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Mycoflora associated with *brachystegia eurycoma* (Achi) seeds and their effects on the biochemical properties of the seed

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Abstract
Mycoflora associated with Brachystegia eurycoma (Achi) seeds and their effects on the Biochemical properties of the seeds were investigated. The fungi pathogen implicated were Aspergillus Niger (68%), Aspergillus flavus (30%), Aspergillus nidulans (40%) Rhizopus stonifer (60%), Alternaria altermata (10%). All the fungal isolates were found to be pathogenic to boiled healthy seeds of B. eurycoma. The results of proximate analysis of fungal infected and non-infected seeds (control) carried out revealed that there was an increase in moisture content from 15.30% in the healthy seeds to 19.30% in the infected sample. Protein content had also increased from 6.80% in the healthy seeds to 18.50% in the infected seeds. Meanwhile the following parameters were found to be decreased in the infected than in the healthy B. eurycoma seeds, *Viz* Ash 1.50%, Fibre 1.20%, Lipid 9.80% and Carbohydrate content 49.80%. Mineral analysis results showed that there was a significant increase in phosphorus content (3.20% to 25.2%) in infected seed relative to healthy ones. Calcium and Iron content had also increased, though not significantly. Sodium, Potassium and Magnesium content had decreased in infected seed sample, however the difference was not significant. All the Plyto chemical content measured; Tannin, Total oxalate, Saponins, Hydrogen cyanide increased in the infected seed sample compared to the healthy ones.

Keywords: Mycoflora, brachystegia eurycoma, seeds, proximate composition, phytochemical

Introduction

Brachystegia eurycoma belongs to fabaceae family. In Nigeria, they have different names; In Edo State, it is known as Okuen, Cross River State calls it Okung and in the Igbo speaking State, it is known as Achi. It is a less important legume, used to lessen the problem of malnutrition in developing countries (Ikegwue *et al*., 2010). It is an economic tree crop with large twisted branches that grows up to 35m tall (Oyen 2012) [22]. It is used as shade for ornamental tree, especially in the dry season. The seed is rich in protein and carbohydrate and it flowers from April-May and matures from September – January (Oyen, 2012) [22]. The plant has flat brown edible seed that are disk-shaded, 2cm in diameter. When mature, their seeds are thrown out of their pods explosively. The seeds are spicy and consumed as condiment. They are used to increase the viscosity of soup, (soup thickner) in Nigeria. It is also used to make food wrappers and gums. According to researchers, it helps in maintaining heat within the body when consumed, in other words, it is good source of nutrient and helps in the control of the body temperature. (Onimawo and Egbekun 1998) [21]. B. eurycoma is one of the underutilized food crops in Nigeria. Most often, cocoyam and melon are popularly used in southern Eastern Nigeria as soup thickner. Their high carbohydrate contents and oil contents respectively could increase one's risk of weight gain. On the other hands, B. eurycoma which could be used as a substitute because of its relatively high protein is most often neglected the tree is used as food and medicine. Exudates from the stem are used as antibacterial for the treatment of wounds and infections. Then the seed tincture has useful anti- inflammatory compounds. When the seeds are sundried and stored in the same container with other spices and stimulants or exposed in the markets, it is liable to harbor propagules of many common fungi associated with post – harvest deterioration and toxin production since humid climate favour fungi. Recent studies have shown that post-harvest commercial B. eurycoma are laden with several fungi species Ikechi-Nwogu and Chime (2017) [15]; Emiri and Chuku (2017) [10], some of which are known to produce mycotoxins, making their consumption a potential health hazard. This study is aimed at identifying fungal species responsible for the spoilage rot of B. eurycoma seeds as well as investigating the effects of the fungal isolates on phytochemical, proximate and mineral composition of B. eurycoma seeds

Material and Methods

Collection of Samples

Boiled healthy and partially infected B. eurycoma (Achi) seeds were purchased from oil mill market in Port Harcourt, Rivers State, Nigeria and taken to the plants pathology laboratory for further studies.

Media Preparation

The medium used for fungal isolation was the Sabouraud Dextrose Agar (SDA). This was prepared by weighing 32.8g of Sabouraud Dextrose Agar (SDA) into a 500ml conical flask. Distilled water (500ml) was added into the flask with a measuring cylinder amended with chloramphenicol and stirred to homogenize. The mouth of the conical flasks was plugged with sterile cotton wool and wrapped with foil. The conical flask with its contents was autoclaved for 15 minutes at 121^0C at $1.1kgcm^{-3}$

pressure. Sterile Petri dishes were prepared and the mixture dispensed into them while still hot and allowed to solidify.

