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Research Article

Effect of Fungal Pathogens on the Nutritional Qualities of Kola Nuts (*Cola nitida*)

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Abstract

This study was conducted to evaluate the effect of fungi on the nutritional quality of *Cola nitida* (also known as kola nuts) collected from two States in Nigeria namely: Ondo and Osun. Samples of apparently healthy nuts (AHN) and infected nuts (IN) were collected from the two States and subjected to proximate and mineral analyses. Fungi associated with the AHN and IN were isolated and identified. Morphological characterization of the isolates was carried out using fungi identification guides. The result showed that twelve fungi were associated with at least one of the two States. Six fungi: *Aspergillus niger* (*A. niger*), *A. flavus, A. fumigatus, Fusarium oxysporum* (*F. oxysporum*), *Lasiodiplodia theobromae* (*L. theobromae*) and *Rhizopus stolonifer* (*R. stolonifer*) were commonly associated with kola nuts from the two location; three fungi: *Botrytis cinerea, Colletotrichum gloeosporioides* (*C. gloeosporioides*) and *C. lindemuthianum* are specific to kola nuts from Ondo State while *Fusarium solani* and *Penicillium expansum* were peculiar to kola nuts from Osun State.

F. oxysporum (25%) and *C. lindemuthianum* (24%) had the highest percentage occurrence for IN and AHN respectively. Fungi infection on the kola nuts caused loss of 44.88% and 44.16% crude protein on nuts from Osun and Ondo States respectively. In addition, AHN from Osun had significantly higher crude fat and crude fibre than its Ondo counterpart while infected Osun nuts had the least crude fibre (4.1%).There was significantly lower mineral nutrients in all IN than their healthy counterparts from the two locations. Care must be taken while harvesting, handling, storing and transporting kola nuts to minimize the prevalence of fungi which induces rot.

Keywords: Kola Nut; Rot; Proximate Composition; Mineral

Abbreviations

A. niger: Aspergillus niger; A. flavus: Aspergillus flavus; C. nitida: Cola nitida; C. gloeosporioides: Colletotrichum gloeosporioides; C. lindemuthianum: Colletotrichum lindemuthianum; R. stolonifer: Rhizopus stolonifer, L. theobromae: Lasiodiplodia theobromae; F. oxysporum: Fusarium oxysporum

Introduction

Cola nitida (commonly called kola) is a specie of plant belonging to the *Sterculiaceae* family, it is a plant native to tropical West Africa [1], having about 125 species of trees native to the tropical rainforests of Africa. *Cola nitida* and *C. acuminata* are the two most commonly cultivated species, of this two *C. nitida* is the most acceptable of the cola species for social and traditional uses.

C. nitida is cultivated from Senegal to Nigeria, in the West Indies and South America [2]. In West Africa forest areas, kola is second in importance to the oil palm as an indigenous cash crop. It has therefore been an important article of international trade in many parts of Africa [3]. The nut from *Cola nitida* is a very important item used in social and ceremonial activities by Africans. It contains about two percent caffeine and is chewed by many people as a stimulant [4]. The nut has many usages, for the production of soft drinks, wines, beverages and candies [5]. The caffeine in the nuts also acts as a bronchodilator, expanding the bronchial air passages, hence kola nuts are often used to treat whooping cough and asthma [3]. The nuts are source of antioxidant and contain a wide array of complex secondary plant metabolites such as *theobromea, D-catechin, L-epicatechin* and *kolatine* [4].

Preserving the freshness of kola nuts to prevent fungi growth in storage has been a delicate and laborious task, which most traders seek to solve. Most kola nut traders control spoilage by removing infested nuts at intervals during the storage period [2]. This method does not control fungi which spread rapidly in the nuts, and most traders and consumers do not discard fairly mouldy (fungi infected) nuts. A study conducted by [6] in South Eastern Nigeria revealed the presence of *Fusarium oxysporum, Penicillium italicum, Aspergillus niger, Aspergillus tamarii, Rhizopus stolonifer, Rhizopus arrhizus, Aspergillus ochraceous* and *Mucor mucedo* in apparently healthy kola nuts and these fungi have been observed to be associated with spoilage of the nuts in storage. This might affect the nutritional quality of the kola nuts. A loss of up to 50% per ton due to storage mould had been recorded in poorly stored kola nuts [1]. In view of these, this research is aimed at evaluating the effect of fungi infestation on the nutritional and biochemical qualities of kola nuts.

