Food Science and Technology 4(2): 23-30, 2016
DOI: 10.13189/fst.2016.040202 http://www.hrpub.org

Mycological Deterioration and Pathogenicity Studies of Post-harvest Cassava

Agu Kingsley Chukwuebuka[1,*], Awah Nsikak Sunday[1], Okeke Chidi Benjamin[1], Nwodo Martin Tochukwu[1], Anaukwu Chika Gladys[1], Iloanusi Confidence Ada[1], Archibong, Etim Joseph[1], Ngenegbo Uche[2], Mgbemena Ijeamaka Charity[3], Umeoduagu Nnamdi[4]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria
[2]Department of Parasitology and Entomology, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria
[3]Department of Business Administration, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria
[4]Department of Microbiology, Tansian University Umunya, Nigeria

Abstract Post-harvest deterioration is the most important cause of loss of cassava (*Manihot esculenta*) production and this is mainly due to fungal diseases. This research was conducted to identify the major fungi responsible for post-harvest deterioration of cassava. A total of twenty four cassava tubers were harvested from a farm in Okpuno, Awka South LGA, Anambra State, Nigeria and used for the study. The weight loss of the tubers were determined. Rot causing fungi were isolated and characterized and the pathogenicity testing of the isolates were performed. Three genera of fungi were identified from the spoilt cassava samples including *Fusarium*, *Aspergillus* and *Lasiodiplodia*. *Aspergillus* spp. were found to be the chief causal agents of cassava rots. Three species of *Aspergillus* were isolated viz. *Aspergillus terreus, Aspergillus tamarii* and *Aspergillus* sp; two species of *Fusarium* viz. *Fusarium solani* and *Fusarium oxysporium* were isolated. All the fungi identified were pathogenic to fresh cassava. The percentage severity of rots and percentage weight loss ranged from 115.38% to 145.38% and 7.68% to 25% respectively for both methods.

Keywords Post-harvest Loss, Cassava, Fungal Rots, Pathogenicity, Percentage Weight Loss

I. Introduction

Cassava (*Manihot esculenta)* is a major food crop in Nigeria, supplying about 70% of the daily calorie of over 50 Million people. It has also been estimated that cassava provides food for over 500 Million people in the world. It is essentially a carbohydrate food with low protein and fat [12]. Edible part of fresh cassava root contains 32-35% carbohydrate, 2-3% protein, 75-80% moisture, 0.1% fat, 1% fibre and 0.75 - 2.50% ash [12]. Cassava tubers are an important source of carbohydrates. The production of cassava for human consumption has been estimated to be 65% of cassava products while 25% is for industrial use, mostly starch (6%) or animal feed (19%) and 10% lost as waste.

Next to yam, cassava (*Manihot esculenta*) is a commonly produced tuber crop in Africa [19], [20], [2], [3]. Cassava is grown mostly by small-holder farmers. According to FAO's estimates, the average fresh tuber yield of cassava under traditional farming practices in sub-Saharan Africa ranges between 5 and 8 tonnes per hectare. This is much lower than its potential yield capacity of 40 to 60 tonnes per hectare. Although cassava is an important crop with multiple uses, it does not receive the much needed attention during its production. Cassava roots are highly perishable and most post-harvest losses of cassava occurs during storage owing to physiological and microbial factors. These microbes enter through bruises received during harvesting as well as the inherent high moisture content of fresh roots which promote both microbial deterioration and unfavourable biochemical changes in the commodity [27].

A major drawback to cassava utilization is that the root starts to spoil after 48 hours of harvest. Some investigators reported an even shorter period before the tuber become unusable. High post-harvest spoilage and poor post-harvest handling leads to uneven quality of the processed cassava and results in contamination by fungi respectively. Poor and inadequate infrastructural facilities for milling and storage, and poor access roads, which are vital for adding value, further increase the postharvest handling challenges. Infections by the cassava leaf mosaic disease, the cassava brown streak disease and the cassava mealy bugs and scales further reduce crop yield.

