

RESEARCH ARTICLE

# Seed Health, Quality Test, and Control of Seed-borne Fungi of Some Improved and Local Cultivars of Rice (*Oryza sativa* L.) in Kano, Northwestern Nigeria

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## Abstract

This research was carried out to evaluate the health and quality of rice seed. The germination of seed and presence of rice seed related fungi were recorded and used to evaluate the effect of seed dressing chemicals on germination and vigor index over untreated rice seeds. Seven cultivars commonly grown in Kano, Kano State, Northwestern Nigeria “FARO 52” (WITA), “FARO 44” (SIPI), “FARO 60” , (improved varieties), “Kwandala” , “Jamila” , Ex-china, and “JIF” (local varieties) were used in this study. The seed dressing chemicals used were Apron Star 42 WS, Dress Force 42WS and ZEB-Care 80%WP. This study was performed under three main tests, i.e dry inspection, blotter tests, agar plate and microscopic examination. The highest number of healthy seeds (94.16%) was recorded from “JIF” variety and lowest (64.77%) from “Jamila” . The highest number of deformed seeds was observed from variety “FARO 44” whereas the lowest noted on “JIF” . The identified fungi were *Fusarium spp.*, *Bipolaris oryzae*, *Aspergillus flavus*, *Curvularia lunata*, *Aspergillus niger*, and *Nigrospora oryzae.*, *Rhizoctonia spp.* and *Rhizopus spp.* Highest seed infection was recorded for *A. flavus*, *A. niger*, and *Fusarium spp.*, and the least with *C. lunata* and *N. oryzae*. Treated seeds with Zeb-care (Mancozeb 80% WP), increased their vigor index over untreated by 62.78% and can be recommended as seed dressing chemical for optimum control of rice seed-borne pathogens.

**Keywords:** quality seed, seed germination, vigor index, seed-borne fungi, seed dressing chemical

## Introduction

Rice is considered as one of the major staple cereal foods and forms a significant component of the energy resource of the human race. Rice is an economically and scientifically important crop and mostly produced and consumed in Asia and African countries (Islam et al. 2000; Dede et al. 2019). Currently, 35–40 % more yield productivity is required to meet global demands (FAO 2017). The available rice stock in these countries is stored to adjust the demands and supply for both seed and food (Islam et al., 2000). To date, the seed of other cereals crops like wheat, maize, and barely have received adequate attention for storage and post-harvest, however, the research for rice is still not completely yet (Misra et al., 1995).

Storage facilities in Asia and Africa are still far from satisfactory. The farmers keep their stock in various ways, some of the means of storage are out-of-date; therefore, weak and practically unserviceable. Increasing rice production and subsequent reduction of its international importation and endemic plant pathogens continue to be a challenge in safeguarding plant health in Nigeria. Therefore, early and accurate diagnosis and pathogen surveillance is the most important approach to tackle this matter. The assessment of seed health standard in rice is very important for farmers and food security as it is a first line approach in managing seed-borne diseases of plants.

Furthermore, the quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full yield potential and value (McGee 1995). The importance of seed quality in understanding the full potential of a variety is well

known. The three significant aspects of seed quality are genetic and physical purity, high germination percentage and vigor, and free from seed-borne diseases and insects (Seshu and Dadlani 1989). Seed vigor is recognized as an essential seed quality parameter distinct from germination (Seshu et al., 1988). Seed-borne diseases may not only introduce new pathogens to affect the quantity or quality of the crop yield but also contaminate the soil permanently (Anselme, 1981).

Fungi are commonly identified as causal agents of many seed-borne diseases (Nsemwa and Wolffhechel 1999). Studies from Biruma et al. (2003) and Kanobe et al. (2004) showed a wide range of seed-borne fungi pathogens on the farmer's stored rice seeds, of which many farmers mainly rely on to propagate the next planting session. Most seed-borne diseases caused by the fungi pathogens are disastrous as they may decrease seed germination, causing seed discoloration and produce toxins that may be detrimental to man and domestic animals. Distribution of some seed-borne fungi associated with rice seeds has been reported in many countries, including Nigeria (Suleiman and Omafè 2013); Pakistan (Butt et al. 2011); Egypt (Madbouly et al. 2012); Bangladesh (Ora et al. 2011); Cameroon (Nguefack et al. 2007) and Chad Republic (Serferbe et al. 2016).

