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Phytochemical Evaluation of the Insecticidal Potential of Some ethnobotanicals against Bean Weevil (*Acanthscelides obtectus*)

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Abstract: Based on ethnobotanical use as insecticidal plants in Ibadan, ethanol extracts of the leaves of *Sida acut*a Brum., *Nicotiana tabacum* L., *Annona muricata* L., *Gliricidia sepium*(Jacq.) Kunth*, Boerhaavia diffusa* Linn. and *Paullinia pinnata* L. were screened for secondary metabolites. The leaf powder of each plant was screened for antiinsecticidal activity against bean weevil (*Acanthscelides obtectus*). Phytochemical screening of the leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins and terpenoids being the highest quantity in most of the plants investigated. The highest total percentage mortality was observed with *A. muricata, N. tabacum* and *P. pinnata* (93%) followed by *S. acuta* and *G. sepium* (86%) while the least percentage mortality was recorded for *B. diffusa* (79%). The percentage mortality indicated that the plant samples caused significant mortality on the target insects and that the observed overall mortality also increased with increase in time intervals after treatment. All the plant samples proved effective although with slight differences in the mortality rate of the bean weevils. These results show that the plants tested should be incorporated into the grain protection practice of resource-poor farmers. However, there is need to ascertain the shelf life of the powders and if repeated application would be needed after a given period.

Key words: Screening · Secondary metabolites · Leaf samples · Antiinsecticidal activities · *Acanthscelides obtectus*

INTRODUCTION

Many of the reported indigenous plants have come under scrutiny, leading to extraction and characterization of their active constituents which accounted for various uses. The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins [1]. Bisht and Kama [2] observed that there is strong need to investigate their ability to be used as fungicides or insecticides. Phytochemicals derived from plant sources can act as larvicide, insect attractant and have different activities observed by many researchers [3].

Plants are considered as rich sources of bioactive chemicals and may be alternative source of insect control agents [4]. In view of the increasing demand for pest control agents, especially those that are effective, cheap and environmentally non-harzadous, crude plant extracts have played an important role in this aspect [5]. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticidal properties [6]. Although, insecticides of plant origin have extensively been used on agricultural pests, their use has been relatively very limited against insect vectors of public health importance [7].

Generally, the protection of stored grains from insect damage is currently dependent on synthetic pesticides [8]. The repeated use of synthetic insecticides for insect pest and vector control has disrupted natural biological control systems. It has also resulted in the resistance development, undesirable effect on target organism and fostered environmental and human health concern, which initiated a search for low cost, environmentally safe, indigenous control measures [8, 9 -11]. As some plants are used for their insecticidal properties by the indigenous people of Ibadan, Nigeria, the present study was undertaken. This was with a view to screening *Sida acuta, Boehaavia diffusa, Gliricidia sepium, Annona muricata, Paullinia pinnata* and *Nicotiana tabacum* leaf powder for their phytochemical constituents and ascertaining the insecticidal potential of their leaf powder.

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MATERIALS AND METHODS

Plant Materials: Fresh leaves of *Sida acuta, Boehaavia diffusa, Gliricidia sepium, Annona muricata, Paullinia pinnata* and *Nicotiana tabacum* were collected within the premises of Forestry Research Institute of Nigeria (FRIN). The plant materials were identified and authenticated at the herbarium (FHI) of the Institute.

The leaves were air-dried at room temperature (26°C) for 6 weeks, after which the samples were milled into uniform powder using an electric grinder. The samples were then taken to the laboratory for the analysis of the phytochemical constituents.

Methods of Phytochemical Screening: The processed materials were exposed 10 times to hexane for fat removal. After this procedure, materials were kept under hexane for 24 hours, filtered and left for 5 to 7 days to allow the residual hexane to evaporate. The materials were covered with ethanol and maintained for 24 hours. Chemical tests were carried out on the milled leaves for the qualitative determination of the phytochemical constituents using standard procedures to identify constituents as described by Harbone [12] and Trease and Evans[13] and Sofowora [14].

Qualitative and Quantitative Analysis of the Plant Samples: Forty ml of water was added to 1g of sample and kept in the oven at 100°C for 15 minutes. Four ml of petroleum ether was added to 1ml of extract above and 3ml of concentrated H_2SO_4 was also added. A reddish brown colouration of the interface indicated the presence of terpenoids.

