Seed-Borne Mycoflora of Selected Rice Varieties in Mubi, Adamawa State Nigeria

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Abstract: ***The main objective of the study was to investigate the incidence of seed-borne mycoflora of rice seeds from Mubi. Rice varieties were obtained from marketers at Kasuwan Dole, Mubi. The varieties use included Garuwa, Chaina, Hurda, Kotaina, Faro 44 and Gudai. Blotter paper and agar plate method were used to isolate fungal species associated with the rice varieties. A total of nine fungal genera associated with the rice seed samples were identified in both blotter paper and agar plate methods. The fungus Alternaria padwickii (15.59 %) was the most predominant in the blotter method, while Curvularia oryzae in the agar plate method. The blotter method yielded a higher percentage of seed mycoflora in Kotaina with 19.10 %, while the lowest was observed on Gudai with 12.94 %. The agar plate method yielded a higher percentage of seed mycoflora in Kotaina with 20.44 %, and the lowest was observed on Hurda with 11.35 %. The highest vigour index was obtained in Hurda (471.28) and the lowest in Kotaina (155.83). A negative correlation was observed in the four varieties, while a positive correlation was obtained in Garuwa (0.585%). This study revealed diverse species of fungi associated with rice seed varieties. Further research on the economic importance of these seed-borne fungi with regards to seed germination and seedling infection resulting from these fungi is thus recommended.***

Keywords: pathogen, agar, infestation, mycoflora, farmers

1. Introduction

Rice (*Oryza sativa* L.) is a self-pollinated cereal crop, belonging to the family Poaceae. There are about 20 different species, of which only two have been cultivated for staple food, namely *Oryza sativa* L. which is native to tropical and subtropical Southern Asia and the African rice (*Oryza glaberrima*) which is native to West Africa (Habib *et al.,* 2012). Rice is one of the most important staple foods for more than half of the world's population (IRRI, 2006) and influences the livelihoods and economies of several billion people. It is grown in almost all the tropical and subtropical regions of the world. Rice is cultivated in an area of about 165 million hectares globally, thus giving a total production of about 744.4 million tonnes (496.4 million tonnes, milled basis), {FAO, 2014}. Mohammed (2014) reported that before the advent of the Second World War, the predominant rice variety cultivated in Nigeria was the African originated red-grained species of the *O. glaberrima*. However, with the begining of World War II and its attendant, there was a greater requirement of food for fighting troops this resulted in the introduction of Guyana varieties, of which BG 79 was the most widely cultivated (Salako,). Since then, a lot of progress has been made in the development and release of new rice varieties, by a host of Research Institutes in the country especially the National Cereals Research Institute (NCRI) Badeggi (Mohammed, 2014).

Demand for rice in Nigeria is growing faster than that of any other staples with consumption broaden across all socio-economic classes. Nigeria has been identified to consume about 5.4 million metric tons of rice annually while the remaining 3.1 million metric tons is imported (Mohammed, 2014). According to Omofonmwan and Kadiri (2007), the second most serious problem confronting Nigeria rice farmers is the devastation caused by pests and diseases coming after the problem of finance. The North-Eastern agro-ecological zone has the potential for achieving national self-sufficiency in rice production. However, the yield of this crop has never been able to meet the growing demand as a result of disease infestation.

Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases (Ora *et al*., 2011). It identifies the cause of seed infection that affects the planting value of seed lots for seed certification by seed growers to supply seeds to farmers. Seed testing affects policies on seed improvement, seed trade, and plant protection. Of the various modes of transmission of plant diseases, seeds play an important role in the transmission of pathogens and development of plant diseases. The seed-borne pathogens may be externally or internally or extra or intra embryonal associated with the seeds (Somda *et al*., 2008). Of these, fungi are the largest group among such microorganisms, and this includes saprophytic and weak parasitic fungi which lower the quality of seeds by causing discoloration thus reducing the commercial value of seeds (Ibrahim and El-Dahab, 2014).

These pathogens are disastrous as they reduce seed vigour 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. Many diseases of economically important crops are seed-borne like bakanae disease of rice (*Fusarium moniliforme*), loose smut [*Ustilago tritici* (Pers.) Rostrup], flag smut (*Urocystis tritici* Koern.), karnal bunt [*Neovossia indica* (Mitra) Mundkar] and ear cockle of wheat (*Anguillulina tritici* Gerv. & Bened.) (Javaid and Anjum, 2006). If seed infected or contaminated by pathogens were sown in non-infested soil, the pathogens may be established in that soil (Javaid *et al.*, 2011).

