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IN VITRO CONTROL ON FUNGUS ASSOCIATED WITH BIO – DETERIORATION OF SWEET POTATO (*IPOMOEA BATATAS* (L.) LAM) TUBERS

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ABSTRACT
Apparently healthy and rotted sweet potato tubers (*Ipomoea batatas* (Linn.) Lam) were obtained from selected markets and farms from Anyigba, Egume, Ejule and Dekina, Dekina Local Government Area, Kogi State, Nigeria. Isolation and identification of fungi associated with rotten sweet potato tuber was carried out. Diseased portion of the rotted tubers were scraped and inoculated on Potato dextrose agar (PDA) at 27 ± 2^0C, sub-cultured to obtain pure culture. *Aspergillus niger* was isolated from the rotten portion of the tubers and identified based on morphological characters and pathogenicity tests. Leaf aqueous extracts of neem (*Azadirachta indica* (Linn.) and bitter leaf (*Vernonia amygdalina*) were evaluated for their inhibitions at concentrations of 10%, 20%, 30% and 40% on the isolated fungus. The study showed that neem leaf extract was effective in suppressing mycelial growth of *A. niger* at higher concentration (30% and 40%); which could be employed in the biocontrol of infections caused by *A. niger*. The percentage inhibition at their various concentrations was also determined.
Key words: Sweet potato, tubers, *Aspergillus niger*, Extract, rotten, *Vernonia amygdalina, Azadirachta indica*

INTRODUCTION

The major important root crop of tropical regions, including Nigeria, includes cassava, yam, sweet potatoes and Irish potatoes. These tubers suffer from pest harvest losses resulting from physical, physiological or pathological factors or the combination of all the three (Booth, 1974). The late blight disease of potatoes in Ireland in 1845 caused by *Phythophthora infestans*, was responsible for the famous Irish famine of 1845 – 46; resulted in the death and migration of million of people from the country to other countries such as United State of America (Onuegbu, 2002).

The sweet potato (*Ipomoea batatas* (L.) Lam) is a dicotyledonous plant that belongs to the family Convolvulaceae. It is a large, starchy, sweet tasting tuberous root. The young leaves and shoots are sometimes eaten on greens. There are approximately 50 genera and more than 1000 species. Davison (1978) recognized two species in the section batatas, which includes the sweet potatoes and subsequently, three species, have been added and one removed. The closest with relative of the sweet potato appears to be *Ipomoea trifida* and *Ipomoea tabascan*.

Sweet potatoes are a creeping plant and the only economical species of the family Convolvulaceae (Cobley and Steel, 1976). The starchy tuberous roots are the major source of food for millions of people; the leaves are also useful sources of vegetable in some countries (Croft, 2007). The plant originated in tropical America and they have spread throughout the world (Yen, 1974). Sweet potato is ranked seventh in the world production after wheat, maize, rice, Irish potatoes, barley and cassava (FAO, 1982). Sweet potatoes are of particular importance as food crop throughout sub topics

and tropical region. The crop is one of the important carbohydrate sources for many millions of people, particularly those in the developing nations. Sweet potato has a high nutritive value than Irish potato, especially in vitamins A, B and C and in calcium. China and Japan are the major consumers of sweet potatoes (Martin, 1985).

Post harvest losses of all perishable tropical produce have been conservatively estimated at 25% of production (Booth, 1974). Attack by fungi, bacteria and viruses are probably the most serious courses of post-harvest losses of between 25% and 60% of the initial weight depending on variety when stored for six and half months in a semi subterranean (Booth, 1974). Ameinyo and Ataga (2006) reported *Rhizopus oryzae* and *Aspergillus niger* as being responsible for sweet potato rot. Surkova (1978) reported *Fusarium oxysporium, Fusarium trichothecoides* and *Fusarium radicicola* to cause potato tuber rot under different condition of temperature and humidity. The three species were susceptible to rot with higher relative humidity and higher temperature. The use of dry as well as cool storage is therefore important in reducing the loss from *Fusarium* rots of potatoes.

However, Wheeler (1979) reported many storage disease of tuber rots caused by *Rhizopus* spp; it causes soft rot of fleshy parts which proceeds rapidly at lower temperature. The most common post – harvest storage disease of sweet potatoes as reported by Ameinyo and Ataga (2006) include *Rhizopus* soft rot (*Rhizopus stolonifer*), bacteria soft rot (*Erwinia chrysentheli*), Fusarium surface rot (*Fusarium oxysporum*) and black rot (*Ceratocystis fimbriata*).

