



ANTI-FUNGAL EFFECTS OF GINGER RHIZOME EXTRACTS ON MYCELIAL GROWTH OF SOME FUNGAL PATHOGENS OF *Dioscorea rotundata* IN TARABA STATE, NIGERIA

^{1*}Aji, P.O. & ²Tunwari, B.A.

^{1*}Department of Biological Science, Federal University Wukari, Katsina - Ala Road, P.M.B. 1020, Wukari, Taraba State, Nigeria.

²Department of Crop Production and Protection, Federal University Wukari, Katsina - Ala Road, P.M.B. 1020, Wukari, Taraba State, Nigeria.

Corresponding Author's e-mail address: ajipheobi@gmail.com

ABSTRACT

The objective of this study is to isolate, identify and to determine the effect of various concentrations of aqueous and ethanol extracts from the rhizome of the test plant *Zingiber officinale* in the control of fungal isolates from yam rot in Wukari Nigeria. Rotted yam tubers were obtained from three markets in Wukari. The ingredients of test plant were extracted by aqueous and ethanol solvents. Rhizome extracts at different concentrations (0%, 20%, 40% and 60%) of aqueous and ethanol extraction of the test plant was poisoned to growth media prior to inoculation. The fungi associated with the spoilage of the sample of the yam tuber were identified base on their morphological characteristics. Of all the samples studied, three species of fungi were found to be associated with the yam rots. The most commonly isolated fungi were *Aspergillus niger* and others are *Aspergillus flavus* and *Rhizopus stolonifer*. All concentrations used suppressed the mycelia growth of the tested pathogens except the control treatment. The effect was proportional to concentrations and inhibition value was highest at 60% concentration, for aqueous extraction, *Zingiber officinale* was more effective on *Aspergillus flavus*, for both aqueous and ethanol extractions, *Zingiber officinale* was more effective on *Rhizopus stolonifer*. Phytochemical analysis showed that the extracts contain, tannin, saponins, terpenoids, alkaloid, steroids and those absent phenols, glycosides, anthraquinones, anthracenes and flavonoids. The presence of these compounds supports the use of the extracts as antimicrobial agents which can prolong the shelf-life of yam under storage.

KEY WORDS: Antifungal effects, Extracts, Phytochemicals, *Zingiber officinale*.

INTRODUCTION

The yam tuber belongs to the genus *Dioscorea* in the family of Dioscoreaceae and is monocotyledonous. It is one of the highly rated and commonest food crops of the tropical world. The edible varieties of yam are important food crop and serve as an important carbohydrate staple for millions of people in both the tropical and subtropical countries even in West Africa, The Caribbean, Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania (Coursey, 1967; Okigbo and Ikediugwu, 2000). The FAO (1989) estimated that the world production is around 20 million tons per year. Nigeria alone produces three quarter of the world total output of yams. Of the ten cultivated species, the six most important in Nigeria are *Dioscorea rotundata* Poir (white yam). *Discorea cayenensis* Lam (yellow yam). *Discorea alata* L. (Water yam). *Discorea dumetorum* (Cluster, or bitter yam). *Discorea esculenta* (Loir) bark (Chinese yam) and *Discorea bulbifera* L. (aeria yam) (Adeniji, 1970; Okigbo, 2004). The principal microorganisms associated with yam in Nigeria include *Aspergillus niger*, *Van*, *Tiegh*, *Hendersonula rotuloidea*, *Macrophomina phaseoli*, *Rhizopus nodosus* Namyslowski, *Botrodiploida theobrome*, *Fusarium monoliform varsubgluctinanus* Wollenw and *Reinking.Pencilium sclerotigenum* Yamamoto, and *Rosellina bundodes* (Berk and Br) Sacc. (Ogundana *et al.*, 1970; Adeniji, 1970;

