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Efficacy of extracts of some aromatic medicinal plants on cowpea bruchid, *Callosobruchus maculatus* in storage

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Abstract

The aqueous and the ethanoic extracts of four aromatic medicinal plants, *Eugenia caryophyllus* (Sprengel) Bullock et Harrison, *Bryophyllum pinnatum* (Lamarck) Oken, *Eucalyptus camaldulensis* Dehnhardt and *Xylopia aethiopica* (Dunal) A. Rich were tested for their protectant abilities on cowpea seeds and their toxicity on cowpea bruchid, *Callosobruchus maculatus* (F.). At the doses of 1 ml, 2 ml and 5 ml of the extracts per 25 g of cowpea seeds, the aqueous extracts of the plants could not effectively control the weevils. At higher doses of 5 ml the aqueous plant extracts could induced insect mortality as follow: 71.21 ± 0.25% by *E. caryophyllus*, 81.42 ± 0.25% by *B. pinnatum*, 80.00 ± 0.23% by *E. camaldulensis* and 100.00 ± 0.00% by *X. aethiopica*. The ethanoic extracts of *E. caryophyllus* caused 80.28 ± 0.11%, 100.00 ± 0.00% and 100.00 ± 0.00% insect mortality at 1 ml, 2 ml and 5 ml doses respectively within 7 days. Likewise, the ethanoic extracts of *B. pinnatum* caused insect mortality of 42.36 ± 0.30%, 100.00 ± 0.00% and 100.00 ± 0.00% within 7 days while *E. camaldulensis* gave 53.70 ± 0.24%, 74.27 ± 0.22% and 100.00 ± 0.00% and *X. aethiopica* gave 80.10 ± 0.50%, 100.00 ± 0.00% and 100.00 ± 0.00% insect mortality.

The seed losses in 4 weeks observed from the aqueous extract treated seeds ranged from 2.50 ± 0.13 by *E. caryophyllus* to 32.50 ± 0.50 by *X. aethiopica*. The seeds treated with ethanoic extracts of *E. caryophyllus* gave no seed loss at all doses used while the highest doses of 5 ml also gave no seed losses by *B. pinnatum* and *E. camaldulensis.* The seed loss recorded at lower doses ranged from 0.63 ± 0.02% by *B. pinnatum* to 12.63 ± 0.10% by *E. camaldulensis.*

Key words: ethanoic extracts, egg deposition, *Callosobruchus maculatus*, cowpea, seed losses.

Introduction

Cereals (maize, rice and sorghum) and pulses (cowpea, soybeans and groundnuts) are the most important sources of food to man, animals and they are important sources of revenue in tropical countries. These farm produce are bedevilled by insect pests. The major constraints facing effective and efficient storage of cereal grains are the insect and vertebrate pests (Adedire, 2001). Heavy losses of food crops of over 50% have been found to occur during storage while the overall global loss of 10% have been observed in cereals and legumes (De Lima, 1987). This problem is further aggravated by the simple reason that the main bulk of cereal grain production and storage in the third world is in the hands of small-scale farmers, who have little or no resource to procure adequate chemicals and facilities for protection against pests.

Stored farm produce have been known to be targets of various pests, which caused severe quantitative and qualitative losses (Thanthianga and Mitchell, 1990). The bruchid beetle is the most notorious insect pest of cowpea, which starts its attack right from the field prior to harvest to storage where the insect population is built up to damaging levels (Patnaik *et al*., 1986; De Lima, 1987).

Many studies have been conducted to show that plant products are very effective in protecting stored legumes from damage by bruchids and storage fungi (Don-Pedro, 1990; Huis, 1991; Lale, 1994). Ofuya *et al*. (1992) reported that insecticidal plant powders may be more potent in the form of extract using an appropriate solvent. The aromatic medicinal plants used in this work have been used extensively in treating chronic piles, headaches and cough in man by local herbalists, but no work has been done to determine whether these plants have insecticidal properties. It is against this backdrop that this work was done to find out the insecticidal and the protective abilities of the plants. Thus this paper reports the effects of the aqueous and ethanoic extracts of 4 selected plants on cowpea bruchid: *Callosobruchus maculatus* (F.) (Coleoptera Chrysomelidae Bruchinae).

Materials and methods

Insect culture

The insects used in this work were obtained from naturally infested cowpea seeds bought from Oba's Market in Ado-Ekiti, Ekiti State of Nigeria. The insects were raised on uninfested Kano local white cultivar of cowpea that had been sterilized in an oven at 40 °C for 4 h (Santhoy and Rejesus, 1975). Subsequent insects were drawn from this culture.

