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Antifungal Effects of *Carica Papaya* and *Azadirachta indica* on Cocoyam (*Colocassia esculentus* L.) corm rot Disease in Umudike, Nigeria

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Abstract
Antifungal potency of two crude extracts (*A. indica* and *C. papaya*) was studied using four concentrations of aqueous and ethanoic plant extracts at 25, 50, 75 and 100% on cocoyam corms. The extracts were separately amended in potato Dextrose agar (PDA) in *in-vitro* control of fungal rot causing agents isolated from cocoyam corms (*Botryodiplodia, Aspergillus niger, Trichoderma* and *Rhizopus stolonifer*). Infected cocoyam corms were collected from cocoyam program of National Root Crop Research Institute, Umudike and taken to Plant protection laboratory of the Institute for analysis. *Botryodiplodia, A. niger, Trichoderma* and *R. Stolonifer* proved to be pathogenic from the pathogenicity test carried out since they all incited rots in cocoyam corms. The results further revealed that all the test botanicals were able to significantly ($P<0.05$) inhibit the mycelia growth of all the four test fungi. *A. indica* was consistently observed to be more potent to all the four test fungi and followed closely were ethanoic extracts of *C. papaya* (42.33-61.00%). The findings have shown the potential of plants extracts in the control of cocoyam corm rot caused by *B. theobromae, A. niger, Trichoderma sp.* and *R. stolonifer*. These botanicals *A.indica* and *C.papaya* will therefore, reduce the use of synthetic chemicals by farmers in controlling pathogens of stored cocoyam corms, cost of production and environmental pollution.

Keywords: Synthetic, Botanicals, Antifungal, Extract and Pathogenicity

Introduction

The medicinal and antimicrobial activities of plant botanicals are now being employed by researchers worldwide in combating or alleviating the menace of disease attack of all agricultural products. Plant extracts and essential oils are effective in controlling plant pathogens on field and in storage (Tripathi *et al*., 2008). The science of application of botanicals for treatment of diseases is currently called ethno-pharmacology. For example, bear berry (*Actostaphylos ura-ursi*) and cranberry juice (*Vaccimium macrocarpon*) was used to treat urinary tract infections. Garlic (*Allium sativum*) and tee tree (*Melaleucaaternifolia*) were described as broad-spectrum antimicrobial agents (Heinrich *et al*., 2004).

Cocoyam is a perennial monocotyledonous herb (Onwueme, 1978). It consists of a central corm from which cormels, roots and shoots arise (Onwueme, 1978), with each leaf consisting of a long erect petiole and large lamina (Williams *et al*., 1982). Edible cocoyam (*Colocassia esculenta*) is a major stable carbohydrates food in sub-Saharan Africa. It is nutritionally superior to other root and tubers, in terms of digestible crude protein and minerals like Ca, Mg and P contents (Green, 2003). Cocoyam is the third most important crop in root and tuber family cultivated in Nigeria after yam and cassava (Igbozulike, 2015). The use of cocoyam in Nigeria includes; as soup thickener, consumption as porridge and can equally be used by processing into shelf food in the following ways through roasting, cooking, boiling, baking, frying, milling and pounding. Amand-Vinas and Lorenz (1999). It is used as an anti-poisoning and wound healing agents by applying the fresh mash in the sores surfaces, (being an antibiotic agent). This tuber crop is consumed both by domestic animals and human beings. Cocoyam has some percentage of oxalic acid content which made it dangerous to consume raw for animal and humans because it can cause itching if consumed raw. It must be boiled, roasted or fermented before consumption (NRCRI, 2015). In 2008, Nigeria produced about 5.39 million metric tonnes of cocoyam out of the world production. The problems encountered in cocoyam production are deterioration of corms during storage and harvest which always resulted to loss in the quality of the corms. *Carica Papaya* and *Azadirachta indica* are both medicinal plants that have been prominently used by researchers in controlling fungal infection of postharvest loss due to rot in root and tuber crops (Mahmoud *et al*., 2011 and Anukworji *et al*., 2012). These botanicals are biodegradable, cheap, readily

available and environmental friendly (Okigbo and Nmeka, 2005c). It is on this backdrop that the study focused on the antifungal potency of these extracts against the in vitro mycelia growth of spoilage fungi isolated from infected cocoyam corms in storage.

Materials and Methods

Experimental Site

The study was carried out at the Plant Protection Laboratory, National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria.

