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ELIMINATING SEEDBORNE FUNGI OF MONODORA *MYRISTICA* (AFRICAN NUTMEG) USED IN SOUTHERN NIGERIA.

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ABSTRACT

Seedborne fungi of shelled seeds of the African nutmeg *Monodora myristica*, "Ehuru" were isolated and identified using the standard blotter method. These included *Aspergillus flavus, A. niger, A. tamarii, Alternaria terreus, Fusarium oxysporium, F. semitectum, Curvularia lunata, Trichoderma vivide, Phoma sp, Rhizopus stolonifer, Botryobiplodia theobroneae, Penicillium oxalicum.* *Monodora* seeds from Yenagoa (Bayelsa) and Port Harcourt (Rivers) states had the highest percentage occurrence of fungi (47.6% and 46.1%), respectively. Those from Akure (Ondo State) and Calabar (Cross Rivers State) gave the least (20.1% and 21.2% respectively). The most frequently occurring fungus was *A. flavus* (71.3%), while the least was *R. stolonifer* (9.7%). Seeds of *Monodara* from Lagos State harboured 98.4% *T. vivide* but totally absent in seeds from other states like Anambra and Ondo, respectively. Disinfection of shelled seeds with hot water at 100°C, 1% chlorox, or 5% Brine solution for 5 minutes each and grinding into powder all reduced the percentage of fungal species recovered after treatment compared with the untreated seeds. Roasting of seeds in fire before grinding into powder totally eliminated all fungal species from treated seeds.

Key words: Seed, fungi, African nutmeg, elimination

INTRODUCTION

Monodara myristica (Gaertn) Dunal-FTA, otherwise called African nutmeg, commonly known as "Ehuru" (Ibo), has a round or oblong smooth shiny seed with a hard brownish coat and belongs to the family *Annonaceae*. The seed is variously used in different parts of Southern Nigeria in flavouring foods or as a preservative or as components of various native medicinal concoctions (Burkhill, 1985). The seed has a very pleasant sweet aroma which is usually enhanced by roasting in fire. Since it is usually sundried and stored with other spices and stimulants in containers or exposed in heaps in the market stalls awaiting sale or use, it is liable to harbour propagules of many of the common fungi some of which are associated with post harvest deterioration and toxin production. These fungi are also highly favoured by the humid tropical climate prevalent in Southern Nigeria. Reports abound in literature of various fungi commonly inhabiting and contaminating fruits, vegetables, foodstuff, seeds condiments, stored products and even spices and stimulants in different parts of Nigeria (Ogundana and Ajulo, 1980; Ekundayo, 1987; Nwachukwu and Umechuruba, 1991; Edwards *et al*, 1994; Chiejina, 1994; Ekpo, 1994; Elenwo, 1996, 1997; Osuide and Igbinovia, 1999).

The *Aspergillus* group of fungi in particular, *Fusarium*, *Penicillium*, *Trichoderma* etc known to produce some mycotoxins which are dangerous to the health of consumers of such products contaminated by

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these genera (Abramson, 1980; Ekundayo, 1987; Edwards *et al,* 1994) are among the fungi usually identified. The mycoflora of some spices and stimulants consumed or variously used in several states around the Niger Delta and Southern States of Nigeria have been reported (Elenwo, 1996, 1997) but those of *Monodora* had not been reported.

This work therefore is on the fungi isolated and identified from shelled seeds of African nutmeg from twelve states of Southern Nigeria and also examines some methods of eliminating such fungi by simple treatments in order to ensure the safety of consumers.

MATERIALS AND METHODS:

Seed Acquisition: Dried seeds of *M. myristica* (African nutmeg) commonly called "Ehuru" (Ibo) were bought from Local Market in Aba (Abia), Owerri (Imo), Yenagoa (Bayelsa) Calabar (Cross Rivers), Enugu (Enugu), Onitsha (Anambra), Warri (Delta), Benin City (Edo), Akure (Ondo), Lagos (Lagos), Port Harcourt (Rivers), Uyo (Akwa Ibom) respectively.

Monodora seeds were shelled before use, that is, the outer shiny hard cover was removed to expose the cotyledons. Seeds were not previously disinfected nor surface sterilized but were plated directly into 3 layers of wet filter paper in 9 cm autoclaved Petri Dishes. According to the International Seed Testing Association (ISTA) standard blotter method (Neergaad, 1979), 10 seeds from each state sample were plated and incubated at room temperature (29 ± 1°C) on surface sterilized laboratory benches at an illuminated area of the laboratory for 7 days. Each plated seed was examined visually and by means of a binocular stereomicroscope (25 x 50) magnification for the presence of fungi growing on the seeds. Pure cultures of fungi were made on Potato Dextrose Agar and where necessary, cultures were referred to the International Mycological Institute, Kew, Surrey, in England for identification.

