Bioscience Research Journal
Vol. 32, No. 1, February 29, 2020
Printed in Nigeria

0795-8072/2020 $10.00 + 0.00

http://www.niseb.org.ng/journals

BRJ 32101

Fungicidal Activities of Methanolic Plant Extracts on Mycelia Growth of *Fusarium oxysporum* Associated With Sweet Orange (*Citrus sinensis* (Linnaeus) Osbeck)

M. O. Adebola*, T. S. Bello, A. E. Oyelade, M. B. Aremu, M. J. Egubagi and S. M. Umar

Department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria
* Corresponding author Email: adebolamo@gmail.com. Phone:08033821297

(Received July 5, 2019; Accepted December 2, 2019)

ABSTRACT: Post-harvest degradation is a major challenge of sweet orange (*Citrus sinensis*) production worldwide. The deterioration in the fruit quality from bloom to harvesting stage is greatly caused by microbial infection. The present study was therefore, carried out to identify and control *in vitro*, the deteriorating fungus responsible for fruit rot disease of sweet orange during storage. Samples of spoilt orange fruits were collected fortnightly from the market. *Fusarium oxysporum* was isolated and identified from infected sweet orange fruits. Methanoic extracts of *Vernonia amygdalina, Hyptis suaveolens* and *Zingiber officinale* were used as biological agents against fungal isolates. Phytochemicals analysis revealed the presence of flavonoids, alkaloid, steroid, saponin, tannin, phenol and terpene in these extracts. All the extracts inhibited mycelia growth of *F. oxysporum.* The potency of all the extracts increased with increasing concentration in the order, 25mg/ml < 50mg/ml < 75mg/ml. *In vitro* studies of these extracts revealed that the highest percentage growth inhibition (89.6%, 72.6% and 48.9%) in *Z. officinale*, *V. amygdalina* and *H. suaveolens* respectively were obtained at 75mg/ml. Also, *Z. officinale* gave the highest percentage growth inhibition of *F. oxysporum* pathogen (75.1%, 87.0% and 89.6% at various concentrations of 25,50 and 75mg/ml respectively) compared to control, followed by *V. amygdalina* (22.8%, 36.2% and 72.6%) while extracts of *H. suaveolens* caused relatively lower inhibitory effect on mycelia growth. These results revealed that the leaf extract from *Z. officinale* was the best of the extracts studied, as an antifungal agent of the postharvest rot pathogen of sweet orange fruit. Therefore, it is recommended that further studies should be carried out to test the *in vivo* efficacy of these plant extracts in store houses since these plants are readily available, the extracts are easy to prepare and not expensive compared to conventional fungicides.

Keywords: Phytochemicals, Rot diseases, Fungicides, Disease control, *Citrus sinensis, Fusarium oxysporum*

Introduction

Sweet orange (*Citrus sinensis* (Linnaeus) Osbeck) commonly called orange is a member of the family Rutaceae and a major source of vitamins, especially vitamin C, folacin, calcium, potassium, thiamine, niacin and magnesium (Ehler, 2011; Muhammad *et al*., 2013). The global orange acreage

according to FAO statistics in 2016 was about 74.42 million tonnes in total production, this was dropped to 73.31 million tonnes in 2017 (FAOSTAT, 2017).

According to Barth *et al*. (2009), it has been calculated that about 20% of all fruits and vegetables produce is lost every year due to spoilage by pest and diseases. *Fusarium* species are widespread capable of surviving for a long period of time as chlamydospores and as mycelium in plant and its parts (Cristea, 2005). Fusarium diseases of forest crops, horticultural and ornamental crops are; wilts, blights, cankers, rots, etc. It secretes toxic substances that can infect agricultural products, making them undesirable for consumption (Desmond *et al*., 2008). *Fusarium oxysporum* reduced seed germination and seedling survivability by 40% and caused pre emergence damping off of seedlings (Begum *et al.,* 2007).

