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CONTROL OF CASSAVA ROOT ROT WITH LEAF EXTRACTS OF *COSTUS AFER* AND *MIMOSA PUDICA*

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ABSTRACT

Fungitoxic effect of methanol extracts of *Costus afer* (Ker) and *Mimosa pudica* (L.) were investigated *in vitro* on causative agents of cassava root rot using methanol extraction method. The microbial pathogens were *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium solani* and *Rhizopus stolonifer*. The methanol extracts of the plants were prepared by adding separately 100g, 150g and 200g of the leaf powder of *Costus afer* and *Mimosa pudica* into 100ml of methanol. Inhibition zones of the test organisms varied at various concentrations. Analysis of Variance (ANOVA) was employed and Duncan's new multiple range test (DNMRT) was also used to test the difference among treatment. The result of this study revealed that the plant extracts showed antifungal activity against the test organisms at various concentrations. Methanol extract of *Mimosa pudica* showed higher antifungal activity against the test pathogens than the methanol extract of *Costus afer*. The most fungitoxic extract on *Rhizopus stolonifer* was obtained with *Mimosa pudica* at 100g/ml. The antifungal potentials of *C. Afer* and *M. pudica* as observed in this study therefore raises hope in the use of biopesticides to control fungal pathogens.

Keywords: Antifungal activity, Cassava, Root, Leaf, Extracts.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous root crop belonging to the Euphorbiaceae family. It is a major food crop in the tropics, particularly in the developing countries of the Sub-Saharan region of Africa (Hahn *et al*., 1979). Cassava is a root crop grown on an estimated land area of $10.8x10^6$ ha in some African countries (FAO, 1999). In most of these countries, cassava is grown mainly for its starchy roots, which are valuable sources of cheap calories, particularly for the low-income earners (IITA, 1990). The mode of cassava utilization varies from one place to another. Among the common products of cassava are gari, fufu, starch, cassava flour etc. Also, leaves and tender shoots are consumed as vegetable in some countries (Dahniya, 1994). In addition to human consumption, the crop is used for the production of ethanol, animal feed and starch for various industrial uses particularly in Thailand and Vietnam (IITA, 1990; FAO, 2000). Several workers have reported the isolation of many different types of fungi from rotted cassava root in storage. Some of the fungi found to be pathogenic on cassava roots after re-inoculation include *Sclerotium rolfsii* (IITA, 1990), *Fusarium oxysporum* Schlecht, *Botryodiplodia theobromae* Pat, *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link, *Rhizopus* spp; *Fusarium solani* (Mart) Sacc., and *Macrophomina phaseolina* (Tassi) Goidanich (Booth, 1978, Okigbo *et al*., 2009a). Different control measures so far suggested for post-harvest cassava root rot diseases include reduced temperature, pre-harvest practices, storage techniques, natural resistance and use of chemicals (Booth, 1976). However, the use of synthetic fungicides, apart from their potential danger to both farmers and environment are unaffordable by most of them (Obagwu *et al*., 1997; Amienyo and Ataga, 2007). Recent studies on the use of plant extract have opened a new opportunity for the control of plant diseases. Plant extracts have been reported to be safe, non-toxic to man, but effective against plant pathogens (Okigbo *et al*., 2009b). In Nigeria, plant extracts have been used to control fungal diseases of plants such as cowpea (Amadioha and Obi, 1999), banana (Okigbo and Emoghene, 2004), yam (Onifade, 2000; Okigbo and Nmeka, 2005), sweet potato (Amienyo and Ataga, 2007) and maize (Awuah, 1989) but have been sparsely used in the control of cassava diseases. The objective of this study, therefore, was to identify the different root rot pathogens affecting cassava, and use of extracts of *Costus afer* and *Mimosa pudica* to control root rot pathogens of cassava.

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MATERIALS AND METHODS

Sources of Materials

Samples of cassava (Crantz) with root rot diseases were randomly selected from recently harvested cassava from a farm at Umudike, Abia State, Nigeria. The leaves of *Costus afer* (Ker) and *Mimosa pudica* (L.) used in this study as botanicals were collected from the wild near the National Root Crops Research Institute, Umudike, Abia State. The plant identities were verified and authenticated by the Horticultural Unit of the Research Institute.

Isolation and identification of fungi associated with rotted cassava roots

Each root sample was washed in clean running tap water and sections of approximately $2mm^2$ were cut from the tissue, using a sterile scapel, at the interface between healthy and infected portions of the tuber. The pieces of tissue were surface-sterilized with 1% sodium hypochlorite for 2minutes, and rinsed in sterile distilled water. The pieces were tapped dry with a sterile paper towel. Five sections of the cleaned root were plated out on potato dextrose agar (PDA). The inoculated Petri dishes were sealed with parafilm to prevent contamination and then incubated at 28± 2°C for 7 days and observed daily for fungal development. The various fungal isolates from each of the samples were sub-cultured by transferring hyphal tips from the colony edges to fresh PDA plates using a flame sterilized needle to obtain a pure culture, and incubated at 28°C. Cultures were identified using a compound microscope and identification keys described by Sulton (1973) and Nelson *et al*. (1983).