Preparation of aqueous and ethanoic extracts of the tested plants

The plant parts (table 1) were bought from the market, and their identities were confirmed at the Department of Plant Science and Forestry herbarium, University of Ado-Ekiti, Nigeria. The selected plant parts and parts were dried under the sun. The plant parts were pulverized separately with an electric blender and passed through a sieve with the mesh size of 0.25 mm. Each powdered material was kept separately in an airtight container until required. One gramme of each sample separately extracted was dissolved in 10 ml of hot water and left for 24 h to infuse. The filtrates were separated from the mixtures by passing the solution through a sieve with a mesh size of 0.05 mm. The same method was used to obtain the ethanoic filtrate of the plants.

Toxicity assay

One millilitre of the filtrate was admixed with 25 g of sterilized, uninfested Kano local white variety of cowpea and tumbled for 10 minutes to ensure thorough mixing. The cowpea seeds were then oven-dried at 40 °C for 4 h as reported by Santhoy and Rejesus (1975) before being kept with 10 newly emerged adults (5 copulating pairs) of *C. maculatus* in rearing boxes (12x10x8 cm). Nylon mesh was used to cover the set up and the mesh was held in place with rubber bands. The same procedure was adopted for the doses of 2 ml and 5 ml per 25 g of cowpea. In the control trials, uninfested cowpea seeds were oven dried as reported by Santhoy and Rejesus (1975). Then only 1 ml or 2 ml or 5 ml of water or ethanol was separately mixed with 25 g of the seeds and tumbled for 5 min. to ensure thorough mixing. The cowpea seeds were then oven-dried at 40 °C for 4 h. Ten newly emerged adults (5 copulating pairs) of *C. maculatus* were introduced into the rearing boxes together with the seeds in each treatment and covered with nylon mesh. The mesh was held in place with rubber bands. Each of the experiments was performed in triplicate. The experiments were monitored for 7 days. Data were collected daily on insect mortality. Percentage mortality was calculated using the following formula:

$$\% \text{ mortality} = \frac{\text{No of dead insects x 100}}{\text{Total number of insects}}$$

Seed loss assay

The assays were maintained at room temperature (i.e. 25 ± 5 °C) in laboratory and prepared as explained above. Then 5 copulating pairs of adult weevils were introduced into each of the boxes and left for a week to allow egg deposition. After egg deposition, adult insects were removed from the boxes and the development of the eggs was monitored for another 4 weeks. In the control trials, ethanol alone was used to treat cowpea seed at the doses of 1 ml, 2 ml and 5 ml before 5 copulating pairs of the weevils were introduced into the boxes. The controls were also left for a week to allow egg deposition before adult weevils were relocated. The developments of the eggs were monitored for another 4 weeks. Each of the experiments was performed in triplicate. At the end of the fourth week, the components of each box were poured in a dish and cowpea seeds were sorted out of the plant powders. The seeds were further sorted into those with developing eggs or insects or holes and those without. Those having developing eggs/insects/holes were weighed on an analytical scale. The percentage seed loss was calculated by using the following formula:

$$\% \text{ seed loss} = \frac{\text{Weight of seeds with eggs or insect or holes x 100}}{\text{Initial weight of seeds}}$$

Table 1. List of plant species and parts tested for toxicity against *C. maculatus*.

Plant species	Family	Parts used
Eucalyptus camaldulensis	Myrtaceae	Leaves
Eugenia caryophyllus	Myrtaceae	Fruits
Bryophyllum pinnatum	Crassulaceae	Leaves
Xylopia aethiopica	Annonaceae	Fruits

Results

Toxicity assay

The effects of the aqueous and the ethanoic extracts of the tested plants on mortality of the cowpea bruchid, *C. maculatus,* at different doses are presented in tables 2 and 3, respectively. Aqueous extract of *Eugenia caryo-*

Table 2. Insect mortality percentage in aqueous extract treated seeds of cowpea within 7 days.

Plants	% mortality within 7 days		
	1 ml	2 ml	5 ml
Eugenia caryophyllus	42.50 ± 0.10	50.00 ± 0.15	71.21 ± 0.25
Bryophyllum pinnatum	50.56 ± 0.31	52.03 ± 0.20	81.42 ± 0.25
Xylopia aethiopica	71.65 ± 0.11	100.00 ± 0.00	100.00 ± 0.00
Eucalyptus camaldulensis	40.10 ± 0.21	51.34 ± 0.32	80.00 ± 0.23
Control (water)	0	0	0

Each value is a mean of triplicate ± standard error of the mean.

Table 3. Insect mortality percentage in ethanoic extract treated seeds of cowpea within 7 days.