Different diseases arise after potatoes are harvested, this is because the storage organs are essentially dormant structure and their cells are physiologically unlike those of the growing plants. The numerous diseases which occur in transit and storage result mainly from the activity of fungi and bacteria. Investigations carried out by various workers clearly indicate that the real cause of the spoilage of tubers in transit or storage is the high temperature and injuries sustained by the tubers during the process of marketing (Amienyo and Ataga, 2006). Such diseases considerably reduce the commercial value of the produce.

Harvested tubers are vulnerable to attack by microorganisms because of their moisture content and rich nutrient. Due to harvest, packing and transportation, injuries of various kinds are caused which facilitate the entry of certain pathogens. Some of the pathogens produce extracellular enzymes and start degenerative process in advance of the fungal hypae or bacterial cells of the attacking pathogens. As a result of infection, the market and nutritive value of the tubers are reduced, either due to its ugly appearance or the changes in the stored products of the tubers (Oyewale, 2006).

The role of higher plants as sources of fungicides and insecticides and their importance in controlling different plant pathogens are gaining prominence in view of the hazards and cost of agro – chemicals. Plant extracts with their degradable and eco-friendly nature have shown some promise in recent years. Amadioha and Obi (1999); Kumar and Schan (1979) reported the control of *Curvularia lumata* and *Alternaria tenus* of cereals with fungicides of plant origin. Distillate from the leaves of *Ocimum gratissimum* was equally reported to reduce the incidence of *Fusarium moniliforme*. The extract of leaves of *Vernonia amygdalina* successfully reduces the incidence of fusarium. Similarly, Owolade *et al* (2000) reported that *O. gratissimum* and *Vernonia amygdalina* effectively protected maize seed from seed pathogen of *Fusarium moniliforme*. Ziv (1997) reported that adding extract of inula vicose to PDA caused long delay in the development of various species of pathogenic fungi. According to him, in two of the fungi, *Botrytis cinera* and *Rhizopus stolonifer*, sporulation was delayed up to 12 days after inoculations. Dangvietis (2001) similarly reported that an extract from pine and spruce needle called fitokols was found to be effective against different fungi species (*Cladosporium, Botrytis, Fusarium* and *Phytophthora*).

Remenzani *et al* (2002) has explored the effect of volatile oils from *Eucalyptus citriodera* and its major constituents, against two well known pathogens, *Rhizoctonia solani* and *Helminthosporum oryzae. Azadirachta indica* extract significantly reduce the mycelial growth of *Pyricularia oryzae*, in the development and spread of blast disease in rice plant in green house (Amadioha and Obi

(1999). Olufolaji (1999) reported the antifungal activities of extracts of *A. indica* in controlling the wet rot disease of *Amaranthus* sp. caused by *Choanephora cucurbitarum*. Also, fungitoxic activity of extracts from *A. indica* and *Xylopia aethiopica* on *C. lindemuthianum* in cowpea has been reported by Amadioha (1998). Some of these workers reported that attack by fungi; bacteria and viruses are probably the most serious causes of post harvest losses in perishable and tropical crops.

In Nigeria, it appears that little attention has been paid to the problems of post-harvest tuber rot of potatoes. In an attempt to curb and control this disease, various chemicals have been used. Chemicals have negative effect on the environment because they are not biodegrable, hence the need for alternative to chemicals. This formed the basis of this research which focus on the use of an aqueous extracts of leaves of neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) in control of diseases caused by *Aspergillus niger* on sweet potato

MATERIALS AND METHODS

Collection of samples

Apparently healthy and rotted potatoes were collected from Anyigba and its environs, kept in clean polythene bags for further laboratory investigations. Scrapings were made from rotted potato tubers, mounted on clean glass slides and observed under compound microscope to determine the associated fungi. Fresh leaves of neem (*Azadirachta indica*) and bitter leaf (*Vernonia amygdalina*) were collected within Kogi State University premises, washed and air dried in the laboratory.

Pathogen isolation

Direct isolation was applied using sterile scarpel to pick small pieces of affected portion of the rotten potato at the periphery of the rot. The pieces were place on Petri dish containing PDA. The plates were incubated at ambient temperature (27± 2^{o}C) for 8 days and observed for fungal colonies. Discrete colonies observed were subcultured to obtain pure cultures.

Pathogenicity tests

Eight fresh apparently healthy tubers were used for the pathogenicity test. Out of the eight tubers, two served as the control. The tubers were washed in a tap water and then rinsed in sterile distilled water and allowed to dry. Pure culture of the isolates was inoculated in the clean tubers, using flammed cork borer of 4mm to create wound on the tubers. A sterile cork borer of 3mm diameter was used in depositing about 3mm of each pure isolate in the wound created. Vaseline jelly was used to seal each inoculum on the wounds to prevent contamination by other microorganisms. The control was left blank and kept in a clean dish in the laboratory for daily observation. They were covered with a large polythene bag to provide a humid environment and to prevent entry of other pathogens following the methods of Amoo *et al.* (2007). Re-isolation and re-examination of fungi was carried out according to Koch's Postulate and Agrios (2005).