More than 50 fungal pathogens have been reported to be seed-borne causing pre and post-infections and considerable quality losses such as seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and nutritive value (Kavitha *et al.*, 2005). Farmers often experience losses in rice due to storage pathogens. The extreme seed-borne pathogens of rice are associated with infection of rice and cause yield reduction, quality deterioration, and germination failure. Farmers generally use different hybrids of rice varieties as a result of which they are faced with difficulties of many diseases. The cultivation of imported rice varieties in Nigeria has increased rapidly. With this in mind, therefore, assessment of the incidence of seed-borne mycoflora associated with selected rice varieties in Mubi was necessitated.

2. Materials and Methods

Study Area

Mubi is located on latitude 10^{o} 12 N and longitude 13^{o} 10 E. It has a tropical climate with a temperature range between 15 and 42^{o}C and relative humidity of 10 to 45% in the dry season. The annual rainfall in Mubi is about 1056mm (Adebayo, 2004).

Collection of Sample

The samples of six (6) rice varieties were collected randomly from Kasuwan Dole. The samples were each enclosed in polythene bags with proper labeling and kept in the laboratory pending assessment.

Preparation of Potato Dextrose Agar (PDA)

Two hundred grams (200 g) of peeled and sliced potato was boiled in 500 ml water in a beaker for 8 minutes. Then the extract was filtered in a conical flask using cheesecloth. Twenty grams (20 g) of dextrose and 20 g agar was added to the extract and the volume was made up to 1000 ml. The prepared standard PDA was sterilized for 10 minutes in an autoclave (Amza, 2018).

Inoculation of Media

Under the aseptic condition, the sample was immersed in a 2 % sodium hypochlorite contained in a sterile 9 cm diameter petri dish for surface sterilization for 30 seconds using sterile forceps. The sterilized sample was rinsed in three changes of sterile distilled water and then dried between sterile filter paper. The sample was then plated aseptically on 9 cm diameter petri dish containing sterile solidified potato dextrose agar and incubated at room temperature for 7 days (Patel and Solanki, 2017).

Isolation of Fungi from the Rice Seed

Isolation of fungi from the six different varieties of the rice was carried out using blotter paper and agar plate methods as described by ISTA (2006).

Identification of Fungal Isolates

Identification was carried out through microscopic examination of spores and fruiting bodies such as colony growth, colour, sporulation type and other basic characteristics as suggested by John *et al*. (2007) using a compound microscope and relevant books (Barnett and Hunter,1992).

The percent seed mycoflora and percentage frequency of various fungal species were calculated using the formula below;

$$\%\ \text{seed mycoflora} = \frac{\text{total number of seed on which the fungi were encountered}}{\text{Total number of seed tested}} \times 100$$

$$\text{Frequency of occurrence (\%)} = \frac{\text{number of seeds on which a fungi species occurs}}{\text{Total number of seed examined}} \times 100$$

Seed germination percentage

The hundred (100) seeds from each of the samples were placed on a damp 20 cm filter paper and covered with another filter paper. The base was folded in and whole rolled up and secure with elastic bands and incubated upright in plastic trays for 8 days. After the incubation period, the number of germinated seeds was recorded and the relationship between seed percentage mycoflora and germination was determined using correlation analysis.

Seed vigour

The seedling vigour test was determined on sand (ISTA, 2006). One hundred seeds were selected at random and sown on the sand in each plastic tray in four lines (25 seeds /line). Percentage germination was taken at 14 Days after Sowing (DAS). After 20 days, the shoot and root length were measured. Fifteen seedlings from each tray were randomly selected for measurement of shoot and root length. The seedling vigour was determined by using the following formula

Vigour index = (Mean of root length + Mean of shoot length) x percentage of seed germination. (Haque *et al.*, 2007).

3. Results

The blotter method yielded a higher percentage of seed mycoflora in Kotaina with 19.10 % whereas, the lowest percentage of seed mycoflora observed on Gudai with 12.94 %. A total of nine fungal genera namely *Aspergillus niger*, *verticillium*, *Curvalaria*, *Fusarium*, *Bipolaris*, *A. flavus*, *Alternaria*, *A. candidus*, *Fusarium* were found to be associated with the seed samples of different rice varieties (Table 1). The fungus *Alternaria padwickii* (15.40 %) was the most predominant fungi associated with rice varieties, while the lowest was *A. niger* (6.57%).