Pathogenicity test of the isolated fungi

Fresh, healthy roots from 8-12 month old cassava plants were washed in running tap water to remove soil and other debris from the root surface. The tubers were surface-sterilized in a 1% solution of sodium hypochlorite by immersing them for 2mins after which they were rinsed with sterile distilled water and left to dry under a laminar flow hood for 30 minutes. Each root was bored at two edges (upper and lower parts) to a depth of 1cm, using a flame-sterilized 8mm diameter cork borer. A disc of 7 days old PDA culture of the test isolates was washed into sterile beakers with sterile distilled water and 1ml of each isolate was inoculated into the hole and sealed with the root piece removed from the hole. The points of inoculation were sealed with parafilm to prevent entry of external contaminants. The same procedure was used for the control except that 1ml of sterile distilled water was inoculated inside of each hole made in the roots. Each inoculated root was placed at air environment for 8 days and examined for rot development.

Preparation of Crude extracts of the plant part

Fresh leaves of *Costus afer* and *Mimosa pudica* were washed thoroughly under running water and soaked in a 1% solution of sodium hypochlorite for 2 minutes, rinsed severally with sterile distilled water and air dried at room temperature for 2 hours. Three different extract concentrations were prepared by weighing 100g, 150g and 200g of each plant part on a balance and were blended in a blender and 100ml of methanol was added to obtain extract concentrations of 100%, 150% and 200%. The extracts were sieved through four layers of sterile cheese cloth. One 1ml of each extract concentration was dispensed per Petri dish and 9ml of molten PDA was added. The plates were gently rotated to ensure even dispersion of the extracts. The agar-extract mixture was allowed to solidify and used for the inhibition of mycelia growth of the tested fungi.

Effect of the leaf extracts on fungal growth

The method of Sangoyomi (2004) was used to determine the effect of the extracts on fungal growth. This was done by inoculating at the centre of the Petri dishes a 4mm diameter mycelia disc obtained from the colony edge of 7 days old culture of each of the four tested fungi. The controls were set up using blank agar plates (no extracts). Three replicates plates of PDA-extract per isolate were incubated at 27°C and radial growth was measured daily for 7 days. Colony diameter was taken as the means along two directions on two perpendicular lines drawn on the reverse of the plates. The percentage of inhibition was calculated according to the method described by Whipps (1987) as:

$$\text{Percentage of inhibition} = \frac{R_1 - R_2}{R_1} \times \frac{100}{1}$$

Where R_1: radial distance of pathogen in control plates, and R_2: radial distance of pathogen in extract incorporated agar plates.

The percentage of inhibition was determined as a guide for selecting the minimum inhibitory concentration that will be effective in controlling rot causing fungi. Extracts were rated for their inhibitory effects using the scale described by Sangoyomi (2004): $\leq$ 0%; not effective, >0-20%; slightly effective, >20-50%; moderately effective, >50-100%; effective and 100% inhibition; highly effective

Statistical analysis

Experimental design used was Completely Randomized Design with three replicates. Data were analyzed using analysis of variance (ANOVA) via Statistical Analysis System (SPSS) of Version 21 and means were separated with Duncan's new Multiple Range Test (DNMRT) at $P<0.05$.

RESULTS

Isolation and identification of fungi causing rot on cassava roots

The fungal pathogens isolated from the sample of rotted cassava roots were *Aspergillus niger*, Van Tieghem, *Fusarium solani* Mart, *Botryodiplodia theobromae* Pat and *Rhizopus stolonifer* Vuill. The frequency of isolation varied with different fungi associated with the rotted root. *Botryodiplodia theobromae* had the highest frequency of occurrence (71%) while the least frequency of occurrence was *Fusarium solani* (25%) (Table1).

Table 1. Percentage occurrence of fungi isolates associated with rotted cassava root

Fungi isolates	Percentage Occurrence
Aspergillus niger	40
Botryodiplodia theobromae	71
Fusarium solani	25
Rhizopus stolonifer	62

The pathogenicity test of the fungal isolates revealed that *Botryodiplodia theobromae* was the most virulent, causing 20% rot on the cassava root while the least virulent was *Rhizopus stolonifer*, causing 2.5% rot on cassava root (Table 2).

Table 2. Pathogenicity test of the fungal isolates from rotted cassava root

Fungi isolates	Percentage rot
Aspergillus niger	**10**
Botryodiplodia theobromae	**20**
Fusarium solani	**7**
Rhizopus stolonifer	**2.5**

Effect of crude extracts of *Costus afer* and *Mimosa pudica* on the mycelia growth of the test fungi

All the plant extracts showed varying degrees of inhibition on the tested fungi. This was dependent on the concentration of the extracts. At 100g/ml concentration, the methanol extract of *Mimosa pudica* had the highest inhibitory effect of 63.65% (effective) on *Rhizopus stolonifer*, this was significantly higher than other interactions, while the least inhibitory effect of 50.70% (moderately effective) was recorded against *Aspergillus niger*. Whereas, the methanol extract of *Costus afer* gave the highest inhibitory effect of 57.66% (effective) against *Fusarium solani* and the least inhibitory effect of 37.87% (moderately effective) on *Botryodiplodia theobromae* (Table 3).

