

# Incidence of Seed-borne Fungi of Rice in Ghana and Antifungal Activity of Three Botanical Extracts

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## Abstract

*A questionnaire-based survey on rice farmers' source of seeds, cultivar planted, storage conditions and practices employed in the storage of rice seeds and pests and diseases encountered in storage was conducted in three selected rice-growing districts of Ashanti Region, Ghana. Seed-borne fungi associated with farmer-saved rice seeds were assessed. Again, the efficacy of ginger rhizome, lemon grass and chili pepper aqueous extracts (100% w/v), and Mancozeb (check) were evaluated against seven seed-borne fungal species of rice in-vitro. Sixty seed samples collected from the interviewed farmers were examined for the presence of seed-borne fungi using the blotter method. Seed sample with high incidence of fungal pathogens selected after seed health test was soaked separately in the aqueous extracts, Mancozeb and water for 24 h. The seeds were later plated on blotter papers in Petri dishes. From the survey, majority of smallholder rice farmers interviewed (71.7%) saved their own seeds for planting and only 18.4% of the farmers treated their seeds during storage and before sowing. Twenty seed-borne fungal species were recovered from the rice seed samples from the three districts, the major pathogenic ones including *Bipolaris oryzae*, *Fusarium spp.*, *Colletotrichum sp.*, *Curvularia spp.* and *Cercospora sp.* Soaking the seeds in aqueous lemon grass extract (100% w/v) was effective in managing the seven seed-borne fungal species when compared with the other aqueous extracts.*

**Keywords:** Botanicals, farmer-saved seeds, rice, seed-borne fungi, seed treatment

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## Introduction

Rice (*Oryza sativa* L.) is the second highest produced cereal grain globally after maize (Awika, 2011). It provides considerable calories of energy for many people than any other crop, accounting for 23 % of the world's supply of calories (FAO, 2005). It is estimated that Africa produced an average of 24 million metric tons of rice in 2020/2021 trade year (Faria, 2022). In Ghana, total domestic production of milled rice in 2017 was 460,000 metric tonnes and the production available for human consumption was 300,000 metric tonnes (SRID-MOFA, 2017).

Rice production, however, is constrained by several factors such as unavailability of quality improved seeds, lack of appropriate farming implements, pests and diseases. Diseases notably blast, brown spot, sheath blight and

bakanae reduce yields of rice significantly in major rice producing countries including Ghana (Savary *et al.*, 2000). Fungal diseases such as brown spot and blast, affecting rice are seed-borne and seed transmitted. It is reported that majority of rice farmers in Africa use their own saved seeds which may carry a number of seed-borne pathogens (Plantlet, 2019). In Ghana, transmission of diseases through seeds could be very high because most farmers use their own saved seeds for cultivation (Kormawa *et al.*, 2004). Thus contribute to high levels of diseases on the field with resultant high yield losses. High disease pressures and yield losses are common when farmers do not control diseases and also depend on their saved seeds for planting. Even though effective and efficient control of seed-borne fungi can be achieved by the use of synthetic chemical fungicides, the

same cannot be applied to grains for reasons of pesticide toxicity (Harris *et al.*, 2001). Chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon., 2005). It is therefore important to identify alternative means to manage seed-borne pathogens. The use of botanical materials has been reported to be nontoxic in the environment and cheap (Akunne *et al.*, 2013). Plant extracts have also been reported to possess a broad spectrum antifungal activity against plant pathogens and are successfully used to control diseases in plants (Okigbo and Nmeke, 2005; Okigbo and Emoghene, 2004). Ginger has been reported to contain biocompounds such as zingerone, shogaols and gingerols (Chrubasik *et al.*, 2005; Grzanna *et al.*, 2005), lemon grass; citronellol, citral and citronellal (Mukarram *et al.*, 2022) and pepper; capsaicin (Goci *et al.*, 2021; Buitimea-Cantua *et al.*, 2020). These botanicals have been found to be effective in the management of rice blast disease and other seed-borne pathogens such as *Bipolaris oryzae*, *Fusarium verticillioides* and *Curvularia lunata* (Tzortzakis and Economakis, 2007; Hasan *et al.*, 2005; Nguefack *et al.*, 2005).