Synthetic fungicides have been used to control spoilage by *Fusarium oxysporum*. Its residues however, have effects on target crops, creating resistance in pathogens, reducing soil fertility and hazardous to the health of animal and humans alike (Riaz *et al*., 2008). The use of botanicals as fungicides has emerged as one of the prime means to protect crops and their products from fungi pathogens (Adebola *et al*., 2019), and are considered to be ecofriendly, minimum residual toxicity to the ecosystem (Ogbo and Oyibo, 2008), cost effectiveness, efficient as an inhibitor to fungal growth mostly within a few days (Adebola *et al*., 2019) and sometimes even within a few hours (Abdel-Kader *et al*., 2012).

Therefore this study investigated *in vitro* the antifungal activities of ginger (*Zingiber officinale*), bush mint (*Hyptis suaveolens*) and bitter leaf (*Vernonia amygdalina*) extracts against mycelia growth of *Fusarium oxysporum*; causal agent of fruit rot disease of sweet orange.

Materials and Methods

Collection of Materials

Spoilt sweet orange fruits were collected fortnightly between April and May, 2019 from orange sellers at Mobil fruit and vegetable market, Minna, Niger State. Healthy and mature rhizomes of *Z. officinale* (Ginger), leaves of *V. amygdalina* (Bitter leaf) were purchased from Central Market Minna, whereas, leaves of *H. suaveolens* (Bush mint) were collected from Botanical Garden of Federal University of Technology, Minna, Niger State. The plants were authenticated with voucher numbers Fut/plb/fmz/075, Fut/plb/fmlm/005 and Fut/plb/fmast/105 respectively at Department of Plant Biology, Federal University of Technology, Minna, Niger State.

Preparation of plant materials

The mature rhizomes of *Z. officinale,* leaves of *H. suaveolens* and leaves of *V. amygdalina* were washed and air-dried at room temperature for fifteen days. The dried materials were ground separately to a fine particle using a suitable blender.

Extraction of Samples

The modified methods of Odey *et al*. (2012) were used in the extraction process. One hundred grams (100 g) each of the plant parts were weighed separately and soaked in 400 ml of methanol (98% BDH) at a ratio of 1:4 (powder: solvent). The mixtures were preserved in air-tight containers and left for 48 hours at room temperature. Filtrations was done to remove residue using double layer muslin cloth followed by another stage of filtration using WhatMan No 1 filter paper (24 cm). The filtrate was then separately concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of the original volume at 37°C - 40°C. The final concentrations to dryness were done by evaporating to dryness in water bath at 60°C.

Phytochemical tests

Using the procedures described by Obasi *et al*. (2010), the powdered sample were subjected to phytochemical tests for Steroids, Alkaloids (Hager's test), Flavonoids (Alkaline reagent test), Saponins (foam test), Phenol (Ferric chloride test), Tannins (Gelatin test) and Terpene (Salkowski's test).

Preparation of Potato Dextrose Agar (PDA)

Thirty-nine (39) grams of Potato Dextrose Agar was suspended in conical flask containing 1000 ml of distilled water and heated to dissolve completely. The medium was sterilized and autoclaved at 15 lbs pressure (121°C) for 15minutes before dispensing for growth of fungi (United States Pharmacopoeia, 2016).

Isolation and Identification of Fungal Isolates

This was carried out according to little modifications to the procedures described by Jonathan *et al*. (2017). Samples of apparently spoilt sweet orange fruits were surface sterilized by wiping with 75% ethanol. They were rinsed in three changes of sterile distilled water and were later blotted dry. Portions from the advancing edges of lesions were cut using sterile knives. About one gram of each cut portion taken at random was aseptically placed in a test tube containing 9 ml of sterile distilled water. The mixtures were then homogenized using a sterile glass rod. Plates containing about 15ml of Potato Dextrose Agar, that have been mixed with Chloramphenicol (30mg/l), were inoculated with 1ml of the mixture and incubated at 27±2 ^{0}C for 7 days. Upon culture maturation, macroscopic identification of *F. oxysporum* was achieved using cultural and morphological features such as; colony texture and colour on agar (Jonathan *et al*., 2017). Also, slide culture was prepared for microscopic identification of the fungus. Lactophenol cotton blue stain was used to stain the slide in order to view the characteristics of the isolate using the Olympus model light microscope (Akinmusire, 2011). Fungi isolates was identified using fungi family of the world mycological monographs (Cannon and kirk, 2007; Amadi and Adebola, 2012).

