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Original Research Article

Assessment of Effects of Postharvest Tuber Rot of Yam (*Dioscorea spp L (Poir)*) on Proximate Composition and Control of Isolated Pathogens with Some Plant Extracts and Synthetic Fungicide in Awka South, Nigeria

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Abstract

Investigation was carried out to assess the effects of post-harvest tuber rot and the proximate composition and control of isolated pathogen with some plant extract and synthetic fungicide in Awka. The experiment was conducted in the Pathology Laboratory of Department of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka. The varieties used include; white yam (*Dioscorea rotundata),* water yam *(D. alata)* and yellow yam *(D. cayensis).* The infected portion of the yams was sliced into smaller portions of 0.4cm diameter and surface sterilized in 5% methylated spirit and distilled water for 3 minutes and was rinsed twice. It was later incubated in acidified PDA for four days. The fungi growth observed was isolated and sub-cultured two times to get a pure culture which was later used for identification. *In-vitro* experiment was carried out to test the effect of two plant extracts *Aloe vera* and Lime *(Citrus aurantifolia*) and a synthetic fungicide (Aldrex T.). This experiment was laid out in a factorial in Complete Randomized Design (CRD) and replicated three times. Proximate analysis of the infected and uninfected yam tubers was also carried out. The result of isolation and identification of the rot pathogens from the infected yam tuber showed that the rot causing organism was only *Aspergillus flavus.* Plant extracts and synthetic fungicide (Aldrex T) were both effective in reducing the radial growth of the fungi pathogen. The highest percentage radial growth inhibition 75.00% was obtained in both *A. vera* and synthetic fungicide (Aldrex T) while the least (30.06%)was obtained in C. *aurantifolia* plant extract. Uninfected yam tubers had higher proximate values except in Moisture and Ash. It is therefore recommended that plant extracts tested in this study should be used as alternative to synthetic fungicide since they showed similar effectiveness in reducing yam tuber rot in Awka. Yam tubers should be properly harvested and handled to avoid injury to avoid spoilage and deterioration in their nutrient composition as well as loss of their market value.

Keywords: Assessment fungal pathogen, Plant extracts, Postharvest rot, Proximate analysis, Yam tubers

INTRODUCTION

Yam belongs to the genus *Dioscorea* in the family Dioscoreacea and is one of the most important staple foods in the world, especially some parts of Tropics and Subtropics (Okigbo and Ogbonnaya, 2006). The edible

varieties of yam are important food crops and serve as an important carbohydrate staple for millions of people in both the Tropical and Subtropical countries in West Africa, the Carribeans, the Northerrn and Central part of South - East Asia including parts of China, Malaysia, Japan and Oceania (Coursey, 1967). The FAO (1989) estimated that the world production is around 20 million ton per year. Nigeria alone produces three quarter of the world total output of yams. Okigbo (2004) noted that the ten cultivated species in Nigeria are *D.rotundata* Poir (White yam), *D. cayenensis* Lam (yellow yam), *D. alata* L. (water yam), *D. dumetorum* (cluster or bitter yam), *D. esculenta* Bark (Chinese yam) and *D. Builbifera* L. (aerial yam). Besides their importance as food source, yams also play significant role in socio-cultural lives of some producing regions. The ritual and superstition often surrounding yam and utilization in West Africa is a strong indication of the antiquity of use of this crop (Norman *et al,* 1995; Ogbo and Agu, 2014). Coursey, 1967, reported that yam tubers are of a very high value, as in food, where it is a major source of carbohydrate, minerals of Calcium, Phosphorus, Iron and Vitamins such as Riboflavin, Thiamine and vitamins B and C. Although yams are grown throughout Africa, including countries like Cameroon, Togo, Ghana, Nigeria and Ivory Coast. Nigeria is said to be the world's largest producer of yam accounting for over 70-76 percent of the world total output (FAO, 1985, Frank and Kingsley, 2014).