Collection of Infected Cocoyam Corms

Infected corms of cocoyam showing disease symptoms of soft and dry rots were obtained from Cocoyam Program of the Institute. The infected corms were packaged in different sterile polyethylene bags, taken to the laboratory for isolation and identification of pathogens. Both botanical were collected from the staff quarters of NRCRI, Umudike.

Sterilization of Materials

All glassware used in this study were washed with detergent, rinsed, dried and sterilized by autoclaving at a temperature of 121°C and 15psi for 20min. The scalpel, cork borer and inoculating needle were sterilized by dipping them in 70% ethanol and passing them over a Bunsen burner flame until red hot.

Isolation of Fungi

The isolation technique used by Okigbo and Nmeka (2005b) was used in this study. A small section of the cocoyam tissue showing advancing margin of rot and adjoining healthy tissue were cut using sterilized scalpel whose surface were sterilized with 70% ethanol and flamed to red hot. The cut pieces were soaked in 70% ethanol for 1min for surface sterilization. The pieces (1mm-2mm) were then rinsed in three successive changes of sterile distilled water (Okigbo and Nmeka (2005b). The cocoyam pieces were placed on sterile paper towels in the laminar Air flow cabinet to dry for 5 minutes.

Inoculation

The dried infected tissues were aseptically planted onto Petri dishes containing acidified sterile solidified potato dextrose agar (PDA) with the aid of sterile forceps and the plates were incubated at room temperature $\pm$27°C for 5 days. Fungi associated with the cocoyam rot affected tissue were observed and the frequency of isolation determined using method of Okigbo and Ikediugwu (2000a). Sub-culturing was done to obtain pure cultures of the isolates.

Identification of Fungal Isolates

Pure colonies growing on the plates were identified macroscopically and microscopically. Features observed were colony colour, type of hyphae, texture, shape and growth pattern. Direct observation of culture under the light microscope by careful preparation of slides stained with cotton blue-in-lactophenol was done. Detailed drawings of the diagnostic features and identification were noted and compared to existing manual guides according to Ahmed and Ravinder (1993) and Burgess *et al.* (2008).

Pathogenicity Test

Healthy cocoyam corms were washed with running tap water, rinsed in three successive changes of sterile distilled water. Thereafter, the corms were disinfected with 70% ethanol for 1min and again rinsed with sterile distilled water 3 times. The tubers were allowed to air dry. A flamed 5mm cork borer was used to bore hole into healthy cocoyam corm, a 5mm diameter disc from the purified isolate of 5 days old culture was cut and replaced into the hole created in the healthy corm. The same procedure was used for the control, except that sterile agar discs were used instead of the place of the purified cultures (inoculum) in the holes created in the corms. Blue Vaseline was used to completely seal the holes (Okigbo *et al*., 2013). Two whole corms of cocoyam were inoculated per fungus. The inoculated corms were placed in sterile transparent polythene bags whose inside has been moistened with cotton wool soaked in sterile distilled water to maintain a high humidity. The inoculated corms were kept in the laboratory at room temperature for about 14 days and assessed for rot development by cutting through the point of inoculation where rots developed. The pathogens were re-isolated as previously described and their cultural and morphological characteristics compared with those of the original isolates.

Preparation of Extracts

The fresh leaves of *Carica papaya* and *Azadirachta indica* were thoroughly washed with tap water and then with sterile distilled water (SDW), sun dried for 7 days before milling. The dried samples were separately grinded in a laboratory mill (Thoms Wiley Model ED 5 USA) after which the grinded samples were sieved to obtained powdered samples used for the extraction.A cold solvent extraction method (Harbone, 1973) was used for extraction of plant extract. Exactly 25g, 50g, 75g and 100g portions of each grounded sample were mixed separately with 100ml of each solvent (water and ethanol) in a 1000ml conical flask to produce 25%, 50%, 75% and 100% extract concentrations respectively. The extract solutions were sieved using four layers of sterile cheese cloth and stored in sterile conical flask which were later used for mycelial growth inhibition.

Antifungal Activity of the Extracts

Effect of plant extracts on mycelial growth of the test fungi was studied using the food poisoning technique (Sangoyomi, 2004). One milliliter (1ml) of each plant extract was dispensed per plate and 9ml of the media (molten PDA) added to each of the Petri-dish containing extract and carefully spread evenly over the plate, giving rise to 10% extract concentration. This was used for examining the growth inhibition of mycelia of each fungi. The plates were gently swirled to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a 5mm diameter mycelia disc obtained from the edge of 5-day old pure cultures of each test fungi. Each treatment consists of control 1 agar plate (no extract)

inoculated with a test fungi as described above. Petri-dishes dispensed with molten PDA and 1ml of grisovid dissolved in distilled water inoculated with each test fungus served as the commercial fungicides (control 2). All the plates were incubated at 28°C for 5 days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whipps (1987) as modified by Amadioha (2003).

$$\text{Percentage growth inhibition} = \frac{R1-R2}{R1} \times \frac{100}{1}$$

Where; R1 is the radial growth of the pathogen in control plate, while R2 is the radial growth of the pathogen in extract incorporated agar plates.