The objectives of this study were (i) to document rice farmers' source of seeds, knowledge on rice cultivar planted, storage conditions and practices employed in the storage of their own saved seeds and pests and diseases encountered in storage (ii) identify seed-borne fungi associated with farmer-saved rice seeds and (iii) evaluate the effectiveness of ginger (*Zingiber officinale*), lemon grass (*Cymbopogon citratus*) and chili pepper (*Capsicum frutescens*) aqueous extracts in managing seed-borne fungal

pathogens of rice.

## Materials and methods

### Baseline survey

A survey was conducted to document rice farmers' source of seeds, rice cultivar planted, storage conditions and practices employed by these farmers and pests and diseases encountered in storage. It was carried out in nine (9) communities in three selected rice-growing districts in the Ashanti Region of Ghana using a well-structured questionnaire from June-September, 2011. Selected districts and the communities include; Ejisu Municipality: Besease, Ampabame and Nobewam; Ahafo-Ano South District: Camp, Barniekrom and Asuodei; and Afigya Kwabre District: Apropronso, Abankroso and Nyabesu Nkwanta. A total of 60 farmers, 20 from each district, were randomly selected and interviewed (Table 1). Response of the questionnaire on rice farmers' source of seeds, cultivar planted and storage conditions and practices employed in the storage of their own saved seeds were summarized in percentages and presented in pie charts, bar charts using Microsoft Excel and Tables.

### Collection of rice seed samples

A total of 60 farmer-saved rice seed samples were collected from the 60 interviewed farmers, 20 samples from each district. The sampled seeds were stored in brown envelopes, labelled, sealed and sent to the Plant Pathology Laboratory of CSIR-Crops Research Institute for seed health testing. The well-sealed rice samples were kept in a refrigerator before the health testing.

**Table 1: Districts, communities and number of samples collected**

District/Municipality	Community	Number of samples	Total
Ejisu	Nobewam	10	20
	Ampabame	2	
	Besease	8	
Ahafo-Ano South	Barniekrom	12	20
	Camp	5	
	Asuodei	3	
Afigya Kwabre	Nyabesu Nkwanta	5	20
	Abankroso	5	
	Apropronso	10	



seed-borne fungi identified based on the mycelia and shape and other characteristics of the conidia produced. Description book by Mathur and Kongsdal (2003) was used in the identification. The incidence of each fungus and percent germination of the seeds were recorded. The incidence of each fungus and percent germination of rice seeds were calculated using the formulae;

$$\text{Incidence of fungus} = \frac{\text{number of seeds infected by fungus}}{\text{total number of seeds tested}} \times 100$$

$$\text{Percent germination} = \frac{\text{number of germinated seeds}}{\text{total number of seeds tested}} \times 100$$

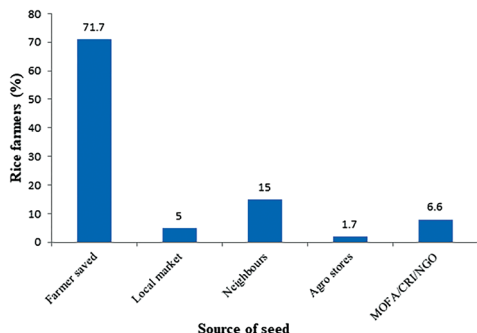
The data obtained on the evaluation of antifungal activity of aqueous botanical extracts against seed-borne fungi and rice seed germination were subjected to analysis of variance (ANOVA) using GenStat statistical package, edition 14.2. Differences in treatment means were compared for significance using the honest significance difference (HSD) at a probability level of 5 %. Count data  $\sqrt{(x + 0.5)}$  was transformed by the square root transformation before analysis.

