Postharvest quality of commercial *Irvingia* kernels and the potential use of *Ocimum gratissimum* (Scent leaf) against fungal spoilage

Ebimieowei Etebu[1*] & Emiri Uchechi[2]
[1]Department of Biological Sciences,
Niger Delta University, Wilberforce Island,
Bayelsa State, Nigeria.
[2]Department of Agricultural Education,
Isaac Jasper Boro College of Education, Bayelsa State,
Nigeria
*Corresponding author: eetebu@gmail.com

Abstract
Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) is an important tree plant whose seed kernels are widely used in Nigeria, but commercial Irvingia kernels pose a potential health hazard to its numerous consumers, owing to postharvest fungal infections. The choice of chemicals in the control of plant diseases have shifted from application of synthetic fungicides to botanicals (plant extracts), owing to the health and environmental hazards associated with the use of the former. Hence in this research, postharvest qualities of commercial Irvingia kernels were investigated, and the antifungal activity of Ocimum gratissimum extract against spoilage fungi was studied using well-in-agar diffusion method. Results showed that mean postharvest qualities of commercial Irvingia kernels in Bayelsa State, Nigeria were not significantly different across different markets. Overall postharvest disease incidence and severity were 47.78% and 34.67%, respectively. Three fungal species Aspergillus, Fusarium and Mucor were isolated, and crude extracts of Ocimum gratissimum inhibited the growth of all three fungi. Antifungal activity measured as diameter of zone of inhibition showed that O. gratissimum extract at 20% concentration was very active against Aspergillus (17.00mm) and Mucor (16.00mm) species whilst being moderately active against Fusarium species (15.00mm). O. gratissimum could serve as a potentially viable alternative to chemical fungicides in the preservation of postharvest Irvingia kernels.

Keywords: *Postharvest, spoilage, Irvingia, Aspergillus, Fusarium, Mucor, Ocimum gratissimum*

INTRODUCTION

The spoilage of agricultural plant produce arising from postharvest diseases and disorders is a serious challenge undermining public health and developmental efforts of many countries all over the world. Postharvest diseases are responsible for heavy losses of agricultural produce during storage, reduce food quality and render them unfit for human consumption (Doyle, 2007). Generally, microorganisms are adjudged the most notorious culprits, amongst other factors, responsible for postharvest diseases of crops (Ray *et al.*, 2000) and fungi in particular, have been found to be one of the principal causes of postharvest losses in many zones of the world, ranking alongside insects and pest (Greer, 1990). 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 a huge health hazard. This is because some fungi implicated with postharvest diseases are able to produce mycotoxins known to be highly toxic, carcinogenic, and are able to also suppress one's immune system, cause growth retardation, liver disease, and death in both humans and domestic animals (Miller, 1991;

Prelusky *et al.*, 1994; Bankole *et al.*, 2005). Seeking ways to effectively control postharvest spoilage of agricultural produce is therefore undeniably essential, especially for crops such as *Irvingia* kernels that are widely consumed in Nigeria and other parts of the world.

Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) sometimes called bush mango is a dominant tropical forest tree of west and Central Africa, and is one of the most domestically consumed wild fruit tree (Ladipo, *et al.*, 1995). The kernels, considered to be the valuable part of the fruits, are used as a sauce thickening agent in the preparation of a famous 'Ogbono' soup which is widely consumed in Nigeria (Matos *et al.*, 2009; Oyakhilome, 1985). Locals usually harvest the fruits for their kernels which are sun dried and stored in bags until when needed for sale or consumption. Recent studies have shown that postharvest commercial *Irvingia* kernels are laden with several fungal species (Etebu and Bawo 2012a), some of which are known to produce mycotoxins. Although, there is yet no report of illness arising from consumption of *Irvingia* kernels, other workers have demonstrated the occurrence of mycotoxins among *Irvingia* kernels in storage (Adebayo-Tayo et al., 2006), making their consumption a potential health risk. There is therefore the need to seek ways that would control the proliferation of potentially harmful spoilage fungi associated with the kernels.

The use of synthetic chemicals in the preservation of postharvest agricultural produce in storage has proven over the years to be very effective in controlling pathogenic fungi (Frazier and Westhoff, 1998; Manczinger *et al.*, 2002). Notwithstanding the pedigree of effectiveness, their use is increasingly becoming undesirable because they are themselves carcinogenic, teratogenic, highly toxic with long degradation periods, and are able to induce chemical poisoning, as well as fungal resistance (Lingk, 1991; Adegoke *et al.*, 2002). As a result, the search for postharvest control strategies has recently been directed towards the use and implementation of natural preservatives that may have a positive effect on human health (Burt, 2004; WHO 2002).