FAO reported that Nigeria alone in 1989 produced 18.3 million tonnes of yam from 1.5 million hectares, representing 73.8 percent of 28.8 million tonnes of yams produced in Africa. Yam can be grown in nearly all the tropical countries provided water is not the limiting factor (Pius and Odjuvwuederhie*,* 2006).

Rot is a major factor limiting the post-harvest life of yams and losses can be very high. Losses due to post harvest rot significantly affect farmers' and traders' income, food security and seed-yams stored for planting (Okigbo, 2004). The quality of yam tubers are affected by rots which makes then unappealing to consumers (Ogbo and Agu, (2014); Agu *et al. (*2015). Losses of yams in storage mostly to rot are considered to be heavy in Nigeria; as a result the demand for yam tubers has always exceeded its supply (FAO, 1997). Statistics have shown that an average of over 25% of the yield is lost annually to diseases and pests (FAO, 1997). Over 50% of yam tubers produced and harvested in Nigeria are lost in storage. Most rots of yam tubers are caused by pathogenic fungi such *as Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Fusarium solani, Botryodiplodia theobromae, Penicilllium chrysogenum, Rhizoctonia spp, Penicillium oxalicum, Trichoderma virideandRhizopus nodosus* (Okigbo and Ikediugwu, (2000); Okigbo, (2004); Aidoo, (2007); Frank and Kingsley, (2014(b); Agu *et al. (*2014). Therefore, the objectives of this study was to investigate the effect of the fungi pathogen on nutrient composition of yam and to isolate, identify and control the spoilage pathogens of yam tubers in Awka South Local Government Area, Anambra State.

MATERIALS AND METHODS

The study was carried out in the laboratory of the Department of Crop Science and Horticulture and at the Biotechnology Research Laboratory, located at Nnamdi Azikiwe University, Awka. *D.rotundata* (White yam), *D. cayenensis* (yellow yam) were obtained from the Eke-Awka market in Awka, Anambra State. *Dioscorea alata* (water yam) was obtained from Amaenyi market in Awka, Anambra State. *Aloe vera* leaves, *Citrus aurantifolia* (lime fruit) and synthetic fungicides (Adrex T.) were also obtained from Eke-Awka market in Awka, Anambra State. The yams obtained were taken to the Pathology Laboratory of Department of Crop Science and Horticuture, Nnamdi Azikiwe University Awka, Anambra State. The infected portions of the yams were sliced into smaller portions of 0.4cm diameter and surface sterilized in 5% methylated spirit and distilled water for 3 minutes and was rinsed twice with distilled sterile water. It was later incubated in acidified potato dextrose agar (PDA) for four days. The fungi growth observed was isolated and cultured and sub-cultured two times to get a pure culture which was later used for identification.

Preparation of PDA for *in-vitro* experiment

Twenty grams of PDA was weighed with the electronic weighing balance and was mixed in 500 ml of distilled water in a conical flask. The mixtures were stirred vigorously until it became homogeneous. It was then corked using cotton wool wrapped with aluminum foil before being placed into the autoclave to boil.

The conical flask containing PDA was placed into the autoclave and was properly sealed. The autoclave boiled to a temperature of 120°C and pressure of 15±1 Psi for 20-25 minutes after which it was ready for use.

Incubation and Isolation of Fungal Pathogens

A sterile inoculating loop was used to place small portions of infected yam tubers into sterile petri dishes containing the 20mls of PDA with two drops of lactic acid. This was done to inhibit the growth of bacteria, after which it was properly sealed and labelled. The plates with three replicates were incubated and left for 4 days and was observed daily for fungal growth.

The initial culture was sub-cultured two times to obtain a pure culture. The method used was the spot method

where an inoculating loop was used to slightly touch the culture, then put at the center of the Petri dish containing fresh plate of the prepared PDA. The sub-culture was left for three days and observed daily for fungal growth. The pure cultures were used for identification of the fungi with the aid of a compound microscope and identification guides.

