

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 effect of Lemon grass 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 were collected separately from Farin-Gada market Jos, fungi species were isolated from rotten cassava 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 lemon grass 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 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 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 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].

38 The main constraints in cassava production are diseases and sometimes pest. The extent of losses
39 may be as high as 80%, the spoilage of cassava tuber arises from combination of physiological and
40 pathological factors [5]. Biochemical analysis of infection process showed that the microbial pathogen
41 must produce a set of enzymes capable of attacking the carbohydrate polymer and protein composition of
42 the infected plant's cell wall [6].

43 Fungi also play an important role in producing amylase which is capable of degradation of starch
44 tissue of the plant [7]. Different studies have shown fungi from rotted cassava tubers and roots after re-
45 inoculation in storage included *Fusarium solani*, *Rhizopus stolonifera*, *Phytophthora drechslera*,
46 *Aspergillus niger* and *Botryodiplodia theobromae* [8]. These fungi cause discoloration in the
47 surrounding tissue of infected cassava tubers, resulting in change in appearance, deterioration of texture
48 and flavor or taste of cassava product. Rot fungi result in post-harvest losses and reduction in market
49 value of tubers [9]. Knowledge of geographical distribution of root rot pathogens may be useful to
50 breeders targeting root rot resistance.

51 **2.0 MATERIAL AND METHODS**

52 **2.1 Study Area**

53 The study was carried out in biology laboratory at Federal College of Forestry, Jos North Plateau
54 State from March to May, 2019. Plateau state is located between latitude 8.5⁰-10.46⁰ North and
55 longitudes 8.20⁰-10.36⁰ East in the north central zone of Nigeria [10]

56 **2.2 Collection of Cassava tuber**

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

62 **2.3 Isolation of Fungal organisms**

63 Small portion of diseased cassava tubers were picked under aseptic conditions using sterile
64 scissors and sterilized by dipping inside 70 % ethanol for 5 minutes. The picked diseased ~~portion~~
65 ~~were~~portions were then placed in a Petri dishes containing autoclaved solidified potato dextrose agar
66 (PDA). The solidified plates were incubated in a locker at a room temperature (28-32⁰C) for 3-5 days.
67 Fungal colonies from the incubated plates were purified by sub culturing into fresh medium until pure
68 ~~culture were~~cultures were obtained [11]. Percentage frequency occurrence of the organisms from the
69 samples site was calculated using the follows formula;

$$70 (\%) = \frac{\text{Individual fungi isolate}}{\text{Total number of fungi isolated}} \times 100$$

71 2.4 Fungi Identification

72 The method of John *et al.* [12] was used. ~~A small portion of freshly grown colony were~~Small
73 ~~portions of freshly grown colony were~~ picked from the plate using a sterile inoculating needle into a glass
74 slide. One to two drop of lacto phenol cotton blue was dropped. The slide was covered with the cover slip
75 and sealed using petroleum jelly. The slide was then viewed under a compound microscope using ×10 and
76 ×400 magnification. The fungi cell morphology identified under the microscope were compared with the
77 observed feature of conidia and conidiophores as adopted by Barnett and Hunter [13].

Comment [S1]: Better identification method is DNA

78 2.5 Preparation of Lemon Grass Extract

79 The fresh leaf of Lemmon grass was used. The Collected leaf were first washed with tap water to
80 remove the trace of sand and dirty and rinsed severally with sterile distilled water and air dried on the
81 laboratory bench. The dried plant leaf were pulverizing using a wooden pestle and mortar, the pulverized
82 powder was then soaked in 250ml absolute ethanol for 48 h and the solution filtered in to a beaker. The
83 ethanol was allowed to evaporate and solution dried to powder by heating at low temperature in an oven.
84 The powder was dissolved in sterile distilled water to give 25% concentrate of the leaf extract and kept in
85 a fridge wrapped properly with aluminum foil paper to prevent contamination. From the stock solution
86 (25%), subsequent concentrations (10, 15 and 20 %) were prepared by serial dilution.

87 2.6 Pathogenicity test

88 Apparently healthy cassava tuber were washed with sterile distilled water and followed by
89 | surface sterilization using 70 % alcohol. A 4H hole (5mm diameter) was made on the tubers with a sterile
90 | cork borer. Fresh Mycelia cell were picked from cultures plates and used for the inoculation of cut part.
91 | The cut portions were sealed with petroleum jelly to prevent contamination by other microorganisms [12].
92 | The inoculated tubers and the control (inoculated) were placed separately in sterile polythene bags
93 | containing cotton wool soaked in sterile distilled water to provide humid environment [14]. The bags
94 | were properly labelled and incubated at a room temperature. Disease symptoms induced by artificial
95 | inoculation after the incubation period were recorded after 10 days and the experiment was repeated trice.

