Potential of combining neem (*Azadirachta indica* A. Juss) seed oil with varietal resistance for the management of the cowpea bruchid, *Callosobruchus maculatus* (F.)

N.E.S. Lale*, A. Mustapha

Department of Crop Science, Faculty of Agriculture, University of Maiduguri, P. M. B. 1069, Maiduguri, Nigeria

Accepted 1 July 1999

Abstract

The efficacy of different rates (25, 50, 75 and 100 mg/5 g seed) of application of neem (*Azadirachta indica*) seed oil (NSO) was assessed on four cowpea varieties (Kanannado, IT89KD-391, Borno brown and IT89KD-374) with differing susceptibilities to *Callosobruchus maculatus*. The different rates of NSO significantly interacted with cowpea varietal resistance and reduced oviposition and percentage adult emergence of *C. maculatus*. The interaction of the strategies also significantly reduced percentage of cowpea seeds infested by *C. maculatus*. Treatment of seeds with NSO at the rates of 50 mg/5 g and 75 or 100 mg/5 g reduced seed damage from over 25% in controls to less than 10% and less than 5%, respectively, in all varieties. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Neem; Cowpea variety; Integrated bruchid management; *Callosobruchus maculatus*

1. Introduction

The cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea, *Vigna unguiculata* (L.) Walpers in the tropics (Jackai and Daoust, 1986; Ogunwolu and Odunlami, 1996). It causes substantial quantitative and qualitative losses manifested by seed perforation, and reductions in weight, market value and germinability of seeds (IITA, 1989). About 4% of the total annual production or about 30,000 tonnes valued at over 30 million US dollars is lost
annually in Nigeria alone to this bruchid (Caswell, 1980; Singh et al., 1983). Under traditional storage conditions, 100% infestation of cowpea occurring within 3 to 5 months of storage is common (Booker, 1967; Caswell and Akibu, 1980).

The integration of insecticidal natural products from locally-available plants for use in storage, and the growing of varieties of cowpea with some resistance to \( C. \text{maculatus} \) may lead to the sustainable management of the bruchid especially in subsistence agriculture. Oil and powder obtainable from neem (\textit{Azadirachta indica} A. Juss) seeds have been reported to provide sustained protection of stored grains (Jotwani and Scircar, 1965; Ivbijaro, 1983a, 1983b; Makanjuola, 1989; Ogunwolu and Idowu, 1994; Lale and Ajayi, 1996; Ogunwolu and Odunlami, 1996). The oil obtainable from neem seeds has been shown to be more effective than the powder formulation in reducing egg-laying and adult emergence of the bruchid (Lale and Abdulrahman, 1999). Nevertheless, total reliance on the application of neem seed oil for the control of cowpea bruchids may result in the emergence of resistant strains of the bruchid through selection. Apart from resistance, the rapid recovery of a pest population from an insecticidal treatment can arise by one of three other mechanisms (Lale, 1996). First, it could be due to the development by mutation or genetic expression of a new biotype with a higher biotic potential. Secondly, it may be caused by the release of the pest from biological control agents due to a disproportionate mortality of its natural enemies. Thirdly, it could arise as a result of hormoligosis, the inducement of females of certain pest species to lay more viable eggs by sublethal doses of pesticides (Luckey, 1968; Kurdyukov, 1972; Croft and Brown, 1975; Chelliah et al., 1980; Reissig et al., 1982; Yokoyama and Pritchard, 1984; Lale, 1991).

Varietal resistance in the crop species (complete or partial), though a relatively cheap and safe method of controlling \( C. \text{maculatus} \), is also vulnerable to the emergence of biotypes of the bruchid that are tolerant of the resistant mechanism(s) of the host plant. Dick and Credland (1986a, 1986b) reported that TVu2027, a resistant variety of cowpea, had become ineffective against certain biotypes of \( C. \text{maculatus} \).

The integration of these two tactics for bruchid control is likely to be more successful than a sole dependence on either of them. In an integrated bruchid control programme, a resistant or partially resistant cowpea variety may improve the effectiveness of insecticides. This study reports the findings of the integration of neem seed oil and four cowpea varieties with different levels of resistance to \( C. \text{maculatus} \).

