

1           **EFFICACY OF EXTRACTS OF SOME PLANTS AGAINST POST**  
2           **HARVEST FUNGAL DETERIORATION OF CASSAVA ROOT (*Manihot***  
3           ***esculenta* Crantz) IN NIGERIA**

4  
5           **ABSTRACT**

6           Fungitoxic potentials of *Piper guineense*, *Ocimum gratissimum*, *Casia alata*, and *Tagetes erecta*  
7           extracts in the management of postharvest fungal deterioration of cassava root were investigated.  
8           Pathogenicity tests revealed *Aspergillus niger*, and *Trichoderma viride* as causal organisms of  
9           root rot of cassava which utilized the substrate for their growth and development. *A. niger* was  
10          found to be more virulent having the highest rot incidence of 75% followed by *T. viride* which  
11          depicted a lower pathogenic effect with rot incidence of 33.3%. The result of the inhibitory  
12          potentials of the water and ethanolic extracts of *P. guineense*, *O. gratissimum*, *C. alata*, and *T. erecta*  
13          against the two fungal pathogens showed significant differences ( $p \leq 0.5$ ) in their rates of  
14          fungitoxicity on *A. niger*, and *T. viride*. The ethanolic extract of *T. erecta* gave the highest mean  
15          inhibitory effect of 63.8% on *A. niger* while the least mean growth inhibition of 9.20% was  
16          recorded by water extract of *P. guineense* on *T. viride*. The results of *in vivo* test of the plant  
17          extracts applied before and after inoculation with spore suspension of test fungi indicated high  
18          significant effect on the rot incidence and severity. *Piper guineense* water extract was less  
19          effective in controlling the development and spread of the pathogens during pathogenesis hence  
20          the highest percentage disease incidence and severity when it was applied after the inoculation  
21          with spore suspension of *A. niger*. The lowest incidence of rot was recorded with water and  
22          ethanol extracts of *T. erecta* applied before inoculation of *T. viride*. *A. niger* showed a stronger  
23          resistance to the plant extracts than *T. viride* in the control of cassava root rot in storage.

24          *Key words: cassava root rot, Piper guineense, Ocimum gratissimum, Casia alata, and Tagetes*  
25          *erecta.*

26  
27          **INTRODUCTION**

28          Cassava (*Manihot esculenta* Crantz), is a basic staple food and main source of ready and cheap  
29          carbohydrate for food, feed for livestock and raw material for industries in Africa and many  
30          countries of the world (Markson *et al.*, 2012; Amadioha, 2012; Bua and Okello, 2011). It is a  
31          reliable and convenient source of food for millions of rural and urban dwellers in developing  
32          countries in its processed forms; gari, lafun, bread, flakes, flour, tapioca, fufu, etc (IITA, 2010;  
33          Denton *et al.*, 2004; Amadioha and Markson, 2007a). Cassavas roots are used for the production

34 of bioethanol and starch for industrial products hence an important engine for economic growth  
35 and development in many cassava producing countries of the world ((Plucknett *et al.*, 2003;  
36 Dixon, 2016; Sani, 2016). Despite the importance of cassava in the world, its production  
37 potentials is still undermined by the activities of various disease agents which constitute serious  
38 production challenge that greatly reduce yield in many developing countries of the world  
39 (Chalwe *et al.*, 1999; Onyeka, 2002; Bua and Okello, 2011; Onyeka *et al.*, 2005). Cassava root  
40 unlike yam does not store well when harvested as it rapidly deteriorates due to invasion by  
41 microbial agents that render the roots unfit for human consumption (IITA, 1996). Cassava root  
42 rot diseases which occur as dry, soft or wet rots have caused enormous postharvest losses due to  
43 fungal deteriorations which either infect the produce on-farm or develop during storage and they  
44 include: *Botrydiplodia theobromae*, *Fusarium solani*, *F. oxysporium*, *Aspergillus niger*, *Rhizopus*  
45 *stolonifer*, *Diplodia manihotis*, *Cylindrium clandestrium*, *Macrophomina phaseolina*, *Penicilium*  
46 *oxalicum* (Okigbo, 2002, 2003; Shukla *et al.*, 2012; Arya, 2010).

