



(RESEARCH ARTICLE)



Efficacy of oils from nine plant species as protectants against infestation by *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae)

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Abstract

Cowpea seeds bruchid (*Callosobruchus maculatus* F.) is an important pest of cowpea with a reputation of causing high grain loss both in quantity and quality. The insecticidal efficacy of nine plant oils [neem (*Azadirachta indica* A. Juss), groundnut (*Arachis hypogaea* L.), Castor (*Ricinus communis* L.), Palm fruit mesocarp and kernel (*Elaeis guineensis* L.), coconut (*Cocos nucifera* L.), Olive (*Olea europaea* L.), Soybean [*Glycine max* (L.) Merr.] and melon (*Cucumeropsis mannii* Naudin.) against this arthropod was evaluated under laboratory temperature of 26 ± 3 °C and 74 ± 4 % R.H at the Crop and Environmental Protection Laboratory of the Federal University of Agriculture, Makurdi, Nigeria. The experiment involved exposing adult *C. maculatus* to 3 mLkg⁻¹ of the oils admixed with Ife brown cowpea seeds. The set up was a completely randomized design with four replicates. Results show that all the plant oils tested had a significant ($p < 0.0001$) insecticidal effect on *C. maculatus*. Adult bruchid mortality was >70% at 72 hours after exposure to the oils. Oviposition, F₁ progeny, the number of damaged seeds and weight loss were significantly reduced ($p < 0.0001$) by the plant oils compared with the control (untreated cowpea seeds). For all the parameters evaluated in the study, oils from *A. indica*, *R. communis*, *E. guineensis* kernel and *G. max* appeared to provide better protection against *C. maculatus* than the other plant oils tested. We conclude that seed treatment with these oils could be an important pest control options for *C. maculatus* in smallholder's cowpea storage facilities.

Keywords: Plant oils; Cowpea storage; Bruchid mortality; Seed damage.

1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, is one of the most economically and nutritionally important legumes and an inexpensive source of protein for both rural poor and urban consumers in Africa [1]. The fresh or dried seeds, pods and leaves are commonly used as food for humans and livestock [1, 2]. Cowpea by-products such as cowpea seed waste and cowpea hulls have also been used to replace conventional feedstuffs in some developing countries [3].

Cowpea production and storage are affected by several biotic and abiotic constraints. Insect infestation is one of the most important biotic factor affecting the crop in both field and storage. Almost every part of the cowpea plant is infested by one or many insect species. Some of the important arthropods causing considerable damage to the crop in the field are foliage beetle (*Ootheca mutabilis* Sahib), aphids (*Aphis craccivora* Koch.), flower bud thrips (*Megalurothrips sjostedti* Trybom) legume pod borer, (*Maruca vitrata* Fabricius), pod sucking bugs (*Clavigralla tomentosicollis* Stal., *Anoplocnemis curvipes* Fabricius, *Riptortus dentipes* Fabricius, *Nezara viridula* Linnaeus, among others), etc [4]. In storage, the seed bruchids, *Callosobruchus maculatus* Fabricius, causes the major losses. Infestations of stored cowpeas can be as high as 90% in markets and village stores [5, 6]. Almost 80% of cowpea crops are produced by small scale farmers and stored in small storage facilities [7, 8, 9]. These storage structures and conditions usually encourage bruchid infestation leading to severe losses in the quality and quantity of stored cowpea grains annually. Quantitative assessment of losses is usually difficult because of the high variability in infestation from one year to another. However, estimates from several

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countries in Africa indicate an intense impact of *C. maculatus* infestation and damage in the continent. In West Africa, up to 100% damage to cowpeas may happen in a few months after storage due to the infestation of *C. maculatus* [7, 10, 11].

The use of synthetic insecticides for the control of *C. maculatus* and other storage pests of cowpea has often proved to be very effective. These insecticides may be applied as a powder formulation (e.g. Actellic® (Pirimiphos-methyl), liquid formulation (such as Karate®), or as fumigant/ gas formulation (phosphine) depending on the intended use of the insecticide [12]. However, the expensive nature of these chemicals discourages most farmers from using them [13, 14]. A report by Prakash *et al.* [15] also shows that the use of synthetic insecticides in crop protection programs resulted in disturbances of the environment, pest resurgences, pest resistance to pesticides and lethal effect to non-target organisms in the agro-ecosystems. Insecticides also affect human health directly or indirectly by disrupting ecological systems that exist in rivers, lakes, oceans, streams, wetlands, forests and fields around areas they are used [16].

