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Moulds and Mycotoxins Associated with Cashew Fruits (*Anacardium Occidentalel.)* Deterioration

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Abstract:
A survey was carried out on moulds and mycotoxins associated with deterioration of the cashew fruits in Keffi, Nasarawa State. The disease survey covered four locations in Keffi. The locations include AngwanLambu, G.R.A, DadinKowa and Cross-3. The four locations were visited four times and a total of 80 cashew fruits were collected and sampled. Forty-five (45) fruits were infected with different fungal diseases while thirty-five (35) were free from fungal infection. The fungal species isolated and identified were Aspergillus flavus, Aspergillus oryzae, Aspergillusversicolor and Rhizomucorpusillus. Their percentages of occurrence were 46.7 %, 20.0 %, 13.3% respectively and are independent of the different locations. G.R.A has the highest percentage of occurrence (46.7 %) and Cross-3 has the least occurrence (13.3 %). Analysis of the nutritional contents of infected cashew fruits shows a reduction in the moisture content, crude protein, and fibre and carbohydrate level compared with the uninfected fruits. The relative humidity of the survey area was very high thereby predisposing the fruits to fungal growth and mycotoxin production. Aspergillus flavuswas the most virulent, while Aspergillusversicolorwas the least virulent. The result showed the occurrence of Aflatoxins B1 in three of the sample fruits with their RF values; 0.51, 0.51, 0.51. Therefore, it is necessary to avert the situation in this area by controlling factors which enhances the spread of the pathogens.

Keywords: *Fungal infection, deterioration, mycotoxin, pathogenicity, fruit, cashew*

1. Introduction

The cashew (*Anacardium occidentale* L.) is a small to medium sized tree (7-20 m length) belonging to the family *Anacardiaceae*. The fruit is a kidney–shaped achene about 3cm long with a hard gray–green pericarp about 0.025 - 0.03m length and 0.02 - 0.025m width. Also, it has a red or yellow pseudo-fruit with dimensions of 0.04 -0.12m length. [20]. originally a native of North South America, it is now widely grown in the tropical climates for its cashew fruits and cashew seeds. The leaves are spirally arranged, leathery texture, elliptic to obviate. The flowers are produced in a panicle or corymbs up to 26cm long. The largest cashew tree covers about 7,500 square meters (81,000sq ft.). The fruits of the cashew tree are accessory fruits (sometimes called Pseudocarp or false fruits). The cashew tree can handle temperatures above 40° C (105° F) well. An average day temperature for growing cashew is around 25° C (77° F) which is mostly ideal. Cashew can be grown either by grafting or planting of seeds and can grow like weeds as long as water is available. They are fairly drought resistant and can grow well even on marginal soil for where other fruit trees would fail. But the best soil for growing cashew is the sandy soils, well drain and free from strong wind. The plants are perennial and they produce

fruits once a year before the onset of rainy season. Although the cashew tree has a well-developed root system and can tolerate drought condition

Blossoming takes place between November and January. A typical cashew tree starts fruiting at the three years of planting, while grafted cashew, fruits within 18 months. The trees are genuinely tropical and very frost sensitive. The cashew trees grow in a wide spectrum of climates regions between the 25^{o} N and S latitudes [1]. The growth and production of cashew trees can be enhanced by establishing clonal orchards, and improving fertilizing and irrigational paction.

Globally, the cashew nuts are esteemed and highly priced food delicacy because of their pleasant taste and flavour. Medicinally, the cashew is a useful tree as different parts of it are used either individually or collectively to treat several diseases [13]. The fresh or hot water extract of different plant part is useful orally as aphrodisiac, anti-dysenteric, anti- Hemorrhagic and externally as anti-inflammatory.

In Nigeria however, despite the cultivation of cashew in plantations and the establishment of cashew processing factories, shortages are still being experienced on the finished products due to fungal attack [23]. All plants weather diseased or healthy is host to a variety of fungi that can be categorized broadly in several biological groups. On the fungi associated with cashew nuts bio-deterioration, 14 fungi belonging to 5 genera were recovered at varying levels [3,23]. Most of the cashew fungi are obligate parasites or biotrophs. These fungi spread from one plant to another and from one location to another in several ways. The pathogens produce spores, either through asexual or sexual reproduction that aid in the dissemination of the fungus. Fungi have serious impacts on the economic, social and ecological realms. They have also led to cashew losses, increased cost of breeding program and scarcity of cashew caused by epidemics of cashew rot [16].