Amongst natural preservatives, the use of natural essential oils obtained from plants has been promising. They have been shown to reduce microbial and chemical spoilage among agricultural produce (Guenther, 1948; Pessoa, 2002) and more importantly, they are eco-friendly, with no proven detrimental effect on human health and the environment even at high concentration (Nychas, 1995; Smid and Gorris, 1999). One of such proven botanicals is *Ocimum gratissimum*. Extracts of *Ocimum gratissimum* have been shown by numerous workers to control postharvest microbial infestations and aflatoxin contamination of food commodities (Bankole and Somorin, 2010; Ikheorah and Okoye, 2005; Nguefack *et al*., 2004; Okigbo, 2004; Okigbo and Ogbonnaya, 2006).

In this work, the postharvest quality of commercial *Irvingia* kernels sold in selected community markets in Bayelsa State, Nigeria was studied, and the potential use of *Ocimum gratissimum* in the preservation of *Irvingia* kernels was also studied by investigating the antifungal activity of the test plant extract against spoilage fungi isolated from postharvest *Irvingia* kernels. The findings of this work would provide simple ways to reduce the health risk hazards potentially associated with the consumption of postharvest *Irvingia gabonensis* kernels.

Materials and methods

Experiment 1: Survey of postharvest quality of commercial *Irvingia* kernels

One cup of *Irvingia* kernels (about 60 kernels) were purchased from 3 retailers from a community market situated at Amassoma (4°58′N 6°06′E) of Bayelsa State, Nigeria.

Thereafter, 30 kernels were randomly, separately selected from each replicate and assessed for weight, Percentage incidence and severity of postharvest disease/spoilage. The procedure was repeated with *Irvingia* kernels purchased from two other community markets, Agudama-Epie and Edepie (as shown in fig. 1)

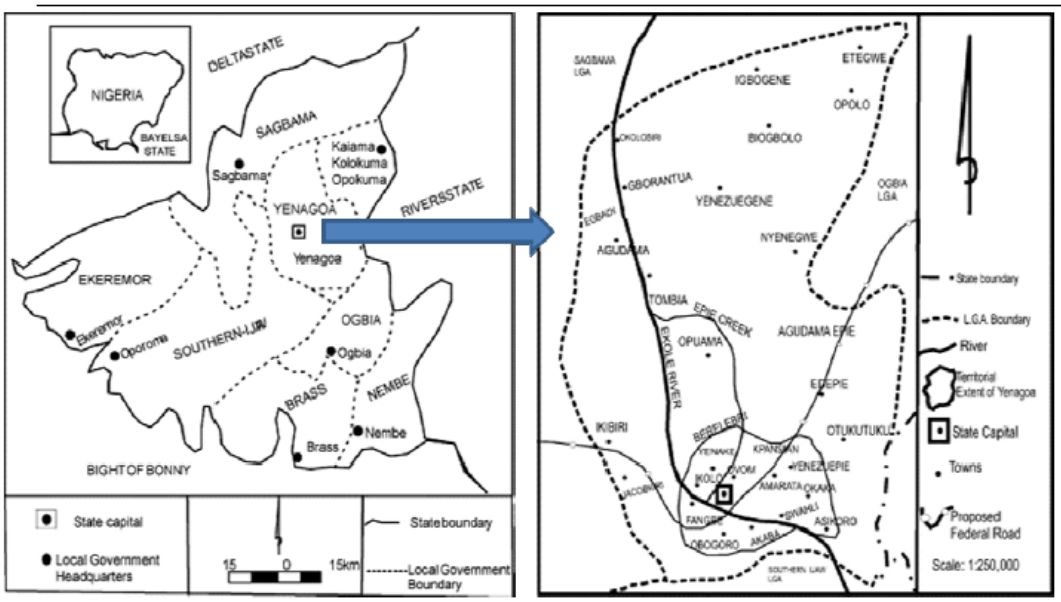

Fig. 1: Map showing two sample markets (Agudama-Epie and Edepie)
Source: National Population Commission, 1996

Postharvest spoilage of Irvingia kernels was identified by the appearance of brownish to black colouration on the kernels. Percentage incidence of infected kernels was calculated thus:

$$\text{Percentage disease incidence} = \frac{\text{Number of discoloured kernels}}{30} \times 100\%$$

Severity of postharvest spoilage was determined visually by the proportion of kernel area affected by brownish to black discolouration and expressed in percentage as according to Etebu et al. (2003) and Etebu (2013).

The mean score of 30 kernels from a cup of kernels purchased from a retailer was considered to represent a replicate. Percentage data were arcsine transformed according to Gomez and Gomez (1985), and all data sets were subjected to ANOVA using Generalized Linear Model of SPSS version 16.0 Statistical software.