Preparation of leaf extracts and bioassay.

Fresh matured leaves of neem and bitter – leaf were thoroughly rinsed in running tap water before they were air – dried in the laboratory and pound with pestle in a mortar to facilitate extraction. Cold – water extraction was obtained by infusing 10g, 20g, 30g and 40g each of neem and bitter – leaf powder separately in 100ml of sterile distilled water, using 250ml conical flask. They were thoroughly mixed together using sterile glass rod and left for 24 hours to allow for extraction of the active ingredients as cold extraction before filtered into a fresh 500ml flask using four – fold cheese cloth as described by Wokocha and Okereke (2005), corked with sterile cotton wool and exposed to U/V light for further sterilization. These preparations represented 10%, 20%, 30% and 40% concentrations respectively. Thirty – nine grams (39g) of potato dextrose agar was dissolved in one litre of distilled water and the medium was autoclaved at 1.02kg/cm^3 pressure for 15 minutes. Six milliliters (0.1%) of streptomycin was added to the 1 litre of the sterilized media just before pouring into Petri-dishes, to prevent bacterial growth and allowed to cool and solidify.

The bio assay fungicidal properties of each plant extract were tested on the mycelia growth of the isolated fungus by growing it on the PDA medium containing 2ml of 10%, 20% 30% and 40% of each plant extract separately spread on the surface of the solidified PDA Petri – dishes. A disc of 4mm diameter (using a sterile cork – bore) of each pure culture of the isolated fungus was placed on the thin film

formed on the PDA just at point of intersection of two lines at the bottom of each Petri – dish. Three plates were treated with extract of each plant. The control experiments had distilled water in place of plant extracts. The treatments and control were incubated for five days at ambient temperature (27 ± 2°C). As soon as the control plates are filled up, the results were collated and analyzed.

RESULTS

The test fungus, which was identified as *Aspergillus niger*, was isolated from infected potato tubers. And they were found to be pathogenic using Koch's postulate. The fungus produced a black, powdery colony on PDA. The hyphae was septate, narrow and highly branched, with upright conidiophores producing a vesicle on which are arranged two rows of phialids which radiate from the apex. Conidia are one – celled, spiny, thick walled and fairly spherical.

The results showed that the two plant extracts had fungicidal properties, with neem leaf generally more effective in retarding vegetative growth than bitter – leaf extract. The *in vitro* retardation growth of the fungus observed on the extracts increased with increase in concentration compared with control (Fig. 1).

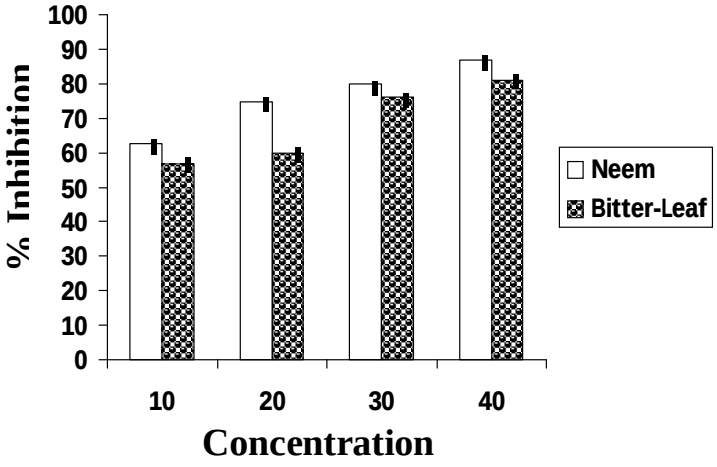

Figure 1: Mean Percentage inhibition effects of two plant extracts on mycelial growth of *Aspergillus niger*

The result on table 1 shows that maximum retardation was achieved at 40% concentration of neem extract even though with the visible mycelia noticed. At the end of the incubation period, about 87% inhibition of the mycelial growth of the isolate was achieved; with a significant difference compared with untreated plates (control). Also noticed at 30% and 40% concentration was a clear decline in sporulation of the isolate. At lower concentration (10%, 20%), there was fluffy mycelia growth which declined at the end of the incubation period. Visible spore production was equally noticed during the incubation at lower concentration.

Table 1: Percentage inhibition of neem – leaf extract on *Aspergillus niger*.