**Results**

**Rice farmers’ source of seeds, cultivar planted, storage conditions and practices employed in the storage of farmer-saved rice seeds**

**Source of seeds utilized by smallholder rice farmers**

Majority of the farmers interviewed either

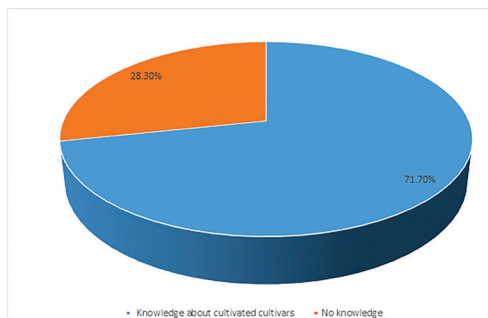


**Figure 2: Sources of seeds utilized by smallholder rice farmers in the three selected districts**

use their own saved seeds (71.7 %) or obtained seeds from friends (Fig. 2).

**Rice farmers’ knowledge on cultivar planted**

Majority of the farmers (71.7 %) knew the type of rice cultivar they planted, while 28.3 % did not know the names of the cultivar they used (Fig. 3). The most cultivated ones were Jasmine 85, Lapete and Sikamoo.



**Figure 3: Percent distribution of farmers’ knowledge on rice cultivars planted in the three selected districts in Ashanti Region**

**Storage practices and conditions employed by rice farmers in the three selected inland valley rice growing districts**

**Storage materials and environment used by rice farmers for storage**

Majority of rice farmers (53.3 %) in the three selected districts stored their rice seeds in nylon fertilizer sacks, followed by jute sack (43.3 %). The least used storage container was polythene bags (3.3 % of interviewed farmers). All the farmers kept their rice seeds in the various containers in their rooms under ambient environment (Table 2).

**Seed treatment before storage and sowing of farmer-saved rice seeds**

Majority of rice farmers (88.3 %) did not treat their rice seeds before storage. The few that treated their rice seeds used rodenticides during storage. Only 6.7 % of the farmers treated their saved seeds with fungicide (Mancozeb) before sowing (Table 3).

**Pests and diseases encountered by rice farmers in storage**

The rice farmers encountered pest problems

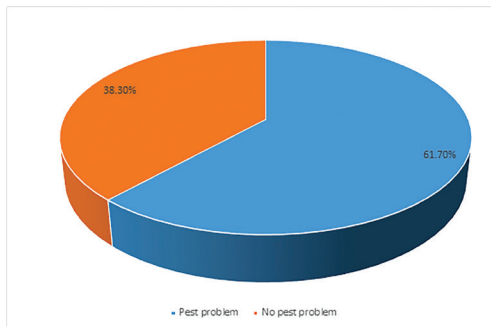
**Table 2: Storage materials and environment used by rice farmers in the three selected districts**

Storage container	Per cent farmers (n=60 farmers)	Storage condition
Nylon sacks	53.3	Ambient temperature (21-37°C)
Jute sacks	43.3	Ambient temperature (21-37°C)
Polythene bags	3.3	Ambient temperature (21-37°C)

**Table 3: Per cent farmers' seed treatment potential prior to storage and before sowing**

Treatment	% Rice farmers response (n = 60 farmers)	
	Prior to storage	Before sowing
No seed treatment	88.3	93.3
Seed treatment with pesticides	11.7	6.7

mostly such as grain weevils and mice in storage with 61.7 % of the respondent farmers encountering that problem. No farmer observed diseases of rice in storage (Fig. 4).

**Figure 4: Pests and diseases of rice in storage**

#### Identified seed-borne fungi on samples from the three selected districts

*Aspergillus flavus* recorded the highest fungal incidence (100%) followed by *Bipolaris oryzae* and *Fusarium verticillioides* identified on 95% each on the seed samples from Afigya-Kwabre District. *Fusarium pallidoroseum* and *Sarocladium oryzae* were the least fungi identified with an incidence of 5 % each.

With regards to samples from Ahafo-Ano South District, *Bipolaris oryzae* was the most predominant fungal pathogen associated with the seeds. *Alternaria padwiickii*, *Alternaria* sp., *Curvularia* sp., *Aspergillus niger* and *Stemphylium* sp. were the least identified, with an incidence of 5% each. *Rhizopus* sp. and *Aspergillus flavus* recorded the highest frequency of infection and were identified on 100% and 95% respectively on samples from Ejisu Municipality. *Fusarium solani* and *Phoma*

sp. were the least identified (Table 4).

