Antifungal activity of extracts of *Ocimum gratissimum* and *Aframomum danielli* against moulds isolated from stored rice

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Abstract

The fungitoxic effect of extracts of *Ocimum gratissimum* and *Aframomum danielli* on some moulds isolated from rice grains were determined *in vitro*. Aqueous extracts of *Aframomum danielli* inhibited the radial growth of the moulds at different levels between 46.4 - 56.7%. *Aspergillus niger* (56.7%) was the most sensitive to *Aframomum danielli* while *Cladosporium sphaerospermum* (46.4%) was the least sensitive. *Ocimum gratissimum* extract inhibited the radial growth of the moulds between 46.4 – 59.7% with *Penicillium citrinum* showing the highest sensitivity and *C. sphaerospermum* being the least sensitive. There was no significant difference ($p>0.05$) in the effect of *Ocimum gratissimum* and *Aframomum danielli* on all the moulds. *Ocimum gratissimum* showed the greater antifungal activity against the storage fungi (mean = 53.4%) compared to *Aframomum danielli* (mean = 51.9%). However, there was no significant difference ($p>0.05$) in the effect of *Ocimum gratissimum* and *Aframomum danielli* on the storage fungi. Both plant products showed varying levels of fungitoxic activities and could be potentially used in the storage of *Ofada* and *Abakaliki* rice against moulds.

Keywords: Plant extracts, Antifungal activity, Storage fungi, Moulds, Rice,

1. Introduction

Rice (*Oryza sativa* L.) is a cereal grain that meets many nutritional needs. Rice is a major source of income and nutrition in many developing countries where there is insufficient food supply (Janick, 2002). Rice is a predominant staple food in about eight African countries including Nigeria (FAO, 2004). Although the largest producer of rice in West Africa, the yield of rice in Nigeria is still very low and is reducing due to the problems of storage (WARDA, 2002). Storage of rice is done traditionally in baskets, sacks and bamboo-made structures and this exposes rice to many pests, parasites and microorganism causing the loss of a considerable proportion of stored grains. Fungi have been found to be one of the principal causes of grain loss and deterioration (Greer, 1990). The presence of moulds in stored grains may lead to various forms of deterioration (Aboaba and Amasike, 1991), decreased nutritive value (Maxiya-Dixon, 2004) and mycotoxin production (Bankole et al., 1999).

Over the years, control of pathogenic fungi in foods have drawn considerable attention with the use of industrial chemicals such as propionic acid and ammonia in the storage of grains against fungal attack (Frazier and Westhoff, 1998). These chemicals have shown to be effective in preventing fungal growth. However, when they are concentrated on the grains there could induce chemical poisoning, environmental toxicity and development of resistance by fungi to the chemical agent. Some tropical aromatic plants have shown to exert high antimicrobial activities and since they are natural products, mostly consumed by man, there is little or no fear of poisoning even at very high concentrations (Adegoke et al., 2002). Some of these plants include *Azadirachta indica* (neem) (Bankole and Adebanjo, 1995), *Cymbopogon citratus* (Bankole et al., 2005) and *Thymus vulgaris* (Ngueface et al., 2004).

Ocimum gratissimum L. has been shown to have several medicinal uses (Ojeifo and Denton, 1993). Leaves of *O. gratissimum* have been found to exhibit high antifungal activities against *Fusarium moniliforme*, *Aspergillus flavus* and *Aspergillus fumigatus* (Ngueface et al., 2004) and based on convincing *in vitro* evidence, it is said to be a potential food preservative (Tagne et al., 2000). *Aframomum danielli* has been reported to show antimicrobial activities and also to reduce aflatoxin concentration in maize (Adegoke et al., 2000a, b; Ikehorah and Okoye, 2005). It has also been used in the storage of maize and soybean (Adegoke et al., 2002). The present study assesses the fungitoxic activity

of *O. gratissimum* and *A. danielli* on some moulds isolated from stored rice grains *in vitro*. This is to serve as an indicator of the potential of these natural plant products for use in the storage of rice.

2. Materials and methods

2.1. Collection of plant materials

Seeds of *A. danielli* and leaves of *O. gratissimum* were collected from Ibadan, Nigeria. Botanists in the Department of Plant Science and Applied Zoology of the Olabisi Onabanjo University, Ago-Iwoye, Nigeria confirmed the identity of the plant materials. The plant materials were air-dried under the shade at 25-29ºC until they became dry and crispy after five days. The dried leaves of *Ocimum gratissimum* were ground using a vegetable blender and sieved to remove coarse particles. Also, the dried seeds of *A. danielli* were grounded into powder using a sterile mortar and pestle.

2.2. Extraction

The powder of both plants was extracted as follows: 250 g of the powdered leaves were put in separate round bottom flasks. One L of sterile distilled water was added into each flask, covered with aluminum foil and allowed to stand for about five days. The mixture was thoroughly shaken and filtered.

2.3. Concentration

The filtrate was then concentrated by heating over a water bath until all the moisture had evaporated. The crude extract was then obtained in a beaker. The crude extracts of both plants were kept in the refrigerator at 4ºC until it was used.

2.4. Re-propagation of fungal isolates

Fungal isolates from *Ofada* and *Abakaliki* rice preserved at the culture bank of the Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria were used for the study. A sterile needle was used to pick spores of the stock culture of each organism and inoculated on a fresh Potato Dextrose Agar (PDA) plate and incubated for seven days. Sterile cork borer was used to make discs on the culture of each isolate. The mycelial discs were then used to inoculate fresh PDA plates. The plates were incubated at 28°C for seven days. The radial growth of each isolate was recorded at 24 hours interval for the seven days period.