Concentrations (%)	Mean (cm)	Inhibition	% Inhibition
10	1.68	2.82	62.7
20	1.14	3.36	74.7
30	0.90	3.60	80.0
40	0.59	3.91	86.9
Control	4.50	-	-

The *in vitro* bioassay of bitter-leaf extract on the isolate show similar trend, the extract was less effective in controlling or retarding the mycelial growth in *Aspergillus niger* (Table 2). The aqueous extract from bitter – leaf was inhibitory to mycelial growth of the isolate during the period of incubation with about 81% inhibition. There was however, a significant difference between the control and other concentration.

Table 2: Percentage inhibition of bitter – leaf extract on *Aspergillus niger*.

Concentrations (%)	Mean (cm)	Inhibition	% Inhibition
10	1.95	2.55	56.7
20	1.48	2.70	60.0
30	1.08	342	76.0
40	0.85	3.65	81.1
Control	4.50	-	-

DISCUSSION

The results clearly revealed that *Aspergillus niger* was found to be the fungus responsible for the rot of sweet potato in the study area. *A. niger* is a filamentous ascomycetes fungus that is umbiquitous in the environment and has been implicated in opportunistic infections of humans. In addition to its role as an opportunistic human pathogen, *Aspergillus niger* is economically important as a fermentation organism used for the production of citric acid (Amadioha, 1998). Several workers have reported tuber rot of sweet potato caused by several fungi in storage (Clark and Hoy 1994; Onuegbu, 2002). This is in agreement with the present findings on potato rot. In most cases, fungi gain entrance into sweet potato tubers through natural openings and wounds created during harvesting, transporting, handling and marketing. However, Okigbo and Nmeka (2006) noted that tubers at time of harvest may already be infected by pathogens derived from disease foliage, roots or other tubers.

Synthetic pesticides have been used in controlling fungal diseases which has gulped millions of dollars in tropical and sub-tropical countries in importing them. There is equally a reported complex of health and ecological problems caused by an improper use and over use of pesticides. This has prompted the search for an alternative plant control. The two leaves extracts from each of the plants used for the study recorded retardation of vegetative growth of the fungus when compared with the pure culture. This may be as a result of the presence of active water solution antifungal principles associated with each of the plant leaves (Akinpelu, 1999). Also, this deduction could offer a partial reason for the popular use of bitter-leaf and neem leaf in traditional health care practices especially in all part of Nigeria for the treatment of diarrohea and fever and for the treatment of other various ailments (Gill, 1992; Iwu, 1993).

The neem tree was reported to have over 100 compounds with pesticidal properties. One of the best known is azadirachtin, found in all parts of the tree, but more in fruit and seeds. Neem has been used to protect stored roots as well as tubers against the potato moth. Neem powder is said to extend the storage life of potatoes for three months (Akinpelu, 1999). Neem, generally, has a great potential to control various phytopathogenic fungi and therefore, has much prospect to be used as a good fungicide. The use of neem cake and neem leaves as a soil treatment measure have produced good result against various soil borne fungi such as *Pythium aphanidermatum* and *Rhizoctonia solani* (Suleiman and Emua, 2009). The mean radial growth values of the

fungus on media plates with different concentrations supported the percentage inhibition noticed, which is a reflection in the water soluble antifungal element in their respective leaves. From the result, it was observed that the aqueous extracts used for the study recorded retardation or inhibition of mycelial growth of the fungus *in vitro*. The water-soluble antifungal principles in the plants are responsible for the antifungal activities. The use of plant extract on fungi that cause rots and other diseases of plants should be encouraged as they have been found to be able to reduce the damaging effect of fungi on the plant produce and even stored produce. The cultivation of these plants that have the antifungal properties should be highly encouraged in order to reduce the damaging effect on fungi. The facts that these plants used in this study are easily available, with easy method of extraction, they can be exploited in the control of sweet potato rot disease.

REFERENCE

Agrios, G. N. (2005). Plant Pathology. Academic press, New York. 803pp.

Akinpelu, D. A. (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. Fitoterapia Journal Study Medicinal Plants 70: 432 – 435.

Amadioha, A. C. (1998). Control of powdery mildew in pepper (*Capsicum anum*) by leaf extracts of pawpaw (*Carica papaya*). Journal of Herbs Species and Medicinal Plants, Haworth Press Inc. pp. 41-47.

Amadioha, A. C. and Obi, V. I. (1999). Fungitoxic activity of extracts of *Azadirachta indica* and *Xylopia aethiopica* on *Colletotrichum lindermuthianum* in cowpea. Journal of Herbs, Species and Medicinal Plants 6(2): 33-40.