For the quantitative test, 1g of sample was weighed into 10ml of petroleum ether and allowed to extract for 15 minutes. It was then filtered and the absorbance at a wave length of 420µm was read in a spectrophotometer [14].

Test for Flavonoids: Twenty five ml of water was added to 1g of sample and kept in the oven for 100°C for 15 minutes. Two ml extract + 5 ml of NH_4OH + 1 ml concentrated H_2SO_4 were added together. A yellow colouration indicated presence of flavonoids [12].

For the quantitative test, 10ml of 80% methanol was used to extract 1g of sample and left for 2 hours. It was filtered and weighed in a Petri dish and left to dry in the oven at 40°C. The Petri dish was weighed when it has dried to constant weight [12].

Test for Tannins: Twenty five ml of water was added to 1g of sample kept in the oven at 100°C for 15 minutes. Ten ml of water was added to 1 ml extract and was boiled. Few drops of 0.1% $FeCl_3$ were added and observed for green or blue-black colouration indicating the presence of tannins [13].

For the quantitative test, 1g of the sample was weighed and extracted with 25ml of the solvent mixture of 80:20 acetones and poured into 10% glacial acetic acid for 5 hours. It was then filtered and the absorbance was measured at 500µm. the absorbance of the reagents blank was also measured. A standard graph was made with 10, 20, 30, 40, 50mg/ 100g of tannic acid. The concentration of tannin was read and dilution factor was taken into consideration [13].

Test Alkaloids: Twenty five ml of water was added to 1g of sample and kept in the oven at 100°C for 15 minutes. 1 ml of extract was added to 10 ml acid ethanol and filtered. To 5ml of the filtrate were added 2ml of NH_4OH and 5 ml of petroleum ether and then shaken, separated in separating funnel. 10 ml acetic anhydride was added to petroleum ether layer. Dragendorff and Meyer reagents were added to each portion. Intense colour formation indicated the presence of alkaloids.

For the quantitative test, 1g of sample was weighed and 20ml of 100% acetic acid in ethanol was added. It was shaken and allowed to stand for 4 hours and then filtered. The filtrate was evaporated to a quarter of its original volume. One drop of concentrated Ammonia was added. The precipitate formed was filtered through a Whatman (W1) filter paper. The filter paper was weighed after it has dried to constant weight (W2)

$$\%\text{Alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

One gram of sample was weighed and 15ml and ethanol was added and placed in a water bath at 55°C for 4 hours. It was filtered and the residue was washed with 20% ethanol twice. The extract was reduced to about 5ml in the oven. 5 ml of petroleum ether was added to the concentrated extract inside a separating funnel. The petroleum ether layer was discarded and 3ml of butanol added to it and washed with sodium chloride. The butanol was later poured into a weighed Petri dish. It was then placed in the oven to evaporate to dryness and the residue was weighed [13].

Test for Saponins: Sample was boiled in water and filtered. 10ml of the filtrate was shaken vigorously and observed for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. The presence of emulsion of the oil indicated the presence of saponins [14].

For the quantitative test, 1g of sample was weighed and 15ml ethanol was added and was placed in a water bath at 55°C for 4 hours. It was filtered and the residue was washed with 20% ethanol twice. The residue was reduced to about 5 ml in the oven. 5ml of petroleum ether was added to the concentrated extract inside a separating funnel. The petroleum ether layer was discarded and 3 ml of butanol was added to it and washed with 5ml of 5% sodium chloride. The butanol was later poured into a weighed Petri dish and placed in the oven to evaporate to dryness and the residue was weighed [14].

Collection and Culturing of Adult Bean Weevils: Adult bean weevils were collected and cultured from infested stored beans obtained from Dugbe market in Ibadan, Nigeria. The culturing was done on whole grain of bean for *Acanthscelides obtectus*. Freshly emerged adults of *A. obtectus* from the laboratory cultures were then taken and used for the experiment.

Methods of Treatment: Leaf powder of the six plant samples (15 g each) was thoroughly admixed with 60 g disinfested food materials (bean seeds) contained in separate plastic dishes with lid. Fifteen newly emerged beans weevils were introduced into each plastic dish containing beans seeds and replicated three times. The control which was void of the plant powder also had 15 newly emerged adult beans weevil introduced.

Period of Observation: Mortality rate of the adult bean weevils was recorded at 24, 48 and 72 h, respectively for each treatment and recorded accordingly; the mean percentage mortality of *A. obtectus* was then calculated.