Experimental Design and Data Analysis

The Design used was Completely Randomized Design (CRD) with three replicates. Test of variance was calculated using Analysis of variance (ANOVA) and statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatment means for each measured parameter were further separated using fishers least significance difference (LSD) to determine levels of significance according to Cochran and Cox (1992).

Results and Discussion

The fungi that were isolated from rotten cocoyam corm (*Colocassia esculenta*) resulting from the sampling survey above included *Asergillus niger, Rhizopus stotonifer, botryodiplodia theobromae, Trichoderma spp* and *Pencillium sp.* The most frequently occurring was *Aspergillus niger* 28.05% while the least occurring was *Pencillium sp.*10.00% (Table 1). The fungi tested included *A. niger, R. stolonifer, B. theobromae and Trichoderma spp* and were confirmed to cause the similar disease and rot type noticed on the rot infected sample..*Trichoderma* incited dry rot at minimal level at 21.00%. The fungus *Botryodiplodia sp* was the predominant, causing 79% rot on the *colocassia esculenta* while *Trichoderma* the least with 21.00% (Table 2). Both aqeous and ethanol extract of C. papaya has inhibitory affect on the mycelia growth of the four test fungi (Tables 3 and 4). One hundred percent (100%) aqeous extract had the highest of 52.13% on *Trichoderma* with the mean value of 28.64, while the lowest inhibition was 0.90% on *A. niger* with mean value of 23.74 (Table 3). The higher the percentage concentration of aqeous extract, the higher the inhibition percentages of the four test fungi (Table 3). The ethanol extract at 100% concentration had the highest inhibition percentage of 61.00% on *rhizopus* with the mean value of 45.93. The lowest percentage inhibition was 7.03% on *A. niger* at the concentration of 25% with mean value of 27.47 (Table 4). Both ethanol and aqeous extract of *A. indica* had inhibitory effects on all the pathogenic fungi (Tables 5 and 6). The highest inhibitory (79.27%) from 100% concentration was recorded on *A. niger* with mean value of 62.84, while the lowest inhibitions percentage from 25% concentration was 44.46% on *R. stolonifer* with mean value of 60.90 (Table 5). The ethanol extract had the highest inhibitory 85.07% on *A. niger* with the mean value of 69.04 at 100% concentration, while the lowest inhibition 47.37% at 25% concentration with the mean value of 69.04 on the same *A. niger* was recorded (Table 6). The ethanol extract of *C. papaya* gave the highest growth inhibition of *R. stolonfer* (61.00%) which is significantly ($P \leq 0.05$) greater than 58.97% recorded on *Trichoderma.* There is significant difference $P \leq 0.05$ in the inhibition percentage between 44.70% (*A. niger*) and *Botrydiplodia*; 42.53%) in ethanol extract of *C. papaya* (Table 4). From the aqeous extract of *C. papaya,* there is significant ($P<0.05$) difference between the inhibition on *Trichoderma* (52.13%) and Rhizopus (49.57%). Equally there is significant ($P<0.05$) difference between 52.13% inhibition on *trichoderma* and (44.13%) on *A. niger* (Table 3). The ethanol extract of *A. indica* had an inhibitory significant ($P<0.05$) difference on *A. niger* (85.07%) against *Trichoderma* (82.90%). *Botryodipladia* had a significant ($P<0.05$) difference on *Trichoderma* (82.90%) and *Rhizopus* (79.63%) (Table 6). The aqeous extract of *A. indica* has a significant ($P<0.05$) difference on *A. niger* (79.27%), *Botryodiplodia* (48.73%) and *Aspergillus niger* (47.73%) even though there is a level of inhibition percentage.

