Fungi Associated with Seeds of *Irvingia gabonensis*is and their Effect on Shelf Life

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Abstract
Irvingia gabonensis is a species of African tree commonly known as "bush mango". The seeds are valued due to its nutritive and medicinal values. The ground seeds of I. gabonensis easily loose its thickening ability due to improper preservation and fungi attack. This quality reduction causes loss of flavor and deterioration and as such loss of economic value. It is on this note that research into the use of leaf powder of moringa, bitter leaf and scent leaf of different concentrations were added to the ground seeds of I. gabonensis and their effects on the general storage quality were assessed as measures of shelf life. The samples were stored for a period of six months, and fungal isolation was carried out on monthly basis. Proximate composition was done in the Department of Food Science and Technology while the mycological study was carried out in Biological Science laboratory in Rivers State University. The proximate composition of the I. gabonensis revealed the presence of moisture, ash, fibre, lipid, carbohydrate and protein while the mineral content found were iron, phosphorus, potassium, sodium and magnesium. Ten different fungi were isolated viz: Aspergillus niger, Aspergillus terreus, Trichophyton rubrium, Scedosporium Prolificans, Fusarium oxysporum, Penicilium spp, Rhizopus stolonifer, Mucor spp, Aspergillus fumigatus, and Aspergillus flavus to be associated with the spoilage of the seed of I. gabonensis with varying degrees of incidence. Results also showed that applications of moringa leaf powder increased the nutritional value of powdered seeds Irvingia gabonensis by stabilizing most of the proximate and mineral values. O. gratissimum however, had a greater inhibitory effect on the fungal isolate of Irvingia gabonensis stored for six months compare to all the other botanicals.

Key word: *Fungi, Irvingia gabonensis, Shelf life and Botanicals*

Introduction

*Irvingia gabonensis*is is an economic tree, native to most tropical forests in west and central Africa, in the genus *Irvingia* (Lowe *et al.,* 2010, Chuku, 2002). *Irvingia gabonensis* is sometimes called "African mango" or "bush mango" because the trees bore mango-like fruits (Matos *et al.,* 2009). *Irvingia gabonensis can* grow up to a height of 130ft and 3ft in diameter with a smoothie outer bark and grey to yellow- grey color. *Irvingia* fruits are gathered around the tree or harvest from the tree, the fruits are most times allowed to decompose for few days before breaking the nut with a hammer, or the fruits can be cut with a knife for extraction of the seeds immediately after harvest, the seeds are sun dried for few days. Sun-drying the *Irvingia* helps to preserve the shelf life of the seeds. A well dried and preserved *Irvingia* seed can be used for more than a year. *Irvingia gabonensis* is highly demanded due to its nutritional, economical and medicinal worth. Anegbeh *et al.,* (2003) concord that *Irvingia* fruits can be used both for modern and traditional medicine to treat several illnesses. The seed is highly regarded because it serves as soup thickener (Matos *et al.,* 2009). Duguma *et al.,*1990, Ndoye *et al.,* 1997 studies shows that the seed extract of *I. gabonensis* can cause a

great significance in body weight reduction among obese in Cameroon, also similar work by Ngondi *et al.*,(2005) valuated the effect of *I.gabonensis* seeds in the management of obesity. Further study by Sulaimon *et al*., (2015) also valuated anti-diabetic properties of *Irvingia gabonensis* leaf and bark extracts on alloxan induced diabetic rats.

Materials and Methods

Sample collection

5kg of freshly harvested dry *Irvingia gabonensis* was purchased, from three different markets. Mile 3 market, mile one market Port Harcourt city LGA and Rumuokoro market Obio/Akpor LGA all in Rivers State. Composite sample was made and then oven dried before grinding with a sterile grinder. A new grinder was also purchased from Mile 3 market; it was washed with water and soap then rinsed in distilled water.

Collection of *Moringa* leaf, Scent leaf and bitter leaf Samples

Moringa fresh leaves were harvested from a matured *Moringa* plant beside the green house, close to old biology building, Rivers State University. The *Moringa* leaves were washed in tap water and rinsed in distilled water as described by (Chuku, and Chuku, 2013) and was dried in a botanical way (drying under shade that is not directly under sun) for four days, which later was ground into powdery form, and was kept in a cellophane bag for further use. Freshly harvested scent leaf and bitter leaf samples were purchased from Rumuokoro market, it was washed in tap water and rinsed in distilled water as described by (Chuku, and Chuku, 2013) then was dried in a botanical way (drying under shades not directly under the sun) for four days, which was later grinded with a sterile grinder into powdery form and was then kept in bag for further use.