The main objectives of this study were i) to identify seed-borne fungi associated with rice and evaluate its role for seedling abnormalities ii) to investigate the possibility of combining germination and seed health tests to assess the influence of seed treatments on germination and seedling vigor.

## Materials and Method

### *Experimental Site*

The experiment was carried out at the Research and Teaching laboratory and screen house of the Department of Crop Protection, Faculty of Agriculture, Bayero University, Kano, (11° 58' N, 8° 25' E and 47m above sea level) Northwestern, Nigeria.

### *Source of Experimental Materials*

The study was conducted using seven rice varieties commonly grown in the local area. These are "FARO 52" (WITA), "FARO 44" (SIPI), "FARO 60" (improved varieties), "Kwandala", "Jamila", Ex-china, and "JIF" (local varieties). Out of the seven cultivars, four rice seed samples collected from Green spore Agric. Limited ("FARO 44", "FARO 52", "FARO 60" and

"Jamila") while the remaining three varieties ("JIF", "Kwandala", and Ex-china) collected from rice farmers in Kura and Bichi local government areas, respectively. The three seed dressing chemicals used were Apron Star 42 WS, Dress Force 42WS, and ZEB-Care 80%WP.

### *Detection of Seed-borne Fungi using Blotter Method*

To perceive the seed-borne fungal pathogens associated with the seeds using blotter method was used following the international rules for seed testing as used by Ahmed et al. (2013), modified from ISTA to perceive the seed-borne fungal pathogens associated with the seeds. In this method, three layers of blotting papers (Whatman™ filter paper No.1) were soaked in sterile distilled water and placed at the bottom of the 90 mm diameter plastic Petri dishes. Four hundred seeds from each sample were taken randomly and then put on the moist filter paper at the rate of 25 seeds per plate. Incubation was conducted at  $28 \pm 2^\circ\text{C}$  under 12/12 alternating cycles of light and darkness in the incubation room for seven days. During the incubation period, Petri dishes were checked and wetting of filter paper done as the need arise. After incubation, seeds were examined under the stereo-microscope for the presence of seed-borne fungi. Number of infected seeds expressed in percentage. Two selected rice varieties within the highest disease incidence were tested on agar plate method and seed treatment.

### *Identification of Fungi*

Each seed was observed under stereo-microscope at  $\times 16$  and  $\times 25$  magnifications to identify the seed-borne fungi. The most associated fungi were detected by observing their growth characters on the incubated seeds and identified following the identification keys from (Ahmed et al., 2013). Temporary slides were also prepared and observed under a compound microscope for proper identification. The fungi were identified up to species level, following the identification keys of (Fakir et al., 2002). The results presented as percent incidence for the individual fungal pathogen identified.

### *Agar Plate Method*

In this technique, one hundred seeds tested for each variety and replicated ten times. Surface sterilized seeds as above were plated (10 seeds/Petri dish) on the potato dextrose agar (PDA) medium. The plated seeds were incubated for seven days at  $28 \pm 2^\circ\text{C}$  in darkness. At the end of the incubation period, fungi developed from the seeds on the agar medium, and then were sub-cultured into fresh medium for

sporulation. The fungi were examined and identified based on colony characteristics, and morphology of fruiting bodies under a compound microscope after seven days incubation.

#### *Examination of Incubated Materials*

The slide of various fruiting structures of the fungi was prepared and observed under a compound microscope ( $\times 100$ ) for identification following (Barnett and Hunter, 1998).

