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Incidence of seed borne fungi in farm saved rice seeds, quality declared seed and certified seed in Morogoro Region in Tanzania

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Rice (*Oryza sativa* L.) is the second most important food crop after maize in Tanzania. It faces many challenges like diseases, pests and physical damages, which reduce the seed quality. This article identified microorganisms and the district where they occurred. 13 fungal species comprising of 11 pathogenic and 2 saprophytic fungi were detected and the incidence level varied among sample. Mvomero District showed the highest incidence recorded (64.5%) for sample collected from Hembeti and Dakawa/Msufini villages. In Kilombero, the fungal specie which detected highest incidence was *Fusarium equiseti* (31%) followed by *Fusarium moniliforme* (28.5%). *Verticillium cinnabarinum* and *Curvularia inaequalis* had the least incidence of 1 and 1%, respectively. The study recommends that before planting season, seeds should be tested to minimize spread of fungal species.

Key words: Pathogens, quality declared seed (QDS), farmer saved seed (FSS), rice seed, seed quality, Kilosa, Kilombero, Mvomero.

INTRODUCTION

Rice (*Oryza sativa* L.) is a globally important staple food for more than half of the world's population (Khush et al., 2013). The crop is increasingly becoming an important staple food and cash crop in Africa (Tanko et al., 2016). In this continent, 15 million tons of rice is produced annually (Ronald et al., 2014). Eastern and Southern Africa contributes 16.1% of rice where the major contributors are Madagascar and Tanzania (Food and Agriculture Organization (FAO), 2013; United Republic of Tanzania (URT), 2012).

In Tanzania, rice is the second most important and popular food crop after maize (URT) The major rice producing regions in Tanzania include Morogoro, Shinyanga, Mbeya, Mwanza, Rukwa and Tabora, respectively. The trend of increase or decline of rice yield in Tanzania has not been clear for the past 20 years, due to numerous factors including emergence and poor management of production. Rice yields may fluctuate

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depending on the effects of climate change, poor agricultural practices, and inadequate standard of harvest and post-harvest techniques deployed.

Seed is one of the three basic elements for crop production and help to increase agricultural productivity as it provides the maximum limit of crop yield for all other production inputs. Unlike fertilizers and pesticides, farmers cannot produce without seed (Miva et al., 2017).

However, seeds carry pathogens such as fungi, bacteria, nematodes and viruses responsible for transmitting seed-borne diseases, which often cause partial or total crop losses (Barret et al., 2015). When seed has good physical, physiological, health and genetic qualities, farmers have greater prospects of producing a good crop (Miva et al., 2017).

The present investigation has been carried out to establish baseline information on quality of farm saved rice seed by smallholder farmers, to enhanced strategy of yield improvement. This is important as, many farmers' uses own stored seeds for the next cropping season, though seed borne diseases can be transferred easily and hence other quality attributes may be sub-standard. Proper assessment of the quality of seed, stored by farmers from their previous crop will establish the broad picture of seed quality of locally produced rice as compared to Quality Declared Seed (QDS) and Certified Seed. Therefore, this paper aims to identify the seed borne fungi and evaluate their incidence in farm saved rice seeds in Morogoro Region in Tanzania.

MATERIALS AND METHODS

Description of the study area and collection of rice samples for laboratory tests

The rice samples were collected from 45 farmers, 15 from each district in the region. Laboratory investigations were carried out from mid-December 2017 to June 2016 at the African Seed Health Centre and Tanzania Official Seed Certification Institute (TOSCI) by Sokoine University of Agriculture (SUA) in Tanzania.

Multi-stage and cluster-sampling techniques were used to identify the village samples, which ensure good representativeness of rice farming population in the study areas. Rice seed samples of 1.5 kg were collected from each farmer packed in a paper bags, labeled and transported to the African Seed Health Laboratory at Sokoine University of Agriculture then stored in the refrigerator at 5°C (to avoid further development of microorganisms), for laboratory test.

