MEDWELL PUBLISHING
Journal of Molecular Genetics 1 (1): 11-17, 2009
ISSN: 2070-4267

Antifungal Effect of Aloe-Vera Gel on Fungal Organisms Associated with Yam (Dioscorea Rotundata, Poir) Rot

I.O. Ezeibekwe, M.I. Opara and F.N. Mbagwu
Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria

Abstract: Investigations were carried out to test the potency of Aloe vera gel extract on fungal organisms associated with yam rot. These fungi were isolated and identified as *Fusarium oxysporium* Schlech ex. Fr., *Rhizopus oryzae*, *Went*, *Botryodiplodia theobromae Pat* and *Fusarium solani.* Mart sacc. Pathogenicity test was carried out to confirm these organisms as the pathological agents of the yam rot. The Aloe vera gel at the different concentrations of 25, 50 and 100% did not inhibit the growth of these fungi when tested for its antifungal potency, p = 0.05. The result of this study showed that Aloe vera gel did not actually inhibit fungal growth as the there was progression in the growth and development of the fungi.

Key words: Antifungal, effects, Aloe vera, fungal, organisms, yam, rot

INTRODUCTION

Aloe vera (*Aloe barbadensis*) is one of the natures wonder plants (Duke, 1985). The botanical kingdom has about one and half million species. It is a herb of great medicinal potential, some of the claims include improvement of conditions of debility, relief of headaches, healing of skin injuries. Aloe vera works in synergy with other medications increasing their efficacy without conflict in desired results (Atherton, 1998). It has no adverse side effects (Tumlinson, 2003). It is desirable to search for new types of fungicides of chemical origin for the control of yam rot. Fungicides of plant origin are specific biodegradable, cheap, readily available and environmentally safe than synthetic chemicals (Okigbo and Ogbonna, 2006). The importance of the crop yam (Dioscorea rotundata) to the economy of Nigerians as food and medical values cannot be over emphasized. This research is therefore, aimed at investigating the potency of Aloe vera as an antifungal agent against yam rot organisms.

Aloes are like Cactus in appearance but are actually perennial, succulent plant with yellow flowers. They belong to the family Liliaceae from the Corrolliferaceae division of the monocotyledon (Hutchinson and Howel, 1974). Historical evidence suggests that Aloe plant originated in the warm dry climates of Africa, Latin America and the Carribean. However, because the plant is readily adaptable and man has been eager to carry it with him from place to place, it is now found in many lands. Th-Uphof and Cramer (1968) have reported the following species, *Aloe keayi*, *Aloe ferrox*, *Aloe variegate*, *Aloe saponaria*, *Aloe marcrocarpa* and *Aloe barbadensis* (Aloe vera).

Aloe vera (*Aloe barbadensis*) is a stemless or very short stemmed succulent plant growing up 80-100 cm tall spreading by offsets and root sprouts. The leaves are lanceolate, thick and fleshy, green to grey green with serrated margin. The flowers are produced on a spike up to 90 cm tall, each flower pendulous with yellow tubular corolla 2-3 cm long. The matured plant buds off young seedlings from the root and detaches from the plant (Atherton, 1998).

Aloe vera is relatively easy to care for in cultivation in frost-free climates. The species require well drained sandy potting soil in moderate sunlight.

Aloe vera contains a broad spectrum of free amino acids, steroids, polysaccharides, saponins, lignin, Anthraquinones, salicylic acid and vitamins A, B, B_2, B_3, B_6, B_{12}, C, E, folic acid and minerals such as calcium, copper, iodine, iron, magnesium etc. (Waterworth, 1976). Aloe vera has been used externally to treat various skin conditions such as cuts, burns and eczema (Farrar, 2005).

Yam (Dioscorea rotundata): Yam is the common name for some species in the genus Dioscorea (family Dioscoreaceae). These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in West Africa, Asia, Latin, America, Oceania, Islands of the south pacific, South East, Asia, India, the carribean and parts of Brazil. Nigeria alone accounts for considerably more than half of the world's total production of yam

Corresponding Author: I.O. Ezeibekwe, Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria

(Kay, 1987). Yam tubers can grow up to 2.5 m in length and weigh up 70 kg (150 pounds). Dioscorea rotundata, the white yam is the most important cultivated yams and mainly found in Africa. There are over 200 cultivated varieties between them. They are large plants, the vines can be long as 10-12 m. The tubers most often weigh about 2.5-5 kg (6-12 pounds). After 7-12 months growth, the tubers are harvested. In Africa, they are pounded into paste to make traditional dish fufu (Kay, 1987). Yams can be cooked in various ways by boiling, roasting and frying. Yam in Nigeria is also produced into various staple, intermediate end product (Okaka *et al*., 1991) which are used for direct consumption by animals, used as basic ingredients for snacks or made into flour. Yam is of very high value as food where it is a major source of carbohydrates, minerals, vitamins B6 and C and dietary fibre (Coursey, 1983).