Discussion

Fungal pathogens are the major causative agents of rot in root and tuber crops, reducing the yield, and quality per annum (IITA, 1985). The fungal organisms indicated in this study for causing cocoyam rot were *Botryodiplodia theobramae, Aspergillus niger, Rhizopus stolonifer* and *Trichoderma sp.* (William *et al*., 1982 and IITA, 1985). These organisms were responsible for cocoyam corm rot in the store and equally in the soil on the field. The pathogenicity test showed that the inoculated fungi causes rot on the cocoyam corm; this was due to the ability of the pathogenic fungi to utilize the tissues of cocoyam corm as nutrients aiding its growth and development. This result is similar to the fungi associated with Nigeria cocoyam (Igbozulike, 2015). Some other botanicals have been used to control pathogens of cocoyam corms (Anukworji *et al*., 2012) and (Bhagwat and Datar, 2014). In this study, ethanol extract of *A. indica* produced a significant inhibition growth on pathogenic fungi in post-harvest cocoyam compared with that achieved by ethanol extract of *Carica papaya*. The aqeous extract of *A. indica* equally had significant inhibition in all the test organisms when compared to aqeous extract of *C. papaya.* The difference in percentage inhibition of both extracts on test fungi could be as a result of different percentage of phytochemicals: alkaloids, flavonoids, phenols, saponin, tannins and oxalate and the medium used for extraction (Okigbo and Nmeka, 2005c).

Conclusion

The leave extracts of *A. indica* and *C.* papaya have the

potential to control rots in post-harvest cocoyam corms. Both botanicals can provide alternative means of reducing rot in stored cocoyam corms to the use of chemical fungicides. The botanicals are readily available, cheap and environmentally friendly, which are all advantages over synthetic fungicides.

References

Ahmed, K.M. and Ravinder, Reddy, C.H. (1993). A pictorial guide to the identification of seed borne fungi of sorghum, pear, millet, chick pea, pigeonpea and groundnut information. Bulletin no. 34: (In En summaries in Fr, Es and Ar.) Parancheru, A.P. 502324, India: International Crops Research Institute for Semi-Arid Tropics. Pp. 200.

Amadioha, A.C.,(2003).Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in Cowpea.*Acta Phytopathologica et Entomologica* Hungarica, 38:259-265.

Anukworji, C.A., Ramesh, R.P. and Okigbo, R.N. (2012). Isolation of fungi causing rot of cocoyam (*colocassia esculenta* (*L.*) Schott) and control with plant extracts: (*Alluium sativum*, L., *Garcinia Kola, Heckel, Azadiractita indica, L.* and *Carica papaya, L.*). *Glob Adv. Res. J. Agric. Sci*., (1): 202-217.

Arnaud-Vinas, M.D.R. and Lorenz, K. (1999). Pasta products containing taro (*Esculenta*, L. Schott) and Chaya (*Cnidoscuolus chavamansa* L. Mcvaugh). *Journal of food Processing and Preservation*, 23:1- 20

Bhagwat, M.K. and Datar, A.G. (2014). Antifungal activity of herbal extracts against plant pathogenic fungi. *Arch Phytopathology Plant Protect*, 47(8).

Burgess, L.W., Knight, T.E., Tesoriero, L. and Phna, H.T. (2008). Diagnostic manual for plant disease in Vietnam. ACIAL Momograph No. 129, ACIAR: Canberr, Pp. 210.

Cochran, G.W. and Cox, G.M. (1992). Experimental designs. Second edition. (Wiley classic library). John Wiley and Sons Inc. 1992; 611.

FAOSTAT (2010). Food and Agricultural Organization Agricultural Statistics. Rome: FAO, 2020.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedure for Agricultural Research, 2nd Edn, John Wiley and Sons. Pp. 680.

Green, B.O. (2003). Taxonomic and nutritional analysis of certain tuber crops in the Delta of Nigeria. *African Journal of Environmental Studies*, 4(1/2): 120-122.

Harbone, J.B. (1973). *Phytochemical methods.A guide to modern technique of plant analysis.*Chapman and Hall, New York.

Heinrich, M., Barnes, J., Gibbons, S. and Williamson, E.M. (2004). Fundamentals of Herbal Medical Practice. *Journal of Biological Research and Biotechnology*, 2(1): 34-46.

Igbozulike, A.O. (2015). Evaluation of cocoyam corms processing and storage in Nigeria. *Int. J. Sci. Eng. Res*., 6(7): 259-63.

IITA (2009). International Institute of Tropical Agriculture. Yam production in Africa,

Mahmoud, D.A., Hassanein, N.M., Youssef, K.A. and Abouzeid, M.A. (2011). Antifungal activity of different neem extracts and the nimonol against some important human pathogens. *Braz J. Microbial*., 42:1007-16.