Mycotoxins are toxic secondary metabolites produced by aerobic, mycelial, microscopic fungi, especially from the genera *Aspergillus*, *Fusarium*, and *Penicillium* [7, 8, 10]. They are increasingly recognized as threat to food and nutritional security globally [5]. The risk assessment applied to Mycotoxin research has predicted that the geographical risk area for crop contamination is expanding and there is a need for inclusion of another variable which is the effect of climate change [2, 5, 11, 14, and 15].

2. Materials and Methods

2.1. Study Area

The study was carried out in Plant Science and Biotechnology Unit Laboratory, Department of Biological Sciences, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria.

2.1.1. Survey and Sample Collection

A total of 80 cashew samples were collected from four different locations (20 each), in Keffi Local Government Area of Nasarawa State. All the fruits obtained were stored in a cold temperature at -4^{o} C until when required for the investigation. These locations include: Angwan Lambu, G. R. A, DadinKowa and Cross-3. These selected locations form part of the Guinea Savannah region of Nigeria tropical climate.

2.1.2. Samples Preparation

The cashew fruits that were obtained from the four different locations in Keffi Local Government Area of Nasarawa State were taken to the Laboratory for disinfection. The cashew fruits were washed with tap water to remove dirt. Then put in 70 percent of ethanol for 5 minutes, it was also put in sodium hypochloride solution for 20 minutes and rinsed with distilled water for three times in other to remove all microorganism. These procedures were carried out for all cashew fruits collected from the four different locations [6, 24]. Then the disinfected cashew fruits were kept in a sterilized bottle and covered for fungi growth and development forfive days. After three days, there was fungi structures development on the cashew fruit in form of mycelium and spores indicating that they can be cultured. They were cut into small fragments since the cashew fruit is large and cannot properly enter the plates.

2.1.3. Culturing of Fungal Isolates in the Media (SDA)

To ensure a successful culturing of the fungi isolates, certain aseptic conditions were observed as follows;

- The dip containing the blade holder and forceps contains 70 percent ethanol.
- Spirit lamp flame was also used to flamed these tools for further sterility, before inoculated into the medium, the medium was placed close to the spirit lamp flame before covering. The procedure was repeated for all until culturing was completed. Lastly, the medium was sealed using a masking cello tape to prevent the entry of other microorganisms. The method used in the isolation of these fungi is the direct surface Agar plating method [24]. Finally, the cultured media were kept in an incubator at a temperature of about 37 ^{o} C for three days, for further investigation.

2.1.4. Identification of Fungal Isolates

The identification of fungi was done by studying the cultural characteristics of each of isolate [21, 19]. Five days after which the fungi structures have been well developed in each plate the fungi structures were collected stained with lacto phenol on a slide and then mounted on a microscope for examination. An identification key was used to identify correctly the established fungus on the fruits in each media [4, 9]. The morphological features (keys) used for the identification of fungi species are as follows: Shape of their hyphae, types of conidia, color characteristic of spores. A total

number of four fungi species were isolated from the cashew samples obtained from the four different locations in Keffi Local Government Area, Nasarawa State. The species of these fungi isolated from the cashew samples are:*Aspergillus flavus, Aspergillus oryzae, Aspergillusversicolor, Rhizomucor pusillus*. These four fungi species were further incubated at a temperature of 30^0 C for 12 weeks for Mycotoxin examination [12].

2.1.5. Test for Pathogenicity of Fungal Isolates

To establish that the fungal isolate causes the disease condition (i.e., cashew Fruits deterioration) two fresh cashew fruits were washed with 10 percent (10 %) sodium hypochloride and rinsed in distilled water and allow to dry. A hole 8-10mm in diameter was made on healthy cashew fruits with a cork borer and each isolate inoculated into each hole. The samples incubated for the characteristic symptom to develop. Symptoms of the disease were seen to develop on the fresh fruits (cashew) after five days of inoculation. The isolate was re-isolated and re-identified.