Onayemi (1983) reported that over 50% of the yam tubers produced and harvested in Nigeria are lost in storage. The disease causing agents reduce the quantity of yam produced and also reduce the quality by making them unappealing to the consumer. Yam is prone to infection right from the seedling stage through harvesting and even after harvesting in storage (Amusa *et al*., 2003). Yams are subjected to several diseases. There are different genera of fungi that have been reported in association with storage deterioration in yam tubers (Noon, 1978; Okigbo and Ikediugwu, 2000). Plant extracts have been successfully used to control diseases in plants and tuber crops (Amadioha and Obi, 1999; Onifade, 2002; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005). This study was therefore, undertaken to investigate if Aloe vera gel has any antifungal property against fungal organisms associated with yam rot.

MATERIALS AND METHODS

Sample collection: Ten yam tubers with symptoms of rot were obtained from Eke-Ukwu market in Owerri, Imo State. The diseased yam tubers were packaged in polyethylene bags and taken to the laboratory of Imo State University, Owerri, where they were assessed for microbial (fungal) presence.

Five stands of Aloe vera used in the experiment were collected from Uncle Ray market garden, Owerri, Imo State (Fig. 1).

Media preparation: The medium used was Potato Dextrose Agar (PDA) prepared according to the manufacturer's instruction. About 39.6 g of powdered PDA medium was dissolved in 1 L of sterile distilled water and sterilized by autoclaving at 121°C at 15 p.s.i for 15 min and allowed to cool before pouring carefully into 20 sterile Petri dishes. Two drops of lactic acid was added to the solution to inhibit any bacterial growth. The Petri dishes that contained the medium was incubated for 24 h at room temperature (28°C) to check for sterility before use as described by Cheesbrough (2004).

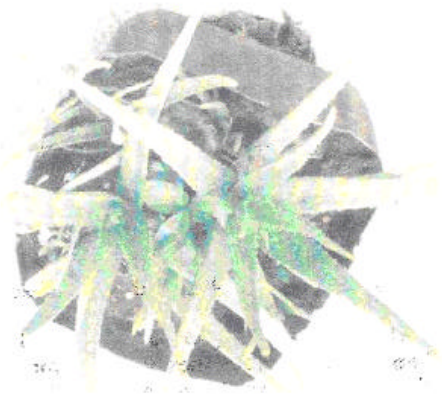

Fig. 1: Aloe vera plant: Source of plant material

Isolation of pathogens: Rotted yam tubers were rinsed in distilled water, surface sterilized with 70% ethanol and cut open with a sterilized knife. About 20 pieces (3 mm in diameter) of the infected yam tissues were picked from the point of advancement of rot with a flamed sterilized forceps and inoculated on a solidified Potato Dextrose Agar (PDA) medium. Two replicates were made for each of the ten yam tuber samples and twenty plates inoculated on. The inoculated plates were incubated at room temperature (28°C) and observations made daily for possible fungal growth. Subculturing was done to obtain pure cultures of the isolates. Stock cultures were prepared using slants of Potatoe Dextrose Agar (PDA) in McCarteny bottles and stored in a refrigerator at 4°C. Cultural characteristics of the fungi were observed and recorded. Occurrence of the organisms was recorded as follows:

$$\frac{\text{Total number of plates}}{\text{Total number of fungal occurrence}} \times \frac{100}{1}$$

Identification of organisms: The identification of the isolates was done by examining the isolates macroscopically and microscopically. The colony characteristics, spores, mycelium either septate or not, conidium, were taken note of. These structural features were matched with standards in Barnet and Barry (1972) and Booth (1971).