Isolation and Identification of Fungi

Five partially infected seeds of B. eurycoma used were washed in tap water, and surfaced sterilized with 5% Sodium hypochloride and rinsed twice in sterilized water after which they were asceptically plated into chloramphenical-amended SDA in petri dishes equidistantly, in triplicate. The inoculated plates and their contents were incubated for 7 days at room temperature of 28± 2°C. Pure culture of fungi growing in mixtures was obtained thereafter. Pure cultures of the isolates were made after series of isolation. The fungi were later identified based on colour, spore morphology and the nature of the mycelia according to the key of olds (1983) [19].

Mean Percentage Incidence of Fungi

The mean percentage incidence of fungi was calculated using the formula:

$$\text{Mean percentage} = \frac{\textit{Total number of occurence of a particular fungi}}{\textit{Total number of plated sample}} \times \frac{100}{1}$$

Pathogenicity Studies

Healthy samples of B. eurycoma were washed in tap water and surfaced sterilized in 5% sodium hypochloride. Axenic cultures isolated from infected seeds were aseptically inoculated onto the healthy seeds on damp blotter papers in petri dishes and incubated at room temperature of 28 ± 2°C for five days. Petri dishes containing seeds of B. eurycoma without the fungal isolates served as control. The methods of Agrios (2005) [2] and Trigiano *et al*., (2004). The set up was monitored regularly for fungal growth. At the end of this period, morphological characteristics and growth pattern observed in each one were compared with the ones of the original isolates.

Determination of Phytochemical, Mineral and Proximate Composition of Healthy and Infected Seeds of B. eurycoma

Healthy samples of B. eurycoma were taken to the laboratory for the determination of their proximate compositions comprising of Ash, Moisure, Fibre, Lipid, Carbohydrate and Protein, as well as their mineral contents and phytochemical. These parameters were determined according to the method of association of Official Analytical; chemists (AOAC, 1990) [3]. Similarly B. eurycoma seeds inoculated with Rhizopus stolonifer, Aspergillus flavus, Aspergillus Niger, Alternaria altermata collectively as combined isolates were also sent to the food science and Technology laboratory to ascertain the effect of phytochemical, mineral and proximate composition of the seed. Data obtained were subjected to statistical analysis.

Table 1: Mean Percentage Incidence of Fungi Isolated From Brochystegia Eurycoma Seeds

S/n	Fungal isolates	Boiled seed (b. Eurycona)
1	Aspergillu niger	68.0 ± 0.44
2	Aspergillus Flavus	30.0 ± 0.22
3	Aspergillus nidulans	40.0 ± 0.53
4	Rhizopus stolonifer	60.0 ± 0.75
5	Alternaria altermata	10.0 ± 0.32

P > 0.05

The results revealed Aspergilus Niger (68%), Aspergilus flavus (30%), Aspergillus nidulans (40%) and Rhizopus stolonifer (60%) were isolated from boiled seeds of B. eurycoma.

Proximate composition of healthy and infected seeds of B. eurycoma is presented in Table 2.

Table 2: proximate composition of healthy and infected seeds of b. eurycoma

S/n	Parameter	Values% healthy Sample	Values% infected sample
1	Moisture	15.30± 0.21	19.3±0.06
2	Ash	3.85± 0.36	1.50 ± 0.02
3	Fibre	3.50 ± 0.54	1.20 ± 0.01
4	Lipid	16.90 ± 0.18	9.80 ± 0.36
5	Carbohydrate	53.64 ± 0.72	49.80 ± 0.40
6	Protein	6.80 ± 0.58	18.50 ± 0.15

P > 0.05

Results of the proximate composition showed an increase in the values of moisture and protein in infected seeds sample while Ash, Fibre. Lipid and Carbohydrate decrease in infected sample.

Table 3: Mineral contents of healthy and infected seeds of b. Eurycoma

S/N	Parameter	Values % Healthy Sample	Values % Infected Sample
1	Calcium	2.13 ± 0.50	7.30 ± 0.26
2	Phosphorus	3.20 ± 0.12	25.2 ± 0.29
3	Sodium	0.44 ± 0.05	0.20 ± 0.05
4	Potassium	4.11 ± 0.58	3.00 ± 0.30
5	Iron	0.04 ± 0.02	0.25 ± 0.12
6	Magnesium	0.91 ± 0.42	0.25 ± 0.18

P > 0.05

Mineral contents revealed an increase in the values of calcium, phosphors and iron in infected seeds relative to the healthy ones. While sodium, potassium and magnesium decreased in values in infected seed relative to the healthy ones.

