Pesticidal Indications Of Crude Aqueous Leaf Extracts Of *Vernonia Amygdalina* (Dell*)* On Tuber Rot Microbes

Ijato, J. Y

Department Of Plant Science, Faculty Of Science, Ekiti State University, Ado- Ekiti P.M.B 5363, Ekiti State, Nigeria
E-mail: considerureternity@gmail.com, GSM: 08067335124

Abstract: Biological control approach of phyto-diseases on the field and in store (postharvest) is gaining attention as a practice in plant disease management system due to the negative resultant effects of synthetic chemicals application on the ecosystem as they accentuate in the plant tissue, thereby eliciting mutagenic, carcinogenic and teratogenic effects on man and animal. The aqueous leaf extract of *Vernonia amygdalina* was screened using poisoned medium technique for its fungitoxic effects on four isolated fungal rot microbes of yam tubers viz: *B. theobromae, A. niger, A. glaucus* and *A. flavus.* All the concentrations of both cold and hot extracts remarkably inhibited the mycelia extension of the rot pathogens. Forty grams in 100ml of hot water inhibited *B. theobromae* and *A. flavus* by 99.05% and 98.05% respectively. Also, as low as 10 and 20g in 100ml of cold water inhibited *A. flavus* to 95.82 %. In an era where consumers request chemical-free product and still want food devoid of corruptions as a result of microbial growth, toxins as well as other qualities-deteriorating factors, using aqueous leaf extract of *Vernonia amygdalina* to checkmate yam rot as an alternative to petrochemical-based fungicide, becomes an appreciable alternative for food security.
[Ijato, J. Y. **Pesticidal Indications Of Crude Aqueous Leaf Extracts Of *Vernonia Amygdalina* (Dell*)* On Tuber Rot Microbes.** *World Rural Observ* 2019;11(3):40-43]. ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). http://www.sciencepub.net/rural. 7. doi:10.7537/marswro110319.07.

Key word: *V. amygdalina,* fungitoxic, crude aqueous leaves extract, yam tuber rot pathogens

Introduction

Vernonia amygdalina commonly called bitter leaf belongs to the family *Astereacea,* it is a perennial shrub of 2-5m in height, and it grows quite well in humid environment in the tropical Africa. It has an elliptic leaves that are about 6mm in length; a characteristic odor, bitter taste and rough bark (Anyasor *et al*., 2010; Ijeh and Ejike, 2011). It thrives on a range of ecological zones and it is used as a hedge plant in some communities due to its drought tolerance ability (Uzoigwe and Agwa, 2011). In many parts of West Africa, the plant has been domesticated and being consumed (Okigbo and Nmeka, 2008, Mensah *et al.,* 2008). It is known as "Grawa" in Amharic, "Ewuro" in Yoruba, "Etidot" in Ibibio, "Onugbu" in Igbo, "Ityuna in Tiv, "Oriwo" in Edo and Chusadoki" in Hausa (Egedigwe, 2010). A wide array of phytochemicals of antifungal factors such as oxalates, phytates and tannins have been reported to be present in *V. amygdalina* (Ejoh, 2007; Eleyinmi, 2008).

Stigmastane-type saponins such as vernonioides A1, A2, A3, A4, B2, B3 C, D and E phytochemicals have been found abundantly in the leaves of *Vernonia amygdalina* some of the identified sesquiterpene lactones are vernolide, vernodalol (Erasto, 2006), vernolepin, vernodalin and hydroxyl vernolide (Ijeh and Ejike, 2011). Igile, *et al*., (1994 and 1995) reported the presence of the flavonoids, lutelin, luteolin 7-0-β-glucononiside and luteolin 7-0-β-glucoside, in the leaves of *V. amygdalina*. Other researchers have confirmed the presence of flavonoids in the plant (Ijeh and Ejike, 2011).

Other phytochemicals reported to be present in the leaves of *Vernonia amygdalina* are terpenes, coumarins, phenolic acids, lignans, xanthones and anthraquinones (Ejoh, 2007). Izevbigie (2003) reported the presence of bio-active peptides called edotides in the leaves of *V. amygdalina.*

These phytochemicals (and possibly some more yet to be identified) are believed to be responsible for bio-activities exhibited by the plant. These bio-active principles may act singly or synergistically to produce the results for which the medicinal values of *V. amygdalina* have been associated and vigorously studied (Melariri, *et al*., 2011).