Isolation and identification of the fungi

Seedborne fungi collected from rice sample were detected by Blotter method (Yu et al., 2015). Sterile blotter papers with 9 cm diameter were placed on the Petri dishes. 200 seeds were evaluated from each sample. 8 Petri dishes is used per sample. Twenty five seeds placed in each Petri dish in a radial manner on the blotter papers were incubated at 25°C for 24 h, of an alternating cycle light and darkness for 7 days. After 7 days, seeds were examined using a stereomicroscope to determine the presence or absence of fungal growth.

Fungal conidia and conidiomata were detected by light microscope. Various magnifications were used to identify conidia and mycelia produced by each group of fungi. Individual genus was classified to species level, using respective keys (Senbeta and Abdella, 2014).

The referenced two hundred seeds were used to determine the incidence of fungal microorganisms. Fungal species, found growing on the surface of seeds were identified and their incidence was calculated as:

$$\text{Incidence} = \frac{\text{No.of rice seeds on which fungus appear}}{\text{Total No.of seeds examined}} \times 100$$

Data analysis

Difference in the various samples, representing pathogens and types of sources of seeds (Farmer Saved Rice, certified and (QDS)) were established, through mean separation using Turkey's test after significant ANOVA results at P≤ 0.05. Correlation among the incidence of different microorganisms detected during health test was also analyzed using Viera et al. (2016) method.

RESULTS

Fungal contamination

Generally 13 fungal species comprising of 11 pathogenic and 2 saprophytic fungi were detected (Table 1). Mvomero District showed the highest fungal incidence recorded (64.5%), for sample collected from Hembeti and Dakawa/Msufini villages. In Kilombero, the fungal specie which detected the highest incidence was *Fusarium equiseti* (31%) followed by *Fusarium moniliforme* (28.5%). *Verticillium cinnabarinum* and *Curvularia inaequalis* accounted for 1% respectively (Table 1).

Fungal incidence of seeds infestation per district surveyed

Table 2 gives a summary of the fungal incidence of seeds infestation per district surveyed. Sample seeds observed were all infested with fungal at different level. 3000 rice seeds were analyzed from each district for the presence of, seed-borne microorganisms. Mvomero district recorded the highest percentage of contaminated seeds (16.36%), followed by Kilosa District (13.10%) and the lowest in Kilombero District (12.43%). The highest number of fungi species detected was 10, 9 and 8 in Kilosa, Kilombero and Mvomero, respectively.

Conidia of identified fungal species; the most prevalent species

Characteristics of the identified fungal species and conidia of few species are summarized in Table 2 and Plates 1 to 3. The characteristics generally ranged from, appearance of colony (colour or shape) and morphology of species structures under microscope. Plates 1, 2 and 3 further illustrate conidial characteristics detected.

Table 1. Fungi incidence in rice seed samples detected from Morogoro Region.