Ogundana, 1972; Okigbo and Ikediugwu, 2000). Other fungi which have been reported as secondary invaders are *Fusarium oxysporum* Schlecht, *Cladosporium spearo spermum*, *Fusarium solani*, *Geotrichum candidum* (Okafor, 1966; Coursey, 1967; Adeniji, 1970). *Fusarium solani*, *Rhizopus stolonifera*, *Botrodiploida theobrome*, *Geotrichum canacidum* (Okafor, 1966; Coursey, 1967; Adeniji, 1970) some such as *Trichoderma* and *Billus subtilis* are also effective in the control or reducing storage rot in yam (Okigbo, 2005). The uses of synthetic chemicals such as sodium orthiophenylphenate, borax, captan, thiobendazole, benomyl, bleach (sodium hypochlorite) have been found to significantly reduce storage rot in yam (Booth, 1974; Noon, 1978.). Other control methods involve the use of microorganism such as *Trichoderma viride* and *Bacillus subtilis* (Okigbo and Ikediugwu, 2000; Okigbo, 2002). However, farmers in developing economies such as Nigeria have hardly adopted these findings, because the majority of them cannot afford the financial cost. Moreover, chemical pesticides have the additional potential disadvantages of accumulation in the ecosystem and of induction of pesticide resistance in pathogens (Adeniji 1970; Okigbo and Ikediugwu, 2000; Okigbo, 2004). There is also the problem of lack of expertise in the safe handling of pesticides among most of the farmers.

The use of synthetic chemicals such as sodium orthiophenylphenate and borax has been found to reduce storage rot yam (Booth,1974).But biological control is generally favoured as a method of plant disease management (Okigbo and Ikediugwu, 2000; Okigbo, 2002; 2005). Kuhn and Hargreaves (1987) observed that substances found fungicidal *in vitro* in almost all cases kill the fungus *in vivo*. Plant extracts have been used to control yam diseases (Okigbo and Ogbonnaya, 2006), Plants with such fungicidal properties include *Zingiber officinale* (Maurice, 1993). *Z. officinale* (family: *Zingiberaceae*) is a herbaceous perennial plant which has an upright stems and narrow medium, green leaves arranged in two ranks on each stem.

MATERIALS & METHODS

Collection of yam

Ten yam tubers with symptoms of rot were obtained from new market, Old market of Wukari, Taraba state, Nigeria,

Isolation of spoilage fungi from rotted yam

Potato dextrose agar were routinely used for culturing fungi respectively during the study, laco-phenol cotton blue stain was also use for microscopic examination of fungi ,All materials used were adequately sterilized

Collection and Preparation of Plant Extracts

The method of Ijato (2011) was used to prepare both aqueous and ethanol extracts. *Zingiber officinale* rhizomes (Plate 1) were collected from New Market of Wukari local Government, Taraba State. The plants were taken to the

Biological Sciences Department, Federal University Wukari, Taraba State. The collected plant parts were washed thoroughly under running tap water and were allowed to air dry for 7 days. These were being grinded separately. Thirty grams of each sample was added to 1 ml of distilled water in separate conical flasks. This was vigorously shaken and left to stand for 24 hours. The samples were filtered with 3 layers cheese cloth and filtrate extract preparation of 60%, 40% and 20% concentrations were used as the aqueous extract. The same procedure was used for 60%, 40% and 20% ethanol extract.

Pathogenicity Test

Pathogenicity test was carried out using techniques of Okigbo *et al.* (2009). Healthy yam tuber was washed with sterile distilled water, wiped dry using Whatman No.1 filter paper and surface sterilized with 0.1 % mercury chloride solution to remove surface contaminants and rinsed in three changes of sterile distilled water. A sterile 2 mm cork borer was used to make a 2 mm cut on the yam tuber and then culture of the isolates were inoculated into the open cut surface and the removed tissue was replaced with the core borer and sealed with Vaseline jelly. Yam tuber was inoculated in three replicates. The yam tuber was incubated for 5 days. On establishment of disease symptoms, the infected yam tissue was taken and cultured until pure cultures were obtained (Pates 2 and 3). The morphological and microscopic characteristics of the Isolates were compared with the original isolate.