Materials And Methods

Collection of Plant Materials and Preparation of Extracts

Healthy and rotten yam tubers with softness of tissue identified as being rotten were purchased from Aba-Ebira markets along Ekiti State University road, Ilokun, Ado-Ekiti.

Fresh leaves of *V. amygdalina* were collected from a residential compound at Ilokun, Ado-Ekiti, Ekiti State, and these were taken to herbarium unit, Department of Plant Science, Ekiti State University,

Ado-Ekiti for identity authentication and analysis. The leaves were rinsed with tap and distilled water, air-dried at room temperature, pulverized separately, powder weighed into 10-50g and added to 100ml of cold and hot water separately. This was then filtered with four fold of cheese-cloth and extract used.

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. 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 observations for emergence of fungal colonies were made daily, pure culture of the isolates was obtained. Stock cultures were prepared on slants in McCartney bottles and stored in a refrigerator for use.

Pathogenicity Test

Yam tubers were kept in sterile polyethylene bags and taken to the Microbiology laboratory of Ekiti State University, Ado-Ekiti. The tubers were washed with tap water. The surface of the infected yams was sterilized with 70% ethanol, washed with several changes of sterile distilled water and left to stand for ten minutes in other to dry before isolation of the pathogens. Five mm cork borer was driven to a depth of 4mm into the healthy yam tubers and the bored tissues were removed. Four mm diameter disc of the pure culture of *B. theobromae, A. niger, A. glaucus, A. flavus* each was cut and placed in each holes respectively and the earlier removed tissues were replaced back. The holes were sealed with petroleum jelly. The control was set up in the same manner except that sterile agar disc was used instead of fungal inoculums. The inoculated yam tubers were placed in two replicates at room temperature, under sterile condition. The inoculated yam tubers were observed for infection after 14, 21 and 32 days.

Effects of aqueous extract on fungal mycelia radial growth

The method of Amadioha and Obi (1999) was adopted to determine the effects of extracts on fungal growth. This involved creating four equal sections on each Petri dish by drawing two perpendicular lines at the bottom of the plates, the point of intersection indicated the centre of the plates. One mm of varied concentrations of cold and hot aqueous extract of *V. amygdalina* was separately introduced into Petri dish containing the media (PDA). A disc (4mm diameter) of the pure culture of each isolate was placed on the medium containing the extract just at the point of intersection of the two lines drawn at the bottom of the Petri dish. Control experiment was set up without plant extract.

The levels of toxicity of the extracts on the fungi (growth inhibition) were determined using the following formula by Okigbo and Ikediugwu, (2000).

$$\% \text{ growth inhibition} = \frac{DC - DT}{DC} \times 100$$

Where: DC = average diameter of control

DT = average diameter of fungi colony with treatment

Result

The pathogens isolated from rotten tissues of yam during storage were *B. theobromae, A. glaucus, A. flavus* and *A. niger*. The isolation of these pathogens corroborated the finding of Tedela and Ijato (2013), pathogenicity test showed that all the pathogens caused yam tuber rot. Rot was observed by softness of yam tissues. Data were collected on the basis of percentage inhibition of radial mycelial growth of rot organisms treated with hot and cold plant extracts. The phytotoxic effects of aqueous extract of *V. amygdalina* on fungal rot organisms differed significantly ($p<0.05$) from untreated control and standard. The efficacies of aqueous extracts of *V. amygdalina* on the rot pathogens increased with the increase in concentration.

Effects of aqueous leaf extract (hot and cold) of *V. amygdalina* on mycelial growth of fungal rot organisms

The antimycotic effects of both cold and hot water leaf extracts of *V. amygdalina* on the fungal pathogens are presented in Table 1. Hot water leaf extract of *V. amygdalina* at 10-50g/100ml was antimycelial on *B. theobromae* ranging between 88.57% and 99.05%. The highest mycelial reduction effect of hot water leaf extract of *V. amygdalina* at 50g/100ml was on *B. theobromae,* exhibiting 99.05%, followed by antifungal effects of 97.14%, 95.26% and 93.81% by hot water leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml against *B. theobromae.* The lowest biocidal value of hot water leaf extract of *V. amygdalina* at 10g/100ml was recorded against *B. theobromae* (88.57%). Cold water leaf extracts of *V. amygdalina* at 10-50g/100ml exhibited antimycotic potentials on *B. theobromae* ranging from 65.95% to 93.57%. The most phytotoxic capacity of cold water leaf extract of *V. amygdalina* at 50g/100ml was found against *B. theobromae* (93.57%). Also, antifungal effects of 86.19%, 85.71% and 84.76% were recorded against *B. theobromae* by cold water leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml respectively.