Determination of the proximate and nutritional composition of *Irvingia gabonensis, Moringa oleifera, Ocimum gratissimum* and *Vernonia amygdalina*.

The samples were taken to the Department of Food Science and Technology Rivers State University, where the proximate and mineral composition were analyzed. The parameters determined were the protein, Carbohydrate, Ash, fibre, lipid etc. the method of analysis used was according to the Association of Official Analytical Chemists (AOAC, 2010) standard methods of analysis.

Mycological studies

Preparation of mycological medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120^{o}C for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask containing the mycological medium was autoclaved at 121^{o} C and pressure of 1.1kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40 ^{o} C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of fungi from *Irvingia gabonensis*

One gram of *Irvingia gabonensis* sample showing visible signs of spoilage by moulds was inoculated onto Sabouraud Dextrose Agar in Petri dishes onto which ampicillin was added to

hinder the growth of bacteria in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25^{o} C ± 3^{o} C (Baudoni, 1988, Chuku, 2009, Samson *et al*, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates was obtained after a series of isolations**.**

Identification of fungal organisms from *Irvingia gabonensis*

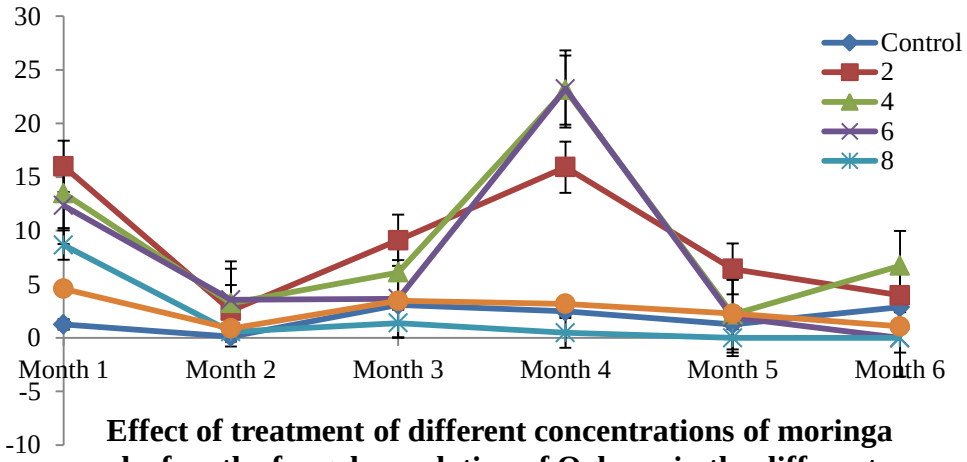
Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Samson *et al*, 1981, Olds, 1983, Barnett and Hunter, 1972).

Pathogenicity studies

Pathogenicity studies was carried out on *Irvingia gabonensis* to check if the fungi isolated from *Irvingia gabonensis* were capable of causing spoilage of the freshly harvested fruits. The methods of (Agrios, 2005, and Trigiano, 2004) were basically followed. The fungal isolates were introduced into *Irvingia gabonensis* and observed for seven days. The set up was monitored regularly for growth.

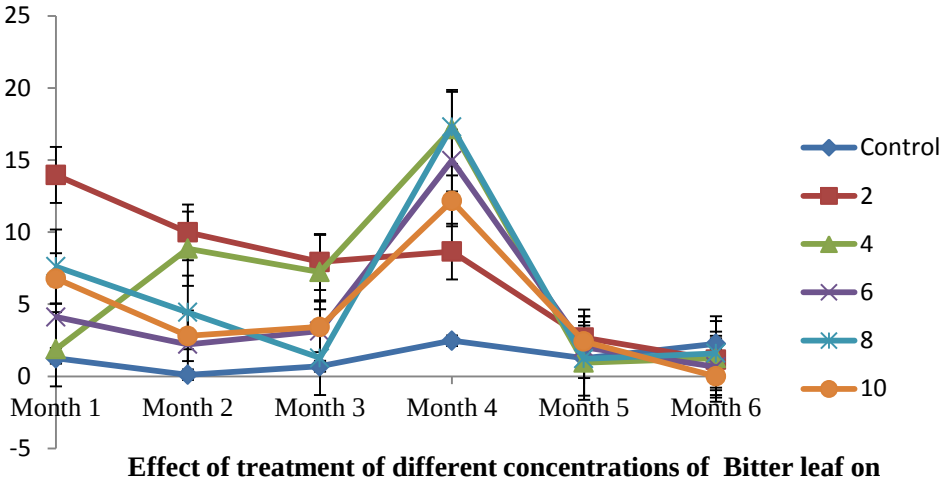
Result and Discussion

Figure 1: Effect of treatment of moringa leaf powder on the fungal population of powdered seeds of *I. gabonensis*