Pathogenicity Test of *F. oxysporum* on Sweet Orange

Pathogenicity test was carried out as described by Adebola *et al*. (2019) where isolates were tested on healthy fruits for its ability to initiate spoilage. Clean matured healthy fruits of sweet orange were washed with tap water and rinsed with distilled water after which they were surface sterilized with 75% ethanol. A sterile 4 mm cork borer was used to make holes in each of the fruits. *F.* oxysporum isolates were used to inoculate some randomly selected sets of the sweet orange fruits. Controls were also inoculated, but, with Sterile Distilled Water. In each case, the point of inoculation was sealed with petroleum jelly to prevent contamination. Both sets of inoculated fruits were separately placed in sterile polyethylene bags, each, moistened with wet balls of absorbent cotton wool to create a humid environment. These were then incubated at 27 ±2^{0}C for 5 days. Afterwards, the inoculated fruits were observed for symptom development. The causal agents were re-isolated from the infected orange fruit and compared with the original isolates. This experiment was replicated three times.

Assay Test of Plant Extracts

Concentration of plant extracts for antifungal activities

Plant extracts were re-dissolved in methanol, which was the extracting solvent. The prepared extract solutions were sterilized using Millipore (0.22 µm pores) before the tests were carried out. One (1) gram of each extract was dissolved in 10 ml methanol to yield a concentration of 100 mg/ml.

Agar dilution technique

The modified methods of Kumar *et al*. (2008) were used. The re-dissolved, sterilized plant extracts using concentration 25mg/ml, 50mg/ml and 75mg/ml were mixed with sterile Potato Dextrose Agar medium (PDA) to obtain the final concentration of 100 mg/ml for each plant extract. These were then poured into sterile Petri dishes (90 mm diameter). The control was set up by substituting sterile distilled water for plant extracts. A Four (4) mm disc was used to collect *F. oxysporum* from the periphery of 6

days-old cultures. These were inoculated at the center of the Petri dishes and incubated at 27±2°C for 7 days. Mycelia growth of the inoculum been examined in each of the extracts, set up in triplicate, was recorded on a daily basis for 7 days. The percentage of mycelia inhibition was calculated thus:

$$\frac{\mathrm{R}_1 - R_2}{R_1} \times 100$$

Where; R_1 and R_2 are the growth diameter (mm) in control and treatment respectively.

Data Analysis

Statistical analyses of inhibition of radial growth was subjected to one way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 17.0 and means were separated according to Duncan's Multiple Range Test (DMRT) at 5% probability level.

Results

Pathogenicity Test

Pathogenicity test results showed that *Fusariuum oxysporum* is the causative organism of sweet orange with characteristic lesions found on the fruit as shown in Plate I.

Plate I Pathogenicity tests on healthy orange (*Citrus sinensis*) fruit

4.1 Phytochemical Screening

Phytochemical analysis (Table 4.1) showed the presence of alkaloid, saponin, tannin, flavonoid, steroid, phenol and terpene in the three plants screened. Alkaloid, flavonoid and steroid were found in all the plant extracts. The phytochemical analysis showed that *V. amygdalina* did not contain terpene. Only tannin was not found in *Z. officinale* but all other phytochemicals were present. Also, saponin was not found in *H. suaveolens.*

Table 1: Phytochemical constituents of three medicinal plants screened

Phytochemical Constituents	*Hyptis suaveolens*	*Zingiber officinale*	*Vernonia. amygdalina*
Tannin	+	-	+
Flavonoid	+	+	+
Saponin	-	+	+
Steroid	+	+	+
Alkaloid	+	+	+
Terpenes	+	+	-
Phenols	-	+	+

Key: + = Present - = Absent

4.2 Antifungal Activities of *Hyptis suaveolens* leaf extract on Mycelial Radial Growth(mm) of *Fusarium oxysporum*

Table 2 shows antifungal activities of the methanolic leaf extract of *Hyptis suaveolen* on mycelial radial growth inhibition of *F. oxysporum.* At end of period of incubation (day 7), the highest reduction in growth (2.12mm) was observed at 75mg/ml and was significantly different ($p< 0.05$) from other concentrations which also showed some reductions. However, the least reduction in mycelial growth (4.11mm) was observed in control. The same trend was observed throughout the days of incubation.