Treatment of Seeds for Elimination of Fungi:

Simple treatment methods were applied for elimination or reduction of fungi from *Monodora* seeds. Only seeds from Port Harcourt (Rivers State) were used for this test. Shelled seeds were treated as described below, plated and incubated as earlier described.

(a) **No treatment:** 400 shelled seeds were plated, 10 seeds per plate with no treatment at all. These served as control.

(b) **1% Chlorox (NaOCl + 95% ethanol 1:1 ratio v/v):** 400 shelled seeds were soaked for 5 minutes in 1% chlorox solution in a beaker and removed individually using sterile forceps and placed on moist filter paper in Petri dishes.

(c) **5% brine Solution:** Seeds were soaked in a solution containing 5g of cooking salt (NaCl) in 100mls of water for 5 mins, removed and then plated as in (b) above.

(d) **Hot water:** Seeds were soaked in hot water at 100°C for 5 minutes and plated as described above.

(e) **Grinding into powder:** 400 shelled seeds were ground into powder using a Moulinex dry blender. One gram each of the powder was weighed out and by means of a sterilized spatula were heaped on wet filter paper in Petri dishes (10 heaps per plate) and incubated.

(f) **Roasting on fire before grinding into powder:** 400 shelled seeds were spread on an oven tray and roasted in the oven at 200°C for 30 minutes, then allowed to cool and ground into powder using a Mounlinex dry blender. One gram of each powder was heaped as described in (e) above and

incubated similarly.

The Moulinex blender was wiped clean with cotton wool soaked in ethanol before and after grinding.

RESULTS:

Monodora seeds from the twelve states had fungal species growing out of them (Table I). All the 400 seeds from three states Delta, Lagos and Rivers had 100% fungal growth. There was no state with seeds having less than 50% infection. The highest mean percentage occurrence of fungi species was recorded with seeds from Rivers State (46.1%). By simple test for significant difference, it was found to be positive at P = 0.05% level.

A total of eleven (11) fungi species were isolated and identified from seeds of *Monodora myristica* (Table 2). The *Aspergillus* group of fungi *A. niger, A flavus,* A. *tamarii* occurred in the highest number of seeds from all twelve states. *Fusarium* sp, *Alternaria* sp, *Penicillium, Trichoderma* were also frequently isolated but *Curvularia, Botryodiplodia* and *Rhizopus* were less frequently isolated (Table 2). *T. viride* though present on seeds from all other states was absent on seeds from Anambra, Enugu and Ondo states. However it was most prevalent on seeds from Lagos states to the tune of 98.4%. Similarly *Curvularia* sp was absent on seeds from four states - Abia, Edo, Enugu and Ondo respectively. Table 3 shows the effect of the different treatment methods in eliminating fungal species from *Monodora*

Table I. Percentage (%) Incidence of fungi on shelled seeds of African nutmeg (*Monodora myristica*) from 12 states in Southern Nigeria.

S/NO	Source of Seeds (State)	** % Seeds with fungal Growth	***Mean % fungal occurrence per state
1	Abia (Aba)	91.0	32.7
2	Anambra (Onitsha)	81.0	25.0
3	Akwa Ibom (Uyo)	90.0	33.0
4	Bayelsa (Yenegoa)	94.0	38.0
5	Cross Rivers (Calabar)	88.0	21.2
6	Delta (Warri)	100.0	40.6
7	Edo (Benin)	78.5	25.8
8	Enugu (Enugu)	71.0	25.5
9	Imo (Owerri)	84.0	34.6
10	Lagos (Lagos)	100.0	40.0
11	Ondo (Akure)	77.0	20.1
12	Rivers State (Choba)	100.0	46.1

Results are based on 400 shelled seeds

** Values represent average percentage of 4 replicates per state

*** Mean % occurrence of all the fungi identified per state.

seeds. All the methods reduced or eliminated fungi to various extents compared with untreated seeds. Dipping of seeds in 1% chlorox, the usual laboratory disinfectant, gave 58.5% control while treatment with 5% brine solution gave 55.8% reduction in fungal growth. Soaking in hot water (100°C for 5mins) eliminated most of the seedborne fungi, leaving only two species *Penicillium* and *Rhizopus* sp. This gave 98% control. Although grinding of seeds into powder gave 52.6% control, it seemed to encourage the growth of some otherwise latent fungi – *Rhizoctonia* , *Collectotrichum* and *Phoma* sp in addition to those recorded from untreated seeds. The most effective treatment was

Table 3: Effect of Treatment on elimination fungal species on shelled seeds of African Nutmeg (Monodora myristica) from 12 States of Southern Nigeria.