Identification of Isolated Pathogen

A compound microscope of the model (Olympus-XN 50) was used to view the organisms. A drop of distilled water was placed on the slide, and a small portion of the culture from the three- day culture was collected from the growth using a sterile needle, it was then covered with the slide cover and placed under the microscope for viewing. The identification of the fungal was based on the morphology of the culture and the fruiting bodies. An illustrated Manual on the identification of fungi by (Barnet and Hunters, 1999) and Alexoplus *et al.* (2002) were used for identification.

Extraction by Cold Maceration of Aloe Vera Leaves

The leaves were chopped in pieces and were soaked with ethanol in a 2.5l reagent bottle for sixty-two hours and the bottle was agitated intermittently for proper extraction. After 62 hours the soaked plant was filtered using cotton wool and further filtered using filter paper. The filtrate was then concentrated using rotary evaporator.

Extraction by Cold Maceration of Lime fruits

The lime fruits were sliced in bits and were soaked with petroleum ether in a 2.5l reagent bottle for sixty –two hours and the bottle was agitated intermittently for proper extraction. After 62 hours the soaked plant material was filtered using cotton wool and further filtered using filter paper. The filtrate was then concentrated using Rotary Evaporator.

Analysis of moisture Content

The crucibles were cleaned and dried in the oven before taking an initial weight, then the samples were also weighed and place inside the crucible. The weight of the sample and crucible was noted. The crucible containing the sample was then place in the oven to dry at 105 °C for at least 8 hours. It was allowed to cool after drying in a desiccator and weighed again to get the final weight.

Crude Protein

One gram of the sample was weighed and placed in the Kjeldahl flask, l0g of potassium sulphate, 0.7 g of selenium (catalyst) and 20 ml of concentrated Sulphuric acid was added to the flask. The flask was placed in the digester and was heated to boiling until the solution is clear. After about 1 hour of cooling about 90 ml of distilled water was added. Twenty-five (25) ml of Sodium sulphate solution was also added and stirred. Eighty (80) ml of 40% Sodium hydroxide solution was also added and quickly connected to the distillation unit. Distillate was collected up till 50 ml in an indicator solution. At the end of the distillation, the flask and the condenser were removed and rinsed. This was titrated against 0.1 N HCL solution.

Crude lipids

The fats were extracted from the sample with petroleum ether and evaluated as a percentage of the weight before the solvent is evaporated. The extraction flasks were removed and dried in an oven before weighing to the nearest milligrams. 20 g of the dry sample was then weighed and placed in an extraction thimble, before connecting the flask containing petroleum ether at 2/3 of total volume to the extractor. The setup was brought to boiling at about 90 °C to obtain about 10 refluxes. The flask containing the ether extract was then dried again to evaporate the ether leaving the extract. The flask was then reweighed.

Crude fibre

The crude fibre content of the sample were determined after it has been digested in sulphuric acid and Sodium hydroxide solutions and the residue boiled. The difference in weight after calculation indicates the quantity of fibre present..Two grams of the defatted, dry yam samples was weighed out, placed the flask and 200 ml boiling sulphuric acid was added and was boiled for 5 minutes. The content was then filtered using Buchner funnel with the filter paper. The filtrate was washed with boiling water to removed excess acid. The filtrate was then transferred into another flask containing boiling sodium hydroxide solution and boiled for another 5 minutes. Same procedure was also carried out to get the filtrate washed and was then placed in a pre-heated weighed crucible and dried for about 1 hour in an oven. The weight of the crucible and residue was noted and placed in a furnace at 550 °C for 3 hours. They were left to cool in a desiccator and weighed again.

Ash

Three grams of dry sample of the yam was place in a previously weighed crucible. The crucible was placed in a furnace and heated at 550°C for 6 hours. This was left to cool in a desiccator before weighing.

