Control of the cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), on stored cowpea using vegetable oils

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ABSTRACT

Coconut oil and two types of palm oil ("Palmin oil" and "Frytol oil") were evaluated for control of the cowpea beetle, *Callosobruchus maculatus* (F.) on stored cowpea, *Vigna unguiculata* (L.) seeds. The oils were applied at the rate of 4, 6 and 10 ml per kg cowpea. Oil treatment reduced oviposition and progeny emergence, but did not affect the longevity of the adult insects. The highest dosage of 10 ml kg^{-1} seed protected the seeds for 5 months. Palmin oil appeared superior to coconut and frytol oils. The oils had no adverse effect on seed viability after 5 months' storage.

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RÉSUMÉ

OSEKRE, E. A. & AYERTEY, J. N.: *Contrôle de coléoptère de dolique* Callosobruchus maculatus *(F.) (Coleoptera: Bruchidae) sur la dolique stockée utilisant l'huile de légumes.* Huile de coco et deux types de huile de palmier ("Palmin oil" et "Frytol oil") étaient évaluées pour le contrôle de coléoptère de dolique, *Callosobruchus maculatus* (F.) sur la dolique stockée, les graines *Vigna unguiculata.* Les huiles étaient appliquées à la proportion de 4, 6 et 10 ml kg^{-1} dolique. Le traitement d'huile reduisait la ponte et l'emergence de progéniture mais n'a pas influencé la longévité des insectes adultes. La dose la plus élevée de 10 ml kg^{-1} graine protégeait les graines pour 5 mois. Huile Palmin semblait supérieure à la noix de coco et les huiles de Frytol. Les huiles n'avaient d'effet défavorable sur la viabilité de graine après 5 mois de stockage.

Introduction

Cowpea represents the legume of choice for many homes in many developing countries, in the face of the high cost of animal protein. However, the crop is damaged by the cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in storage. Owusu-Akyaw (1991) reported that grain damage could reach 100 per cent if the insects are not controlled. The serious damage caused to the grain by the pest has led to extensive use of synthetic insecticides to control it. However, problems associated with the development of resistance by pests, toxic pesticide residues in treated foods, prohibitive cost and erratic supply of these insecticides have stimulated, over the years, interest in finding alternatives to their use.

Vegetable oils are reported as one of such viable alternatives in the control of some insect pests of stored products, including *C. maculatus* (Singh *et al.*, 1978; Don-Pedro, 1989; Murdock *et al.*, 1997). The use of plant oils in the control of storage insect pests is an ancient practice (Yun-tai & Burkholder, 1981), and is particularly appropriate in small-scale storage systems. The potential of these oils for the control of *C. maculatus* had prompted several trials to determine their efficacy as protectants of cowpea against pest damage (Mital, 1971; IITA, 1975; Singh *et al.*, 1978; Golob & Webley, 1980;

Messina & Renwich, 1983). However, these reports show considerable variations in the effectiveness of the oils in controlling the pest and in their effect on seed viability. The method of preparing the oils has been suggested as one of the reasons for the differences (Obeng-Ofori, 1995). However, this study used oils that were prepared with standard methods by the manufacturers. It therefore had the advantage of observing how these oils compared to those that were prepared with traditional methods as reported by others. They will also serve as standard oils for similar work in future.

This study aimed at evaluating the efficacy of three oils commonly available in Ghana. The use of these oils by the small-scale farmer can be encouraged, if found suitable, to protect cowpea against infestation by *C. maculatus*.

Materials and methods

Grains and oils

The cowpea variety (Asontem) used for this study was bought from the Ghana Food Distribution Corporation in Accra. The vegetable oils–coconut oil (produced in the Nzema area of the Western Region of Ghana from *Cocos nucifera* Linn.) and two types of palm oil produced from *Elaeis guineensis* Jacq. ("Palmin" and "Frytol" oils) manufactured by Lever Brothers Ghana Limited–were used. "Palmin" is a purified industrially manufactured palm oil with the carotenoids present, while "Frytol" is a purified, deodorised and bleached, industrially manufactured palm oil with the carotenoids removed. Coconut was bought from the Makola Market in Accra. The oils were stored in a laboratory maintained at 25 ± 2 °C and 65 - 70 % r.h.

