Efficacy of plant extracts on the toxicity, ovipositional deterrence and damage assessment of the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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ABSTRACT

A laboratory experiment was conducted to investigate the efficacy of different plant derivatives against the development of the cowpea weevil, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) fed on cowpea, *Vigna unguiculata* (W) seeds. The leaf extracts of aromatic plants, *Murraya koenigii* and *Azadirachta indica* (A.Juss) were evaluated for their growth, adult mortality and oviposition inhibition of *C. maculatus*. The results revealed that the extracts of the two plant species caused a considerable reduction in the number of weevils. The combination of neem seed kernel extract and leaf extract of *M. koenigii* was the most effective in checking insect infestation and allowing the least number of F1 adults to emerge from the seeds over other treatments. Acetone leaf extracts of *M. koenigii* were more toxic to adult beetles compared to ethanolic extracts. Thus, the botanicals acted as insect antifeedant and the order of toxicity of various treatments on cowpea weevil were: combination of neem seed kernel extract + *M. koenigii* leaf extract > neem > *M. koenigii*.

Keywords: Growth, toxicity, ovipositional repellency, *Callosobruchus maculatus*, *Murraya koenigii*, *Azadirachta indica*.

1. Introduction

Pulses form an important part of Indian dietary. Being an important source of protein and essential adjuncts to a predominantly cereal-based diet they enhance the biological value of the protein consumed. Pulses constitute an important source of income to resource-poor farmers. In the field, the crop is susceptible to many insect pests [19] which are a major constraint to cultivation. In India, the stored products are heavily infested by insects, causing as much as 20-50% post-harvest loss.

The cowpea weevil, *Callosobruchus maculatus* (Fabricus) (Coleoptera: Bruchidae) is a pest of cowpea, *Vigna unguiculata* (L) [24, 25]. Infestation by this beetle is common in field, but most damage occurs during storage [6]. Over 90% of the insect damage to cowpea seeds is caused by *C. maculatus* which is the main constraint to increased cowpea production.

Synthetic insecticides are expensive for subsistence farmers and may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. However their increasing use in recent years has created a range of ecological problems such as bio-magnification, resurgence and the development of insecticide tolerant strains of pest species. As such, farmers have reverted to the usage of natural products that include plant extracts, powders, ashes, cow dung and oils to control pests with varying level of effectiveness [13].

Insect damage in stored grains and pulses may amount to 40% in countries where modern storage technologies have not been introduced. Currently, the measures to control pest infestation in grain and dry food products rely heavily upon the use of gaseous and liquid insecticides, which pose possible health hazards to warm-blooded animals and a risk of environmental contamination. One alternative to synthetic insecticides is insecticidal plants [26, 27]. The use of plant products has assumed significance as an important component of insect pest management because of their economic viability and eco-friendly nature. They hold promise as alternatives to chemical insecticides to reduce pesticide load in the environment.
The plant kingdom contains a huge array of chemical substances; many of these are used by plants for their defense against insect attack [1]. Phytochemicals possess a wide spectrum of biological properties against insects. They may act as antifeedants, repellents, growth inhibitors, attractants, chemosterilants or as insecticides. The naturally occurring phytochemicals are usually biodegradable and non-toxic to plants, warm-blooded animals and the environment. They offer great potential as safer, more effective and economic pesticides [21, 28].

With growing awareness of the hazards associated with the use of synthetic organic insecticides, there is a greater need to explore suitable alternative methods of pest control against stored products [17, 18]. In view of the fact that the use of the indigenous plants will be a promising approach to reduce the bruchid population very effectively, an attempt has been made to study the growth, toxicity and ovipositional repellency of cowpea weevil, C. maculatus after treating with extracts of neem seed kernel and M. koenigii leaves.

