An Evaluation Of Fungicidal Capacity Of Ethanol Extract Of *Vernonia amygdalina* (DELL) On Yam Tuber (*Dioscorea rotundata* Poir) Rot

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Abstract: The fungicidal activities of *Vernonia amygdalina* extracts were evaluated on the rot causing pathogens of yam tubers *in vitro*. The ethanol extracts of *V. amygdalina* were prepared and 1ml of the extract was dispensed into plate and allowed to solidify along with molten PDA. The isolated rot organisms: *Botryodiplodia theobromae, Aspergillus niger, Aspergillus flavus* and *Aspergillus glaucus* from rotten yam tubers were inoculated and incubated separately for 5days, inhibition was determined by measuring with metre rule along two perpendicular lines drawn at the bottom of the plate. The results showed that all the concentrations of the extracts had remarkable reduction effects on radial mycelial growth of the isolates. Ethanol extracts of 10% and 20% of *V. amygdalina* at 10g and 20g/100ml inhibited *A. niger* and *B. theobromae* by 92.95 and 97.14% respectively, similarly, ethanol extracts of 30% of *V. amygdalina* at 20 and 30g/100ml inhibited *A. flavus* by 100%. Ethanol extracts of 40% of *V. amygdalina* at 50g/100ml inhibited *B. theobromae* by 92.38 %, ethanol extracts of 50% of *V. amygdalina* at 50g/100ml inhibited *B. theobromae* by 90.48%. The results from this finding herald the appealing fortunes of a potentially viable antifungal agent of plant source.
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Key word: fungicidal, ethanol extract**,** *V. amygdalina,* tuber rot pathogens.

Introduction

Yam is a vital source of carbohydrate to man and animals; it contains a considerable amount of protein and vitamin C (Obilo *et al*., 2005, Oyelana *et al*., 2011). The countries that produce yam in Africa include: Nigeria, Benin, Togo, Ghana, Ivory Coast and Kenya. Nigeria alone produces three quarters of the world total output of yam, for instance, in 1980; Nigeria produced about 70% of all yam tubers (FAO, 2008).

Water yam is therapeutic to diabetic patients (Okigbo and Ogbonnaya, 2006). Industrially, yam can be processed to obtain alcohol; it is also processed into biscuits in some communities (Obilo *et al*., 2005). In Philippines, *Dioscorea alata* is prepared into most popular delicacies such as ice cream, jellies and candies (Burton, 1996).

Fungal infection of *D. rotundata* occurs from the farm land, after harvest and during storage as a result of several factors/agents like mechanical injury, natural openings, insect pest, bacteria and plant parasitic nematodes pathway (Undie and Akungbuwe, 1987). Fungi are abundantly present in the soil and are about 90% higher than other soil pathogens (FAO, 2001). In view of the havoc linked with inorganic chemicals, there is a need to develop alternative pesticides especially of plant origin to eradicate the menace of pathogenic rot, plant extracts are most effective and desirable alternatives for controlling rot disease of yam tuber (Amadioha and Obi, 1999). Plant extracts compared with agrochemicals are non-phytotoxic, easily biodegradable, specific, cheap, readily available and environmentally safer than the synthetic fungicides (Amadioha, 2000). Active principles in plants are influenced by plant age, extractant, extraction mode, and time of plant materials harvesting (Amadioha and Obi, 1999). The objectives of this research are to:

1 >determine the fungitoxic ability of the ethanol plant leaf extracts of *V. amygdalina.*

2 >find the concentration that is most effective for controlling yam tuber rot.

Materials And Methods

Collection of Plant Materials and Preparation

Healthy and rotten yam tubers with softness of tissues identified as being rotten were collected from a nearby market at Aba-Ebira, Ilokun, Ado-Ekiti. Fresh leaves of *V. amygdalina* were collected from forest reserve in Ado-Ekiti, rinsed with tap and distilled water, air dried at room temperature and ground separately. Each of 10, 20, 30, 40 and 50g of *V. amygdalina* powder was added to 100ml of ethanol separately. These were filtered using four-fold cheese cloth.

Isolation of Spoilage Fungi from Rotten Yam Tubers

Rotten yam tubers were washed in running water, rinsed in several changes of sterilized water, surface sterilized with 70% alcohol and cut open. About 3 pieces (3mm diameter) of the infected tissues were picked with a flamed forceps and inoculated on the sterilized solidified Potato dextrose agar (PDA) medium in different plates. The inoculated plates were incubated at room temperature and observed daily for emergence of fungal colonies, sub-culturing was done to obtain pure isolates. Stock cultures were prepared on (PDA) slants in McCartney bottles and stored in a refrigerator till needed for use.