Materials and Methods

Sample collection and separation

Samples of kola (*Cola nitida*) nuts were randomly collected from local markets in Atakunmosa East (7030'N 4049'E), Atakunmosa

West (7035'N 4040'W) and Odo Otin (80 01'N 4042'E) Local Government Area (LGA) in Osun States while Ile-Oluji (7013'N4052'E) and Ifedore (7023'N 504'E) LGA in Ondo States. Apparently healthy nuts (AHN) and infected nut (IN) were collected from all the locations. AHN obtained from each State were bulked together as well as the IN.

Isolation of fungal pathogens

Fungi were isolated from the both the apparently healthy nuts (AHN) and infected nut (IN) samples. The nuts were cut into small pieces of about 3 mm using a sterilized scalpel, the cut samples were surface sterilized in 2% sodium hypochlorite for 2 minutes and then rinsed in five changes of sterile distilled water and blotted dry on sterile filter paper The samples were inoculated on freshly prepared potato dextrose agar augmented with streptomycin to suppress any bacterial growth. The PDA plates were incubated at 28 ± 2°C in the incubator. Colony growth was subsequently subculture into freshly prepared PDA until pure culture was obtained. Characters of the emerged colony and conidia were observed at 6 days after inoculation and compared with fungi descriptors [7]. The occurrences of the isolated fungi were determined using the formula

$$\text{Fungi occurence} = \frac{\text{Frequency of occurrence of isolate}}{\text{Total occurence of all the isolates}} \times 100 \quad (1)$$

Proximate composition of healthy and infected kola nuts

Proximate characteristics of the apparently healthy and infected kola nuts (*Cola nitida*) were determined as enumerated below:

Moisture content determination

Each of the kola sample was weighed and oven dried at 105oC for about 24 hours. The samples were cooled in the desiccators for about 1hour and re-weighed. This was repeated until a constant weight was obtained [8]. Moisture content was calculated using the formula:

$$\%\ \text{Moisture content} = \frac{\text{Loss in weight}}{\text{Weight of sample before drying}} \times \frac{100}{1} \quad (2)$$

Total ash determination

Ground samples of the apparently healthy and infected kola nuts were weighed and placed in separate crucibles. The crucibles were placed into muffle furnace and slowly heated from 200oC to 450oC. The samples were heated until they became whitish in color. The samples were thereafter removed from the furnace and placed in the desiccator to cool. The samples were then re-weighed and ash content was determined according to the protocol described by [8].

Determination of fat content

Samples of the ground kola nuts were weighed into the thimble. A 500 ml round bottom flask was weighed and filled up to 2/3 of content with petroleum ether. The Soxhlet extractor was fit up with a reflux condenser. The heat source was adjusted so that the solvent could boil gently for about 5 to 6 hours after which it (solvent) was distilled. The flask containing the fat residue was dried in an air oven at 100°C for 5 minutes, cooled in the desiccators and weighed. The thimble was then placed in a beaker in an oven at 50°C, dried to constant weight with sample, cooled in the desiccators and weighed. The per cent fat was then calculated accordingly [8].

Determination of crude fibre

About 50 ml of boiling 1.25% tetra-oxosulphate (VI) acid was added to 0.4g ground kola nut samples. After boiling for about 30 minutes, the mixture was filtered. About 50 ml of 1.25% sodium hydroxide was added to the residue and further boiled for 30 minutes using cooling finger and vegetable oil as anti-foaming agent. The mixture was filtered again and washed with hot distilled water and once with 10% hydrochloric acid, four times with hot water, twice with methylated spirit and thrice with petroleum ether. The residue was collected into crucible, dried in an oven at 105°C and eventually placed into muffle furnace at about 3000°C for 30 minutes and later in the desiccators to room temperature and weighed [8].

Crude protein and carbohydrate determination

In order to determine the crude protein content of the apparently healthy and infected kola nuts, the samples were digested and distilled. Crude protein was calculated by multiplying the Kjeldahl nitrogen with a factor of 6.25 [8]. The carbohydrate contents of the kola beans were also calculated by difference.