2.5. Bioassay

The antifungal property of the extracts on the isolates was determined using the procedure described by Tagne et al. (2000). The crude extracts were diluted with distilled water at 5:10 w/v. Five mL of each extract solution was dispensed into 10 mL molten agar and poured into sterile Petri dishes. The agar was then allowed to solidify. Sterile cork borers (6 mm diameter) were used to cut each isolate culture which was about one week old. Mycelial disc of each isolate was inoculated on separate plates. The plates were then incubated at 28°C for seven days. The radial growth of each isolate was measured at 24 h interval for the seven-day period. Two controls were set up, one with chloramphenicol and the other with PDA only. The inhibition was calculated as percentage of the difference between the radial growth of the isolate when inhibited and the radial growth when uninhibited, for each isolate. The percentage of inhibition (PI) for radial growth was calculated

$$PI = \frac{(a - b)}{a} \times 100$$

a - radial growth when uninhibited
b - radial growth when inhibited

3. Results and discussion

Both extracts of *O. gratissimum* and *A. danielli* inhibited the radial growth of the fungi on PDA after seven days of incubation at different levels. The extracts of *A. danielli* exhibited the highest activity on *Aspergillus niger* (56.7%) while the lowest activity was observed on *C. sphaerospermum* (46.4%) (Table 1). This result is similar to a study by Adegoke et al. (2000b) who reported that *A. danielli* inhibited the growth of *A. flavus*, *A. fumigatus* and some other food spoilage yeasts. Lipid extracts of the spice was also reported to reduce aflatoxin B_1 in maize even at concentration as low as 50 ppb (Ikheorah and Okoye, 2005).

Table 1 Percentage Inhibition of moulds by extract of *Aframomum danielli.*

Fungi Type	Isolates	Radial growth (mm) * a	Radial growth (mm) * b	Percentage inhibition (%)
Storage	*Aspergillus flavus*	72	38	47.2
	Aspergillus niger	83	36	56.7
	Aspergillus tamarii	80	35	56.3
	Penicillium citrinum	72	35	51.4
	Penicillium oxalicum	75	37	50.7
	Rhizopus nigricans	74	35	52.7
	Rhizopus oryzae	66	34	48.5
	Mean			51.9
Field	*Cladosporium sphaerospermum*	69	37	46.4
	Fusarium compacticum	77	35	54.5
	Fusarium oxysporum	78	38	51.3
	Fusarium proliferatum	80	35	56.3
	Mean			52.1

* - values given are mean values of triplicates; a - radial growth when uninhibited; b - radial growth when inhibited

The aqueous extracts of *O. gratissimum* showed its highest antifungal activity on *Penicillium citrinum* (59.7%) while it was least on *C. sphaerospermum* (46.4%) (Table 2). This is similar to the report by Tagne et al. (2000) that essential oils of *O. gratissimum* at 10 ppm inhibited the growth of *Fusarium moniliforme* whereas at 200 ppm, the growth was completely inhibited. In another study by Nguefack et al. (2004) essential oils of *O. gratissimum* at 800 ppm completely inhibited the growth of *F. moniliforme, A. flavus* and *A. fumigatus*.

Table 2 Percentage Inhibition of moulds by extract of *Ocimum gratissimum.*

Fungi Type	Isolates	Radial growth (mm) *a	Radial growth (mm) * b	Percentage inhibition (%)
Storage	*Aspergillus flavus*	72	36	50.0
	Aspergillus niger	83	39	56.0
	Aspergillus tamarii	80	35	56.3
	Penicillium citrinum	72	29	59.7
	Penicillium oxalicum	75	37	50.7
	Rhizopus nigricans	74	35	52.7
	Rhizopus oryzae	66	34	48.5
	Mean			53.4
Field	*Cladosporium sphaerospermum*	69	37	46.4
	Fusarium compacticum	77	35	54.5
	Fusarium oxysporum	78	38	51.3
	Fusarium proliferatum	80	35	56.3
	Mean			52.1

* - values given are mean values of triplicates; a - radial growth when uninhibited; b- radial growth when inhibited

From the result of this study, there is a great similarity in the activities of *A. danielli* and *O. gratissimum* extracts, and there was no significant difference ($p > 0.05$) in the activities of the two extracts on the radial growth of the moulds. Though the aqueous extracts inhibited the growth of the fungi, there are usually greater efficacies recorded when essential oils are used. It could hence be thought that the active ingredients causing the inhibition of growth are concentrated in the essential oil. Based on the fungitoxic activity observed in this study, *A. danielli* and *O. gratissimum* can be potentially used in the protection of rice grains against storage fungi. Although *A. danielli* has already been used successfully to preserve maize and soybean in storage (Adegoke et al., 2002), *O. gratissimum* had more inhibitory effects on the storage fungi used in this study (53.4%) compared to *A. danielli* (51.9%) thus suggesting that *O. gratissimum* could have more potential in the control of moulds in stored rice.

4. Conclusion

Natural plant products which have shown to be useful in protecting agricultural commodities against fungal infection and consequent mycotoxin production were shown to retard fungal growth in this study. If these plants are used in the storage of rice, they could reduce the loss of grains in storage and also the consumption of mycotoxin-contaminated foods especially in populations where grains constitute a major portion of the diet. The use of natural plant products in storage will also eliminate the problem of chemical poisoning that could arise from use of synthetic chemicals in the storage of grains. However, the best form of the plant product which could offer the best protection against moulds should be determined and exploited in the storage of agricultural produce.

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