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Insecticidal Activity of the Aqueous Extracts of Four Under-utilized Tropical Plants as Protectant of Cowpea Seeds from *Callosobruchus maculatus* Infestation

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Abstracts: The test plants species, namely *Crotaria retusa*, *Hyptis suaveolens*, *Ricinus communis* and *Tithonia diversifolia* were extracted with water. The extracts were evaluated on *Callosobruchus maculatus* for mortality, oviposition and adult emergence effects. The long-term protectant ability and viability were also investigated. The results showed that the aqueous extracts from *T. diversifolia* were most effective on *C. maculatus*, followed by extract from *Ricinus communis*. The least potent extracts were those extracted from *Crotalaria retusa* and *Hyptis suaveolens*. Also, the extracts considerably reduced oviposition by *C. maculatus*. Extracts from *T. diversifolia* and *R. communis* drastically reduced infestation and subsequence damage of the treated cowpea seeds for a period of three months. Most of the treated seeds germinated after 90 days storage period. The results from this study revealed that aqueous extracts from all the four plants species were effective in controlling cowpea bruchid, *C. maculatus* and could serve as an alternative to synthetic insecticides for protection of stored cowpea seeds against bruchids.

Key words: Insecticidal activity, aqueous extracts, Cowpea, *Callosobruchus maculatus*

INTRODUCTION

The cowpea storage bruchid, *Callosobruchus maculatus* is a serious field-to-store pest of cowpea seeds, *Vigna unguiculata* in the tropic where the crop is cultivated and utilized as a cheap dietary protein source for human and livestock. The weevils prefer dried cowpea seeds but will attack other beans and peas in storage.

C. maculatus infestation normally starts with female laying eggs on ripening cowpea pods in the field (Casewell, 1976). During inclusion, the larva burrow through the chorion of the egg, directly into the pod wall and then into the seed, where the larvae develop and pupate (Prevett, 1961; Booker, 1967; Caswell, 1984). The larva stage causes considerable damage to cowpea seed in the store through the consumption of the cotyledon and then the embryo. Also, the seed may be contaminated by insect fragments, excreta and chemical secretions (Howe, 1965).

The destructive activities of insects and other storage pests have been adequately subdued by synthetic insecticides. The problems of many synthetic insecticides include high persistence, poor knowledge of application, cost, non-availability, genetic resistance and hazard to health (Sighamony *et al*., 1986; Adedire and Ajayi, 1996).

Attention is currently being focused on the use of natural compounds of plant origin for stored crop protection because they are readily biodegradable, often of low mammalian toxicity and thus pose no danger to the environment if used in small amount (Keita *et al*., 2001). The use of plant derived bio-pesticides such as plant crude extracts and oils that are relatively cheaper and ecologically more tolerable than convectional chemical pesticides are being encouraged among resource-poor farmers in developing nations (Okosun and Adedire, 2010).

The purpose of this investigation is to evaluate the insecticidal activities of the aqueous extracts from some under utilized plants as protectant of cowpea seeds from *C. maculatus* infestation.

MATERIALS AND METHODS

Insect cultures: Parent stock of *Callosobruchus maculatus* was obtained from the food store at Ekiti Anglican Diocesan High School, Ado-Ekiti, Ekiti State, Nigeria. The insects were reared in the laboratory on cleaned cowpea seeds at ambient temperature and relative humidity. From this stock, new generation of *C. maculatus* was raised. The cultures were maintained by continually replacing the devoured and infested seeds with fresh, uninfested ones. During the process of replacement, copulating pairs of adult *C. maculatus* were introduced into the containers.

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Extraction procedure: Test plants species evaluated for insecticidal activity are stated in Table 1. Extraction of each plant material was carried out in the laboratory by soaking 2.0 g of the plant powder in 100 mL of water for 24 h. The solution was then heated at 60°C for 30 min in a heating mantle and percolated through muslin cloth in order to remove the debris. The resulting filtrate was stored in a plastic container and refrigerated until ready for use.

Insect mortality bioassay: An aliquot of 0.8 mL which is equivalent to 4.0% v/w of each plant extract was added to 20 g of clean uninfested cowpea seeds and thoroughly mixed together with the aid of a spatula. The seeds were air-dried for about 2 h in the laboratory. An untreated control was also included for comparison. Each treatment was replicated four times.

Twenty adult weevils were introduced to each petri dish. Weevil mortality was observed daily for 4 days. After every 24 h, the number of dead weevils were counted and recorded. The insects were confirmed dead when there was no response to probing with sharp pin at the abdomen.