2.1.6. Mycotoxin Extraction from the Deteriorated Cashew Samples

Mycotoxin extraction was carried out using 12 weeks deteriorated cashew samples from which fungi species have been identified. All the four fungal isolates from the four different locations were kept at room temperature (27^0C-30^0C) according to [17,22]. The laboratory procedure involves; crush mill pulverized samples were weighed into 500ml Erlenmeyer flask. Twenty-five milliliters of 1M phosphoric acid and twenty-five milliliters of methylene chloride were added. The Erlenmeyer flask was shaken for 30 minutes in a rotary shaker and contents filtered with 3cm wide rapid filtrate paper in a Buckner funnel. At least 200ml of the filtrate were collected. 50 milliliter aliquots were placed into separate 100ml flask and were evaporated to dryness in a water bath at 60^0C. For assay of Mycotoxin, the dried extracts were re-dissolved in 250ml chloroform, centrifuged for 30 minutes and 10 milliliter vials and evaporated to dryness. The residues were re-dissolved in 200ml benzene and shaken thoroughly for 30 seconds to ensure complete dissolution of toxins.

2.1.7. Chromatographic Running of the Extracted My cotoxins

Pre-coated chromatographic plate (20 cm × 20cm silica gel) were used and each was activated at 110^0C for 30 minutes before spotting, then the stock solutions of standard toxins were thoroughly shaken twice besides the sample spots, 5µl of standard Mycotoxin were spotted at the bottom of the plate. Three samples extracted were spotted.

2.1.8. Detection of Mycotoxins in Spotted Samples

The developing plates were allowed to dry and examined visually under ultra-violet light. The Mycotoxin samples were detected by matching the fluorescence color, intensities and the RF values of the sample spot with those of standard spot. Sample spots whose fluorescence intensities, color and RF value matched those of the standards were presumptively considered positive and were used for confirmation.

2.1.9. Confirmation of Mycotoxins Presence

The TLC plates that were presumptively considered positive were sprayed, firstly dried and viewed again at 365nm to confirm the presence of Mycotoxin detected.

3. Results

During the study, 80 cashew fruits were collected and sampled for fungi species (Table 1). Forty-five 45 fruits had fungi species while 35 fruits sample were without fungi species. The fungal isolates were *Aspergillus flavus, Aspergillusversicolor, Aspergillus oryzae* and *Rhizomucorpusillus*. Their percentage occurrences were 46.70, 20.00, 20.00, and 13.33 respectively (Table 2).

Locations	Number of Locations	Number of Fruits	Total Number of Fruits Collected
AngwanLambu	4	5	20
G.R.A	4	5	20
DadinKowa	4	5	20
Cross-3	4	5	20
Total	20	20	80

Table 1: Specimen of Cashew Fruits Collected for Sampling
G.R.A= Government Reserve Area

Location Cashew Fruits	Total No. of species (+)	No. with fungi species (-)	No. without
A/Lambu	20	12 (15.00)*	8 (10.00)*
G.R.A	20	15 (18.75)*	5 (6.25)*
D/ Kowa	20	10 (12.50)*	10 (12.50)*
Cross-3	20	8 (10.00)*	12 (15.00)*
Total	80	45 (56.25.00)*	35 (43.75)*

Table 2: Incidence of Fungal Isolates with Relation To Locations in Keffi Local Government Area
**Values in Parenthesis Are in Percentage.*
(+) = Positive, (-) = Negative

From the four different locations (AngwanLambu, G.R.A, DadinKowa and Cross-3), G.R.A has the highest incidence of fungal infection (18.75) while cross-3 had the least fungi infection. (10.00). The Pathogenicity test showed that *Aspergillus flavushad the* highest percentage of infection and was observed as the most virulent after 14 days of inoculation, while *Aspergillus versicolor* showed the least virulent traits(Table 3). Proximate compositions showed the percentage of infected and uninfected fruits as each value is a replicate of three measured values. The result also revealed that the nutritional contents of the infected cashew fruits showed a reduction in the crude protein, crude fibre and crude carbohydrate level compared tothe uninfected cashew fruits (Table5).