Isolation of spoilage fungi from commercial kernels

All the 270 kernels previously selected at random from all retailers across all three markets were thoroughly mixed and about 10 kernels were further picked at randomly, and surface sterilised in 0.7% sodium hypochlorite according to Etebu *et al.* (2003) and plated onto PDA medium previously prepared according to Manufacturer's prescription and amended with 50µg ml^{-1} each of streptomycin and tetracycline according to Etebu *et al.* (2003). The plates were incubated at room temperature of about 25°C for 3 days. Fungal colonies were repeatedly sub-cultured after every three days until pure cultures were obtained. Pure fungal colonies were thereafter transferred onto Sabouraud dextrose agar (Oxoid Ltd, Hampshire, UK) and sporulating colonies were identified based on macroscopic and microscopic examination according to Alexopoulos (1962) and Barnett and Hunter (1972).

Three fungal isolates (*Aspergillus, Fusarium* and *Mucor* species) so obtained were further

exposed to *Ocimum gratissimum* extract with a view of studying the inhibitory effect of the latter on the fungal isolates.

Experiment 2: Potential use of *Ocimum gratissimum* (Scent leaf) against postharvest fungal spoilage of *Irvingia* kernels

The inhibitory effect of aqueous leaf extracts of *Ocimum gratissimum* on growth of three test fungi (*Aspergillus* sp. *Fusarium* sp. and *Mucor* sp.), previously isolated from postharvest kernels of *Irvingia* fruits was studied, using the well-in agar diffusion method. Leaves of *Ocimum gratissimum* were collected in June 2013 from a natural forest located in Amassoma (4°58′N 6°06′E) of Bayelsa State, Nigeria, and washed thoroughly under running water and further with sterile distilled water. The leaves thereafter were homogenized into paste using a vegetable blender. Two portions of paste were thereafter separately mixed with sterile distilled water to arrive at 5 and 20% of extract, respectively. The resulting mixtures were stirred vigorously and allowed to stand for one hour, and were thereafter separately filtered through folds of sterile cheese cloth. The resultant crude plant extracts (20% and 5%) were then kept in separate containers in a refrigerator at 4°C until used.

Sabouraud dextrose agar (SDA) (Oxoid Ltd, Hampshire, UK) was prepared according to manufacturer's prescription, and integrated with 50µg ml^{-1} each of two antibiotics (streptomycin and tetracycline) according to Etebu et al., (2003) and Etebu and Bawo (2012). Three media plates were separately inoculated with suspension of *Aspergillus* sp. *Fusarium* sp. and *Mucor* sp. respectively by spread plate method in three replicates. Thereafter, a sterile Cork borer (8 mm diameter), was used to bore hole in each of the inoculated solid agar, and 1ml of the 5% *O. gratissimum* extract was dispensed into the hole on each plate. The plates was allowed to stand for 30mins for diffusion of the extract to occur and then incubated at room temperature for 5 days. This procedure was separately repeated for the 20% *O. gratissimum* extract, a fungicide (Ketoconazole 500µgml^{-1}) suspension, and distilled sterile water. The fungicide and water were to serve as positive and negative controls, respectively.

The inhibitory effect of the test plant extracts and control treatments were ascertained by a clear zone of inhibition of fungal growth around the well after 5 days of incubation. Data on postharvest qualities of commercial *Irvingia* kernels and diameter of zone of Inhibition were first transformed where appropriate according to Gomez and Gomez (1985) and subjected to ANOVA using the generalized linear model of SPSS version 16.0 computer software. Mean diameter of zones of inhibition were further subjected to Tukey's mean separation test and comparisons were made with respect to the test plant extracts and controls.

Results and Discussion

Commercial *Irvingia* kernels sold in Bayelsa State, Nigeria were infected by postharvest spoilage fungi but pathological qualities of kernels sold in the different markets were not significantly ($P=0.05$) different. Although postharvest qualities of kernels sold in the different markets were not significantly ($P=0.05$) different, a substantial proportion of the kernels were observed to have disease symptoms in all three markets investigated.

Overall mean percentage disease incidence and severity across all three markets were 47.78% and 34.67%, respectively (Table 1).

Table 1: Postharvest quality of commercial *Irvingia* kernels sold in some selected community markets in Bayelsa State

Community	Mean weight of kernels	Mean percentage disease incidence	Mean % disease severity (Arcsine transformed)	Weighted Mean % disease severity
Agudama-Epie	46.70	47.77	33.00	29.66
Amassoma	43.57	52.23	35.01	32.92
Edepie	46.47	43.33	40.20	41.66
Overall mean	35.58	47.78	36.07	34.67

*No significant difference was observed between any two community markets regarding any of the postharvest quality parameters measured in this work.