Oviposition and adult emergence bioassay: Each plant extract was admixed with 20 g of clean and uninfested seeds of susceptible Ife-brown in a petri dish, at the rate of 4.0% v/w. The cowpea seeds and extracts were thoroughly mixed with a glass rod to ensure uniform coating of the extracts and the seeds. The seeds were air dried for about 2 h, followed by the introduction of a pair of newly emerged adult *C. maculatus*. A control experiment comprising untreated seeds was also setup. The petri dishes were covered with petri plates and left on the shelf for 7 days or until the death of the insects. The total number of eggs laid was counted and recorded. The experiment was kept inside the wooden cage for another 30 days to allow for the emergence of the first filial (F_1) generation. The number of adult that emerged was calculated thus:

$$\text{Percent adult emergence} = \frac{\text{No. of adult emerged}}{\text{No. of eggs laid}} \times 100$$

Grain damage bioassay: Fifty grams of clean uninfested cowpea seeds were measured into transparent plastic cups and admixed with 2.0 mL of extracts which corresponds to 4.0% v/w. An untreated control experiment without any extract was included in the setup. Each treatment was replicated four times. The plastic containers were covered with muslin cloth held tightly in place by rubber band and then kept in the wooden cage in the laboratory. After 90 days, each replicate was assessed for seed damage and weight loss. Percentage seed damage was determined thus:

Table 1: Test plants species evaluated for insecticidal activity

Scientific name	Common	Family name	Parts used
Crotalaria retusa	Rattlepods	Papilionaceae	Leaves
Hyptis suaveleons	Bush mint	Lamiaceae	Leaves
Ricinus communis	Castor	Euphobiaceae	Seeds
Tithornia diversifolia	Tree marigold	Asteraceae	Bark

$$\text{Percent Damage} = \frac{\text{No. of seeds damaged}}{\text{Total No. of seeds}} \times 100$$

Viability bioassay: Germination test was conducted on 20 g of cowpea seeds per replicate with 4.0% v/w of the plant extracts and kept under ambient condition for 90 days in order to assess the effect of the plant extracts on the cowpea seeds. Fifty seeds were randomly selected and germinated on a tissue paper soaked in water in petri dishes. The germination process lasted for seven days. Thereafter, the number of germinated seeds in the petri dishes were counted and recorded.

Statistical analysis: Data obtained were converted to percentages. Arcsin transformation was carried out on the percentage value. ANOVA was performed on transformed data and the means separated by turkey's test.

RESULTS

Effect of aqueous extracts of test plants species on mortality of adult *C. maculatus*: Mortality of *C. maculatus* in seeds treated with different plant aqueous extracts was significantly different from weevil mortality in untreated seeds (Table 2). Adult mortality increased with length of exposure. All extracts showed weevil mortality ranging from 23.75 to 100%. Aqueous extracts of *Hyptis suaveolens, Ricinus communis* and *Tithonia diversifolia* were most effective against *C. maculatus*, evoking 66.25, 82.50 and 100% mortality, respectively by 96 h of exposure while extracts from *C. retusa* was least, causing 53.75% weevil mortality by 96 h of exposure.

Table 2: Cumulative percentage mortality of *Callosobruchus maculatus* exposed to 4.0% v/w aqueous extracts of the test plants species

	Percentage mortality at hours of post treatment			
Test plant species	24	48	72	96
Crotalaria retusa	23.75 ± 1.25^{ab}	35.00 ± 2.04^{a}	42.50 ± 1.44^{b}	53.75 ± 2.39^{a}
Hyptis suaveolens	36.25 ± 1.25^{c}	51.25 ± 1.25^{c}	63.75 ± 1.25^{bcd}	66.25 ± 1.25^{b}
Ricinus communis	48.75 ± 3.15^{d}	62.50 ± 3.23^{d}	71.25 ± 427^{cd}	82.50 ± 1.44^{cd}
Tithonia diversifolia	60.00 ± 0.00^{e}	83.75 ± 2.39^{e}	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}
Untreated control	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 1.25^{a}	5.00 ± 2.04^{a}

Each value is the Mean±Standard error of four replicates. Means in the same column followed by the same letter are not significantly different at $p\geq0.05$ by Turkey's test

Effect of aqueous extracts of test plant species on oviposition and adult emergence of *C. maculatus*: All the different extracts in this study reduced the number of eggs laid by *C. maculatus* (Table 3). Oviposition by *C. maculatus* was significantly lowered in extract-treated seeds than in untreated seeds. The percentage adult emergence in the untreated seeds was significantly different from percentage adult emergence in the treated seeds. Extract from *T. diversifolia* was most effective because it evoked the least percentage adult emergence followed by extracts from *Hyptis suaveolens*, *C. retusa* and then *R. communis*, respectively.

Protectant ability of aqueous extracts from test plants species during storage for a period of 90 days: The most effective extracts were those of *T. diversifolia* and *R. communis* because it caused drastic reduction in seed damage and weight loss, followed by *Hyptis suaveolens* and then *C. retusa* respectively (Table 4). In the untreated seeds, 81.20% damage occurred as revealed by emergent holes of the bruchids as a result of the feeding activities of *C. maculatus* larvae on the cowpea seeds.