Pathogenicity studies: Ten fresh healthy tubers of yam were washed with tap water and distilled water respectively and thereafter sterilized with 70% ethanol. Cylindrical discs (4 mm) were removed from the tubers with a sterile 4 mm cork borer. The 4 mm discs of 5 days old cultures of the isolates were used to plug the holes created in the tubers, respectively. The discs of the tuber in the cork borer was replaced and then sealed with Vaseline jelly to make it air tight. Sterile PDA disc used in place of the culture discs served as the control. This was done for all the isolates obtained in pure culture (Okigbo and Nmeka, 2005; Okigbo and Ogbonna, 2006).

Two replicates were made for each organism. The inoculated tubers and the control were each enclosed in a sterile polyethylene bag and incubated for eight days at room temperature (25-37°C). A micro-humid environment was provided by enclosing a sterile water soaked aseptic cotton wool in each set up.

Measurement of rot: The extent of rot was determined by using a sterilized and flamed knife to cut open the inoculated yam tuber from the point of inoculation to obtain identical halves. A sterilized transparent ruler was used to measure the depth of rot. The actual depth was determined by substracting the initial depth (2 cm) from the final depth (i.e., final depth-initial depth = actual depth. Also in determining the degree of rot in diameter, the initial diameter (4 mm) was subtracted from the final diameter of rot to get the actual diameter of rot. The area of rot was calculated as follows:

Πdl = Area of diameter
D = Diameter
L = Depth
Π = 22/7 (constant)

The spread of dry or wet rot was calculated for both lengths, depth and width i.e diameter and recorded.

Reisolation was carried out using a sterilized and flamed scapel. Small pieces of rotted tissue about 1 mm from the advancing edge of the rot were removed from the infected tuber and asceptically inoculated into PDA media in Petri dishes and were incubated for 72 h at room temperature. Subculturing was done to get pure cultures of the organisms. The colony and microscopic features of the organisms identified according to the standards of Barnet and Barry (1972) and Booth (1971).

Preparation of plant materials (Extracts): Four large fresh leaves of Aloe vera collected from the lower portion of plant were thouroughly washed under running water and sunsequently with distilled water and air dried at 25°C for 2 h in the laboratory. Thereafter they were dipped into 70% ethanol for surface sterilization.

Crude extraction of Aloe vera gel: The fresh leaves of Aloe vera were slit lengthwise with flamed sterilized knife and the gel removed by pressing gently into a sterilized glass. The gel was used immediately in the preparation of the media (Tumlinson, 2003).

The raw gel was used as 100, 50 and 25% concentrations; for 50% concentration 50 mL of raw gel was dissolved in 50 mL of distilled water and for 25% concentration, 25 mL of raw gel was dissolved in 75% mL of distilled water (Mallam and Hones, 1945).

Activity of extract on fungal growth: The method of Amadioha and Obi (1999) was used to determine the effect of the extract on fungal growth. Two perpendicular lines were drawn at the bottom of the plate, the point of intersection indicating the center of the plate. This was done before dispensing PDA into each of the plants.

About 5 mL of the extract was dispensed into the Petri-dish after which 20 mL of melted PDA was poured into each plate, shaken together and allowed to solidify. Two replicates were made for each gel concentration i.e., 3 treatments.

Control experiments were set up without the addition of the Aloe vera gel. The fungi tested were *Fusarium oxysporium*, *Botryodiplodia theobromae*, *Rhizopus oryzae* and *Fusarium solani* and were repectively inoculated at the aforementioned point of intersection of the prepared medium. Six plates were prepared for each organism and one control experiment each.

Daily readings were taken for 5 days measuring the diameter of growth on the plates.

Experimental design: Experimental design used involved Randomized Complete Block Design (RCBD). Analysis of Variance (ANOVA) to separate the means using Least Significance Difference (LSD) to determine levels of significance.

RESULTS

The isolation and identification of the causative organisms of the rotted yam tuber, four fungi were identified according to the standards of Barnet and Barry (1972) and Booth (1971) as *Fusarium solani* Mart sacc. *Rhizopus oryzae* Went, *Batryodiplodia theobromae Pat.* and *Fusarium oxysporium* Schelechr Ex Fr (Table 1, Fig. 2-5). The percentage occurrences of the isolates

Table 1: Macro and micro features of the fungi (Isolates)