Culturing of experimental beetles

About 750 g of heat-sterilized (at 60 °C for 4 h) cowpea grains were put in each of 10 previously cleaned 2-l Kilner jars. One hundred adult *C. maculatus*, previously collected from heavily infested cowpea from the market, were placed in each of the jars and covered with muslin cloth held in place by the covers of the jars.

The cultures were maintained in a laboratory at 25 ± 2 °C and 65 - 70 % r.h. On the 20th day after the cultures were set up, by which time adequate number of eggs would have been laid, the jars were emptied and all the adult insects (both dead and alive) were separated from the grains by sieving. The jars were then filled with the same grains. From the 30th day of culturing onwards, adults began to emerge and these were used for the different experiments.

Grain treatment with vegetable oils

The oils were admixed with the seeds at rates of 4, 6 and 10 ml kg^{-1} seed by pouring 4, 6 or 10 ml of each of the oils on 1 kg grain in separate glass bowls. A glass rod was used to stir the mixtures for 5 min, besides intermittent shaking of the bowl, to ensure complete and even coating of the seeds. The oil-coated seeds were then used for the various experiments, which were conducted in a completely randomised design with various replicates and maintained in a laboratory at 25 ± 2 °C and 65 - 70 % r.h.

Effect of oils on oviposition

Eight batches of 40 oil-coated cowpea seeds for each oil type were put in 250-ml Kilner jars. Three pairs of 0-1 day-old adult insects from the cultures were put into each of the jars, and covered with muslin cloth held in place by the jar covers. Three pairs of 0-1 day-old adults were also put into each of eight jars containing 40 cowpea grains, which had not been treated with the oils, to serve as the control. Since the adults are short-lived, and the females lay eggs (soon after mating) which take about 6 days to hatch, seeds from each jar were picked individually and the egg cases were counted 8 days after infestation.

A further investigation was conducted to determine whether already mated females would oviposit on oil-treated grains. For this study, a pair of freshly emerged adults were put into each of 25 separate small vials (5 cm × 2 cm diameter) and the open end sealed with cellotape. Four holes were punctured in the cellotape on each vial to ensure aeration within the vial. The paired insects were closely observed until each pair had mated. Forty oil-treated cowpea seeds were then put into four separate Petri dishes. Five already mated females were placed in each of the Petri dishes and another five put into a Petri dish containing 40 untreated seeds. These were kept for 8 days after which the egg cases in each dish were counted.

Effect of oils on emergence of F_1 progeny

The experimental set-up described above for oviposition was maintained for 30 days after which F_1 progenies started to emerge. On the 40th day after the start of this experiment, emerged adults (dead and alive) in each jar were counted. These numbers, expressed over the number of eggs laid in the respective jars, gave the percentage emergence. The efficacy of each treatment was determined by comparing numbers of F_1 progenies in the treated seeds with those in the untreated control.

Effect of oils on the longevity of insects

A separate set-up, similar to that described for the study on oviposition, was established in Petri dishes. Here, the Petri dishes were inspected daily, and the insects that had died in each Petri dish were removed and recorded. Insects that did not move or had uncoordinated movement when probed with a hairbrush were recorded as dead. A cumulative number of insects found dead was taken on the 4th, 7th and 14th day after the insects had been placed in the Petri dishes.

Protection offered by oils against C. maculatus *on stored cowpea under various conditions*

Cowpea seeds treated with 4, 6, and 10 ml of the oils kg^{-1} cowpea seed were exposed to the insect under four different storage conditions. Each treatment was replicated five times. About 250 g of the untreated seeds, which were put in five Kilner jars covered with muslin cloth held in place by jar covers, were maintained as control.