PLATE 1: *Zingiber officinale* rhizomes



PLATE 2: Infected Yam Tubers



PLATE 3: Yam Tubers showing rot after 14days of inoculation

Effect of Plant Extracts on Fungal Mycelia Growth

The approach of Ijato (2011) was used to evaluate the effect of the extract on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. This was done before dispensing PDA into each of the plates. The extracts were poured into the flask, plugged with cotton wool and wrapped with aluminum foil to avoid contamination (Madari and Singh, 2005). About 2 mls each of extract of *Zingiber officinale* was separately introduced into the Petri-dishes containing the media and the pure isolates (poisoned food method). Control experiments were without addition of any plant extract but sterile distilled water. Fungitoxicity was determined in terms of percentage colony inhibition % (Nene and Thalpiyal, 2000).

Percentage Colony inhibition =

Where;

DC = Average Diameter of fungal colony in control

DT = Average diameter of fungal colony with treatment.

Phytochemical Analysis of Plant Materials

Qualitative analyses of the constituents of the plant extracts were carried out. The presence of biological active ingredients in the *Zingiber officinale* was investigated using standard methods as described by Edeoga *et al.* (2012).

Test for tannins

Two millilitres each of the aqueous and ethanolic extracts were separately boiled for ten minutes in 10 ml of water in a test tube. A few drops of 0.1 % ferric chloride were added to each test tube and observed for 10 minutes for a brownish green or a blue black coloration (Okwu, 2005). This test was repeated once to confirm the results.

$$\frac{DC - DT}{DC} \times 100$$

Test for phlobatannin

Two millilitres each of the aqueous and ethanolic extracts of the plant samples were boiled for 10 minutes with 1 % aqueous hydrochloric acid. The deposition of a red precipitate was taken as evidence for the presence of

phlobatannins (Okwu, 2005). The test was repeated once as above to confirm results.

Test for saponins

Frothing test according to Trease and Evans (1989) was adopted. About 5 ml each of the aqueous and ethanolic extracts of the samples were shaken with equivalent amount of water in a test tube for 5 minutes. This was boiled in the water bath for 5-10 minutes. Frothing that persists on warming was taken as evidence of the presence of saponins (Trease and Evans, 1989). The procedures were repeated as well to confirm the results.

Test for flavonoids

About 5 ml of dilute ammonia solution was added to 3 ml each of the aqueous and ethanolic extracts, followed by the addition of concentrated tetraoxosulphate (VI), (H₂S₀4). A yellow coloration was taken as evidence for the presence of flavonoids (Okwu, 2005). This test was repeated again to confirm results.

Test for alkaloids

About 2 ml each of the aqueous and ethanolic extracts were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath for 10 minutes; 1 ml of the extract was treated with a few drops of Mayer's reagent, precipitation with these reagents was seen as evidence for the presence of alkaloids. The method was repeated again to confirm the results (Sofowora, 1993).

Test for steroids

One millilitre each of the aqueous and ethanolic extracts was dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish brown colour formed at the interphase indicates the presence of a steroid ring (Sofowora, 1993). The same procedures were repeated once to confirm the results.

Test for terpenes

One millilitre each of the aqueous and ethanolic extracts were added to 2 ml of chloroform and treated with five drops of acetic anhydride along with 2 drops of concentrated sulphuric acid. A pink colour formed at the interphase indicated a positive test of terpenes (Sofowora, 1993). This test was repeated once to confirm results.

Experimental Design and Data Analysis

The experimental layout was completely randomized design containing aqueous and ethanol extract treatments each at 0%, 20%, 40% and 60% concentrations. The

experiment was replicated three times. All the data was analyzed using analysis of variance (ANOVA) according to Gomez and Gomez (1984). Least Significant Difference (LSD) according to Scheff (1953) was being used to separate the means where there was significant difference.