Table 3. Inhibition of test fungi with methanol extracts of *Costus afer* and *Mimosa pudica* at 100g/ml

Plant extracts	A. *Niger*	B. *theobromae*	*F. solani*	*R. stolonifer*
Costus afer	39.96^{a}	37.87^{c}	57.66^{ab}	53.68^{a}
Mimosa pudica	50.70^{a}	55.50^{bc}	57.50^{a}	63.65^{c}

Means with same letter in the same column are not significantly different at P>0.05 using Duncan's new
Multiple Range Test (DNMRT)

At 150g/ml concentration, the methanol extract of *Mimosa pudica* had the highest inhibitory effect of 50.10% (moderately effective) on *Aspergillus niger* and the least inhibitory effect of 47.20% (moderately effective) against *Fusarium solani*. Whereas, the methanol extract of *Costus afer* gave the highest inhibitory effect of 45.08% (moderately effective) against *Aspergillus niger* and the least inhibitory effect of 34.06% (moderately effective) on *Botryodiplodia theobromae* (Table 4).

Table 4. Inhibition of test fungi with methanol extracts of *Costus afer* and *Mimosa pudica* at 150g/ml

Plant extracts	A. *niger*	B. *theobromae*	*F. solani*	*R. stolonifer*
Costus afer	45.08[a]	34.06[ab]	35.50[a]	42.03[a]
Mimosa pudica	50.10[a]	49.50[b]	47.20[a]	48.53[a]

Means with same letter in the same column are not significantly different at P>0.05 using Duncan's new
Multiple Range Test (DNMRT)

At 200g/ml concentration, the methanol extract of *Mimosa pudica* gave the highest inhibitory effect of 51.33% (effective) against *Aspergillus niger* and the least inhibitory effect of 31.05% (moderately effective) on *Rhizopus stolonifer*. Whereas, the methanol extract of *Costus afer* gave the highest inhibitory effect of 49.05% (moderately effective) against *Aspergillus niger* and the least inhibitory effect of 38.34% (moderately effective) on *Fusarium solani* (Table 5).

Table 5. Inhibition of test fungi with methanol extracts of *Costus afer* and *Mimosa pudica* at 200g/ml

Plant extracts	A. *niger*	B. *theobromae*	*F. solani*	*R. stolonifer*
Costus afer	49.05[a]	42.33[a]	38.34[a]	39.23[a]
Mimosa pudica	51.33[ab]	43.31[a]	40.25[ab]	31.05[c]

Means with same letter in the same column are not significantly different at P>0.05 using Duncan's new
Multiple Range Test (DNMRT)

DISCUSSION

The organisms associated with post-harvest rot of cassava roots in this study were *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium solani* and *Rhizopus stolonifer*. These were frequently isolated from rotten cassava and their involvement in pathogenesis were also confirmed. Several workers have also reported the isolation of these fungi from post-harvest cassava root (Booth, 1976; Rickard and Coursey, 1981). The pathogenicity tests revealed that all of the four tested fungi induced rot in cassava root, with *B. theobromae* being the most virulent one. This agrees with the reports of Sangoyomi (2004), Okigbo and Nmeka (2005), Amienyo and Ataga (2007) and Okigbo and Ogbonnaya (2006). In most cases, fungi gain entrance into cassava roots through natural openings and wounds created during harvesting, transporting, handling and marketing (Amienyo and Ataga, 2007). However, Okigbo and Nmeka noted that at time of harvest, roots may already be infected by pathogens derived from foliage diseases or mother roots. The fungicidal effects of plant extracts on the inhibition of different pathogens of crop plants have been widely reported by several workers (Amadioha and Obi, 1999; Olufolaji, 1999; Onifade, 2000; Udo *et al*. 2001; Okigbo and Emoghene, 2004; Okigbo and Ogbonnaya, 2005; Okigbo *et al*. 2009b). In this study, crude extracts of *C. afer* and *M. pudica* were used in order to develop a cheap and simple method of controlling post-harvest cassava root rot for farmers' use. The fungitoxic effects of the plant extracts against mycelia growth varied with plant species, concentrations and with each fungus tested. The present observations showed that *C. afer* and *M. pudica* were effective against mycelia growth of most of the tested fungi. The most fungitoxic extract on *R. solani* was obtained with *M. pudica* at 100g/ml concentration. This study has shown that both *C. afer* and *M. pudica* have the potential to control post-harvest rot of cassava, indicating that they could be an alternative way of reducing and controlling rot by farmers, since they are less expensive, environmentally safe, non-phytotoxic and easy to prepare.

Conclusion

This study demonstrated that *C. afer* and *M. pudica* showed antifungal activity against the tested organisms. This finding is important from the point of view of controlling diseases associated with cassava without the use of chemicals which cause environmental pollution. The antifungal potentials of *C. afer* and *M. pudica* as observed in this study therefore raise hope in the use of natural plants to control fungi pathogens and to replace the synthetic dangerous and expensive chemicals used at present.

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