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FUNGAL PATHOGENS ASSOCIATED WITH FOREST FRUIT *Dialium guineense* (ICHEKU) IN PORT HARCOURT METROPOLIS

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ABSTRACT

A study was carried out on Dialium guineense (Icheku) fruit for the growth of fungal pathogen. The fruits were collected from four different locations of Port Harcourt; namely Borokiri, Choba, Oyigbo and Rumuokoro. Standard Blotter Method and Agar method for the growth of pure culture were used for the isolation of the following organisms; Aspergillus niger, Aspergillus flavus, Botrydioplodia theobroma, Fusarium oxysporum, Penicillium chrysogenum and Rhizopus stolonifer. Rumuokoro had the most organisms, while Borokiri had the least organisms. Aspergillus niger occurred in all the locations but was most frequent in Oyigbo with a frequency of occurrence of 8.1%. The frequency of occurrence of Aspergillus flavus was 4.1% in all the locations. For Penicillum chrysogenum Oyigbo had the highest frequency of 10.8%. Fusarium oxysporium occurred in three locations with Rumuokoro having the highest frequency of 5.4%. For Rhizopus stolonifer, Oyigbo had the highest occurrence frequency of 12.2%. Botrydioplodia theobromae was found in two locations Rumuokoro 9.5% and Choba 4.1%.

Key words: *Dialium guineense and fungi.*

INTRODUCTION

Common Names

(English): black velvet, velvet tamarind
(Igbo): icheku
(Yoruba): awin

Botanic Description

Dialium guineense is a tree of 30 m high, shrubby with a densely leafy crown. Bole without buttresses, Bark smooth, grey; slash reddish, yielding a little red gum. Leaves sometimes are finely hairy, with a common stalk of 5-13 cm long, with an odd terminal leaflet and usually 2 pairs of opposite or alternate leaflets, the lower pair being somewhat smaller; leaflets is mostly 3.5-10 x 2.5-5 cm, elliptic to broadly elliptic, sometimes slightly obviate; blunt at the apex or abruptly and shortly acuminate, symmetrical and rounded leathery, glabrous above and with the midrib slightly

sunken. Flowers are usually whitish, in large terminal, or occasionally auxiliary, panicles up to 30 cm long; branches spreading out widely and more or less horizontally; the whole inflorescence at first covered with very short, brownish hairs; individual flowers are with short stout stalks, the buds are about 2 mm long. Fruits are densely velvety, black; each fruit with a stalk of about 6 mm long with a little collar near the apex and a brittle shell enclosing 1 seed (or exceptionally 2), embedded in a dry, brownish, sweetly acidic, edible pulp.

Figure 1: *Dialium guineense* (Black velvet) Fruits.

Ecology and Distribution

Natural Habitat

Dialium guineense grows in dense savannah forests, shadowy canyons and gallery forests. It is found from Senegal to Sudan along the southern border of the Sahel. This is the most common and widespread Dialium in Nigeria. In Ghana, it is found along transition zones bordering high forest, in riverian forest of the savannah woodland, in coastal scrub, and in riparian vegetation of the Volta.

Geographic Distribution

Native : Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Equatorial Guinea, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Niger, Nigeria, Sao Tome et Principe, Senegal, Sierra Leone, Sudan, Togo.

Biophysical Limits

Soil type: Naturally found on moist, sometimes brackish soils.

Reproductive Biology:

In Nigeria the tree flowers from September to October and fruits from October to January. In Ghana, in September to November the tree is covered with small white flowers in panicles; fruit ripens in March to May but may be earlier and may persist longer. Animals, which like to eat the pulp in which the seeds are embedded, help disperse the fruit. However, the fruit can also be transported by water since it floats; transport by sea currents may lead to long-distance dispersal.

Propagation and management

Propagation Methods:

They are buried just below the soil surface and a layer of sawdust is spread on top. The beds should be shaded and watered regularly (twice a day). During germination, the testa breaks at soil level, exposing the creamy white, thick cotyledons.