RESULTS & DISCUSSION

Isolation and Identification of the Pathogens

Three fungi were found associated with rotting of the yam tubers in all the three markets surveyed. The isolated fungi from Yam tubers were identified as, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus stolonifer*. The frequency of occurrence from the three markets (Table 1) Shows that *Aspergillus niger* has the highest percentage frequency of occurrence of 40 %, followed by *Rhizopus stolonifer* (33.33 %). These fungal species isolated and identified in this study corroborate those reported by

Ogaraku and Usman (2008). The pathogenicity test (Table 2) confirmed the natural pathogens responsible for the rot disease in the sampled yam tubers. The intrinsic ability of some exposed yam tubers has equally been reported (Okigbo and Ogbonna, 2006; Oyelana *et al.* 2011). The average spread of the rotted area at 14 days after incubation (1.10 – 6.80cm) was observed for all the fungal species. *Aspergillus niger* exhibited a wider area (6.80 cm) followed by *Aspergillus flavus* with 5.70 cm spread (Table 2). A significantly different result was reported by Oyelana *et al.* (2011) in which they observed that *Penicillium chrysogenum* exhibited a 62 mm spread and a 60 mm and 55 mm spread by *Fusarium solani* and *Aspergillus flavus* respectively. These implicated organisms posed a significant threat to the revenue of farmers and the health of consumers.

TABLE 1: Frequency of Occurrence (%) of the Pathogens Isolated from the Yam Tubers

Fungal isolates	Frequency of occurrence in different locations				Percentage (%) occurrence
	New Market	Old Market	Yam Market	frequency	
<i>A. niger</i>	3	5	4	12	40
<i>A. flavus</i>	4	3	1	8	26.67
<i>R. stolonifer</i>	3	2	5	10	33.33
Total	10	10	10	30	100

LSD = Least Significant Difference

TABLE 2: Pathogenicity Test of the Pathogens Isolated from Yam Tuber

Days	Mycelia growth of fungal organisms		
	<i>A. niger</i>	<i>R. Stolonifer</i>	<i>A. flavus</i>
1	0.00 ⁱ	0.00 ^h	0.00 ⁱ
2	0.00 ⁱ	0.00 ^h	0.00 ^h
3	0.00 ⁱ	0.00 ^h	0.00 ^h
4	1.50 ^h	0.00 ^h	0.00 ^h
5	2.30 ^g	0.00 ^h	0.85 ^g
6	3.60 ^f	0.00 ^h	1.10 ^{fg}
7	4.50 ^c	0.00 ^h	1.50 ^f
8	5.20 ^d	0.95 ^g	1.87 ^f
9	5.95 ^c	1.30 ^g	2.35 ^e
10	6.10 ^b	1.97 ^e	3.64 ^{cd}
11	6.35 ^a	2.70 ^d	3.97 ^c
12	6.50 ^a	3.20 ^c	4.30 ^c
13	6.55 ^a	3.70 ^b	5.10 ^b
14	6.80 ^a	4.32 ^a	5.70 ^a
LSD(P=0.0001)	0.47	0.43	0.46

Effect of the *Zingiber officinale* extract at different concentrations on the fungal isolates

Z. officinale extracts at various concentrations on the growth of pathogens are shown in Table 3. The results showed that the extracts *Z. officinale* significantly ($p < 0.05$) reduced the radial growth of the pathogens in both aqueous and ethanol media (Table 3). All concentrations of aqueous and ethanol ginger rhizome extract suppressed the mycelial growth of the 3 tested pathogens. The effect was proportional to concentration of the extract. The inhibition was highest at 60% concentration and the lowest at 20 % concentration. This is in agreement with the works of Trease and Evans (1989); Amadioha and Obi, (1999)

and Udo *et al.* (2001) who reported the high potency of plant extracts for the control of pathogenic fungi of other crops. Results of the phytochemical analysis revealed the presence of Tannins, phlobatannins, steroids, terpenes Saponins, flavonoids and alkaloids in *Zingiber officinale* extracts (Table 4). The presence of these phenolic compounds in this extract indicates that this plant can serve as antimicrobial agent. This is because phenol and phenolic compounds have been extensively used in disinfection and remain the standard with which other fungicides are compared (Doherty *et al.*, 2010). Phenolic compounds act as electron donors and are readily oxidized to phenolate ion or quinone, an electron acceptor (Doherty

et al., 2010). The antifungal activity of the oil is believed to be associated with the phytochemical components of these plants (Matasyoh *et al.*, 2007) which diffuse into and damage cell membrane structures. Velluti *et al.* (2004) highlighted that generally, one of the critical things to consider for commercial applications is that the levels of essential oils and their compounds necessary to inhibit the microbial growth were higher in foods than in culture media. This is due to interactions between the phenolic compounds and the food matrix (Nuchas and Tassou, 2000). Extracts from rhizomes of *Z. officinale* therefore have potent antiseptic, bactericidal and fungicidal properties. These findings support the use of these extracts

for prevention of infections as also reported by Okwu (2004). Results of this work suggest that fungitoxic compounds are present in *Z. officinale* extracts since they were able to control the growth of the fungal pathogens tested. This is in agreement with the work of Udo *et al.* (2001) who worked on the inhibition of growth and sporulation of fungal pathogens in