2. Materials and Methods
2.1 Laboratory rearing of Callosobruchus maculatus
The parental stocks of Callosobruchus maculatus were reared and bred under laboratory conditions on the seeds of cowpea (Vigna unguiculata) inside a growth chamber at 30±2 °C, 12:12 L: D and 70% RH. Initially, 50 pairs of 1-2 day old male and female adult insects were placed in a jar containing cowpea seeds. The jars were sealed and a maximum of 7 days were allowed for mating and oviposition. Then parent stocks were removed and cowpea seeds containing eggs was transferred to fresh cowpea seeds in the breeding jars covered with pieces of cloth fastened with rubber band to prevent contamination and escape of beetles. The subsequent progenies of beetles were used for further experimentation.

2.2 Test plant materials
The leaves of Murraya koenigii and Azadirachta indica were collected from plants growing in and around the Bharathiar University Campus, Coimbatore, Tamil Nadu, India. The leaves were thoroughly washed and air-dried in the shade for a period of 3-4 weeks; the dried leaves were manually ground into powder with the help of a mortar and pestle. The resulting powder was passed through a mesh sieve to obtain a fine dust.

The extracts of acetone were prepared by mixing 100 ml of acetone with the marc five times with a 1.5 g of powdered leaves in a covered glass beaker to make 100% stock solution. This powder was similarly extracted with acetone to obtain an acetone extract of neem seed kernel.

3. Bioassays
3.1 Toxicity bioassay
3.1.1 Fumigant toxicity effect
A filter paper strip (1.5 cm diameter) was impregnated with appropriate amount of ethanolic and aceton M. koenigii extract separately and the solvent was allowed to evaporate for 10 minutes[14]. Then the filter paper was attached to the undersurface of the screw cap of a glass vial (6.5 ml), and five pairs of 5-10 hour old bruchids were introduced into the bottle. The neck of the bottle was blocked with a metal the cap of each vial was screwed and incubated for 24 hours. Subsequently, insects were transferred into a clean vial with 50 fresh, untreated cowpea seeds. Parallel experiments with aceton and ethanolic neem seed kernel treatment and control were also conducted. Experimental design was a CRD with six replicates per treatment.

3.1.2 Contact toxicity effect:
Contact effects on bruchid oviposition and F1 emergence were tested using a modified method of Huang et al. appropriate dose of Murraya koenigii plant extract dissolved in ethanol was separately applied on the inner surface of glass vials (6.5 ml) and under surface of screw caps. The solvent was allowed to evaporate (10 minutes) and five pairs of 5-10 hour old bruchids were introduced into the vial and the screw cap was tightened. After 24 hour of incubation period, bruchids were transferred to clean vials with 50 untreated, fresh cowpea seeds. Parallel experiments with aceton and ethanolic neem seed kernel treatment and control were also included for comparison purposes. Experimental design was a completely randomized design (CRD) with six-replicates per treatment. Number of eggs laid were recorded daily during the incubation period of 10 days in both contact and fumigant toxicity tests. Whenever the first generation adults (F1) emerged, the number of F1 adults was also counted in both contact and fumigant tests.

3.2 Ovipositional bioassay
3.2.1 Choice ovipositional test (Choice chamber bioassay):
The choice chamber consisted of eight transparent plastic bottles (300 ml), placed equidistant to each other. The bottles were connected to a large transparent bottle (1-liter) placed in the center of the chamber through glass tubes (1 cm diameter and 8 cm long). The experimental apparatus was placed in a plastic basin having a diameter of 42 cm and the height of 18 cm and the sidewalls were covered with black paper. The plant extract samples were placed on blotting paper strips (2.5 × 3.0 cm) separately and the solvent was allowed to evaporate and each strip was placed in an appropriate bottle containing 50 cowpea seeds. Two bottles containing 50 cowpea seeds without any treatment were considered as control. Two hundred and fifty adult bruchids (unsexed, 1-3 d old) were introduced to the central bottle, and the chamber was placed in a dark room. After 24 hours, the number of bruchids moved in to each bottle and the number of eggs deposited in each bottle were recorded. Experiment design was Complete Randomized Design (CRD) with five replicates.