Pathogenicity Test

Purchased healthy and rotten yam tubers were conveyed in a sterile polyethylene bags to the Microbiology laboratory of Ekiti State University, Ado-Ekiti. The tubers were washed with tap and distilled water. The surface of the rotten yams tubers were sterilized with 70% ethanol and left for ten minutes to dry before the isolation of the pathogens. Five millilitres cork borer was driven to a depth of 4mm into the healthy yam tubers and the bored tissues were removed. Four millilitres diameter disc of the pure culture of isolated and identified as *B. theobromae, A. niger, A. glaucus* and *A. flavus* each was placed in each hole respectively and the removed tissues were replaced back. The holes were sealed with candle wax. The control was set up in the same manner except that sterile agar disc replaced fungal inocula. The inoculated yam tubers were placed in 2 replications at room temperature under sterile condition. These were observed for fungal infection after 14days.

Effect of the extract on fungal mycelia growth

One mm of varied concentrations of *V. amygdalina* ethanol leaf extract was introduced into plate containing (PDA). Four mm diametre disc of pure culture of each isolate was placed on the medium containing the extract at the point of intersection of the two lines drawn on the bottom of the Petri dish. Control experiment was set up without adding plant extract to the pure culture of each isolate (Amadioha and Obi, 1999). The inhibition level of the extracts on the fungi was determined using the following formula by (Ijato, 2011) = $\frac{DC\ DT}{DC} \times 100$

Where: DC = average diameter of control

DT = average diameter of fungi colony with treatment

Results

The antimicrobial effects of 10% ethanol extract of *V. amygdalina* on rot fungi differed significantly ($p<0.05$) from the untreated control and standard. In addition, the efficacy of 10% ethanol extract of *V. amygdalina* on the rot organisms increased with the increase in concentration.

Effect of 10% ethanol leaf extracts of *V. amygdalina* on mycelial growth of fungal rot organisms

The antimycotic effects of 10% ethanol leaf extract of *V. amygdalina* on the fungal pathogens were presented in Table 1. The effects of 10% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml were inhibitive on *B. theobromae* ranging from 50.48% to 90.71%. The mycelial proliferation of *B. theobromae* was most retarded by 10% ethanol leaf extract of *V. amygdalina* at 50g/100ml to 90.71%, followed by 87.14% inhibition on *B. theobromae* by 10% ethanol extract of *V. amygdalina* at 40g/100ml. Antimicrobial capacities of 10% ethanol leaf extract of *V. amygdalina* at 30g and 20g/100ml manifested 85.74% and 85.48% on *B. theobromae.* The antimycelial capacities of 10% ethanol leaf extracts of *V. amygdalina* at 20-50g/100ml were not significantly different on *B. theobromae.*

Antimicrobial effects of 10% ethanol leaf extracts of *V. amygdalina* at 10-50g/100ml on *A. flavus* ranged from 75.19% to 86.90%. 10% ethanol leaf extract of *V. amygdalina* at 50g/100ml of was most effective on *A. flavus* by 86.90%, followed by 10% ethanol leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml, impeding the radial mycelial growth of *A. flavus* to 84.39%, 79.38% and 75.47% respectively, while the lowest antimycotic effect of 75.00% was elicited by 10g/100ml of 10% ethanol leaf extract of *V. amygdalina* against *A. flavus*. There were no significant differences in the antimicrobial capacities of 10% ethanol leaf extract of *V. amygdalina* at 10-30g/100ml on *A. flavus* but significantly different from the effects of 40g and 50g/100ml of 10% ethanol leaf extract of *V. amygdalina* on *A. flavus* the effects of 10% ethanol extract of *V. amygdalina* at 10-50g/100ml reflected portents fungicidal effects on *A. glaucus* ranging from 75.19% to 86.90%. The most antimycotoxigenic effect was exhibited by ethanol leaf extract of *V. amygdalina* at 50g/100ml of 10% against *A. glaucus* (86.90%), followed by 10% ethanol extract of *V. amygdalina* at 40g, 30g and 20g/100ml, eliciting 84.39%, 79.37% and 75.48% antirot property against *A. glaucus* respectively, while the least phytotoxic effect by 10% ethanol leaf extract of *V. amygdalina* at 10g/100ml against *A. glaucus* was 75.19%. There were no significant differences in the antimicrobial capacities of 10-30g/100ml of 10% ethanol leaf extract of *V. amygdalina* on *A. glaucus*. High fungicidal effects of 10% ethanol leaf extract of *V. amygdalina* at 10g-50g/100ml was exhibited on *A. niger* ranging between 73.54% and 92.95%. The antimicrobial activity of 10% ethanol leaf extract of *V. amygdalina* at

50g/100ml was greatest on *A. niger* by 92.95%, followed by 10% ethanol leaf extract of *V. amygdalina* at 40g and 30g/100ml, exhibiting inhibitory potential of 88.33% and 82.29% on *A. niger* respectively, 10% ethanol leaf extract of *V. amygdalina* at 20g/100ml induced fungicidal effect of 75.00% against *A. niger*.

