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Asian Journal of Plant Science and Research, 2016, 6(1):1-5

ISSN : 2249-7412
CODEN (USA): AJPSKY

Detection of Fungi and Aflatoxin Contamination in Shelved Fruits of *Dialium guineense* Wild, Rivers State, Nigeria

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ABSTRACT

Detection of fungi and Aflatoxin contamination in shelved fruits of Dialium guineense Wild was carried out in the Department of Forestry and Environment, Rivers State University of Science and Technology and National Agency for Food and Drug Administration and Control (NAFDAC) Laboratories. The fruits for the experiments were obtained from three markets in Port Harcourt Metropolis; Choba, Rumuwoji (Mile 1) and Rumukwurushi (Oil mill) and stored in sterile black polyethene bags. The experiment was laid out in a Completely Randomized Design (CRD) with four treatments and three replications. The fungal pathogens isolated from the infected fruits of D. guineense were Aspergillus niger Van Tieghn, Rhizopus microcrosporus var. microsporus Stephen & Mondo, Penicillium purpureum Stolk & Samson and Penicillium minioluteum Dierckx. There was significant difference ($P \leq 0.05$) among the most frequently occurring fungus in the three markets; A. niger (884 ± 2.03-998 mg ± 4.81) followed by R. microsporus var. microsporus (576 ± 0.55-688 mg ± 0.83) P. purpureum (144 ± 0.03-251 mg ± 0.34) and P. minioluteum, (84 ± 0.02-132 mg ± 0.01). The use of Moringa leaf extracts significantly ($P \leq 0.05$) reduced the growth of test fungi when compared to PDA (0% ppm) without leaf extract. The use of biopesticides such as moringa leaf extracts can help in protecting our fruits from pathogen attack. These plant leaf materials are cheaper for the poor-resource farmers and also environmentally friendly. Aflatoxin assay revealed that healthy looking fruit of D. guineense was 3.7 ± 0.021 iμ/kg, infected, 3.8 ± 0.01 iμ/kg and the standard value was 4.0 ± 0.15 iμ/kg. The study has also shown that healthy looking fruits of D. guineense contain some amount of mycotoxins though not in hazardous quantity.

Keywords: Honey, Sucrose, Maltose, Specific gravity, Acid equivalent

INTRODUCTION

The growth of some fungal spp. in fruits and vegetables under conducive environmental conditions results in the production of mycotoxins (CAST, 2003). There are generally three main genera of fungi that produced mycotoxins; Aspergillus, Fusarium and mycotoxins. The detection of mycotoxins in grains shipments can cause rejection by importing countries resulting in a loss in consumer confidence in the importing country and severe economic losses for the exporting country [1]. Mycotoxin affects several agricultural products such as cereals, oil seeds, pulses, root crops, fruits from most developing countries in Africa [2,3]. The toxicological effects of aflatoxins are close-dependent, at high doses. They are lethal if consumed causing liver, myocardial and kidney tissue damage while at low lethal exposure they cause potent human hepatocellular carcinogen [4-6] reported that more than 300 fungal metabolites are toxic to man, animals and pose serious health hazard besides, mycotoxins alter regular metabolism, induced physiological and biochemical changes in host cells resulting abnormal proliferation of plant cells.

Kpodo and Halm [7] and Diop [8] reported that aflatoxin contamination of maize/peanut stored in some silos and warehouses in parts of Ghana revealed that all the samples contained aflatoxins (>30 ng/g). Pollet [9] and Wyers [10] also reported that in Cote D'lvoire aflatoxin studies have been conducted on maize and peanuts of about 360 ng/g. Several studies have been conducted in Nigeria on aflatoxins in food [1] while Opadokun and Ikeorah [11] revealed

that over 20% of 145 maize samples from market in Kano and Plateau State of Nigeria were assayed of aflatoxin B_1. Similarly Aja-Nwachukwu and Emejuaiwe [12] observed that 80% of maize sample from different locations in South Eastern Nigeria were positive for aflatoxins. Therefore it is imperative for the detection of fungal contaminants to ensure safe and high quality fruits of *D. guineense*. The objective of the present study therefore was aimed at isolating the fungal species from infected fruits of *Dialium guineense*, identifying the presence of mycotoxin in the infected fruits of *D. guineense* and evaluating the effects of Moringa leaf extracts on the mycelial growth of test fungi.

MATERIALS AND METHODS

Collection of *D. guineense* fruits

Three thousand (3000) fruits of *Dialium guineense* each were obtained from three markets in Port Harcourt and its environs namely, Choba, Rumuwoji (Mile One) and Rumukwurushi (Oil Mill). The fruits were obtained twice per month and kept in sterile polythene bags in refrigerator for isolation of fungi, and mycotoxin assay in the Department of Forestry/Environmental Laboratory, Rivers State University of Science and Technology, Port Harcourt, Nigeria.