RESULTS AND DISCUSSION

As shown in Tables 1 and 2, it is observed that alkaloids are present in trace amounts in *A. muricata* and present in moderate amount in *G. sepium*. Flavonoid is present in appreciable amount in all plant samples except in *P. pinnata* and *B. diffusa* where it is present in moderate amount. Saponin is present in moderate amounts in *P. pinnata*, *S. acuta* and *G. sepium* while it is present in trace amounts in *A. muricata*, *B. diffusa* and *N. tabaccum*. It is also observed that tannins and terpenoids are also present in appreciable amount in all the plant samples.

The presence of terpenoids in all the plant samples is quite significant. According to Khalid *et al*. [15], the presence of terpenoids in a plant enables such a plant to have high toxicity against an insect pest. The high levels of terpenoids observed in the present study may account for the high insecticidal activity of the plant samples.

The use of indigenous plant products and other locally available materials to protect stored cereals and legumes have been reported by some workers [16-18].

The present study has shown that the leaf powders of the plants tested were effective against the bean weevil *Acanthscelides obtectus*. The percentages of mortality shown differed with the plant species, the highest total percentage mortality was observed with *A. muricata, N. tabacum* and *P. pinnata* (93%) followed by *S. acuta* and *G. sepium* (86%) while the least percentage mortality was recorded for *B. diffusa* (79%). This result agreed with the report of Asawalam *et al.* [19] who reported that the leaf powder of *N. tabacum* was effective in killing adult insect of *Sitophilus zeamais* as the maize grains were treated with the powder. It was also reported that 2.0 g of *N. tabacum* leaf powder applied on 50 g of maize grains resulted in 100% mortality of *S. zeamais* [20].

Overall mean mortality of *A. obtectus* also increased with increase in time intervals after treatment. This was observed with slight differences in the mortality rate for the different plant applications as shown in Table 3.

Table 1: Qualitative analysis of the plant samples

Parameters	*Annona muricata*	*Paullinia pinnata*	*Sida acuta*	*BoerhaaviaDiffusa*	*Nicotiana tabacum*	*Gliricidia Sepium*
Alkaloids	+	++	++	++	++	+++
Flavoniods	+++	++	+++	++	+++	++++
Saponins	+	++	++	+	+	++
Tannins	+++	++++	++++	+++	+++	++++
Terpenoids	++++	++++	++++	+++	++++	+++

KEY:

+ ----	Presence in trace amount
++ ----	Presence
+++ ---	Moderately present,
++++--	Abundant

Table 2: Quantitative analysis of the plant samples

Parameters	*Annona muricata*	*Paullinia pinnata*	*Sida acuta*	*Boerhaavia Diffusa*	*Nicotiana tabacum*	*GliricidiaSepium*
Alkaloids (mg/100g)	373.4	600.0	548.4	445.0	633.4	740.0
Flavoniods (mg/100g)	296.7	715.0	828.4	775.0	1333.4	1528.4
Saponins (mg/100g)	220.0	360.0	426.7	71.6	131.7	533.4
Tannins (mg/100g)	963.4	1646.7	1251.7	1171.7	1226.7	1106.4
Terpenoids (mg/100g)	1651.7	1720.0	4310.0	1133.4	1822.7	1126.7

Table 3: Mean percentage mortality of *A. obtectus*

Treatment	24 h	48 h	72 h	Total
Control	0.0	0.0	13.3	13.3%
S. acuta	13.3	33.3	40.0	86.8%
A. muricata	13.3	26.6	53.3	93.2%
N. tabacum	20.0	26.6	46.6	93.2%
G. sepium	13.3	33.3	40.0	86.3%
B. diffusa	13.3	26.6	40.0	79.9%
P. pinnata	6.66	40.0	46.6	93.3%

Although the insecticidal activity of the different plants could be attributed to their phytochemical constituents, the mortality could also be due to another reason. The plant powders probably contributed to death as a result of physical barrier effect of the leaf powders. This is because the powder could block the spiracles of insects, thus impairing respiration. *N. tabacum* was reported to possess contact, stomach and respiratory poisoning properties attributed to the active constituent nicotine [17].

CONCLUSION

This study revealed that all the plants tested possess insecticidal properties and they may be potentially useful in the controll of *Acanthscelides obtectus*. the present study is a contribution to sourcing low cost and natural alternative insecticides from plants. However further studies will determine the shelf life of the powders to find out if repeated application is needed after a given period.

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