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INVESTIGATION ON FUNGI ASSOCIATED WITH THE SPOILAGE OF *Chrysophyllum albidum* (G.Don) FRUITS.

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ABSTRACT

Chrysophyllum albidum (African star apple) fruit is among the well known indigenous fruit trees in the tropical rainforest zone of the Western region of Africa. The fruits are much cherished by many people including the young and old and are of large commercial interest in these countries. The fruits often deteriorate within a very short period and become spoilt as a result of fungi attacks. The study therefore carried out the survey of fungi attacks on *C. albidum* and investigates the etiology of post harvest deterioration. Fresh fruits were procured from purposely selected markets in Ogun State for microbial analysis. Descriptive and cross tabulation analysis were used to analyze the data and these include: percentages, frequency distribution and cross tabulation. The results of this study showed that five fungal genera were associated with African star apple fruits. The fungi found on the surface of the fruits were *Fusarium solani, Aspergillus niger, Aspergillus fumigatus, Penicillium citrinum, Lasiodiplodia theobromae* and *Rhizopus spp. Fusarium solani* was the most abundant and prevalent in almost all the localities. The incidence of the occurrence of fungi found associated with highest percentage of Lasiodiplodia theobromae (40.37%) and *Fusarium solani* (26.23%) respectively. The least occurrence of all the fungal isolates is *Aspergillus flavus. Aspergillus niger, Penicillum citrinum* and *Aspergillus fumigatus* were found to be pathogenic to *C.albidum* fruits and responsible for their spoilage. Therefore the use of local preservatives (plant extracts) like *Afromomum danielli, Afromomum melegueta* and chemical disinfectants like paraozone, jik, sodium chloride and sodium benzoate at mild form were recommended to reduce the losses due to storage moulds. More so, early harvesting of the fruits, prevention of over ripening and keeping the fruits dry and cool at all time should be ensured.

Keywords: *Chrysophyllum albidum,* fungi attacks, etiology, microbial analysis, preservatives

INTRODUCTION

Chrysophyllum albidum (G.Don.) belongs to the family Sapotaceae. It is primarily a forest tree species and its natural occurrence have been reported in diverse ecozones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'lvoire (Bada, 1997). The plant often grows to a height of 36.5m though it may be smaller (Bada, 1997). The African star apple fruit is a large berry containing 4 to 5 flattened seeds or some times fewer due to seed abortion (Keay, 1989). It is classified as a dehiscent berry with seeds ranging from one to several with bony often-shining testa and large broad hilum.

African star apple fruit is among the well known indigenous fruit trees in the tropical rainforest zone of the Western region of Africa (Sierra Leone) to Eastern region of Africa (Uganda). The fruits of *Chrysophyllum albidum* are much cherished by many people including the young and old and are of large commercial interest in these countries. The African star apple fruit has been found to have highest content of ascorbic acid with 1000 to 3,330 mg of ascorbic acid per 100gm of edible fruit or about 100 times that

of oranges and 10 times of that of guava or cashew (Asenjo, 1946). It is reported as an excellent source of vitamins, irons, flavours to diets and raw materials to some manufacturing industries (Adisa, 2000; Bada, 1997; Okafor and Fernandes, 1987; Umelo, 1997).

In addition, its seeds are a source of oil which is used for diverse purposes. The seeds are also used for local games (Bada, 1997). The fruits also contain 90% anacardic acid, which is used industrially in protecting wood and as source of resin while several other components of the tree including the roots and leaves are used for medicinal purposes (Bada, 1997).

It makes significant dietary contribution which ameliorates food insecurity in developing countries, since it is available at most seasons including strategic period of the year when conventional staples that are difficult to store are scarce (Okigbo, 1977). *Chrysophyllum albidum* has been reported by Okigbo (1977) to contain 8.8% protein, 17.1% oil, 20.9% sugar, 11.0% starch and several minerals. Recent research initiatives have shown that the economic prospect of this fruit has increased tremendously.

The medicinal uses of this plant appear to be limited to areas of its natural habit at lowland rain forest belt of Nigeria (Olopade, 1997). The bark of the tree is used in preparation of medicines for the treatment of fever and black coated tongues called *Efunfun* in Yoruba. The leaves are very important in the preparation of various medicines to make anti-malaria drugs and when combined with honey, it is used in the treatment of dry cough (Olopade, 1997). It is important to note that the most important part of the plant in ethno medicine is the seeds, which are often discarded. The cotyledons within the hard coat are useful in the preparation of medicines for the treatment of secondary amenorrhea in women and infertility due to oligospermia in males (Olopade, 1997).

C. albidum fruit is common in both urban and rural center especially during the months of December to April. The fruits are not usually harvested from the trees, but left to drop naturally to the forest floor where they are picked. Allowing the fruits to drop before picking promotes fungal infections. Recent market survey revealed that the fruits often deteriorate within a very short period. According to Adebisi (1997), *C. albidum* actually becomes bad within a period of 5 days, with the deterioration starting with changes of normal pinkish colour to one with patches and followed by shrinking of the fruit. The study therefore undertaken to survey fungi attacks on *C. albidum* and investigate the etiology of post harvest deterioration and as well as establishing the pathogenicity of fungi isolated during its storage.