However, cassava remains a desirable crop because of its many advantages. It is easy to produce, adaptable to many

environments, has minimal labour requirements and is comparatively less susceptible to pests and diseases than most other crops. This implies that there is need to address the above challenges in order to increase productivity, marketing opportunities and profitability of cassava production.

This research is therefore aimed at isolating and identifying spoilage molds associated with post-harvest loss of cassava tubers (*Manihot esculenta*). With a view of classifying the various types of rots observed during the study and to determine the percentage occurrence of the spoilage molds, thus carrying out comparative pathogenicity tests in order to ascertain the pathogenicity index of the rot-causing molds and the percentage severity of rots caused.

2. Materials and Methods

Sample Collection

A total of twenty four diseased cassava tubers *(Manihotesculenta)* were obtained from a local farm in *Okpuno*, Awka South LGA, Anambra State, Nigeria and transported to the Microbiology Laboratory of NnamdiAzikiwe University, Awka for analyses.

Isolation of Rot-causing Molds

Diseased tissues were obtained from spoiled cassava with the use of sterile knife and forceps and cultured on Saboraud's Dextrose Agar (SDA) supplemented with 0.1 ml Chloramphenicol to inhibit bacteria and incubated for 48 hours at room temperature.

Characterisation and Identification of Spoilage Molds

The isolated molds were identified based on the gross morphological appearance of fungal colonies on SDA medium. The microscopic features were studied by slide culture technique with reference to the Manual of Atlases of fungi [10].

Pathogenicity Testing

Two methods were employed in this test and a comparison drawn from both methods. The method of Okigbo and Nmeka (2005) was employed. In this method, test tubers were washed with sterile distilled water and thereafter, disinfected with 70% ethanol. Cylindrical discs were removed from the disinfected tubers with a sterile 4 mm cork borer, in which test molds were inoculated. The cylindrical discs were replaced and the inoculation points sealed with Petroleum jelly. Incubation was done for two weeks.

In the second method, disinfected whole tubers of appreciably similar weights were placed in an enclosed sterile vessel containing 150 ml of Saboraud's Dextrose Agar which was allowed to gel prior to inoculation with the rot-causing molds' mycelia and incubated for a period of two weeks. Thus, tissue traumatisation of tubers by Okigbo and Nmeka (2005) was avoided.

In both experiments, controls were set up with un-inoculated agar.

Weight Determination

Cassava weights in grammes (g) were taken on a weekly basis, throughout the entire duration of the experiment prior to the pathogenicity testing.

Determination of Rot Severity

This was done aseptically by cutting out the diseased portions of the cassava tubers, obtaining their weights and calculations done with the formula [3].

$$\text{Percentage rot severity} = \frac{W-w}{w} \times 100$$

Where:
W = Final weight of tubers
w = Weight of rotted part

Determination of Percentage Weight Loss

This was conveniently done by obtaining the percentage difference in weights of the fresh cassava and the rotted cassava.

$$\text{Percentage weight loss} = \frac{FW-Rw}{Rw} \times \frac{100}{1}$$

FW = Weight of fresh cassava
Rw = weight of rot cassava

3. Results

A total of six molds were isolated from twelve diseased cassava tubers that were sourced from *Okpuno* farm, Awka, Anambra state, Nigeria. The molds and their features are presented in Table 1.