This showed that about half of *Irvingia* kernels sold in these markets in the month June had abnormal discolouration, and on the average, over one-third of the surface area of all commercial *Irvingia* kernels were completely discoloured in these markets, about one year in storage (June, 2012- June, 2013). Results from a recent work showed that a gram of commercial *Irvingia* kernels could be contaminated with as much 1.17E+05 to 6.92E+05 of fungal isolates (Etebu and Bawo, 2012a), and postharvest disease incidence and severity of agricultural produce such as *Irvingia* kernels have been reported to be dependent on treatment method, storage conditions and period (Etebu and Bawo, 2012b; Niranjana *et al*., 2009). Differences in postharvest qualities measured in this work were not significantly ($P \leq 0.05$) different with respect to the different community markets because Locals who trade in this commodity are from the same ethnic, cultural and possibly educational background, and this would have made them treat and store the kernels about the same way. Although the form of treatment adopted prior to storage of *Irvingia* kernels was not investigated in this work, earlier Workers have reported that the kernels are usually spread on platforms often with no pre-treatment and are turned regularly until sufficiently dried so that they can be stored until needed for consumption (Etebu and Bawo, 2012b; Latapi and Barret, 2006; Berinyuy et al., 2012). Kernel discolouration and fungal attack are said to be the major determinants of *Irvingia* quality in marketing (Ladipo, 1999). Irvingia kernels become discoloured due to fungal attack if they are not sufficiently dried soon after harvest.

Members of three genera of fungi (*Aspergillus, Fusarium* and *Mucor* species) were isolated from commercial *Irvingia* kernels sold in all the three community markets studied in this work. A fairly recent similar work showed that *Irvingia* kernels sold in Yenagoa metropolis of Bayelsa State, Nigeria were often contaminated with *Aspergillus* and *Mucor* species amongst others (Etebu and Bawo, 2012a). *Fusarium* species were not encountered then. However, an equally recent work showed that *Fusarium* species could indeed be implicated in postharvest spoilage of the *Irvingia* kernels depending on the pre-storage treatment methods, storage conditions and period (Etebu and Bawo, 2012b). It therefore suggests that at least one of the retailers in any of the markets sampled would have exposed his/her kernels in ways that necessitated the proliferation of *Fusarium* species.

Result on the potential use of *Ocimum gratissimum* in the preservation of postharvest *Irvingia* kernels showed that aqueous crude extracts of *Ocimum gratissimum* at concentrations of 5% and 20% separately inhibited the growth of all test fungi in comparison to the control (Water) treatment. *Ocimum gratissimum* is found throughout the tropics and subtropics, including Nigeria (Sulistiarini et al., 1999; Orwa et al., 2009), and numerous workers have shown that its extracts could be applied to control postharvest microbial infestations and mycotoxin contamination of food commodities (Bankole and Somorin, 2010; Ikheorah and Okoye, 2005;

Nguefack *et al*., 2004; Okigbo, 2004; Okigbo and Ogbonnaya, 2006). However, this work is the first report where the potential use of *O. gratissimum* in the preservation of postharvest quality of *Irvingia* kernels was investigated.

Antimicrobial properties of *O. gratissimum* are attributed to phytochemicals and essential oils which they possess. Phytochemicals and essential oils are secondary metabolites that are non-nutritive in themselves but have been found to confer on plants that possess them the ability to resist microbial infections, and sometimes environmental stress (Prabhu *et al.*, 2009; Ladipo *et al*., 2010; Matasyoh *et al*., 2007; Pandey and Chowdhury, 2000; Terezinha *et al.*, 2006; Dubey *et al.*, 1997; Jirovetz *et al.*, 2005; Offia and Chikwendu 1999; Njoku *et al.*, 1997; Lawrence, 1997). Phytochemicals and essential oils inherent in *O. gratissimum* have been shown to possess a broad range of properties including antibacterial, (Oussalah *et al*., 2007, Prabhu *et al*., 2009) antifungal (Terezinha *et al.*, 2006, Matasyoh *et al*., 2007), antiviral (Schnitzler *et al*., 2011), insecticidal (Essam, 2001), antioxidant properties (Chu *et al*., 2002; Ganiyu, 2006) etc.

The fungal inhibitory effect of extracts of *O. gratissimum* in this study was observed to be dose dependent. Whilst the overall mean diameter of zone of inhibition across all three test fungi was 28.69mm, the plant extract at 20% concentration had a greater inhibitory effect on the fungi (16.00mm) than at 5% concentration (8.77mm) (Table 2).