2. Materials and methods

Three (Kanannado, IT89KD-391 and IT89KD-374) of the four cowpea varieties used for the study were obtained from the sub-station of the International Institute of Tropical Agriculture, Ibadan, Nigeria at Kano. The fourth variety (Borno brown) was obtained from a local market in Maiduguri, Nigeria. Three varieties of cowpea had resistance to \( C. \text{maculatus} \); Kanannado was highly resistant, IT89KD-391 and Borno brown were moderately resistant, while IT89KD-374 was susceptible. To safeguard against any unwanted infestation, the cowpea varieties were stored under freezing conditions for four weeks prior to screening. They were, however, equilibrated before use for seven days to laboratory conditions (25-30°C and 20-40% r. h.) under which the experiment was conducted. Sound seeds free of infestation and any noticeable
defect were then selected from each batch for the experiment. Adults of *C. maculatus* used for
the experiment were obtained from a culture that had been established two months earlier on a
susceptible variety of cowpea (Yar Chad) from a colony obtained from an infested batch of
cowpea procured from a local market.

2.1. Extraction of neem seed oil

Mature, fallen neem seeds were gathered from a neem plantation in Maiduguri, Nigeria.
These were washed, sun-dried, decorticated and pulverised using a manually operated mill.

Eight-hundred grammes of the pulverised neem seed were weighed into a 5-litre plastic bowl
and pre-boiled water was added, a little at a time, and mixed into the powder. This procedure
was repeated until a dough-like material was formed. The oil was pressed out manually into a
250 ml beaker.

2.2. Experimental procedure

Four dosages (25, 50, 75 and 100 mg) of the neem seed oil (NSO) were applied separately in
0.2 ml of analytical grade of acetone to 5 g seeds of each of the four cowpea varieties in 100
ml glass jars. Treated seeds were stirred with a glass rod until seeds were uniformly coated and
until the acetone was completely evaporated. Control seeds were treated with 0.2 ml of acetone
only and stirred as described above. The experiment was laid out in a randomised complete
block design replicated four times under laboratory conditions (25-30°C and 20-40% r. h.).

Five females and two males (2-day old) *C. maculatus* were then introduced into each glass
jar with the aid of a pooter and allowed to lay eggs for 5 days. On the 5th day, all insects were
removed and the eggs (hatched and unhatched) on seeds in each glass jar were counted. Adult
bruchids that emerged and damaged seeds in each replicate were also counted. Thereafter, all
seeds in each replicate were opened and unemerged adults, pupae and late instar larvae within
the cotyledons were counted. The number of unhatched eggs and dead first instar larvae was
determined as the difference between the total number of eggs laid and the number of emerged
and unemerged adults, pupae and late instar larvae according to the method of Lale and Kolo

Adults that emerged and unhatched eggs/dead first instar larvae were expressed as
percentages of the total number of eggs laid and damaged seeds were expressed as a percentage
of the total number of seeds in each replicate. Percentage data were transformed to their
arcsine equivalent values prior to statistical analysis. All data were then subjected to two-way
ANOVA. Means were compared using the least significant difference statistic (*P* ≤ 0.05).

3. Results

Table 1 shows that the interaction of neem seed oil and the resistance status of the cowpea
varieties had a significant negative effect on the number of eggs laid on three (IT89KD-391,
Borno brown and IT89KD-374) of the four cowpea varieties. The effect of seed oil dose level
on the number of eggs laid was significant in all varieties. Significantly fewer eggs were laid on
seeds treated with as little as 25 mg NSO than on untreated seeds, and in three varieties, IT89KD-374, Borno Brown and IT89KD-391, significantly fewer eggs were laid on seeds treated with 100 mg than with 25 mg. In all treatments fewer eggs were laid on Kanannado than other varieties but the effect of variety on egg numbers was not significant. No adults emerged from the controls or any treatment of the Kanannado seeds. In the other cowpea varieties, significantly higher percentages of adults emerged from untreated seeds than from treated seeds and in Borno brown, significantly lower percentages emerged from seeds treated with 100 mg of NSO than from seeds treated with 25 mg. With an occasional exception, suppression of adult emergence was dose-dependent, decreasing with increasing dosage (Table 2). The difference between the percentages of adults that emerged from the controls of the three cowpea varieties allowing complete development was not significant. When the number of unhatched eggs and first instar larval deaths were assessed, only 3.5% of eggs laid on Kanannado seeds had progressed to the large larval stage in the controls, and none in any treatment. This variety was therefore omitted from further analyses.