In Test 1, 250 g of oil-treated seeds were put separately in Kilner jars covered with muslin cloth held in place by jar covers and placed in trays containing industrial oil. Fifteen pairs of 0-1 day-old beetles were put into each jar. These were left for 6 months without interruption.

In Test 2, the same procedure as outlined above was followed, but here, the treatments were re-infested with 15 pairs of the beetles after 40 days' intervals and kept for 6 months.

In Test 3, in which only palmin oil was used (because it seemed slightly superior to the others in easiness to work with), the treated and untreated seeds were kept in 11cm × 20 cm × 1 cm cotton sacks and infested with 15 pairs of 0-1day-old insects. The bags were tied tightly at the open ends, and after every 40 days, they were untied and the seeds were re-infested with another 15 pairs of the insects and maintained for 6 months. This test was an attempt to simulate bag storage, as farmers normally store their produce in sacks.

In Test 4, which was designed to simulate farm storage conditions, where insects move freely in a farm store to infest produce, palmin oil-treated seeds and untreated controls were kept in 11 cm × 20 cm × 1 cm sacks and infested with 15 pairs of 0-1day-old beetles. The sacks were loosely tied with twine so that the insects could easily escape and / or enter every sack through the tied ends. These were stored for 6 months and percentage infestation was determined. All seeds with one or more holes were counted as infested.

Effect of oil on seed viability

For seed viability test, the cowpea seeds were treated with the oils at the rate of 10 ml kg^{-1}. The viability test was conducted on seeds treated with that dosage only because that was what had provided the seeds adequate protection after 5 months storage. Five seeds were collected from each of 36 jars containing either oil-treated or untreated seeds. These were bulked according to the type of oil. Four replicates were maintained for each treatment. The bulked samples were placed separately on moist Whatman No.1 filter paper (9-cm diameter) in glass Petri dishes (10-cm diameter). After 5 days, they were inspected and the number of seeds that germinated in each dish was noted. The remaining treated and untreated seeds were put in Kilner jars, covered with muslin cloth which was held in place by jar covers, and left free from insect infestation for 5 months. The viability test was again carried out after the period.

The data were transformed to either square-root values or Arcsine (where values were in percentages) and the transformed data were subjected to analysis of variance. Fisher's protected Least Significant Difference (LSD) was used to compare treatment means.

Results

Effect of oils on oviposition

The mean number of eggs laid in the control was 47, while the maximum recorded for the treated grains was 23.5. The results of this experiment showed that the oils significantly reduced ($P>0.05$) oviposition (Table 1). Frytol oil had the highest percentage reduction (54 ± 0.62) in oviposition at 4 ml per kg while palmin oil recorded the highest percentage reduction (88.6 ± 0.0) in oviposition when 10 ml kg^{-1} was used. The reductions in oviposition as dosage increased for each oil type were also significant.

Effect of oils on progeny emergence

Oil treatment significantly reduced progeny emergence. The mean percentage progeny emergence recorded for the control was 93 per cent. The reduction in progeny emergence as the oil dosage increased was also significant ($P>0.05$), and no adults emerged when the treatment was increased to 10 ml kg^{-1} (Table 2).

Effect of oils on longevity of the insects

The results showed that oils had no significant effect on the longevity of adult *C. maculatus*. The highest percentage mortality of 67.5 per cent was recorded in the control, compared to 65.0, 40.0 and 65.0 per cent recorded for palmin, coconut and frytol oils, respectively, after 4 days of storage (Fig. 1). After 7 days, mortality in the control treatment was 86.25 per cent, and nearly 90.0 per cent by the 14th day.