Nitrogen – free extract (NFE)

These include all the nutrients not assessed by the prior methods of proximate analysis. These are composed mainly of digestible carbohydrates, vitamins and other non-nitrogen soluble organic compounds. Since the result is obtained by subtracting the percentages calculated for each nutrient from 100, any errors in evaluation will be reflected in the final calculation.

Calculations

Nitrogen-free extract (%) = 100 - (A + B + C + D + E). Where: A = moisture content (%), B = crude protein content (%), C = crude lipid content (%), D = crude fibre content (%), E = ash content (%),

In-Vitro Experiment

Twenty grams of PDA was weighed with an electronic weighing balance and was mixed in 500ml of distilled water in a conical flask. The mixture was stirred vigorously until it became homogenous. It was then corked using cotton wool wrapped with aluminum foil. The medium was then placed into the autoclave. The conical flask containing PDA was placed into the autoclave and was properly sealed. The autoclave boiled to a temperature of 120°C and pressure of 15±1 Psi for 20-25 minutes after which it was ready for use The plant extracts and synthetic fungicide which were prepared at different concentrations of l0%, 20% and 30% were mixed separately with l0mls of molten PDA with two drops of lactic acid in the petridish to inhibit the growth of bacteria. A sterile cork borer of diameter 0.4 was used to cut culture of the isolated pathogen. The discs were carefully placed in the center of each Petri dish containing PDA and different concentrations of the plant extracts and the synthetic fungicide after which each was properly sealed and labelled. Water (distilled sterile water) was used as control. Twelve Petri dishes were used for each plant extract against the test fungus at a time. Another twelve dishes were used for the synthetic fungicides. The Petri dishes were incubated at 27°C with 12 hours of alternating light and darkness. Daily observations and measurements of the radial growth were carried out. The diameter of the fungal colony was determined by measuring from the back sides of the plates with the use of meter rule. The colony growth was measured taking the average of the largest and shortest diameter of the same colony. This is because the colony growth of fungus is not always a regular circle. The fungitoxicity of the extracts was calculated in terms of percentage colony inhibition using the formula (Amadioha, 2003).

$$\text{Percentage growth inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where dc is the average diameter of fungal colony with control, dt is average diameter of fungal colony with treatment.

The experimental design used in this investigation was factorial in a Complete Randomized Design (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated using least significant difference (LSD) at 5% probability level. The Genstat Release 10.3 version was used for all the statistical analysis.

RESULTS

Result of Isolation and Identification of Fungi Pathogen from the Different Varieties of Yam

The result of isolation and identification of fungi pathogen from the different varieties of yam showed that there was only one fungi pathogen found in the yam. The only fungi pathogen identified and confirmed using the illustrated fungi pathogen was *Aspergillus flavus* which was common in all the yam incubated and assessed. Plate 1 and 2

Effects of *A. vera, C. aurantifolia* extracts and synthetic fungicide and their concentrations on percentage growth inhibition of *Aspergillus flavus* in culture

Table 1 showed that the plant extracts and the synthetic fungicide had significant (P>0.05) effect on the growth inhibition of *Aspergillus flavus* in culture where the synthetic fungicide produced the highest growth inhibition (75.00%) in all the days in culture followed by *A. vera* which had similar inhibition effect (75.00%) with the synthetic fungicide in day 2 and 3.

Table 1 also showed that *C. aurantifolia* had the least fungitoxic effect on the test organism with values (45.25, 37.63%, 34.92% and 20.06%) for days 2 and 5 respectively which were also significantly (P > 0.05) lower than values in *A. vera* and synthetic fungicide.

It was observed that while the effect of the synthetic fungicide was seen to be consistent in all the days in

Mag x75%

Plate 1. A culture of *Aspergillus flavus* from infected white yam.