Ameinyo, C. A. and Ataga, A. E. (2006). Post harvest fungal diseases of sweet potato (*Ipomoea batatas* (L.) Lan) tubers sold in selected markets in River State, Nigeria. Scientia Africana 5(2): 95-98.

Amoo, B. G., Balogun, O. S., Oyedanmade, E. E. A. and Fawole, O. B. (2007). Survey of rhizospheric mycoflora associated with sorghum in Kwara State, proceedings of 5th International Conference Nigeria Society for Experimental Biology (NISEB) 11-12.

Booth, R. H. (1974). Post-harvest deterioration of tropical root crops, and their control. Tropical Science 16: 49–63.

Clark, C. A. and Hoy, M. W. (1994). Identification of resistances in sweet potato to rhizopus soft rot using two inoculation methods. Plant Disease 78(11): 1078 – 1081.

Cobley, C. A. and Steel, D. B. (1976). An introduction to botany of tropical crops (2nd Ed) Longman Group Ltd London 651pp.

Croft, B. Y. (2007). Root rot of cowpea caused by *Pythium myriotylum* in Northern Queensland. Journal of Australasan Plant Pathogen 18(1) 8-9.

Dangvietis, M. (2001). Experience of Pine and Spruce Needles extractives used for plant protection. Proceedings of the international workshop Estonia Agricultural University, 19- 23.

Davision, R. S. (1978). Elements of Food Technology AV Publishing Company, Inc. West Port. Connecticut, 772Pp.

Food and Agricultural Organization of United Nations (1982). Production Statistics Year Book. Vol. 30.

Gill, L. S. (1992). Ethnomedicinal uses of plant in Nigeria. University of Benin Press Benin, Nigeria, 276pp.

Iwu, M. M. (1993). Handbook of African Medicinal Plants. Boca Raton: CRS Press Inc., Nigeria. pp223-224.

Kumar, R. G. and Sohan, S. N. (1979). Effectiveness of some plant extracts on conidia germination of *Curvularia pallescen.* Phythopathology 32(3):489 – 491.

Martin, F. W. (1985). Differences among sweet potato in response to shading. Tropical Agriculture (Trinidad) 62: 161 – 163.

Okigbo, R. N. and Nmeka, I. A. (2005). Control of tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. African Journal of Biotechnology 4(8): 804-807.

Olufolaji, D. B. (1999). Control of wet rot disease of *Amaranthus* sp caused by *Choanephora cucurbitarum* with extracts of *Azadirachta indica*. Journal of Sustainable Agriculture and Environment 1(22): 183-190.

Onuegbu, B. A. Fundamentals of crop protection, Agro-science consult and extension. River State University of Science and Technology 237-240.

Owolade, B. F., **Amusa, A. N.** and **Osikanlu, Y. O. K.** (2000). Efficiency of Certain Indigenous Plant Extracts against seed-borne infections of *Fusarium moniliforme* on

Maize (*Zea mays*) in South Western Nigeria. Cereals Research Communications 28(3): 323 – 324.

Oyewale, M. O. (2006). Fungal diseases of sweet potato. http://acs.Convex.Com/acs/gree06/techprogram/P26999.HTM

Ramenzani, H. P., Batish, D. R., Kow, R. K. and **Dragon, J. S.** (2002). Fungal Effects of volatile oils from *Eucalyptus citriodora* and its constituents citronelle. A paper at the 55th conference proceedings News and Plant Protection Society Incorporated, 329 – 330.

Suleiman, M. N. and **Emua, S. A.** (2009). Efficacy of four plant extracts in the control of root rot disease of cowpea (*Vigna unguiculata* (L.) Walp). African Journal of Biotechnology 8(16): 3806-3808.

Surkova, T. A. (1978). Features of the pathogenesis of Fusarium rots of potatoe tubers. Review of Plant Pathology 57: 225-235.

Wheeler, B. E. J. (1979). Plant Pathology. McGraw Hill Book Company, New York, 819pp.

Wokocha, R. C. and Okereke, V. C. (2005). Fungitoxic activity of extracts of some medicinal plants on *Sclerotium rolfsii* causal organisms of the basal stem rots diseases of tomato. Nigerian Journal of Plant Protection 22: 106 – 110.

Yen, D. E. (1974). The Sweet Potato and Oceania: An Essay in Ethnobotany, Honolulu, Hawaii, Bishop Museum Press, 389pp.

Ziv, O. (1997). Extracts of *Inula viscose* for controlling Plants Diseases in Fresh and Dry Post-Harvest Products. Proceedings of the 18th congress of the Israeli Phytopathological Society 20-22.