TREATMENT	** FUNGI ISOLATED AFTER TREATMENT	% ELIMINATION OF FUNGAL OCCURRENCE DUE TO TREATMENT
(1) No treatment	*Aspergillus tamarii* *Aspergillus niger* *Aspergillus flavus* *Alternaria terreus,* *Fusarium oxysporium,* *Curvularia* sp *Trichoderma viride* *Penicillium* sp *Fusarium semitectum* *Botryodiplodia theobromeae* *Botrytis cinerea* *Rhizopus sp*	0.0
(2) 1% Chlorox (NaOCl + 95% of Ethanol: 1:1 v/v for 5 minutes	*Aspergillus flavus* *Alternaria terreus* *Fusarium semitectum* *Penicillium* sp *Trichoderma sp*	58.5
(3) Hot water (100°c) for 5 minutes	*Penicillium sp* *Rhizopus stolonifer*	98.0
(4) 5% Brine Solution (NaCl + H_2O) for 5 minutes	*Aspergillus flavus* *Aspergillus tamarri* *Penicillium,* sp *Curvularia* sp	55.8
(5) Grinding Seeds into powder	*Curvularia* sp, *A. niger* *A. Flavus, Penicillium sp,* *Alternaria tereus, Rhizopus stolonifer, Fusarium oxysporium, Rhizoctonia sp, Colletotrichum sp, Phoma sp*	52.6
(6) Roasting seeds before grinding into powder	No fungus at all up to 7 days after incubation	100

** Only *Monodoa myristica* seeds from Port Harcourt (Rivers State) were used for this test. All tests were repeated at least twice.

roasting of seeds in the oven and grinding into powder. Total or complete elimination of fungi (100% control) was recorded as no fungus at all was found growing on any heap of roasted ground sample even after incubation for 7 days.

Incidentally, roasting of *Morodora* seeds on fire before use is the common practice when it is used for flavouring foods. These results seem to have confirmed the safety of this practice.

DISCUSSION

Like most other food condiments, seeds, fruits and even spices and stimulants often studied, shelled seeds of *Monodora myristica* were found to harbour such common storage fungi like *Aspergillus, Fusarium, Penicillium* etc many of which produce mycotoxins (Abramson et al, 1980). *Monodora* seeds from all twelve sample states had at least nine out of the eleven fungal species identified. Many of these fungi have been previously reported by several workers in the tropics and southern Nigeria in particular as being responsible for contamination of foods fruits, seeds, vegetables, stored products and spices and stimulants. (Ogundana and Ajulo, 1980; Opaneye, 1984; Ekundayo, 1986, 87; Edward *et al*, 1994; Chiejna, 1994; Elenwo, 1996 and 1997).

The various forms in which the dry shelled seeds of *M. myristica* are used in Southern Nigeria are important enough to cause concern to mycologist and nutritionists who are aware of the increasing levels of microbial contamination of foods, even spices and their consequent effects on consumers. The abundant rich and fluffy growth of *Fusarium, Aspergillus* and *Trichoderma* species on shelled seed of *Monodora* even after surface sterilizaion and disinfection indicates the abundance of both fragments, vegetative hyphae and even spores of these fungi in the seeds.

Treatment of seeds with 1% Chlorox, 5% Brine solution, hot water or grinding into powder appeared to reduce the number of fungal species isolated to a considerable extent compared with the untreated seeds. Nevertheless, grinding of seeds into powder seemed further pre-dispose or activate propagules of some apparently dormant fungi which were not isolated from the untreated samples (Table 3). This is consistent with earlier results using spices and stimulant like *Xylopia aethiopicum, Afromomum, melegueta,* and *Dennetia tripetala* (Elenwo, 1996, 1997). Although washing of M*onodora* seeds in hot water (100°C) for 5 minutes greatly reduced the number of fungal species isolated to only two, roasting of M*onodora myristica* seed before grinding into powder totally eliminated all fungal species and even prevented the usual external fungal contaminants (Table 3). It is note worthy that roasting and grinding is the most popular way of using *M. myristica* seeds, both for flavouring, cooking and medicinal purposes. This fact has further been confirmed in this work. Our results therefore suggest that seeds of M*onodora* except for propagation purposes should never be used in any other form without first roasting and grinding into powder. This would ensure a very high safety level for both consumers and other users.

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