Sample no.	Variety	Seed category	Village	Fungal spp	No. of seed infested	Incidence (%)
Kilombero District						
N.1	TXD 306	QDS	Michanga	*Curvularia lunata*	31	15.5
N.2	TXD 306	FSS		*Fusarium moniliforme*	57	28.5
N.3	Mbawambili	FSS		*Fusarium pallidoroseum*	24	12
N.4	Mbawambili	FSS		*Verticillium cinnabarinum*	2	1
N.5	TXD 306	Certified 1		*Curvularia. inaequalis*	2	1
N.6	Mdomo wa fisi	FSS	Mbasa	*Alternaria. padwickii*	7	3.5
N.7	Dai pesa	FSS		*Alternaria alternate*	5	2.5
N.8	Mkia wa Fisi	FSS		*Fusarium equiseti*	62	31
N.9	Ngome	FSS		*Fusarium pallidoroseum*	29	14.5
N.10	Supa india	FSS		*Curvularia lunata*	41	20.5
N.11	Supa india	FSS	Idandu	*Fusarium pallidoroseum*	12	6
N.12	Supa Malolo	FSS		*Bipolaris oryzae*	26	13
N.13	Masamtula	FSS		*Curvularia lunata*	46	23
N.14	Supa india	FSS		*Exserohilum rostratum*	24	12
N.15	Kisegese	FSS		*Bipolaris oryzae*	24	12
				Total % sum		13.07
Kilosa District						
N.16	Supa india	FSS	Rudewa	*Fusarium moniliforme*	10	5
N.17	Mdomo wa fisi	FSS		*Bipolaris oryzae*	7	3.5
N.18	Supa india	FSS		*Curvularia inaequalis*	7	3.5
N.19	Mbawambili	FSS		*Alternaria alternate*	12	6
N. 20	Lawama	FSS		*Curvularia lunata*	17	8.5
N.21	Lawama	FSS	Kimamba- B	*Fusarium pallidoroseum*	15	7.5
N.22	TXD 306	FSS		*Alternaria alternata*	20	10
N.23	TXD 306	FSS		*Fusarium moniliforme*	13	6.5
N.24	TXD 306	QDS		*Alternaria padwickii*	12	6
N.25	Kabangala	FSS		*Fusarium moniliforme*	51	25.5
N.26	Masamtula	FSS	Chanzuru	*Aspergillus* spp.	12	6
N.27	Supa india	FSS		*Alternaria alternata*	4	2
N.28	Supa india	FSS		*Bipolaris oryzae*	2	1
N.29	Muunguja	FSS		*Exserohilum rostratum*	20	10
N.30	TXD 306	Certified 1		*Aspergillus* spp.	24	12
				Total % Sum		7.53
Mvomero District						
N.31	Shingoyamwaline	FSS	Wami	*Phoma* spp.	10	5
N.32	TXD 306	QDS		*Fusarium pallidoroseum*	22	11
N.33	Mbawambili	FSS		*Fusarium moniliforme*	22	11
N.34	Supa India	FSS		*Exserohilum rostratum*	10	5
N.35	Muunguja	FSS		*Curvularia lunata*	33	16.5
N.36	TXD 306	FSS		*Phoma* spp.	15	7.5
N.37	Supa Malolo	FSS	Hembeti	*Bipolaris oryzae*	6	3
N.38	Masamtula	FSS		*Alternaria padwickii*	12	6
N.39	TXD 306	FSS		*Curvularia lunata*	15	7.5
N.40	TXD 306	FSS		*Fusarium pallidoroseum*	25	12.5
N.41	TXD 306	FSS		*Fusarium moniliforme*	129	64.5
N.42	626	FSS	Dakawa/Msufini	*Curvularia lunata*	45	22.5
N.43	Domola-fisi	FSS		*Alternaria padwickii*	16	8
N.44	Shingoyamwalinene	FSS		*Exserohilum rostratum*	4	2
N.45	TXD 306	Certified 1		*F. pallidoroseum*	6	3
				Total % Sum		12.33

FSS = Farmer saved-seed; QDS = Quality declared seed.

Table 2. Summary of fungal species and their incidence on farmer saved rice seed collected from rice growing districts in Morogoro Region*.

District	No. of contaminated seeds	Seed contamination (%)	No. of detected species	Most prevalent species
Kilombero	373	12.43	9	*F. moniforme* (1) *C. lunata* (2) *F. pallidoroseum* (3)
Kilosa	393	13.10	10	*F. moniliforme* (1) *C. lunata* (2) *F. pallidoroseum* (3)
Mvomero	491	16.36	8	*F. moniliforme* (1) *C. lunata* (2) *F. pallidoroseum* (3)
Mean	419	13.96	9	

*The number of samples were 15 and the number of tested seeds by District were 3,000 in Kilombero, Kilosa and Mvomero. Number in bracket means the first (1), second (2) or third (3) most prevalent among samples from each districts.