Table 3: Effect of aqueous extracts of test plants species on oviposition and adult emergence of *C. maculatus*

Test plant species	No. of eggs laid	Adult emergence (%)
Crotalaria retusa	14.50±0.65[f]	62.02±6.21[ab]
Hyptis suaveolens	7.50±0.65[c]	57.09±9.04[b]
Ricinus communis	4.00±0.58[a]	65.97±5.90[ab]
Tithonia diversifolia	10.75±0.48[de]	29.47±5.26[a]
Untreated control	24.25±0.85[h]	86.45±1.55[h]

Each value is the Mean±Standard error of four replicates. Means in the same column followed by the same letter(s) are not significantly different at p≥0.05 by Turkey's test

Table 4: Effects of 4.0% v/w aqueous extracts of the test plants species on long-term storage of cowpea seed

Test plant species	Mean No. of seeds	Mean percentage of seed damage	Percentage weight loss
Crotalaria retusa	174.75	40.34±1.45[b]	20.05±0.22[h]
Hyptis suaveolens	171.75	18.76±1.18[e]	16.80±1.10[cd]
Ricinus communis	175.75	6.84±0.42[bc]	4.95±0.43[a]
Tithonia diversifolia	174.75	4.580.42[ab]	3.45±0.05[a]
Untreated control	171.25	81.20±1.01[a]	37.95±1.39[d]

Each value is the Mean±Standard error of four replicates. Means followed by the same letters in the same column are not significantly different at p≥0.05 by Turkey's tes

Table 5: Percentage germination of cowpea seeds treated with 4.0% v/w aqueous extracts of test plants species after storage for a period of 90 days

Test plant species	Mean percentage germination
Crotalaria retusa	90.00±2.04[ab]
Hyptis suaveolens	91.25±3.15[ab]
Ricinus communis	82.50±1.44[a]
Tithonia diversifolia	92.50±1.44[ab]
Untreated control	100.00±0.00[a]

Each value is the Mean±Standard error of four replicates. Mean in the same column followed by the same letter(s) are not significantly different at p≥0.05 by Turkey's test

Effect of aqueous extracts of the test plant species on grain viability: Percentage germination of all treated seeds after seven days was generally high (Table 5). Almost all the treated seeds germinated. The untreated seeds had the highest percentage germination of 100%, followed by extracts from *T. diversifolia*, *H. suaveolens*, *C. retusa* and *R. communis*, evoking 92.50, 91.25, 90 and 82.50% germination, respectively.

DISCUSSION

The results obtained from this study showed that aqueous extracts from all the test plant species caused high mortality of adult *C. maculatus*. The cowpea seeds treated with extracts from *Tithonia diversifolia* was the most toxic of all the extracts tested, followed by that of *R. communis*, *H. suaveolens* and then *C. retusa*, evoking 100, 71.25, 63.75 and 42.50% mortality, respectively by 96 h of exposure. The results from this investigation are similar to the observation of Adedire *et al.* (2011) who obtained 97.50% mortality of *C. maculatus* in cowpea seeds treated with acetone extracts from cashew kernels at 0.5% v/w. Kayode and Obembe (2012) had also reported the effective protection of cowpea seeds against *C. maculatus* with aqueous extracts from seven tropical trees.

The insecticidal effect of the plants aqueous extracts on *C. maculatus* in the treated cowpea seeds might be as a result of contact toxicity. Most insects breathe by means of trachea which usually open at the surface of the body through spiracles. The extracts that were mixed with the seed might have blocked these spiracles thereby leading to suffocation and death of the insect (Adedire and Akinkurolere, 2005; Rahman and Talukder, 2006; Akinkurolele *et al.*, 2006).

Oviposition by female *C. maculatus* was significantly lower in extract-treated cowpea seeds as against oviposition in the untreated seeds. It was also observed that the percentage adult emergence was drastically reduced by 30 days of exposure to the aqueous extracts. The fact that the plant extracts induces reduction of oviposition by female *C. maculatus* and mortality of the development stages had been reported by a number of authors and fairly well documented (Boeke *et al.*, 2001). The effect of the extracts on oviposition in the present study could be linked with respiratory impairment which probably affects the process of metabolism and consequently other systems of the body of the bruchids (Ossisiogu and Agbakwuru, 1978; Onolemhemhem and Oigiangbe, 1991).

The plants extracts possibly inhibited locomotion; hence, the weevils were unable to move freely, thereby affecting mating activities and fecundity. The inability of the eggs to stick to the treated cowpea seeds due to the presence of the extracts may also reduce survival after adult emergence.

The ability of some plant extracts to protect cowpea seeds from damage by *C. maculatus* over a long-term storage period had been tested with positive results. All the plant extracts considerably reduced seed damage. Some plant extracts have been tested for long time protectant ability on seeds and grains with positive results (Pereira, 1983). Shaaya *et al.* (1997) reported that oil extracted from crude palm kernel and rice bran at the rate of 1.5 and 3 g kg^{-1} cowpea seeds offered full protection from *C. maculatus* for a period of 4 to 5 months.

CONCLUSION

In conclusion, results obtained from this study confirmed that the aqueous extracts of the test plants species, especially that of *T. diversifolia* and *R. communis* were highly effective in controlling the population of *C. maculatus* in treated seeds. The aqueous extracts of *T. diversifolia* and *R. communis* could serve as alternatives to synthetic insecticides for use by resource-poor farmers who store small quantities of the seeds for their consumption, sales and planting. In addition, cost as regards the purchase of solvents such as n-hexane, pet-ether acetone, ethanol methanol acetone etc. which are expensive may not be accessible to small holder farmers could be eliminated.

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