Table 2: Antifungal activities of methanolic leaf extract of *Hyptis suaveolens* at different concentrations on mycelial radial growth of *Fusarium oxysporum*

Treatments (mg/ml)	Day 1 (mm)	Day 2 (mm)	Day 3 (mm)	Day 4 (mm)	Day5 (mm)	Day6 (mm)	Day 7(mm)
25	0.84 ± 0.17^{c}	1.34 ± 0.04^{b}	1.79 ± 0.02^{b}	2.34 ± 0.04^{c}	2.80 ± 0.08^{c}	3.29 ± 0.07^{c}	3.71 ± 0.04^{c}
50	0.49 ± 0.05^{a}	0.92 ± 0.02^{a}	1.33 ± 0.03^{a}	1.70 ± 0.04^{b}	2.09 ± 0.06^{b}	2.46 ± 0.06^{b}	2.76 ± 0.07^{b}
75	0.40 ± 0.04^{a}	0.88 ± 0.05^{a}	1.16 ± 0.05^{a}	1.43 ± 0.02^{a}	1.66 ± 0.06^{a}	1.86 ± 0.12^{a}	2.12 ± 0.17^{a}
Control	0.69 ± 0.07^{b}	1.58 ± 0.22^{b}	2.03 ± 0.26^{c}	3.11 ± 0.38^{d}	3.31 ± 0.25^{d}	3.89 ± 0.14^{d}	4.11 ± 0.07^{d}

Values followed by the same superscript alphabets in the same column are not significantly different at $p>0.05$. Values are presented in mean ± standard error of three replicates.

4.3 Antifungal Activities of *Zingiber officinale*(Ginger) extracts on Mycelia Growth (mm) of *Fusarium oxysporum*

The results (Table 3) on antifungal activities of ***Z. officinale*** at end of period of incubation (day7), showed that there was no significant difference ($p>0.05$) in the fungal mycelial radial growth between 50mg/ml (0.53mm) and 75mg/ml (0.47mm) treatments compared to the control (4.11mm).

Table 3: Antifungal activities of methanolic leaf extract of *Zingiber officinale* at different concentrations on radial mycelia growth of *Fusarium oxysporum*

Treatment (mg/ml)	Day 1 (mm)	Day 2 (mm)	Day 3 (mm)	Day 4 (mm)	Day 5 (mm)	Day 6 (mm)	Day 7 (mm)
25	0.25±0.04^{b}	0.40±0.05^{b}	0.53±0.08^{b}	0.71±0.11^{b}	0.83±0.11^{b}	0.96±0.12^{b}	1.03±0.15^{b}
50	0.28±0.03^{b}	0.31±0.03^{b}	0.37±0.02^{a}	0.40±0.02^{a}	0.43±0.04^{a}	0.51±0.01^{a}	0.53±0.01^{a}
75	0.18±0.03^{a}	0.26±0.04^{a}	0.31±0.06^{a}	0.37±0.04^{a}	0.40±0.05^{a}	0.45±0.07^{a}	0.47±0.07^{a}
Control	0.69±0.07^{c}	1.58±0.22^{b}	2.02±0.26^{c}	3.11±0.38^{c}	3.31±0.25^{c}	3.89±0.14^{c}	4.11±0.07^{c}

Values followed by the same superscript alphabets in the same column are not significantly different at p>0.05. Values are presented in mean ± standard error of three replicates.