Fungi Isolates	No. of Cashew fruits	Percentage Infection after Inoculated14 days
Aspergillus flavus	5	100
Aspergillus oryzae	5	80
Aspergillus versicolor	5	20
Rhizomucor pusillus	5	60
Total	20	100

Table 3: Percentage Infection of Cashew Fruits Artificially Inoculated with Fungi Diseased Samples (Pathogenicity Test)

Locations species (negative)	No. without fungi species (positive)	No. with fungi samples	Total No. of
AngwanLambu	12 (11.25)*	8 (8.75)*	20
G.R.A	15 (11.25)*	5 (8.75)*	20
DadinKowa	10 (11.25)*	10 (8.75)*	20
Cross-3	8(11.25)*	12 (8.75)*	20
Total	**45**	**35**	**80**

Table 4: Chi-Square Analysis on the Incidence of Fungi Species in Different Locations in Keffi Local Government Area
**Number in Parentheses Is the Expected Frequencies*

There was no significant difference (P< 0.05) in the incidence of different isolates in relation to locations.
The Mycotoxin result of the 12-week deteriorated cashew samples showed the multi-thin layer chromatographic analysis of benzene acetonitrile extracts of fungi infested fruits compared with that of standard Mycotoxin (Table 6). Extract of the four different cashew samples gave different colors under ultra-violet light with RF values; 0.51, 0.51, 0.51 and 0.10. Under the ultra-violet light at 365nm, standard Aflatoxins B1 fluoresced.

Cashew fruits Condition	Moisture content	Crude protein	Crude fat	Crude fibre	Crude ash	Crude carbohydrates
Uninfected	84.00	0.10	0.05	6.75	2.00	7.10
Infected	89.30	0.01	0.05	4.50	2.00	4.14

Table 5: Proximate Composition (in %) of Cashew Fruits
**Each value is a replicate of three measured values*

Samples	Moulds	RF values	Mycotoxin Detected
1Aspergillus flavus	0.51	Aflatoxins	B1
2 Aspergillus oryzae	0.51	Aflatoxins	B1
3 Aspergillus versicolor	0.10		Not detected
4Rhizomucor pusillus	0.51	Aflatoxins	B1

Table 6: Mould Growth and Mycotoxins Extracted from Cashew Fruits after 12 Week Inoculation

**RF = Retention Factor of Standard Mycotoxins Availability in A Sample*

4. Discussion

This study revealed a range of mycotoxic fungi are associated with the deterioration of cashew fruits in Keffi Local Government Area of Nasarawa State, Nigeria. High relative humidity and temperature was observed in the locations surveyed; this may be responsible for the fungal infection of the fruits. This agrees with the findings of [18] that very high relative humidity may have aided in the spread of the organisms in these areas. This study revealed that there was high incidence of *Aspergillus flavus* in the different locations when compared to others fungi isolates in the same locations. Also, the non-detection of fluorescence in one of the fungal infected sample (*Aspergillusversicolor*) indicates the absence of any mycotoxins in the sample.

5. Conclusion

Cashew fruits are naturally perishable under unfavorable conditions therefore caution should be taken to ensure that they are handled properly at the field and during storage so that the infestation of fungus on this plant via natural opening and injuries inflicted on the plant are avoided to the barest minimum. Farmers should have better understanding on the farming system suitable for cashew production for proper disease management (usually plantation). The use of proper chemical control against fungus causing deterioration on fields and store cashew crops should be enhanced and the adoption of natural cooling method during preservation of the cashew fruits can help farmers to control the growth of fungus as most of them are thermophilic.

6. References

i. Alexander, H.T. (2008). *A Nutty Chemical' Chemical and Engineering News*.86(36):26-27.

ii. Baranyi, N. Kocsubé, S., &Varga, J. (2015). Aflatoxins: Climate change and biodegradation. Curr.Opin.*Food Science*. 5, 60–66. 237.

iii. Bennert, J.W. (2010). An over view of the Genus*Aspergillus*: Molecular Biology Genomics, Caister Acad. Press, London, pp 89 – 90.

iv. Cheesbrough, M. (2000). District Laboratory Practicein Tropical Countries Part 2, Cambridge University Press, Cambridge. P. 47-54.

v. Fabio, G.C., Mauricio R.S., & Daniela, J.V.(2018).Mycotoxin Contamination of Beverages Obtained from Tropical Crops, 83, 1-36.