Table 2: Comparative effects of different concentrations of plant (*Ocimum gratissimum)* extracts and fungicide on postharvest spoilage fungi of *Irvingia* kernels samples

Treatment	Diameter of Zone of inhibition (mm)			
	Aspergillus sp.	*Fusarium* sp.	*Mucor* sp.	Mean
Water	0.00^{a}	0.00^{a}	0.00^{a}	0.00
Plant Extract* (5%)	8.90^{b}	8.80^{b}	7.80^{b}	8.77
Plant Extract* (20%)	17.00^{c}	15.00^{c}	16.00^{c}	16.00
Ketoconazole (0.5mg/ml)	90.00^{d}	90.00^{d}	90.00^{d}	90.00
Mean	28.95	28.68	28.45	28.69

*Plant Extract = *Ocimum gratissimum* Extract

This pattern of correspondingly greater diameter of zone of inhibition with respect to increase in concentration of the plant extract was similar to results reported by several other workers (Amadioha and Obi 1999; Udo *et al.*, 2001; Ijato, 2011). These workers separately reported that an increase in the concentration of an antimicrobial plant extract achieved a better result in the control of pathogenic fungi. Similarly, Tagne *et al.*, (2000) also reported that plant extracts at 10ppm exhibited a considerable degree of antifungal activity but test fungi were completely inhibited when concentration of extract was increased to 800ppm.

On the contrary, other works have equally shown that the growth of some fungi, *Fusarium* species in particular, are inhibited more by plant extracts when the latter are used in smaller concentrations that at higher concentrations (Zunera *et al.*, 2012). This occurrence could possibly be explained thus: It could be that the plant extracts in question are not antifungal, but contain substances that are more or less sources of nutrients exploited by the fungi. Since abundance of food would naturally lead to increased growth rate, increase in concentration of such plant extracts would also favour the growth and increase of such groups of fungi and

vice versa.

Results from this present work also showed that the degree of antifungal activity of extracts of *O. gratissimum* was different for the different test fungi. Antifungal activity of the extract was most active against *Aspergillus* species and least on *Fusarium* species (Table 2). The differential antifungal effect of *O. gratissimum* on *Aspergillus* and *Fusarium* could not easily be fathomed. A long-standing debate has been whether or not *Fusarium* and *Aspergillus* spp. are antagonistic pathogens (Robertson-Hoyt *et al.*, 2006), and an earlier work by Marín *et al.* (1998) seem to suggest that the two species of fungi are indeed antagonistic. Specifically, they showed that the growth of *Aspergillus* spp. was slowed in the presence of *Fusarium* spp. Similarly, different workers at different times have shown that *Fusarium* and *Aspergillus* species respond differently to antifungal agents. An antifungal substance could be active against *Aspergillus* species but not *Fusarium* species and vice versa. In particular, an earlier work aimed at investigating the susceptibility of different test fungi to different antifungal drugs showed that Itraconazole and caspofungin that were clearly active against *Aspergillus* species were not active against *Fusarium* species (Lalitha *et al.*, 2007). Conversely, Day and Associates (2009) showed that whilst *Fusarium* species were susceptible to two antifungal drugs (moxifloxacin and tobramycin), the drugs were not active against *Aspergillus* species. The results of this present work suggest that *O. gratissimum* may be more effective if used in the control of postharvest diseases occasioned by *Aspergillus* species than by those caused by *Fusarium* species.

Johnson and Case (1995) asserted that an organisms could be considered resistant if diameter of zone of inhibition is $\leq$ 10mm, intermediate (moderately susceptible/resistant) if diameter of zone of inhibition is between 11-15mm and susceptible if the zone of inhibition is $\geq$ 16mm. Going by this assertion, 20% *O. gratissimum* extract could be said to be very active against *Aspergillus* and *Mucor* species whilst being moderately active against *Fusarium* species.

Conclusion

Aqueous crude extracts of *O. gratissimum* inhibited the growth of *Aspergillus, Fusarium* and *Mucor* species especially at 20% concentration. *Ocimum gratissimum* at this concentration was considerably active against *Aspergillus* and *Mucor* species whilst being moderately active against *Fusarium*. This botanical would potentially be effective in the control of postharvest spoilage of *Irvingia* kernels in storage, especially diseases/spoilage occasioned by *Aspergillus* and/or *Mucor* species. It would serve as a viable alternative for chemical fungicides, being readily available to all and sundry and possesses little or no threat to our environment.

References

Adebayo-Tayo B.C., A.A. Onilude, AA Ogunjobi, J.S. Abolagede and M.O Oladopo (2006), Detection of fungi Aflattoxin in shelved Bush mango seeds Urangia Spp). Stored for sale in Uyo Eastern Nigeria. *Electronic J. Environ Agric Food chem. 5(5) 1969-1574.*

Adexopoulus, C.J. 1962. Introducing Mycology: 1-613. *John Wiley & Sons, New York*

Adegoke, G.O., Gbadamosi, R., Evwoerhurhima, F., Uzo-Peters, P., Falade, K., Itiola, O., Moody, O., Skura, B., (2002). Protection of maize (*Zea mays*) and Soybean (*Glycine max*) using *Aframomumdanielli*. *Eur. J. Food Res and Tech***. 214**: 408-411.