Biopesticides, derived from plants with insecticidal properties, are attractive alternatives to synthetic chemical insecticides for insect pest management. They have gained the reputation of being cheap, easy to prepare, non-poisonous to humans, readily available and they have more than one active ingredient which works synergistically making it difficult for pests to develop resistance [16]. Consequently, their role in the production and postharvest protection of food crops in most developing countries is growing. Over 120 plants and plant products have demonstrated insecticidal or deterrent activity against stored product pests [17]. Many farmers use these botanical extracts, powders, ashes, oils to protect their legumes from bruchid infestations, with varying degrees of success [7, 11, 17, 18, 19, 20].

Currently, the use of oils from plant sources is part of a new trend for controlling storage pests. It is therefore imperative to direct more research in this area to provide farmers, grain merchants, grain warehouse managers, etc with information on viable options for the protection of cowpea seeds/ grain from bruchids attacks. We therefore, set up this experiment to evaluate the efficacy of nine plant oils as cowpea seed protectants against infestation and damage caused by *C. maculatus*.

2. Material and methods

2.1. Experimental site

The study was carried out at Crop and Environmental Protection Laboratory of the Federal University of Agriculture, Makurdi, (FUAM) Nigeria (coordinates: Latitude 7° 47' 45.0" N Longitude 8° 36' 56.8" E).

2.2. Preparation of the plant oil samples

Nine plant oils (from seeds, kernels, and fruits) were locally sourced and the oil was extracted at the Laboratory. Depending on the plant type, the seeds were decorticated to separate the kernels, mesocarp removed from kernel, etc. They were then pan-grilled on fire and pounded to form a paste. The pastes were then pressed (using an expeller) to give off the oil. Neem oil was extracted from kernels of *Azadirachta indica* (A. Juss) obtained from the neem trees within the University, groundnut oil was obtained from kernels of *Arachis hypogaea* (L.) while castor oil was extracted from beans of *Ricinus communis* (L.). Palm oil and palm kernel oil were obtained from the mesocarp (reddish pulp) and kernel of *Elaeis guineensis* (L.) fruits respectively. Coconut oil was extracted from drupe endocarp of *Cocos nucifera* (L.), while olive oil was obtained from fruits of *Olea europaea* (L.). Soybean and melon oils were extracted from the seeds of *Glycine max* (L.) Merr. and *Cucumeropsis mannii* Naudin respectively.

2.3. Preparation of cowpea seeds

Ife brown variety of cowpea (*Vigna unguiculata* L.) obtained from the Institute of Agricultural Research, Ibadan was used for the experiment. The seeds were sorted and seeds with holes and cracks were discarded. Wholesome cowpea seeds selected were disinfested by refrigerating for 25 days at -4°C and then dried under the sun to ensure that it was free from insect infestation and to also ensure that the minimum moisture content for storage was not exceeded. The seeds were packed afterward in airtight jars until they were used for the experiment.

2.4. Insect culture

The test bruchid (*C. maculatus*) adults were collected from previously infested cowpea seed purchased from the North bank cereal market in Makurdi, Nigeria. They were brought to the laboratory and cultured on Ife brown variety of cowpea variety. Twenty pairs of adult *C. maculatus* were introduced into two 4-liter plastic jars containing 4 kg of the

cowpea seed each. The jars were covered with a fine muslin cloth to allow air into the jar for bruchid respiration. The culture was maintained in the laboratory at room temperature (26 ± 3 °C and $74 \pm 4\%$). The parents' stocks were removed after 7 days. The seeds containing the eggs were left in the jar for 30 days. The emerging F_1 progenies from the cultures were used for the experiment.