Table 1. Characterisation and Identification of Fungal Isolates

Isolates	Colony morphology	Microscopy	Identity
PC1	Colonies are fast growing. Aerial mycelium, White/ grayish colouration Incubated at 30°C for 5 days	Microconidia are avoid in shape. Macroconidia are borne of phialide on branched or unbranched.	*Fusarium solani*
PC2	On SDA, colonies were greyish to mouse grey to black, fluffy with abundant Aerial mycelia, the reverse black. Incubated 30°C for 5 days5ays	Conidiophores were hyaline, Simple, cylindrical to holoblastic and annelidic	*Lasiodiplodia theorbromae*
PC3	Yellowish–brown pigmentation consisting of a dense felt conidiophores Incubated 30°C for 5 days reverse side is also brawny	Conidiophores were hyaline Loosely columnar, hyaline has Conidial heads. (300- 400 µm in diameter) Conidia were globose to subglobose (4-7 µm in diameter)	*Aspergillus terreus*
PC4	Aerial hyphae present, pinkish brown in colour. The reverse side is light brown Incubated 30°C for 5days	Yellowish-brown double Walled conidia (300- 400 µm in diameter)	*Aspergillus tamari*
PC5	Powdery mass of yellow-green spores on the surface and light yellow in the reverse side. Incubated 30°C for 5 days	Hyphal growth are thread-like Branching and producing Mycelium (3-6 µm in diameter)	*Aspergillus flavus*
PC6	Whitish orange colonies The reverse was creamy. Incubated 30°C for 5 days	Terminal chlamydospores with non septate hyphae, fusiform, slightly curved macroconidia	*Fusarium oxysporium*

Table 2. Physiological Changes of Tubers during Pathogenicity Testing by the Method of Okigbo and Nmeka, 2005

Rot-causing Molds	Cassava Samples	Observed Symptoms	Rot Category
Fusarium solani	Cassava 1	Infected tissues appeared milky with greyish discolouration and smelled fermented silage	Dry rot
Lasiodiplodia theorbromae	Cassava 2	Grey to black discolouration	Dry rot
Aspergillus terreus	Cassava 3	Grey to brown discolouration	Dry rot
Aspergillus tamari	Cassava 4	Infected tissues turned orange in colour	Dry rot
Aspergillus flavus	Cassava 5	Grey to green discolouration	Dry rot
Fusarium oxysporium	Cassava 6	Grey to black discolouration with pinkish margins	Dry rot

Table 3. Physiological Changes of Tubers during Pathogenicity Testing by the Method of Agu *et al.,* 2015

Rot-causing Molds	Cassava Samples	Observed Symptoms	Rot Category
Fusarium solani	Cassava 10	Infected tissues appeared milky with greyish discolouration and smelled fermented silage	Dry rot
Lasiodiplodiatheobr omae	Cassava 11	Grey to black discolorations with tuber becoming soft	Soft rot
Aspergillus terreus	Cassava 12	Grey to brown discolouration	Dry rot
Aspergillus tamari	Cassava 13	Infected tissues turned orange in colour	Dry rot
Aspergillus flavus	Cassava 14	Grey discolouration with tuber becoming soft	Soft rot
Fusarium Oxysporium	Cassava 15	Grey to black discolouration with pinkish margins	Dry rot