Table 1: Frequency occurrence of associated fungi with seeds of various rice varieties (Blotter paper method)

S/NO	Fungus/ Variety	Gar	Chai	Hur	Kot	F. 44	Gu	Total	Frequency (%)
1.	*Aspergillus niger*	11	9	1	5	4	2	32	6.57
2.	*Verticillium sp*	10	8	4	7	8	5	42	8.62
3.	*Curvularia lunata*	14	7	8	8	11	3	51	10.47
4.	*Fusarium oxysporum*	6	3	6	9	17	11	52	10.68
5.	*Bipolaris specifera*	13	16	11	12	9	9	70	14.37
6.	*Aspergillus flavus*	8	15	10	14	7	11	65	13.35
7.	*Alternaria padwickii*	16	14	7	19	13	6	75	15.40
8.	*Aspergillus candidus*	7	9	9	8	16	12	61	12.53
9.	*Fusarium moniliforme*	5	4	12	11	3	4	39	8.00
	Total	90	85	68	93	88	63	487	
	Freq. (%)	18.48	17.45	13.96	19.10	18.07	12.94		

Key: gar = garuwa, chai = chaina, hur = hurda, kot = kotaina, F. 44 = Faro 44 and gu = gudai

The seed mycoflora as determined by the agar plate method (Table 2) revealed Kotaina with 20.44 % as the highest while on Hurda with 11.35 % as the least. Nine (9) fungal genera were found to be associated with the seed while *Curvularia oryzae* (19.69 %) was the most predominant. The effect of the mycoflora on the seed was determined and revealed Hurda as the highest (471.3), chaina (338.3), faro44 (254.9), Garuwa (230.4) and the least Kotaina (155.8) (Table 4)

Table 2: Frequency occurrence of associated fungi with seeds of various rice varieties (Agar plate method)

S/NO	Fungus/ Variety	Hur	F. 44	Gar	Kot	Gu	Chai	Total	Frequency (%)
1.	*Aspergillus candidus*	2	6	8	5	4	9	34	8.58
2.	*Aspergillus niger*	4	8	7	7	3	6	35	8.84
3.	*Alternaria padwickii*	4	6	6	9	5	5	35	8.84
4.	*Fusarium oxysporum*	6	2	4	6	4	4	26	6.56
5.	*Aspergillus flavus*	2	11	12	10	7	8	50	12.63
6.	*Curvularia lunata*	5	9	8	9	11	12	54	13.64
7.	*Verticillium sp.*	8	4	3	8	4	5	32	8.08
8.	*Curvularia oryzae*	11	14	13	15	9	16	78	19.69
9.	*Bipolaris specifera*	3	10	11	12	7	9	52	13.13
	Total	45	70	72	81	54	74	396	
	Freq. (%)	11.35	17.68	18.18	20.44	13.64	18.69		

Key: gar = garuwa, chai = chaina, hur = hurda, kot = kotaina, F. 44 = Faro 44 and gu = gudai

Table 3: Effects of mycoflora on seed vigour index

S/NO	Variety	Seed vigour index
1.	Kotaina	155.8
2.	Faro 44	254.9
3.	Hurda	471.3
4.	Garuwa	230.4
5.	Chaina	338.3
6.	Gudai	427.45
SE±		0.45
C.V. (%)		3.55

Table 4.3 shows the effect of mycoflora on seed vigour index. The highest vigour index was recorded in Hurda (471.28), followed by Gudai (427.45), Chaina (338.33), Faro 44 (254.94), Garuawa (230.41) and the lowest vigour index was recorded in Kotaina (155.83).

The correlation of percentage of seed mycoflora with seed germination indicated that the highest percentage of seed germination was recorded in Garuwa (0.585 %), followed by Faro 44 (-0.157%), Gudai (-0.157%), Hurda (-0.061%), Chaina(-0.061%). The lowest seed germination percentage was recorded in Kotaina(-0.002%) (Table 4).

Table 4: Correlation of percent seed mycoflora with seed germination

S/NO	Variety	Germination (%)
1	Kotaina	-0.002
2	Faro 44	-0.157
3	Hurda	-0.061
4	Garuwa	0.585
5	Chaina	-0.061
6	Gudai	-0.157

A positive significant was observed in Garuwa while the rest showed a negative significant difference.

4. Discussion

In this study, the seed health test method was used, two common methods blotter paper and agar plate methods for the detection of seed mycoflora. The mycoflora obtained in the blotter method were inconsonant with the report of Archana and Prakash (2013) who found seven genera of fungi *viz*., *Acremonium Alternaria*, *Aspergillus*, *Bipolaris*, *Curvularia*, *Fusarium*, *Microdochium* and *Verticillium* comprising twenty-seven species were found to be associated with the seed samples of rice varieties. However, high numbers of fungi were obtained using the agar plate method compared to blotter paper method. This contradicts the report of Ashfaq *et al*. (2015) who reported a high number of fungi with blotter paper.