2.5. Bioassay of oils from nine plant species on cowpea seed bruchids

The experiment was laid out in a completely randomized design with four replications. The treatments consist of oil extracts from nine (9) plant species and untreated cowpea seeds (control). Cowpea seeds were admixed with the plant oils at the rate of 0.3 ml per 100 g. The same weight of seed in an untreated jar served as the control. Five (5) pairs (1:1 male: female) of one-day-old adult bruchid were introduced into each jar of treated and untreated cowpea seeds. The jars were examined at 24 hours interval and data on bruchid mortality were recorded for seven days (168 hours after exposure). At 7 days after infestation, all the bruchids were removed and the mean number of eggs per jar was counted ($n = 10$ seeds per jar). Adult emergence (F_1) were monitored and recorded until there is no emergence for 3 consecutive days. After 12 weeks of treatment, the number of grains perforated ($n = 100$ seeds) was counted and express them as a proportion of the total number of grains. The content of each jar was then sifted and sieved to remove dust (from bruchid feeding activities) before taking the final gain weight data. Percentage of seed weight loss was calculated using:

$$\% \text{ weight loss} = \frac{w_i - w_f}{w_i} \times 100$$

Where: w_i = initial weight and w_f = final weight.

2.6. Statistical analyses

Data on bruchid mortality were transformed to arcsine values, while data on bruchid egg counts and F_1 progeny were transformed using the square root model $(x + 0.5)^{1/2}$ before F -test to meet the assumptions of homogeneity of variance. Mortality, infestation and damage data collected were subjected to ANOVA using SAS [21]. Where F -statistics was significant, mean values were separated using Student Newman Keul's Test (SNK) at a 5% level of probability. Pearson's correlation between infestation (number of eggs laid and F_1 progeny), damage parameters (Number of perforated seeds and seed weight loss) were also carried out.

3. Results

The results show that all the plant oils evaluated in the study had a significant ($p < 0.0001$) effect on cowpea seed bruchid. More than 70% of adult bruchid mortality occurred at 72 hours of exposure to all the plant oils (Table 1). Neem oil-treated seeds brought about 100% adult bruchid mortality at 72 hours after exposure (HAE) followed by palm kernel oil at 120 HAE. Adult bruchid mortality caused by the other plant oils reached 100% at 168 HAE except for oils from *C. nucifera*, *C. mannii*, *E. guineensis* (fruit mesocarp), *O. europaea* and *G. max* where the bruchid mortality at 168 HAE were 90%, 90%, 83%, 95% and 93% respectively. Adult bruchid mortality caused by neem oil treated seeds was significantly higher ($p < 0.0001$) compared with that of the other plant oils and the control. However, among the plant oils, palm oil-treated jars recorded the lowest ($p < 0.0001$) adult mortality. There was no mortality in the untreated (control) jars all through the 168 hours of mortality assessment.

All the tested plant oils brought about 70.9% – 98.6% reduction in the number of eggs laid by parental generation (Table 2). About 79.8 %– 100.0% reduction in F_1 progenies also occurred as a result of the plant oil treatment of the cowpea seeds. The number of eggs laid by parental generation and adult emergence was significantly lower ($p < 0.0001$) in neem oil treated jars compared with the other plant oils and the control. Among the plant oils, palm oil-treated seeds recorded the highest infestation (oviposition and F_1 progeny) values ($p < 0.0001$). About 91.5 – 100.0% reduction in cowpea seed damage occurred as a result of the plant oil treatment of the seeds (Table 3). The oils also saved about 78.9 – 98.5% weight of cowpea that could have been lost to seed perforation by the bruchid larvae. Seed damage (Seed perforation and weight loss) was significantly lower ($p < 0.0001$) in neem oil-treated seeds, while palm oil-treated seeds had the highest seed damage values when compared with other plant oils evaluated. However, seed damage observed in palm oil-treated seeds was not significantly different ($p > 0.05$) from that of seeds treated with coconut oil. There were strong positive associations between bruchid infestation and cowpea seed damage ($r > 0.950$; $n = 40$; $p < 0.0001$) (Table 4).

Table 1 Contact toxicity of oils from nine plant species on cowpea seed bruchids (*Callosobruchus maculatus* F.)