Protection offered by the oils against C. maculatus *on stored cowpea under various conditions*

The results of these experiments showed similar degree of infestation of the seeds, whether in Kilner jars or in cotton sacks. The reduction in infestation of the oil-treated seeds as the oil dosage increased was significant ($P>0.05$) (Tables 3 & 4). When the oil-treated seeds were stored in Kilner jars, 100 per cent infestation was recorded for all the three types of oils at 4 and 6 ml kg^{-1}. However, only about 10 per cent infestation was recorded at 10 ml kg^{-1} (Table 3). No damage was recorded when seeds were stored for 6 months after only one set of the insects had been initially introduced.

Effect of oil treatment on cowpea seed viability

Oil treatment had no effect on the viability of the seeds under the experimental conditions used. Seed viability after 5 months storage compared

TABLE 1

Mean Number of Eggs and Mean Percentage Reduction in Oviposition of C. maculatus *by Different Oil Treatments*

Treatment	4 ml kg^{-1}		6 ml kg^{-1}		10 ml kg^{-1}	
	No. of eggs laid	% reduction	No. of eggs laid	% reduction	No. of eggs laid	% reduction
Palmin oil	23.5	42.8 ± 1.02^{a}	9.0	58.7 ± 0.38^{bc}	0.9	88.6 ± 0.0^{d}
Coconut oil	16.2	48.0 ± 0.78^{b}	7.0	60.3 ± 0.42^{c}	1.0	86.7 ± 1.16^{d}
Frytol oil	13.2	54.0 ± 0.62^{b}	10	57.2 ± 0.81^{bc}	7.0	65.3 ± 2.53^{c}
Control			47			

Values followed by the same letter(s) are not significantly different ($P>0.05$) using LSD test. CV = 4.8 %

TABLE 2

Percentage Emergence and Percentage Reduction in Emergence of C. maculatus *F1 Progeny Under Different Oil Treatments*

Treatment	4 ml kg⁻¹		6 ml kg⁻¹		10 ml kg⁻¹	
	% emergence	% reduction	% emergence	% reduction	% emergence	% reduction
Palmin oil	41.2	55.5 ± 0.31[a]	0.0	100 ± 0.0[c]	0.0	100 ± 0.0[c]
Coconut oil	27.9	69.9 ± 0.20[a]	17.1	81.5 ± 0.16[b]	0.0	100 ± 0.0[c]
Frytol oil	32.2	65.2 ± 0.21[a]	23.4	75.3 ± 0.12[b]	0.0	100 ± 0.0[c]
Control			93			

Values followed by the same letter(s) are not significantly different (P>0.05) using LSD test. CV = 7.6 %

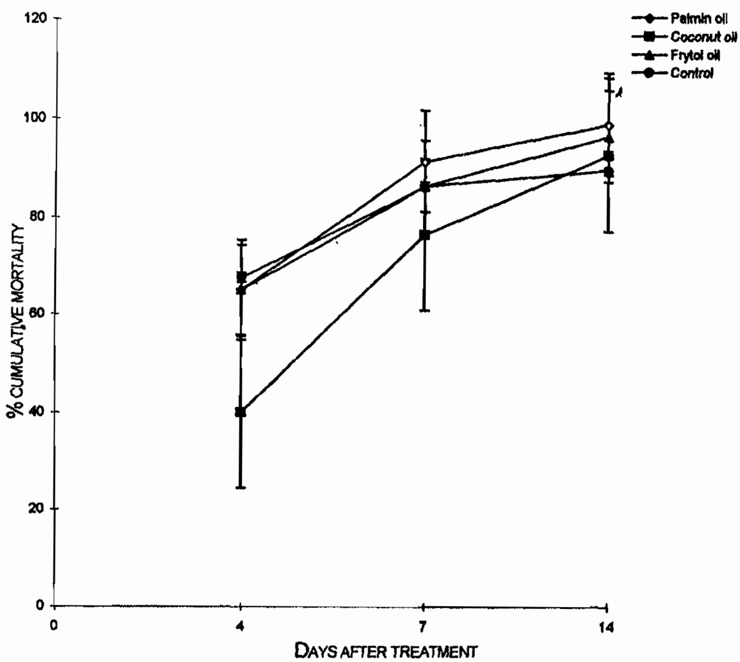

Fig. 1. Percent cumulative mortality of *C. maculatus* on oil-treated cowpea seeds.

favourably with the initial viability (Table 5).

