

# EFFECTS OF PATHOGENIC FUNGAL INFECTION ON THE NUTRIENT AND ANTI-NUTRIENT CONSTITUENTS OF POST HARVEST *Irvingia gabonensis* (OGBONO) SEEDS

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**Abstract:** Effects of pathogenic fungal infection on the nutrient and anti-nutrient constituents of post-harvest *Irvingia gabonensis* seeds were evaluated. The fungi pathogens isolated from partially rotted seeds of *I. gabonensis* seeds include *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium italicum*. These fungi were used as test fungi to inoculate healthy *I. gabonensis* seeds aseptically. The results of the proximate analysis in (%w/w)/100g of seeds inoculated with test fungi and uninoculated (control) seeds carried out revealed that there was a significant increase ( $p < 0.05$ ) in Moisture, Ash and Lipid content of seeds inoculated with the test fungi, relative to uninoculated (control) seeds. While fiber carbohydrate and protein content decreased in fungi inoculated seeds relative to uninoculated seeds. There was a significant difference ( $p < 0.05$ ) in mineral content evaluated. Calcium, Phosphorus and Iron increased in seeds inoculated with the test fungi relative to the uninoculated, while Potassium, Sodium and Magnesium decreased in seeds inoculated with the test fungi. Anti-nutrient (phytochemical) content; Tannin, Saponin, Total oxalate and Cynogenic glucoside increased in the fungi inoculated seeds relative to uninoculated seeds. Significant differences also existed among the test fungi. Apparently, *Penicillium italicum* induced more changes in the anti-nutrient (Phytochemical) content measured. Hence higher values were recorded by *Penicillium italicum* in Tannin, Saponin, Total oxalate and Cynogenic glucoside,

Keywords: *Irvingia gabonensis*, Nutrient, Anti-nutrient, Fungi, Inoculated

## Introduction

*Irvingia gabonensis* is a highly, economically important tree native to most tropical forest in west and central Africa (Lowel *et al.*, 2002) *I. gabonensis* (Ogbono) is sometimes called bush mango or African mango because the tree bears mango like fruit (Matos *et al.*, 2009). The kernel of the seeds, 3cm in diameter, is enclosed in a fibrous testa, and is especially valued for their fat and protein rich nuts which serves as a soup thickening agent and oil (Matos *et al.*, 2009). In addition to its nutritional and economic benefits *I. gbanonsisis* highly valued for its health and medicinal benefits. Studies have shown that seed extract of *Irvingia gbanonsisis* caused a significant reduction in body weight among obese people in Cameroon (Ngondi *et al.*, 2005). Post-harvest diseases are responsible for heavy losses of agricultural produce during storage, reduce food quality and render them unfit for consumption, (Doyle 2007). Generally, microorganisms among other factors, are adjusted the most notorious culprits

responsible for storage diseases of crops (Ray *et al.*, 2000) and fungi in particular have been found to be one of the principal causes of post-harvest losses in many zones of the world. The occurrence of fungi in food does not only render the food undesirable in terms of palatability but consumers of fungal infected food stand the risk of huge health hazards. This is because some fungi implicated with post-harvest diseases are able to produce mycotoxins known to be highly toxic and carcinogenic and are able to also suppress one's immune system, cause diseases of both human and domestic animals (Bankole *et al.*, 2005). Recent studies have shown *I. gbanonensis* in storage are laden with the several fungal species (Emiri and Chuku 2017), some of which are known to produce mycotoxins. Other researchers have demonstrated the occurrence of mycotoxins among *Irvingia gabonensis* in storage (Adebayo-Tayo *et al.*, (2006) making their consumption a potential health risk. Although harvest mycodeterioration on proximate composition of *I. gabonensis* has been reported (Sanyaolu *et al.*, 2014), however there is dearth of information on the effect of pathogenic fungal infection on the nutrient and anti-nutrient constituents of *I. gabonensis*. It is against this backdrop that this research was carried out. The objectives of this study therefore are (1) Isolate and identify Pathogenic fungi associated with *I. gabonensis* seeds (2) Determine the effects of the pathogenic fungi infection on the nutrient and anti- nutrient properties of the seed. Findings from this study will add to the body of knowledge and information reported by earlier researchers.