47 Different control measures have been suggested and used for the control of post-harvest cassava  
48 root rot diseases especially, curing, use of resistant variety, and use of chemicals. However,  
49 farmers in developing countries cannot afford the cost of curing equipment and they lack the  
50 expertise to maintain the required temperature and relative humidity. Also, the use of synthetic  
51 fungicides, apart from their potential danger to both farmers and environment are unaffordable  
52 by resource poor farmers (Obagwu *et al.*, 1997; Amienyo and Ataga, 2007). Therefore, selection  
53 of some plant extracts for the management of the disease will be a preferred option since they are  
54 readily available, with little or no toxicity to humans, biodegradable, with less complex  
55 preparation and application procedures (Shenge, 2002; Amadioha, 2012; Awurum and  
56 Enyiukwu, 2013). Evaluation of extracts of *P. guineense*, *O. graticimum*, *C. alata*, and *T. erecta* in the  
57 control of storage rot of cassava root caused by *A. niger* and *T. viride* is presented in this paper.

## 58 MATERIALS AND METHODS

59

### 60 Source of Plant Material

61 The cassava roots (TME 419 Variety) were obtained from the National Root Crops Research  
62 Institute, Umudike, Abia State, Nigeria. The leaves of *Ocimum gratissimum* and *Piper guineense*  
63 were obtained from open market stalls in Umuahia, Abia State while *Cassia alata*, and *Tagetes*  
64 *erecta* were collected from the University community, Umudike, Umuahia, Abia State, Nigeria

65

### 66 Preparation of Culture Medium

67 The culture medium used was Potato Dextrose Agar (PDA) prepared by dissolving 39g of the  
68 PDA into one liter of distilled water in a conical flask, thoroughly mixed and heated in an  
69 electric water bath until the agar melted and then sterilized by autoclaving at 212°C for 15  
70 minutes. The sterile medium was allowed to cool (46°C) and 15ml portions dispensed into sterile  
71 Petri-dishes and allowed to solidify (Amadioha and Markson, 2007a).

72

### 73 Isolation and identification of Fungal Pathogen:

74 The rotted cassava roots were washed with tap water, surface sterilized with 70% ethanol  
75 solution and rinsed in sterile distilled water. Pieces of rotted tissues (3 mm diameter) were taken  
76 from the boundary of the infected and healthy portions of the root and placed on the culture  
77 medium. The inoculated plates were transferred into microhumidity chamber and incubated at  
78 28°C. The plates were examined daily for any mycelial or colony growth and the emerging  
79 colonies were subcultured to obtain pure cultures of the isolates. Pathogenicity test was carried  
80 out on the isolates (Amadioha, 2001). Re-isolation was made to obtain pure cultures of the  
81 inoculated isolates that established rot condition and their pure cultures compared with the

82 original cultures of the isolates. The isolates that caused the root rot of cassava were regarded as  
83 pathogens and were characterized and identified as pathogenic organisms (Barnette and Hunter,  
84 1987; Bua and Okello, 2011)).

85

### 86 **Preparation of Leaf Extracts**

87 Fresh leaves of *O. gratissimum*, *P. guineense*, *C. alata* and *T. erecta* were washed under running  
88 tap water and rinsed with sterile distilled water, air dried at room temperature (27°C) and then  
89 dried in an oven set at 60°C for 24 hours. The dried leaves were ground into powder and  
90 weighed out separately (10g, 20g, 30g, 40g and 50g) into a beaker before adding 100ml of the  
91 extracting solvent (ethanol or sterile distilled water). Each solution was thoroughly mixed and  
92 left to stand for 24 hours and then filtered separately using a four –fold cheese cloth into a  
93 beaker. The filtrates constituted 10%, 20%, 30%, 40% and 50% concentrations of cold water or  
94 ethanol leaf extracts of the test plant materials (Amadu and Akpa, 2014). The purity of the  
95 extracts was confirmed (Cheesbrough, 2000).