Discussion

Palmin, frytol, and coconut oils protected cowpea seeds against attack by *C. maculatus*. Oil treatment significantly reduced oviposition and progeny emergence at all the three-dosage levels used. No progeny emerged when grain was treated at 10 ml kg⁻¹. All three oils showed similar protection and effectiveness. These results confirm the effectiveness of oils in protecting food grains against attack by some stored product insects (Singh *et al.*, 1978; Hill & Schoonhoven, 1981; Tikku, Koul & Saxena, 1981; Don-Pedro, 1989; Pacheco *et al.*, 1995). The similar protection offered by all the oils showed that the presence or absence of carotenoids in oils does not significantly affect their action as grain protectants.

On the effect of oils on oviposition, the results agree with those reported by Singh *et al.* (1978), Messina & Renwich (1983), Sujatha & Punnaiah (1985), Yadav (1985), Babu, Reddy & Hussaini (1989) and Pacheco *et al.* (1995), but they contradict the observation by Don-Pedro (1989) that in a no-choice experiment, oils had no significant effect on total oviposition. The reduction in oviposition in the oil-treated seeds may be caused by an oil film on the seeds which presented an unsuitable surface for oviposition. Although Don-Pedro (1989) showed that oils had no significant effect on total oviposition in a no-choice experiment, a contrary

TABLE 3

Percent Infestation and Percentage Reduction of C. maculatus *Infestation After Six Months Storage in Kilner Jars Under Various Infestation Regimes Treated With Different Doses of Plant Oils*

Infestation regime/oil	*4 ml kg⁻¹* % infestation	*4 ml kg⁻¹* % reduction	*6 ml kg⁻¹* % infestation	*6 ml kg⁻¹* % reduction	*10 ml kg⁻¹* % infestation	*10 ml kg⁻¹* % reduction
*Artificial re-infestation**						
Palmin oil	100.0	0.0 ± 0.0[a]	100.0	0.0 ± 0.0[a]	10.2	89.7 ± 0.64[c]
Coconut oil	100.0	0.0 ± 0.0[a]	100.0	0.0 ± 0.0[a]	10.3	90.2 ± 0.81[c]
Frytol oil	100.0	0.0 ± 0.0[a]	100.0	0.0 ± 0.0[a]	10.0	90.0 ± 0.12[c]
Control		100				
No artificial re-infestation+						
Palmin oil	100.0	0.0 ± 0.0[a]	86.4	16.2 ± 0.81[b]	0.0	100.0 ± 0.0[d]
Coconut oil	100.0	0.0 ± 0.0[a]	89.5	15.1 ± 1.23[b]	0.0	100.0 ± 0.0[d]
Frytol oil	100.0	0.0 ± 0.0[a]	84.6	17.2 ± 1.08[b]	10.0	100.0 ± 0.0[d]
Control		100				

Values followed by the same letter(s) are not significantly different (P>0.05) using LSD test. CV = 24.5 %
* Artificial re-infestation every 40 days after initial infestation with 15 adult insects.
\+ No artificial re-infestation after initial infestation with 15 insects.

TABLE 4

Percent Infestation and Percentage Reduction of C. maculatus *Infestation Treated With Different Doses of Palmin Oil and Stored for 5 Months in Cotton Sacks Under Various Infestation Regimes*

Infestation regime/oil	*4 ml kg⁻¹* % infestation	*4 ml kg⁻¹* % reduction	*6 ml kg⁻¹* % infestation	*6 ml kg⁻¹* % reduction	*10 ml kg⁻¹* % infestation	*10 ml kg⁻¹* % reduction
*Artificial re-infestation**						
Palmin oil	100.0	0.0 ± 0.0[a]	92.2	7.8 ± 0.05[b]	1.2	98.8 ± 0.13[c]
Control		100				
Insects exposed to natural infestation+						
Palmin oil	100.0	0.0 ± 0.0[a]	88.6	11.4 ± 0.62[b]	0.0	100.0 ± 0.0[c]
Control		100				

