Antimycelial Properties Of Ethanol Leaf Extracts Of *Chromolaena Odorata* On Yam Tuber Soft Rot Fungi

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Abstract: The menace of yam tuber rot calls for an alternative control approach such as using antimicrobial agents of plant origin. The ethanol extract of *Chromolaena odorata* was prepared by adding 10-50g of its powdered leaves into100ml of varied concentrations of ethanol 10, 30 and 50%, 1ml of the plant leaf extract was mixed with molten PDA into the Petri dish and allowed to solidify together. The isolates: *Botryodiplodia theobromae, Aspergillus niger, Aspergillus flavus* and *Aspergillus glaucus* from rotten yam tubers were inoculated and incubated separately for 5 days. Mycelial inhibition was determined by measuring with metre rule along two perpendicular lines drawn at the base of the Petri dishes. The results showed that all the concentrations of the plant leaf extracts had remarkable reduction effects on radial growth of the isolates. This finding proved the efficacy of the plant leaf extracts for the control of post harvest rot of yam tuber.
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Introduction

In Africa, annual crop losses of about 500,000 tons have been estimated to be due to disease attack in the field by nematodes, fungi, bacteria, and viruses (Ikotun, 1989). Thirty-six different fungi are known to attack yam tubers in storage across the continents of Africa, Asia and America (including the Caribbean) but only 16 species have been reported in association with severe storage deterioration (Ikotun, 1989). Rots of yam tubers generated by fungi often start in the field and progress in storage (Ikotun, 1989). Fungal rot of yam tubers are differentiated into three groups: dry rots, soft spots and wet rots, which disintegrate tissues into watery mass; and hard rots which toughen the rot tissues.

Storage of yam tubers in Nigeria can be improved by avoiding crop failure due to rot as increasing productivity of yam tubers is intensified. The human initiatives to curb post harvest crop losses by fungal pathogens to ensure availability of food for the teeming population as an approach for food security using botanicals of fungicidal principles, is a well thought decision of laudable perspective.

Materials And Method

Collection and preparation of plant materials

Yam tubers that showed symptoms of softness of tissues and healthy ones were collected from a market at Ado–Ekiti, packed into a polyethene bag and conveyed to the laboratory for further studies, *C. odorata* was as well collected, taken to the herbarium unit of Ekiti State University, Ado-Ekiti for proper identification.

Isolation of spoilage fungi from rotten yam tubers

Piece of yam tuber (3 x 3 x 2 mm) were cut from advancing edge of rotten yam tubers, surface sterilized in 70% alcohol for a minute, dried on sterile tissue paper and plated out on Potato dextrose agar (PDA) already incorporated with streptomycin. Three replicates from each of the rots were plated out. The plates were incubated at room temperature for five days and fungi associated with rot affected tissue were identified and their frequency of occurrence determined using methodology of Okigbo and Ikediugwu (2000).

Pathogenicity test.

The method of Okigbo and Ikediugwu (2000) was used. Cylindrical, 1cm deep were removed from various spots of a healthy yam tuber with sterile 5mm cork borer and 4mm discs taken from edge of a colony of test fungus was placed downward into each of the hole in the tuber. The core of the yam tuber was replaced after 2 mm piece has been cut off to compensate for the thickness of the agar inoculum and the replaced core sealed with melted paraffin wax. Sterile PDA was used in place of the culture disc, 2ml of the extract of various plant materials were separately introduced into the Petri-dish containing the media (PDA). A dish (4mm diameter) of the pure culture of each isolate was placed on the extract just at the point of intersection of the two lines drawn at the bottom of the Petri-dish. Control experiments were set up without the addition rot fungi. Fungal toxicity was recorded in terms of percentage colony inhibition and calculated according to this formula.

$$\text{Growth inhibition (\%)} = \frac{DT - DC}{DC} \times 100$$

Where DC – Average Diameter of control

DT – Average Diameter of fungal colony with treatment.

Preparation of extracts

C. odorata (leaves) where air–dried and ground separately, varied grams of sample were added to 100ml of varied concentrations of ethanol in separate flasks. This was vigorously stirred and left to stand for 12 hr. The sample was filtered with four- fold cheese cloth and the filtrate used as the extract.

Effects of the extract on fungal mycelia growth

Four equal sections were created on each Petri-dish by drawing two perpendicular lines at the base of the plate; the point of intersection indicated the centre of the plate. This was done before dispensing PDA into each of the plates. The fungal radial growth was determined along these axes (Amadioha and Obi, 1999).