96

### 97 **Effect of Extracts on the radial growth of fungal pathogens *in vitro***

98 The antifungal effects of test plant extracts against the fungal growth was evaluated *in vitro*  
99 (Amadioha and Obi (1998). 2 ml each of the extract concentrations (10%, 20%, 30%, 40% and  
100 50%) was separately transferred into a sterile Petri dish and freshly prepared molten PDA (15ml)  
101 was aseptically poured into each of the plates. The content of the plates were mixed to obtain the  
102 PDA-extract media which were allowed to solidify. A 5mm diameter disc of each pathogen was  
103 dropped separately at the centre of the solidified extract-PDA medium in culture plates. The  
104 treatments were replicated three times. The control plates were made up of PDA (15 ml) + 2ml  
105 of water or ethanol (no plant extracts), inoculated with the test fungi. The inoculated Petri dishes

106 were incubated at 27<sup>0</sup>C and observed daily for fungal growth. The fungal radial growth in each  
107 plate was measured with a ruler along the two directions of the perpendicular lines drawn on the  
108 reverse side of the plates after the growth in the control experiment had reached the edge of the  
109 plate. The mean colony diameter of the three replicates was taken as the mean growth of each  
110 treatment. Fungitoxicity was calculated as percentage colony inhibited by the extracts using the  
111 formular by Amadioha (2004) as shown below:

$$112 \quad \% \text{ Fungal Growth inhibition} = \frac{DC - DT}{DC} \times \frac{100}{1}$$

113

114 Where DC = Average diameter of colony in control experiment.

115 DT = Average diameter of fungal colony with extract treatment.

116

### 117 ***In vivo* Screening of Plant Extracts against fungal pathogens**

118 The 50% extract concentration of the water and ethanol plant extracts that gave the highest  
119 inhibitory effects *in vitro* was used in this experiment. Two sets of ten surface sterilized healthy  
120 cassava roots were each treated as a group with spore suspension (1 x 10<sup>5</sup> spores/ml of distilled  
121 water) of the test fungal pathogens (Amadioha and Markson, 2007b) as follows:

122 **Group A** – a set of ten surface sterilized uninfected (healthy) cassava roots each dipped into the  
123 extract concentration of test plants and allowed to dry for 2 hrs before spray-inoculating with the  
124 spore suspension of the test fungal pathogens.

125 **Group B** - a set of surface sterilized ten uninfected cassava roots each spray-inoculated with the  
126 spore suspension, air dried for 2 hours and then dipped into the plant extracts.

127 The control experiments were treated as A and B above but dipped in the respective extracting  
128 solvents. Each of the treated cassava roots including the control was enclosed separately in  
129 polyethylene bags with cotton wool soaked with distilled water (micro humidity chamber) and

130 incubated at  $27\pm 2^{\circ}\text{C}$ . The experiment was replicated two times. The samples were observed  
 131 daily for rot development for 14 days. The disease incidence and severity were assessed.

$$132 \quad \text{Disease incidence (\%)} = \frac{\text{No. of rotted cassava roots}}{\text{Total No. of cassava roots}} \times \frac{100}{1}$$

135

136 Disease Severity was assessed (Murugan and Luaina, 2013) on a 0-5 scale as follows:

- 137 0. - No infection  
 138 1. - Slight infection ( $\leq 10 - 20\%$  of root infected)  
 139 2. - Moderate infection (21 - 40% of root infected)  
 140 3. - High infection (41 - 60% of root infected)  
 141 4. - Extensive infection (61 - 80% of root infected)  
 142 5. - Complete rot (81 - 100% of root infected)

$$1 \quad \text{Disease severity index} = \frac{\text{Sum of all scores}}{\text{Number of plants scored (N) x Highest score (5)}} \times \frac{100}{1}$$

145 Where; N is the total number of cassava root assessed; 5 - the maximum score of the scale used

## 146 **Statistical Analysis**

147 The values are means of three replicates in two separate experiments in complete randomized  
 148 design (CRD). The data obtained were analyzed using Analysis of Variance (ANOVA) and Least  
 149 Significant Difference (LSD) at 5% level of probability was used to separate the means..

