Original Research Article

BIOASSAY OF LEMON GRASS ON FUNGI PATHOGEN ASSOCIATED WITH CASSAVA TUBERS ROT IN FARIN GADA MARKET, JOS

ABSTRACT

The aim of this study was to determine the effects of Lemon grass on fungal pathogens associated with cassava tuber rot. The study was carried out in the biology laboratory of the Federal College of Forestry Jos, Plateau state from March to May, 2019. Rotten and healthy cassava were collected separately from Farin-Gada market Jos, fungi species were isolated from rotten cassava by direct inoculation of the spoiled part on sterile Ppotato Ddextrose Agar medium and incubated for 3-5 days, the isolated fungi were identified microscopically and macroscopically. The identified fungi were used for pathogenicity test. The antifungal effect of different concentrations of ethanol extract of lemon grass was investigated. Data collected were analyzed using one way ANOVA and the means were separated using Lleast Ssignificant Ddifference (LSD) at ($p \geq 0.05$). The fungi isolated included *Fusarium* spp, *Penicillium* spp, *Geotrichum candidum*, and *Aspergillus flavus*, the fungi isolated indicated the frequency distribution value of 30, 15, 35 and 20 % respectively. 20 mL of the tested extract gave the highest inhibition of 19.07, 20.57, 18.17 mL and 18.00 mL on *Fusarium* spp, *Penicillium* spp, *Geotrichum candidum* and *Aspergillus flavus* respectively. At the 5thth day of incubation the results of the pathogenicity showed that *Aspergillus flavus* gives the highest deterioration of 9.17 mm. The length of deterioration showed significantly different. The lemon grass extract indicated anti-fungal effect on the fungi isolates, therefore could be used to control cassava tuber rot caused by fungi.

Keywords: cassava, lemon grass, fungi, rot, tubers

1.0 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major commercial and staple crop in the tropical and sub-tropical world, which Nigeria is currently one of the largest world producers [1]. The mode of cassava utilization varies from one place to another, studies revealed that cassava is one of the most important crops in Nigeria. Cassava is a major source of carbohydrates for millions of people in several regions, particularly in developing countries. The cassava crop plays a vital role in reducing poverty and rural exodus because the use of technology required is minimal [2]. In addition to the social impact, cassava has attracted the interest of the agriculture business due to its multiple industrial uses of starch [3]. Nigeria alone currently produces over 14million tones annually, representing about 25% important role in the rural economy southern agro ecological zone and is increasingly gain importance in other parts of Nigeria [4].

The main constrains in cassava production is diseases and sometimes pest. The extent of losses may be as high as 80%, the spoilage of cassava tuber arises from combination of physiological and pathological factors [5]. Biochemical analysis of infection process showed that the microbial pathogen must produce a set of enzyme capable of attacking the carbohydrate polymer and protein composition of the infected plants cell wall [6].

The fungi also play important role in producing amylase which is capable in degradation of starch tissue of the plant [7]. Different studies has shown fungi from rotted cassava tubers and root after re-i~~n~~noculation in storage included *Fusarium solani, Rhizopus stolonifera*, *Phythophthora drechslera*, *Aspergillus niger* and *Botryodiplodia theobromae* [8]. These fungi cause discolouration in the surrounding tissue of infected cassava tubers, resulting in change in appearance, deterioration of texture and flavor or taste of cassava product. Rot fungi result in post-harvest losses and reduction in market value of tubers [9]. Knowledge of geographical distribution of root rot pathogens may be useful to breeders targeting root rot resistance.

2.0 MATERIAL AND METHODS

2.1 Study Area

The study was carried out in biology laboratory at Federal College of Forestry, Jos North Plateau State from March to May, 2019. Plateau state is located between latitude 8.5^0-100 46^0 North and longitudes 8.20^0 -10.36^0 East in the north central zone of Nigeria [10]

2.2 Collection of Cassava tuber

Rotten cassava tubers (50) were collected separate sellers from Farin gada market, Jos. The samples were packed in sterile polythene bags, labeled properly and taken to the laboratory for further study. Hundred grams (100 g) of lemon grass leaves~~f~~ were collected and packed in a polythene bags. Collected lemon grass were taken to the herbarium at Federal College of Forestry for proper identification.