Determination of mineral content

Macro and micro elements (calcium, potassium, magnesium, sodium, phosphorus, iron, manganese and zinc) in both the apparently healthy nuts (AHN) and infected nuts (IN) were determined using instrumental method (with the aid of Atomic Absorption Spectrophotometer Buck Scientific Model 2010VGP) following Beer Lambert's principle. Standard solutions of the minerals were prepared for each sample and the absorbance values were read using individual cathode lamp meant for each of the metals. After linearity/calibration was achieved, the ash samples dissolved in hydrochloric acid were aspirated through capillary action into flame and read. Extrapolation from the calibration curve was used to determine the amount (mg/g) of each of the tested mineral [8].

Statistical Analysis

Data obtained were analyzed using Analysis of Variance (ANOVA).Means were compared by Duncan's Multiple Range Test (DMRT) and significance was accepted considered significant at P = 0.05

Results and Discussion

Occurrence of the fungi isolates

From the result a total of twelve fungi were isolated from both AHN and IN. Morphological characters of the isolates were matched with fungi identification guides. The twelve fungi were associated with at least one of the two States. Six fungi namely: *A. niger, A. flavus, A. fumigatus, F. oxysporum, L. theobromea* and *R. stolonifer* were commonly associated with nuts from the two locations (Table 1) while *Botrytis cinerea, C. gloeosporioides* and *C. lindemuthianum* were specific to kola nuts from Ondo State and two fungi: *Fusarium solani* and *Penicillium expansum* to kola nuts from Osun State.

Each fungal pathogen induces rot in agricultural produce. The findings by [9] revealed the occurrence of *A. niger, Penicillium* sp, and *Fusarium* sp on decayed vegetables, also [10], isolated L. theobromae from kenaf seed as the black rot pathogen of kenaf seeds, [6] isolated *F. oxysporum, A. niger, R. stolonifer* among others from cucumber and water melon. The percent occurrence of each fungal

isolates was observed on both the AHN and IN (Table 1). More than 10% occurrence was indicated for *C. lindemuthianum* (24.82%), *F. oxysporum* (18.18%), *P. expansum* (13.64%) and *A. flavus* (13.09%) on the AHN, while *Fusarium oxysporum* (25.18%), *L. theobromae* (16.09%) and *C. lindemuthianum* (15.02%) and *F. solani* (11.49%) had high occurrence on the IN.

Fungi	Occurrence (%)	
	Apparently Healthy nuts	Infected nuts
A. flavus	13.09	10.1
A. niger	6.38	1.15
A. fumigatus	A	1.25
B. cinerea	A	8.09
C. gloeosporioides	4.55	A
C. lindemuthianum	24.82	15.20
F. oxysporum	18.18	25.41
F. solani	9.09	11.49
L. theobromae	9.00	16.39
P. expansum	13.64	4.00
P. atramentosum	A	7.02
R. stolonifera	A	1.15

Table 1: Percentage occurrence of fungi isolated from the kola nuts (*C. nitida*) collected from Osun and Ondo states. A: Not present

Plate a: Apparently healthy kola nuts.

Plate b: Infected kola nuts.

Effect of the fungi infection on the proximate and organic content of kola nuts (*C. nitida*)