#### *Dry Inspection of Seed Samples*

Four hundred seeds from each sample were visually inspected and graded into nine categories. The categories were: (1) good seeds, (2) spotted seeds, (3) discolored seeds, (4) deformed seeds, (5) varietal mixture (6) broken seeds, (7) insect damaged seeds, (8) chaffy seeds and (9) inert matter. The result of different seed categories was expressed in percentage.

#### *Fungicide Seed Treatment*

Seeds were rinsed with sterile distilled water in a clean plastic bowl to remove impurities before chemical seed treatment. Fungicides were applied to the seeds following the manufacturer's recommendation. The three fungicides used are Apron Star 42 WS (20% w/w thiamethoxam + 20% w/w metalaxyl-M + 2% w/w difenoconazole), Dress Force 42WS (20% Imidacloprid + 20% metalaxyl-M + 2% tebuconazole) and ZEB-Care 80%WP (mancozeb 80%WP). 0.025g of each chemical used in treating 10g of rice seeds of "Jamila" and "FARO 44" varieties with a high percentage of disease incidence that had been selected for this experiment. The control consisted of untreated rice seeds, that were allowed to dry after treatment.

#### *Germination of Treated-Seeds in Plastic Pots*

Planting media used in the screen house was sterilized sandy soil so as to meet ISTA regulations for grain size, pH, and conductivity (only recommended for seeds >5mm). Plastic pot (30 cm diameter) 10 L in volume and about 16.5 cm depth was used. The treated seeds have been supplied with sufficient water to prevent from drought condition.

#### *Vigor Test*

A vigor test was done using the sand pot method (ISTA 2001). Shoot and root length were measured using a ruler (30 cm length) after 14 days of sowing. Fifteen seedlings (30 seedlings per treatment) were

randomly selected for the measurement of shoot and root length. The seedling vigor was determined using the formula of (Abdul-Baki and Anderson, 1972): Vigor index = (mean of root length + mean of shoot length)  $\times$  % of seed germination.

## **Data Analysis**

All the collected data was analyzed using descriptive statistics and presented as percentage (%).

## **Results and Discussion**

#### *Dry Inspection of Rice Seed Samples*

The highest number of visually healthy seed (94.16 %) recorded from "JIF" variety and lowest (64.77 %) from "Jamila", respectively (Table 1). The highest number of deformed seed (1.8 %) recorded from variety "FARO 44", and lowest (0.47 %) recorded for "JIF". The highest number of apparently healthy seed and lowest deformed seeds were observed in the seeds of "JIF" variety (Table 1), suggesting it as the best among all the varieties.

Survey and determination of collected seed health status from different regions in Bangladesh also have been conducted by previous studies. Fakir et al. (2002) recorded that collected seeds in Bangladesh consist of 91.20 to 98.89 % pure seed, 3.72 to 37.71 % spotted seed, and 8.46- 15.50 % deformed seed. Following study from Fakir et al. (2003) also reported a wide variety of components among the collected seed samples of rice from different regions of Bangladesh. Another study from Uddin (2005) showed that farmer's seeds (variety Upazilla) in Noakhali can be categorized into healthy seed (44.33 to 59.42 %), spotted seed (27.84 to 44.77 %), discolored seed (3.93 to 8.94 %), partly filled seed (0.43 to 2.35 %), deformed seed (0.91 to 3.98%), unfilled seed (0.001 to 0.68%), varietal mixture (0.26 to 2.22%), other plant parts (0.001 to 0.36%), inert matter (0.50 to 0.34%) and insect damaged seed (0.05 to 0.75%). To date, seed health survey at Bogra district, Bangladesh showed that seed from farmer's storages can be grouped into good seed, spotted seeds, deformed seeds, discolored seeds, chaffy grains and insect damaged seeds recording: 77.84 %, 16.32 %, 3.22 %, 1.03 %, and 0.56 % respectively (Akter and Hossain, 2016).