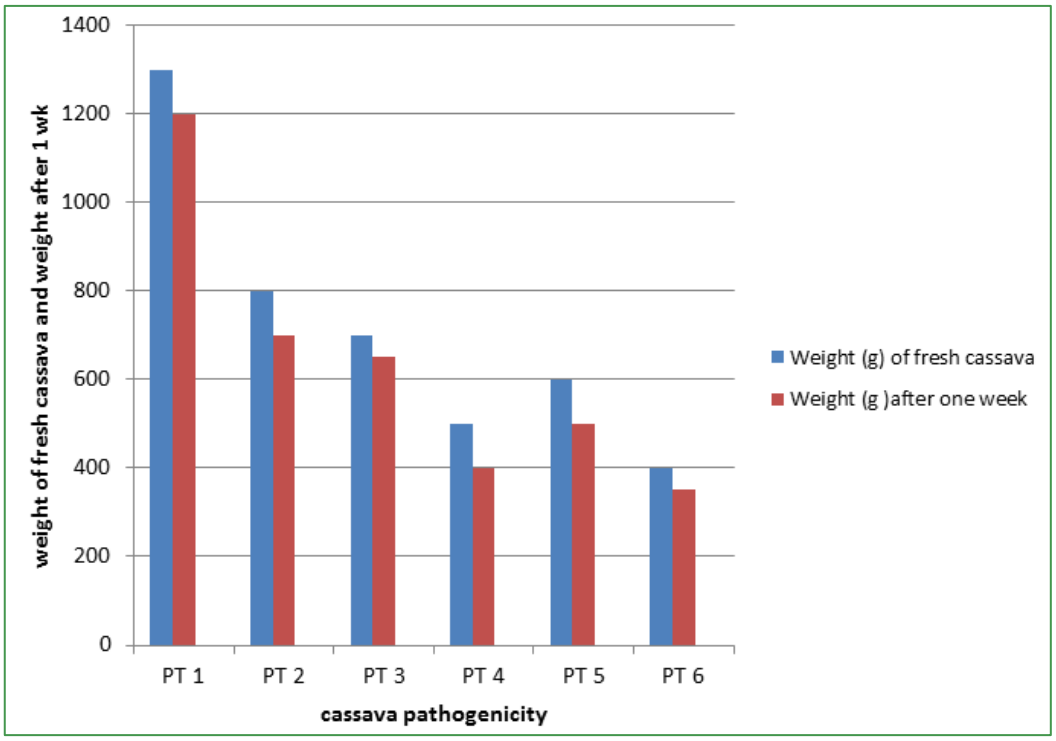

Figure 1. Weight Changes of Tubers during Pathogenicity Test

Key. PT= Cassava Pathogenicity Test

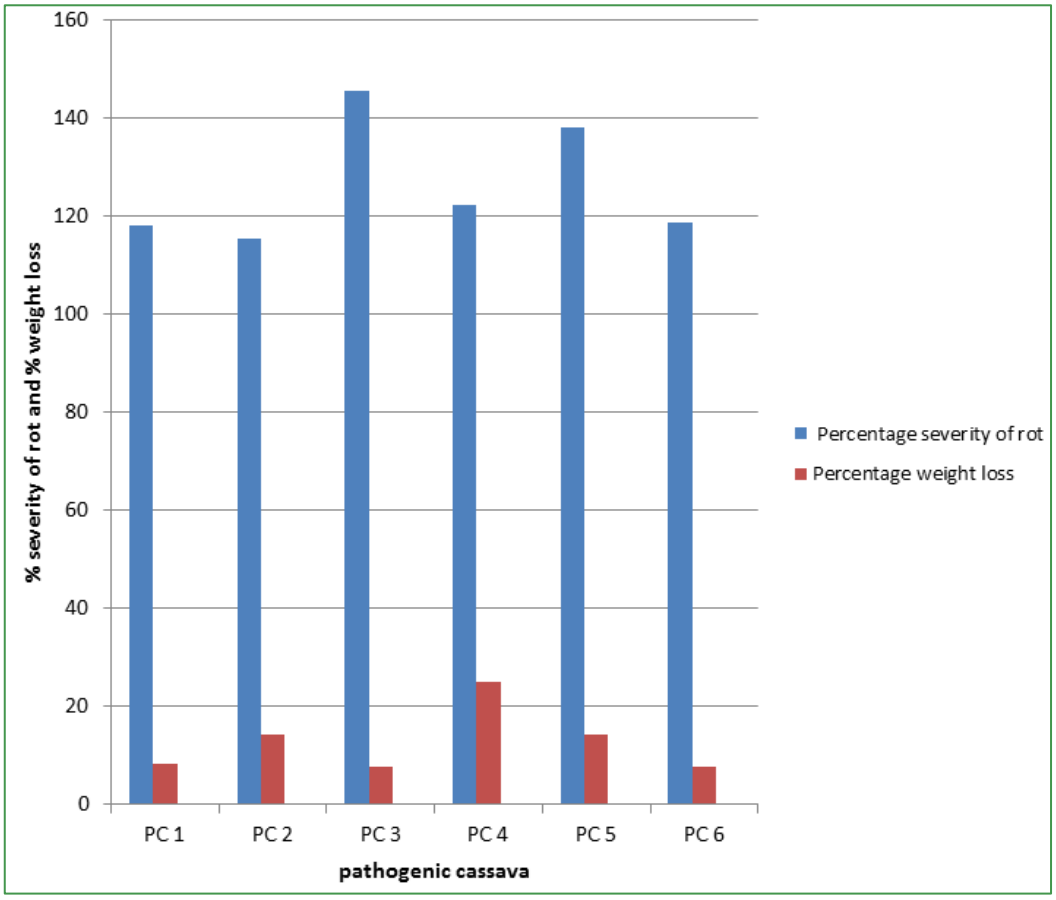

Figure 2. Percentage Severity of Rots and Percentage Weight Losses Observed during Pathogenicity Test

PC = Cassava Pathogenicity Test

4. Discussions

The isolation and characterisation of molds from spoilt cassava tubers were carried out and six molds viz*: Fusarium solani, Lasiodiplodia theorbromae, Aspergillus terreus, Aspergillus tamari, Aspergillus flavus,* and *Fusarium oxysporium* were isolated. This is in consonance with those isolated from spoilt yam tubers and potatoes by various researchers [19], [20], [2], [3], [27].

Two different methods were employed in infecting the healthy susceptible tubers with different rot-causing molds that were isolated from infected tubers. These methods include the method of Okigbo and Nmeka (2005) and the method of Agu *et al*., 2015. Furthermore, the results of the method of Okigbo and Nmeka, recorded some inconsistencies as none of the isolated organism elicited same symptoms with the original symptoms reported in Table 1. However, the result of the method showed that all the test molds elicited the same symptoms that were observed in the diseased tubers as shown in Table 1, this may be as a result of the fact that the test tubers were stored in sterile air-tight containers during the entire period of the experiment. The percentage severity of rots observed from the tubers using both methodologies is shown in Table 2.