## **Material and Methods**

**Source of Sample:** Visually healthy and partially rotted *Irvingia gabonensis* seeds were purchased from a open market in Port Harcourt metropolis, Nigeria. The research work was conducted at the mycology/plant pathology unit of the department of plant science and biotechnology, Rivers State University, Port Harcourt, Nigeria.

**Media Preparation:** The medium used for fungal isolation was the Potato Dextrose Agar (PDA). This was prepared by weighing 32.8g of Potato Dextrose Agar (PDA) 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 flask was plugged with sterile cotton wool and wrapped with foil. The conical flask with its contents was autoclaved for 15 minutes at 121oC at 15 PSI pressure. Sterile Petri dishes were prepared and the mixture dispensed into them while still hot and allowed to solidify.

### ***Isolation and Identification of Fungi from Partially Rotted Irvingia gabonensis Seeds***

Five partially rotted seeds of *Irvingia gabonensis* used were washed in tap water, rinsed in distilled water and surface sterilized with 5% sodium hypochlorite for 5 minutes and rinsed twice in sterilized distilled water after which they were aseptically introduced into the PDA in Petri dishes equidistantly, replicated three times.



*Visually healthy seeds of Irvingia gabonensis*



*Partially rotted seeds of Irvingia gabonensis*

Pure cultures of fungi growing in mixtures were obtained thereafter. Pure cultures of the isolates were made after series of isolation. Microscopic examination of fungal isolates was carried out. The fungi were identified based on colour, spore morphology and the structure of the mycelia, according to the key of (Olds 1983) The fungi isolated; *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium italicum*, were used as test fungi for the study.

### **Inoculation of *Irvingia gabonensis* seeds with Test Fungi**

One hundred grams of visually healthy *Irvingia gabonensis* seeds were weighed out and cleaned aseptically. The cleaned seed were then put into 25ml conical flasks, plugged with sterile non-absorbent cotton wool and covered with the foil and then autoclaved at 121°C for 15 minutes at 15 PSI to eliminate any internal and external seed borne micro-organism Nwukwu, Ijeoma and Ikechi-Nwogu, (2012). After autoclaving, the flasks were allowed to cool and 100ml of sterile distilled water was added to each flask and shaken gently to wet all the seeds and to create a humid and conducive environment for the fungi to be inoculated and to have an even distribution. Each flask containing seeds was inoculated with a disc of 7 days old mycelium spores of each fungus obtained from the pure culture of fungi isolated from infected seeds. This was done with a 1.5cm diameter sterile cork borer. The flasks were gently shaken for about 15minutes to obtained uniform distribution of the mycelium among the seeds. The control flask received the same treatment, but there were no fungi added to it. The entire flasks which include the fungi inoculated and uninoculated seeds were incubated at room temperature in complete darkness for 14 days.

A total of 15 flasks were used, 3 flask replicate for each set of fungi inoculated seeds and uninoculated (control) seeds. They were incubated for 14 days in a dark incubation room. At the end of the incubation period, the flasks of each fungal treatment and flasks for control were harvested for biochemical analysis. The seeds in each flask were transferred into a pre-weighed watch glass, dried at 45°C for 24 hours and the spores and mycelia of the fungi removed by sieving Nwukwu, Ijeoma and Ikechi-Nwogu, (2012). Nutrient analysis of various proximate compositions, Moisture, Lipid, Protein, Fiber, Ash and carbohydrate as well as

mineral, calcium, potassium, phosphorus, sodium, iron and magnesium composition in both fungi inoculated and uninoculated seeds at the incubation period of 14 days were determined. Also, analysis of the anti-nutrient content (Tannin, Saponin, Total oxalate and Cynogenic glucoside) of the seed was carried out following procedures recommended by the Association of Official Analytical Chemists (AOAC), 2000). The results of each component were subjected to statistical analysis using the software statistical package SAS (2005) at 5% level of significance and mean separation was done using least significant difference (LSD) test.