4.4 **Antifungal Activities of *Vernonia amygdalina* extracts on Mycelia Growth (mm) of *Fusarium oxysporum***

The results (Table 4) on antifungal activities of ***V. amygdalina*** at end of period of incubation (day7), showed that there was significant difference (p>0.05) in the fungal mycelial radial growth between all the concentrations used 25mg/ml(3.18mm) > 50mg/ml (2.63mm) >75mg/ml (1.10mm). The highest Mycelia radial growth (4.11mm) was recorded in control..

Table 4: Antifungal activities of methanolic leaf extract of **Vernonia amygdalina** at different concentrations on radial mycelia growth of *Fusarium oxysporum*

Treatment (mg/ml)	Day 1 (mm)	Day 2(mm)	Day 3 (mm)	Day4 (mm)	Day5 (mm)	Day6 (mm)	Day7 (mm)
25	0.78±0.00^{b}	1.15±0.03^{b}	1.50±0.05^{c}	1.82±0.05^{c}	2.28±0.07^{c}	2.70±0.04^{c}	3.18±0.05^{c}
50	0.52±0.01^{a}	0.80±0.01^{a}	1.03±0.04^{b}	1.33±0.06^{b}	1.72±0.11^{b}	2.23±0.12^{b}	2.63±0.16^{b}
75	0.44±0.03^{a}	0.77±0.05^{a}	0.92±0.03^{a}	0.98±0.04^{a}	1.03±0.04^{a}	1.07±0.02^{a}	1.13±0.29^{a}
Control	0.69±0.07^{b}	1.58±0.22^{c}	2.03±0.26^{d}	3.11±0.38^{d}	3.30±0.25^{d}	3.89±0.14^{d}	4.11±0.10^{d}

Values followed by the same superscript alphabets in the same column are not significantly different at p>0.05 Values are presented in mean ± standard error of three replicates.

4.5 Antifungal Activities of the Three Plant Extracts on Percentage Mycelial Radial Growth Inhibition (%) of *Fusarium oxysporum*

The results of the effects of methanolic extracts of the three plants tested at different concentrations on percentage growth inhibition of *F. oxysporum* is shown in Table 5.

Table 5: Effects of the Plants Extracts on Percentage Growth Inhibition of *Fusarium oxysporum*

Concentrations (mg/ml)	*Hyptis suaveolens* (%)	*Zingiber officinale* (%)	*Vernonia amygdalina* (%)
25	10.57±1.24^{b}	75.10±3.55^{c}	22.83±1.29^{a}
50	33.17±1.69^{a}	87.03±0.17^{c}	36.17±3.85^{b}
75	48.90±4.13^{a}	89.60±0.96^{c}	72.60±0.69^{b}

Values followed by the same superscript alphabets in the same row are not significantly different at p>0.05. Values are presented in mean±standard error of three replicates.

The results revealed that *Z. officinale* extracts gave the highest significant difference (p<0.05) in percentage growth inhibition of the pathogens in all the different concentrations at the end of incubation

period. At 25mg/ml (75.10%), 50mg/ml(87.03%) and 75mg/ml(89.60%).The least percentage inhibition was observed in *H. suaveolens* and was significant difference ($p<0.05$).

Discussion

The expensive and hazardous nature of synthetic antifungal agents in the control and management of fungal pathogens, *F. oxysporum* inclusive, have diverted the attention of pathologists to an environmentally safe and cost effective measure; use of plant extracts as an antifungal agent (Gupta and Dicksit, 2010). In this study, the pytochemical screening revealed variations in the active compounds present in each of the plant extracts. Such variation could best explain the varying inhibitory effects observed.

This study revealed that as the concentration of the extracts increased, the mycelia growth of *F. oxysporum* was proportionally checked has observed and earlier reported by Adebola *et al*. (2019) that the highest concentration of the extract used gave the highest inhibition of the pathogen mycelia growth which decreased with decrease in concentrations of the extract.