Hot water leaf extract of *V. amygdalina* at 10-50g/100ml indicated antifungal effects on *A. flavus*

ranging between 89.66% and 98.97%. The fungitoxicty of hot water leaf extract of *V. amygdalina* at 50g/100ml was highest on *A. flavus* (98.97%), followed by antifungal effects of hot water leaf extract of *V. amygdalina* at 40g/100ml inducing 97.99% against *A. flavus*, hot water leaf extract of *V. amygdalina* at both 30g and 20g/100ml caused 97.21% inhibition on *A. flavus*. Hot water leaf extract of *V. amygdalina* at 10g/100ml, evoked the least antimycotic effect on *A. flavus* (89.66%). Cold water extract of *V. amygdalina* at 10-50g/100ml inhibited biocidal effects on *A. flavus* ranged from 74.64% to 95.82%. Cold water extract of *V. amygdalina* at 50g/100ml had highest phytotoxic potential on *A. flavus* by causing 95.82% inhibition, followed by antimycelial capacity of 93.59% on *A. flavus* by cold water extract of *V. amygdalina* at 40g/100ml, cold water leaf extract of *V. amygdalina* elicited fungicidal effect of 91.36% against *A. flavus* at both 30g and 20g/100ml. Hot water leaf extracts of *V. amygdalina* at 10-50g/100ml exhibited high antimycotoxigenic effects on *A. glaucus* ranging from 59.72% to 96. 11%. The highest antifungal capacity of 96.11% was recorded against *A. glaucus* by hot water leaf extract of *V. amygdalina* at 50g/100ml, followed by 92.78% and 91.39% by hot water leaf extract of *V. amygdalina* at 40g and 30g/100ml on *A. glaucus* respectively, hot water extract of *V. amygdalina* at 20g/100ml retarded the radial mycelial growth of *A. glaucus* to 91.36%. Cold water leaf extracts of *V. amygdalina* at 10-50g/100ml expressed high biocidal effects on *A. glaucus* ranging between 85.83% and 90.28%. Cold water leaf extract of *V. amygdalina* at 50g/100ml performed most by causing 90.28% inhibition on *A. glaucus,* closely followed by cold water leaf extract of *V. amygdalina* at both 40g and 30g/100ml exhibiting 88.06% antimycotic effects on *A. glaucus.* Similarly, cold water leaf extract of *V. amygdalina* at 20g/100ml elicited microbecidal effect of 86.39% against *A. glaucus.* The lowest antiparasitic effect of 85.83% was elicited by 10g/100ml of cold water leaf extract of *V. amygdalina* on *A. glaucus.* Hot water leaf extracts of *V. amygdalina* at 10-50g/100ml reflected high antimycotic effects on *A. niger* ranging from 24.76% to 80.63%. *A. niger* was most inhibited by hot water leaf extract of *V. amygdalina* at 50g/100ml by 80.63%, followed by exhibition of antimycotic effects of 75.00%, 67.50% and 65.83% by hot water leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml against *A. niger* respectively. The antifungal effects of cold water leaf extracts of *V. amygdalina* at 10g-50g/100ml on *A. niger* ranged from 82.08% to 93.75%, antimicrobial effect of 93.75% was exhibited by cold water leaf extract of *V. amygdalina* at 50g/100ml on *A. niger,* followed by 92.29%, 87.84% and 85.42% by cold water leaf extract of *V. amygdalina* at 40g, 30g and 20g/100ml against *A. niger* respectively.