Mag. x100

Plate 2. Micrograph of *Aspergillus flavus* isolated from different infected yam tubers

Table 1. Effects of *A. vera, C. aurantifolia,* synthetic fungicide and their concentrations on the percentage (%) growth inhibition on *Asperillus flavus* in culture

	Incubation period (days) and Growth inhibition (5)			
Treatments	**Day 2**	**Day 3**	**Day 4**	**Day 5**
AVE	75.00	75.00	59.33	57.33
LPE	45.25	37.63	34.92	30.06
SF	75.00	75.00	75.00	75.00
LSD (5%)	**3.117**	**3.301**	**4.750**	**2.241**
Concentration(g/ml)				
0	0.00	0.00	0.00	0.00
10	81.19	81.87	70.69	67.53
20	87.41	81.79	73.90	70.51
30	91.73	86.42	81.08	78.49
LSD (5%)	**3.600**	**3.812**	**5.482**	**2.588**

NOTE: AVE = *Aloe vera* extracted with ethanol, LPE= Lime *(Citrus aurantifolia)* extracted with petroleum ether, SF = Synthetic Fungicide (AldrexT), Trt=Treatment, Conc. = Concentration

Table 2. Interaction effects of extracts of A*. vera, C. aurantifolia, synthetic fungicide* and concentrations on the percentage growth inhibition of *Aspergillus flavus* in culture

Day 2					**Day 3**				**Day 4**				**Day 5**			
Conc. Trt.	0	10	20	30	0	10	20	30	0	10	20	30	0	10	20	30
AVE	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0
LPE	0.00	43.57	62.23	75.20	0.00	45.90	45.37	59.27	0.00	40.93	42.43	56.30	0.00	31.73	34.50	54.00
SF	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0	0.00	100.0	100.0	100.0
LSD (0.05)			6.235				6.602				9.500				4.482	

culture, that of the plant extract were depreciating with time in culture.

Also table 1 showed that there was significant differences in the effect of the various concentrations, where concentration level of 30g/ml had the highest inhibition value in all the days in culture followed by the effect of 20g/ml while the least inhibition values were obtained in l0g/ml. All the concentration levels did better than the control.

The result also showed that the effect of 20g/ml and l0g/ml had statistically same inhibition values (81.79%, 81.97%) and (73.90%, 70.69%) in days 3 and 4 respectively. In the fifth day 30g/ml had the highest inhibition effect (78.49%) followed by 20g/ml with value (70.51%) while l0g/ml had the least inhibition value (67.53%).

Interaction effects of *A. vera, C. aurantifolia* and synthetic fungicide (Aldrex T) and their concentrations on the percentage growth inhibition of *Aspergillus flavus* that cause yam rot in culture

The result of the of the interaction effects of *A. vera, C. aurantifolia* and synthetic fungicide (Aldrex T) and their concentrations showed significant interaction effect on percentage growth inhibition of *Aspergillus flavus* that cause yam rot in culture, where *A. vera* plant extract and Aldrex T. has consistently significantly (P>0.05) higher interaction effect than *C. aurantifolia* plant extract in days 2 to 5 (Table 2). Also *A. vera* plant extract and Aldrex T. has statistically the same inhibition values of 100.00% in days 2 and 3 respectively (Table 2). It was observed in Table 2 that the highest inhibition value for *A. vera* in days 4 and 5 were 86.93% and 81.47% obtained in concentration levels of 30g/ml for the two days while that of synthetic fungicide had perfect inhibition of 100% values. *C. aurantifolia* plants