Amadioha AC and Obi VI (1999). Control of anthracnose disease of cowpea by *Cymbopogon citrates* and *Ocimum gratissimum*. *ACTA. Phytopathol. Entomol. Hungarica*.**34**:85-690

Bankole SA, Joda AO and Ashidi JS (2005). The use of powder and essential oil of *Cymbopogon citratus* against mould deterioration and aflatoxin contamination of

'egusi' melon seeds. *J. Basic Microbiol***45**: 20-30

Bankole SA and Somorin, YM (2010) Antifungal activity of extracts of *Ocimum gratissimum* and *Aframomum danielli* against moulds isolated from stored rice. *J. Stored Product Protection. **425**:167.019*

Barnett, H.L and Hunter, B.B. (1972). Illustrated genera of fungi, Imperfecti 3rd edition, Burgess Publishing Company, Minnesota, U.S.A, 1972.

Berinyuy, JE, Tangka, JK and Fosto GMW (2012). Enhancing natural convection solar drying of high moisture vegetables with heat storage. *Agric. Eng. Int: CIGR J.* **14:** 141 - 148

Burt S. (2004). Essential oils; their antimicrobial properties and potential application in foods. *Int. J.Food Microbial*. **94**: 223-253

Chu Y.F., Sun J., Wu X. and Liu R.H. (2002). Antioxidant and anti-proliferative activities of common vegetables. *J. Agric. Food Chem*. **50:** 6910-6916

Doyle, M. (2007). Microbial Food Spoilage - Losses and Control Strategies. FRI BRIEFINGS, Food Research Institute, University of Wisconsin–Madison. Http://fri.wisc.edu/docs/pdf/FRI Brief Microbial Food Spoilage 7 07.pdf. Accessed July, 2013

Dubey NK, Kishore N, Varma J and Lee SY (1997). Cytotoxicity of the essential oils of *Cymbopogoncitratus* and *Ocimum gratissimum*. *India J. Pharm Sci*. **59**:263-264

Essam E. (2001). Insecticidal activity of essential oils: Octopaminergic sites of action. *Comp. Biochem. Physiol. Toxicol. Pharmacol*. **130:** 325-337

Etebu E. (2012). Postharvest pathology and phytochemicals of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) fruit and waste. *Agric Sci. Res*. **2(1)**:235-250

Etebu E. (2013). Differences in the fruit size, postharvest pathology and phytochemicals between *Irvingia gabonensis* and *Irvingia wombolu. Sustain. Agric Res*. **2(1)**:52-61

Etebu E. and Bawo D.D.S. (2012a). Fungal quality and phytochemicals of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) kernels sold in Yenagoa metropolis of Bayelsa State, Nigeria. *J.Biol.Agric. and Healthcare.* **2(11):** 41-50

Etebu E. and Bawo D.D.S. (2012b). The effect of treatment methods and storage conditions of postharvest disease and fungal quality of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) kernels. *Food Sci. and quality manag*. **10:**17-27

Frazier WC and Westhoff DC (1998). Food Microbiology. Third edition. Tata McGraw Hill publishing Company Ltd. New Delhi. pp 17-21.

Ganiyu O. (2006). Antioxidant and antimicrobial properties of ethanolic extract of *Ocimum gratissimum* leaves. *J. Pharmacol. Toxicol***. 1:** 45-53

Gomez, G.; Valdivieson, M., (1985). Cassava foliage: Chemical composition, cyanide content and effect of drying on cyanide elimination *J.Sci, Food Agric, 36(6): 433-441*.

Guenther E. (1948).The essential oils. New York, USA: Van Nostrand 56pp

Ijato, JY (2011). Inhibitory effect of two indigenous plant extracts of *Zingiber officinale* and *Ocimum gratissimum* on post-harvest yam (*Dioscorea rotundata*) rot in vitro. *J. Am. Sci*. **7(1)**:43-47.

Ikheorah, J and Okoye,W (2005). Four decades of research on aflatoxins in Nigeria: A review of NSPRI experience.In: Book of Abstracts, Regional Workshop on Mycotoxins, 7-11 February, 2005. Lagos, Nigeria.

Irina Matos, Antonio J. Pereira, Mariana Lince-Faria, Liza A. Cameroon, Edward D. Salmon, Helder Maiato. (2009) Synchronizing chromosome segregation by flux dependent force equalization at kinetochones. *J.C.B. The Journal of Cell Biology*

Jirovetz L, Buchbauer G, Ngassoum MB, Ngamo LT and Adjoudj O (2005) Combined investigation of the chemical composition of the essential oils of *Ocimum gratissimum* and *Xylopiaaethiopica* from Cameroon and their insecticidal activities

against stored maize pest, *Sitophiluszeamais. Ernähr***. 29**:55-60

Johnson T. and Case C (1995). Chemical Methods of Control," adapted from Laboratory Experiments in Microbiology, Brief Edition, 4th ed. Redwood City, CA: Benjamin/Cummings Publishing Co., available online from The National Health Museum, Access Excellence Activities Exchange [accessed September 11,2006] http://www.accessexcellence.org/AE/AEC/CC/chance_activity.html.