This antifungal study showed that *Z. officinale* gave the highest percentage growth inhibition of *F. oxysporum* compared to the other extracts studied. This result might probably be as a result of phytochemical variations in the extracts. This was in agreement with Atai *et al*. (2009) and Supreetha *et al*. (2011) in separate reports, that ginger, containing phytochemical which makes it fungicidal against *Candida albicans*. Kubra *et al*. (2013) added that the presence of dehydrozingerone, a phytochemical in ginger with antifungal properties, might be responsible for its activities against some fungi pathogens.

Similar opinion was shared by Moreira *et al*. (2010) who reported that the phytochemical constituents and the essential oils obtained from plant could be fungicidal against pathogenic fungi. Fadina (2010) and Chiejina (2013) have also reported similar reports on the potential of extracts from *V. amygdalina* and *Z. officinale* in the control of various fungal pathogens.

The inhibitions recorded against fungi by *V. amygdalina* could be as a result of active components such as alkaloids, saponins and tannins present in the extract. Adebola *et al*. (2018) explained similarly, that the leaves extracts of *A. indica* containing alkaloids, saponins, tannins, have fungicidal properties.
Increase in the concentration of the plant extracts correspondingly decreased radial growth of *F. oxysporum*. This could be that an increase in the concentration of the extract, has increased the quantity of the active ingredients present in the extracts, thereby increasing their inhibitory effects on the fungus as opined by Akshada (2017).

Conclusion and Recommendations

The study showed that plant parts contain active antifungal phytochemicals that are able to inhibit fungal growth of *F. oxysporum*. It was observed that the higher the concentration of the extracts, the higher the inhibitory effects on growth of *F. oxysporum*. Extract obtained from rhizomes of *Z. officinale* showed the highest percentage inhibitory effect at all concentrations, while leaves extract of *H. suaveolens* showed the least.

On this note, the specific active phytochemicals responsible for the antifungal activities of these plant extracts should be investigated.

References

Abdel-Kader, M.M., N.S., El-Mougy, N.G., El-Gamal, R.S., El-Mohamdy, Y.O. & Fatouh. (2012). *In vitro* assay of some plant resistance inducers, essential Oils and plant extracts on antagonistic ability of fungal bio-agents. *Journal of Applied Sciences Research*, 8(3), 1383-1391.

Adebola, M.O., Ayeni, O.B., Aremu, M.B., Kalesanwo, A.O. (2018). Evaluation of leaf extracts of four plant species against rice blast pathogen ((*Magnoporthe oryzae* (T.T. Hebert) M.E. Barr.). *Nigerian Journal of Mycology* 10: 88-101.

Adebola, M.O., Bello, T.S., Yusuf, H. & Egubagi, J.M. (2019). Efficacy of Three Selected Botanicals in the Control of *Botrytis cinerea* Associated With Damping Off in *Citrullus lanatus* (Thumb.) Matsum and Nakai. *BIU Journal of Basic and Applied Sciences*, 4(1), 16-26.

Akinmusire, O.O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri, North Eastern Nigeria. *Advances in Environmental Biology*, 5(1): 157-161.

Akshada, K. (2017). Phyto-active compounds from herbal plant extracts: Its extraction , isolation and characterization. *World Journal of Pharmaceutical Research*, 6(8): 1186-1205.

Atai, Z., Atapour, M. & Mohseni, M. (2009). Inhibitory effect of ginger extract on *Candida albicans*. *American Journal of Applied Science*, 6(6): 1067-1069.

Barth, M., Hankinson, T.R., Zhuang, H. & Breidt, F. (2009). Microbiological spoilage of fruits and vegetables. In: Sperber, W.H., Doyle, M.P. (eds.), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety. Springer Science+Business Media, LLC pp. 135-183.

Begum, M.M., Sariah,M., Zainal, M.A., Puteh, A.B. & Rahman, M. A. (2007). Histopathological studies on soybean seeds infected by *Fusarium oxysporum* f. sp. *glycines* and screening of potential biocontrol agents. *Research Journal of Microbiology*, 2: 900-909.

