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STUDIES ON FUNGAL DETERIORATION OF MELON (*Colocynthis citrullus* L.) SEEDS IN LAGOS, NIGERIA.

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ABSTRACT

Nine fungi were isolated from diseased melon (*Colocynthis citrullus* L.) seeds collected from three major markets in Lagos State, Nigera. They included three species of *Aspergillus*: *Aspergillus niger, A. flavus, and A.fumigatus*; one species each of *Cladosporium, Curvularia, Penicillium sp, Mucor, Rhizopus oryzae,* and *Absidia corymbifera.* Mycelial growth of these fungi on various media was investigated. The result showed that Malt extract supported the optimum growth of *A. niger, Peniccillium* sp, *A. flavus and Curvularia* sp. Carrot Agar and Corn Meal Agar supported the optimum growth of *Cladosporium* sp. And *Rhizopus oryzae*, *A. fumigatus* and *Mucor* sp respectively while *Absidia corymbifera* had optimum growth on Potato Dextrose Agar. Ability to utilize various oil sources showed that *A. niger, A. flavus*, *Mucor* sp and *Absidia corymbifera* had significant growth on groundnut oil. *Cladosporium* sp and *Penicillium* sp had best growth on palm oil. *Rhizopus oryzae* demonstrated optimum capacity for degradation of coconut oil: whereas *A. fumigatus* showed little or absolutely no growth on the three oils after the seventh day. The results obtained showed that the ability of an organism to maximally utilize various media and its tendency to degrade different oils could be a clear indication of its capacity and tendency to cause deterioration of melon seeds in storage.

Key Words: Melon seeds, deterioration, fungi, oil sources

INTRODUCTION

Melon (*Colocynthis citrullus* L.) is a widely cultivated and consumed oil seed crop in West Africa (Bankole *et al.,* 2005). The seeds, locally called "Egusi" are widely consumed in various forms as a condiment in Nigeria cuisine basically in their local soup (Bankole and Joda, 2004). Some notable Nigerian delicacies include "Egusi soup". Melon ball snacks and ogiri (a fermented condiment) (Bankole and Joda,2004; Bankole *et al.,* 2005). The seeds could also be fried and chewed as snacks (Abaelu *et al.,* 1979). Melon seeds (Egusi) contain about 53% oil, 28% protein (60% in defatted meal), 11% starch and soluble sugars (Oyolu, 1977; Abaelu *et al* .,1979). Melon seeds contain a fairly high amount of unsaturated fatty acid and Linoleic acid, suggesting a possible hypocholesteronic effect (Bankole *et al*., 2005).

One major problem that besets melon seeds is that they deteriorate quickly in storage due to fungal activities (Aboaba and Amasike, 1991; Bankole, 1993). Donli and Gulani in 2001 reported that fungi are the major cause of spoilage of grains and seeds, and probably ranks second only to insects as spoilage organisms. Fungi of the genera *Aspegillus* and *Penicillium* are widely distributed storage fungi of melon seeds, causing seed discolourations, decreased

nutritive value, increase in free fatty acid and peroxide values, decreased seed germination, producing a number of toxic metabolites including aflatoxin (Aboaba and Amasike, 1991; Bankole, 1993; Bankole et al.,2005). Some of the isolated fungi are known producers of aflatoxins. Aflatoxins have been associated with elevated rate of liver cancer, stunted growth and immunotoxicity in West Africa (Gong *et al*., 2002; Turner *et al*., 2003).

The objectives of this study was to isolate and identify fungi that cause deterioration(spoilage) of melon seeds; to test the isolated fungi's ability to degrade lipids from different sources and also to investigate the growth of the isolated fungi on different media.

MATERIALS AND METHODS

Samples of shelled and unshelled melon seeds were randomly connected weekly for three months, between October and December, 2006 from Iyana-Iba market, Mammy market (inside Ojo Barracks) and Ojo central market in Lagos State Nigeria. The seeds from each market were carefully subjected to scrutiny and the discoloured, visibly mouldy, and physically distorted seeds were aseptically separated, packaged and labeled for each market.