Table 1
Mean number of eggs laid by *Callosobruchus maculatus* on seeds of four cowpea varieties protected with neem seed oil (NSO)

<table>
<thead>
<tr>
<th>Cowpea variety</th>
<th>Dosage of neem seed oil (mg/5 g seed)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanannado</td>
<td></td>
<td>8.7</td>
<td>5.3</td>
<td>4.5</td>
<td>4.0</td>
<td>3.0</td>
<td>5.1</td>
</tr>
<tr>
<td>IT89KD-391</td>
<td></td>
<td>11.0</td>
<td>7.3</td>
<td>5.3</td>
<td>4.3</td>
<td>3.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Borno brown</td>
<td></td>
<td>11.7</td>
<td>8.3</td>
<td>6.8</td>
<td>5.3</td>
<td>4.3</td>
<td>7.3</td>
</tr>
<tr>
<td>IT89KD-374</td>
<td></td>
<td>13.7</td>
<td>9.3</td>
<td>7.3</td>
<td>5.3</td>
<td>4.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>11.3</td>
<td>7.5</td>
<td>5.9</td>
<td>4.7</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

a $1 \text{SED} = 3.3, \text{LSD} (P > 0.05)$ Not significant (cowpea variety), $\text{SED} = 1.6, \text{LSD} (P \leq 0.05) = 3.3$ (dosage of NSO), $1\text{SED} = 3.3, \text{LSD} (P \leq 0.05) = 6.5$ (interaction).

Table 2
Mean percentage emergence of *Callosobruchus maculatus* adults from seeds of cowpea varieties protected with neem seed oil (NSO)

<table>
<thead>
<tr>
<th>Cowpea variety</th>
<th>Dosage of neem seed oil (mg/5 g seed)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT89KD-391</td>
<td></td>
<td>91.1 (72.6)</td>
<td>50.5 (45.3)</td>
<td>61.8 (51.9)</td>
<td>33.8 (35.6)</td>
<td>29.7 (33.0)</td>
<td>54.6 (47.7)</td>
</tr>
<tr>
<td>Borno brown</td>
<td></td>
<td>75.7 (60.5)</td>
<td>76.7 (60.5)</td>
<td>64.9 (53.6)</td>
<td>46.1 (42.7)</td>
<td>31.8 (34.3)</td>
<td>59.2 (50.3)</td>
</tr>
<tr>
<td>IT89KD-374</td>
<td></td>
<td>94.4 (76.3)</td>
<td>65.1 (53.8)</td>
<td>51.4 (45.8)</td>
<td>40.5 (39.5)</td>
<td>45.2 (41.3)</td>
<td>60.9 (51.3)</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>88.1 (69.8)</td>
<td>64.1 (53.2)</td>
<td>59.4 (50.4)</td>
<td>40.1 (39.3)</td>
<td>34.9 (36.2)</td>
<td></td>
</tr>
</tbody>
</table>

a $1\text{SED} = 5.0, \text{LSD} (P > 0.05)$ Not significant (cowpea variety), $\text{SED} = 6.0, \text{LSD} (P \leq 0.05) = 13.1$ (dosage of NSO), $\text{SED} = 3.3, \text{LSD} (P \leq 0.05) = 22.6$ (interaction).

b No emergence from Kanannado controls or any treatments.

$^c$ Figures in parentheses are arcsine values to which SED and LSD are applicable.
The combination of varietal resistance and the different rates of NSO in the other varieties had a significant interaction effect on the percentage of unhatched eggs/dead first instar larvae (Table 3). In IT89KD-391, significantly higher egg/larval mortality occurred in seeds treated with 100 mg of NSO than in untreated seeds; in IT89KD-374, significantly higher mortality occurred in seeds treated with 50 or 75 mg of NSO than in untreated seeds; and in Borno brown, significantly higher mortality occurred in seeds treated with 100 mg of NSO than in seeds treated with 25 mg or in untreated seeds. Percentage egg/larval mortality was not significantly different among the varieties; significantly higher mortality occurred in seeds treated with the different rates of NSO than in untreated seeds and 100 or 75 mg on average caused significantly higher mortality than 25 mg but not 50 mg.

The interaction of varietal resistance and the rates of NSO application also caused a significant reduction of the percentage of seeds damaged by \( C. \) \textit{maculatus} (Table 4). In each of the varieties, untreated seeds were significantly more infested than treated seeds; in Borno brown and IT89KD-374, seeds treated with 75 or 100 mg were significantly less infested than seeds treated with 25 mg; and in IT89KD-391, seeds treated with 100 mg were significantly less infested than seeds treated with 25 mg. Treatment of seeds with 50 mg/5 g or more oil reduced damage from over 25% to less than 10% in all three varieties.

4. Discussion

The study has shown that integrating neem seed oil and partial resistance significantly increased percentage of seeds of different cowpea varieties protected against damage by \( C. \) \textit{maculatus}. The highly resistant variety Kanannado was able to prevent complete development of the bruchid in the absence of the oil. It is however, possible that other strains of \( C. \) \textit{maculatus} or other species of Bruchidae may not be as completely controlled by this variety. Protection against infestation by \( C. \) \textit{maculatus} appears to have been achieved mainly through reduced egg-laying and increased mortality of eggs and first instar larvae.