2.3 Isolation of Fungal organisms

Small portion of diseased cassava tubers were picked under aseptic conditions using sterile scissors and sterilized by dipping inside 70 % ethanol for 5 minutes. The picked diseased portion were then placed in a Petri dishes containing autoclaved solidified potato dextrose agar (PDA). The solidified plates were incubated in a locker at a room temperature (28-32^{0}C) for 3-5 days. Fungal colonies from the incubated plates were purified by sub culturing into fresh medium until pure culture were obtained [11]. Percentage frequency occurrence of the organisms from the samples site was calculated using the follows formula;

$$(\%) = \frac{Individual\ fungi\ isolate}{Total\ number\ of\ fungi\ isolated}\ X\ 100$$

2.4 Fungi Identification

The method of John *et al*. [12] was used. A small portion of freshly grown colony were picked from the plate into a glass slide using a sterile inoculating needle ~~into a glass slide~~. One to two drop of lacto phenol cotton blue was dropped. The slide was covered with the cover slip and sealed using petroleum jelly. The slide was then viewed under a compound microscope using ×10 and ×400 magnification. The fungi cell morphology identified under the microscope were compared with the observed feature of conidia and conidiophores as adopted by Barnett and Hunter [13].

2.5 Preparation of Lemon Grass Extract

The fresh leaf of Lemmon grass was used. The Collected leaf were first washed with tap water to remove the trace of sand and dirt~~sy~~ and rinsed severally with sterile distilled water and air dried on the laboratory bench. The dried plant leaves~~f~~ were pulverizing using a wooden pestle and mortar, the pulverized powder was then soaked in 250ml absolute ethanol for 48 h and the solution filtered in ~~-~~to a beaker. The ethanol was allowed to evaporate and solution dried to powder by heating at low temperature in an oven. The powder was dissolved in sterile distilled water to give 25% concentrate of the leaf extract and kept in a fridge wrapped properly with aluminum foil paper to prevent contamination. From the stock solution (25%), subsequent concentrations (10, 15 and 20 %) were prepared by serial dilution.

2.6 Pathogenicity test

Apparently healthy cassava tuber were washed with sterile distilled water and followed by surface sterilization using 70 % alcohol. A hole (5mm diameter) was made on the tubers with a sterile cork borer. Fresh Mycelia cell were picked from cultures plates and used for the inoculation of cut part. The cut portions were sealed with petroleum jelly to prevent contamination by other microorganisms [12]. The inoculated tubers and the control (inoculated) were placed separately in sterile polythene bags containing cotton wool soaked in sterile distilled water to provide humid environment [14]. The bags were properly labelled and incubated at a room temperature. Disease symptoms induced by artificial inoculation after the incubation period were recorded after 10 days and the experiment was repeated trice.

2.7 Determination of Inhibitory Effect of Lemon grass Extract

Different concentrations (10, 15 and 20 mL) of lemon grass extract was poured into a conical flasks containing 100 mL prepare potatoes dextrose agar media and sterilized using autoclave. After autoclaving, the medium was allowed to cool and then poured into Petri dishes and allowed to solidity before inoculation. The medium without lemon grass extract service as control. A 5 day old colony was picked using a sterile inoculating needle and placed aseptically on the centre of the plate and incubated at room temperature in a locker, the treatments were replicated three times. The readings were taken daily.

2.8 Experimental design and Statistical Analysis

A Complete Randomized Design, (CRD) was used, the experiment was replicated 3 times. The data obtained ~~was~~were analysed using Analysis of variance (ANOVA) and the means were separated using least significant difference (LCD) at $p = 0.05$

3.0 RESULTS AND DISCUSSION

Twenty two fungi species were isolated from rotten cassava tubers collected from sample sites, the fungi species were later grouped into four group based on their macroscopic and microscopic characteristic. The result on Table 1 revealed fungi specie isolated and identified were *A.~~spergillus~~ flavus*, *Fusarium* spp, *Geotrichum candidum* and *Penicillium* spp. Among the fungi isolated, *Geotrichum candidum* had the highest frequency of occurrence value of 35 % With respect to localization, this was followed by *Pennicillium* sp. was the least common genus with a 15 % relative prevalence. This current

work collaborate work of Ngobisa *et al*. [5]. The fungi of the genus *Geotrichum* sp. probably play a role in the process of fermentation and post-harvest deterioration of tuberized roots of cassava [15, 16].