Plants	% mortality within 7 days		
	1 ml	2 ml	5 ml
Eugenia caryophyllus	80.28 ± 0.11	100.00 ± 0.00	100.00 ± 0.00
Bryophyllum pinnatum	42.36 ± 0.30	100.00 ± 0.00	100.00 ± 0.00
Xylopia aethiopica	80.10 ± 0.50	100.00 ± 0.00	100.00 ± 0.00
Eucalyptus camaldulensis	53.70 ± 0.24	100.00 ± 0.00	100.00 ± 0.00
Control (ethanol)	0	0	0

Each value is a mean of triplicate ± standard error of the mean.

phyllus (Sprengel) Bullock et Harrison at 1 ml, 2 ml and 5 ml doses gave 42.50 ± 0.10%, 50.00 ± 0.15% and 71.21 ± 0.25% insect mortality respectively within 7 days. Aqueous extract of *Bryophyllum pinnatum* (Lamarck) Oken at 1 ml, 2 ml and 5 ml doses resulted in 50.56 ± 0.31%, 52.03 ± 0.20% and 81.42 ± 0.25% insect mortality respectively within 7 days. Insect mortality of 40.10 ± 0.21%, 51.34 ± 0.32% and 80.00 ± 0.23% were observed in aqueous extract of *Eucalyptus camaldulensis* Dehnhardt within 7 days. While no death was observed in the control within 7 days, 71.65 ± 0.11%, 100.00 ± 0.00% and 100.00 ± 0.00% were obtained with aqueous extracts of *Xylopia aethiopica* (Dunal) A. Rich at 1 ml, 2 ml and 5 ml dosages respectively within 7 days.

Ethanoic extracts of *E. caryophyllus* gave 80.28 ± 0.11%, 100.00 ± 0.00% and 100.00 ± 0.00% insect mortality at doses of 1 ml, 2 ml and 5 ml respectively within 7 days. Insects mortality was higher in ethanoic extracts of *B. pinnatum* resulting in 42.36 ± 0.30%, 100.00 ± 0.00% and 100.00 ± 0.00% deaths at 1 ml, 2 ml and 5 ml doses respectively. At the same doses ethanoic extracts of *E. camaldulensis* gave 53.70 ± 0.24%, 100.00 ± 0.00% and 100.00 ± 0.00% insect mortality, respectively within 7 days, while *X. aethiopica* gave insect mortality of 80.10 ± 0.50%, 100.00 ± 0.00% and 100.00 ± 0.00%. In the control, no dead insect was observed.

Seed loss assay

The results of aqueous extracts against seed losses are shown in table 4. Massive deposition of eggs was observed on both treated and untreated cowpea seeds. At the doses of 1 ml, 2 ml and 5 ml, aqueous extracts of *E. caryophyllus* gave seed losses of 11.25 ± 0.10%, 6.25 ± 0.15% and 2.50 ± 0.13% respectively in 4 weeks. *B. pinnatum* gave 26.88 ± 0.82%, 24.38 ± 0.70% and 8.75 ± 0.71% at 1 ml, 2 ml and 5 ml doses respectively within 4 weeks. Seed losses observed in *E. camaldulensis* at the same doses are 25.00 ± 1.00%, 11.25 ± 0.90% and 10.00 ± 0.85% respectively within 4 weeks. The highest seed losses were observed in *X. aethiopica* at 1 ml (32.50 ± 0.50%) and 2 ml (28.73 ± 0.42%) doses respectively within 4 weeks. The seed loss reported for *X. aethiopica* at 5ml dose was 19.38 ± 0.51% while the control gave 62.50 ± 0.10%.

The results of ethanoic extracts against seed losses are shown in table 5. At all doses, *E. caryophyllus* conferred adequate protection on the seeds, thus no seed loss was observed during the duration of the experiment. While no seed loss was observed at the highest dose of 5 ml, *B. pinnatum* gave 1.88 ± 0.05% and 0.63 ± 0.02% at 1 ml and 2 ml doses respectively in 4 weeks. The highest seed loss of 12.50 ± 0.10% was obtained in the seed treated with *E. camaldulensis* at 1 ml dose while 2 ml dose gave 6.25 ± 0.40%. No seed loss was observed at the highest dose of 5 ml in 4 weeks. *X. aethiopica* gave seed losses of 9.38 ± 0.41%, 4.38 ± 0.55% and 1.25 ± 0.40% at 1 ml, 2 ml and 5 ml dosages in 4 weeks. The seed losses observed in the control experiment gave 50.63 ± 0.50%, 49.50 ± 0.48% and 39.74 ± 0.47% at the same doses of ethanoic treatments respectively in 4 weeks.