Ladipo D.O (1995). Collection and Conservation of the Genetic Resources of Irvingia gabonensis in West and Central Africa. Unpublished Report. ICRAFT. Nairobi, Kenya p. 26. Ndouye O, Reiz M, Ayebe A (1998). NTFPs markets and potential sources for forest Resource degradation, in central Africa. The role of research for a balance between welfare improvement and forest conservation. Paper presented at the international export workshop on NTFPs for central Africa. Limbe Botanic Garden Cameroon.

Ladipo (1999) Evaluation of agro forestry practices in Omo Forest Reserve. Consultant report. FORMECU, Federal Department of Forestry, Abuja, Nigeria.

Ladipo MK, Doherty VF and Kanife UC (2010). Phytochemical screening and anti-bacterial investigation of the extract of *Ocimum gratissimum* (Scent leaf) on selected *Enterobacteriaceae.PAT***. 6 (2)**:75-84

Latapi, G and Barret DM (2006). Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part II. Effects of storage on nutritional and sensory quality of sun-dried tomatoes pretreated with sulphur, sodium metabisulfite, or salt. *J. Food Sci.* **71:** 32 - 37

Lawrence BM (1997). Progress in essential oils: *Perf. flav*.**22**: 70-74

Lingk, W (1991). Health risk evaluation of pesticide contamination. *Drinking water.Gesunde Pflangen*, **1**:43-21.

Manczinger, L., Antal, Z. and Kredics, L. 2002. Ecophysiology and breeding of mycoparasitic *Trichoderma* strains (a review). *Acta Microbiol Immunol Hung*, 49: 1-14.

Marín S, Sanchis V, Ramos AJ, Vinas I and Magan N (1998). Environmental factors, in vitro interactions, and niche overlap between *Fusarium moniliforme*, *F. proliferatum*, and *F. graminearum*, *Aspergillus* and *Penicillium* species from maize grain. *Mycol. Res.* **102:** 831-837.

Matasyoh LG, Josphat CM, Francis NW, Miriam GK, Anne WTM, and Titus KW(2007). Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in eastern Kenya. *Afr. J. Biotech***. 6:** 760-5

Miller, JD (1991). Significance of mycotoxins for health and nutrition. In: Champ, BR, Highley E, Hocking AD and Pitt JI (Eds), Fungi and Mycotoxins in Stored Products. ACIAR Proceedings 36.Australian Centre for International Agricultural Research, Canberra, pp. 126- 135.

Nguefack, J, Leth, V, AmvamZollo, PH, Mathur, SB, (2004). Evaluation of five essential oils from aromatic plants for controlling food spoilage and mycotoxin-producing fungi. *International Journal of Food Microbiology* ***94****: 329-334.*

Niranjana S.R, Sukanya S.L., T. sudishal, J. Hariprasadi P., Eatima S.K, Prakashland, H.S. 2009. Antimicrobial activity of leaf extracts of Indian medical plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology. Vol. 8 (23) PP. 6677-6682.*

Njoku CJ, Zeng L, Asuzu IU, Oberlies NH and McLaughlin JL (1997). Oleanolic acid, a bioactive component of leaves of *Ocimum gratissimum* L. *Int J. Pharmacog*.**35:**134-7

Nychas GJE (1995). Natural antimicrobials from plants. Gould G.W. (editors) New methods of food preservation (1st ed.) New York**. 4**:58-89

Offia VN and Chikwendu UA (1999). Anti-diarrhoea effects of *Ocimum gratissimum* leaf

extract in experimental animals. *J. Ethnopharmcol*.**68:**327- 30

Okigbo RN (2004). A review of biological control methods for postharvest yams (*Dioscorea* spp.) in storage in South Eastern Nigeria.*KMITL Sci J*.**4(1)**: 207 - 215.