150

## 151 **RESULT**

### 152 **Effect of plant extracts on the radial growth of the pathogens in culture**

153 The pathogenicity test revealed *Aspergillus niger* and *Trichoderma viride* as pathogens having  
 154 induced rot on inoculated healthy (uninfected) cassava root. *A. niger* had the highest rot

155 incidence of 75% and was considered the most virulent followed by *T. viride* that depicted a  
 156 lower pathogenic effect with rot incidence of 33.3% (Table 1).

157 The *in vitro* screening of the plant extracts against the radial growth of *A. niger* and *T. viride*  
 158 showed that the extract of the plant materials had significant ( $P \leq 0.05$ ) inhibitory effects on the  
 159 organisms tested. The inhibitory effect of the test plants increased with higher concentration and  
 160 also differed with extracting solvents across the test organisms. *T. erecta* ethanol extract  
 161 recorded the highest mean inhibitory effect of 75.2% on *A. niger* while the least was *P.*  
 162 *guineense* aqueous extract with mean inhibition of 27.5% on *T. viride* which is significantly  
 163 different ( $P \leq 0.05$ ) when compared with the control experiment. The 50% extract concentrations  
 164 recorded the highest inhibitory effect on all the pathogenic organisms across all the plant extracts  
 165 whereas the least mean values were recorded with 10% concentration of test plant materials. The  
 166 ethanol extracts gave more inhibitory effects than water extracts and this was significant at  $P \leq$   
 167 0.05. *T. viride* showed a stronger resistance across all the extracts of the test plant materials  
 168 (Table 2).

169 **Table 1: Percentage mean rot of fungal pathogens**

Fungi	Percentage Rot (%)
<i>Aspergillus niger</i>	75.0
<i>Trichoderma viride</i>	33.3

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177 **Table 2: Percentage growth inhibition of aqueous and ethanol plant extracts on *A. niger***  
 178 **and *T. viride* in vitro.**

Treatment/ Plant Extract Concentration	Fungal Radial Growth Inhibition (%)			
	<i>A. niger</i>		<i>T. viride</i>	
	WE	EE	WE	EE
<i>Piper guineense</i>				
10	10.5	26.8	4.67	19.8
20	28.4	36.2	20.2	29.7
30	41.8	41.8	33.3	36.7
40	52.2	56.3	38.2	41.8
50	60.5	65.3	46.2	46.8
Control	0.00	0.00	0.00	0.00
Mean	<b>37.3</b>	<b>43.9</b>	<b>27.5</b>	<b>33.9</b>
<i>Occimum gratissimum</i>				
10	19.8	30.7	4.83	18.7
20	38.3	44.3	20.8	36.3
30	50.2	62.3	31.8	47.2
40	62.5	70.5	50.8	52.7
50	67.5	75.5	54.4	63.0
Control	0.00	0.00	0.00	0.00
Mean	<b>46.2</b>	<b>55.3</b>	<b>31.5</b>	<b>42.5</b>
<i>Cassia alata</i>				
10	30.5	39.3	18.3	30.5
20	42.5	54.8	24.0	41.7
30	67.5	77.0	55.3	56.0
40	76.0	80.2	61.5	60.2
50	87.5	85.6	77.8	64.7
Control	0.00	0.00	0.00	0.00
Mean	<b>59.4</b>	<b>66.0</b>	<b>46.3</b>	<b>49.5</b>
<i>Tagetes erecta</i>				
10	37.0	50.2	18.7	40.3
20	51.2	73.3	40.7	54.5
30	77.2	81.0	57.5	62.0
40	81.5	87.2	64.5	68.0
50	85.7	91.2	71.2	71.3
Control	0.00	0.00	0.00	0.00
Mean	<b>65.1</b>	<b>75.2</b>	<b>49.4</b>	<b>58.2</b>
LSD (5%) Conc.	<b>3.02</b>		<b>4.37</b>	
LSD (5%) Extract	<b>1.57</b>		<b>2.28</b>	