Tree Management:

Harvesting the trees is difficult because the wood is dense. They often have tall buttresses, which have to be slashed before cutting, as much of the wood would be wasted if the trunk were cut above the buttress. The logs cannot be transported by river as they sink in water.

Uses

Food:

The pulp is red, with a sweet-sour, astringent flavour similar to baobab, but sweeter. It is peeled and eaten raw; it can be a little constipating. The thirst-quenching, refreshing fruit pulp can also be soaked in water and drunk as a beverage.

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The fruits of the plant are chewed among some women in southeast Nigeria to improve lactation and check genital infection. (Nwosu, 2000). **Leaves** are bitter; they may be used to cook 'domoda', a Ghanaian dish that tastes both sweet and bitter. The leaves and stem bark are used as folklore remedies for the treatment of infections such as diarrhoea, severe cough, bronchitis, wound, stomachaches, malaria fever, jaundice, antiulcer and haemorrhoids. (Bero et. al., 2009). It is used as antiulcer and as a vitamin supplement among some tribes in the southern part of Nigeria. (Lawal et. al., 2010).

Fuel: The tree is said to make good firewood and charcoal. **Timber:** Sapwood is white with distinct ripple marks; the heartwood is red-brown. Because of the high silicate content of the timber, axes and saws quickly get blunt. The wood is hard, durable, heavy, light brown, with a fine texture. It is used for vehicles, houses and flooring.

Fungi are members of the kingdom Fungi and are eukaryotes (i. e. Organisms whose cells contain complex structures enclosed within membranes). It can also be referred to as organisms that have nuclei in their cells. This characteristic separates them from bacteria, which are prokaryotes, i.e. they lack nuclei in their cells. The Fungi are classified as a kingdom that is separate from bacteria, plants and animals. In many ways fungi are more closely related to animals than to plants, and they have been thought to share a common protist ancestor with animals. (The Free Encyclopedia, 2009). Fungi are heterotrophic organisms (meaning that they require external sources of organic compounds for food). Fungi grow as multicellular filaments called hyphae forming a mycelium; some fungal species also grow as single cells. They reproduce sexually and asexually and it is commonly via spores, produced on specialized structures or in fruiting bodies. Some species have lost the ability to form specialized reproductive structures, and propagate solely by vegetative growth. Examples of fungi are yeasts, molds and mushrooms.

Occurring worldwide, most fungi are largely invisible to the naked eye, they can live in or on soil, water, insects, human, dead matter, and as symbionts of plants, animals, or other fungi. They perform an essential role in all ecosystems in decomposing organic matter and are indispensable in nutrient cycling and exchange. Some fungi become noticeable when fruiting, either as mushrooms or mould.

Many fungi are used as a direct source of food, such as mushrooms and truffles and in fermentation of various food products, such as wine, beer, and soy sauce. More recently, fungi are being used as sources of antibiotics in medicine and various enzymes, such as cellulases, pectinases, and proteases, important for industrial use or as active ingredients of detergents. Many fungi produce bioactive compounds called mycotoxins, such as alkaloids and polyketides that are toxic to animals including humans. Some fungi are used recreationally or in traditional ceremonies as a source of psychotropic compounds. Several species of the fungi are significant pathogens of humans and other animals. Losses due to fungi diseases of crops and food spoilage caused by fungi can have a large impact on human food supply and local economies.

MATERIALS AND METHODS

The icheku fruits were obtained from hawkers in four (4) different parts of Port Harcourt which are: Rumuokoro, Oyigbo, Borokiri and choba.

Materials

Irish Potatoes, Dextrose, and agar.