#### *Fungal Pathogens Identified from Rice Seed Samples*

The present study has also focused on the survey of seed-borne fungi associated with rice varieties mostly

Table 1. Dry inspection of rice seed samples

Variety	Different grades of seeds (%)								
	HS	DS	SS	BS	IDS	CS	DES	VM	IM
Ex-CHINA	66.29	18.46	6.50	0.00	1.92	0.9	1.11	1.04	3.78
FARO52	86.35	1.52	9.13	0.55	0.00	0.078	1.33	0.00	0.26
FARO44	90.53	1.20	3.18	2.16	0.00	1.13	1.8	0.00	0.00
“JAMILA”	64.77	2.10	28.4	1.18	0.00	1.7	1.6	0.00	0.25
FARO60	82.76	1.10	12.99	0.13	0.00	1.31	1.69	0.00	0.02
“KWANDALA”	77.72	0.96	19.13	0.33	0.00	0.95	0.55	0.00	0.36
“JIF”	94.16	0.99	2.49	0.63	0.057	1.04	0.47	0.00	0.16

Note: HS = healthy seed = good looking seed free from spots and abnormalities, DS = discolored seed, SS = spotted seeds, BS= broken seeds, IDS = insect damaged seeds, CS = chaffy seeds, DES = deformed seeds, VM = varietal mixture and IM = inert matter

Table 2. Fungal pathogens identified from rice seed samples

S/No.	Variety	Fungi identified
1	“KWANDALA”	<i>Fusarium spp.</i> , <i>Rhizopus spp.</i> , <i>A. flavus</i> , <i>A. niger</i>
2	“JIF”	Bacterial growth, <i>Fusarium spp.</i> , <i>A. flavus</i> , <i>Aspergillus niger</i> .
3	“FARO 60”	<i>Rhizopus spp.</i> , <i>A.niger</i> , <i>A. flavus</i> , <i>Rhizoctonia spp.</i>
4	“FARO 44”	<i>A. flavus</i> , <i>A. niger</i> , <i>Fusarium spp</i> , <i>Bipolaris oryzae</i> , <i>Curvularia lunata</i> , <i>Rhizoctonia spp.</i> , <i>Rhizopus spp.</i>
5	“FARO 52”	<i>Rhizoctonia spp.</i> , <i>Fusarium spp.</i> , <i>A. flavus</i> , <i>A. niger</i> , <i>Rhizopus spp.</i> , <i>Curvularia lunata</i> .
6	“JAMILA”	Bacterial growth, <i>Rhizopus spp.</i> , <i>N. oryzae</i> , <i>A. flavus</i> , <i>A. niger</i> , <i>Fusarium spp.</i> , <i>C. lunata</i> .
7	Ex-CHINA	Bacterial growth

grown in Kano state, Nigeria. Some of the fungal species were isolated and identified with the blotter method while others were obtained by the agar plate method. During our investigation, eight species of fungi identified on the seeds of seven rice varieties (Table 2). The identified fungi were *Fusarium spp.*, *Bipolaris oryzae*, *Aspergillus flavus*, *Curvularia lunata*, *Aspergillus niger*, and *Nigrospora oryzae.*, *Rhizoctonia spp.*, and *Rhizopus spp.* Highest seed infection recorded for *A. flavus*, *A. niger*, *Fusarium spp.*, followed by *Rhizoctonia spp* and *Rhizopus spp.*, while *B. oryzae*, *C. lunata*, *N. oryzae* recorded the lowest percentage of seed-borne infection. The *Aspergillus* species, the one recorded with high frequency, tends to produce toxic substances apart from the deteriorating effect of root rotting. This result was in consistence with findings of (Javaid et al.,

2002; Ibiem et al., 2006), that reported the presence of *Aspergillus* species but, not as main pathogen. Many of the isolated fungi from our study have been reported to be associated with seeds of other crops (Tsoyombeng and Fomengia, 2015). Some of them are also known to cause seed rot, decrease seed germination and cause pre and post damping off as well as seedling death (Al-Kassim and Monawar, 2000) when *Fusarium spp* and *Aspergillus flavus* are found in high frequency. This high frequency of the detected seed-borne pathogen from our study has been reported in previous studies on the seeds of rice in India (Archana and Prakash 2013) and Pakistan (Khan et al., 1988; Javaid et al., 2002). Soil borne mycoflora associated with rice and their influence on growth was also recorded in Abakaliki, Ebonyi State in Southeastern Nigeria (Utobo et al., 2011). Although,

no such kind of soil borne pathogens related to rice crops were recorded in our study area.