179 **Values are means of three replicates in two separate experiments.**

180 **WE = water extract, EE = ethanol extract**

181

182 **Effect of plant extracts applied before and after on the incidence and severity of cassava**  
183 **root rot caused by the pathogens**

184 The incidence and severity of cassava root rot incited by the pathogenic organisms was  
185 significantly reduced when treated with extracts of the test plant materials either before or after  
186 inoculation when compared with the control experiment (Tables 3 and 4). Ethanol extracts were  
187 better than aqueous extracts in checking the development and spread of the pathogens during  
188 pathogenesis. However, there were no significant differences in severity index of cassava roots  
189 treated with extracts of the same extracting solvent. *P. guineense* water extract was less effective  
190 in controlling the incidence and severity of cassava root rot especially when it was applied after  
191 the inoculation with spore suspension of *A. niger*. The lowest incidence (14.5%) and severity  
192 (1.17) of cassava root rot were recorded with ethanol extracts of *T. erecta* applied before  
193 inoculation of *T. viride* and *A. niger* respectively. This was followed by ethanol extracts of *C.*  
194 *alata*, *O. gratimum*, and *P. guineense* applied before spray-inoculating with the pathogenic  
195 organisms. Generally, the extracts of *T. erecta* had a stronger inhibitory effect on the pathogens  
196 during pathogenesis whereas *A. niger* showed a stronger resistance to the extracts of the plant  
197 materials.

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202 **Table 3: Effect of aqueous and ethanol plant extracts applied before and after inoculation**  
 203 **on the disease incidence by *A. niger* and *T. viride***

Treatment Plant Extracts	Pathogens and Disease Incidence (%)			
	<i>A. niger</i>		<i>T. viride</i>	
	A	B	A	B
<i>Piper guineense</i>				
Water Extract	45.5	33.7	40.5	33.5
Ethanol Extract	35.5	25.2	30.2	30.7
<i>Ocimum gratissimum</i>				
Water Extract	40.5	26.8	44.2	33.7
Ethanol Extract	33.4	23.1	27.6	26.3
<i>Cassia alata</i>				
Water Extract	37.7	27.7	35.5	25.7
Ethanol Extract	28.6	17.4	25.4	18.5
<i>Tagetes erecta</i>				
Water Extract	35.6	21.8	26.0	14.5
Ethanol Extract	25.3	15.2	26.0	14.5
<b>Control</b>	50.50		66.23	
<b>LSD (5%)</b>	<b>1.86</b>		<b>3.46</b>	

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205 **A – Inoculation after plant extract application.**206 **B – Inoculation before application of plant extract**

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214 **Table 4: Effect of aqueous and ethanol plant extracts applied before and after inoculation**  
 215 **on the disease severity of cassava tuber incited by *A. niger* and *T. viride***

Treatment Plant Extracts	Pathogens and Disease Severity Index			
	<i>A. niger</i>		<i>T. viride</i>	
	A	B	A	B
<i>Piper guineense</i>				
Water Extract	4.10	3.50	3.73	3.30
Ethanol Extract	4.17	2.23	3.30	1.27
<i>Ocimum gratissimum</i>				
Water Extract	4.17	2.83	4.13	3.20
Ethanol Extract	4.10	2.03	3.27	1.23
<i>Cassia alata</i>				
Water Extract	4.27	3.03	3.27	2.83
Ethanol Extract	3.80	1.43	3.03	2.03
<i>Tagetes erecta</i>				
Water Extract	4.60	2.17	3.17	2.57
Ethanol Extract	3.23	1.17	3.10	2.10
Control	4.33		4.27	
LSD (5%)	<b>0.39</b>		<b>0.29</b>	