#### Antifungal effect of botanical aqueous extracts on major seed-borne fungal pathogens

Seed treatment with aqueous lemon grass (*Cymbopogon citratus*) extract (100 % w/v) for 24 h compared favourably with Mancozeb (check) in the management of the major fungi identified on the rice seeds followed by ginger (*Zingiber officinale*) aqueous extract (100 % w/v) (Table 5).

#### Effect of botanical aqueous extracts on germination of rice seeds

Significant differences were observed between the other treatments and water (control). Mancozeb (check) produced the highest seed germination of 95%, which is significantly different ( $P < 0.05$ ) from the aqueous botanical extracts. This was followed by aqueous lemon grass extract (93%), aqueous ginger rhizome extract (88%) and aqueous hot pepper extract (86%) (Table 6).

#### Discussion

##### Source of seeds utilized by smallholder rice farmers

Majority of the rice farmers interviewed in this study used their own saved seeds to start their next season planting. This implies that farmer-saved seeds were the main source of seeds for rice farmers in Ashanti Region. This observation agrees with reports by Vo *et al.* (2001) that over 70% of rice farmers depend on their own saved seeds for the next season's planting.

In sub-Saharan Africa, it is established that

**Table 4: Incidence of fungi on rice seed samples from the three Districts**

Fungi recorded	Incidence of fungi (%)		
	Ahafo-Ano South District (n=20 samples)	Afigya-Kwabre District (n=20 samples)	Ejisu Municipal (n=20 samples)
<i>Alternaria padwickii</i>	5.0	0.0	0.0
<i>Alternaria</i> sp.	5.0	0.0	15.0
<i>Aspergillus flavus</i>	80.0	100.0	95.0
<i>Aspergillus niger</i>	5.0	0.0	15.0
<i>Bipolaris oryzae</i>	100.0	95.0	75.0
<i>Cercospora</i> sp.	60.0	60.0	60.0
<i>Colletotrichum</i> sp.	10.0	0.0	0.0
<i>Curvularia lunata</i>	70.0	40.0	50.0
<i>Curvularia pallescens</i>	30.0	0.0	10.0
<i>Curvularia</i> sp.	5.0	0.0	0.0
<i>Fusarium verticillioides</i>	90.0	95.0	55.0
<i>Fusarium pallidoroseum</i>	55.0	5.0	40.0
<i>Fusarium oxysporum</i>	75.0	15.0	15.0
<i>Fusarium solani</i>	10.0	0.0	5.0
<i>Nigrospora oryzae</i>	35.0	0.0	0.0
<i>Penicillium</i> sp.	30.0	50.0	25.0
<i>Sarocladium oryzae</i>	10.0	5.0	15.0
<i>Stemphylium</i> sp.	5.0	0.0	0.0
<i>Rhizopus</i> sp.	55.0	85.0	100.0
<i>Phoma</i> sp.	0.0	10.0	5.0

**Table 5: Seed treatment effect on major seed-borne fungi after soaking for 24 h**

Seed treatment	Incidence of seed-borne fungi (%)						
	<i>A. flavus</i>	<i>A. niger</i>	<i>B. oryzae</i>	<i>Cercospora</i> sp.	<i>C. lunata</i>	<i>F. verticillioides</i>	<i>Rhizopus</i> sp.
Ginger	1.86c	0.71c	3.08b	0.71b	0.71c	1.86b	0.71c
Pepper	8.54b	8.83b	1.00d	0.71b	1.22b	1.57c	3.94b
Lemon grass	1.22d	0.71c	1.73c	0.71b	0.71c	1.22d	0.71c
Mancozeb	1.00d	0.71c	0.71e	0.71b	0.71c	0.71e	0.71c
Water (control)	10.51a	9.95a	4.51a	1.7a	1.73a	2.36a	5.64a
Lsd (5%)	0.22	0.03	0.04	0.01	0.03	0.20	0.20
CV (%)	3.10	0.58	2.23	0.71	1.80	10.30	9.58

Values with different letters are significantly different at  $p < 0.05$ .

most farmers do not buy seeds. They save their own or trade with other farmers (Anon, 2001; Almekinders *et al.*, 1994). Farmers depend on their own saved seeds because they do not have access to certified seeds, or when certified seeds

are available the prices are too high for the small-scale farmer to afford.

