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Bioassay of Lemongrass on Fungi Pathogen Associated with Cassava Tubers Rot in Farin Gada Market, Jos

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Authors' contributions

This work was carried out in collaboration among all authors. Authors WCJ and OO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MI managed the analyses of the study. Authors NJ and MJM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to determine the effects of Lemongrass on fungal pathogen 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 tubers were collected separately from Farin-Gada market Jos, fungi species were isolated from rotten cassava tubers by direct inoculation of the spoiled part on sterile Potato Dextrose 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 lemongrass was investigated. Data collected were analyzed using one way ANOVA and the means were separated using Least Significant Difference (LSD) at ($p \geq 0.05$). The fungi isolated include, *Fusarium* sp, *Penicillium* sp, *Geotrichum candidum*, and *Aspergillus flavus*. The frequency of occurrence of the isolated fungi indicated *Fusarium* sp, *Penicillium* sp, *Geotrichum*

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candidum, and *Aspergillus flavus* had 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 5th 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 significant difference. The lemongrass extract indicated anti-fungal effect on the fungal isolates, therefore could be used to control cassava tuber rot caused by fungi.

Keywords: Manihot esculenta Crantz; ethanolic extract; Fusarium; Penicillum; Geotrichum candidum.

1. 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 are 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 produce a set of enzyme capable of attacking the carbohydrate polymer and protein composition of the infected plants cell wall [6].

Lemongrass (*Cymbopogon citratus*) belong to family Poaceae which represents an important genus of about 120 species that grows in tropical and subtropical regions worldwide. The plant is used in diverse fields such as pharmaceutical, cosmetics, food and flavor, and agriculture industries. Lemongrass are cultivated on large scale, especially in tropics and subtropics [7]. Lemongrass possesses strong lemony odor due to its high content of aldehyde citral [8]. Lemongrass is commonly used in herbal medicine for treatment of nervous and gastrointestinal disturbances, and as antispasmodic, analgesic, anti-inflammatory, diuretic and sedative [9]. Studies on extracts from Lemongrass leaves have demonstrated antioxidant, anti-microbial and anti-fungal activities [10,11].

Fungi play important role in producing amylase which is capable of degrading starch tissue in plant [12]. Different studies has shown fungi from rotten cassava tubers and roots, these fungi species include, *Fusarium solani, Rhizopus stolonifera*, *Phythophthora drechslera*, *Aspergillus niger* and *Botryodiplodia theobromae* [13]. These fungi cause discolorations 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 [14]. Knowledge of geographical distribution of root rot pathogens may be useful to breeders targeting root rot resistance. This research is therefore, aim at study of effects of Lemongrass on fungal pathogens associated with cassava tubers rot.

2. MATERIALS 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°-100 46° North and longitudes 8.20° -10.36° East in the north central zone of Nigeria [15].

2.2 Sample Collection

Fifty rotten cassava tubers were collected from different sellers from Farin gada market, Jos. The samples were packed in sterile polythene bags, labeled properly and taken to the laboratory for further study. 500 g of lemongrass leaves were

collected and packed in a polythene bags. Collected Lemongrasses were taken to the herbarium at Federal College of Forestry for proper identification.

2.3 Isolation of Fungal Organisms

0.5 g portions of diseased cassava tubers were picked under aseptic conditions using sterile scissors and surface sterilized by dipping inside 70% ethanol for 5 minutes, this was followed by rinsing using sterile distilled water. The picked diseased portions 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°C) for 3-5 days. Fungal colonies from the incubated plates were purified by sub culturing into fresh medium until pure cultures were obtained [16]. 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. [17] was used. Small portions of freshly grown colony were picked from the plate into a glass slide using a sterile inoculating needle. 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 [18].

2.5 Preparation of Lemongrass Extract

Lemongrass leaves were air dried on laboratory bench and pulverized into powder using blender. 200g of the plant powder was weighed into 500 mL conical flasks and was soaked in 70% ethanol. This was left to stand for 48h, then shook for 6h on a rotary shaker. The sample was filtered using a non-absorbent cotton wool on a Buchner funnel-flask using a vacuum pump. The residue was subjected to several rinsing and filtration with fresh ethanol to attain good level of extraction. The collected filtrate was evaporated to dryness using a rotary evaporator and a drying cabinet. The percentage yield of the extract was determined and the extract was transferred into a sterile sample container and preserved in the refrigerator [17].

2.6 Pathogenicity Test

Healthy cassava tuber were washed with sterile distilled water and followed by surface sterilization using 70% alcohol. 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 [17]. The inoculated tubers and the control (un-inoculated) were placed separately in sterile polythene bags containing cotton wool soaked in sterile distilled water to provide humid environment [19]. 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 Lemongrass Extract

Different concentrations (10, 15 and 20 mL) of Lemongrass 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 solidify before inoculation. The medium without lemongrass 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 were analysed using Analysis of Variance (ANOVA) and the means were separated using least significant difference (LCD) at $p = 0.05$.

