Phytotoxic Potentials Of Aqueous Extract Of *Chromolaena Odorata* Against Mycotoxigenic Agents Of Yam Tubers After Harvest.

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Abstract: The present investigation revealed *Botryodiplodia theobromae, Aspergillus niger, Aspergillus flavus* and *Aspergillus glaucus* as causes of post-harvest rot disease of yam tubers on the bases of pathogenicity test. Hot extracts of 10g, 30g and 50g /100ml of *Chromolaena odorata* inhibited *Aspergillus flavus* to 92.19%, 92.19% and 97.77% respectively, while 20g and 40g/100ml inhibited *Aspergillus glaucus* to 95.56% and 92.22% respectively. 10g, 40g and 50g/100ml of cold extract of *C. odorata* inhibited *Aspergillus niger* to 98.08%, 96.69% and 96.92% while 20g/100ml concentration inhibited *B. theobromae* to 96.86% and the least inhibited being *Aspergillus flavus* (93.31%). 30g and 40g/100ml concentration of *C. odorata* inhibited *Aspergillus flavus* to 89.97% and 98.89% respectively, while *Aspergillus niger* (96.16%) was least inhibited. Cold aqueous extract of *C. odorata* at 50g/100ml inhibited *B theobromae* by 95.24%. Both hot and cold extracts of *C. odorata* were found to be very potent against yam tuber rot pathogens. These serve as preferred alternatives to chemicals which usually have negative effects on the environment.

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Introduction

Yam (*Dioscorea rotundata*) belongs to the genus Dioscorea. It is a monocotyledonous angiosperm plant, mainly grown in the tropical and sub-tropical countries of West Africa, Caribbean, Northern and Central part of East Asia, including parts of China, Malaysia, Japan and Oceania (Okigbo *et al*., 2010), it is closely integrated into social, economic, cultural and religious aspect of life in Nigeria. It serves as an important source of minerals such as calcium, phosphorus, iron, riboflavin, thiamin, carbohydrate, and vitamin B and C (Obilo *et al*., 2005). Yam is medically used to treat diseases like diabetes mellitus, to increase coronary flows and to prevent high hypercholesteromia (Okigbo and Ogbonnaya, 2006). Yam rot is one of the major diseases caused by fungi, it occurs when infected tuber do not show any external sign, it starts in the soil/field and progresses in storage. Oyelana, *et al.,* (2011) reported the application of synthetic fungicides like sodium orthiophenate, captan, thiobendazole, benomyl against yam pathogens, they reduced storage rot of yam tubers leading to an increase in crop shelf live but induced resistance from targeted organisms; they accumulated in the ecosystem and lack of expertise among most farmers that handled them.

The use of plant extracts as an approach in phyto-disease management is preferable because it is selective, cheap and lack adverse effect (Amadioha and Obi, 1999). Plant extracts have been successfully used to control tuber crops diseases such as yam, potato and cassava (Onifade, 2002; Okigbo and Emoghene, 2004).

Materials and Method

Collection and preparation of materials

Tubers of yam with symptoms of soft rot and the healthy ones were obtained randomly from Aba-Ebira along Iworoko-Ifaki road, Ado-Ekiti, packed in a polythene bag and taken to the laboratory for further pathological studies.

Leaves of *C. odorata* were collected from the parent plant in the vegetation area of Ado Ekiti, air-dried, pulverized, weighed into 10 to 50g and added to 100ml of cold and hot water in separate conical flasks, stirred vigorously and left to stand for 12 hrs. The sample was filtered with Whatman filter paper and the filtrate was used as the extract.

Pathogenicity test

Thin sections of the surface sterilized-rotted portion of yam were plated on potato dextrose agar (PDA) incorporated with streptomycin in a Petri dishes. The plated Petri dishes were incubated at room temperature (28^0C) in the dark for 72 hours and were observed daily for fungal growth. The developing fungi were characterized, identified and pure-culture prepared and stored in McCartney bottles for pathological assay. Cylindrical cores of 5mm deep were removed from four different points of an healthy yam tuber with sterile cork borer of 5mm and 4mm

disc taken from the colony of the initially isolated sub-cultured organisms and placed downward into the holes created in yam tubers, the surface of the hole was completely smeared with petroleum jelly, a piece of cotton wool was placed on separate sterile covered container and incubated for 72 hrs at room temperature (28^0C).