Table 1: **Effect of 10% ethanol** leaf **extracts of *V. amygdalina* on mycelial growth of fungal rot organisms**

g/100ml of 10% alcohol	% inhibition of mycelial growth			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10	50.48[b]	75.19[b]	75.19[b]	73.54[c]
20	85.48[a]	75.47[b]	75.48[b]	75.00[c]
30	85.74[a]	79.38[b]	79.37[b]	82.29[b]
40	87.14[a]	84.39[a]	84.39[a]	88.33[a]
50	90.71[a]	86.90[a]	86.90[a]	92.95[a]
Standard	40.70[c]	30.20[c]	60.50[c]	50.00[d]
Control	0.00[d]	0.00[d]	0.00[d]	0.00[e]

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 *V. amygdalina* on mycelial growth of fungal rot organisms

The antimycotic effects of 30% ethanol leaf extracts of *V. amygdalina* on the fungal pathogens are presented in Table 2 30% ethanol leaf extract of *V. amygdalina*. antimycotic effects of 30% ethanol leaf extracts of *V. amygdalina* at 10-50g/100ml on *B. theobromae* ranged from 85.71% to 96.50%. The radial mycelial proliferation of *B. theobromae* (96.50%) was most reduced by 30% ethanol leaf extract at 50g/100ml, followed by 30% ethanol leaf extract at 40g/100ml, exhibiting antimycotic ability of 94.57% on *B. theobromae*. Also, 30% ethanol leaf extract of *V. amygdalina* at 30g and 20g/100ml elicited fungitoxic effects of 94.44% and 91.71% on *B. theobromae* respectively, *B. theobromae* was least inhibited by 30% ethanol leaf extract of *V. amygdalina* at 10g/100ml, evoking 85.71%.

Antimycotoxigenic effects of 30% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml on *A. flavus* ranged from 84.95% to 100%. The effects of 30% ethanol leaf extract of *V. amygdalina* at both 50g and 40g/100ml of most efficient on *A. flavus*, exerting 100.00%. Poisonous capacities of 30% ethanol leaf extract of *V. amygdalina* at 30g and 20g/100ml by 95.65% and 90.25% against *A. flavus* respectively. High fungicidal effects from 30% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml were showed on *A. glaucus* ranging from 91.67 to 94.67%. *A. glaucus* was the most inhibited by 30% ethanol leaf extract of *V. amygdalina* at 50g/100ml, evoking 94.67%, followed by exhibition of 94.44%, 92.67% and 91.75% against *A. glaucus* by 30% ethanol leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml of respectively.

Biocidal abilities of 30% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml exhibited ranges between 77.08% and 96.04% on *A. niger*. Highest antimycotic capacity of 96.04% was displayed against *A. niger* by 30% ethanol leaf extract of *V. amygdalina* 50g/100ml, followed by 93.75%, 93.50% and 87.58% on *A. niger* by 30% ethanol leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml respectively.

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

g/100ml of 30% alcohol	% inhibition of mycelial growth			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10	85.71^{c}	84.95^{c}	91.67^{a}	77.08^{b}
20	91.49^{b}	90.25^{b}	91.75^{a}	89.58^{a}
30	94.44^{ab}	95.65^{a}	92.67^{a}	93.50^{a}
40	94.57^{b}	100.00^{a}	94.44^{a}	93.75^{a}
50	96.50^{a}	100.00^{a}	94.67^{a}	96.04^{a}
Standard	40.70^{d}	30.20^{d}	60.50^{b}	50.00^{c}
Control	0.00^{e}	0.00^{e}	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

Effects of 50% ethanol leaf extracts of *V. amygdalina* on mycelial growth of fungal rot organisms