3. 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 groups based on their macroscopic and microscopic characteristic. The result on Tables 1 and 2 revealed fungi species microscopic and macroscopic characteristic. The isolated and identified fungi are *A. flavus*, *Fusarium* specie, *Geotrichum candidum* and *Penicillium* spiece. Among the fungi isolated, *Geotrichum candidum* had the highest frequency of occurrence value of 35 % with respect to localization, this was followed by *Fusarium* sp (30%), *Pennicillium* species were the least common genus with 15 % occurrence (Table 3). This current work collaborate work of Ngobisa et al. [5]. The fungi of the genus *Geotrichum* specie probably play a role in the process of fermentation and post-harvest deterioration of tuberized roots of cassava [20,21].

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* specie
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* specie

Table 2. Morphological views of fungi isolates

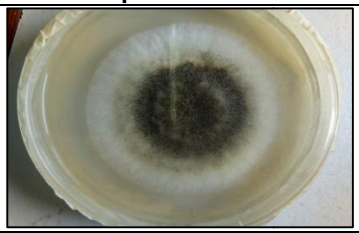
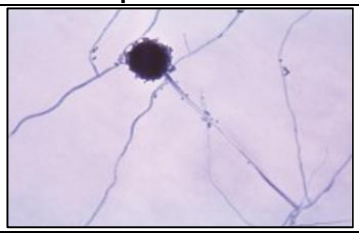
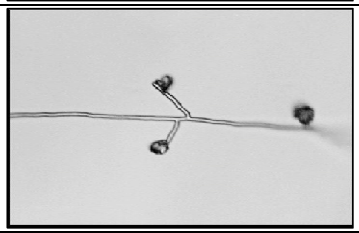
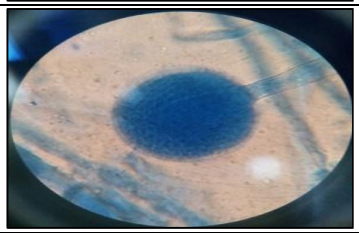
Sample	Macroscopic characteristic	Microscopic characteristic	Probable isolate
A			*Aspergillus flavus*
B			*Fusarium* specie
C			*Geotrichum candidum*
D			*Penicillium* specie

Table 3. Percentage distribution of fungi isolates

Fungi isolated	Frequency occurrence (%)
Geotrichum candidum	35
Fusarium sp	30
Aspergillus flavus	20
Penicillium sp	15

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

The fungal isolates obtained in this 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 [22]. In most studies, *Geotrichum* specie*, Penecillium* specie*, A. flavus* and *Fusarium* sp were found to gain entrance into cassava tubers through natural opening and wounds created during harvesting, transporting, handling and marketing [12].

The presence of various concentrations of leaf extracts of Lemongrass introduced into Potato Dextrose Ager showed reduction in radial growth of the fungi pathogen study. The results in Table 4 to 7 showed that the plant extracts 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 Lemongrass 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 is similar with the study of Amadioha [23] and Tijani et al. [24] who demonstrated the bioactivity of *Azadirachta indica* and *Moringa oleifera* seed against *Erwinia* and *Rhizopus stolonifer* associated with tuber rot.

Twenty milliliter (20 mL) extract of Lemongrass reduced the radical growth of *Fusarium* and *Penicillum* sp by 19.07 mm and 20.57 mm respectively (Tables 4 and 5). Taiga [25] revealed antifungal action of *Nicotinia tabacum* against radial growth of *Fusarium* sp and *Penicillum* sp isolated from yam tuber.