## Results and Discussion

The results of the proximate analysis of *I. gabonensis* seeds inoculated with test fungi, and uninoculated (control) is presented in table 1. The test fungi induced appreciable changes in the proximate composition of the seed. There was a significant increase ( $P < 0.05$ ) moistures, ash and lipid content of the seed inoculated with test fungi compared with the uninoculated (control) seeds. However, there was a significant decrease ( $P < 0.05$ ) in fiber, carbohydrate and protein content of the seeds inoculated with test fungi relative to the uninoculated (control). Significant differences also occurred among the various test fungi with respect to the proximate composition. (Table 1)

Table 1: Effects of Fungal infection on the proximate composition of *I. gabonensis* seeds

Proximate Composition (% w/w)/100g						
Test fungi	Moisture	Ash	Fiber	Lipid	Carbohydrate	Protein
Uninoculated (control)	13.50	4.50	2.05	10.40	49.41	20.13
Rhizopus stolonifer	17.20	5.10	0.65	11.75	47.21	18.70
Aspergillus flavus	16.07	5.62	0.80	11.50	45.48	17.20
Aspergillus niger	14.30	4.80	1.32	11.00	44.00	15.70
Penicillium italicum	19.02	5.81	0.50	12.80	42.60	16.00
LSD ( $p < 0.05$ )	2.03	1.05	0.49	0.72	1.21	1.03

Means are significantly different by LSD at  $P < 0.05$

Results of the effects of fungal infection on the mineral composition of *I. gabonensis* seeds is presented in table 2. There was a significant increase in calcium and phosphorus content. However, increase in iron was not significant. Potassium, sodium and magnesium decreased significantly. Significant difference also existed among the test fungi as seen in table 2.

Table 2: Effects of Fungal infection on the Mineral Composition of *I. gabonensis* seeds

Mineral Composition (% w/w)/100g						
Test fungi	Calcium	Potassium	Phosphorus	Sodium	Iron	Magnesium
Uninoculated (control)	1.20	5.50	3.40	0.45	0.03	0.32
Rhizopus stolonifer	4.20	5.00	4.20	0.25	0.04	0.04

Constituents....

Aspergillus flavus	4.42	4.70	4.40	0.10	0.08	0.10
Aspergillus niger	3.90	5.20	4.60	0.30	0.10	0.08
Penicillium italicum	4.50	5.35	4.70	0.20	0.07	0.10
LSD (p<0.05)	0.04	0.86	0.93	0.002	1.50ns	0.01

Means are Significantly Different by LSD at p<.05

Results on the effects of fungal infection on anti-nutrient (phytochemical) content revealed that all the test fungi appeared to cause significant increase in all the anti-nutrient content examined. The test fungi also differed significantly.

Table 3: Effect of Fungal infection on the Anti-nutrient (Phytochemical) Composition of *I. gabonensis* seeds

Anti-nutrient (Phytochemical) Composition (%w/w)/100g				
Test fungi	Tannin	Saponin	Total oxalate	Cynogenic glucoside
Uninoculated (control)	0.48	1.80	1.11	37.30
Rhizopusstolonifer	0.71	2.85	2.15	40.20
Aspergillus flavus	0.60	3.30	2.00	42.40
Aspergillus niger	0.82	3.41	2.32	41.50
Penicilliumitalicum	1.00	3.80	3.40	44.30
LSD (p<0.05)	0.005	0.056	0.52	0.31

Means are significantly different by LSD at p<.05

## Discussion

Some mould fungi have been reported to have caused an improvement in some nutrient properties in plant produce. Mimmawati and Yetti (2013) reported that two mould fungi, *Eupenicillum javanicum* and *Aspergillus niger* which have manannolitic and cellulolytic anzyme can be used to improve the nutritional quality of palm kernel cake. Their report agrees with the result from this research. *Aspergillus nige* rand *PenicilliumItalicum* were observed to induce more changes in the proximate composition examined.