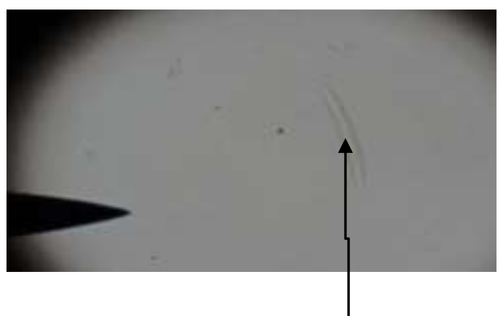

Plate 1. Conidia of Bipolaris oryzae under microscopy (× 750 mignification).

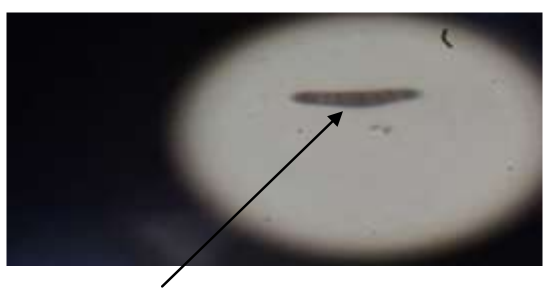

Plate 2. conidia of *Fusarium monliforme* under microscopy (× 40 magnification).

Correlation analysis

Correlation analysis coefficients among physical quality attributes and seedborne fungi incidences, were observed between *Curvularia lunata* and *F. moniliforme* (0.518**), *Fusarium pallidoroseum* (0.545**), *Alternaria padwickii* (0.526**) and *Exserohilum rostratum* (0.515**). Significant (0.01) correlation was observed between *Aspergillus* and *Phom*a (0.0498**), *Penicillium* (0.948**), and *Bipolaris* and *Exserohilum* (0.695**); *Phoma* and *Penicillium* were also positively correlated (0.532**). *F. pallidoroseum* positively correlated with *A. padwickii*

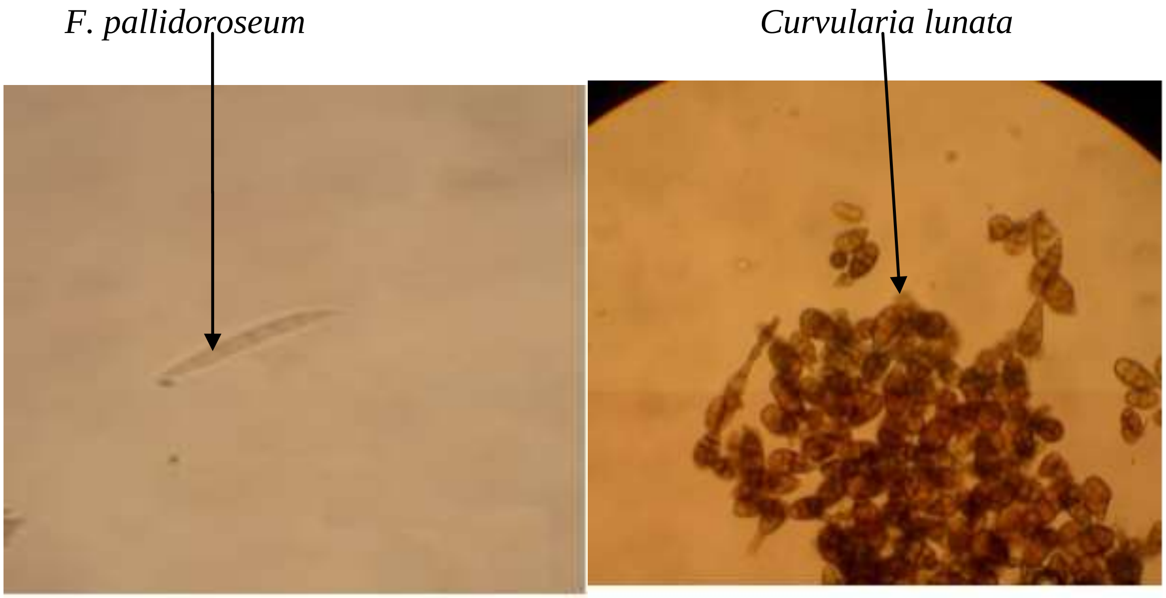

Plate 3. Conidia of *Fusarium pallidoroseum* and *Curvularia lunata* under microscopy (40 x magnifications).