96 **2.7 Determination of Inhibitory Effect of Lemon grass Extract**

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

103 **2.8 Experimental design and Statistical Analysis**

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

107 **3.0 RESULTS AND DISCUSSION**

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

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

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

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

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

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

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

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

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

152 *Fusarium* species are among group of fungi associated with cassava root rot. Crop losses due to
153 root rot ranges from 0.5 to 1 ton/ha but losses greater than 3 ton/ha, an equivalent of 15 to 20% produce,
154 often occur [23]. Many species of *Fusarium* were associated with rotted cassava roots in Nigeria and
155 Cameroon [24]. Of all diseases caused by *Fusarium* on cassava, the economic important one is the
156 vascular wilt disease induced by *Fusarium oxysporum*. Although *Penicillium* has been implicated in
157 postharvest fungus but most pathogenic infections occur before harvest during fruit germination. The
158 genus *Penicillium* includes about 150 species but only a minor fraction of these cause economic
159 infections [25].

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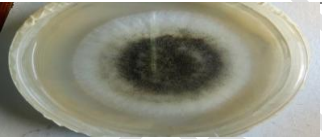
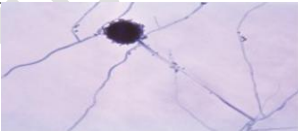


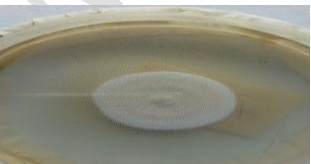
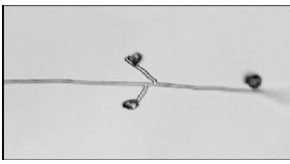
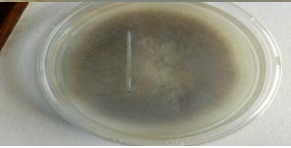
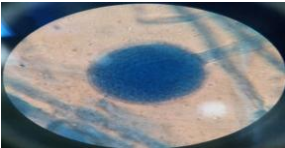
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168 Table 1: Macroscopic and microscopic characteristic of fungi Isolates from cassava

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

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170 Table 2. Morphological views of fungi isolates

Sample	Macroscopic characteristic	Microscopic characteristic	Probable isolate
A			<i>Aspergillus flavus</i>
B			<i>Fusarium spp</i>
C			<i>Geotrichum candidum</i>
D			<i>Penicillium spp</i>

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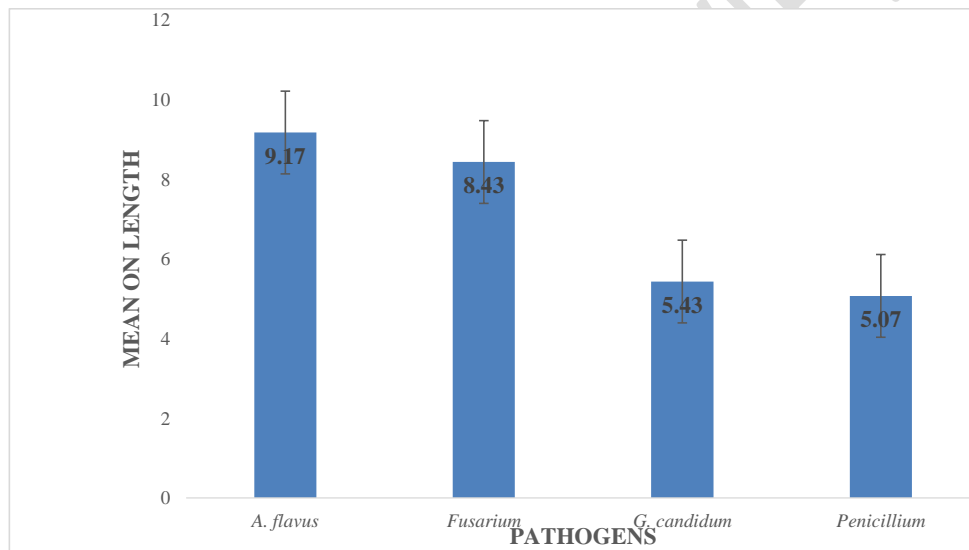
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173 **Table 3: Showing the percentage distribution of fungi isolate**

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

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

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

Comment [S2]: SD is missing in all tables

Plants Extract on the Radial Growth of Fungi Isolated <i>Fusarium</i> 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

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

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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 <i>Penicillium</i> 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

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

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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 <i>Aspergillus flavus</i>					
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

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

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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 <i>Geotrichum candidum</i>					
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

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

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199 4.0 CONCLUSION

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

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

207 **5.0 RECOMMENDATION**

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

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UNDER PEER REVIEW