MATERIALS AND METHOD

Fresh, mature, ripe and infected *C. albidum* fruits were collected from purposefully selected markets in Ogun State, Southwestern part of Nigeria. The selected markets include Mamu, Awa, Ago Iwoye, Oru and Ijebu Ode. The fruits were all conveyed to the laboratory aseptically in a clean and sterile sampling bag which was labelled and ready for microbial analysis.

Preparation of Culture Media

Thirty nine grams of potato dextrose agar (PDA) was weighed and suspended into 1 litre of distilled water which was allowed and bring to boil for 15minutes to dissolve completely and sterilized by autoclaving at 121^0C for 15 minutes. The medium was amended by adding 10ml of lactic acid to 1litre of the medium after cooling to 47^0C to lower the pH to 3.7± 0.2.

Isolation Procedure

Direct Plating Method

The petri dishes, conical flasks, beakers, pipettes used were thoroughly washed with detergent, rinsed several times and sterilized in an autoclave for 15 minutes at 15 pounds per square inch pressure at 121^0C. The materials that cannot stand the heat of the autoclave were surface sterilized in near absolute alcohol (97% ethanol). Isolation of mycoflora with infected mesocarp was done using sterilized scalpel and surface sterilize in 10% sodium hypochlorite solution and rinsed in 3 stages of sterile distilled water, this was then blot dried with Whatman filter paper. The surface sterilized infected portions were plated on

amended sterile potato dextrose agar (PDA) medium in 9cm Petri dishes and incubated at 25±2˚c for 5-7 days in five replicates.

Pour Plate Methods

The infected edges of *C. albidum* fruits were cut and 1g of cut mesocarp from each of the sample was homogenized in 9ml of 0.1% sterile peptone water in 10ml measuring cylinder. The volume of the broth was made up to 10ml mark with peptone water and 0.1ml of the appropriate dilution were pour plated on amended PDA and incubated at 25±20^{0}C for 5-7 days. The isolation was replicated three times. The plate counts were conducted for a total isolation experiment for each African star apple fruits.

Identification of Mycoflora Isolates

Aseptically purified sub cultured discrete colonies were maintained and stored in PDA slants for further characterization. The fungi isolates were under stereo binocular microscope identified and then determined using cultural and morphological characterized as well as the description key provided by Barnett and Hunter (1972), Broth (1971) and Wester (1980). Isolation of mycoflora was done in five replicates for all the locations. The frequency and prevalence of each mycoflora was calculated.

Pathogenicity Test

C. albidum fruits were sterilized by swabbing with 70% alcohol and rinsed in sterile. A sterile cork borer (4mm diameter) was used to remove a tissue from each of the surface sterilized fruits. A second cork borer was used to cut disc of agar 3 day old culture of fungal mycelia of the candidate isolates and used to inoculate the hole created by scooping out the fruit tissue. The scooped out tissues were replaced to cover the inoculated portion of the fruit. The inoculated fruits were then enclosed in polythene bags containing moist cotton wool to maintain high relative humidity and incubated at 25% in an incubator for days. Four *C. albidum* fruits were inoculated per candidate isolates while the control fruits were inoculated with sterile PDA disc. The extent of rot was determined by measuring the size of infection in millimeters. Wet mounts of hyphae or asexual pore obtained from the infected fruits were stained with cotton blue lactophenol and viewed under the compound microscope for the pressure of pathogen that was used in the inoculation.

RESULTS

The results of this study showed that five fungal genera were associated with African star apple fruits (Table 1). The fungi found on the surface of the fruits were *Fusarium solani, Aspergillus niger, Aspergillus fumigatus, Penicillium citrinum, Lasiodiplodia theobromae,* and *Rhizopus spp.*

TABLE 1: Fungal Isolates Associated with *C. albidum* Fruits from various sampled markets

FUNGUS	AGO IWOYE	AWA	ORU	IJEBU ODE	MAMU
Fusarium solani	+	_	+	+	+
Aspergillus niger	+	_	_	_	+
Penicillium citrinum	+	_	_	+	_
Aspergillus flavus	+	_	_	_	_
Lasiodiplodia theobromae	_	+	+	_	+
Aspergillus fumigates	_	_	+	+	+
Rhizopus spp	_	_	_	+	_

KEY: + present - absent

Fusarium solani was the most abundant and prevalent in almost all the localities. Table 2 shows the percentage of fungal pathogens from the sites. *Lasiodiplodia theobromae* was most abundant in Awa, Oru and Mamu, followed by *Fusarium solani* in four different localities as shown in Table 2.

TABLE 2: Percentage of Fungal Isolates from Rotten *C. albidum* Fruits from various Sampled Markets.