vi. Hamed, B., Amacbu, H. B.,&Fasse, S. (2010). *Bacillus pumitus,*A new pathogen on Potato Tubers Storage in Mali. *AfricanJournal of Microbiology Research* Vol. 4 (20), pp. 2068-2071.

vii. García-Moraleja, A, Font, G.; Mañes, J.,& Ferrer, E. (2015). Analysis of mycotoxins in coffee and risk assessment in Spanish adolescents and adults.*Food Chemistry Toxicology*, 86, 225–233.

viii. García-Moraleja, A.; Font, G.; Mañes, J., Ferrer, E. (2015).Simultaneous determination of mycotoxin in commercial coffee.*Food Control*, 57, 282–292.

ix. Ibrahim, S.,&Rahma, M.A. (2009). Isolation And Identification Of Fungi Associated With Date Fruits (*Phoenix dactylifera*, Linn.). *Journal of Pure and Applied Sciences*, 2(2): 127 – 130.

x. Ismaiel, A.A., &Papenbrock, J. (2015).Mycotoxins: Producing fungi and mechanisms of phytotoxicity. *Agriculture*, 5, 492–537.

xi. Jagger, J.W.H. (2018). Feed the Future Innovation Lab for the Reduction of Post-Havest loss. *Mycotoxin Book of Abstract*. Pp 27-28. Sansas, State University, USA.jjharvey@KSU.ed

xii. Klich, M. A. (2002). Identification of Common *Aspergillus* Species, CBS, Netherlands.Medicinal Plant Research, 2018, Vol. 8, No. 1 doi: 10.5376/mpr.2018.08.0001.

xiii. Koehler, F.E. (1887). Medicinal plant.Vol.1.Published by Gera East-Central German City South Leipzig.

xiv. Medina, A., Rodríguez, A.,&Magan, N. (2015). Climate change and mycotoxigenic fungi: Impacts on mycotoxin production. Curr.Opin.*Food Science*. 5, 99–104. 236.

xv. Medina, A., Akbar, A.,Baazeem, A., Rodriguez, A., &Magan, N. (2017). Climate change, food security and mycotoxins: *Fungal Biology*. 31, 143–145.

xvi. Morton, J. (2006). 'Avoid Failures and losses in the cultivation of cashew.*Economic Botany*. 26(3) 1972 – 1974.

xvii. Mohammed S.A. (2012). Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from Saudi Arabia .*Journal of American Science*, 8(12).

xviii. Ogaraku, A.O., Abdulkarim, B.M., Jerry, E.A. &Iyonmahan, I.R. (2012). Fungi associated with deterioration of Cassava (*ManihotesculentaCranta*) tubers in Keffi, Nasarawa State, Nigeria. NasaraScientifique: *Journal of Natural and Applied Science*, 2(1): 81-86.

xix. Oladele O.O., &Fatukasi, O.I.(2018). Control of Postharvest Decay on Cashew Fruit (*Anacardium occidentale* L.) with Aqueous Extract of Cashew Leaf.

xx. Perozo, A., Ramírez, M., Gómez, A.Y., &Buitrago, N. (2006).Germinación y características morfológicas de plántulas de merey (*Anacardium occidentale* L.) tipoamarillo.) Revista de la Facultad de Agronomía (Universidad del Zulia), 23 (1), 134-140. 235.

xxi. Pitt, J.I., & Hocking, A. D. (2009): Fungi and food spoilage. Third Edition.Spinger Science Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from Saudi Arabia Business Media.Pp. 519 .Humboldt kellog Annual Agric. Conference 71– 73.

xxii. Suleiman M.N., & Taiga, A. (2009). Efficacy of aqueous extracts of neem, and sharf for the control of fungi associated with milled and unmilled stored rice.

xxiii. Suleiman M.N. (2010). Occurrence and distribution of fungi associated with bio-deterioration of cashew nuts in the eastern senatorial district, Kogi State.

xxiv. Silue N., Soro S., Kone T., Abo K., Kone, M., &Kone, D. (2017).Parasitical Fungi in Cashew (*Anacardium occidentale* L.)Orchard of Cote d`Ivoire.*Plant Pathology Journal*. 16(2): 82-88. Nigeria. *Archives of Applied Science Research*, 2 (5): 462-465.