(0.312**), *Alternaria alternata* (0.286**), *Fusarium equisti* (0.363**), *Bipolaris* (0.491**) and *Exserohilum* (0.297**).

Results from the current study also revealed significant correlation between seed physical quality and fungi incidences (Table 3). There was a positive correlation between germination and *C. lunata* (0.665**), *F. moniliforme* (0.468**), *F. pallidoroseum* (0.568**), *Aspergillus* (0.382**), *A. alternatka* (0.348**), *F. equisti* (0.188*), *Bipolaris* (0.287**), *Exserohilum* (0.299**) and *Penicillium* (0.198*).

Moisture content was also correlated with *F. pallidoroseum* (0.347*), *C. inaequalis* (0.353**), *Aspergillus* (0.360*) and *Penicillium* (0.35). Conversely, fungi incidence did not affect seed purity, with exception of *A. alternata*.

DISCUSSION

Seedborne fungi observed in this study are usually found in rice seeds, as compared to study conducted by Tokpah et al. (2017). In additional, Azam et al. (2012) reported a rate of 13 to 20% for *A. alternate*, 10 to 17% for *F. moniliforme* and 8.4% for *Curvularia* spp. in rice seeds. Sharma and Kapoor (2016) obtained similar results. In this study, there were many seedborne fungi in rice seeds, as compared to report of Wang et al. (2012).

The highest rate of incidences of seedborne fungi in rice seeds was found in Mvomero, followed by Kilosa. These districts have a wide range of seed varieties compared to Kilombero. Other comparable reasons might be storage practices and seed handing, which might lead to high incidence level. High incidences include *F. moniliforme* (65%) and *F. equiseti* (31%) with *C. lunata* (16.5, 20.5, and 23%) which may have justifications. It is also important that, these fungal species are commonly found in rice seeds worldwide despite the locations and climatic conditions (Azam et al., 2012).

Frequent occurrences of seedborne fungi in farmer's saved seeds were due to the fact that, most seeds are stored in the rooms which result to difficulty in temperature and humidity control. The environmental factors such as temperature, moisture and relative humidity affect the growth of seed-borne. Generally, high temperature of 15 to 30°C is required for growth and survival of many fungi in the seeds and high relative humidity of more than 65% is required for spore germination (Singh et al., 2012).

Seed-borne fungi such as *A. alternata, Bipolaris oryzae, F. moniliforme, A. padwickii, C. inaequalis, C. lunata, F. pallidoroseum, E. rostratum, F. equiseti, V. cinnabarinum, Phoma* species*, Aspergillus flavus, Penicillium* spp. and *F. moniliforme* have also been identified on rice seeds in other studies. In this research, *F. moniliforme*, *F. pallidoroseum* and *C. lunata* were the most important pathogens frequently detected in the rice seeds.

F. moniliforme, which causes Bakanae disease and Brown spot on rice seeds have negative impact on the quality of rice (Singh et al., 2012) and are commonly detected. Since farmers in the study area did not report these diseases as constraints, the species do not cause much yield loss in rice. It was reported that, Brown spot disease in rice cause losses of 3 to 15% of yield which is lower than loss caused by rodents and birds.

Owolade et al. (2011) determined that, cereal crops including rice seeds colonized by fungal species during storage were responsible for low germination and

Table 3. Correlation among physical quality attributes between these attributes and fungal incidences and among the fungi species.