This work also corresponds with the work of Messiga *et al*. (2008) that isolated rot causing fungi from cassava (Manihot esculenta) and concluded that these molds contribute to low yields and post-harvest loss of cassava in Yaounde, Cameroon. Ogaraku and Usman (2008) isolated rot causing fungi such as: *Aspergillus niger, Aspergillus flavus, Fasarium oxysporium, Rhizopus stolonifer and Sclerotium rolfsii,* from yam tubers and found some of the fungi isolated from cassava thus, showing that these fungi can also attack other tubers.

The percentage severity of loss ranged from 115.38 to 145.38% and percentage weight loss ranged from 7.69 to 25%. These molds were able to elicit same symptoms as they did to the initial tubers from where they were isolated. Weight comparison of the test tubers, using both research methods showed a decrease in tuber weight throughout the research period as shown in Figure 1 thus, suggesting that tubers lose weight during storage. This implied that these molds are integer factors that pose a problem to post-harvest storage of cassava tubers and showed the need to create measures to control them in order to avert impending economic loss.

5. Conclusions

Molds contribute to the problem encountered during post-harvest storage of cassava tubers. The method of Okigbo and Nmeka (2005) shows that these molds can attack the tubers when their protective covers have been compromised; this suggests that tubers should be wholesome (wound-free) and firm, without any kind of abrasion of the outer covering, so as not to create a portal of entry for spoilage molds prior to storage. The second method which brought into focus the integrity of the tubers and subsequent colonization and penetration of the tubers by the spoilage molds, suggests that healthy tubers should be stored in dry place, with low moisture content. Thus, research efforts aimed at the improvement of storage techniques will help reduce post-harvest losses during storage and thus improve cassava production.