Effect of treatment of different concentrations of moringa leaf on the fungal population of Ogbono in the different months

Figure 2: Effect of treatment of *Vernonia amygdalina* leaf powder on the fungal population of powdered seeds of *I. gabonensis*



Effect of treatment of different concentrations of Bitter leaf on the fungal population of Ogbono in the different months

Figure 3 Effect of treatment with different concentrations of Scent leaf powder on the fungal population of powdered seeds of *I. gabonensis* for a period of 1-6 months.

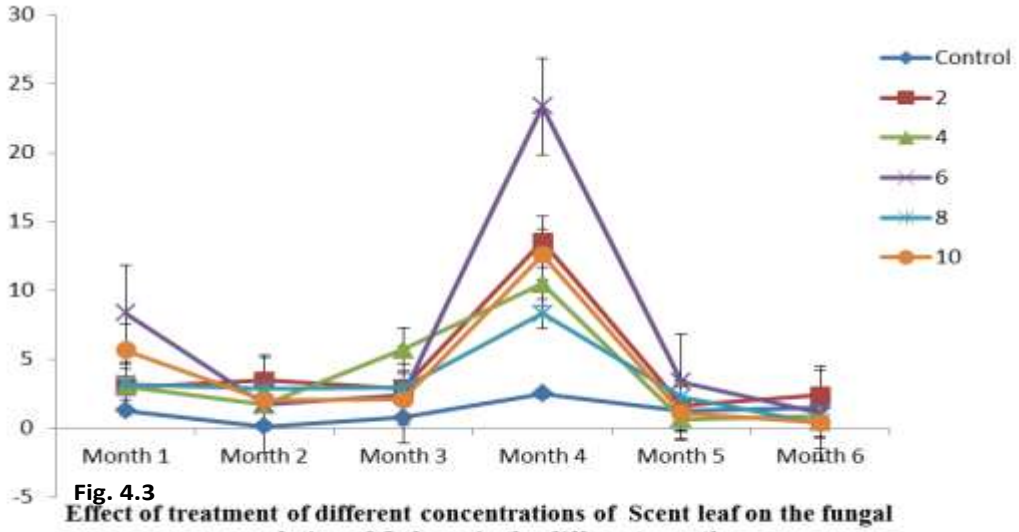


Fig. 4.3

Effect of treatment of different concentrations of Scent leaf on the fungal population of Ogbono in the different months

Table 1: fungal isolates and % incidence from powdered seeds of *I. gabonensis* treated with different botanicals

Plant samples	Fungal isolates	% incidence
Moringa leaf powder	*Aspergillus niger*	100
	Aspergillus terrius	80
	Trichophyton rubrum	20
	Scedosporium prolificans	10
	Aspergillus fumigatus	100
	Fusarium oxysporum	60
	Crytococcus neoformans	100
Bitter leaf	*Aspergillus niger*	100
	Fusarium oxysporum	80
	Cryptococcus neoformans	100
	Trichoderma spp	20
	Penicilum sp	60
	Rhizopus stolonifer	
Scent leaf	*Aspergillus flavus*	30
	Crytococcus neoformans	100
	Penicilum italicum	80
	Scedosporium prolificans	20
	Trichophyton rubrum	40
	Rhizopus stolonifer	80
	Culvularia sp	10
	Fusarium oxysporum	100

Irvingia gabonensis is a species of African tree that goes by the names "bush mango", "ogbono" etc. the seeds are valued due to its nutritive and medicinal values. The ground seeds of *Irvingia gabonensis*, easily loose its viscosity due to improper preservation and fungi attack. This quality reduction causes off flavor and deterioration and at such loss of economic value. It is on this note that research into the use of some botanical leaf powder of different concentration is used to assess its effects on the general storage quality of the ground seeds