The result of pathogenicity test carried out with *Geotrichum, Penecillium* spp, *A.~~spergillus~~ flavus* and *Fusarium* spp shown on Figure 1 revealed that all the fungal~~i~~ isolates caused varying lengthens of rot on cassava tuber. *A.~~spergillus~~ flavus* gave maximum level of deterioration (9.17 mm) based on the lengthen of spoilage recorded, this was closely followed by *Fusarium* sp. This in agreement with the study of Ngobisa *et al*. [5] who isolated *Fusarium* sp and *Geotrichum* sp from cassava tuber. The Penicillum sp specie showed the lowest rate of spoilage (5.04 mm) among the fungal~~i~~ isolates studied. Suleiman and Sule [4] demonstrated that *Penicillum* sp indicated low pathogenicity on Cassava tubers when compared to *Rhizopus stolonifer*.

The fungal~~i~~ isolates obtained in work are regarded as saprophytic and parasitic fungi, their spores are cosmopolitan, found everywhere in the air and are often source of contamination and toxin production [17]. In most studies, these fungi were found to gain entrance into cassava tubers through natural opening and wounds created during harvesting; transporting, handling and marketing [7].

The presence of various concentrations of leaf extracts of Lemon grass introduced into potato dextrose ager showed reduction in radial growth of the fungi pathogen study. The results in T~~t~~able 4 to 7 showed that the plant extract had fungicidal properties comparing with the control. The results showed increase in the extract concentration led to increase in vegetative fungi growth. At 20 mL lemon grass extract, the lowest radial growth (18.17 mm) retardation of *Geotrichum candidum* was observed after 5 days of incubation. The control showed the highest radial growth value of 40.33 mm after 5 days (Table 7). This similar with the study of Amadioha [18] and Tijani *et al*. [19] who demonstrated the bioactivity of *Azadirachta indica* and *Moringa oleifera* seed against *Erwinia* and *Rhizopus stolonifer* associated with tuber rot.

Twenty milliliter (20 mL) of lemon grace reduced the radical growth of *Fusarium* and *Penicillum* sp by 19.07 mm and 20.57 mm respectively (Table 4 and 5). Taiga [20] revealed antifungal action of *Nicotinia tabacum* against radial growth of *Fusarium* sp and *Penicillum* sp isolated from yam tuber. The

study demonstrated that the extracts concentration exhibited varying reduction of the mycelial growth of the fungi; with a significant (0.05) difference compared with the control.

The use of synthetic fungicide apart from their potential danger to both farmers and environment are unaffordable by most of the cassava farmers. Recent studies on the use of plant extracts have opened a new opportunity for the control of plant disease. In Nigeria, plant extracts have been used to control fungal diseases of plants such as tomatoes [12], maize [11], but have been sparsely used in the control of cassava diseases [21].

Works from other researchers indicate majority of the species belonging to the genus *Aspergillus* species are saprophytic fungi and only few species including *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus niger* are said to be weak plant pathogens. These fungi penetrate plant hosts through wounds caused mechanically or by insects [22]. *Aspergillus* spp induces black mould rot that occurs primarily on tuber crops that are injured and kept at high temperature.

Fusarium species are among group of fungi associated with cassava root rot. Crop losses due to root rot ranges from 0.5 to1 ton/ha but losses greater than 3 ton/ha, an equivalent of 15 to 20% produce, often occur [23]. Many species of *Fusarium* were associated with rotted cassava roots in Nigeria and Cameroon [24]. Of all diseases caused by *Fusarium* on cassava, the economic important one is the vascular wilt disease induced by *Fusarium oxysporum.* Although *Penicillium* has been implicated in postharvest fungus but most pathogenic infections occur before harvest during fruit germination. The genus *Penicillium* includes about 150 species but only a minor fraction of these cause economic infections [25].

Table **1:** **Macroscopic and microscopic characteristic of fungi Isolates from cassava**

Samples	**Microscopic characteristics**	**Macroscopic characteristics**	**Probable isolates**
A	produce dark brown spores from their conidial head	White surface later bearing black conidia.	*Aspergillus flavus*
B	Oval shaped microconidia,produced in false heads	Colonies were bright coloured with cottony aerial mycelium.	*Fusarium* spp
C	Hyphae with septa sporangiospores held within the sporangia structure.	Appears as a cottony white structure and then turns black on the surface.	*Geotricum candidum*
D	branched conidiosphores, they form brush like clusters	The plate reverse showed pale to yellowish.	*Penicillium* spp