NRCRI (2015). National Root Crops Research Institute, Umudike. Cocoyam program. http://www.nrcri.org.

Okigbo, R. N. and Ikediugwu, F. E. O. (2000a). Studies on biological control of post-harvest rot of yam (*Dioscorea sp.*) with *Trichoderma viride. J. Phtopathol*., 148 (6):-351-355.

Okigbo, R.N. and Nmeka, I.A. (2005c). *African Journal of Biotechnology*, 4(8): 804-807.

Okigbo, R.N. and Nmeka, I.A. (2005b). Control of yam tuber with leaf extracts of *Xylopia aethiopica and Zingiber officinale. Afr. J. Biotect*., 4: 804-807.

Okigbo, R.N., Anukworji, C.A. and Ezeabara, C.A. (2013). Effect of leaf extracts of *Chromolaenna odorata* (L.) and *Terminalia catappa* (*L.*) on mycelia growth of yam tuber pathoges. *Niger. J. Plt. Prot*., 27(1): 74 – 84.

Onwueme, I.C. (1978).The Tropical tuber crops-Yams,Cassava,Sweet Potato and Cocoyam.John Wiley and Sons, Chichester, 234pp.

Sangoyomi,T.E. (2004). Post-harvest fungal deterioration of yam (*Discorea rotundata* poir) and its control. An Unpublished Ph.D Thesis submitted to University of Ibadan,Nigeria.179pp.

Tripathi, P., Dubey, N.K. and Shukla, A.K. (2008). Use of the essential oil as postharvest botanical fungicides in the management of grey mould of grapes caused by *Botrytiscinera. World Journal of Microbiology and Biotechnology*. 2008, 24:39-46.

Whipps, J.M. (1987). Effect of media on growth and interactions between a range of soil borne glass pathogens and antagonistic fungi. *New Pathol*., 107:127-42.

William, C., Chew, W. and Rajaratuam, J. (1982). Cocoyam in payne, W. (ed) *Tropical and Field Crop of the wetter regions of the tropics*. Longman Pp. 210-211.

Table1: Frequency of occurrence of fungi isolated from infected Cocoyam corm

Organisms	Occurrence (%)
Aspergillus niger	28.05
Rhizopus stolonifer	19.05
Botryodiplodia sp	20.05
Trichoderma sp	23.01
Penicillium	10.00

Table 2: Percentage incidence of isolated pathogenic fungi

Organisms	Percentage Rot (%)
Aspergillus niger	73.00
Rhizopus stolonifer	55.00
Botryodiplodia sp	79.00
Trichoderma sp	21.00

Table 3: Percentage growth inhibition of Test fungi using extract of *C.papaya* (Aqueous)

Isolates	25	50	75	100	Mean
Botryodiplodia	4.26	20.53	34.17	42.77	25.43
A.niger	0.90	22.50	27.43	44.13	23.74
Trichoderma	5.13	17.94	39.33	52.13	28.64
Rhizopus	18.83	35.90	47.93	49.57	38.06
$LSD_{0.05}$	3.067	3.022	2.106	1.042	

Table 4: Percentage growth inhibition of Test fungi using extract of *Carica papaya* (ethanol)

Isolates	25	50	75	100	Mean
Botryodiplodia	8.40	21.13	34.70	42.33	26.64
A.niger	7.03	26.00	32.13	44.30	27.47
Trichoderma	15.37	33.97	43.73	58.97	38.01
Rhizopus	27.10	44.10	51.50	61.00	45.93
$LSD_{0.05}$	3.105	2.852	2.142	1.042	

Table 5: Percentage growth inhibition of Test fungi using extract of *A.indica* (Aqueos)

Isolates	25	50	75	100	Mean
Botryodiplodia	48.73	53.00	59.83	76.10	59.42
A.niger	47.73	59.33	65.03	79.27	62.84
Trichoderma	46.17	58.83	64.93	70.10	58.76
Rhizopus	44.46	49.57	67.53	77.80	60.90
$LSD_{0.05}$	2.250	2.272	1.970	1.082	

Table 6: Percentage growth inhibition of Test fungi using extract of *A.indica* (ethanol)

Isolates	25	50	75	100	Mean
Botryodiplodia	58.67	61.90	72.00	83.07	68.91
A.niger	47.37	66.67	77.07	85.07	69.04
Trichoderma	51.27	62.40	68.30	82.90	66.22
Rhizopus	49.90	60.17	73.57	79.63	65.82
$LSD_{0.05}$	2.013	1.742	1.608	1.709	