In-vitro investigation of plant extract on fungal growth

Poisoned PDA with plant extract was poured into Petri dishes; the mixture was allowed to solidify. Each isolated fungi viz: *Aspergillus niger, A glaucus Botryodiplodia theobromae, Aspergillus flavus* was inoculated into the Petri dishes using flamed inoculating needle. The plates were incubated for 72hours and observed for fungal growth. Extract-free medium was used as the control. The radial growth of fungi was measured using metre rule from the point of inoculation. The percentage fungitoxity level of aqueous plant extract was determined using the method of Okigbo *et al.,* (2010)

$$\text{Growth inhibition (\%)} = \frac{(LC - LT)}{LC} \times \frac{100}{1}$$

Where LC = average length of uninfected portion of control and,

LT = average length of uninfected portion with treatment

Result

Effect of aqueous extracts (hot and cold) of *C. odorata* on mycelial growth of fungal rot organisms

The antimycotoxigenic effects of both cold and hot water extracts of *C. odorata* on the rot organisms are presented in Table 1. Hot water extracts of *C. odorata* at 10-50g/100ml showed high inhibitory capacities on *B. theobromae* ranging between 82.38% and 90.48%. The mycelial growth of *B. theobromae* (90.48%) was most reduced by hot water extract of *C. odorata* at 50g/100ml, followed by exhibition of 89.52%, 88.57% and 86.95% by hot water extract of *C. odorata* at 40g, 30g and 20g/100ml against *B. theobromae* respectively, the least inhibitory effect of 82.38% was elicited by hot water extract of *C. odorata* at 10g/100ml on *B. theobromae.* Cold water extracts of *C. odorata* at 10-50g/100ml had antimycotic effects on *B. theobromae* that ranged between 94.28% and 97.14%. The mycelial growth of *B. theobromae* (97.14%) was most inhibited by cold water extract of *C. odorata* at 50g/100ml, followed by inhibitory effects of cold water extract of *C. odorata* at 40g and 30g/100ml, exhibiting 96.86% and 95.23% on *B. theobromae* respectively, cold water extract of *C. odorata* at 20g/100ml elicited antifungal effect of 94.94% against *B. theobromae,* while cold water extract of *C. odorata* at 10g/100ml was least inhibitive against *B. theobromae* (94.28%). Hot water extracts of *C. odorata* at 10-50g/100ml showed antifungal effects on *A. flavus* ranging between 91.08% and 97.19%. Hot water extract of *C. odorata* at 50g/100ml was most antimycotic on *A. flavus* (97.19%), hot water extract of *C. odorata* at 40g/100ml induced inhibitory effect of 92.19% against *A. flavus,* followed by exhibition of antimycotic effect of 92.10% by hot water extract of *C. odorata* at both 30g and 20g/100ml against *A. flavus,* while the least mycelial reduction effect was recorded against *A. flavus* (91.08%) by hot water extract of *C. odorata* at 10g/100ml. The effects of cold water extract of *C. odorata* at 10-50g/100ml on *A. flavus* ranged between 89.96% and 98.88%. Cold water extract of *C. odorata* at 50g/100ml reduced the growth of *A. flavus* by 98.88%, followed by cold water extract of *C. odorata* at 40g and 30g/100ml, eliciting biocidal effect of 96.55% and 93.31% on *A. flavus,* while cold water extract of *C. odorata* at both 10g and 20g/100ml, evoked least antimycelial effect of 89.96% on *A. flavus*.

Hot water extract of *C. odorata* at 10-50g/100ml showed high microbecidal effects on *A. glaucus* ranged from 77.78% to 95.56%. *A. glaucus* (95.56%) was most inhibited by hot water extract of *C. odorata* at 50g/100ml, followed by inhibitory capacity of hot water extract of *C. odorata* at 40g, 30g and 20g/100ml by 94.78%, 94.55% and 88.89% on *A. glaucus* respectively, while hot water extract of *C. odorata* at 10g/100ml exhibited the least inhibition of 77.78% against *A. glaucus.* Cold water extract of *C. odorata* at 10-50g/100ml high fungicidal effect on *A. glaucus* ranging between 92.55% and 98.22%. The mycelial growth of *A. glaucus* (98.22%) was most inhibited by cold water extract of *C. odorata* at 50g/100ml, followed by inhibitory effects of 95.55%, 94.55% and 93.33% by cold water extract of *C. odorata* at 40g, 30g and 20g/100ml on *A. glaucus* respectively, while cold water extract of *C. odorata* at 10g/100ml expressed the lowest biocidal effect against *A. glaucus* by 92.55%. In Hot water extract of *C. odorata* at 10-50g/100ml was fungitoxic on *A. niger* ranging from 81.67% to 91.92%. *A. niger* was most affected with the inhibition of 94.78% by hot water extract of *C. odorata* at 50g/100ml, hot water extract of *C. odorata* at both 40g and 30g/100ml had 90.00% on *A. niger,* followed by least inhibitive effect of hot water extract of *C. odorata* at 10g/100ml on *A. niger* by 81.67%. Cold water extract of *C. odorata* at 10-50g/100ml had antimicrobial effects on *A. niger* ranging from 95.83% to 98.08%. Cold water of *C. odorata* at 50g/100ml recorded the highest antifungal effect of 98.08% on *A. niger*, followed by cold water extract of *C. odorata* at 40g, 30g and 20g/100ml, inducing antimycotic effects of 96.91%, 96.66% and 96.16% against *A. niger* respectively, while *A. niger* was least inhibited to 95.83% by cold water extract of

C. odorata at 10g/100ml. The inhibitory effects of cold and hot water extract of *C. odorata* on the fungal rot organisms differed significantly ($p<0.05$) from the untreated control and standard. The effectiveness of both cold and hot water extracts of *C. odorata* on the rot organisms increased with increase in concentration.