Plate 1. Pathogenicity Test by the Method of Okigbo and Nmeka, 2005

Plate 2. Pathogenicity Test by the Method of Aguet al., 2015

Plate 3. Cassava Tuber Spoilt by *Aspergillus tamari*

Acknowledgements

We acknowledge the efforts of the entire Laboratory Staff of Microbiology Department, Nnamdi Azikiwe University for helping out with the preparation of the culture media for the Mycological Analyses of the various fungi used in this study.

REFERENCES

[1] K. C. Agu, M. O. Ogbue, H. U. Abuchi, A. U. Onunkwo, L.C. Chidi-Onuorah, N. S. Awah, Lipase production by fungal isolates from palm oil-contaminated soil in Awka, Anambra State, Nigeria. International Journal Agricultural Biosciences, Vol. 2, No. 6, 386-390, 2013.

[2] K. C. Agu, N. S. Awah, P. G. Sampson, M. O. Ikele, A. E. Mbachu, K. D. Ojiagu, C. B. Okeke, N.C.N. Okoro, O.I. Okafor. Identification and Pathogenicity of Rot-Causing Fungal Pathogens Associated with Xanthosoma sagittifolium Spoilage in South Eastern Nigeria. International Journal of Agriculture Innovations and Research, Vol. 2, No. 6, 2319-1473, 2014.

[3] K.C. Agu, G.U. Nweke, N.S. Awah, B.C. Okeke, I.C.C. Mgbemena, R. N. Okigbo, U. C Ngenegbo. Fungi Associated with the Post-Harvest Loss of Sweet Potato. International Journal of Research Studies in Biosciences, Vol. 3, No. 9, 32-37, 2015.

[4] A. C. Amadioha, A. A. Markson. Postharvest control of cassava tuber rot caused by Botryodiplodia acerinausing extracts of plant origin. Phytopathology, Vol. 40, No. 5, 359-366, 2007a. A. C. Amadioha, A. A. Markson. Control of storage rot of cassava tuber caused by Rhizopusoryzae using some plant extracts. Phytopathology, Vol. 40, No. 6, 381-388, 2007b.

[5] C.A. Anukworji, R. R. Putheti, R.N. Okibgo. Isolation of fungi causing rot of cocoyam (Colocasia esculenta (L.) Schott) and control with plant extracts: (Allium sativum, Garcinia kola, azadirachta indica and Carica papaya). Global Advanced Research Journal Agriculture Science, Vol. 1, No. 2, 33-47, 2012.

[6] A. Arya. Recent advances in the management of plant pathogens: Botanicals in the fungal pest management. In management of fungal plant pathogen. Arya A. and perello, A.E. (Editors). UK, International, Pp.1-25, 2010.

[7] A. N. Awurum, D. N. Enyiukwu. Evaluation of the seed-dressing potentials of phytochemicals from Carica papaya and Piper guineense on the germination of cowpea (Vigna unguiculata) seeds and incidence of the seed-borne fungi. Continental Journal of Agricultural Science, Vol. 7, No.1, 29-35, 2013.

[8] A.N. Awurum, P.O. Uchegwu. Effect of duration of contact of seed extract of Piper guineense, Monodora myristica and Xylopia aethiopica on the germination and incidence of seed-borne fungil of stored cowpea (Vigna unguiculata) seeds. Continental Journal of Biological Sciences, Vol. 6, No. 1, 37-42, 2013.

[9] S.A. Bankole, A. Adebanjo. Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. African Journal of Biotechnology, Vol. 2, No. 9, 254-263, 2003.

[10] D. Bennett. Farm Press- New study shows the benefits of fungicides use.www.westernfarmpress.com\new=study-shows. 2005

[11] T.H. Booth, T.S. Buckle, O.S. Cardenas, G. Gomez, E. Herras. Changes in quality of Cassava roots during storage. Journal of Food Technology. Vol. 11,149-163, 1976.