The results in figure 1 showed the proximate and organic composition of apparently healthy and infected *Cola nitida* nuts from Ondo and Osun States. From the results, Osun AHN (Osun H) had significantly higher crude protein (4.01%), crude fat (2.4%) crude fibre (6.92%) and carbohydrate (89.7%) than Ondo AHN (Ondo H). However, all the IN from the two states had significant loss of proximate nutrients. Although, the IN from both states had a comparable range of value for the crude protein but Ondo IN had the least values for crude fat, moisture and carbohydrate while Osun IN had the lowest amount of crude fibre and ash. The proximate values obtained from the AHN from Ondo and Osun kola nuts were significantly lower than the reported data on C. nitida by [2] which stated that *C. nitida* had 15.4% protein, 11.90% fat, 10.70% fibre and 4.3% ash. However, this result partly agreed with the literature reports [11,12] which recorded 2.40% and 3.95% ash content of *C. nitida*. The prevalence of these pathogenic microbes may be the cause of both morphological damage and nutritional losses in kola nuts. This finding is in consonance with the publication by [13] which revealed that agricultural products like cereals can be very susceptible to toxigenic fungal growth in the field, during storage or during processing and their presence in stored products can significantly decrease quality and economic value of the harvested grain. In the light of this, variation in climatic, edaphic and disease factors of kola nuts (*C. nitida*) have been identified to be the major cause of variation in the nutritional properties of AHN [2]. The result showed that higher moisture content was observed for healthy kola nuts from both states than their respective infected counterparts. This finding strongly supports the publication report by [3] and [2] highlighted that low moisture content of kola nuts is good for their long preservation as it could prevent early spoilage of the nuts. Also, [14] reported that fruit with about 80% water content, low pH, highly rich in mineral elements and sugars served as suitable medium for microbial growth. On the other hand [12] stated that the high moisture content of kola nuts (*C. nitida*) was beneficial to the traders/sellers who often sell by volume and not by weight. The highest organic matter was recorded in AHN from Osun (Osun H) followed by Ondo H, and then Osun I while Ondo I had the least. There was no significant difference (P = 0.05) in organic carbon content of all the infected kola nuts (*C. nitida*) from both states where as Osun AHN (6.41%) was slightly higher than its Ondo healthy (6.30%) counterpart. From this result, it could be inferred that the abundance of organic carbon in the AHN may potentially encourage the fungi activity and this observation was in agreement with the report by [15] which revealed that the presence of carbon sources are the important substrates for bacteria and fungi activities. In addition, the results of this study were at par with publication report by [16] which stated that *Rhizopus stolonifer* was possibly the cause of rapid rot and discoloration of stored tomato fruits in Nigeria but *B. cinerea* and *P. atramentosum* appeared to be most active fungi with high degrading capacities on the *C. nitida* nuts than other fungi pathogens. Fungi infection of *C. nitida* nuts may occur during harvesting, post-harvest handling, processing, packaging, storage, transportation and distribution at various selling outlets where the fungi are prevalent.

Effect of the fungi infection on the nutrients content of *C. nitida* nuts

The macro-nutrients composition of apparently AHN and infected *Cola nitida* nuts obtained from Ondo and Osun States were as shown on figure 2. From the results, Osun AHN had a comparable calcium content with its Ondo apparently healthy counterpart whereas there was no significant difference in the amount of calcium from both infected Osun and Ondo nuts. Conversely, Ondo healthy nuts (Ondo H) contained higher amount of potassium (11.63 mg/g) and sodium (4.79 mg/g) than Osun AHN with 11.57 mg/g for K and 4.69 mg/g for Na and these values were significantly higher than their infected counterparts. However, Osun AHN recorded higher levels of phosphorus (5.81 mg/g) and magnesium (3.77 mg/g) than Ondo healthy kola nuts (*C. nitida*) while the fungi infection had caused drastic reduction of these macro-nutrients in the nuts from the two states.

Figure 1: Proximate and organic composition of kola nuts from the two states.
Where HE: Healthy Kola Nuts; I: Infected Kola Nuts

Figure 2: Macro-nutrients content of kola nuts from the two locations/states.
Where H: Healthy Kola Nuts and I: Infected Kola Nuts

The impact of fungi rot on the micro-nutrients content of kola nuts from Ondo and Osun state was shown in figure 3. It was obvious that Osun H contained higher amount of iron (3.61 mg/g) and zinc (2.79 mg/g) than Ondo H (3.53 mg/g for Fe and 2.69 mg/g for Zn), while its infected counterparts had marginal loss of these nutrients. The range of values for iron and zinc obtained from this study was significantly lower than those reported for *C. nitida* and *C. acuminata* by [2]. In view of the relevance of nutrient elements to human body system for instance, iron is a component of hemoglobin while zinc stimulates enzyme activities, [3] recommended 8 mg/kg iron intake for men and 18 mg/kg iron intake for pre-/menopausal women. It implied therefore that healthy kola nut (*C. nitida)* is not only a stimulant but can meaningfully help to alleviate anaemia.

Figure 3: Micro-nutrients content of kola nut from the two locations/states.
Where H: Healthy Kola Nuts: I: Infected Kola Nuts

Conclusion

High fungal infection on kola nuts from the two locations under investigation had shown that the disease pathogens are not location specific although, these two states belong to the same agro ecology. The high moisture content of healthy *C. nitida* nuts from Ondo and Osun state may be responsible for the fungi rot. However, fungi growth on the kola nuts from Ondo and Osun states had rendered the nuts unhealthy for human consumption and loss of its nutritional and market qualities. Adequate care must be taken during harvesting, packaging, handling and storage of kola nuts to minimize the post-harvest losses. It is also important to create more awareness on the potential health hazards of consuming relatively cheaper and pathogen contaminated kola nuts.

Conflict of Interest

The authors declared that no conflict of interest exists.

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