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

ABSTRACT

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

Key words: cassava root rot, Piper guineense, Ocimum graticimum, Casia alata, and Tagetes erecta

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Source of Plant Material

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

Preparation of Culture Medium

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

Isolation and identification of Fungal Pathogen:

The rotted cassava roots were washed with tap water, surface sterilized with 70% ethanol solution and rinsed in sterile distilled water. Pieces of rotted tissues (3 mm diameter) were taken from the boundary of the infected and healthy portions of the root and placed on the culture medium. The inoculated plates were transferred into microhumidity chamber and incubated at 28^{0}C. The plates were examined daily for any mycelial or colony growth and the emerging colonies were subcultured to obtain pure cultures of the isolates. Pathogenicity test was carried out on the isolates (Amadioha, 2001). Re-isolation was made to obtain pure cultures of the inoculated isolates that established rot condition and their pure cultures compared with the

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

Preparation of Leaf Extracts

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

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

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

were incubated at 27^0C and observed daily for fungal growth. The fungal radial growth in each plate was measured with a ruler along the two directions of the perpendicular lines drawn on the reverse side of the plates after the growth in the control experiment had reached the edge of the plate. The mean colony diameter of the three replicates was taken as the mean growth of each treatment. Fungitoxicity was calculated as percentage colony inhibited by the extracts (Amadioha, 2004).

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

Where DC = Average diameter of colony in control experiment.

DT = Average diameter of fungal colony with extract treatment.

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

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

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

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

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

incubated at 27±2^{0}C. The experiment was replicated two times. The samples were observed daily for rot development for 14 days. The disease incidence and severity were assessed.

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

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

0. - No infection
1. - Slight infection (≤10 - 20% of root infected)
2. - Moderate infection (21 - 40% of root infected)
3. - High infection (41 - 60% of root infected)
4. - Extensive infection (61 - 80% of root infected)
5. - Complete rot (81 - 100% of root infected)

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

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

RESULT

Effect of plant extracts on the radial growth of the pathogens in culture

The pathogenicity test revealed *Aspergillus niger* and *Trichoderma viride* as pathogens having induced rot on inoculated healthy (uninfected) cassava root. *A. niger* had the highest rot incidence of 75% and was considered the most virulent followed by *T. viride* that depicted a lower pathogenic effect with rot incidence of 33.3% (Table 1).

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

Table 1: Percentage mean rot of fungal pathogens

Fungi	**Percentage Rot (%)**
Aspergillus niger	75.0
Trichoderma viride	33.3

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

Treatment/ Plant Extract Concentration	Fungal Radial Growth Inhibition (%)			
	A. niger		*T. viride*	
	WE	**EE**	**WE**	**EE**
Piper guineense				
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	**37.3**	**43.9**	**27.5**	**33.9**
Occimum graticimum				
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	**46.2**	**55.3**	**31.5**	**42.5**
Cassia alata				
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	**59.4**	**66.0**	**46.3**	**49.5**
Tagetes erecta				
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	**65.1**	**75.2**	**49.4**	**58.2**
LSD (5%) Conc.	**3.02**		**4.37**	
LSD (5%) Extract	**1.57**		**2.28**	

Values are means of three replicates in two separate experiments.

WE = water extract, EE = ethanol extract

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

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

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

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

A – Inoculation after plant extract application.

B – Inoculation before application of plant extract

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

Treatment Plant Extracts	Pathogens and Disease Severity Index			
	A. niger		*T. viride*	
	A	**B**	**A**	**B**
Piper guineense				
Water Extract	4.10	3.50	3.73	3.30
Ethanol Extract	4.17	2.23	3.30	1.27
Ocimum graticimum				
Water Extract	4.17	2.83	4.13	3.20
Ethanol Extract	4.10	2.03	3.27	1.23
Cassia alata				
Water Extract	4.27	3.03	3.27	2.83
Ethanol Extract	3.80	1.43	3.03	2.03
Tagetes erecta				
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%)	**0.39**		**0.29**	

A – Inoculation after plant extract application/

B – Inoculation before application of plant extract

DISCUSSION

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

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

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

Significant differences ($p \leq 0.05$) were recorded on the incidence and severity of cassava root rot incited by the pathogens in the *in vivo* screening of the plant extracts applied before and after inoculation with spore suspension of test fungi. The application of the extracts before inoculation recorded a lower disease incidence and severity than application of the extracts after inoculation even though both showed some high levels of rot reduction when compared with the control experiment. The least percentage root rot incidence and severity was recorded in cassava roots treated with *T. erecta* ethanol extract applied before inoculation of *A. niger* while the highest

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

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