The phytotoxic effects of 50% ethanol leaf extracts of *V. amygdalina* on the fungal pathogens are presented in Table 3. The antimycotoxigenic potentials of 50% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml on *B. theobromae* ranged from 75.00% to 90.24%. The most phytotoxic effect of 50% ethanol leaf extract of *V. amygdalina* at 50g/100ml was elicited against *B. theobromae* (90.24%), followed by growth reduction effects of 90.23% by 50% ethanol leaf extract of *V. amygdalina* at 40g/100ml on *B. theobromae*. Also, 50% ethanol leaf extract of *V. amygdalina* at both 30g and 20g/100ml induced 87.38% on *B. theobromae*. The effects of 50% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml on *A. flavus* ranged from 74.92% to 88.85%. Phytotoxic potential of 50% ethanol leaf extract of *V. amygdalina* at 50g/100ml exhibited 88.85% inhibition on *A. flavus*, followed by 50% ethanol leaf extract of *V. amygdalina* at 40g and 30g/100ml of, expressing antimycelial efficacies of 85.23% and 79.09% on *A. flavus* respectively. The mycelial growth of *A. flavus* was reduced by 50% ethanol leaf extract of *V. amygdalina* at 20g/100ml on *A. flavus* to 76.31%.

High phytotoxic effects of 50% ethanol leaf extract of *V. amygdalina* at 10-50g /100ml on *A. glaucus* ranged from 81.11% to 90.00%. The most fungicidal exploit of 90.00% was against *A. glaucus* by 50% ethanol leaf extract of *V.* amygdalina at 50g/100ml, followed by induction of 87.22% on *A. glaucus* by 50% ethanol leaf extract of *V. amygdalina* at both 40g and 30g/100ml. Harmful effect of 50% ethanol leaf extract of *V. amygdalina* at 20g/100ml inhibited *A. glaucus* to 84.44% while the least of 10g/100ml of 50% ethanol leaf extract of *V. amygdalina* was exerted against pathogenic mycelial of *A. glaucus* (81.11%*)*

The antifungal indications of 50% ethanol leaf extract of *V. amygdalina* at 10-50g/100ml on *A. niger* ranged from 84.38% to 91.04%. Highest exhibited antimycotic value of 50% ethanol leaf extract of *V. amygdalina* at 50g/100ml was on *A. niger* (91.04%), followed by inhibitory potential of 90.21% against *A. niger* by 50% ethanol leaf extract of *V. amygdalina* at 40g/100ml. The mycelia growth of *A. niger* was impeded by 50% ethanol leaf extract of *V. amygdalina* at both 30g and 20g/100ml to 86.88% and 86.25% respectively.

Table 3: Effect of 50% ethanol leaf extracts of *V. amygdalina* on mycelial growth of fungal rot organisms.

g/100ml of 50% alcohol)	% inhibition of mycelial growth			
	B. theobromae	*A. flavus*	*A. glaucus*	*A. niger*
10	75.00[b]	74.92[c]	81.11[b]	84.38[b]
20	87.38[a]	76.31[bc]	84.44[ab]	86.25[ab]
30	87.38[a]	79.09[b]	87.22[a]	86.88[ab]
40	90.23[a]	85.23[ab]	87.22[a]	90.21[a]
50	90.24[a]	88.85[a]	90.00[a]	91.04[a]
Standard	40.70[c]	30.20[f]	60.50[f]	50.00[f]
Control	0.00[d]	0.00[g]	0.00[g]	0.00[g]

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 studies showed 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* that have been previously reported as rot pathogens of yam tubers, this tends to occur when infected tubers do not reveal visible external symptoms (Tedela and Ijato, 2013). The fungicidal activity of some plant extracts used for controlling different plant pathogens have been reported (Nahed, 2007, Nahunnaro, 2008). Investigations into the antifungal properties of *V. amygdalina, O. gratissimum, C. odorata, S. alata* among other extracts have been found to reduce the mycelial growth of *Erysiphe cichoracearum, Collectotrichum capsici* and *Protomycopsis phaseoli,* which compete favourably with the chemical pesticide benlate and ridomil. Ijato *et al;* (2011) reported significant inhibitory effect of neem (*A. indica*) and *O. gratissimum* extracts on mycelia growth of *Aspergillus niger* and *Rhizopus* spp respectively. The variation noted in the antifungal effects of the various concentrations of the extracts may be due to solubility of the active substances in ethanol and the presence of inhibitor (s) against fungicidal principles, this agreed with Amadioha (2000). In conclusion, it is always better to prevent tubers infection than seeking control. Therefore, varied concentrations ethanolextracts of *V. amygdalina* (10-50g/100ml) could serve as bio-protective agents against rot fungi of yam tubers. This is an alternative approach to synthetic fungicide as it is eco-friendly, economically viable and non-toxic to plants and animals. *V. amygdalina* is readily available for the peasant yam growers and marketers who may not be able to afford the cost of chemicals fungicides for the control of yam tuber rot.

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