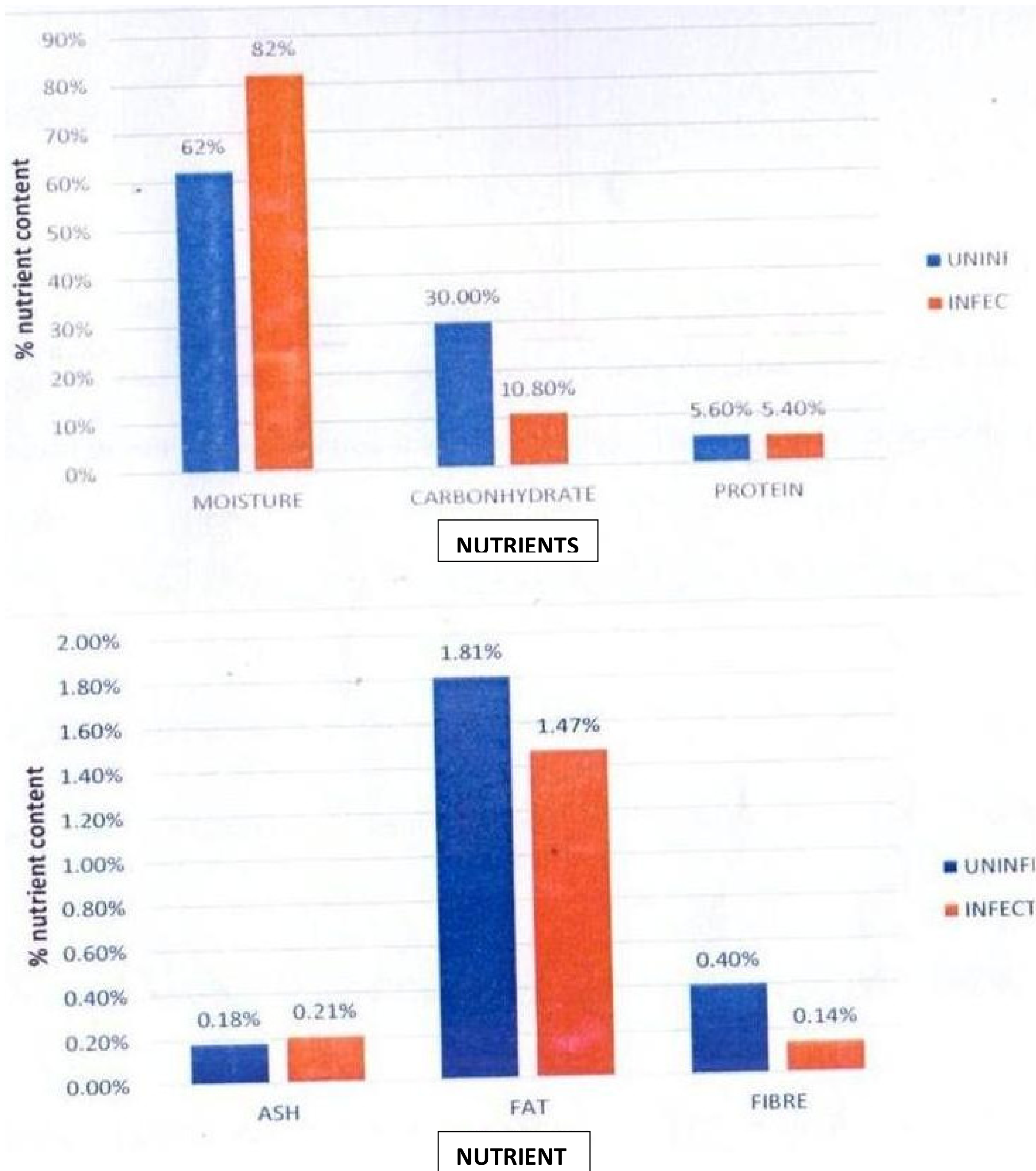

Figure 1 and 2. Showing the graphical representations of the proximate analyses of infected and uninfected yam tubers

extract had the least inhibition in day 2 in 30g/ml concentration level. The result also showed that all the plant extract x concentration interaction effect performed better than the control (Table 2).

