BB Hunter (1972). Illustrated genera of Imperfect Fungi.3rd Edn. Burgees Publishing Co., Minneapolis Minn, pp 241.

Dash C (2013). Seed health and quality test of three rice varieties for the detection of fungi associated with seed samples. *Universal Journal of Plant Science*. 1(2): 37-42.

Deka, B., and M.S. Ali, (1995). Rice grain fungi: occurrence and role over storage seed. *Plant Health* 1: 26-28.

Gill MA and MG Khan (2000). Seed borne fungi of rice from central Punjab and their control. *Pakistan Journal of Phytopathology*.12(1): 12-14.

Islam, M.R., Ali, A., and Mia, M.A.T. (1994). Survey on seed borne pathogens of rice in fifteen districts of Bangladesh. *Bangladesh Journal of Plant Pathology* 10(1/2): 23-26[En,20 ref.]

ISTA. (1985). International rules for seed testing. *International Seed Testing Association* 13: 255-299.

Javaid MS, Wahid A. Idress M, Gill MA, and A Saleem (2002). Seed mycoflora studies in rice. *Pakistan Journal of Phytopathology* 14: 132-34

Laiba, M.T. (1992). The Geography of Manipur. Public Book Store, Imphal, Manipur.p.440.

Main, I.H. and G.A Fakir. (1989). Fungi moisture content and germinability of rough rice seed during storage. *Seed Research* 12(2): 169-173.

Mathur SB., and O. Konsdal (2003). Common laboratory seed health testing methods for detecting fungi. Bassersdorf, Switzerland, ISTA, 425pp.

Mew TW. and J.K. Misra, (1994). A Manual of Rice Seed Health Testing. IRRI. Manila, Philippines.

Mew TW and Gonzales (2002). A handbook of rice seed-borne fungi. International rice research institute, Los Banos, Phillipines, 83.

Mishra, J.K., Gergon, E.B., and T.W. Mew, (1995). Storage fungi and seed health of rice: a case study in the Philippines. Mycopathologica 131(1): 13-24.

Nguefack J, Nguikwie SK, and D Fotio (2007). Fungicidal potential of essential oils and fractions from *Cymbopogon citrates, Ocimum gratissimum* and *Thymus vulgaris* to control *Alternaria padwickii* and *Bipolaris oryzae*, two seed-borne fungi of rice (*Oryza sativa* L.) *Journal of essential Oil Research.* 17: 581-587.

Prasad, R.N. (1987). Degradation of soil and water resources and crop production in NEH Region. *Indian Journal of Hill Farming* 1: 1-8.

Raj MH, Niranjana SR, Nayaka SC, and HS Shetty (2007). Health status of farmer's save paddy, sorghum, sunflower, cowpea seeds in Karnataka, India. *World Journal of Agricultural Sciences* 167-177.

Singh, M.T. (2000). Seed quality assessment of rice with special reference to seed mycoflora in Manipur Valley. Ph.D. Thesis. Manipur University, Imphal, Manipur.

Soave, J., Prabhu, A.S., Ricci, M.T.de, T., Barros, L.G.de., Souza, N.R.G.; Courvo, R.C.V., Ferreira, R.P., Sobral, C.A.M. (1997). Etoligia de manchas de sementes de cultivaras de arraz de sequeiro no centrooeste brasileiro. *Summa Phytopathologica* 23(2): 122-127.

Uma V and EG Wesely (2013). Seed-borne fungi of rice from South Tamil Nadu. *Journal of Academia and Industrial Research.* 1: 612-614.

Utobo EB, Ogbodo EN, and A.C. Nwogbaga (2011). Seed borne mycoflora associated with rice and their influence on growth at Abakaliki, Southeast Agro-Ecology, Nigeria, *Libyan Agriculture Research Centre Journal International* 2: 79-84.

Wahid A, Javed MS Idrees M, and MA Gill, (2001). Studies on the association and effect of *Fusarium solani* on rice seeds in Panjab, Pakistan. In Integrated plant disease management. Proceedings of 3rd National Conference of Plant Pathology, NARC, Islamabad, 1-3 Oct.2001. (pp.70-72). Pakistan Phytopathology Society.

Zope AV, and VS Thrimurty (2004). Effect of botanical pesticides on seed, rhizosphere microflora and seedling vigour in rice. *Journal of Mycology and Plant Pathology* 34: 576-578.

Table 2. Seed infestation level of important rice pathogens in five hill districts of Manipur.

Fungal Pathogen	Churachandpur			Chandel			Senapati			Ukhrul			Tamenglong			Total		
	P.F	Max	Mean	P.F	Max	Mean	P.F	Max	Mean	P.F	Max	Mean	P.F	Max	Mean	P.F	Max	Mean
Alternaria padwickii	15	16	4.22 (± 0.479)	17	19	4.32 (±0.53)	15	23	5.42 (±0.656)	13	17	5.46 (±0.538)	9	20	5.6 (±0.52)	69	23	5.004
Cercospora oryzae	50	-	-	50	-	-	44	4	0.36 (±0.140)	50	-	-	45	4	0.24 (±0.112)	239	4	0.12
Curvularia lunata	46	2	0.18 (±0.088)	45	7	0.3 (±0.159)	39	4	0.72 (±0.200)	43	5	0.46 (±0.167)	39	6	0.8 (±0.231)	212	7	0.492
Drechslera oryzae	19	16	3.88 (±0.510)	18	20	4.14 (±0.584)	12	16	5.18 (±0.515)	8	20	5.68 (±0.52)	5	24	6.22 (±0.547)	62	24	5.02
Fusarium moniliforme	29	12	2.16 (±0.413)	28	10	2.44 (±0.493)	28	13	2.24 (±0.440)	35	6	1.04 (±0.24)	36	9	1.34 (±0.34)	156	13	1.91
Pyricularia oryzae	50	-	-	50	-	-	48	3	0.1 (±0.070)	50	-	-	48	2	0.06 (±0.40)	242	3	0.032
Rhizoctonia solani	50	-	-	50	-	-	42	7	0.6 (±0.220)	47	2	0.10 (±0.058)	42	4	0.46 (±0.159)	231	7	0.232

P.F. = No. of pathogen free samples (out of 50)

Max. = Maximum infestation level (in % frequency) recorded

Mean = Total mean infestation level (in % frequency) in each districts.

Table 3. Smut ball (*Ustilaginoidea virens*) contamination of rice seed samples in hill districts of Manipur

No. of Seed Samples						
Contamination Ranges*	Churachandpur	Chandel	Senapati	Ukhrul	Tamenglong	Total
0	47	49	46	47	50	95.6
1	1	1	1	-	-	1.2
2	2	-	3	3	-	3.2
3	-	-	-	-	-	-
>4	-	-	-	-	-	-
Total	50	50	50	50	50	100

*Number of Smut balls counted from 40g. seed sample