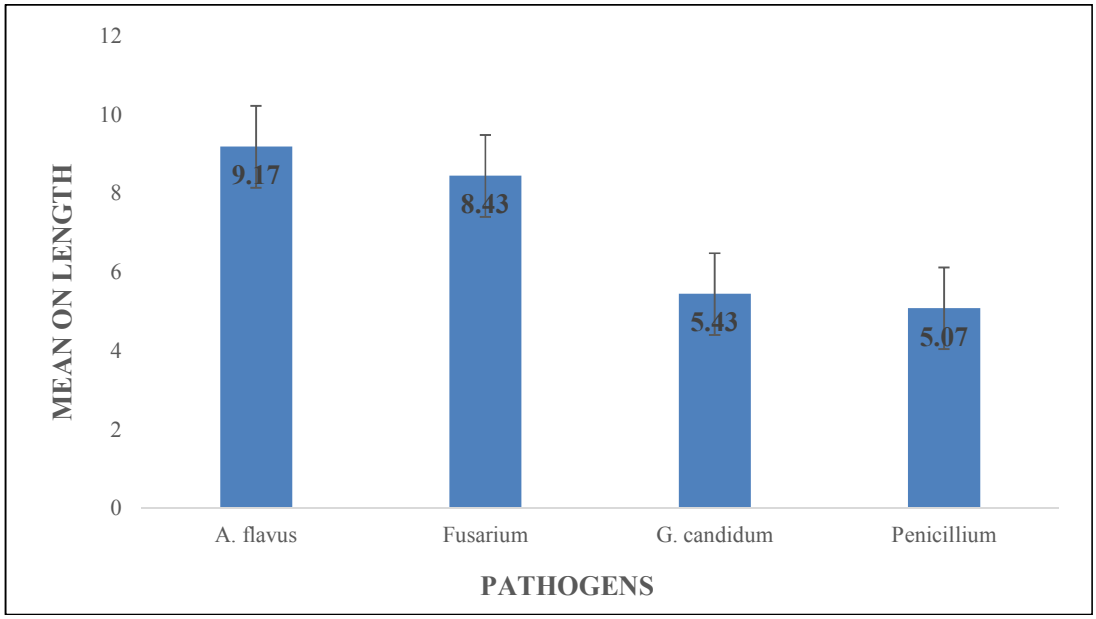


Fig. 1. Bar chart on pathogenicity test of organisms on length (mm)

Table 4. Effect of different concentration of plants extract on the radial growth of fungi isolated by *Fusarium* sp

Plants extract on the radial growth of fungi isolated *Fusarium* sp					
Treatment (mL)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
10	13.33±1.53[a]	14.00±1.00[b]	19.00±1.00[b]	22.20±1.70[b]	24.33±1.37[b]
15	10.33±1.15[b]	11.47±0.50[c]	15.67±1.22[c]	19.37±1.48[c]	23.47±1.26[b]
20	8.17±1.04[b]	9.00±1.00[d]	9.33±1.54[d]	14.00±1.00[d]	19.07±1.10[c]
Control	15.00±1.00[a]	19.00±1.01[a]	22.60±0.44[a]	25.33±1.19[a]	28.00±1.00[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* sp

Plants extract on the radial growth of fungi isolated *Penicillium* sp					
Treatment (mL)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
10	13.93±0.90[ab]	15.33±0.58[ab]	20.13±1.63[ab]	24.00±2.00[b]	27.33±1.24[b]
15	11.47±0.81[b]	13.90±0.86[bc]	17.17±1.50[b]	23.23±1.31[b]	24.87±1.55[b]
20	12.07±1.90[b]	12.50±1.32[c]	11.60±2.17[c]	16.67±1.11[c]	20.57±1.84[c]
Control	16.33±1.52[a]	17.53±1.72[a]	21.67±2.08[a]	26.86±0.76.[a]	31.00±1.00[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±1.51[b]	13.33±1.06[a]	22.00±2.00[ab]	25.00±1.00[b]	31.00±1.00[b]
15	6.00±2.00[b]	9.53±0.50[b]	18.73±1.42[b]	20.80±0.72[c]	25.67±3.12[c]
20	3.97±0.35[b]	7.07±0.85[b]	11.47±1.29[c]	13.33±1.53[d]	18.00±2.10[d]
Control	10.33±1.66[a]	15.00±2.00[a]	25.33±3.21[a]	28.33±1.15[a]	36.00±1.67[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±1.43[a]	12.00±1.06[ab]	14.67±2.52[b]	21.33±2.42[b]	28.00±2.00[b]
15	9.33±1.52[a]	10.33±2.04[bc]	13.70±2.56[bc]	17.33±1.53[c]	25.00±2.00[b]
20	5.00±1.00[b]	8.33±1.18[c]	10.00±1.00[c]	13.33±0.58[d]	18.17±1.26[c]
Control	11.67±2.08[a]	15.00±1.77[a]	19.67±3.31[a]	36.67±2.51[a]	40.33±1.93[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)

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 [17], maize [16], but have been sparsely used in the control of cassava diseases [26].

Works from other researchers indicate most species of *Aspergillus* are saprophytic fungi and only few species including *A. flavus*, *A. parasiticus* and *A. niger* are said to be weak plant

pathogens. These fungi penetrate plant hosts through wounds caused mechanically or by insects [27]. *Aspergillus* sp 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 [28]. Many species of *Fusarium* are associated with cassava roots rot in Nigeria and Cameroon [29]. Of all diseases caused by *Fusarium* on cassava, the economic important one is the vascular wilt disease induced by *Fusarium oxysporum. Penicillium* has been implicated in postharvest loses 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 [30].

4. CONCLUSIONS

This study revealed that most fungi that are associated with cassava rot in Faringada market include, *Fusarium* sp, *Penicillium* sp, *Aspergillus flavus*, and *Geotrichum candidum*. The study also found out that, the highest concentration (20 mL) of Lemongrass extract gave the best radial growth inhibition value of 18.05 mm at day 5. The finding showed that fungal isolates studied in this work are capable of causing deterioration. The result of the pathogenicity test showed varying length of deterioration, with *A. flavus* producing the highest deterioration of 9.17 mm. The use of Lemongrass extract could serve as antifungal agent in mitigating fungal growth on stored cassava tubers.

5. RECOMMENDATION

Further studies on Lemongrass extracts should be done *in vivo* to ascertain their efficacy against *A. flavus, Fusarium* sp*, Penicillium* sp and *Geotrichum candidum* rots during cassava tubers storage. Also high concentrations of Lemongrass extract should be further exploited.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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