216

217 A – Inoculation after plant extract application/

218 B – Inoculation before application of plant extract

219

220 **DISCUSSION**

221 *Aspergillus niger* was found in this study to be more virulent causing the highest percentage root  
 222 rot of cassava than *Trichoderma viride* which has not been previously linked with the storage  
 223 root rot of cassava in Nigeria. *A. niger* has been reported as the leading cause of postharvest  
 224 fungal root rot of cassava especially in South–East Nigeria (Okigbo *et al.*, 2014; 2009a, b) which  
 225 is at variance with the findings of Amadioha and Markson (2007a, b) where *Botryodiplodia*

226 *acerina* and *Rhizopus stolonifer* were recorded as the most important pathogens inciting storage  
227 root rot of cassava in Nigeria. The differences may be due to varietal and age of the test plants,  
228 processing methods employed or edaphic and climatic differences.

229  
230 The plant extracts showed significant differences ( $p \leq 0.5$ ) in their rate of fungitoxicity on *A. niger*  
231 and *T. viride*. The ethanolic extract of *T. erecta* gave the highest inhibitory effect on *A. niger*  
232 while the least growth inhibition was recorded by water extract of *P. guineense* on *T. viride*  
233 indicating that the rot causing organisms showed differences in their rates of resistance to the  
234 plant extracts with *T. viride* being less susceptible (Umana *et al.*, 2016). Ethanol extracts of the  
235 plant materials recorded more inhibitory effects on the pathogens than water extracts *in vivo*  
236 which is line with reports of Suleiman (2010) and Nwinyi *et al.*, (2009). This observation  
237 suggests that ethanol as extracting medium dissolved more active compounds present in the plant  
238 materials than water which probably dissolved less active principles or compounds (Anukworji  
239 *et al.*, 2012; Amadioha, 2000; Okigbo *et al.*, 2009a). *T. erecta* and *C. alata* were more fungitoxic  
240 than *O. graticimum*, and *P. guineense* suggesting that they contained more active compounds or  
241 phytochemicals which dissolved more readily in the extracting solvents thereby causing the  
242 highest radial growth inhibition of the pathogens *in vitro*.

243  
244 Significant differences ( $p \leq 0.05$ ) were recorded on the incidence and severity of cassava root rot  
245 incited by the pathogens in the *in vivo* screening of the plant extracts applied before and after  
246 inoculation with spore suspension of test fungi. The application of the extracts before inoculation  
247 recorded a lower disease incidence and severity than application of the extracts after inoculation  
248 even though both showed some high levels of rot reduction when compared with the control  
249 experiment. The least percentage root rot incidence and severity was recorded in cassava roots

250 treated with *T. erecta* ethanol extract applied before inoculation of *A. niger* while the highest  
 251 incidence and severity was recorded with *P. guineense* water extract applied after inoculation of  
 252 *T. viride* suggesting that the plant extracts could better be used as protectants than as eradicants  
 253 in reducing the development and spread of the pathogens in infected cassava roots. It was  
 254 generally observed that *T. erecta* and *C. alata* had more inhibitory effects on the pathogens than  
 255 *P. guineense* and *O. gratificimum* whereas *T. viride* had more resistance to the plant extracts than  
 256 *A. niger*. These observations corroborated the studies of Umana *et. al.*, (2016) on the control of  
 257 postharvest rot of oranges with plant extracts. The test pathogens also caused necrosis of cassava  
 258 root tissues which is likely a function of toxins produced by the pathogens during pathogenesis  
 259 (Isaac, 1992).

## 260 CONCLUSION

261 The growth of the pathogens, *Aspergillus niger*, and *Trichoderma viride* causing post harvest  
 262 root rot of cassava were inhibited by both ethanoic and water extracts of *Piper guineense*, *Ocimum*  
 263 *gratificimum*, *Casia alata*, and *Tagetes erecta* both *in vitro* and *in vivo*. The fungitoxic potentials of  
 264 extracts of these plant materials which are readily available and cost effective could be exploited  
 265 as potent biopesticides in the management of postharvest fungal deterioration of cassava root  
 266 especially in developing countries where synthetic fungicides are not only scarce but expensive  
 267 when available for resource poor farmers.

268

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