#### **Rice farmers' knowledge on cultivars cultivated**

Majority (71.7 %) of rice farmers in the

**Table 6: Seed treatment effect on germination of rice seeds**

Treatments	Percentage seed germination
Mancozeb	95 <sup>a</sup>
Lemon grass	93 <sup>b</sup>
Ginger	88 <sup>c</sup>
Hot pepper	86 <sup>d</sup>
Water (control)	85 <sup>d</sup>
Lsd (5%)	1.2
CV (%)	0.9

Values with different letters are significantly different at  $p < 0.05$ .

selected districts knew the name of cultivar they planted, while 28.3 % did not have any knowledge on their rice cultivar. Some of the cultivars mentioned were Jasmine 85, Sikamoo, Lapete, Uncle Bens and Vita 7 but the most cultivated were Jasmine 85, Lapete and Sikamoo. According to the farmers, Jasmine 85 and Lapete were long grain, early maturing, have good aroma and marketable while Sikamoo is a short grain local cultivar but was resistant to harsh conditions, pests and diseases. These findings may therefore, validate the reason why most smallholder farmers find it difficult in adopting new varieties as was reported by Ifie, *et al.*, (2022).

#### Seed storage practices and conditions adopted by rice farmers

Most farmers interviewed stored their seeds in nylon sacks and kept in their homes under ambient environmental conditions (21-37°C). However, storage under ambient conditions has been observed to affect seed quality and germination. Ashish *et al.* (2018) reported that farmers in the developing world store their produce, including seed, under ambient environment. A good number of containers are used for storing seeds but their suitability depends on the kind or type of seed and the protection the container can offer the seed in storage. However, rice seed is hygroscopic and would develop equilibrium moisture content with that of the physical environment where it is placed (Copeland and McDonald, 2001). Juliano *et al.* (1990) reported that rice

attained equilibrium moisture content with the environment when stored under ambient conditions.

#### Seed health status of farmer-saved rice seeds

The present study revealed that none of the seed samples was free from seed-borne fungal pathogens. The detection of different fungal pathogens agrees with Javaid *et al.* (2002) and Wahid *et al.* (2001) who reported the presence of different fungi on rice seeds. *Bipolaris oryzae* was the most predominant fungus identified on the rice seeds. This pathogen causes brown spot disease. Gerken *et al.* (2001) reported brown spot disease as one of the serious constraints to rice production in Ghana. Brown spot is one of the important diseases of rice capable of causing losses of 3–15 % (Savary *et al.*, 2000). Its high incidence of 75–100 % in the districts is of great concern. *Fusarium verticillioides* can cause bakanae disease in the sub-tropical regions of the world (Bashyal *et al.*, 2016). Educating farmers to adopt better seed treatment practices is necessary to sustain yields of farmers. Controlling the pathogenic and saprophytic seed-borne fungi would go a long way to increase plant establishment during planting and thus contribute to increase in yields.

#### Bio-efficacy tests of ginger, lemon grass and chili pepper aqueous extract

Conducting experiments to develop appropriate and environmentally friendly ways of controlling seed-borne pathogens, aqueous extract of lemon grass (100% w/v) was as effective as Mancozeb in controlling the major seed-borne fungi identified on the rice samples. This was followed by ginger rhizome aqueous extract (100% w/v). Mohamed *et al.* (2006) reported lemon grass extract to be among the most significant of the newly uncovered, nontoxic therapies and one of the most useful antimicrobial agents successfully used for the treatment of all kinds of infections arising from fungi, viruses, bacteria, parasites and other microscopic invaders. Aqueous extract of lemon grass completely reduced seed infection by *Macrophomina phaseolina*, *Fusarium verticillioides* and *Botryodiplodia theobromae*

(Bankole and Adebajo, 1995). Cold water extract of lemon grass checked the spread of anthracnose of cowpea (Amadioha and Obi, 1999). Somda *et al.* (2007) also found that lemon grass extract reduced *Fusarium verticillioides* infection in sorghum. Lemon grass was also reported to possess antifungal activity capable of controlling postharvest pathogens (Tzortzakis and Economakis, 2007).