Method of isolation

Twenty diseased melon seeds from each sample were disinfected using 1% Sodium hypochlorite solution (1% NaOCl) for one minute, followed by three successive rinses in sterilised distilled water. Eight seeds were then aseptically transferred using a sterile wire loop to each of three plates of Malt Extract agar (MEA) and Nutrient Agar (NA), each of which contains 0.25mg/ml of Ampicillin necessary to impede bacterial growth. The inoculated media plates were incubated at temperature of $28^0C \pm 2^0C$ and observed daily for fungal growth. The behaviour of fungal colonies on culture plates was noted. Hyphae of fungi growing from the seeds were aseptically transferred into freshly prepared MEA and NA media. Fungi associated with various zones of deterioration of the diseased melon seeds were noted.

Radial growth measurement of isolated fungi on various media

The isolated fungi were inoculated separately into each of the four various media; Malt Extract Agar (MEA), Carrot Agar (CA), Corn Meal Agar (CMA) and Potato Dextrose Agar (PDA); and incubated at $28^0C \pm 2^0C$ for seven days. Radial growth was measured daily from four cardinal points on the culture plate, and results were recorded, while observations were noted.

Lipid degradation analysis of fungi isolated from diseased melon seeds (using various oils)

Some quantity (100ml) of each oil was added to 900ml of the already sterilized plain agar, the mixture was heated for 5mins to facilitate dissolution, after allowing the prepared medium to cool to $45\text{-}50^0C$, the medium was aseptically dispensed into sterile Petri dishes and allowed to solidify. Fungal isolates were separately inoculated onto the prepared media containing individual lipid sources. The lipid sources used were coconut oil, groundnut oil and palm oil. A control was set for each lipid sources using ordinary plain agar. The cultured plates were incubated at temperature of $28^0C \pm 2^0C$ for seven days. Radial growth measurement was taken and other observations were noted.

Identification of isolated fungi

Seven day old cultures of the required fungus was used. A drop of lactophenol in cotton blue stain was placed at the centre of a clean slide. The inoculating pins were sterilized in a spirit flame, allowed to cool; and used to aseptically transfer a little of the mycelium into a drop of the stain solution on the slide. The hyphae were carefully teased out with the pin, and covered with a cover slip (practically avoiding formation of air bubbles). The slides were then examined under the microscope (Olutola *et al*., 2000), and description was aided by the use of fungal descriptive features given by Emmons *et al*., 1977; Alexopoulus and Mims, 1979 and Ellis and Ellis, 1987.

RESULTS

Nine species of pathogenic fungi were isolated from diseased melon (*Colocynthis citrullus* L.) seeds, they included *Aspergillus niger, Rhizopus oryzae, A. fumigatus, A. flavus, Penicillium* sp, *Curvularia* sp, *Mucor* sp, *Cladosporium* sp and *Absidia corymbifera.*

Shelled healthy melons seeds are usually white to creamy-white in colour, while unshelled melon seeds are usually brownish in colour. Eight sites of deterioration were observed based on the characteristic zones of infection and colour of the affected melon seeds.

From Table 1, *Curvularia* sp, *Cladosporium* sp. and *Penicillium* sp, were isolated from four different diseased zones; while *A. niger* and *A flavus* were isolated from three different zones of infection..

A. fumigatus, and *Absidi corymbifera* were isolated from two zones of infection, while *Mucor* sp was generally the least isolated from a single zone of infection. The zones of infection with highest documented fungal isolates include seeds with deteriorated basal ends and unshelled melon seeds with total internal ramification.

Table 2 clearly shows the growth of isolated fungi on different oils after seven days. *Asperlligus niger* exhibited a maximum growth on the three oils, while *A. fumigatus* showed little or absolutely no growth on the three oils.