Isolates	Colony characters	Microscopy	Identified organism
A	Arial mycelium, dense, was sparse, white/grayish with tinge of bluish brown colouration	Many micro and macro conidia present. Chlamydospores present, abundant and oval, terminally and intercalary positioned	*Fusarium solani* (Fig. 2)
B	Fast and rapid growth. Whitish with black spores and milky underneath.	Coenocytic (non-septate) has sporangio head (sporangium), flat collumella and spores are single celled	*Rhizopus oryzae* (Fig. 3)
C	White to dirty white, black underneath	Mycelium are septate, chlamydospores are intercalary and terminal, conidia are 2 celled	*Botryodiplodia theobromae* (Fig. 4)
D	Growth of PDA is rapid. White aerial mycelium tinged with pink purple colour	Micro and macro conidia are present, macro conidia slightly sickle celled with apical cell and foot shaped basal cell, chlamydospores are present, single and some in pairs	*Fusarium oxysporium* (Fig. 5)

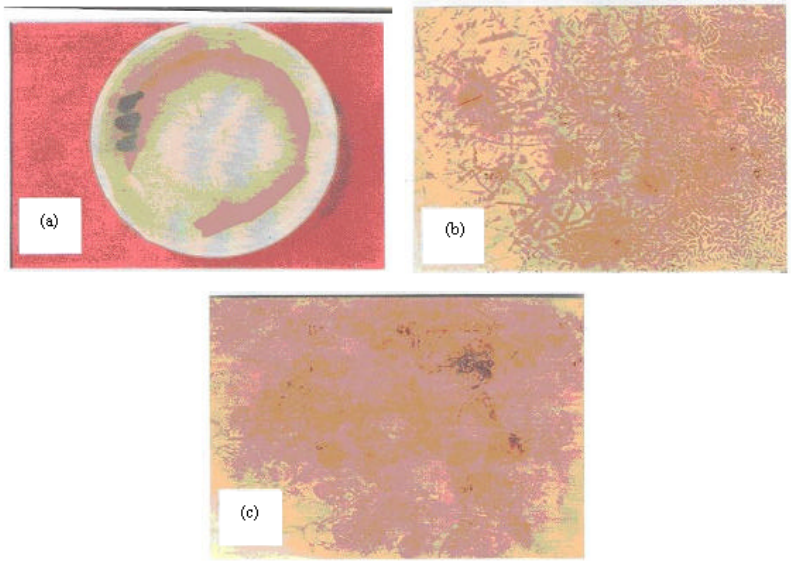

Fig. 2: a) Culture Plate of *Fusarium solani*, b) shows the micro and macro spores, c) Shows chlamydospores and large macrospores

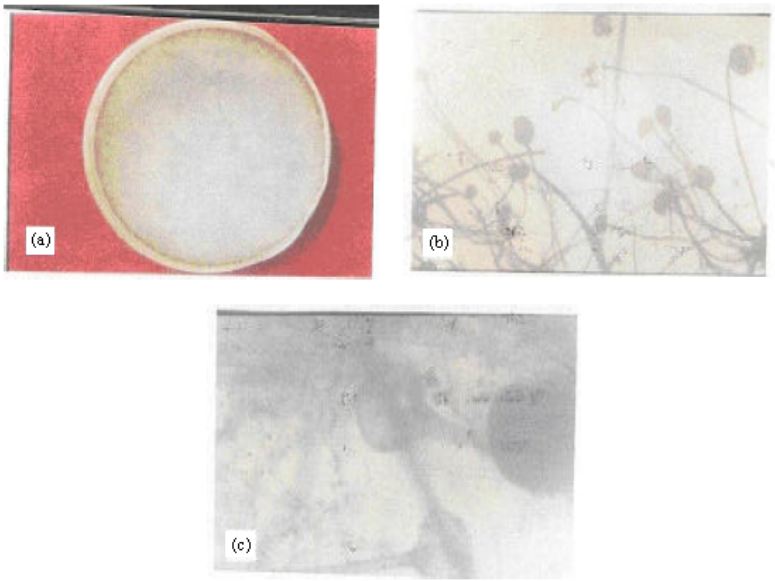

Fig. 3: a) Culture plate of *Rhizopus oryzae*, b) Shows mycelium and sporangium, c) Shows the columella, sporangium and spores