Isolation and identification of fungal

Samples of *D. guineense* from different markets have been screened for isolation of fungal flora following standard blotter paper as well as agar plate technique [13]. The colonies developed from the seeds were counted, isolated and identified after sub-culturing on PDA. The species were identified on the basis of macro- and micro morphology and fungal infestation level authenticated as a percentage frequency of occurrence of mycoflora using the methods of Chukunda et al. [14] and Elenwo et al. [15].

% Frequency of fungi=No. of fruits with fungus/Total no. of fruits assessed × 100

Mycotoxin extraction from *D. guineense* fruits

The mycotoxin extraction and cultification was done at National Agency for Food and Drug Administration and Control (NAFDAC) in Port Harcourt following the methods of Oyo and Adebayo. 15 g of infected fruits of *D. guineense* were transferred to 500 ml Erlenmeryer flasks containing 150 ml of chlroform and placed in a shaker (200 rpm) for 15 h, then filtered through what man No. 1. The chloroform extract was dried over anhydrous sodium sulphate. The remaining samples were dried at 50°C overnight followed by re-extraction by 150 ml of 80% methanol water by chemical detection of mycotoxins. The thin layer chromatography technique was carried out using precoated silica geltype 60, F254 (Merck Germany) the developed plates were then viewed under UV light (366 nm) and sprayed with reagents for identification according to Leito et al. [16].

Effect of moringa leaf extracts on mycelial growth of test fungi

The effect of moringa leaf extract on mycelial growth was carried out using ten Petri dishes containing PDA amended with 20, 60 and 80 (Part per million) of the leaf extracts for each fungus. Each PDA was inoculated with 5 mm of the pure culture obtained from 7 days old cultures of the test fungi. The PDA were then incubated at 27°C for 7 days after which the mycelial growth were measured using transparent ruler and then calculated using the methods of Chukunda et al. [14] and Elenwo et al. [15] to ascertain the percentage inhibition of moringa leaf extract on fungi growth.

Statistical analysis

The experiment was set up in a Completely Randomized Design (CRD). Data were analysized using (ANOVA). Significant means were determined by standard deviation (SD) and Least Significance Difference (LSD).

RESULTS AND DISCUSSION

The results of mycoflora pathogens of *D. guineense* sampled from the three markets Choba, Rumuwoji (Mile one) and Rumukurushi (oil mill) in Port Harcourt Metropolies, Rivers State, Nigeria implicated the following fungi; *Aspergillus niger* Van Tieghn, *Rhizopus microcrosporus* var. *microsporus* Stephen & Mondo, Penicillium purpureum Stolk & Samson and *Penicillium minioluteum* Dierckx (Table 1). However, most of these mycoflora pathogens isolated from the fruits of *D. guineense* have earlier been implicated with fruits decay by some researchers. Arinze [17] reported that some of these fungi, *Aspergillus niger*, *Rhizopus* spp. and *Fusarium* spp. caused serious rotting in tomato fruits. Similarly, Onuegbu [18], isolated *Aspergillus niger* from rotted onions, while Chukunda [14] also found *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Fusarium pallidoroseum*, *Botryodiplodia theobromae*, *Colletotrichum*

gloeosporoides, *Penicillium expansum* and *Botrytis cinera* to be responsible for the serious decay or rotting in avocado pears obtained in the Niger Delta Ecosystem. Adebayo-Tayo et al. [19] and Chuku [20] observed the presence of *Fusarium* spp., *Aspergillus niger*, *Botryodiplodia theobromae* and the *Rhizopus* spp. in African star apple were responsible for the fruit decay. Generally, the fungi organisms isolated from seeds of Vevet tamarind in the present study are known to be spoilage organisms associated with many agricultural products.

In the contrary, Periera et al. [21] and Sutton [22] reported the presence of Bellulcauda dialli in *D. guineense* fruits of Brazil and Sierra Leone. This is not consistent with the present findings.

The result on the effect of total aflatoxin found in the fruits of *D. guineense* indicate that there was no significant difference in the amount of aflatoxin found in both infected and healthy looking fruit (Figure 1). Moses [23] had earlier reported that mycotoxins of Aspergillus and other filamentous fungi were responsible for serious grain deterioration. Also Muhammed et al. [24] in his study implicated aflatoxin contamination as a major source of tomato fruits deterioration. Similarly, Gong et al. [25] in his findings reported that aflatoxin impaired growth in young children, while, Groopman et al. [26] and Hudson et al. [27] isolated aflatoxin from foods consumed in Gambia which caused serious human cancer. However, Alport et al. [28] had earlier investigated on aflatoxin content of food in Uganda. The European Scientific Committee for Food in its report expressed its opinion that aflatoxins are genotoxic carcinogens. EFSA [29] reported that *Mucor pusilus* secreted citrinin and Penetrem-A, *Cladosporium fulvum* produce glycosyl moiety, Cercospora secreted cercosporin. All these toxins are known to create physiological disorders to consumer. Similarly Malik et al. [4] reported that fungi of Deuteromycota secrete toxic metabolites which may be one of the reasons to spoilage of stored fruits/seeds.