Table 4: Phytochemical Contents of Healthy and Infected Seeds of B. eurycoma

S/N	Parameter	Values% Healthy Sample	Values% Infected Sample
1	Tannin	0.03 ± 0.01	0.50 ± 0.10
2.	Total oxalate	0.45 ± 0.05	5.37 ± 0.25
3.	Saponins	0.55 ± 0.02	2.00 ± 0.05
4.	Hydrogen cyanide	0.30 ± 0.03	2.85 ± 0.27

P > 0.05

All the phytochemical contents were seen to increase in value in the infected seeds relative to the healthy ones.

Discussion

Pathogen Identification

The result of fungal isolates presented in Table 1, implicated five organisms namely Aspergillus Niger, Aspergillus flavus, Aspergillus nidulans, Rhizopus stolonifer, Alternaria altermata. The pathogenic ability of these organisms proved positive as they were able to cause spoilage when inoculated with fresh healthy seeds of B. eurycoma. The high percentage incidence of fungi (Aspergillus Niger, (68%), Rhzopus stalonifer, (60%) and Aspergillus nidulans (40%) was not unexpected, because of the moisture content of the boiled seeds which encourage fungal infection. Fungi thrive in moisture. More so, fungal infection on plants and plant products are greatly influenced by factors such as handling, transportation and processing methods (Chuku *et al*., 2009). The result from this study agrees with Ikechi Nwogu and Chime (2017) [15]. Emiri and Chuku (2017) [10] who isolated Aspergillus, Penicillium and Rhizopus spp from B. eurycoma. Similarly, Gbarabe *et al*., (2014) [11] isolated Aspergillus species from the same seed, which confirms the findings from this study. It is important to note that the climatic conditions prevalent in an open market has been reported to favour the survival of some fungi isolated from other crops (Etebu and Emiri 2016).

Proximate Analysis Composition

The result of proximate analysis of fungal infected seeds of B. eurycoma showed that there was an increase in the Moisture and Protein content of the fungal infected seeds relative to the healthy ones control, while there was a decrease in the Ash, Fiber, Lipid and Carbohydrate content of the fungal infected seeds relative to the healthy ones (control), as presented in Table 2. Moisture content increased from 15.30% in the healthy seed sample to 19.3% in the infected sample, protein increased from 6.80% in the healthy seed sample to 18.50% in the infected seed. This observation agrees with the findings of Umana *et al*., (2014) [6] who reported an increase in moisture of fungal infected beans of cocoa. It could therefore be deduced that the relative increase of moisture and protein in the infected seeds may be caused by the digestion, degradation and dissolution of the seed tissue into a mush (water rot) by the pathogens. These degradation activities by pathogens might have also resulted to the relative reduction in the Ash, Fibre, Lipid and Carbohydrate contents of the infected seeds. The Lipid, Fibre, Carbohydrate and Ash might have been broken down by the fungi into smaller molecules that they absorbed (Nweke and Ibiam, 2012) [17]. Bonner (1997) [4] reported that complex molecules such as polysaccharide and protein are required by fungi to build the hyphal wall (chitin, glucan and cellulose) and for respiration to obtain energy. This suggests that these pathogens might have denied man of these essential nutrients upon consumption through their degradation activities, thereby causing some great damaging effects on human health. While the following parameters were found to decrease in the infected than in the healthy seeds. They include Ash 1.50%, Fibre 1.20%, Lipid 9.80%, Carbohydrate 49.80%. Earlier research by Umana *et al*., (2014) [6] showed a decrease in Ash, Fibre and Lipid content of fungal infected cocoa seeds. The result from this study confirms their assertion. The decrease in the ash content may be due to the infecting fungal that have used up part of the mineral content of the seed. The findings from this study is also comparable to the work Onifade and Agboola (2002) who evaluated the effect of fungal infection on proximate nutrient composition of coconut (Cocos nucifera) fruit and found that protein content increased in the fungal infected fruits while carbohydrate, fibre and ash content decreased in fungal infected fruits relative to the healthy ones. The protein content of the healthy seed sample 6.80% was significantly lower than 14.48% reported by Igwenyi and Azoro (2019) on B. eurycoma and similar seeds from the open market. The carbohydrate content of the healthy seeds 53.64% was lower than the report of Igwenyi and Akubugwo (2010); Igwenyi and Azoro (2014) on seeds of B. eurycoma. The value was comparable to 60.17% in B. eurycoma and 51.03% in Dracaena fragrancen (Ukpo/Ibaba) reported by eddy and Udoh (2005). The value of lipid content 16.90% (healthy seeds) negates the result of Igwe and Azoro who reported 7.91% in B. eurycoma seeds, however the result agrees with the report of Uhegbu *et al*., (2009) [24] who reported 16.5% lipid in the same seed. These variations in the oil content may be attributed to differences in climatic conditions, soil properties, average rainfall, freshness and storage conditions/time of the seeds. The fiber and Ash contents 3.50 and 3.85% respectively in healthy seeds agrees with the findings of Igwenyi and Azoro (2014) who reported 3.3 and 4.30% for fiber and ash respectively. Fibre regulates bowel actions and may help to guard against colon and rectal concer as well as diabetes. Fiber supplements or fibre-rich foods may function as normal dietary agents by modulating the digestive and absorptive process (Okaka *et al*., 2006) [18]. They are very important in promoting a range of physiological effects, including increased fecal bulk, water holding capacity, absorption of organic molecules such as bile acids, cholesterol and toxic components (reduced bile acid and plasma cholesterol levels), reduction of minerals and electrolytes (Igwenyi 2008) [13].