Results

Effects of 10% ethanol leaf extracts of *C. odorata* on mycelial growth of fungal rot organisms.

The antimycotic effects of 10% ethanol leaf extracts of *C. odorata* on the fungal pathogens are presented in Table 1. Inhibitory effects of 10% ethanol leaf extracts of *C. odorata* at 10-50g/100ml were on *B. theobromae* ranging from 82.85% to 97.42%. The mycelial of *B. theobromae* was most reduced with 10% ethanol leaf extracts of *C. odorata* at 50g/100ml by 97.42%, followed by 97.14% and 89.52% inhibitions on *B. theobromae* by 10% ethanol extracts of *C. odorata* at 40-30g/100ml respectively. Also, 10% ethanol leaf extracts of *C. odorata* at 20g/100ml exhibited antimicrobial effect of 89.52% against *B. theobromae*. The least exhibited inhibition was 10% ethanol leaf extracts of *C. odorata* at 10g/100ml was on *B. theobromae* (82.85%).

Phytotoxic effects of 10% ethanol leaf extract of *C. odorata* at 10-50g/100ml on *A. flavus* ranged from 93.31% to 97.78%. The effect of 10% ethanol leaf extracts of *C. odorata* at 50g/100ml was most effective on *A. flavus* by 97.78%. Similarly, 10% ethanol leaf extracts of *C. odorata* at 40g, 30g and 20g/100ml elicited antifungal effects of 96.99%, 94.42% and 93.31% on *A. flavus* respectively, followed by 10% ethanol leaf extracts of *C. odorata* at 10g/100ml, exhibiting the lowest mycelial reduction effect against *A. flavus* (93.31%). Fungicidal effects of 10% ethanol leaf extracts of *C. odorata* at 10-50g/100ml on *A. glaucus* ranged from 83.33% to 96.67%. The effect of 10% ethanol leaf extracts of *C. odorata* 50g/100ml was recorded against *A. glaucus* (96.67%), followed by 95.56%, 94.78% and 92.22% by 10% ethanol leaf extracts of *C. odorata* at 40g, 30g and 20g/100ml on *A. glaucus* respectively, while 10% ethanol leaf extracts of *C. odorata* at 10g/100ml least affected *A. glaucus,* exhibiting 83.33% inhibition.

Fungitoxic effects of 10% ethanol leaf extracts of *C. odorata* at 10-50g/100ml on *A. niger* ranged from 86.67% to 98.00%. Antifungal activity of 10% ethanol leaf extracts of *C. odorata* at 50g/100ml was highest on *A. niger* (98.00%), followed by exhibition of inhibitory values of 96.67% and 93.58% by 10% ethanol leaf extracts of *C. odorata* at 40g and 30g/100ml against *A. niger* respectively. The least fungicidal effects were against *A. niger* (86.67%) by 10% ethanol leaf extracts of *C. odorata* at both 20g and 10g/100ml. The antimicrobial effects of 10% ethanol leaf extract of *C. odorata* on *A. niger* differed significantly ($p<0.05$) from the untreated control and standard. The efficacy of 10, 30 and 5% ethanol leaf extracts of *C. odorata* on the rot organisms increased with the increase in concentration.

Table 1: Effects of 10% ethanol leaf extracts of *C. odorata* on mycelial growth of fungal rot organisms.

	% inhibition of mycelial growth			
g/100ml of alcohol	*B. theobromae*	*A. flavus*	*A. glaucus*	*A. niger*
10	82.85^{c}	93.31^{a}	83.33^{a}	86.67^{b}
20	89.52^{b}	93.31^{a}	92.22^{a}	86.67^{b}
30	89.52^{b}	94.42^{a}	94.78^{a}	93.58^{a}
40	97.14^{a}	96.99^{a}	95.56^{a}	96.67^{a}
50	97.42^{a}	97.78^{a}	96.67^{a}	98.00^{a}
Standard	40.70^{d}	30.20^{b}	60.50^{c}	50.00^{c}
Control	0.00^{e}	0.00c	0.00^{d}	0.00^{d}

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

Effects of 30% ethanol leaf extracts of *C. odorata* on mycelial growth of fungal rot organisms.