Dick and Credland (1984, 1986b) have shown that the number of adult \( C. \) \textit{maculatus} which

<table>
<thead>
<tr>
<th>Cowpea variety</th>
<th>Dosage of neem seed oil (mg/5 g seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>IT89KD-391</td>
<td>4.8 (12.7)</td>
</tr>
<tr>
<td>Borno brown</td>
<td>17.1 (24.4)</td>
</tr>
<tr>
<td>IT89KD-374</td>
<td>0.5 (3.9)</td>
</tr>
<tr>
<td>Mean</td>
<td>5.6 (13.7)</td>
</tr>
</tbody>
</table>

\( ^\text{a}\) SED = 5.4, LSD (\( P > 0.05 \)) Not significant (cowpea variety), SED = 7.0, LSD (\( P \leq 0.05 \)) = 14.2 (dosage of NSO), SED = 12.2, LSD (\( P \leq 0.05 \)) = 24.6 (interaction).

\( ^\text{b}\) Figures in parentheses are arcsine values to which SED and LSD are applicable.
can emerge from cowpea seeds depends amongst other things on the number of eggs initially present. Successful infestation is, however, determined by the number of eggs that hatch as well as the number of first instar larvae that are able to penetrate the cotyledons. Interfering with any of these processes leads to a reduction in the population of the bruchid and the degree of seed damage. It has been shown that the seed coats of the varieties used in the present study contain varying levels of ovicidal and/or larvicidal compounds (Lale and Kolo, 1998). Plant extracts are also known to cause significant mortality of first instar larvae when used to protect grains against infestation (Boughdad et al., 1987; Lale, 1995).

The combination of applications of neem seed oil and use of resistant cowpea varieties appears to have great potential for the management of this bruchid in stored cowpea. In partially resistant varieties, their joint use resulted in a possible synergism that may provide a sustainable control of *C. maculatus* in stored cowpea. In addition, their joint use in bruchid control would be likely to delay the emergence of biotypes of the bruchid that are capable of breaking down resistance in cowpea varieties or strains of *C. maculatus* with resistance to neem seed oil. It has been reported, for example, that biotypes of *C. maculatus* capable of utilizing the resistant variety TVu2027 and developing normally emerged following continuous selection relying solely on the resistance of this particular variety (Dick and Credland, 1986a, 1986b).

Neem seed oil has good prospects of being readily adopted especially by subsistence farmers in the tropics: this is because NSO is relatively cheap and readily available. Moreover, it is unlikely to leave harmful residues in stored produce as may occur with synthetic insecticides particularly in situations where the prescribed waiting periods are not strictly adhered to.

**Acknowledgements**

The supply of samples of IT89KD-391 and IT89KD-374 by the International Institute of Tropical Agriculture, Ibadan, Nigeria (Kano sub-station) is gratefully acknowledged. This work was partly funded under the World Bank-Assisted National Agricultural Research Project of Nigeria.

<table>
<thead>
<tr>
<th>Cowpea variety</th>
<th>Dosage of neem seed oil (mg/5 g seed)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT89KD-391</td>
<td>28.6 (32.3)b</td>
<td>10.2 (8.6)</td>
<td>7.1 (15.4)</td>
<td>4.7 (12.5)</td>
<td>2.9 (9.8)</td>
<td>9.3 (17.7)</td>
<td></td>
</tr>
<tr>
<td>Borno brown</td>
<td>25.9 (30.6)</td>
<td>13.1 (21.2)</td>
<td>7.1 (15.5)</td>
<td>5.6 (13.7)</td>
<td>4.6 (12.5)</td>
<td>10.3 (18.7)</td>
<td></td>
</tr>
<tr>
<td>IT89KD-374</td>
<td>32.6 (34.8)</td>
<td>15.2 (22.9)</td>
<td>9.3 (17.8)</td>
<td>4.0 (11.6)</td>
<td>2.8 (9.6)</td>
<td>11.0 (19.3)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>29.0 (32.6)</td>
<td>12.7 (20.9)</td>
<td>7.8 (16.2)</td>
<td>4.7 (12.6)</td>
<td>3.4 (10.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a SED = 1.5, LSD ($P > 0.05$) Not significant (cowpea variety), SED = 1.7, LSD ($P ≤ 0.05$) = 3.5 (dosage of NSO), SED = 3.4, LSD ($P ≤ 0.05$) = 6.8 (interaction).

b Figures in parentheses are arcsine values to which SED and LSD are applicable.
References


(Rutaceae) root bark powder when compared to neem seed powder and pirimiphos-methyl. Crop Protection 15, 603–607.