Table 2. Morphological views of fungi isolates

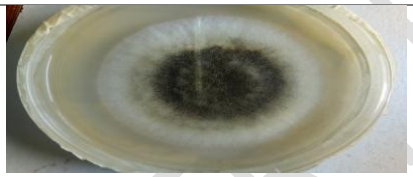
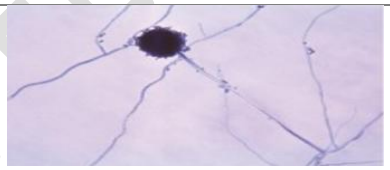
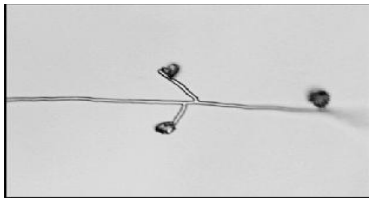
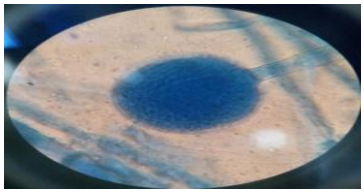
Sample	Macroscopic characteristic	Microscopic characteristic	Probable isolate
A			*Aspergillus flavus*
B			*Fusarium* spp
C			*Geotrichum candidum*
D			*Penicillium* spp

Table 3: Showing the percentage distribution of fungi isolate

Fungi Isolated	Frequency Occurrence (%)
Geotrichum candidum	35
Fusarium spp	30
Aspergillus flavus	20
Penicillium spp	15

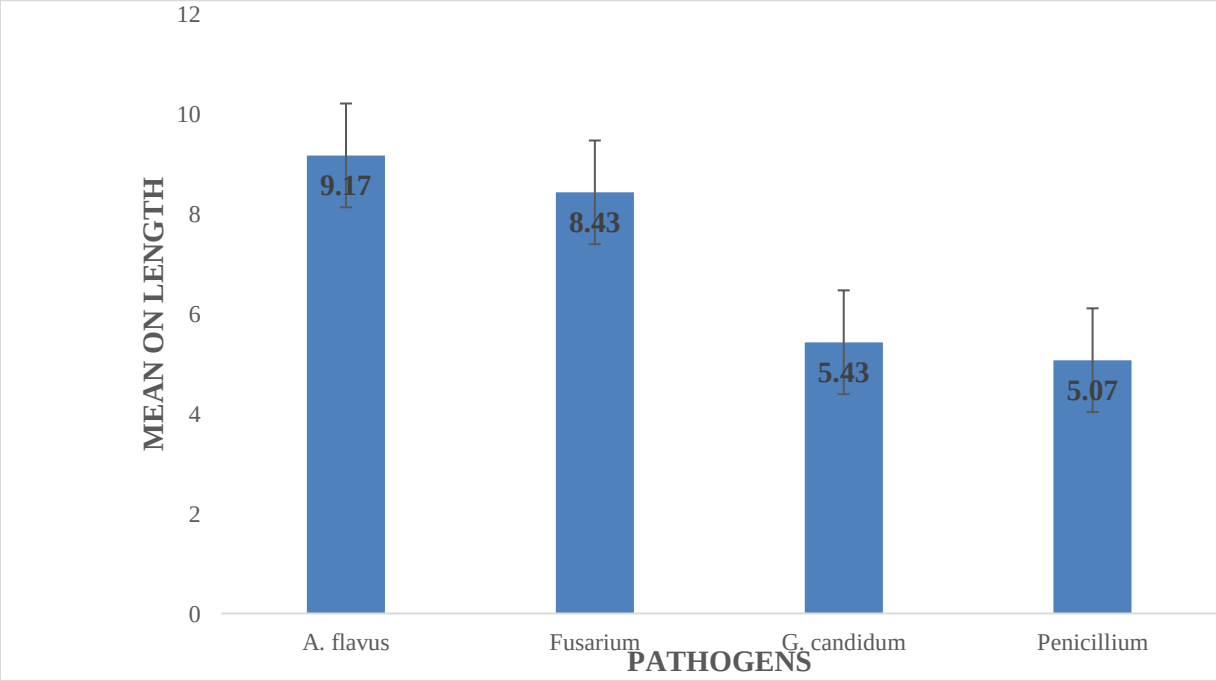


Figure 1: Bar Chart on Pathogenicity Test of Organisms on Length (mm)

Table 4: Effect of different Concentration of Plants Extract on the Radial Growth of Fungi Isolated by *Fusarium* spp

Plants Extract on the Radial Growth of Fungi Isolated *Fusarium* spp					
Treatment (mL)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
10	13.33[a]	14.00[b]	19.00[b]	22.20[b]	24.33[b]
15	10.33[b]	11.47[c]	15.67[c]	19.37[c]	23.47[b]
20	8.17[b]	9.00[d]	9.33[d]	14.00[d]	19.07[c]
Control	15.00[a]	19.00[a]	22.60[a]	25.33[a]	28.00[a]
SE	**0.69**	**0.52**	**0.70**	**0.79**	**0.72**

Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

Table 5: Effect of different Concentration of Plants Extract on the Radial Growth of Fungi Isolated by *Penicillium* spp

Plants Extract on the Radial Growth of Fungi Isolated *Penicillium* spp					
Treatment (mL)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
10	13.93^{ab}	15.33^{ab}	20.13^{ab}	24.00^{b}	27.33^{b}
15	11.47^{b}	13.90^{bc}	17.17^{b}	23.23^{b}	24.87^{b}
20	12.07^{b}	12.50^{c}	11.60^{c}	16.67^{c}	20.57^{c}
Control	16.33^{a}	17.53^{a}	21.67^{a}	26.86^{a}	31.00^{a}
SE	**0.79**	**0.69**	**1.04**	**0.85**	**0.87**

Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

Table 6: Effect of different Concentration of Plants Extract on the Radial Growth of Fungi Isolated by *Aspergillus flavus*

Plants Extract on the Radial Growth of Fungi Isolated *Aspergillus flavus*					
Treatment (mL)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
10	5.33^{b}	13.33^{a}	22.00^{ab}	25.00^{b}	31.00^{b}
15	6.00^{b}	9.53^{b}	18.73^{b}	20.80^{c}	25.67^{c}
20	3.97^{b}	7.07^{b}	11.47^{c}	13.33^{d}	18.00^{d}
Control	10.33^{a}	15.00^{a}	25.33^{a}	28.33^{a}	36.00^{a}
SE	**0.86**	**0.78**	**1.23**	**0.66**	**0.83**

Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

Table 7: Effect of different Concentration of Plants Extract on the Radial Growth of Fungi Isolated by *Geotrichum candidum*

Plants Extract on the Radial Growth of Fungi Isolated by *Geotrichum candidum*					
Treatment (mL)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
10	11.33^{a}	12.00^{ab}	14.67^{b}	21.33^{b}	28.00^{b}
15	9.33^{a}	10.33^{bc}	13.70^{bc}	17.33^{c}	25.00^{b}
20	5.00^{b}	8.33^{c}	10.00^{c}	13.33^{d}	18.17^{c}
Control	11.67^{a}	15.00^{a}	19.67^{a}	36.67^{a}	40.33^{a}
SE	**0.91**	**0.94**	**1.16**	**1.05**	**0.10**

Means on the same column with the same letter do not differ significantly from each other (P = 0.05).

4.0 CONCLUSIONS

This study revealed that the most fungi are associated with cassava rot in Farin Gada market were *Fusarium* spp, *Penicillium* spp, *Aspergillus flavus*, and *Geotrichum candidum*. The study also found out the highest concentration (20 mL) gave the best radial growth inhibition value of 18.05 mm at day 5. The finding showed that fungal isolates are responsible for causing deterioration. The result of the Pathogenicity test showed varying length at deterioration with *A. flavus* producing the highest

deterioration of 9.17 mm. The use of lemon grass could go a long way in mitigating fungal growth on stored crops and prolonging the storage life of the cassava tubers particularly during off or dry season.

5.0 RECOMMENDATION

Further studies on these plants extracts should be done to ascertain their chemical activities against *A. flavus*, *Fusarium* sp, *Penicillium* sp and *Geotrichum candidum* and other rots agents of cassava. Also high concentrations of lemon grass extract should be further exploited.

REFERENCES

[1] Nyerhovwo JT. Cassava: A future of starch. African Journal of Biotechnology. 2004; 7(1): 5-8.

[2] Akinbo O, Labuschagne M and Fregene M. Introgression of whitefly (*Aleurotrachelus socialis*) resistance gene from F1 interspecific hybrids into commercial cassava. Euphytica. 2012; 183: 19-26.

[3] Tonukari NJ. Cassava and the future of starch. Biotechnology Issues for Developing Countries. 2004 7: 5-8.

[4] Suleiman MN and Sule A. Bioassay of plant extracts on two fungal pathogens of cassava tuber rot in Kogi state, Nigeria. FUTA Journal of Research in Sciences. 2016; (1): 156-166.

[5] Nyaka NAIC, kammegne DP, Ntsomboh NG, Mbenoun M, Zok S and Fontem D. Isolation and identification of some pathogenic fungi associated with cassava (*Manihot esculenta* rantz) root rot disease in Cameroon. African Journal of Agricultural Research. 2015; 10(50):4538-4542.

