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FUNGI ASSOCIATED WITH STRORED RICE GRAINS AND THEIR IMPLICATIONS

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ABSTRACT

Studies were carried out to isolate and identify the fungi associated with unshelled and par-boiled rice grains in storage. Aspergillus niger, Aspergillus flavus, Rhizopus oryza, Thamnidium elegans and Penicillium notatum were isolated. Germination of rice was drastically affected by R. oryza and A flavus. The implications of these fungi on rice grains were discussed.

INTRODUCTION

Rice is one of the most staple food worldwide and it provides nourishment for a large number of people (Purseglove, 1972). It consists of a relatively large number of species. A large quantity is produced in Nigeria and it is consumed in all parts of Nigeria (Broadbent, 1971). Variety of dishes are obtained from rice in Nigeria. It is also used as supplement in livestock feed (Purseglove, 1972).

A large number of fungi are known to cause postharvest spoilage in stored grains (Christensen and Kaufman, 1965). The fungi implicated by the report include Aspergillus sp. Mucor sp., Rhizopus sp., Penicillium sp. Monilia sp., and Oidium sp. Christitensen (1974) reported Aspergillis niger, A. glaucus and A terrus to be very common on rice. Several species. of Penicillium and Aspergillus have been isolated from rice imported into Japan (Hirayama and Udagawa, 1958).

Two major conditions have been identified to promote deterioration of stored grains. These include inadequate drying and large number of insects. Normally, fungal attack is prevalent when the moisture content is above 70% relative humidity. Due to these reasons, a very good proportion of rice is yearly lost to fungal attack. The unshelled (husked) rice is normally kept for planting in the subsequent season.

In Nigeria, fungi associated with rice (parboiled and husked) have been given attention, but not as extensive as necessary, considering the importance of rice in the nutrition of several people in Nigeria. Oyeniran (1973) reported Mucor sp., Aspergillus sp. and Rhizopus sp. as the most frequently associated fungi with parboiled rice grains. Little attention has been given to fungal attack on husked rice grains, which are normally stored for propagation.

The storage fungi destroy both the shelled and unshelled grains. Since both the shelled and unshelled grains are of considerable economic importance, destruction and spoilage of these grains affect both man and other animals. Hence, the aim of this study was to isolate and study the fungal organisms associated with shelled and husked rice grains in storage; and also study the effect of these organisms on the germination of husk rice grain.

MATERIALS AND METHOD

(i. Sterilization of Glassware: All glassware were cleaned and drip dry; and they were wrapped in aluminum foil before they were sterilized. Sterilization was carried out inside an oven at 160°C for two hours. After that, the glassware were cooled before use.

(ii. Media: Four media were used to carry out the isolation. These include Cornmeal Agar (CMA); Malt extract Agar (MEA); Water Agar (WA); and Carrot extract Agar (CEA). CAM and MEA were prepared according to the instruction of the manufacturers. However, WA and CEA were formulated during the experiment. In case of CEA, a fresh and healthy sample (200g) of carrot was weighed and cut into

small pieces and washed in sterile distilled water. The sample was boiled in a beaker containing 400 ml of distilled water to softness. The carrot extract was filtered using filter paper. Another 600 ml of sterile distilled water was added to the filtrate. Then 2% w/v agar-agar was added to the filtrate, and sterilized at 121°C for 15 minutes. This was dispensed into Petri-dishes for further use. In case of WA, a portion (2% w/v) of Plain agar was prepared and also sterilized like CEA.

(iii. Collection of rice grain: Par-boiled and unshelled (husked) rice grains were used for experiment. The par-boiled rice were collected from four markets in Lagos: Alaba, Ojo; Alaba-Suru; Ijesatedo and Daleko markets. The husked rice were obtained from Ifo market in Ogun state and from Alaba-Ojo market in Lagos State. Each sample was collected into separate sterile polyethylene bag.

(iv. Isolation: The grains showing symptoms of damage were selected from the physically healthy grains. These were disinfected with 0.02% mercuric chloride for 60 seconds and they were rinsed instantly with two changes of sterile distilled water. Some grains (14) of the unshelled rice were carefully place in Petri-plates of CMA, WA and CEA. Another set of par-boiled grains of rice were similarly treated. All the Pert-plates and their contents were incubated at 28± 2°C and were observed daily for any fungi growth.

Purification of all isolates was carried out and microscopic examination was carried out for each of the pure isolate. Reference was made to various mycological textbook (Ingold, 1973; Alexopoulos, 1979; and Wheeler, 1964) for identification.

(v. Pathogenicity test: Pathogenicity test was carried using wounded husked grains, unwounded husked grains and par-boiled grains. They were physically examined and selected to be free from fungal particles. The selected grains were surface-disinfected aseptically with 0.02% mercuric chloride solution for 60 seconds. Each set of the grain (20) was selected for each Petri-plate of the isolate. These grains were aseptically placed inside each Petri-plate of the isolate. All plates were incubated at 28±2°C for 24 hours, after which the rice grains in each Petri-plate were collectively removed and placed aseptically onto water agar and incubated at 28±20°C for seven days.