FUNGUS	AGO IWOYE	AWA	ORU	IJEBU ODE	MAMU
Fusarium solani	24.0		42.85	37.5	16.66
Aspergillus niger	68.0		14.28		16.66
Penicillium citrinum	4.0			37.5	
Aspergillus flavus	4.0				
Lasiodiplodia theobromae		100.0	28.57		50.0
Aspergillus fumigatus			14.28	12.5	16.66
Rhizopus spp				12.5	

TABLE 3: Frequency of Occurrence of Fungi Associated with *C. albidum* Fruits

CANDIDATE FUNGUS	FREQUENCY OF OCCURRENCE (%)
Fusarium solani	26.23
Aspergillus niger	16.49
Penicilliumcitrinum	6.91
Aspergilllus flavus	0.66
Lasiodiplodia theobromae	40.37
Aspergillus fumigates	7.24
Rhizopus spp	2.06

DISCUSSION

The fungi isolated have been reported to be associated with post harvest deterioration of agricultural products in Nigeria (Onwuzulu, 1991). However, *Fusarium solani* was the most abundant of all the isolates, followed by *Lasiodiplodia theobromae*, *Rhizopus spp* and *Aspergilllus flavus* were the least encountered (Table 1).

Fungi are ubiquitous, and they are spore formers and filamentous. Their spores are single to few reproductive structures that may be dispersed by wind or water as well as by animals and equipments (Mahovic *et al*, 2004). Since most microbial spores are small in size and light, they could settle on the surface of African Star Apple fruits resulting in the range of microbial group isolated from them.

The dominance of fungi in most agricultural fruits is due to the fact that the fruit is acidic. The high acid content of African pear fruits makes it more ideal substrate for fungi than bacteria, since fungi tends to dominate in moderately acidic environment (pH 3.0- 3.5) (Nwanekewi and Onyeagba, 2007). *Lasiodiplodia theobromae* was the most abundant contaminant in Awa and Mamu and this has been reported to be among some of the post harvest causes of rot which naturally inhibit the surface of foodstuffs (Akija and Ejale, 2004).

Rhizopus stolonifer sporangiophores are air borne throughout the year and germinate upon contact with wounds, floral openings or freshly harvested fruits (Agrios, 1997). *Aspergillus* and *Penicillum spp* are soil borne microorganisms, they are the dominant genera found on stored agricultural products. *Mucor* and *Aspergillus spp* have been isolated from soil by Ogboona *et al,* 1983. *Aspergillus niger* and *A.fumigatus* are only known for their lypolytic abilities (Akija,2004), they accelerate the breakdown of fats by lipases into free fatty acid and glycerol thereby lowering the oil content of the fruits of *Dacroydes edulis* which

has been reported to contain 31.9 of fat. *Aspergillus niger* is capable of utilizing, enormous variety of substrate because of the large number of enzymes they produce (Alexopopulous and Mims, 1979). All the identified microorganisms were not restricted to any particular market sample site of the southwestern states of Nigeria. The microorganisms were isolated generally from all the localities where the *Chrysophyllu .albidum* fruits were obtained. The incidence of the occurrence of fungi found associated with highest percentage is found *Lasiodiplodia theobromae* 40.37% and *Fusarium solani* with 40.37% and 26.23% respectively. The least occurrence of all the fungal isolates is *Aspergillus flavus.*
Aspergillus niger, Penicillum citrinum and *A. fumigatus* were found to be pathogenic to *C.albidum* fruits and responsible for their spoilage. *Penicillum spp* has also been reported to be associated with post harvest deterioration of rubber seeds in Nigeria (Okhuoya and Ige, 1986). *Aspergillus flavus* and *Rhizopus spp* were least abundant on culture media but have been reported to be among some of the post harvest causes of rots which naturally inhabit the surface of foodstuffs. It has been reported by Alexopoulous and Mims (1985) that *Rhizopus spp* have very light spores, which are capable of utilizing enzymes they produce. *Dacryodes edulis* also is known for its high nutrient value and fungal spores will germinate and grow on it under favourable conditions, nutrients and environmental conditions (Akija and Ejale, 2004).

Fruits and vegetables are highly perishable crops due to their high water content (70-90%) and their cultural physiological activities (enzymatic oxidative, respiratory etc.) at low level and proven to be attacked by pest and diseases which leads to high spoilage. The ambient tropical and sub tropical temperature and relative humidity conditions also accelerate these activities. All these call for preventive measures from the time of harvest, consumption and processing. Food preservative or disinfectant is essential at the peak of production to prevent or reduce losses and also to ensure freshness in appearance, odour, flavour and nutritional values.

African star apple storage has always been a laborious and delicate task because the farmers are still using traditional method of storage. Much of these fruits are better eaten or consumed in fresh state, even though the bulk of fruits are picked from the forest floor. Preserving the freshness of these fruits for many days or months is therefore the problem which most farmers and the traders seek to solve. Therefore employing the use of local preservatives (plant extracts) like *Afromomum danielli, Afromomum melegueta* and chemical disinfectants like paraozone and jik, sodium chloride and sodium benzoate at mild form can reduce the losses due to storage moulds. Harvesting the fruits as soon as possible to prevent over ripening is one of the best approaches and prevention of the fruit droppings on the forest floors which serve as mycobank of the inoculums should be looked into. Keeping the fruits dry and cool at all time should also be ensured.

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