Discussion

This study has shown that the aqueous and the ethanoic extracts of the tested plants are good insecticides. Their actions on tested insects can be attributed to the active components inherent in these plants. The best results obtained from the aqueous extract were observed in *X. aethiopica* at 2 ml and 5 ml doses where 100.00 ± 0.00% of the insect died within 7 days. The results of higher doses of *E. caryophyllus, B. pinnatum* and *E. camaldulensis* show that the plants are very good as insecticides because they adequately controlled the bruchid. Ethanoic extracts of the tested plants performed better than water extracts as they caused higher mortal-

Table 4. Seed loss percentage in aqueous extract treated seeds of cowpea within 4 weeks.

Plants	% seed loss within 4 weeks		
	1 ml	2 ml	5 ml
Eugenia caryophyllus	11.25 ± 0.10	6.25 ± 0.15	2.50 ± 0.13
Bryophyllum pinnatum	26.88 ± 0.82	24.38 ± 0.70	8.75 ± 0.71
Xylopia aethiopica	32.50 ± 0.50	28.73 ± 0.42	19.38 ± 0.10
Eucalyptus camaldulensis	25.00 ± 1.00	11.25 ± 0.85	10.00 ± 0.85
Control (water) 62.50 ± 0.10			

Each value is a mean of triplicate ± standard error of the mean.

Table 5. Seed loss percentage in ethanoic extract treated seeds of cowpea within 4 weeks.

Plants	% seed loss within 4 weeks		
	1 ml	2 ml	5 ml
Eugenia caryophyllus	0	0	0
Bryophyllum pinnatum	1.88 ± 0.05	0.63 ± 0.02	0
Xylopia aethiopica	9.38 ± 0.41	4.38 ± 0.55	1.25 ± 0.40
Eucalyptus camaldulensis	12.50 ± 0.10	6.25 ± 0.40	0
Control (ethanol)	50.63 ± 0.50	49.50 ± 0.48	39.74 ± 0.47

Each value is a mean of triplicate ± standard error of the mean.

ity of the insects within 7 days. Both lower and higher doses of the extracts controlled the weevils with the exception of *B. pinnatum* and *E. camaldulensis* which at 1 ml caused the mortality of 42.36 ± 0.30% and 53.70 ± 0.24% respectively. Some medicinal plants have been reported to show insecticidal properties and they have variously been used as crop protectants by various workers (Adedire and Lajide, 1999; Ashamo and Odeyemi, 2001; Omotoso, 2004). Extractions of insecticidal plant powder with appropriate solvents have been observed to often concentrate the active materials and make their potency readily detectable by pests (Benner, 1993; Makanjuola, 1989). Ofuya *et al*. (1992) demonstrated that crude ether extract of seeds of *Monodora myristica* (Gaertner) Dunal is far more effective as legume seed protectant against *C. maculatus* infestation than the ground seeds. The higher mortality of ethanoic extracts over aqueous extracts are in accordance with the findings of Aku *et al*. (1998) who also reported a higher toxicity of extracts of *Annona senegalensis* Persoon root bark on *C. maculatus*.

The best results of the aqueous extracts were observed in *E. caryophyllus* at 5 ml (2.50 ± 0.13%) and 2 ml (6.25 ± 0.15%) doses. The results of the seed loss assays show that ethanoic extracts performed better than aqueous extracts. *E. caryophyllus* gave no seed losses at all doses. Higher doses of *B. pinnatum* and *E. camaldulensis* also gave no seed losses. The losses recorded in lower doses of these plants ranged from 0.63 ± 0.02% to 12.50 ± 0.10%. These results are still better than the results of the control which ranged from 39.74 ± 0.47%-50.63 ± 0.50%. The massive egg deposition observed in both treated and untreated seeds show that the plant extract had no effect on egg deposition by the bruchid. Similar observations have been reported in *Zingiber officinale* Roscoe and *X. aethiopica* treated seeds of cowpea by Ofuya (1990). The reduced seed losses observed in ethanoic extract treated seeds is a reflection of the ovicidal activity and the egg development inhibition properties of the plant extracts. *Caryedon serratus* Olivier) suffered reduced egg laying ability and great egg mortality when exposed groundnuts that have been treated with some plant materials (Delobel and Malonga, 1987). The ovicidal properties of the powders of *Cymbopogon citratus* (de Candolle) Stapf and *Ocimum gratissimum* L. against the eggs of *C. maculatus* have been reported by Ofuya (1990). Lale (1995) reported that the mechanisms of action of plant powder include their toxicity to adults, reduction of oviposition, ovicidal activity and toxicity to immature stages of the insect.

An appreciable level of insect mortality and control of seed losses were achieved with the plant extracts especially the ethanoic extracts. However, further trials are recommended to determine the possibility of using the plants extracts in controlling other insect pests of farm produce.

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