Ipomoea batatas and *Dioscorea* sp by garlic extracts. The antimicrobial activity of these plants also agrees with the work of Adejumo and Langenkamper (2012), which showed that methanolic extracts of leaves of botanicals possessed antimicrobial properties.

TABLE 3: Effects of Extracts of *Zingiber officinale* on the Growth of the Isolates

Concentrations (%)	Solvent	<i>A. flavus</i>	<i>A. niger</i>	<i>R. stolonifer</i>
0	Aqueous	68.95 ^a	74.32 ^a	65.04 ^a
0	Ethanol	50.63 ^b	63.26 ^b	50.87 ^b
20	Aqueous	57.50 ^b	64.55 ^b	57.30 ^b
20	Ethanol	41.03 ^{cd}	54.00 ^c	44.56 ^c
40	Aqueous	52.11 ^b	55.20 ^c	50.90 ^b
40	Ethanol	39.76 ^d	40.00 ^d	35.36 ^d
60	Aqueous	39.00 ^{de}	40.12 ^d	43.00 ^c
60	Ethanol	31.15 ^e	30.78	28.77 ^e
LSD (p=0.0001)		8.10	6.31	5.25

LSD = Least Significant Difference

TABLE 4. Quantitative Determination of phytochemical Groups of Extract of Test Plant Leaves

S/N	Compounds	<i>Zingiber officinale</i>	
		<i>Aqueous</i>	<i>Ethanol</i>
1.	Tannins	++	++
2.	Alkaloids	+	++
3.	Flavonoids	-	+
4.	Glycoside	-	-
5.	Saponins	+	+
6.	Phlobatannins	-	+
7.	Terpenes	+	+
8.	Steroids	+	++
9.	Anthraquinones	-	-

++ = Present in high amount; + = Present in moderate amount; - = Absent

Also Okigbo and Nmeka (2005) used leaf extracts of *Xylopiya aethiopicum* and *Z. officinale* to control yam tuber rot caused by *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*. *A. melegueta* extract was also used by Okigbo and Ogbonnaya (2006) in the control of *F. oxysporum* and *A. niger* rot in yam tubers. Also the effect of aqueous extract of ginger was evaluated by Stangarlin *et al.* (2011) at the concentrations of 1, 5, 10, 15, 20 and 25 % on *Sclerotinia sclerotiorum* mycelial growth and sclerotia production, *in vitro*. The efficiency of protection of *Z. officinale* was also verified in lettuce plants. Besides the reduction in disease incidence, the authors reported that, the crop yield and the peroxidase induction were also analyzed in the plant tissues. This antimicrobial property of ginger in reducing the mycelial growth of fungal pathogens is in line with the results of this study. Ijato (2011) reported that extracts of *Z. officinale* and *Ocimum gratissimum* were mycotoxic to *Fusarium oxysporum*, *Botrydioploida theobromae*, *Aspergillus flavus* and *Aspergillus niger* of postharvest rot of yam tubers and that the effectiveness of the extracts increased with increase in

concentration as was observed in this study (Table 3). Further studies on these effective botanical should gear towards fractionation of the extracts which will lead to the isolation of the compounds that is showing considerable antifungal activity. The continuation of study on the plant is essential to isolate, identify, characterize and elucidate the structure of the bioactive compounds responsible for the observed antifungal activities. From this result, it is essential to investigate the specific constituents which are responsible for this observed activity. The *in vivo* study is also required to confirm the usefulness of the obtained results.