Preferentially, it was deduced from the Table that groundnut oil supported the maximum growth of five organisms which includes *A. niger, A. flavus, Mucor* sp, *Absidia corymbifera* and *Curvularia* sp. Palm oil supported the maximum growth of only two organisms which are *Cladosporium* sp. and *Penicillium* sp., while *Rhizopus oryzae* exhibited an exceptional growth on coconut oil. *Aspergillus fumigatus* gave no growth reactions to the oils.

Table 3 shows a comparative study of the growth of fungal isolates on various media. Majority of the fungal isolates yielded maximum growth on Malt Extract Agar (MEA). Carrot Agar (CA) and Corn Meal Agar (CMA) supported the maximum growth of two organisms each, which are *Rhizopus oryzae* and *Cladosporium* sp; *A. fumigatus* and *Mucor* sp. respectively. Potato Dextrose Agar (PDA) supported best the growth of *A. niger*.

Table 1: Deterioration And Characteristic Colour Change Of Diseased Melon Seeds With Associated Fungal Isolates

S/N	Colour of seed parts	Type of fungi isolated from the infected
1.	Unshelled melon seed with black hilium	*Cladosporium* sp, *Aspergillus fumigatus*
2.	Dark brownish-black unshelled melon seeds	*Aspergillus flavus, Curvularia* sp, *Cladosporium* sp.
3.	Shelled melon seeds with transparent basal end	*Cladosporium* sp.
4.	Half melon seeds with deteriorated basal end	*Rhizopus oryzae, A. niger, A. flavus. Curvularia* sp, *Penicillium* sp, *Mucor* sp.
5.	Unshelled melon seed with total internal ramification	*A. niger, A. flavus, Penicillium* sp., *A. fumigatus*
6.	Half melon seeds with deteriorated apical end	*Cladosporium* sp, *Curvularia* sp
7.	Total blackening of unshelled seeds	*Absidia corymbifera, Penicillium* sp
8.	Unshelled seeds spotted or doted black	*Absidia corymbifera, Penicillium* sp, *Curvularia* sp

Table 2: **The Growth Of Isolated Fungi On Different Oils After Seven Days of Incubation**

Source of Lipid	**Radial growth measurement of fungi isolated from diseased melon seeds (cm) lipid**								
	Aspergillus niger	*Cladosporium* sp.	*Penicillium* sp.	*Aspergillus flavus*	*Mucor* sp.	*Aspergillus fumigatus*	*Absidia corymbifera*	*Curvularia* sp.	*Rhizopus oryzae*
Palm oil	4.09 (±0.34)a	2.86 (±0.69)[a]	4.08 (±0.13)[b]	3.55 (±1.02)[a]	2.21 (±0.20)[a]	0.00 (±0.00)[a]	0.46 (±0.10)[b]	3.53 (±1.12)[b]	0.00 (±0.00)[b]
Groundnut Oil	4.20 (±0.11)[a]	2.53 (±0.97)[a]	(±0.88)[a]	4.13 (±0.13)[a]	2.79 (±0.59)[b]	0.00 (±0.00)[a]	2.25 (±0.39)[a]	3.93 (±0.11)[b]	1.20 (±0.35)[c]
Coconut Oil	4.15 (±0.11)[a]	2.19 (±0.22)[a]	2.75 (±0.30)[a]	3.88 (±0.33)[a]	1.85 (± 0.11)[a]	0.00 (±0.00)[a]	1.55 (±0.34)[a]	0.80 (±0.30)[a]	4.00 (±0.37)[a]

Figure along vertical column followed by the same alphabets are not significantly different according to Duncan's multiple range Test (DMRT) at the 95% confidence level.