The microorganisms observed to be associated with deterioration of ground seeds of *Irvingia gabononsis* in this research were similar to (Adebayo *et al*., 2006, Sanyaolu *et al*., 2014). They reported the presence of *Aspergillus sp., Penicillum spp, Rhizopus spp* etc. the presence of these organisms causes easy deterioration of ground seeds of *Irvingia gabononsis* making it tasteless and then losing its nutritional values when used for cooking. Sanyaolu *et al.,* (2014), Chuku *et al*., (2004) reported that *Aspergillus spp*., *Penicillum spp* and *Rhizopus spp* were amongst the fungi that reduces the nutritional and storability of *Irvingia gabonensis.* Examination of the effects of the some botanical leaf powder on the fungal population, suggests that the botanicals possess antimicrobial effect capable of hindering the growth of fungi, this result agreed with the antimicrobial effect found in moringa, *O.gratissimum* and *V. amygdalina* as reported by (Ahamad *et al..*, 2014; Clement *et al..*, 2014; Terezenha *et al..*, 2006).

The stability of the proximate and mineral composition and the reduction in fungal load of the seed of *I. gabonensis* as measures of shelf life stability in this study using different botanicals was of paramount importance. Early researchers had reported the use of ascorbic

acid for shelf life preservation of the powdered *I. gabonensis* with positive impact in terms of viscosity and mould growth (Chuku *et al*., 2002). However, the emphasis on the use of botanicals which are ecologically friendly without side effects on man is highly advocated. The ability to increase the nutrient values of powdered seeds of *I. gabonensis* such as Ash, Lipid, CHO, protein and Fibre is a breakthrough in research. The value addition to the powdered *I. gabonensis* seed is also highly commended for improved human dietary needs.

The nutritional and mineral compositions were assessed in this study; the moisture content in moringa was highest when compared with *O. gratissimum* and *V. amygdalina*, high moisture content is an avenue of spoilage during storage, while relatively low moisture content would prolong storage life. Carbohydrate content were lower in moringa when compared with *O. gratissimum* and *V. amygdalina*, it suggest the botanicals are good source of energy. The protein content in moringa is higher in value than that of *V. amygdalina* and *O. gratissimum* though it meets the recommended daily dietary needs. Nutritionally protein is important in building and maintaining the body tissues, any plant that provide more than 12% protein value is consider a good source of protein. The ash content obtained in this research suggests that the botanicals are good source of inorganic minerals which helps to retard the growth of microorganism. The lipid value obtained in this study were lower than that of *Moringa* and *Ocimum*, this could be as a result of lipid lowering effect of *V.amygdalina* as reported by (Ekpe *et al*.., 2007).

The samples analyzed in this study revealed the presence of minerals such as Ca, P, Na, Fe, Mg and K. The calcium value obtained in this study is below dialy recommended dietary, calcium plays a role in the building and maintaining strong bone and teeth. Potassium values in Moringa and V. amygdalina were higher than *Ocimum*. Nutritionally, potassium regulates the water balance and acid-base balance in the blood and tissues, it is also important for normal growth and for building muscles. Iron plays important role in immune function, temperature regulation and energy metabolism. Phosphorus content found in this study was higher in moringa leaf sample than bitter leaf and scent leaf powder used in this study indicates healthy for the bones and teeth and is found in every cell and maintains normal growth. It balances sugar level and heartbeat. Sodium plays important role in the body. It helps to maintain healthy fluid balance; it also contributes to build muscles, though the body need far less sodium than the average person actually consumes. The magnesium values obtained in this study is lowering in comparable to. Nutritional, magnesium is needed for the body to stay healthy; it helps in regulating muscles and nerve function, blood sugar and blood pressure. It helps in making protein, bone and DNA respectively. Although the amount observed in the samples were below recommended daily intake. It could be due to grams of the samples used in this study. The iron content of the samples in this study shows significantly higher value in comparable to (Clement *et al*.., 2014; Terezenha *et al*.., 2006). Iron is needed for body to transport oxygen throughout the body also iron is capable of maintaining a healthy cell, skin, hair and nails. Therefore, the samples in this work contain a moderate quality of iron good for consumption.

Conclusion

This work has revealed that ground seeds of *I. gabonensis* play host to numerous fungi which when consumed in large amount can be injurious to health because of the bioaccumulation of toxins. However, the addition of powdered leaf sample of *Moringa oleifera* increased the nutritional values of the product as it stabilized most of the proximate and mineral contents of the ground seeds. The addition of powdered leaf of *O. gratissimum* recorded a higher inhibition of the fungal isolates over a period of six months irrespective of the various

concentrations. For maximum benefits on the utilization of the ground seeds of *I. gabonensis*, it is therefore recommended that the powdered leaf samples of moringa and scent leaf be incorporated into the ground seed samples for quality improvement and enhanced shelf life.

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