#### Quality Test: Purity Analysis of Rice Seed Samples

A seed purity analysis was conducted and concluded: 100 % pure seed in “FARO 44” rice variety, and the lowest pure seed (95.18%) was recorded in variety Ex-China (Table 3). The lower purity of cultivar Ex-China compared to other cultivars was caused by lousy storage practices by the farmers while high purity “FARO 44” was obtained from applied seed technology in commercial company. Another study from Chowdhury’s (2012) about seed quality status reported that HYV has the highest percentage of purity. Haque et al. (2007) also found 99.01 % pure seed from the trained farmers and minimum (96.19 %) in untrained farmers. Percentage of seed purity was determined by Uddin (2005) in Upazilla under Noakhali district that ranged from 95.59 to 99.39 %. In convex with our findings, Fakir et al. (2002) categorized the percentage of pure seeds ranging from 91.20 to 98.89 % collected from trained farmer’s stored rice seed.

#### Germination Test Using Blotter Method of Rice Seed Samples

The result in Figure 1 shows the mean percentage of germination of different rice seeds, ranged from 0 % to 90.50%. The highest germination was recorded in variety “JIF” (90.50%), while the lowest germination found in variety Ex-China (0%). The variety “Jamila” has the highest percentage incidence of fungal mycelia growth examined using blotter method (14.25%), and variety Ex-China recorded the lowest rate (1.25%). This research clearly showed that “JIF” has the highest germination percentage even though it has been sourced from farmer-stored seed within the study area. The zero germination in the variety Ex-China could be a result of poor storage conditions that lead to the development of seed-borne pathogens,

insects, mechanical damage at both handling and storage, or aging which renders the seed to be not viable or dormant. Some of these fungal pathogens are known to cause seed rot, decreasing of seed germination, pre and post damping off, and seedling death (Al-Kassim and Monawar, 2000). According to Lamrani et al. (2013), *Alternaria padwickii* colonizes variety of seeds thus reducing the percentage germination and causes seed rot. Species of the genus *Curvularia*, in particular, *C. lunata* has been reported to infect the embryo of the seeds, therefore, reducing the percentage germination of rice seeds (Imolehin, 1987; Bautista and Opina, 1991). *C. lunata* was reported in different rice varieties and identified as one of seed-borne pathogens (Butt et al., 2011; Utobo et al., 2011; Ashfaq et al., 2015).

#### Effect of Seed Treatment on Seed Vigor Index

The variety “FARO 44” and “Jamila” were selected as test sample of seed dressing trial using three different chemicals due to the facts they are most commonly grown variety in Kano. “FARO 44” is an improved variety while “Jamila” is local. Another consideration is these both varieties have higher mycelia growth than others and this indicator is suitable to measure the increasing of vigor index based on the chemical treatment.

The vigor index of treated seeds using Zeb-care was increased up to 62.78% over the untreated control (Table 4). The control (untreated variety) from “Jamila” has a vigor index score of 928.71, while “FARO 44” has the vigor index of 904.11. Variety “Jamila” treated with Zeb-care has 991.49 vigor index and it indicated that there was an increase of vigor index with 62.78% followed by Zeb-care treatment on “FARO 44” with an increase of 31.78%. The lowest increase in vigor index (5.28%) was found in variety “FARO 44” treated with the Apron star (Table 4). The variety “FARO 44” treated with Dress force shown a negative value of -6.11 over the untreated which directly implies that there is a decrease in vigor index over control.