3.3 Damage assessment and F1 progeny bioassay
Another experiment was performed with the infested and treated grains left for 49 days (i.e. 7 weeks). At the end of the 49-day observation period, the extent of weevil damage was assessed using the exit-hole counted as a measure of damage to the grains.
Grains that were riddle with exit-holes were counted; the percentage damage (PD) and weevil perforation index (WPI) of the weevils to the grains were calculated \[P.D = \frac{\text{Total number of treated grains perforated}}{\text{Total number of grains}} \times 100\].

\[\text{WPI} = \frac{\% \text{ of treated grains perforated}}{\% \text{ of control grains perforated} + \% \text{ of treated grains perforated}} \times 100\]

3.4 Statistical analysis

Data obtained during choice and no-choice ovipositional tests were analyzed statistically using One-way ANOVA and the means were compared using Turkey’s Pair wise Comparison test. Data obtained during damage assessment were subjected to analysis of variance and where significant differences existed, treatment means were compared at 0.05 level of significance.

4. Results

Mean number of eggs laid by female bruchid and the progeny production during the fumigant effects of plant extracts is given in Table 1. At 0.75 g/l concentration, a significant oviposition and progeny production deterrency effect was observed in all treatments as compared to control (p < 0.05). The leaf extracts of M. koenigii and Neem completely inhibited oviposition. Neem seed kernel extract showed a stronger effect on oviposition (40.67) and progeny production (34.00) than M. koenigii (53.67 and 40.67 respectively). However, control showed highest mean oviposition and progeny production, the two being at par with each other.

**Table 1: Ovipositional deterrence and progeny production of C. maculatus on cowpea seeds treated with different plant extracts in fumigant test.**

<table>
<thead>
<tr>
<th>Treatment (0.75 g/lit)</th>
<th>Number of eggs laid</th>
<th>% deterrency</th>
<th>F1 progeny emerged</th>
<th>% deterrency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKLE + NSKE</td>
<td>0.00 ±0.00a</td>
<td>100.00</td>
<td>0.00 ±0.00c</td>
<td>100.00</td>
</tr>
<tr>
<td>MKLE</td>
<td>53.67 ± 4.95b</td>
<td>74.79</td>
<td>40.67 ± 3.73b</td>
<td>65.36</td>
</tr>
<tr>
<td>NSKE</td>
<td>40.67 ± 8.81c</td>
<td>81.20</td>
<td>34.00 ± 5.54b</td>
<td>71.04</td>
</tr>
<tr>
<td>Control</td>
<td>161.67 ±12.95c</td>
<td>125.33 ± 8.44c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each data point represents the mean of 6 replicates ± S.E.

MKLE = M. koenigii leaf extract, NSKE = Neem Seed Kernel extract

In contact toxicity test, all the plant extracts significantly reduced (p<0.05) oviposition and progeny production compared to control. In Table 2, the oviposition and progeny production was zero in the leaf extracts of M. koenigii and Neem indicating maximum deterrency effect on oviposition. The ovipositions and progeny productions due to the combined leaf extracts of Murraya koenigii and Neem were significantly lower than that of neem seed kernel extract (50.17 and 33.00 respectively) and leaf extracts of Murraya koenigii (42.00 and 27.16 respectively) treatments.

**Table 2: Oviposition deterrence and progeny production of C. maculatus on cowpea seeds treated with different plant extracts in contact test.**

<table>
<thead>
<tr>
<th>Treatment (0.50 g/litre)</th>
<th>Number of eggs laid</th>
<th>% deterrency</th>
<th>F1 progeny emerged</th>
<th>% deterrency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKLE + NSKE</td>
<td>0.00 + 0.00a</td>
<td>100.00</td>
<td>0.00 +0.00c</td>
<td>100.00</td>
</tr>
<tr>
<td>MKLE</td>
<td>42.00 + 4.18b</td>
<td>58.92</td>
<td>27.16 + 2.86b</td>
<td>61.70</td>
</tr>
<tr>
<td>NSKE</td>
<td>50.17 + 10.77c</td>
<td>50.93</td>
<td>33.00 + 5.54b</td>
<td>53.46</td>
</tr>
<tr>
<td>Control</td>
<td>103.00 + 8.21b</td>
<td>71.00 ± 6.13c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each data point represents the mean of 6 replicates ± S.E.