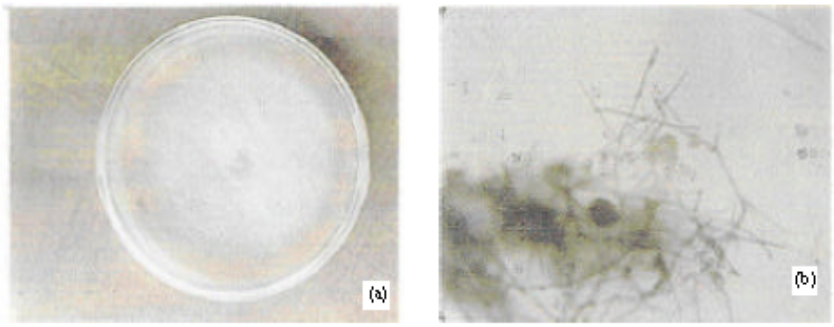

Fig. 4: Culture. a) Hyphae, conidium and Chlamydospores, b) of *B. theobromae*

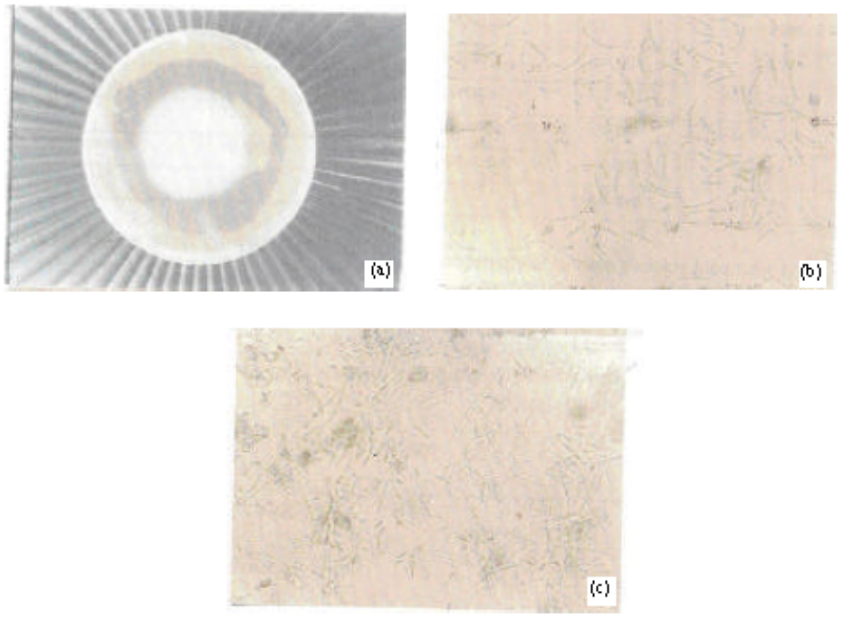

Fig. 5: Culture. a) Conidia, b) and c) Hyphae of *Fusarium oxysporum*

(Table 2) showed that *Fusarium oxysporium* and *Botryodiplodia theobromae* occurred more than others, 33.3% each. The mean diameters of rots exhibited by the respective fungi were shown in Table 3, *Rhizopus oryzae* exhibited a wider rotted area (25.37 cm) in the pathogenicity fests followed by *Botryodiplodia theobromae* (17.20 cm). *Fusarium solani* (12.65 cm), *Fusarium oxysporium* (1.38) and control (0.0 cm). The Fusarium species produced dry rots light brown to orange brown in colouration. Rhizopus oryzae produced extensive wet rot light brown in colour while *B. theobromae* also produced wet rot coffee to black colouration but not as extensive as that of *R. oryzae*. The control produced a brownish thickened layer around the cut area probably due to healing process in response to the cut.

The antifungal effects of different concentrations of Aloe vera gel extracts were shown in Table 4 and 5 representing the first day growth measurements and the 5th day growth measurements, respectively. The daily readings of fungal growth measured in centimeter increased each day in arithmetic progression irrespective of gel concentration in the experimental and the control.