Conclusion

Varied concentrations of cold and hot water as well as ethanol extracts of *Vernonia amygdalina* extracts (10-50g/100ml) could serve as bio-protective agents against rot fungi of yam. These have potentials as alternatives to synthetic fungicides and pesticides. This method of plant disease control is eco-friendly, economically viable and not toxic to plants and animals. *Vernonia amygdalina* is readily available to the peasant yam growers and marketers that may not be able to afford the cost of chemicals fungicides.

References

1. Amadioha, A.C and Obi, V.I. (1999): Control of anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum*. *Acta Phytopathol Entomol. Hungerica*. 34(2): 85-89.
2. Anyasor, G.N, Ogunmenwo, K.O, Ogunowo, A.A and Alao-sanni, O (2010): Comparative antioxidant, Phytochemical and proximate analysis of aqueous and methanolic extract of *Vernonia amygdalina* and *Talium triangulare. Pakistan J. of Nutrition.* 9 (3): 259-264.
3. Egedigwe, C.A (2010): Effect of Dietary Incorporation of *Vernonia amygdalina* and *Vernonia colorata* in Blood Lipid Profile and Relative Organ Weights in Albino Rats. M.Sc. Dissertation Dept. Biochem., Michael Opara University of Agriculture Umudike, Nigeria.
4. Ejoh, R.A, Nkonga, D.V, Innocent, G and Moses, M.C (2007): Nutritional components of some none conventional Leafy Vegetables consumed in Cameroun. *Park. J. Nutri*; 6:712-717.
5. Eleyinmi, A.F, Sporns P, Bressler D.C (2008): Nut ritional Composition of *Gongronoma latifolium* and *Vernonia amygdalina. J.* of *Nutr. Food Sci;* 38:99-109.
6. Erasto, P, Grierson, D.S and Afolayan A.J (2006): Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina. J. Ethnopharmacol*; 100: 117-120.
7. Igile, G.O, Oleszek W, Jurzysta M, Burda, S, Fanfunsom and Fasanmade A.A (1994): Flavonoids from *Vernonia amygdalina* and their antioxidant Activities. *J. Agric. Food Chem*. 42:2445-2448.
8. Igile, G.O, Plaszek, W. Jurzysta, M, Aquino, R, De Tommasi, N and Pizza, C. (1995): Vernonioides D and E two novel Saponins from *Vernonia amygdalina. J. Nat. Prod.*, 58:1438-1443.

9. Ijeh, I.I and Ejike, E.C.C.C (2011): Current perspective on the medicinal potentials of *Vernonia amygdalina* Del. *J. of Med Pl. Res;* 5 (7): 1051-1061.
10. Izevbigie E.B (2003): Discovery of Water Soluble Anticancer agents (Edotides) from a vegetable found in Benin City, Nigeria. *Exp. Biol. Med*; 228: 293-298.
11. Melaariri, P, Campbell, W, Etusim, P and Smith, P (2011): *In vitro* and *in vivo* antiplasmodial activities of extract of *Vernonia amygdalina* Del leaves and *Cymbopogum citratus* Staph. *J of Nat. Prod.,* 4: 164-172.
12. Mensah, J.K, Okoli, R.I, Ohaju-obodo, J.O and Eifediyi, K (2008): Phytochemicals, nutrition and medical properties of some leafy vegetable consumed by Edo people. *Afr. J. Biotech.,* 7: 2304-2309.
13. Okigbo, R.N and Ikediugwu, F.E.O (2000): Studies on Biological Control of Post Harvest rot of Yam (*Dioscorea* spp) with *Trichoderma viride*. *J. Phytopathology*. 148:351-355.
14. Okigbo, R.N and Nmeka, E.C (2008): Antimicrobial effects of three tropical plant extracts on S*taphylococcus aureus*, *Eschericia coli* and *Candida albican. Afri. J. Tradit. Complement Altern. Med*., 5(3): 226-229.
15. Tedela, P.O and Ijato J.Y (2013): Antimicrobial activities of ethanol leaf extracts of *S. alata* Linn on post-harvest yam (*Dioscorea rotundata* Poir) tuber rot pathogens. *International Journal of Innovative Technology and Creative Engineering*. 3 (2): 2045-8711 .
16. Uzogwe, C.I and Agwa, O.K (2011): Antimicrobial activity of *Vernonia amygdalina* on selected urinary tract pathogens. *Afr., J. of Microbiol. Research*. 5(12): 1467- 1472.

9/25/2019