Mineral Composition

The result of the mineral; analysis of fungal infected seeds of B. eurycoma revealed that there was a significant increase in the phosphorus and calcium content of the fungal infected seeds relative to the healthy ones (control), while there was decrease sodium, potassium and magnesium content of the fungal infected seeds relative to the healthy ones (control). However, the difference was not significant, as seen in Table 3. Phosphorus content increased from 3.20% in the healthy seeds sample to 25.2% in the infected seed sample, calcium increased from 2.13%

in the healthy seeds to 7.30% in the infected seed. However, the following parameters were found to decrease in the infected than in the healthy seeds. They are sodium 0.20%, potassium 3.00%, magnesium 0.25%. The result of mineral content of healthy seed samples is comparable to the findings of Emiri and Disegha (2017) [9] on the comparative studies on proximate, mineral and mycological analysis of Citrullus Colocynthis (melon). Minerals are essential elements that exist in non-organic form and are normally required in small amounts, hence they, like vitamins are tagged micro-nutrients (Underwood, 1997). Calcium plays a role in supportive structures of the body and its dietary deficiency together with phosphorus and vitamin D causes rickets in children, osteoporosis and osteomalecia in adults in organic phosphates are necessary in the generation of the energy currency of the body (ATP) (Voet and Voet, 2004) [26].

Phytochemical Content

The values of all the Phytochemical (anti-nutrient) analysed showed an increase in the fungal infected seed compared to the healthy ones (control). The difference however, was not significant as presented in Table 4. The following parameter decreased in the infected than in the healthy seeds. Tannin 0.50%, total oxalate 5.37%, Saponins 2.00% and Hydrogen cyanide 2.85%. It therefore suggests that the infecting fungi increased anti-nutrient composition. Tannis are known to process health benefits where in they are 15-30 times more efficient in free radical quenching activities than trohox and other simple phenolic (Hurnel *et al*., 1999). They have also been shown to play very significant roles in human medicine and treatment of ailments (Addae-Mensah, 1992) [1].

Conclusion

B. eurycoma has an appreciable yield of carbohydrate that serves both as thickener and fuel source for the generation of energy currency of the cell. The variations in the values of Carbohydrate, Lipid, Fibre and Ash contents Fungal infected seed related to the healthy ones (control) shows that Fungi infection contribute to depleting the values. Fungi infection is also adjudged for increase in the anti-nutrient contents. The percentage incidence of fungi as revealed from this work implies that boiled seeds of B. eurycoma sold in the open market is a good substrate for the growth of pathogenic fungi, most of which are known to produce mycotoxin, which in turn is detrimental to human health because of the associated disease. It is therefore imperative to increase public healthy awareness and to develop suitable management practices of food condiments in order to safe guard the health of the consumers.