Table 2: Percentage occurrence of isolated fungi in the rotted yam

Isolates	Occurrence (%)
Fusarium solani	33.33
Rhizopus oryzae	33.33
Botryodiplodia theobromae	16.66
Fusarium oxysporum	16.67

Table 3: Rot measurement of the pathogenicity tests

Isolates	Mean length of rot (cm)	Mean diameter of rot (cm)	Area of rot (cm)
Fusarium solani	2.3	1.75	12.65
Rhizopus oryzae	1.9	4.25	25.37
Botryodiplodia theobromae	1.5	3.65	17.20
Fusarium oxysporum	0.4	1.1	1.38
Control	0.0	0.0	0.0

Table 4: Antifungal effects of different concentrations of Aloe vera gel extracts

Organisms	100%	50%	25%	Control
Fusarium oxysporum	0.7± 0.1	0.8±0.1	0.65±0.05	1.0±0.2
Botryodiplodia theobromae	2.05±0.05	1.8±0.0	1.8±0.0	2.05±0.05
Rhizopus oryzae	4.85±0.25	3.1±0.1	3.25±0.25	4.5±0.2
Fusarium solani	1.7±0.1	1.85±0.15	1.85±0.05	1.85±0.15

Table 5: Antifungal effects of different concentrations of Aloe vera gel extracts

Organisms	100%	50%	25%	Control
Fusarium oxysporum	2.6±0.4	1.8±0.2	2.8±0.5	3.2±0.3
Botryodiplodia theobromae	8.5±0.0	8.5±0.0	8.5±00	8.5±0.0
Rhizopus oryzae	8.5±0.0	8.5±0.0	8.5±00	8.5±0.0
Fusarium solani	8.5±0.0	8.5±0.00	8.5±00	8.5±0.0

Mean replication±S.D (mm)

DISCUSSION

The organisms associated with the rot of white yam in this study were *Fusarium solani, Rhizopus oryzae, Fusarium oxysporium* and *Botryodiplodia theobromae.* These fungi have been associated with post harvest rots (Ogundana *et al*., 1970; Okigbo, 2004). Rotting in storage probably starts in the soil and progresses in storage. In most cases, microorganisms gain access into yams through natural openings and wounds that occur during harvesting and transportation from field to storage barn (Ogundana *et al*., 1970). The soil adhering to the harvested tubers contain many microorganisms that could be pathogenic to the tubers. Osagie (1992) and Okigbo and Ogbonna (2006) confirmed this ascertion.

The thick layering on the surface of the control tuber in the pathogenicity studies was probably a healing process in reaction to the cut since the tissues were still living and performing physiological functions supported by Ekundaya and Naqvi (1972).

Fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by Tewari and Nayak (1991), Okigbo and Emoghene (2004) and Okigbo and Nmeka (2005).

The present investigation showed that Aloe vera gel in concentrations 25, 50 and 100% proved ineffective against the fungal organisms that caused rot in white yam. This could be due to a possible metabolization of any antifungal elements in Aloe vera. It could also be that Aloe vera gel contains some vitamins that may have encouraged the growth of the fungal organisms or could be due to environmental conditions that could have made the gel ineffective against the pathogens.

According to Serrano *et al.* (2006), Aloe vera gel proved effective in the control of dermatophytes causing skin diseases. The likely reasons according to them included the likelihood for Aloe vera gel to work well under body temperature and may be more fungicidal to pathogenic fungi on human skin than on those causing yam rot. It could also be that the gel does not have a fungicidal effect but a fungistatic effect was able to stop the growth of the pathogenic fungi on the skin while the body immune system destroys them thereby repairing the skin (Farrar, 2005).

CONCLUSION

Aloe vera gel could be tested for a preservative role in yam storage. Aloe vera gel can be used as preservative as the study at the University of Mighel Hernandezin Alicante, Spain by Serrano *et al.* (2006) showed that grapes at 1°C coated with this gel could be preserved for 35 days against 7 days of untreated grapes. According to the researchers, the gel operates through a combination of mechanics preventing the entry and establishment of fungal pathogens.

REFERENCES

Atherton, P., 1998. Aloe vera, Magic and Medicine. J. Nur. Stand, 3 (7-15).

Amadioha, A.C. and V.I. Obi, 1999. Control of anthracnose disease of cowpea by cymbopogan vitratus and occimum gratissimum acta. Phytopathologia et. Entomologival Hungarica, 34 (1-2): 83-89.

Amusa, N.A., A.A. Adegbite, S. Mohammed and R.A. Baiyewu, 2003. Yam disease and its management in Nigeria. Afr. J. Biotech., 2 (12): 497-502.

Barnet, H.L. and B.H. Barry, 1972. Illustrated genera of imperfect fungi. 2nd Edn. Macmillan Publishing Coy. 886 Third avenue, New York, 10002: 216.

Booth, C., 1971. The genus *Fusarium*, common wealth Agric. Bureaux, Central Wales, Farnham Royal Bucks, England, pp: 81-167.