The antimycotic effects of 30% ethanol leaf extracts of *C. odorata* on the fungal pathogens are

presented in Table 2. 30% ethanol extracts of *C. odorata* at 10-50g/100ml of treatment; antimycotic potentials of 30% ethanol leaf extracts of *C. odorata* on *B. theobromae* ranged between 87.62% and 96.19%. The most phytotoxic potency of 30% ethanol leaf extracts of *C. odorata* at 50g and 40g/100ml was manifested against *B. theobromae* (96.19%), followed by growth reduction effects of 94.29% and 88.57% as manifested by 30% ethanol leaf extracts of *C. odorata* at 30g and 20g/100ml on *B. theobromae* respectively, while the lowest mycelial inhibitory effect of 30% ethanol leaf extracts of *C. odorata* at 10g/100ml was against *B. theobromae* (87.62%). Antimycotic capacities of 30% ethanol leaf extracts of *C. odorata* at 10-50g/100ml on *A. flavus* ranging from 93.30% to 96.99%. The antimycotic ability of 30% ethanol leaf extracts of *C. odorata* at 50g and 40g/100ml on *A. flavus* by inducing 96.99%, followed by 30% ethanol leaf extracts of *C. odorata* at 30g and 20g/100ml, causing 96.09% and 93.65% inhibition on *A. flavus* respectively. The least effective extract being 30% ethanol leaf extracts of *C. odorata* at 10g/100ml on *A. flavus,* eliciting 93.30%.

Phytotoxic effects of 30% ethanol leaf extracts of *C. odorata* at 10g-50g/100ml on the degrading organisms ranging between 78.88% and 95.56%. The most fungicidal value of 95.56% was against *A. glaucus* by 30% ethanol leaf extracts of *C. odorata* at 50g/100ml, followed by expression of 94.44% and 93.33% antimycotic effects on *A. glaucus* by 30% ethanol leaf extracts of *C. odorata* at 40g and 30g/100ml respectively, 30% ethanol leaf extracts of *C. odorata* at 20g/100ml exhibited antimicrobial effect of 87.50% against *A. glaucus,* while the least manifested harmful effect of 30% ethanol leaf extracts of *C. odorata* at 10g/100ml was on *A. glaucus* (78.88%).

Antimicrobial effects of 30% ethanol leaf extracts of *C. odorata* at 10-50g/100ml were on *A. niger* ranging between 76.66% and 96.66%. The highest phytotoxic capacity of 30% ethanol extracts of *C. odorata* at 50g/100ml was against *A. niger* (96.66%), followed by 30% ethanol leaf extracts of *C. odorata* at 40g, 30g and 20g/100ml by 95.83%, 95.00% and 87.50% on *A. niger* respectively, while *A. niger* (76.66%) was least affected by 30% ethanol leaf extracts of *C. odorata* at 10g/100ml.

Table 2: Effects of 30% ethanol leaf extracts of *C. odorata* on mycelial growth of fungal rot organisms.

	% inhibition of mycelial growth			
g/100ml of alcohol	*B. theobromae*	*A. flavus*	*A. glaucus*	*A. niger*
10	87.62^{b}	93.30^{a}	78.88^{c}	76.66^{a}
20	88.57^{b}	93.65^{a}	87.50^{b}	87.50^{b}
30	94.29^{a}	96.09^{a}	93.33^{a}	95.00^{a}
40	96.19^{a}	96.99^{a}	94.44^{a}	95.83^{a}
50	96.19^{a}	96.99^{a}	95.56^{a}	96.66^{a}
Standard	40.70^{c}	30.20^{b}	60.50^{d}	50.00^{d}
Control	0.00^{d}	0.00^{c}	0.00^{e}	0.00^{e}

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

Effects of 50% ethanol leaf extracts of *C. odorata* on mycelial growth of fungal rot organisms

The bio-activities of 50% ethanol leaf extracts of *C. odorata* on the fungal pathogens are presented in Table 3. Inhibitive effects of 50% ethanol extracts of *C. odorata* at 10-50g/100ml on *B. theobromae* ranged from 96.19% to 99.52%. The mycelial growth of *B. theobromae* (99.52%) was by 50% ethanol leaf extracts of *C. odorata* at 50g/100ml, followed by exhibition of 97.62% and 97.14% inhibitions by 50% ethanol leaf extracts of *C. odorata* at 40g and 30g/100ml on *B. theobromae* respectively, the least inhibitive concentration being 50% ethanol leaf extracts of *C. odorata* at 10g/100ml on *B. theobromae* (96.19%). Antifungal effects of 50% ethanol leaf extracts of *C. odorata* at 10-50g/100ml were on *A. flavus* ranging from 94.43% to 98.66%. Fungicidal effect of 50% ethanol leaf extracts of *C. odorata* at 50g/100ml was on *A. flavus* (98.66%), followed by 50% ethanol leaf extracts of *C. odorata* at 40g, 30g and 20g/100ml against *A. flavus* by 96.09%, 94.43% and 94.43%, while the least mycelial reduction effect of 94.43% was recorded against *A. flavus* by 50% ethanol leaf extracts of *C. odorata* at 10g/100ml.