Table 3: **Effect Of Different Media On The Growth Of Fungi Isolated From Diseased Melon Seed**

Source of Lipid	**Radial growth measurement of fungi isolated from diseased melon seeds (cm) lipid**								
	Aspergillus niger	*Cladosporium* sp	*Penicillium* sp	*Aspergillus flavus*	*Mucor* sp	*Aspergillus fumigatus*	*Absidia corymbifera*	*Curvularia* sp	*Rhizopus oryzae*
MEA	4.15 (±0.11)[a]	2.86 (±0.69)[a]	4.08 (±0.13)[a]	4.13 (±0.13)[a]	2.79 (±0.59)[a]	0.00 (±0.00)[a]	2.25 (±0.39)[a]	3.93 (±0.11)[a]	4.00 (±0.37)[a]
CA	3.34 (±0.10)[a]	4.14 (±0.29)[a]	3.46 (±0.57)[a]	3.18 (±1.13)[a]	2.16 (±0.79)[a]	1.45 (±0.63)[b]	1.73 (±0.74)[a]	3.16 (±0.20)[b]	4.24 (±0.10)[a]
PDA	3.79 (±0.55)[a]	2.54 (±0.29)[a]	1.53 (±0.35)[b]	1.31 (±0.20)[b]	1.74 (± 0.26)[a]	0.00 (±0.00)[a]	2.66 (±0.66)[a]	2.18 (±0.35)[c]	0.90 (±0.15)[b]
CMA	3.84 (±0.51)[a]	3.00 (±0.53)[a]	3.54 (±0.76)[a]	3.35 (±1.22)[a]	3.14 (± 0.96)[a]	1.80 (±0.76)[b]	0.00 (±0.00)[b]	2.86 (±0.55)[b]	2.40 (±0.18)[c]

Figure along vertical column followed by the same alphabets are not significantly different according to Duncan's multiple range Test (DMRT) at the 95% confidence level.

DISCUSSION

The result shows that various fungi can infect melon seeds from different ingress points (basically when they are in a predisposed state). One basic rationale for this assertion could be in the mode of harvesting of melon fruits and seeds, because according to Abaelu *et al*.,(1979), the matured fruits are mechanically injured and allowed to decompose in the soil. Therefore, by subjective reasoning, an introduction of the mechanically injured melon fruits to the soil predisposes them to fungal attack prior to storage; since according to Alexopoulus and Mims (1979), the aforementioned fungi are ubiquitous in nature.

Another factor in favour of these assertions could be in the mode of drying and storage of melon seeds. According to Bankole *et al.* (2005), the storage deterioration of melon seed is significantly influenced by the moisture content, because the biodeteriogens (fungi) require moisture for their activities.

Post harvest deterioration incidence on melon seed can be minimized by the use of good harvesting and processing techniques, appropriate drying techniques (Bankole *et al*., 2005) and hygienic storage conditions. Finally, from the results obtained from Tables 2 and 3, it is inferential to state that the ability of these organisms to maximally and efficiently utilize the nutrient from various media for growth, and their capacity to degrade oils from various sources, clearly shows their capacity for biodegradation of oil seed crops such as melon seeds and plant materials of organic origin with oil residue. Post harvest deterioration of melon seeds partly results from the cultural and environmental practices which ensued prior to consumption of the melon seeds. An alleviation of the sharp and anachronistic practices will result in drastic decline in the occurrence of these post harvest deteriogens.

Lipase production is associated with most fungi (Cochrane, 1965). Lipase is normally encountered in most culture medium. It has been reported (Cochrane, 1965) that *Aspergillus niger* and *Penicillium roqueforti* produced lipases into the culture medium. *Macrophomina phaseolina* and *Phoma nebulosa* have been reported to produce lipase (Reddy and Reddy, 1983). The current result tallies with previous observations. The effect of these fungi could bring total alteration of the biochemical composition of the melon seeds and a decrease in the nutritive value of the seeds. Melon seeds could harbour some of the carcinogenic metabolites produced by isolated fungi, hence the need for good hygiene during production and storage of melon seeds.

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