Variable	CL	FM	FP	VL	CI	AP	AA	FE	BP	EX	AG	PH	PL	Germ	MC	P
C. lunata																
F. moniliforme	0.518**															
F. pallidoroseum	0.545**	0.358**														
V. cinnabarinum	0.133	0.106	0.182													
A. padwickii	0.526**	0.929**	0.312**	0.149	0.023											
A. altenata	0.213*	0.057	0.286**	-0.073	-0.091	-0.133										
F. quiseti	0.271**	0.227*	0.363**	0.610**	0.281**	0.281**	-0.072									
Bipolaris	0.469**	0.156	0.491**	-0.059	-0.073	0.173	0.126	-0.058								
Exserohilum	0.515**	0.200**	0.297**	-0.073	-0.092	0.081	0.042	-0.072	0.695**							
Aspergillus	-0.100	-0.031	-0.074	-0.048	-0.060	-0.087	-0.007	0.047	-0.050	0.160						
Phoma	0.135	-0.024	-0.161	-0.054	-0.068	-0.099	-0.010	-0.054	-0.081	0.213	0.0498**					
Penicillium	-0.130	-0.091	-0.115	-0.039	-0.048	-0.070	-0.072	-0.038	-0.058	-0.007	0.948**	0.532**				
Germination	0. 665**	-0.468**	0.568**	-0.040	-0.129	0.382**	0.348**	0.188*	0.287**	0.299**	-0.015	0.040	0.198*			
Moisture (mc)	-0.209*	0.161	0.347*	0.045	0.353**	-0.062	0.020	-0.004	-0.023	-0.201*	0.360**	0.113	0.354*	0.263**		
Purity	0.033	0.007	0.110	-0.046	0.079	0.090	0.217*	0.103	-0.074	-0.180	0.096	-0.035	-0.073	0.099	0.140	

*P ≥ 0.05 ** P ≥ 0.01 N = 108, CL = *Curvularia lunata,* FM = *Fusarium moniliforme,* FP = *Fusarium pallidoroseum,* VL = *Verticillium cinnabarinum,* CI = *Curvularia inaequalis,* AP = *Alternaria padwickii,* AA = *Alternaria alternata,* FE = *Fusarium equiseti,* BP = *Bipolaris oryzae,*EX = *Exserohilum rostratum,* AG = *Aspergillus niger,* PH = *Phoma* spp*.,* PL = *Penicillium*, Germ = Germination, MC = Moisture content.

reduction of plant population by 42% in the field. The consequence of such infestation is not only limited to yield losses, but also accounts for the build-up of mycotoxins in infected grains, which had negative impact on seed quality.

Generally, seedborne diseases affect the seed quality of any cultivar (Naqvi and Zeeshan, 2013). The findings of this study are therefore important as, they highlight the need for effective measures aimed at establishing the status of microbes in farm saved rice seed, outfits for quality assurance.

The significant relationship by rice seeds germination capacity with some fungal species such as *C. lunata, F. moniliforme, F. pallidoroseum, A. alternata, E. rostratum, F.equiseti* and *Penicillium* spp is differ. In previous research these fungal species have been observed to cause poor germination in rice, also in contrary, significant correlation between some fungi incidence and moisture content of seed were not the same.

Seed moisture content enhances fungal growth, which increases seed moisture content through microbial respiration. Ok et al. (2014) observed similar result in samples where; moisture content was high, the number of fungal species increased, and the increased number of fungal species was reciprocally responsible for increased moisture.

In this study, *C. lunata* and *E. rostratum* were not significant with moisture content which means that, fungi have more incidences of low moisture content. Perhaps, this implies the inability to compete with other fungi that would grow more where moisture content is higher. Ironically, negative correlation between incidences of different fungal species, though existed abundantly, was never statistically significant. This indicates insignificance of direct antagonistic effects of the different fungal species against each other.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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