Okigbo RN and Ogbonnaya OU (2006). Antifungal effect of two tropical plant extract, *Ocimum gratissimum* and *Afromomum melegueta* on postharvest yam *Discorea spp* rot. *Afr. J. Biotechnol.* **5 (9)**: 727-731

Oparaocha E.I., Iwu I. and Ahanaku J.E. (2010). Preliminary study on the mosquitocidal activities of *Ocimum gratissimum*, grown in eastern Nigeria. *J. Vect. Borne Dis*.**47 (1)**:45-50

Orwa C., Mutua A., Kindt R., Jamnadass R.and Simons A. (2009). A tree reference and selection guide version 4.0. Agroforestree Database (http://www. Worldagroforestry. org/af/treedb/). Retrieved 25/9/2012

Oussalah M., Caillet S. Saucier L. and Lacroix M. (2007). Mechanism of action of Chinese cinnamon and savoury against cell membranes and wall of *Escherichia coli Salmonella typhimurium, Staphylococcus aureus* and *Listeriamono cytogenes. J. Food Prot***. 69:**1046-1055

Oyakhilome, ASO. (1985). Some neglected Nigerian ancient useful tree crops. Need for revival and incorporation into present farming systems. Bendel Agricultural and Rural Development Authority, Benin City, Nigeria.

Pandey A.K. and Chowdhury A.R. (2000). Composition of the essential oil *Ocimum gratissimum* grown in Madhya Pradesh. *J. Med. Aromat. Plant Sci*. **22:**23-28

Pauli A.andKubecka K.H. (2010). Antimicrobial properties of volatile phenylproanes. *Nat.Prod. Commun***. 5:**1387-1394

Pestka J.J. and Bondy G.S. (1994) Immunotoxic effects of mycotoxins. In J.D. Miller and H.L. Trenholm (editors), Mycotoxins in grain.52-8

Pessoa L.M, Morais S.M, Bevilaqua C.M.L, Luciano J.H.S. (2002). Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. And eugenol against *Haemonchus contortus. J. Vet. Parasitol.* **109(1-2)**:59-63

Prabhu K.S., Lobo R., Shirwaikar A.A. and Shirwaikar A. (2009). *Ocimum gratissimum*: A review of its chemical, pharmacological and ethno medicinal properties. *Open Compl. Med. J*. **1**:1-5

Prelusky. D.B., Rotter, B.A. and Rotter. R.G. (1994) Toxicology of mycotoxins. In: Miller J.D. and Trenholm H.L (editors), Mycotoxinsin Grain. Compounds other than Aflatoxin. Eagan Press, St. Paul,**359**- 403.

Ray R.C., Nedunzhiyan M. and Balangopalan C. (2000). Micro-organisims associated with postharvest spoilage of yams. *Annals of Trops. Research*. **22**:31-40

Robertson-Hoyt LA, Betrán J, Payne GA, White DG, Isakeit T, Maragos CM, Molnár TL and Holland JB (2006) Relationships among resistances to *Fusarium* and *Aspergillus* ear rots and contamination by Fumonisin and Aflatoxin in Maize. *Phytopathol.* **97:** 311-317

Schnitzler P., Astani A. and Reichling J. (2011). Screening of the antiviral activity of isolated compounds from essential oils. *Evid. Based. Alternat. Compl*. Med. **13**:120-125 Shukla AC (1997) Fungitoxic studies of some aromatic plants against storage fungi, *Ph D Theis*, University of Allahabad, Allahabad, India.

Smid E.J and Gorris L.G.M. (1999). Natural antimicrobials for food preservation. Rahman M.S. (editor) Handbook of food preservation; (2nd ed.)New York. **9:**285-308

Sulistiarini D.L., Oyen P.A. and Nguyen X.D. (1999). *Ocimum gratissimum* L. in, plant resources of south-east Asia. NO 19: Essential oils of plants. Prosea foundation, Bogor, Indonesia. 140-142

Tagne, A., Nguefack, J., Nangmo, R., the, C., AmvamZollo, P.H., 2000. Natural control of

fungi and mycotoxinsin grains – a possible means of reducing human and animal contamination. *J. App. Sci.in Southern Africa* **6:** 37-43.

Terezinha J.F., Rafael S.F., Lidiane Y., Jose R.P.S. and KI-Aneli M.B. (2006). Antifungal activity of essential oil isolated from *Ocimum gratissimum* L. (eugenolchemotype) against phytopathogenic fungi. *Braz. Arch. Biotech***. 49:**861-7

Tharanathan R.N.and Shamanna D. (1975). Composition of *Ocimum gratissimum* shrubby basil seed mucilage. *India J. Chem*. **13:** 307-8.

Udo, S.E., Madunagu, B.E and Isemin, C.D (2001). Inhibition of growth and sporulation of fungal pathogens on sweet potato and yam by garlic extract. *Nigerian J. Bot* **14**:35-39.

World Health Organization. World health report (2002) Reducing risks, promoting healthy life. Geneva, Suiza: OMS. 2002. Available from: http://www.who.int/entity/whr/2002/en/whr02_en.pdf.

Zunera Z., Shazia S., Sobiya S. and Tahir M. (2012). Antifungal potential of Parthenium hysterophorus L. plant extracts against *Fusarium solani*. *Sci. Res. and Essays.* **7(22):** 2049-2059