Values followed by the same letter(s) are not significantly different (P>0.05) using LSD test. CV = 26.3 %
* Artificial re-infestation every 40 days after initial infestation with 15 insects.
\+ Insects exposed to natural infestation in farm store after initial infestation with 15 adult insects.

observation was made in a two-way choice chamber. This suggests that the insects prefer clean seeds to oil-treated ones when they have choice, but where there is no alternative, they lay eggs on oil-treated seeds.

The results for this experiment further confirm observations by Singh *et al.* (1978), Pereira (1983), Babu *et al.* (1989), Don-Pedro (1989), Khaire, Kachare & Mote (1992), and Pacheco *et al.* (1995) that oils reduced progeny emergence of *C. maculatus*. The findings of Don-Pedro (1989) that groundnut and traditionally prepared coconut oils,

TABLE 5

Effect of Oil Treatment on Germination of Cowpea Seeds Stored for 5 Months

Treatment (10 ml kg^{-1})	*Initial % germination*	*Final % germination*
Frytol oil	84	83
Coconut oil	87	84
Palmin oil	82	80
Control	86	84
LSD (5 %)	NS	NS

when applied at 14 and 17.5 ml kg^{-1} to seeds containing late-stage larvae/pupae, had no significant (P>0.05) effect on adult emergence confirm that oils primarily affect the eggs rather than the larvae or pupae, which live mainly inside the seed and may not come into contact with the oil.

The results, which showed that oils had no effect on the longevity of adult *C. maculatus*, confirm the findings of Singh *et al.* (1978) and Don-Pedro (1989) that vegetable oils are not toxic to short-lived adult beetles. The contradictory reports by Hill & Schoonhoven (1981) that oils had effect on the longevity of the insects was possibly due to the insects drowning in large quantities (>10 ml kg^{-1}) of free surface oil. As explained by Singh *et al.* (1978), this could cause the death of insects by flooding their spiracles and causing asphyxiation, or by entering the tracheae and causing anoxia (Hewlett, 1975). In addition, it is not economical to use such high concentrations of oils. These results confirm the suggestion that control measures should aim at the eggs that are more susceptible to the oil.

The results also showed that oil treatment did not affect the viability of the grains, thus confirming the reports by Mummigatti & Ragunathan (1977), Singh *et al.* (1978), Varma & Pandey (1978), Adu (1986), and Obeng-Ofori (1995). On the contrary, Tembo & Murfitt (1995) reported that oils had effect on the viability of seeds. Yun-tai & Burkholder (1981) also reported that oil treatment reduced germination of wheat. Khaire *et al.* (1992) also showed that when mustard oil was used at the same dose or at 10 ml kg^{-1}, it did not affect the viability of one variety of chickpea, but the oil completely inhibited germination of another variety when it was applied and stored the same way.

The differences in germination of oil-treated seeds observed by different workers may be attributed to the differences in oil type and oil extraction method, or to the technique used and the type or variety of grain. Even if oil treatment adversely affects grains, and thus reduces their viability, they can be used to protect grains that are not meant for seed but are to be consumed.

The results of the study have confirmed that the cowpea beetle, *C. maculatus,* can be effectively controlled by admixing 10 ml of vegetable oils with 1 kg of cowpea seeds for at most 5 months. The admixing should be done before the seeds are infested, since larvae and pupae inside the seeds are unaffected by oil treatment. The use of vegetable oils should be encouraged in small farm storage, as the cost of these oils is low when compared with the losses incurred in untreated seeds. The use of vegetable oils to protect stored cowpea would help the small-scale producers of the crop by reducing the cost incurred in protecting cowpea seeds with synthetic insecticides. 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.

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