Results on proximate composition revealed that moisture content increased significantly in fungi inoculated seeds relative to the unionculated (Control) seed (13.50) *Penicillum italicum* caused the highest increase in moisture (19.02%). The increase induced by fungi is due their utilization of the component of the seeds as food nutrients thereby producing water in the process. This is in agreement with the results obtained by Nwaukwu and Ikechi-Nwogu (2012) who reported increase in moisture content of *Dialium guineense* inoculated with pathogenic fungi. Emiri and Enaregha (2020) reported same in *Mucuna sloanie* seeds infected with similar mycoflora. Isar and Ataga (2019) also reported an increased moisture in *Jatropha curcas* seed inoculated with *Fusarium oxysporum* and *Macrophomina phaseolina* in which they observed that *Fusarium oxysporum* caused the highest increase in moisture of the seed. The result from this study confirms their assertion. It thus suggests that deteriorative reactions occur more readily in seeds at higher moisture content and subsequently, this condition

constitute hazard to the longevity of seed survival. However, results from this study disagrees with the findings of Sanyaolu (2014) who reported a decrease in moisture content of diseased *I. gabonensis* seeds.

Similarly, ash increased significantly in seeds inoculated with the four test fungi compared to the uninoculated seed. *Penicillium italicum* and *Aspergillus flavus* were seen to cause the highest increase in ash content. This confirms the report of Nwaukwu and IkechiNwogu (2012) who reported increase in ash content of *Dialium guineense* inoculated with pathogenic fungi. Emiri and Enaregha (2020) reported an increase in ash content of *M. slonei*. Inoculated with some fungi. Ataga and Umechuruba (1997) resolved that the increase could be attributed to the presence of minerals like Potassium and Phosphorus in the Mycelia of the Fungi. This observation however, negates the assertion of Umana et al., (2014) who reported a decrease in ash content in Mycoflora infected pods and seeds of cocoa (*Theobroma cocoa*). Emiri and Enaregha (2019) reported same on *Brachystegia eurocoma*. Lipid content significantly increased in the seed samples inoculated with the test fungi compared to uninoculated seeds. The various fungi isolates that caused the increase also differed significantly, with *Penicillium italicum* inducing the highest increase in lipid. (Table 1). This confirms the assertion of Isalar and Atagar (2019) who reported an increase in Lipid content of *I.gabonensis* seed inoculated with fungi isolates. However, this observation negates the findings of Ogundero (1992); Nwaukwu and Ikechi-Nwogu (2012) who reported a decrease in lipid content of *Dialium guinense* seeds inoculated with pathogenic fungi. The increase in lipids seems to suggest that these fungi have high lipase activities.

The ash (4.50%) and fiber content (2.05%) obtained for healthy (uninoculated) seeds of *I. ganonesis* in this study is comparable to the result of Sanyaolu *et al.*, 2014 who reported 2.65 and 3.85% ash and fibre contents respectively of healthy *I. gabonensis* seeds. Similarly, it is comparable to what was obtained from some other oil seeds such as *A. hypogea* (Atasieet al.,) and *B. glabrum* (Adeleke and Abiodun, 2010). However, the values of moisture, (13.50%) protein (20.13%) and carbohydrate content (49.41%) obtained from healthy *I. gabonensis* seeds are significantly higher than the result of Sanyaolu et al., who reported 5.07, 10.03 and 24.80% of moisture, protein and carbohydrate respectively for healthy *I. gabonensis* seeds.

The crude fiber content obtained in this work is an indication of the ability of *I. ganonesis* seed to maintain a healthy physiological grip for a normal peristaltic movement of the intestinal tract. Deits low in crude fiber is undesirable as it could cause constipation and such diets have been associated with diseases of colon like piles, appendicitis and cancer (Atasie *et al.*, 2009). On a general note, the changes in the nutritional value of *I. gabonensis* as observed in this study agrees with the findings of Oladimeji and Kolapo (2008), who reported that different micro-organisms that were inclusive of fungi were seen to cause a reduction in the nutritional worth of some oil seeds in Nigeria. Sanyaolu et al., 2014 made a similar observation on *I. gabonensis*.