Cannon, P. F. & Kirk, P.M. (2007). Fungal families of the world, *CAB international*, 250 – 257

Cristea, S. (2005). Cris Book Universal, București. *Romanian Biotechnological Letters*, 20(6), 10921-10940

Chiejina, N. (2013). Efficacy of *Aframomum melegueta* and *Zingiber officinale* extracts on fungal pathogens of tomato fruit. *IOSR Journal of Pharmacy and Biological Sciences*, 4: 13-16.

Desmond, O.J., Manners, J.M., Stephens, A.E., Madens, D.J. & Schenk, P.M. (2008). The Fusarium mycotoxin deoxynivalenol elicits hydrogen peroxide production, programmed cell death and defense responses in wheat. *Journal of Molecular Plant Pathology*, 9: 435-445.

Ehler, S. A. (2011). Citrus and its benefits, *Journal of Botany*, 5,:201-207.

Fadina, O.O. (2010). The antifungal and nematicidal potentials of *Vernonia amygdalina* (DAL). *Acta Horticulturae*, 853: 357-362.

Food and Agriculture Organization, Statistics Division (FAOSTAT). (2017). Orange Production in2017;Region/World/Production/Quantity/Crops from picklists. http://www.fao.org/faostat/en/#data/QC Retrieved 10 June, 2019.

Gupta, S. & Dikshit, A.K. (2010). Biopesticides: An Eco-friendly approach for pest control. *Journal of Biopesticides*, 3(1): 186-188.

Jonathan, S.G, Bello, T. S. and Asemoloye, M. D. (2017). Food values, Spoilage moulds and aflatoxin detection in 'Attiéké' (A Cassava Fermented Product). *Journal Microb Biochem Technology*, 9:244-248.

Kubra, I.R., Murthy, P.S. & Rao, J. (2013). *In vitro* antifungal activity of dehydrozingerone and its fungi toxic properties. *Journal of Food Science*, 78, 64-69.

Kumar, V., Basu, M.S., & Rajendran, T.P. (2008). Mycotoxin research and mycoflora in Some commercially important agricultural commodities. *Crop Protection*, 27, 891-905.

Moreira, A.C.P., Lima, E.O., Wanderley, P.A., Carmo, E.S. & Souza, E.L. (2010). Chemical composition and antifungal activity of *Hyptis suaveolens* (L.) Poit leaves essential oil against *Aspergillus* species. *Brazilian Journal of* Microbiology, 41: 28-33.

Muhammad, N.O., Soji-Omoniwa, O., Usman, L.A. & Omoniwa, B.P. (2013). Antihyper glycemic activity of leaf essential oil of *Citrus sinensis* (L.) Osbeck on alloxan-induced diabetic rats. *Annual Research and Review in Biology*, 3(4), 825-834.

Obasi, N.L., Egbuonu, A.C.C., Ukoha, P.O. & Ejikeme, P.M. (2010). Comparative phyto -chemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. *African Journal of Pure and Applied Chemistry*, 4(9), 206-212.

Odey, M.O., Iwara, I.A., Udiba, U.U., Johnson, J.T., Inekwe, U.V., Asenye, M.E. & Victor, O. (2012). Preparation of plant extracts from indigenous medicinal plants. *International Journal of Science and Technology*, 1, 12.

Ogbo, E.M., & Oyibo, A.E. (2008). Effects of three plant extracts (*Ocimum gratissimum*, *Acalypha wilkesiana* and *Acalypha macrostachya*) on post-harvest pathogen of *Persea American Journal of Medicinal Plants Research*, 2(11):311-314.

Riaz, T., Khan, S.N. & Javail, N. (2008). Antifungalactivity of plant extracts against *Fusarium oxysporum*. The cause of cormrot disease of gladiolus. *Journal of Mycopathology*, 6, 13-15.

Supreetha, S., Mannur, S., Simon, S.P., Jain, J., Tikare, S. & Mahuli, A. (2011). Antifungal activity of ginger extract on *Candida albicans*: An *in vitro* study. *Journal of Dental Sciences and Research*, 2(2): 1-5.

The United States Pharmacopoeia. (2016). The United States Pharmacopoeial Convention. Rockville, MD.