Microbecidal effects of 50% ethanol leaf extracts of *C. odorata* at 10-50g/100ml on *A. glaucus* ranged from 93.33% to 97.66%. The most phytotoxic effect of 50% ethanol leaf extracts of *C. odorata* at 50g/100ml was recorded against *A. glaucus* (97.66%), followed by 95.55% and 95.33% inhibitory effects of 50% ethanol leaf extracts of *C. odorata* at 40g and 30g/100ml on *A. glaucus* respectively. while 50%

ethanol leaf extracts of *C. odorata* at both 20g and 10g/100ml had antifungal effect of 93.33% against *A. glaucus*.

Fungitoxic effects of 50% ethanol leaf extracts of *C. odorata* at 10-50g/100ml on *A. niger* rang from 90.00% to 97.75%. Antifungal activity of 50% ethanol leaf extracts of *C. odorata* at 50g/100ml on *A. niger* (97.75%), followed by exhibition of inhibitory effects of 94.42% and 93.67% by 50% ethanol leaf extracts of *C. odorata* at 40g and 30g/10ml against *A. niger* respectively. Also, 50% ethanol leaf extracts of *C. odorata* at 20g/10ml exhibited 92.75% antifungal effect against *A. niger*. The least fungicidal effect by 50% ethanol leaf extracts of *C. odorata* at10g/10ml of was against *A. niger* (90.00%).

Table 3: Effects of 50% ethanol leaf extracts of *C. odorata* on mycelial growth of fungal rot organisms.

	% inhibition of mycelial growth			
g/100ml of alcohol	*B. theobromae*	*A. flavus*	*A. glaucus*	*A. niger*
10	96.19[a]	94.43[a]	93.33[a]	90.00[b]
20	97.14[a]	94.43[a]	93.33[a]	92.75[b]
30	97.14[a]	94.43[a]	95.33[a]	93.67[ab]
40	97.62[a]	96.09[a]	95.55[a]	94.42[ab]
50	99.52[a]	98.66[a]	97.6[a]	97.75[a]
Standard	40.70[b]	30.20[g]	60.50[b]	50.00[c]
Control	0.00[c]	0.00[c]	0.00[c]	0.00[d]

Mean 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 study showed that a number of fungi were associated with post harvest rot diseases of yam. These fungi included *B. theobromae, A. niger, A. flavus* and *A. glaucus.* These rot organisms have been previously reported as rot pathogens of yam (Tedela and Ijato 2013, Okigbo, *et al*; 2009). A storage rot which may occur when infected tubers do not exhibit visible external symptoms (Okigbo and Ogbonnaya, 2006) is believed to start from the soil/field and progresses while in store. Nahed (2007), Nahunnaro (2008) and Okigbo *et al.*, (2009) have reported the fungicidal activities of some plant extracts against various phytopathogens. Investigation into the antifungal properties of *Chromolaena odorata* and among other extracts in reducing the mycelia growth of *Erysiphe cichoracearum, Collectotrichum capsici* and *Protomycopsis phaseoli,* which compete favourably with the chemical pesticide benlate and ridomil. Nwinuka *et al.*, (2009) also observed that the methanol extracts of *C. odorata* showed positive inhibition of *Bacillus subtilis, Klebsiella Pneumoniae* and *Staphylococcus aureus,* which were associated with superficial infections and indicated a negative inhibition for *Pseudomonas pyrogenes* and *Escherichia coli* which were in enteric forms.

The observed toxicity of *C. odorata* in this study was probably due to the relative presence of the different toxic phytochemicals as corroborated by Kigigha *et al.*, (2013). Moreover, results obtained in this present investigation indicated that *C. odorata* produced a stable phenolic compound with active antimicrobial activity. On this background, the biotechnological potential of *C. odorata* in terms of production of phenolic compound that are inhibitive against phytopathogenic bacteria and fungi is noteworthy. *C. odorata* could serve as bio-protective agent and an alternative to synthetic fungicides against rot fungi of yam tubers.

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