The antimicrobial properties of ginger have been widely studied and reported (Hasan *et al.*, 2005). Ginger is known to have analgesic, sedative and antimicrobial effect (Hibert, 2006). Ginger showed inhibitory effects on *Aspergillus niger*, *Rhizopus* sp. and *Penicillium* spp. on seeds of different sunflower cultivars (Afzal *et al.*, 2010).

The ability of ginger to control seed-borne pathogens was due to the fact that it contains 400 different compounds, a mixture of both volatile and non-volatile chemical constituents such as zingerone, shogaols and gingerols, sesquiterpenoids ( $\beta$ -sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction ( $\beta$ -phelladrene, cineol and citral) (Chrubasik *et al.*, 2005; Grzanna *et al.*, 2005). These several chemical constituents increase its antimicrobial effectiveness. This antimicrobial activity was believed to be attributed to the major compounds in zingiberene, and their activity could be multiple (Singh *et al.*, 2008).

Various reports are available on the antimicrobial property of the rhizomes of ginger (Habsah *et al.*, 2000) and aqueous extract from ginger was studied for antimicrobial activity against *Aspergillus niger*, *Saccharomyces cerevisiae*, *Trichoderma* sp., *Lactobacillus acidophilus* and *Bacillus cereus*.

Aqueous extract of pepper was effective against the pathogenic fungi identified in this study than the saprophytic fungi. A number of reports exist that indicate the effectiveness of aqueous pepper extract against pathogens of humans and plants. Al-Delaimy (1999), reported that the antimicrobial component present in pepper is prescribed for diseases such as diarrhoea and cholera. Pepper has antimicrobial property of actively preventing bacteria such as *E. coli* (Arum, 2001). Pepper extracts have

been shown to reduce aflatoxin production in *Aspergillus parasiticus* IFO 30179 and *Aspergillus flavus* var *columnaris* S46 (Ito *et al.*, 1994).

### Effect of seed treatment on germination of rice seeds

The use of infected seeds without any seed treatment against infecting pathogens is often responsible for the decrease in seedling emergence and germination (Hofs *et al.*, 2004). Low seed quality is a serious problem in crop production especially in most developing countries.

Results of this study revealed that all the treatments improved seed germination. The improvement in seed germination by lemon grass and ginger rhizome aqueous extracts could be attributed to the suppression of the incidence of the seed-borne fungi. This agrees with that of Parimelazhagan and Francis (1999) who established that leaf extracts of *Clerodendrum viscosum* (Vent.) reduced the incidence of seed-borne fungi and improved germination and seedling development of rice seeds. These results strongly suggest seed treatment before planting to escape the infection by seed/soil borne fungal pathogens of seedlings.

### Conclusion

Most farmers utilized their own saved seeds for planting and only few treated their seeds before planting. Twenty (20) seed-borne fungal species were identified from rice seed samples from the three districts. These were *Alternaria padwickii*, *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris oryzae*, *Cercospora* sp., *Colletotrichum* sp., *Curvularia lunata*, *Curvularia pallescens*, *Curvularia* sp., *Fusarium verticillioides*, *Fusarium pallidoroseum*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium* sp., *Phoma* sp., *Rhizopus* sp., *Sarocladium oryzae*, *Nigrospora oryzae* and *Stemphylium* sp. This study also showed a high potential of botanical extracts, especially lemon grass and ginger in managing seed-borne fungi of seeds. The detection of high number of pathogenic fungal species on the seed samples calls for sensitization of smallholder farmers on the need to treat their seeds before sowing.



Again certified seeds should be affordable and readily available to the smallholder farmers to limit the use of own saved seeds for planting.

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