MKLE = M. koenigii leaf extract, NSKE = Neem Seed Kernel extract

Table 3 represents the percentage oviposition at different doses of plant extracts during choice oviposition tests (choice chamber bioassay). The percentage oviposition in all treatments was significantly lower than control. A stronger oviposition deterrency effect was observed for combined extract of leaf extracts of M. koenigii and Neem. However the oviposition deterrency effect of leaf extracts of M. koenigii alone was comparably lower than that of combined plant extracts.

**Table 3: Percentage eggs laid by C. maculatus on cowpea seeds at different doses of plant extracts in choice tests.**

<table>
<thead>
<tr>
<th>Dose</th>
<th>MKLE + NSKE</th>
<th>MKLE</th>
<th>NSKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6.2 ± 1.2a</td>
<td>23.9 ± 4.1c</td>
<td>8.0 ± 1.2a</td>
</tr>
<tr>
<td>20</td>
<td>0.9 ± 0.2b</td>
<td>11.9 ± 2.1a</td>
<td>0.6 ± 0.4b</td>
</tr>
<tr>
<td>40</td>
<td>0.3 ± 0.3b</td>
<td>4.9 ± 0.4b</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>80</td>
<td>0.0 ± 0.0b</td>
<td>2.1 ± 0.2b</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>160</td>
<td>0.0 ± 0.0b</td>
<td>0.3 ± 0.2b</td>
<td>42.0 ± 2.0c</td>
</tr>
<tr>
<td>Control</td>
<td>45.6 ± 3.7c</td>
<td>26.4 ± 6.5c</td>
<td>38.8 ± 1.5c</td>
</tr>
</tbody>
</table>

Each data point represents the mean of 5 replicates ± S.E.

MKLE = M. koenigii leaf extract, NSKE = Neem Seed Kernel extract.
Table 4 represents the effect of various treatments on grain damage. The combined extract of *M. koenigii* and Neem gave the lowest value of 0.42% grain damaged, followed by neem (2.81%) and *M. koenigii* alone (4.22). The percent damage values show the activities of one plant material at different concentrations while the weevil perforation index (WPI) compares the activities of different species of plant extracts used.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Conc% (v/w)</th>
<th>Total no: of grains</th>
<th>No: of perforated grains</th>
<th>Unperforated grains</th>
<th>% grain damage</th>
<th>WPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKLE +</td>
<td>0.50</td>
<td>244</td>
<td>11</td>
<td>233</td>
<td>4.51</td>
<td>10.65</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>236</td>
<td>9</td>
<td>227</td>
<td>3.81</td>
<td>9.15</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>238</td>
<td>1</td>
<td>237</td>
<td>0.42</td>
<td>1.10</td>
</tr>
<tr>
<td>NSKE</td>
<td>0.50</td>
<td>242</td>
<td>27</td>
<td>215</td>
<td>11.16</td>
<td>22.78</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>234</td>
<td>10</td>
<td>224</td>
<td>4.27</td>
<td>10.14</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>237</td>
<td>10</td>
<td>227</td>
<td>4.22</td>
<td>10.04</td>
</tr>
<tr>
<td>NSKE</td>
<td>0.50</td>
<td>249</td>
<td>20</td>
<td>229</td>
<td>8.03</td>
<td>17.51</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>238</td>
<td>10</td>
<td>228</td>
<td>4.20</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>249</td>
<td>7</td>
<td>242</td>
<td>2.81</td>
<td>6.91</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>238</td>
<td>90</td>
<td>148</td>
<td>37.81</td>
<td>50</td>
</tr>
</tbody>
</table>

*Weevil Perforation Index (WPI). A value above 50 is an indication of negative protectant ability.