References

1. Addae-Mensah I. Towards a Rational Scientific Basis for Herbal Medicine. A Phytochemist's two Decades Contribution. An Inaugural Lecture Delivered at the University of Ghana, Legion, Ghana University Press Accra, 1992, 63.
2. Agrios GN. Plant Pathology, 5th edition Elsevier Academic Press U.S.A, 2005, 383-557.
3. AOAC. Official methods of analysis, 13th edition. Association of Official Analysis Chemists Washington, D.C, 1990.
4. Bonner J. Vitamin B1, a Growth Factor for Plant Science. 1997; 85:183-184.
5. Chukwu EC, Osakwe JA. Munonye INC. Mould growth in rice (Oriza sativa) as influence by brand. International Jornal of Agriculture. 2009; 1:76-82.
6. Umana EJ, Ishoro AP, Okay EN, Akpan JB, Effiong PF. Mycoflora Associated with Cocoa (Theobrema Cocoa) pods obtained in the Field and their Effects on Seed Nutritional Contents. Journal of Agriculture and crop Research. 2014; 2(12):236-241.
7. Ebimieowei E, Emiri U. Post-Harvest quality of commercial irvingiakernels and the potential use of ocimumgratissimum (scent leaf) against fungal spoilage. Research journal of food science and quality control. 2016; 2(1):20-24.
8. Eddy NO, Udoh CL. Proximate evaluation of some soup thickeners. Chemclass Journal. 2005; 2:12-14
9. Emiri UN, Disegha GC. Comparative Studies on Proximate, Mineral and Mycological Analysis of Coated and Decoted seeds of Citriullus colocynthis (melon). The International Journal of Science and Technology. 2017; 5(12):28-32.
10. Emiri UN, Chuku EC. Seed Borne Fungi and Biochemical Assesment of Raw and Boiled Brachystegia eurycoma (Achi) Seeds used as Soup thickner on Port Harcourt, Nigeria. Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences. 2017; 4(4):21-28.
11. Gbarabe R, Daye B, Etukudo N. Effects of Relative Humidity on Pathogenicity of Mucuna sloanei by Fungi. Sunmes Journal Organization for Scientific and Engineering Research. 2014; 7(2):30-42
12. Hurrel RF, Reddy M, Cook JD. Inhibition of Non-Iron Absorption in Man by Polyphenolic Containing Beverages. British Journal of Nutrition. 1999; 81:289-295.
13. Igwenyi IO. Biochemistry; an introductory approach. Willyrose & Applessed Publishing Coy. Leach Road Abakiliki, Ebonyi State, Nigeria, 2008.
14. Igwenyi IO, Akubugwo EI. Analysis of four seeds as soup thickeners in the south eastern parts of Nigeria. Conference proceeding of 2010 International Conference on Chemistry and Chemical Engineering (ICCCE, 2010), Kyoto, japan, 2010, 426-430.
15. Ikechi-Nwogu CG, Chime H. Fungal contamination of two food condiments (Brachystegiaeurycoma and Detariummacrocarpum) sold in some local markets in Rivers State. Journal of advances in biology & biotechnology. 2017; 14(1):1-7.
16. Ikegwu JO, Okechukwu PE, Ekumankana EO. Physio-chemical and pasting characteristic of flour and starch from Achi (Brachystegiaeurycoma) seed. Journal of Food Technology. Published by Medwell Journals. 2010; 8(2):58-66.
17. Nweke CN, Ibiam OFA. Studies on Pre and Post-Harvest Fungi Associated with the Soft Rot of the Fruit Anona muricata and their Effect on the Nutrient Content of the Pulp. American Journal of Food and Nurition. 2012; 2(4):78-55.
18. Okaka JC, Akobundu ENT, Okaka ANC. Food and Human nutrition, and integrated approach, OCJ. Academic Publishers, Enugu, Nigeria, 2006, 135-368.
19. Olds RJ. A colour Atlas of Micro Biology. 5th Edition Wolf Medical Publication Limited, London, 1983, 213.

20. Onifade AK, Jeff-Agboda VA. Effect of Fungal Infections on Proximate Nutrient Composition of Coconut (Cocos nucifera Linn) Fruit. Journal of Food, Agriculture and Environment. 2002; 10(2):30-33.
21. Oninawo A, Egbekun MK. Comprehensive Food Science and Nutrition. Revised Edition. Ambik publishers, Benin City, 1998.
22. Oyen LPA. Brachystegiaeurycoma. Records from plant resources of tropical Africa. (PROTA) Wageninngen, Netherlands, 2012.
23. Triangiano RN, Windha MJ, Windham AS. Plant pathology concept and laboratory exercise (RC Press. USA, 2004, 345-359.
24. Uhegbu FO, Onwuchekwa CC, Iweala EJ, Kanu I. Effects of processing methods on Nutritive and Anti-nutritive properties of seeds of Brachystegiaeurycoma and Detariummicrocarpum from Nigeria. Pakistanian Journal of Nutrition. 2009; 8(4):316-320.
25. Under Wood EJ. Trace Elements in Human and Animals Nutrition. 4th Edition. Academic Press New York, 1997, 41-42.
26. Voet D, Voet JG. Biochemistry. 3rd Edition. John Willey and Sons Inc., USA, 2004.