Table 3. Seed quality and purity test among the rice seed samples

Variety	Different components under purity analysis (%)		
	Pure seed	Other seed	Inert matter
Ex-CHINA	95.18	1.04	3.78
“FARO 52”	99.74	0.00	0.26
“FARO 44”	100.00	0.00	0.00
“FARO 60”	99.98	0.00	0.02
“JAMILA”	99.75	0.00	0.25
“JIF”	99.84	0.00	0.16
“KWANDALA”	99.64	0.00	0.36

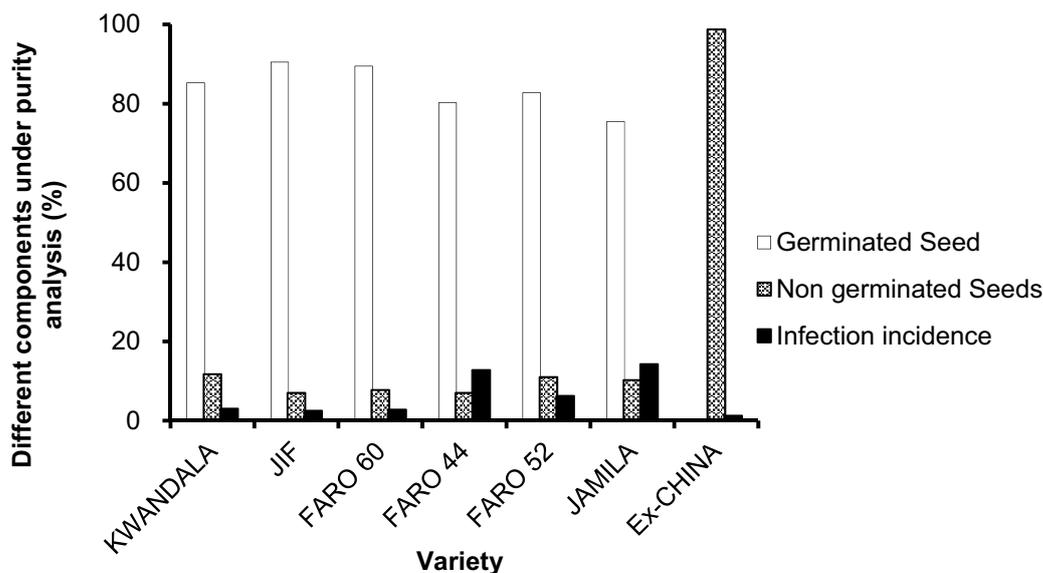


Figure 1: The result of germination test using blotter method among the rice seed samples

Table 4. Effect of seed treatment on increase vigor index over control on rice seed samples

		% Vigor index increased over control		
		Treatment		
Variety	Seed source	Zeb- care	Apron star	Dress force
“FARO 44”	Green spore Agric. Ltd.	+31.78	+5.28	-6.11
“JAMILA”	Green spore Agric. Ltd.	+62.78	+31.17	+22.40

Following the obtained values, the efficacy of seed dressing chemicals was hypothesized benefit the variety “Jamila” more. The Zeb-care dressing chemical displayed a more positive effect (+31.78 and +62.78) on both cultivars (“Jamila” and “FARO 44”) followed by Apron star (+5.28 and +31.17) and lastly Dressed force (-6.11 and +22.40) respectively.

Similar result also has been reported on previous studied by (Bhuiyan et al., 2005; Hossain and Hossain 2012) whereas vigor index increased in vegetable treated seed using BAU Bio-fungicide. Furthermore, Shultana et al. (2009) also evaluated wheat seeds treated with Bavistin and showed higher vigor index on 2843 followed by BAU Bio-fungicide treated seed on 2661.

### Conclusion

Based on the result obtained in this experiment, it can be concluded that farmers are strongly advised to use any of the following varieties “JIF”, “Jamila” and “FARO 44” in Kano State as it has been tested and found to be suitable in both seed quality and purity, it will also help to improve rice production and reduce the threats due to seed-borne fungal diseases. The use of Zeb-care treated seed, which is often neglected

by farmers, is encouraged as the seed dressing fungicide (Zeb-care 80WP) significantly increased seed germination and seedling vigor.

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