[12] A.I. Ihekoronye, N. goddy. Cassava in Tropical Agric. A Reference Manual. Ibadan, Nigeria. Pp87-120.

[13] A.C. Amadioha,. Control of black rot of potato caused by Rhizoctonia bataticola using some plant leaf extracts Pp703, 2004.

[14] A. J.N.A. Messiga, M. Nwangi, R. Bandyopadhyay, C. Nolte. Humid Forest Ecoregional center, International Institute of Tropical Agriculture (IITA), (2008). Messa, Yaounde, Cameroon. PMB 5320, Ibadan, Nigeria.

[15] H. Nahunnaro. Effects of different plant extracts in the control of yam rot induced by Rhizopus stolonifer on stored yam (Dioscorea spp.) in Yola, Adamawa State, Nigeria. Medwell Journal Agriculture Sciences, Vol. 5, 382-387, 2008.

[16] E.O. Nwachukwu, J.O. Osuji. Evaluation of plant extracts for antifungal activity against Selerotiumrolfsii causing cocoyam cormel rot in storage. Research Journal of Agriculture Biological Sciences, Vol. 4, No. 6, 787-793, 2008.

[17] H.N. Nwokocha. Agronomy of sweet potato. Root Crop Research and Technology Transfer Training Course (Manual) NRCRI, Pp77-84, 1992.

[18] A.O. Ogaraku, H.O. Usman. Storage Rot of some Yams (Dioscoreasp) in Keffi Environs, Nassarawa State, Nigeria. Pakistan Journal of Nutrition. Vol. 4, No. 2, 22-27, 2008.

[19] C.O. Frank, C.A. Kingsley. Roles of Fungal Rots in Post-Harvest Storage Losses in some Nigerian varieties of Dioscorea species. British Microbiology Research Journal. Vol. 4, No. 3, 343-350, 2014a.

[20] C.O. Frank, C.A. Kingsley. Proximate Composition, Physiological Changes during Storage, and Shelf Life of Some Nigerian Varieties of Yams (Dioscorea species), Journal of Scientific Research and Reports, Vol. 3, No. 4, 553-562, 2014b.

[21] R.N. Okigbo. A review of biological control methods for postharvest yams (Dioscorea spp.) in storage in South Eastern Nigeria. Science Technology Journal, Vol. 4, No. 1, 207-215, 2004.

[22] R.N. Okigbo, I.A.Nmeka. Control of yam rot with leaf extracts of Xylopia aethiopica and Zingiber officinale. African Journal of Biotechnology, Vol. 4, No. 8, 804-807, 2005.

[23] R.N. Okigbo, P.T. Nwakammah. Biodegradation of white yam (Dioscorea rotundata) and water yam (Dioscorea alata) slices dried under different conditions. Science Technology Journal, Vol. 5, No. 3, 1-10, 2005.

[24] R. N. Okigbo, R. Putheti, C.T. Achusi. Post-harvest deterioration of cassava and its control using extracts of Azadirachta indica and aframomum melegueta, Vol. 6, No.4, 1274-1280, 2009.

[25] R.N. Okigbo. Fungi associated with peels of post-harvest yams in storage. Global Journal of Pure and Applied Sciences, Vol. 9, No. 1, 19-23, 2003.

[26] J. Onyeka, P.M. Chuwang, O. Fagbola. International Journal of Scientific and Technological Research, Vol. 1, No.6, 76-79, 2011.

[27] N.R. Okigbo, C.E. Enweremadu, C.K. Agu, R.C. Irondi, B.C. Okeke, S.N. Awah, C.G. Anaukwu, I. O. Okafor, C.U. Ezenwa, A.C. Iloanusi. Control of white yam (Dioscorea rotundata) rots pathogen using peel extract of water yam (Dioscorea alata). Advances in Applied Science Research, 6(10):7-13, 2015.