Effects of the spoilage pathogen on proximate composition of white yam *(D.rotundata)*

Figs 1 and 2 showed the pictorial representation of the proximate composition of one of the yam varieties used *(D.rotundata)* were the composition of infected yam tissues where compared with the uninfected. It was observed that there were differences in the proximate composition, where the uninfected yam had lower moisture content (62%) than infected (82%) but higher carbohydrate content (30.00%) than the infected (10.80%) and statistically same level of protein content (5.60% and 5.40%) respectively. The result of the proximate composition also showed that uninfected yam

had lower ash content (0.18%) than infected tuber with ash value of 0.21% but uninfected yam had higher fat and fibre content (1.81% and 0.40%) than infected with values of 1.47% and 0.14% respectively. It could be observed that except moisture content, uninfected yam tissues had higher percentage value in all the component tested. From the result it could be inferred that the spoilage micro-organism had a significant deteriorating effect on the yam tubers.

DISCUSSION

The result of isolation and identification of spoilage, fungi that cause post-harvest tuber rot in yam showed that *Aspergillus flavus* was the only fungi pathogen that was isolated from the various spoilt yam varieties that were tested. This result is in agreement with Amusa and Baiyewu (1999); Okigbo and Ikediugwu (2000); Khadijat (2003); Yusuf and Okusanya (2008); Okigbo *et al.* (2013) who also reported that *A. niger, B. theobromae* were also isolated in infected yam tuber. This is also similar to the report of Amusa *et al. (*2002); Nweke and Agbogidi (2008).

Effect of plant extract, their concentration and synthetic fungicide on the growth of *Aspergillus flavus*

The result showed that *A. vera, C. aurantifolia* and synthetic fungicide had significant effect on growth inhibition of *Aspergillus flavus* in culture. This agrees with the report of Yeni (2011) who reported that *Z. officinale* plant extract used were effective in reducing the radial growth of *Aspergillus flavus* that cause post-harvest yam tuber rot. The result also showed that *A. vera* had similar inhibition effect with synthetic fungicide in day 2 and 3 of this investigation. This agrees with the findings of Yoltana and Galon (1995). It was also observed that the effect of the plant extract was depreciating in days in culture. This corroborates with the report of Nweke *et al.* (2015), Okigbo *et al.* (2009) who observed that effectiveness of the plant extract used depreciated after some days indicating that the active ingredient of the plant species were not persistent as synthetic fungicides.

Proximate analysis

The result of proximate analysis of infected and uninfected yam tubers showed that the uninfected yam tubers had higher proximate values in all the component tested except in moisture content. These results is in agreement with reports of Amusa *et al.* (2002) who observed that the moisture and fibre contents of breadfruit *(Artocarpus communis)* increased with deterioration in storage.

CONCLUSION

From the investigation carried out on the isolation and identification of post-harvest spoilage micro-organism of yam tuber and their control using *A. vera, C. Aurantifolia* plant extracts and synthetic fungicide it was found that the only microorganism isolated from tubers of tree infected yam varieties*: D.rotundata, D. alata, D. cayenensis* used in this research was *Aspergillus flavus.* That the plant extracts especially *A. vera* plant extract and synthetic fungicide were very effective in reducing the radial growth of *Aspergillus flavus* in culture. Also, it was discovered that there was significant (> 0.05) difference in the nutrient composition of infected and uninfected yam tuber that were analyzed in this investigation, where it was observed that there was great depreciation in the infected yam tubers compared to that of uninfected.

RECOMMENDATIONS

From the results of this investigation, I recommend that plant extract should be used in alternative to synthetic fungicides since they showed similar effectiveness in reducing yam tuber rot in Awka. This is because the plant extracts are more abundantly available than the synthetic fungicide which is not always available and affordable to farmers coupled with its attendant adverse health effect on the humans.

That tubers should be properly harvested to avoid injuries that serves as entrance points to spoilage micro-organisms which leads to deterioration in nutrient composition and loss of market value.

Conflict of Interest

There is no conflict of interest as there was no sponsorship or support from anybody.

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