CONCLUSION

The conclusion drawn from these studies showed that *A. niger*, *A. flavus* and *R. stolonifer* are common pathogenic fungi which cause tuber rot in yam in the study area. The result from the pathogenicity test indicated that all the isolated fungi are pathogenic and attributed to the cause of yam tuber rot in Wukari. The inhibitory effect of the plant extracts against fungal isolates could be due to the

presence of antifungal substances in the extract. Higher inhibition of fungal growth was observed at higher concentrations of the aqueous and ethanol extracts. The result also indicated that ethanol is better solvent than water for the extraction of active ingredients from this plant; the results of the present investigation are clear indications for the potential of plant extracts to control fungal pathogens. It is also clear from the result that the test plant extract significantly reduce the radial growth of isolated fungi and this finding is first of its kind in Wukari. It seems that the anti-fungi effects are the results of many compounds acting synergistically (Bediakao *et al.*, 2007). This can be formulated and successfully produced as fungicides with local technology, which can be applied at both pre and post-harvest in yam rot /management.

REFERENCES

- Adejumo, T.O. and Langenkämper, G. (2012) Evaluation of botanicals as biopesticides on the growth of fusarium verticillioides causing rot diseases and fumonisin production of maize. *Journal of Microbiology and Antimicrobials*, 4(1):23-31.
- Adeniji, M.O. (1970) Fungi associated with storage decay of yam in Nigeria. *Phytopathology*.**60**: 590-92.
- Amadioha, A.C and Obi, V.I (1999)Control of Anthranose diseases of Cowpea by *Cymbopogon cunitus* and *Ocimum gratissimum*. *Acto Phytopathology and Entomology* 85: 89.
- Booth, R.H. (1974) Post-harvest deterioration of tropical root crops.*Losses and their control.Trop. Sci.* **16**(2).Pp 49-63.
- Bediakao, A., Showemimo, F.A., Asiamo, Y.O. and Amewowor, D. H. A. K. (2007) In vitro analysis of growth media and the control of yam minisett-rot. *Biotechnology* **6**: 1- 4.
- Coursey, D.G. (1967) Yams. Longmans, London. 230pp.
- Doherty, V.F., Olaniran, O.O. and Kanife, U.C. (2010) Antimicrobial activities of *Aframomum melegueta* (Alligator pepper). *International Journal of Biology*. 2(2):126-131.
- Edeoga H.O, Okwu D.E and Mbaebie B.O. (2012) Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. 2005; 4(7):685-688.
- FAO (1989) Roles of rot tuber crop in food security in sub-Saharan Africa. Food production year book.43:14.n
- Gomez, K.A. and Gomez A.A. (1984) Statistical procedure for Agricultural Research .John wiley and sons. Pp 680
- Ijato, J.Y. (2011) Inhibitory effects of Indigenous plant extracts (*Zingiber officinale* and *Ocimum gratissimum*) on post harvest Yam, (*Dioscorea rotundata rot*) *in vitro*, *Journal of American science*. **7**(1): 43-47.
- Kuhn P.J., Hargreaves J.A. (1987) Antifungal substances from herbaceous plants. In: FG Peggs and AG Ayes (eds). Fungal infection of plants. Symp. British Mycol. Soc. CambridgeUni. Press. pp. 48-129
- Madari, S. and Singh, R.P. (2005) Management of mushroom pathogens through botanicals, *India phyto Pathology*.**58**, 189-193.
- Matasyoh, J.C., Wagara, I.N., Nakavuma, J.L and Kiburai, A.M (2007) Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. *African Journal of Food Science*, 5(3):138-142.
- Nene, Y. and Thalplyal, L. (2000) Poisoned Food technique of fungicides in plant disease control 3rd Edn. Oxford abd TBH Publishing Company; New Delhi.
- Nuchas, G.E and Tassou, C.C. (2000) Traditional preservatives-oils and spices. In Robinson, RK, Batt CA, Patel PD (Eds.), *Encyclopedia of food microbiology*. London, UK: Academic Press, pp. 1717–1722.
- Ogundana, S.K., Naqvi, S.H.Z. and Ekundayo, J.A. (1970) Fungi associated with soft rot of yams in storage in Nigeria. *Trans. Br. Mycol. Soc.* Pp 54.
- Ogundana, S.K. (1971)The post-harvest decay of yam tubers and its preminary control in Nigeria.*Biodeteroration of materials* (Eds.) A.N. Walter and E.H. Hueck VAN Plas.2:481-492.
- Okigbo, R.N. and Nmeka, I.A. (2005) Control of Yam tuber rot with leaf extracts of *Xylopiia aethiopicia* and *Zingiber officinale*. *Africa Journal of Biotechnology*, 4(8): 804-807.
- Okigbo, R.N and Ogbonnanya, O.U (2006) Antifungal effects of two tropical plant extracts *Ocimum gratissimum* and *Aframomum melegueta* on post harvest yam *Dioscorea spp* rot. *African Journal of Biotechnology*, 5:727-731.
- Okigbo, R.N. and Ikediugwu, F.E.O. (2002) Evaluation of water losses in different regions of yam (*Dioscorea spp.*) tuber in storage, *Nig. J. Exp. Appl Bio* 3. 320 pp
- Ogaraku, A.O. and Usman, H. O. (2008) Storage Rot of some yams (*Dioscorea spp*) in Keffi and Environs, Nassarawa State, Nigeria. *J. PAT.* 4(2): 22 – 27.
- Okafor. N. (1996) Microbial rotting of stored yam (*Discorea spp*) in Nigeria. *Exp Agric*.**2**:179-182.
- Okigbo, R.N. and Ogbonnaya U.O. (2006) Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam rot.*African Journal of Biotechnology* **5**(9): 727-731
- Okigbo, R.N. (2004) A review of biological control methods for postharvest yams (*dioscorea spp*) in storage in south Eastern Nigeria *KMITL. Sci. j.*, 4(1): 81-85.