Adebayo-Tayo [19] detected aflatoxins B1 and G1 in bush mango seeds with concentrations ranging from 0.2-4.0 ng/g and 0.3-4.2 ng/g respectively. In this study, healthy-looking fruits of *D. guineense* yielded slightly lower values of 3.7 ± 0.02 iμ/kg than infected fruits (3.8 ± 0.01 iμ/kg) of total aflatoxins. Both fell below the standard by NAFDAC of 4.0 ± 0.15 iμ/kg. He further observed that aflatoxin B1 and G1 concentrations ranging from 0.2–4.0 ng/g and 0.3-4.2 ng/kg respectively from shelved bush mango seeds (*Irvingia* spp.) in Akwa Ibom markets as stated earlier. This work supports the finding in this study of aflatoxins in both healthy-looking and infected fruits of *D. guineense*. Ciegler [30] reported that aflatoxin occur in a variety of crops and animal products such as meat, milk and eggs. The production of aflatoxin is affected by several factors which influence the mould growth such as moisture content, relative humidity (RH), temperature, substrate composition and the presence of competing microorganisms.

Table 1. Fungi isolated from *Dialium guineense* fruits sampled from three markets in Port Harcourt metropolis

	Fungi isolates (%)				
Market	***Aspergillus niger***	***Rhizopus microsporus var. microsporus***	***Penicillium purpureum***	***Penicillium minioluteum***	**LSD (P<0.05)**
Choba	884 ± 2.03	688 ± 0.83	238 ± 0.22	132 ± 001	21.07
Rumuwoji (Mile 1)	987 ± 4.12	576 ± 0.55	144 ± 0.03	150 ± 0.06	35.32
Rumukurushi (Oil mill)	998 ± 4.81	620 ± 0.03	251 ± 0.34	84 ± 0.02	43.01
LSD (P<0.05)	21.25	24.00	2.72	2.36	

Table 2. Effect of different moringa leaf extract concentrations and *in vitro* growth of fungal isolates of *D. guineense* fruits (cm) for 20 days.

Organisms	**Leaf extract conc. (ppm)**	**Mycelia growth (mean+SD)**
A. niger	0	8.60 ± 2.05
	20	4.10 ± 1.20
	60	2.00 ± 0.25
	80	0.01 ± 0.02
P. purpureum	0	7.80 + 2.00
	20	3.05 ± 1.02
	60	2.50 ± 0.35
	80	0.20 ± 0.13
P. miniolutum	0	6.30 ± 1.80
	20	4.20 ± 1.28
	60	3.05 ± 1.02
	80	2.00 ± 0.25
R. microsporus var. *microsporus*	0	7.80 ± 2.00
	20	4.30 ± 1.32
	60	2.50 ± 0.35
	80	0.05 ± 0.04

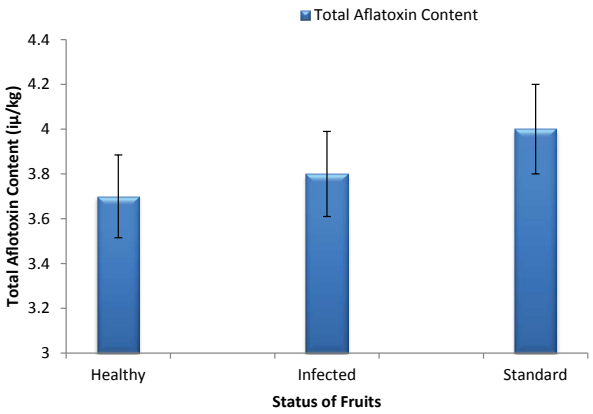

Figure 1: Comparison of trend of changes in babies Apgar in at 1 min and 5 min after birth in the midazolam and propofol groups.

The results of the effect of moringa leaf extract on fungal growth showed significant ($P \leq 0.05$) reduction on *in vitro* mycelial growth of the test fungi (Table 2). Amadioha [31] had earlier screened the use of plant leaf extract in the control of black rot disease of potato caused by *Rhizoctonia bataticola*. Similarly, Ihejirika [32] selected medicinal plants on the post-harvest and biodeterioration of banana fruits. Chukunda et al. [14] also reported that leaf extracts of neem and scent exhibited fungicidal effect on the growth of certain fungi isolated from avocado fruits.

This was in agreement with the previous researchers which showed that percentage inhibition increased with increase in concentration. Ukoima et al. [33] explained that extracts of many higher plants had antifungal properties under laboratory trials. These findings agreed with the present research work. Cuthbertson et al. [34] and Javaid [35] emphasized on the need for alternative environmental friendly use of plant extracts as a measure of controlling plant pests and diseases.

CONCLUSION

In Nigeria, velvet tamarind (*Dialium guineense* Wild) is one of the preferred seasonal fruits especially the South East and South South people. The study has implicated the fruits with aflatoxin comtamination though in a less harmful level. Therefore fruits of *D. guineense* should pass through health regulatory bodies such as NAFDAC to screen and discard infected ones, also to avert mycotoxin production and a good storage method should be encouraged to avoid fungal growth/proliferation.

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