Plant oils	Bruchid mortality count (%)						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Castor (<i>Ricinus communis</i> L.)	50.00 bc	70.00 bc	80.00 b	90.00 b	95.00 a	97.00 a	100.00 a
Coconut (<i>Cocos nucifera</i> L.)	40.00 cd	50.00 de	70.00 c	70.00 b	80.00 a	90.00 ab	90.00 b
Groundnut (<i>Arachis hypogaea</i> L.)	50.00 bc	70.00 b	77.50 b	90.00 a	97.50 a	97.50 a	100.00 a
Melon (<i>Cucumeropsis mannii</i> Naudin)	30.00 d	40.00 e	72.00 bc	72.50 b	72.50 b	90.00 ab	90.00 b
Neem (<i>Azadirachta indica</i> A. Juss)	70.00 a	97.50 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Oil Palm (<i>Elaeis guineensis</i> L.)	30.00 d	40.00 e	70.00 c	70.00 b	70.00 c	80.00 b	82.50 c
Olive (<i>Olea europaea</i> L.)	50.00 bc	60.00 cd	70.00 c	70.00 b	80.00 b	90.00 ab	95.00 ab
Palm Kernel (<i>Elaeis guineensis</i> L.)	60.00 b	77.50 b	80.00 b	92.50 a	100.00 a	100.00 a	100.00 a
Soybean [<i>Glycine max</i> (L.) Merr.]	30.00 d	60.00 cd	70.00 c	72.50 b	80.00 b	90.00 ab	92.50 b
Control (Untreated)	0.00 e	0.00 f	0.00 d	0.00 c	0.00 d	0.00 c	0.00 d
<i>F</i> -value	38.78	82.77	53.36	76.66	160.51	93.80	286.10
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cv (%)	15.42	10.30	11.18	8.78	6.01	7.42	4.00

Means are values of four replicates; Data on insect mortality count were transformed (arcsine) before F-test;
Mean value followed by the same alphabet in a column are not significantly different from each other (SNK: $p > 0.05$);
Cv (%) = Coefficient of variation; *p*-value = Probability value; h = hours

Table 2 Effect of different plant oils on cowpea bruchid oviposition and adult emergence.

Plant oils	Number of eggs per 10 seeds	Oviposition inhibition (%)	Adult emergence	
			F ₁ progeny	Population reduction (%)
Castor (<i>Ricinus communis</i> L.)	8.00 c	92.7	0.25 c	99.5
Coconut (<i>Cocos nucifera</i> L.)	30.00 b	72.7	5.75 bc	89.4
Groundnut (<i>Arachis hypogaea</i> L.)	8.00 c	92.7	0.50 c	99.1
Melon (<i>Cucumeropsis manni</i> Naudin)	30.00 b	72.7	8.50 bc	84.4
Neem (<i>Azadirachta indica</i> A. Juss)	1.50 c	98.6	0.00 c	100.0
Oil Palm (<i>Elaeis guineensis</i> L.)	32.00 b	70.9	11.00 b	79.8
Olive (<i>Olea europaea</i> L.)	28.00 b	74.5	1.25 c	97.7
Palm Kernel (<i>Elaeis guineensis</i> L.)	8.25 c	92.5	1.75 c	96.8
Soybean [<i>Glycine max</i> (L.) Merr.]	29.50 b	73.2	5.50 bc	89.9
Control (Untreated)	110.00 a	0.0	54.50 a	0.0
<i>F</i> -value	1833.01		102.24	
<i>p</i> -value	<0.0001		<0.0001	
Cv (%)	9.91		41.13	

Means are values of four replicates; Square root transformation was carried out on data before *F*-test
Mean value followed by the same alphabet in a column are not significantly different from each other (SNK: $p > 0.05$)
Cv (%) = Coefficient of variation; *p*-value = Probability value

Table 3 Effect of plant oils on cowpea seeds damage and weight loss caused by *Callosobruchus maculatus* (F.).