- Okigbo R.N. (2005) Biological control of postharvest fungal rot of yams (*Discorea* spp.) with *Basillus Subtilis*. *Mycopathologia* 159(2):307-314.
- Okigbo, R.N. and. Emoghene, A.O. (2004) Antifungal activity of leaf extracts of some plant species on *Mycosphaerellafijiensis* Morelet, the causal organism of black sigatoka disease in banana (*Musa acuminata*). *KMITL Science Journal* 4(1): 20-31.
- Okigbo, R.N. and. Ikediugwu F.E.O (2000) Studies on biological control of postharvest rot of yam with *Trichoderma viride*. *Journal.Phytopathol.* 148: 351-355.
- Okigbo, R.N., Anuagasi C.L., Amadi J.E. (2009b) Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plant Research*, 3(2): 86-95.
- Okwu, D.E. (2004) Phytochemicals vitamins content of Indigenous spices of South Eastern Nigeria. *Journal of Sustain Agricultural Environment*.6:30-34.
- Okwu, D.E. (2005) Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. *International Journal of Molecular Medicine and Advance Sciences*, 4:375-381.
- Noon, O. (1978) Fungus Diseases of Plants and their Treatments. *Agricultural Research*, 11, Scheff, H, (1953). A method of judging all contrast in the Analysis of Variance. *Biometric* 40:104-107, 50-55.
- Stangarlin, J.A., Kuhn, O.J., Assi, L. and Schwan-Estrada, K.R.F (2011) Control of plant diseases using extracts from medicinal plants and fungi, 1033-1042pp. In: *Communicating Current Research and Technological Advances*. (Mendez-Vilas Ed).
- Sofowora, A. (1993) Medicinal plants and Traditional medicine in Africa. Spectrum Books limited, Ibadan, Nigeria, 346pp.
- Trease, G.E. and Evans, W.C (1989) *Pharmacognosy*, 13th ed. Balliere Tindall, London, 546pp.
- 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 Journal of Botany* 14:35-39.
- Velluti, A., Marlin, S., Gonzalez, P., Ramos, A.J., Sanchis, V. (2004) Initial screening for inhibitory activity of essential oils on growth of *Fusarium verticillioides*, *F. proliferatum* and *F. graminearum* on maize-based agar media. *Food Microbiology*, 21: 649–656.