Culture medium used: Potato Dextrose Agar

Glass ware and other laboratory equipment: Filter papers, Plastic trays for carrying of Petri dishes, Cotton wool for wiping and plugging of glass wares, Aluminum foil for wrapping of materials to keep them sterile, Sieve for filtering and squeezing out pulps, Knife for cutting, Plastic bowls for washing, Pot for boiling, Microscope, Plastic mortar and pestle for mashing, Forceps for picking, Camera for photographs, Bunsen burner for boiling and flaming, Incubator, cork borer used to bore media to pick up fungal culture for inoculation, Inoculating loop for inoculation, Auto clave for sterilizing, Petri dishes for culturing, Conical flasks, 1000ml Measuring cylinder, 5ml Syringe, Stirring rod, Beakers, Funnel, Cover slip and slides, Mc Cartney bottles, Pipette, Lactic acid, 70% Ethanol, Bleach (*Sodium hypochlorite*), recording materials like; notebooks, markers and pens.

Methods

Isolation

Fungi were isolated from the Icheku pulps using Standard Blotter Method recommended by International Rules for seed Testing (ISTA, 1976) and Agar method (Klement and Voros, 1974). Sterile Petri dishes were lined with 3 layers of sterilized 9cm filter paper. Sterile distilled water was used to wet them and excess water poured out. The fruits used were sorted to remove visible diseased ones then soaked in 70% ethanol for 2 minutes and rinsed twice in sterile distilled water; after which the pods of the fruits were opened to obtain the edible pulps containing the seeds and they were placed in Petri dishes aseptically and incubated at 25^{o}C in the laboratory for 7 days. The following fungi such as; *Aspergillus niger, Fusarium oxysporium, Aspergillus flavus, Botrydioplodia theobromae, Penicillium chrysogenum,* and *Rhizopus stolonifer* were found growing on the Icheku pulps. They were isolated and sub-cultured on Potato (*S. tuberosum*) Dextrose agar from which pure cultures were made.

Preparation of Medium

Potato (*S. tuberosum*) Dextrose Agar

Composition: Potatoes 200g, Dextrose 20g, Agar 20g, Water I litre.

Method of Preparation

Irish Potatoes (*S. tuberosum*) were peeled, weighed, washed and cut in tiny cubes. It was then transferred into a pot containing one litre of water and placed on a Bunsen burner to boil until soft enough to mash. After mashing, it was squeezed through a sieve to obtain the pulp. Which was transferred into a 1 liter/ 1000ml measuring cylinder, 20g of dextrose and agar were dissolved and added. The medium was made up to one litre, dispensed into 250ml flasks, plugged with cotton wool and foil and sterilized with an autoclave at 15PSI at 126^{o}C for 20 minutes. It was allowed to cool and 3 drops of 25% lactic acid was added to inhibit the growth of unwanted microorganisms. After which they were dispensed into 9cm Petri dishes and allowed to solidify. (Ataga, A. E., Elenwo, E. N. and Nwachukwu, E. O. 2010).

Inoculation/ Preparation of Pure Culture

The work benches are first surface sterilized using 70% ethanol and cotton wool. An inoculating needle was flamed until red hot then dip in alcohol to cool. (A hot needle will kill the mould that is to be transferred). With the heat-sterilized needle a small portion of the fungi colony were picked and transferred into a sterile plate containing the solidified Potato Dextrose Agar and the needle flamed again until red hot, to kill all adhering spores and hyphae. (Umechuruba and Elenwo 1997). After which, the culture were allowed to grow in a protected place that has as little air movement as possible. The identification of the isolated fungi was carried out with the aid of manual on the distribution of fungi (Burnett and Hunter, 1972). Frequency of occurrence of fungi was

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determined based on the Score Method recommended by (Ataga and Akueshi, 1986).

RESULT

The following microorganisms were isolated from the *Dialium guinenses* (black Velvet Tamarid) from the different locations of Port Harcourt metropolis which are Rumuokoro, Oyigbo, Borokiri and choba. As shown in the table 1 below:

Table 1: Fungi Isolate from the different study Locations.