Plant oils	Number seeds damaged ($n=100$)	Damage rate (%)	reduction	Seed weight (g)	
				Loss (%)	Save rate (%)
Castor (<i>Ricinus communis</i> L.)	1.00 d	99.0		0.40 c	95.3
Coconut (<i>Cocos nucifera</i> L.)	7.00 bc	93.0		1.75 b	79.3
Groundnut (<i>Arachis hypogaea</i> L.)	1.00 cd	99.0		0.30 c	96.5
Melon (<i>Cucumeropsis mannii</i> Naudin)	8.25 b	91.8		1.60 b	81.1
Neem (<i>Azadirachta indica</i> A. Juss)	0.00 d	100.0		0.13 c	98.5
Oil Palm (<i>Elaeis guineensis</i> L.)	8.50 b	91.5		1.78 b	78.9
Olive (<i>Olea europaea</i> L.)	5.25 c	94.8		0.90 bc	89.4
Palm Kernel (<i>Elaeis guineensis</i> L.)	1.50 d	98.5		0.40 c	95.3
Soybean [<i>Glycine max</i> (L.) Merr.]	5.00 c	95.0		1.00 bc	88.2
Control (Untreated)	100.00 a	0.0		8.45 a	0.0
<i>F</i> -value	1811.49			135.33	
<i>p</i> -value	<0.0001			<0.0001	
Cv (%)	10.41			25.46	

Means are values of four replicates; Mean value followed by the same alphabet in a column are not significantly different from each other (SNK: $p > 0.05$); Cv (%) = Coefficient of variation; *p*-value = Probability value

Table 4 Pearson's correlation between bruchid infestation and seed damage parameters evaluated.

Bruchid infestation	Number of seeds perforated	Percentage of seed weight loss
Number of eggs laid	0.998**	0.977**
F1 Progeny	0.980**	0.951**

** Correlation is significant at the 0.0001 level (2-tailed).

4. Discussion

The study shows that *C. maculatus* infestation and damage in cowpea storage could be severe if artificial control measures are not employed. The results underscore the high risks associated with the presence of this arthropod in any cowpea storage facility. The insecticidal effect of the tested plant oils agrees with previous findings by Ivbijaro [22], Uvah and Ishaya [23], Keita *et al.* [24], Ketoh *et al.* [25], Onolemhemhen [26], Law-Ogbomo and Enobakhare [27] and Haghtalab *et al.* [28].

The adult mortality may be due to the oil's interference with the insect's normal respiration, thereby resulting in their suffocation and death [29, 30]. Furthermore, the adult bruchid mortality may have been caused by saturated (such as lauric, myristic, palmitic and stearic) and unsaturated (*viz*: palmitoleic, oleic, myristoleic and linoleic) fatty acids that make up the chemical profile of plant oils. These acids have been reported to demonstrate bioactivity against some insect pests [31, 32, 33]. Of the different plant oils, neem oils have been the most studied. Neem oil contains *Azadirachtin* which is reported to be toxic to bruchids and many other insect pests [24].

The reduction in oviposition observed in this study could be attributed to the high adult mortality caused by the plant oils. The low number of F₁ progenies in treated jars may also be due to the ovicidal properties of the plant oils. Don-Pedro [18] reported that egg mortality could occur when oil blocks the exchange of air between the egg surface and the environment. Generally, oxygen is needed for insect egg survival especially in the first 7 days after oviposition [34], the use of these plant oils may have caused deficits in the oxygen level of the eggs leading to asphyxiation. The lower percentage weight loss recorded in plant oil-treated seeds may have been due to the lower F₁ population observed in those jars. Feeding activities by bruchids occur during progeny development (larval stage) and it usually results in exit holes on seeds leading to their weight loss. It may be that the plant oils modified the storage micro-environment, thereby discouraging bruchid larvae from perforating the seeds to feed, causing starvation and death of the insects as described by Law-Ogbomo and Egharevba [28, 35].

5. Conclusion

The study shows that the oils from nine plant species were effective against *C. maculatus*. However, oils from *A. indica*, *R. communis*, *E. guineensis* kernel and *G. max* appeared to render better protection to the seeds by significantly destroying various life stages of *C. maculatus* via contact. The plant oils are relatively cheap, accessible to subsistent farmers, and they constitute minimum or no risk to humans, livestock, pets, and other non-target organisms compared to synthetic chemical insecticides. However, further research to determine the bioactive compounds responsible for this insecticidal activity, the mechanism of action and persistence of bioactive compounds in the plant oils are needed to enhance/ facilitate the commercial application of these results.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declared no any conflict of interest.

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