Cheesebrough, M., 2004. Distinct laboratory Practices in Tropical Countries. Part 2. Cambridge University Press, New York, USA., pp: 50.

Coursey, D.D., 1983. Post Harvest Losses in Perishable Food in the Developing World. Post Harvest Physiology and Crop Preservation. In: Usherman, M. (Ed.). Plarnium Publication Cooperation USA., pp: 56-72.

Duke, A.J., 1985. Handbook of Medicinal Herbs. CRS Press. Inc. Florida, pp: 332-333.

Ekundaya, J.A. and S.H.Z. Naqvi, 1972. Pre harvest microbial rotting of yams (*Dioscorea* sp.) in Nigeria. Trans Brmycol. Soc., 58 (1): 15-18.

Hutchinson, J. and F.N. Howel, 1974. A dictionary of useful and everyday plants and their common uses. Cambridge University Press London, pp: 196.

Farrar, P.N., 2005. Medicinal uses of Aloe vera. J. Med., 38: 238-241.

Kay, D.E., 1987. Root Crops Tropical Development and Research Institute. London, pp: 205-206.

Mallam, W.I. and M. Hones, 1945. The use of dilution method in testing disinfectants. J. Biotech., 49: 532-538.

Noon, P.A., 1978. Storage and Market diseases of Yams. Trop. Sci., 20:177-188.

Okaka, J.C.O., P.A. Okorie and O.N. Ozon, 1991. Quality evaluation of sundried yam chips. Trop. Sci., 30: 265-275.

Okigbo, R.N. and F.E.O. Ikediugwu, 2000. Studies on biological control of post harvest rot of yam (*Dioscorea* sp.) with *Trichoderma viride*. J. Phytopathol., 148: 351-355.

Okigbo, R.N., 2004. A review of biological control methods of post harvest yam (*Dioscorea* sp.) in storage in South Eastern Nigeria. KMITL Sci. J., 4 (1): 207-215.

Okigbo, R.N. and I.A. Nmeka, 2005. Control of yam tuber with leaf extracts of *Xylopia aethiopica* and Zingiber officinale. Afr. J. Biotech., 4 (8): 804-807.

Okigbo, R.N. and A.O. Emoghene, 2004. Antifungal activity of leaf extracts of some plant species on *Mycophaerelia figiensis* Morelet, the causal organism of black sigatoka disease of Banana (Musa acuminate). KMITL Sci. J., 4: 20-31.

Okigbo, R.N. and U.O. Ogbonna, 2006. Antifungal effects of two tropical plant leaf extracts (*Occimum gratissimum* and *Afromonum meleguata* on Post harvest yam (*Dioscorea* sp.). Afr. J. Biotech., 5: 727-731.

Onayemi, O., 1983. Observation on the dehydration characteristics of different varieties of yam and cocoyam. Abstract, 6th Symposium of the Int. Soc for Trop. Peru. February, pp: 252-270.

Onifade, A.K., 2002. Antifungal effect of *Azadirachta indica*. A juice extracts on Collectotrichum indemathianum. Global J. Pure Applied Sci., 6 (3): 423-428.

Ogundana, S.K., S.H.Z. Naqvi and J.A. Ekundayo, 1970. Studies on soft rot yams in storage. Trans. Br. Mycol. Soc., 56 (1): 73-80.

Osagie, A.U., 1992. The yam tuber in storage Postharvest Research Unit. Dev. Biochemistry, Univ. Benin, Nigeria, pp: 247.

Serrano, M., J.M. Valverde, F. Gullen, S. Castillo, D. Martinez-Romero and D. Valero, 2006. Use of Aloe vera gel coating to preserve functional properties of table grapes. J. Agric. Food Chem., 54 (11): 3882-3886.

Tewari, S.N. and N. Nayak, 1991. Activity of four plant leaf extracts against three fungal pathogens of rice. Trop. Agric. (Trinidad), 68: 373 -375.

Tumlinson, L.N., 2003. Aloe vera extract. Hiran Aguceutical private limited, Maryland USA., pp: 3.

Th-Uphof, J.C. and J. Cramer, 1968. Dictionary of Economic Plants. Cambridge University Press, New York, London, pp: 26-27.

Waterworth, P.M., 1976. Chemical constituent of plants and their antimicrobial activities. J. Anti-microbial Chemother., 2: 104-108.