Fiber, carbohydrate and protein depleted significantly in seeds inoculated with the test fungi relative to the uninoculated (control) seeds. *Aspergillus niger* depleted the protein content most while *penicillium italicum* depleted fiber content most. This result agrees with the report of (Onifade et al.,2004) about a decrease in crude fiber content of Sweet potato flour enrich

with *A. niger*. In a similar development, Ndife et al., (2013) reported that fiber, and protein content of cocoa beans were seriously depleted when infected with *Phytophthora capsici* and *phytophthora megakarya*. Shehu and Aliero (2010) have also reported that the infected onion leaf showed a significant decrease in the quantity of protein and fiber content. Sanyaolu et al., 2014 also reported a decrease in carbohydrate protein and fibre content of *I. gabonensis* seeds infected with *Aspergillus spp.* It could therefore be deduced that the relative decrease in fiber and protein in the inoculated seeds may be caused by the degradation and dissolution of the seed tissue by the fungi. The protein and fiber might have been broken down by the fungi into smaller molecules that they absorbed. (Nweke and Ibiam 2012).

The mineral content of *I. gabonensis* seed inoculated with the different test fungi revealed that there was a significant increase in Calcium, Phosphorus and Iron compared to the control. (Table 2). Significant differences also existed among the test fungi. *Penicillium italicum* and *A. flavus* took the lead in Calcium. *P. italicum* also induced the highest increase in phosphorus content, while *Aspergillus niger* took the lead in Iron. However, Sodium, Potassium and Magnesium content decreased in seed inoculated with the test fungi, relative to the inoculated seeds. Apparently, *A. flavus* induced the highest decrease in Sodium and Potassium, while *Rhizopus stolonifer* induced the highest decrease in magnesium as seen in table 2.

The value of the various mineral content in uninoculated seeds (control) are comparable to the values reported by Uhegbu *et al.*, (2009) on Nutritive and Anti-nutritive properties of seeds of *Barchystegia eurycoma* and *Detarium microcarpum*. The increase in calcium and phosphorus agrees with the findings of Emiri and Enaregha (2019) on Mycoflora associated with *Brachystegia eurycoma* seeds and their effects on the seed's biochemical properties. The decrease in Sodium, Potassium and Magnesium agrees with the report of Nwaukwu and Ikechi-Nwogu (2012) who reported a significant decrease in the same mineral composition of *Dialium guineense* inoculated with some pathogenic fungi.

There was significant decrease in all the anti-nutrient (phytochemical) content. (Tanin, Saponin, Total Oxalate and Cynogenic glucoside) of seeds inoculated with the test fungi compared to the uninoculated (control) seeds. *P. italicum* took the lead in all the phytochemical examined. It therefore suggest that the test fungi especially *P. italicum* induced an increase in the anti-nutrient (phytochemical) contents. Tannins have been shown to play very significant roles in human medicine and treatment of ailments (Addae Mensah, 1992) Saponins are known to have hypocholesterolemic anti-fungal as well as anti-carcinogenic effect (Koratkar and Roa 1997), this could confer some chemo protection against heart disease to users of *I. gabonensis*

## **Conclusion**

The result obtained in this study emphasized that the activity of pathogenic fungi affects the nutrient and anti-nutrients composition of the seeds of *I. gabonensis*. The important proximate and mineral composition in the seed may also be major contributors to the nutritional value of

the plant. These results lend the seed its credence as an Oil seed rich in protein and carbohydrate. The results from this study also revealed that *Penicillium italicum* appears to be a potent fungus haven recorded the highest increase in tannin, saponin, oxalate and Cynogenic glucoside content. *Aspergillus niger* and *Penicillium italicum* induced more changes in the proximate and mineral composition of *I. gabonensis* seeds compared to other test fungi.

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