(vi. Germination test: Only the husked unwounded grains were used for this investigation. A quantity of rice grains were surface-disinfected as done previously, and were dispensed into each of the Petri-plates containing fungal isolate at the rate of 20 grains per Petri-plate of fungal isolate. A control was set-up using plain agar. All plates were kept at 28±2°C for twenty-four hours after which seeds in each plates were removed aseptically onto water agar. The Petri-plates were incubated at 28±2°C for seven days.

RESULTS

A total of five fungi were isolated from stored rice grains. Two of the fungi belong to the genus Aspergillus. However, the other three belong to other genera. Fungi isolated include: Aspergillus niger, Aspergillus flavus, Rhizopus oryza, Thamnidium elegans. and Penicillium notatum. All the five fungi were found to be pathogenic on wounded and unwounded husked seeds. Par-boiled rice grains were also infected by these fungi (Table 1).

The germination experiment showed that this process was greatly affected as a result of infection (Table 2). Seeds infected with A. niger, Penicillium notatum and Thamnidium sp. The control seeds gave about 80% germination. The data obtained were subjected to analysis of variance and the observed differences in percentage germination were found to be significant.

TABLE 1: PATHOGENICITY OF ISOLATED FUNGI

		Aspergillus niger	Aspergillus flavus	Rhizopus oryza	Thamnidium elegans	Penicillium notatum
1.	Unwounded seeds	+ + +	+ + +	+ + +	+ + +	+ + +
2.	Wounded Seeds	+ +	+ + +	+ +	+ +	+ + +
3.	Parboiled grains	+ + +	+ + +	+ +	+ +	+ + +

+ + + *Highly pathogenic*
+ + *Slightly pathogenic*

TABLE 2: PERCENTAGE GERMINATION OF INFECTED RICE SEEDS AFTER SEVEN DAYS

Organisms	Percentage germination
A. niger	21±5
A. flavus	0.0
R. oryza	0.0
T. elegans	8.5±2
P. notatum	20±4
Control	76±5

Each figure is a mean of five determinations.

DISCUSSION

The result of this investigation revealed Aspergillus sp as the most common fungi associated with rice, especially the husked grains. They are equally found in parboiled rice grains. Oyeniran (1973) in a similar investigation also reported Aspergillus sp. to be among the common fungi that attack grains in storage. Two fungal isolates, Penicillium notatum and Thamnidium elegans were also isolated. Previous reports (Christensen and Kaufmann, 1969; Hirayama and Udagawa, 1958; and Jay, 1992) have also implicated Penicillium as a common mould of grain in storage.

The result obtained also revealed that all rice grains i.e. both husked and parboiled were also vulnerable to attack by moulds. The nutritional content of rice would make rice seeds to be ready habitat for moulds. Traditional parboiling methods could be a reason for the presence of these moulds on the parboiled gains. The parboiled rice is often consumed by direct boiling, but the concurrent development of mycotoxins as a result of parboiling processes will make the grains hazardous to man and livestock.

The germination test carried out revealed that these fungal pathogens had profound effect on the germination of husked rice seeds. Delayed germination was observed in seeds infected with T. elegans and P. notatum and

total inhibition observed in respect of seeds infected with A. Flarus and R. Oryza. This observation could be due to production of metabolites that might have disturbed the processes associated with germination. Perhaps, destruction of the embryo might have occurred as a result of infection.

Apart from mycotoxin production that are often developed during storage, other various forms of spoilage such as discoloration and development of unpleasant odour and flavour are often the norm. Some of these spoilage characters are often aggravated in storage.

Most of the fungal problems of rice often encountered in storage have their origin prior to storage. Thus, proper hygienic handling precautions must be adhered to, in order to minimize the incidence of fungi in rice storage.

REFERENCES

Anon (1978) Postharvest food losses in developing countries. National Academy of Science. Washington. 45-49.

Alexopoulos, C. J. (1979) Introduction to Mycology John Wiley & Sons, London 613 pp.

Broadbent, J. A. (1971) Microbial determination of food stuff during storage. Paper presented at seminar on Grain Storage in humid tropics" University of Ibadan. 23pp.

Christensen, C.M. and H. H. Kaufmann (1969) Grain Storage: The role of fungi in Quality loss. University of Minnesota Press. Minneapolis Minnesota 153 pp.

Christensen, C. M. (1974) Storage of Cereal grains and their product. America Association of Cereal Chemist. St. Paul Minnesota 154 pp.

Hırayama, S. And Udagawa (1958) Tazonomic Studies of fungi on stored rice grains. Jaurnal. Jap. (Bot. 47:290 – 305.

Ingold, C. T. (1973) The biology of fungi ELBS Books, London 227 pp.

Jay, J. M. (1992) Modern Food Microbiology Chapman and Hill, New York 701 pp.

Oyenira, J. O. (1973) The Mycoflora of market rice in Ibadan: A preliminary report on deterioration of rice grain. Rep. Nig. Stored Prod. Res. Inst. (Technical Report).

Purseglove, J. W. (1972) Tropical Crops Dicotyledon I. London Co. Ltd. London pg. 160 – 197.

Wheller, B. E. J. (1964) An Introduction to Plant Diseases. John Wiley & Sons Ltd. Survey 374 pp.