The present study was targeted on the survey of seed-borne fungi associated with some selected rice varieties in Mubi. Nine fungal species were isolated and identified with blotter method while nine were obtained by the agar plate method. These results were in consonance with those of many types of research (Javaid *et al*., 2002; Ibiam *et al*., 2006; Serferbe *et al*., 2016). The isolation of various seed-borne fungi from rice seeds carried out earlier by various researchers, among which were Butt *et al.*, (2011) who reported *Fusarium moniliforme*, *Fusarium* sp, *Cheatomium* sp. and *A. niger* to be associated with rice varieties; Archana and Prakash, (2013) who reported *Aspergillus candidus* and *Aspergillus nidulans*, Gupta, (2010) reported *Aspergillus flavus* and *Aspergillus* sp., *Curvularia lunata* and *Curvularia* sp. to be associated with rice varieties. Similar work was carried out by Farid *et al.* (2002) who tested the twelve seed samples of rice and all were found infected by *Bipolaris oryzae* that cause brown spot disease. Many of the isolated fungi have been reported to be associated with seeds of other crops (Tsopmbeng, 1994; Tsopmbeng and Fomengia, 2015). Some of them are also known to cause seed rot, decrease seed germination and cause pre and post damping-off and seedling death (Al-kassam and Monawar, 2000).

The fungus *Alternaria padwickii* (15.40 %) was the most predominant fungi in the blotter paper method, while the lowest was *Aspergillus niger* (6.57 %) associated with the study sample. This finding contradicts Alam, *et al*. (2014) who recorded highest with *Fusarium sp*. and lowest with *Aspergillus ochraceus* to be associated with rice varieties. *Curvularia oryzae* (19.69 %) was the most predominant, while the lowest was *Fusarium oxysporium* in the agar plate method. This finding also contradicts the report of Makun *et al*. (2006) who found *Aspergillus clavatus* as the most predominant and *Gilocladium spp*. as the lowest fungi associated with rice varieties.

Effect of seed-borne mycoflora on seedling vigor of selected rice varieties were determined and significant results found regarding germination and vigor index. From this study, it was found that seeds of rice varieties infected with seed-borne mycoflora resulted in poor seedling vigor. A significant difference was observed for the percentage of vigour index of various rice varieties. This finding is in agreement with Patel and Solanki (2017) who also observed significant difference for the percentage of vigour index of GR-4, GNR-3, GNR-4, Gurjari, and NAUR-1 rice varieties. Islam *et al*., (2012) reported that *Fusarium moniliforme*, *Rhizopus nigricans* and *Penicillium oxalicum* caused marked reduction in shoot length, whereas *Chaetomium herbasum* and *Fusarium moniliforme* caused marked reduction in root length. *Fusarium moniliforme, F. chlamydosporum* and *Aspergillus niger* caused a reduction in vigour index.

The results obtained on seed vigour were in conformity with the works of Ibrahim and El-Dahab**,** 2014**;** Patel and Solanki (2017). They reported a highly negative significant correlation between seed infection by mycoflora and seed germination in the laboratory for all the cultivars tested. Seed germination decreased with increased in seed infection regardless of the rice cultivars tested was observed. This was in agreement with the work of Islam *et al*. (2012) they reported a similar scenario. Similarly, Mehrotra and Aggarwel (2003) reported that wheat infected with *Tilletia indica* had very little effect on viability irrespective of the age of the seed. While the germination of infected seeds appeared to depend upon the wheat cultivars and the seeds. They added that infected seed had a lower survival rate in storage compared with the healthy seed of the same seed lots. In the same vein, Haikal, (2008) reported that reduction in percentage seed germination of soybean seeds was observed in seeds soaked in filtrates of *Phomopsis phaseoli* and also Soybean seeds soaked in cultures filtrates of *Fusarium solani*, *F. oxysporum*, *Aspergillus flavus*, *A. niger*, *Alternaria tenuis,* and *A. alternate* for 24 hours showed reduction in percentage seed germination.

5. Conclusion

This research revealed various mycoflora associated with some selected rice seeds from mubi and the possibility of the occurrence of disease when such infected seeds are planted. Although the results of the present study may be considered preliminary, they suggest fungi associated with rice seeds are a potential threat to its production. Therefore, research into the economic importance of these seed-borne fungi with regards to seed germination and seedling infection resulting from these fungi is recommended for better seed health management.

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