5. Discussion

Over 130 plants and plant products have been shown to have insecticidal activity against stored product pests [4, 6]. Currently many farmers in parts of Africa and Asia are using botanicals to protect their legumes from attack by bruchids, with varying degrees of success [7]. Neem has been shown to contain compounds like azadirachtin, meliantriol and salanin [8] which are said to be repellant, antifeedant and have growth disrupting effects [22].

Treatment with NSKE increased larval and pupal period and reduced the total oviposition period, adult longevity and fecundity suggesting that the phytochemicals in neem interfere with the neuroendocrine system in insects, which controls the synthesis of edysone and juvenile hormone. The main action of azadirachtin appears to be at the release sites of Prothoracicotropic hormone (PTTH) from the corpora cardiaca. Azadirachtin appears to block the release of neurosecretory material from the corpora cardiaca resulting in a reduced turnover rate. This affects the rate of synthesis of PTTH by brain neurosecretory cells. This suggests that the developmental effect of NSKE and MKLE attribute to disruption of endocrine event in the beetle. It has been indicated by Schmutterer that interference involves the inhibition of the release of edysone or molting hormone. Indication of this was an accumulation of large quantities of stainable neurosecretory material in the corpora cardiaca of the beetle. In this insect, azadirachtin regulated juvenile hormone titer to prevent vitellogenin production in females, causing ovipositional inhibition and sterility.

In the present investigation, it was found that neem seed kernel and *M. koenigii* afford better protection against *C. maculatus*. It was observed that the seed kernel extracts were more effective in checking mortality and oviposition than control. Pradhan [10] reported that neem seed kernel possess an extra ordinary gustatory repellent properties, much higher than neem leaf powder against the desert and migratory locusts. Rouf et al. [18] studied that mixing of neem leaf powder with lentil seeds resulted in reduced oviposition and adult emergence of the pulse beetle. Pandey et al. [14, 15] reported that a petroleum ether extract of neem leaves and twigs mixed with green gram seeds inhibited the oviposition of *C. chinensis*. Butterworth and Morgan [8] revealed that the most active antifeedant is reported to occur in neem seed kernel powder. Further, the results are confirmed by who also reported that Azadirachtin is a major compound in the seed kernel responsible for the reduced oviposition and adult emergence in beetles.

Neem has many other activities against insects disrupting or inhibiting development of eggs, larvae or pupae, preventing the molting of larvae or nymphs, disrupting mating and sexual communication, repelling larvae and adults, deterring females from laying eggs, sterilizing adults, poisoning larvae and adults, feeding deterrent, blocking the ability to swallow by reducing the motility of the gut preventing metamorphosis, thus preventing adult maturation, inhibiting the formation of chitin, the substance essential for the insect to form an exoskeleton [11, 13].

This study also demonstrated the potential of using *M. koenigii* to control *C. maculatus* in stored cowpea but the effect was less pronounced as compared to neem leaves. *M. koenigii* oil contains 39 compounds of which the major is 54.22% followed by caryophyllene (9.49%). *M. koenigii* is known to be the richest source of carbazole alkaloids. It has been reported that carbazole alkaloids possess various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities [12].

6. Conclusion

The results of the study have confirmed that the cowpea beetle, *C. maculatus*, can be effectively controlled by admixing neem seed kernel extract and murraya leaf extract with 1 kg of cowpea seeds for at most 5 months. The use of botanicals should be encouraged in small farm storage, as the cost of these botanicals are low and easily available when compared with the losses incurred in untreated seeds. Additionally, more seeds would be available for use as food and for sale by the farmer as grain infestation would be reduced. Consumers would also get more value for their money, as well as enjoy cowpea seeds that are free from beetle infestation throughout the year.

Thus, the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use. To minimize the severe damage caused by insect pests, the traditional...
use of plant products, proved to be highly effective against stored-product insects. Application of plant products to grain seeds for storage is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. A study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazard.

7. References