Table 1. Effect of aqueous extract (hot and cold) of *C. odorata* on the inhibition (%) of radial mycelial growth of fungal rot organisms

water extracts (g/100ml)	% inhibition of radial mycelial growth							
	hot *B. theobromae*	cold	hot *A. flavus*	cold	hot *A. glaucus*	cold	hot *A. niger*	cold
10	82.38^{b}	94.28^{b}	91.08^{b}	89.96^{b}	77.78^{b}	92.55^{c}	81.67^{b}	95.83^{b}
20	86.95^{ab}	94.94^{b}	92.10^{b}	89.96^{b}	88.89^{ab}	93.33^{bc}	88.33^{a}	96.16^{b}
30	88.57^{a}	95.23^{b}	92.10^{b}	93.31^{b}	92.22^{ab}	94.55^{b}	90.00^{a}	96.66^{b}
40	89.52^{a}	96.86^{ab}	92.19^{b}	96.55^{b}	94.78^{ab}	95.55^{ab}	90.00^{a}	96.91^{ab}
50	90.48^{a}	97.14^{a}	97.19^{a}	98.88^{a}	95.56^{a}	98.22^{a}	91.92^{a}	98.08^{a}
Standard	40.70^{c}	40.70^{c}	30.20c	30.20^{c}	60.50^{c}	60.50^{d}	50.00^{c}	50.00^{c}
Control	00.00^{d}	00.00^{d}	00.00^{d}	00.00^{d}	00.00^{d}	00.00^{e}	00.00^{d}	00.00^{d}

Means with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan Multiple Range Test

Discussion and Conclusion

The present investigation showed that a number of fungi were associated with post-harvest rot of yam. These fungi were: *B. theobromae, A. niger, A. flavus*, and *A. glaucus.* These have been reported as rot pathogens of yam tubers (Tedela and Ijato, 2013). Post harvest rot starts from the soil/field and progresses while in storage. This occurs when infected tubers do not show visible external symptoms (Okigbo and Ogbonnaya, 2006).

Adejumo and Lagenkamper (2012) reported the fungicidal activity of some plant extracts against phytopathogens. Investigations into antifungal properties of *C. odorata,* reduced the mycelia growth of *Erysiphe cichoracearum, Collectotrichum capsici* and *Protomycopsis phaseoli,* which competed favourably with the chemical pesticide benlate and ridomil. The inhibitory effects of *C. odorata* and *A. indica* on yam rot organisms in this study agreed with the report of Okigbo *et al*., (2010) that used *C. odorata* and *A. indica* to inhibit some yam rot pathogens with high inhibitory capacity. Similarly, Titilope (2013) reported the bactericidal effects of dry and fresh leaves of ethanol extracts of *C. odorata* in *vitro* on human pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinectobacter baummani* and *Enterococcus faecalis*. Moreover, the result of antimycotic effects of *C. odorata* in this study agreed with the report of Mbajiuka *et al*., (2014) that reported very high antimicrobial effects of cold water leaves extracts of *C. odorata* on some known human pathogens (*Staphyloccocus aureus, Escherichia coli* and *Candida albicans*).

Similarly, antimycotic effects of *C. odorata* on the rot pathogens in this study agreed with the report of Félicien *et al*., (2012) that the extract of *C. odorata* showed interesting antibacterial effects on *Staphylococcus aureus* and *Escherchia coli* as well as antifungal activity on *Aspergillus ochraceus* and *Penicillium digitatum*. More also, bioactivity of the essential oil of *C. odorata* on *Staphylococcus aureus*; gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* as well as antifungal activity against *Aspergillus niger* was reported by Owolabi *et. al.,* (2010). Sukanya *et.al.,* (2011) also, reported high inhibitory effects of *C. odorata* extracts against clinical bacteria (*Escherichia coli* and *Staphylococcus aureus*) and phytopathogenic bacteria (*Xanthomonas vesicatoria* and *Ralstonia solanacearum*), using methanol, ethanol, ethyl acetate, hexane and chloroform as extractants.

The variation noted in the antifungal effects of various concentrations of extracts may be due to solubility of the active substances in water and the presence of inhibitor(s) against fungicidal principles. This agreed with the investigations of (Ijato, 2011b). Varied concentrations of cold and hot aqueous leaf extracts of *C. odorata* (10- 50g/100ml) could serve as bio-protective agents and an alternative to synthetic fungicides against rot fungi of yam. This method of plant disease control is eco-friendly, economically viable and not toxic to plants and animals.

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