[6] Odebode AC, Salami AO and Osonubi O. Production of cell wall enzyme in pepper seedlings inoculated with arbuscular mycorrhiza (glomus etunicatum). Tanzania Journal of Science. 2001; 27:1-8.

[7] Amienyo CA and Ataga AE. Use of indigenous plant extracts for the protection of mechanically injured sweet potato. Academic Journal Science Research Essay. 2007; 7:51-59.

[8] Amadioha AC. Reducing food losses through sustainable methods of plant disease management: An imperative for actualization of food security in Nigeria. A paper presented at the 13th inaugural lecture mouau, 2012.

[9] Sandielle AVB, Saulo ASO, Carlos ADB, Juliana BR and Eder JDO. Survey of fungi associated with cassava root rot from different producing regions in Brazil. Journal of Science Agriculture. 2017; 74(1):60-67.

[10] Mallo SJ and Wazoh HN. Reclamation of abandoned mined-out areas of Bukuru ray field. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2014; (8)2:25-34.

[11] John WC, Ihum TA, Maipandi MO and Ishaya M. Inhibitory effect of *Vernonia amygdalina* leaf powder on *Rhizopus Stolonifer* and *Fusarium* sp of tomato plants in a greenhouse. Asian Journal of Research in Botany. 2018; 1(2): 1-7.

[12] John WC, Anyanwu NCJ and Ayisa T. Evaluation of the effects of the extract of *Vernonia amygdalina* on fungi associated with infected tomatoes (*Lycopersicon esculentum*) in Jos north local government area, Plateau state, Nigeria. Annual Research and Review in Biology. 2016; 9(4): 1-8.

[13] Barnett HI and Hunter HB. Illustrated genera of imperfect fungi. 4th edn. St. Paul: APS press. 1998; 40-138.

[14] Suleiman MN. Root rot disease of cowpea (vigna unguiculata) and its control using plant extracts and fungicides. Lamp Lambert, Germany 2011; 180.

[15] Raimbault M, Revah S, Pina F and Villalobos P. Protein enrichment of cassava by solid substrate fermentation using moulds isolated from traditional foods. Journal of Fermentation Technology. 1985; 63:395-399.

[16] Oyewole OB and Odunfa SA. Microbiological studies on cassava fermentation for « lafun » production. Journal of Food microbiology. 1988; 5:125-133.

[17] Dutta AC. Botany for degree student 7th edition. Oxford University Press, London. 2005; 563.

[18] Amadioha AC. Control of black rot of potato caused by *Rhizoctonia bataticola* using some plant leaf extracts. Arch Plant Pathology Plant Protection. 2004; (37):111-117.

[19] Tijjani A, Adebitan SA, Gurama AU, Aliyu M, Dawakiji AY, Haruna SG and Mohammed NA. Efficacy of some botanicals for control of wet rot disease of mechanically injured sweet potato caused by *Rhizopus stolonifer* in Bauchi state. International Journal of Science Research Publication. 2013; 3(6):1-10.

[20] Taiga A. Comparative studies of the efficacy of some selected fungicidal aqueous plant extracts on yam tuber dry rot disease. Annual Biology Research. 2011; 2(2): 332-336.

[21] Okigbo RN and Nmeka IA. Control of yam tuber rot with leaf extract of *Xylopiaa ethiopical* and *Zingiber officinale.* African Journal of Biotechnology. 2005; 4: 804-807.

[22] Geiser DM and Lobuglio KF (2001). The *Monophylectic plectomycetes*, *Ascosphaerales*, *Onygenales*, *Eurotiales*. In: the mycota: a comprehensive treatise on fungi experimental systems for basic and applied research, systematics and evolution. Eds. DJ Mclaughen, EG Mclaughen and PA Lenke. Springer-verlag, Berlin, Germany, vii Part A. 2001; 201-219.

[23] Berhanu k. (2017). Isolation, identification and characterization of some fungal infectious agents of cassava in south west Ethiopia. Advances in Life Science and Technology. 2017; 54:6-27.

[24] Bandyopadhyay R, Mwangi M, Aigbe SO and Leslie JF. *Fusarium* species from the cassava root rot complex in West Africa. Phytopathology. 2006; 96: 673-676.

[25] Pitt JI. A laboratory guide to common *Penicillium* species. Commonwealth scientific and industrial research organization, food research laboratory. NSW, Australia 1991.

298
299