Location	Fungi Isolates
Borokiri	*Aspergillus nger, Aspergillus flavus, Penicillium chrysogenum, Fusarium oxysporium*
Choba	*Rhizopus stolonifer, Botrydioplodia theobromae, Aspergillus niger, Aspergillus flavus*
Oyigbo	*Penicillium chrysogenium, Rhizopus stonifer, Aspergillus niger, Fusarium oxysporium*
Rumuokoro	*Botrydioplodia theobromae, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Rhizopus stolonifer, Fusarium oxysporium*

Table 2: Frequency of occurrences of Fungi in the different Locations

Locations	Organisms	Number	% Frequency of Occurance
Borokiri	*Aspergillus nger,*	3	4.1
	Aspergillus flavus,	3	4.1
	Penicillum chrysogenum,	4	5.4
	Fusarium oxysporium	2	2.7
Choba	*Rhizopus stolonifer,*	5	6.8
	Botrydioplodia theobromae,	3	4.1
	Aspergillus niger,	2	2.7
	Aspergillus flavus	3	4.1
Oyigbo	*Penicillium chrysogenum,*	8	10.8
	Rhizopus stonifer,	9	12.2
	Aspergillus niger,	6	8.1
	Fusarium oxysporium	2	2.7
Rumuokoro	*Botrydioplodia theobromae,*	7	9.5
	Aspergillus niger,	5	6.8
	Aspergillus flavus,	3	4.1
	Penicillium chrysogenum,	3	4.1
	Rhizopus stolonifer,	2	2.7
	Fusarium oxysporium	4	5.4
TOTAL		74	100%

The Frequency of occurrences of the organisms in the different locations is shown in Table 2 above. *Aspergillus niger* occurred in all the locations but was most frequent in Oyigbo with a frequency of occurrence of 8.1%. The frequency of occurrence of *Aspergillus flavus* was 4.1% in all the locations. For *Penicillum chrysogenum* Oyigbo had the highest frequency of 10.8%. *Fusarium oxysporium* occurred in three locations with Rumokoro having the highest frequency of 5.4%. For *Rhizopus stolonifer*, Oyigbo had the highest occurrence frequency of 12.2%. *Botrydioplodia theobromae* was found in two locations Rumukoro 9.5% and Choba 4.1%.

DISCUSSION

The above organisms are detrimental to human beings. It is not advisable to buy icheku by the road side and just eat it especially the once without pods. This is because it is a good substrate for the growth of pathogenic fungi, which are infectious. These fungi are found everywhere and anywhere in extremely small quantities due to the minute size of their spores and they cause diseases in humans or other organisms. For example *Aspergillus* which causes diseases in three major ways: through the production of mycotoxins (toxic secondary metabolite); through induction of allergenic responses; and through localized or systemic infections. With the latter two categories, the immune status of the host is pivotal. Allergies and asthma are thought to be caused by an active host immune response against the presence of fungal spores or hyphae. In contrast, with invasive aspergillosis, the immune system has collapsed and little or no defence can be mounted. Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms. Some of the health effects found in animals and humans include death, identifiable diseases or health problems, weakened immune systems without specificity to a toxin, and as allergens or irritants.

The most common pathogenic species are *Aspergillus fumigatus* and *Aspergillus flavus*. Aspergillus flavus produces aflatoxin which is both a toxin and a carcinogen and can potentially contaminate foods such as fruits and nuts. Aspergillosis is the group of diseases caused by *Aspergillus*. The symptoms include fever, cough, chest pain or breathlessness. Usually, only patients with weakened immune systems or with other lung conditions are susceptible.

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*. The most toxic, is carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many humans.

Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A. It has been linked to tumors in the human urinary tract.

Patulin is a toxin produced by the *Aspergillus* and *Penicillium* fungal species. *Penicillium* is especially associated with a range of moldy fruits which causes damage to the immune system.

In conclusion, this study has shown that *Dialium guinenses* (Icheku) is a good substrate for the growth of pathogenic fungi which are infectious and detrimental to human because of

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the diseases they cause such as: damage and weakening of the immune system, to tumors in the human urinary tract, liver cancer, fever, cough, chest pain and breathlessness. From the findings, it is recommended that less of the fruits should be eaten. If we must eat, the sealed pods containing the fruits should be eaten and they must be mould free.

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