Effect of Mahogany *Khaya senegalensis* Seed Oil in the Control of *Callosobruchus maculatus* on Stored Cowpea

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Abstract

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Khaya senegalensis (Desr.) seed oil was evaluated for its effectiveness to control *Callosobruchus maculatus* on stored cowpea. The oil was extracted locally from dry *K. senegalensis* seeds. Its effect was compared with that of Pirimiphos methyl E.C. as a standard. There was almost complete adult mortality of *C. maculatus* within 24 h after treatment with seed oil at 1, 2 and 3 ml/100 g of cowpea. The seed oil significantly reduced the emergence of F_1 and F_2 progeny. The reduction in oviposition was not significant when compared with the control. The damage to cowpea by *C. maculatus* was significantly reduced by *K. senegalensis* seed oil. For all parameters examined, *K. senegalensis* seed oil showed no significant difference with Pirimiphos methyl E.C. in the control of *C. maculatus,* suggesting that *K. senegalensis* seed oil has high potential for use as a botanical resource for control of *C. maculatus.*

Keywords: *K. senegalensis* seed oil; Pirimiphos methyl E.C.; *C. maculatus;* cowpea grains; bioassay

The high cost of synthetic pesticides, the danger of pesticide misuse and of toxic residues in food has resulted in a rapid development and assessment of botanicals as alternatives for chemical control of stored product pests (Shaaya *et al.* 1997). In many storage systems, use of fumigants is the most economical tool for managing stored grain insect pests; however, storage pests are fast developing resistance to phosphine (Bell & Wilson 1995; Chaudhry 1995).

Since there are increasing drawbacks of the continued use of todays conventional fumigants, efforts are needed in the development of new compounds to replace those currently used (Bell & Wilson 1995; Chaudhry 1995; Lee *et al.* 2001).

The toxicity of essential oils extracted from plants to stored-product insects has been of special interest during the last decade (Tunc *et al.* 2000). Most constituents of the essential oils are monoterpenoids that are secondary plant chemicals. The monoterpenes are of interest to industrial markets because of other potent biological activities in addition to their toxicity to insects (Kubo *et al.* 1994; Shaaya *et al.* 1997).

The use of oils as fumigants or contact insecticides to protect grains, especially legumes, against storage insects is a traditional practice in many countries in Asia and Africa. This method is convenient and inexpensive for the protection of stored seeds in households and on small farms (Shaaya *et al.* 1997). Essential oils have a low toxicity to warm-blooded animals, high volatility, and toxicity to stored-grain insect pests (Regnault-Roger & Hamraqui 1993; Shaaya *et al.* 1991).

This paper reports the toxicity of *Khaya senegalensis* (Desv.) seed oil against *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) which is a major pest of stored cowpea causing severe damage to cowpea throughout the world.

MATERIAL AND METHODS

A culture of *C. maculatus* was raised by adding 30 pairs of freshly emerged *C. maculatus* to 500 g of cowpea grains in a large Kilner jar. The culture was maintained in the storage laboratory of the Department of Crop Protection. After 35 days, the freshly emerged F_1 generation adults were used to infest the cowpea samples used for the experiment.

Dry seeds of *K. senegalensis* were collected around the Institute for Agricultural Research where plenty of *Khaya* spp. are growing. The dry seeds were roasted and pounded into a paste. The paste was mixed with cold water and heated to between 60–70°C until the pure oil floated on the surface of the water, where it was collected and kept at room temperature until use.

Cowpea grains, which had been previously fumigated with Aluminium phosphide tablets and aired, were used for the experiment. 100 g of cowpea grains were weighed into Kilner jars. The *K. senegalensis* seed oil was applied at three levels or concentrations: 1, 2 and 3 ml of seed oil to 100 g of cowpea. Pirimiphos methyl E.C. at similar concentrations was used as standard. The control had neither seed oil nor Pirimiphos methyl E.C. Each variant was replicated three times. All the jars, except the control, were vigorously shaken to ensure proper mixing of cowpea grains and plant oil or Pirimiphos methyl E.C.

Each Kilner jar was infested with five pairs of freshly emerged *C. maculatus* adults. The jars were labelled, covered, and arranged on the laboratory bench in a Completely Randomised Design (CRD) at room temperature for 12 weeks.

Mortality was counted in all variants 24, 48 and 72 h after treatment; dead insects were removed and live ones left, until all insects were dead. To determine oviposition, eggs laid on 20 grains taken randomly from each jar in each treatment were counted 14 days after treatment. The number was recorded and the grains returned to the jars.

The progeny count started from 28–35 days after treatment and infestation (DAT) for the F_1, and from 56–63 DAT for the F_2 generation. All newly emerged adults were sieved out and counted. The data collected were subjected to ANOVA and the means were separated using SNK (Student Newman Keuls).

The percentage damage and percentage weight loss were determined by using the Thousand Grain Mass (TGM) method (Golob *et al*. 1982). From each jar of all treatments, 100 grains were randomly taken and separated into holed (damaged) and whole (undamaged) grains. The grains in each category were counted and the numbers used to calculate the percentage damage:

$$\text{Damage (\%)} = \frac{\text{damaged grains}}{\text{total grains}} \times 100$$

For percentage weight loss, the grains in each of the above categories were weighed, and the weights used to calculate the percentage weight loss:

$$\text{Weight loss (\%)} = \frac{(\text{Und}) - (\text{Dnu})}{\text{U (nd + nu)}} \times 100$$

where:
U – weight of undamaged grains
D – weight of damaged grains
nd – number of damaged grains
nu – number of undamaged grains

After 12 weeks the grains were tested for viability or germination. From each jar, 10 grains were randomly taken and placed in Petri dishes lined with moistened filter paper. These were left on the laboratory bench at room temperature for 7 days after which percentage germination was determined.

RESULTS AND DISCUSSION

The results of the mortality count are presented in Table 1. Seed oil of *K. senegalensis* killed nearly all adults of *C. maculatus* within 24 h (9.3–10.0), as did Pirimiphos methyl E.C. (10.0), and both at all concentrations.

The progeny emergence result is presented on Table 2. The treatment with seed oil significantly ($P < 0.05$) suppressed progeny emergence for two generations (28 DAT and 56 DAT). This indicates that even though there was high oviposition, the treatment either prevented the eggs from hatching or prevented the larvae and probably the pupae from completing their development in the cowpea grains. In addition to action against adult insects, plant oils generally have an ovicidal action. Egg mortality is caused by the physical properties of the oil coating, blocking respiration, rather than by a specific chemical effect. The larvae hatching from the eggs of *C. maculatus* must penetrate the seed to survive, but are unable to do this unless the egg is firmly attached to the seed surface. Eggs on oil-treated seeds are less firmly attached than on the controls, suggesting that the oil may inhibit successful larval penetration into the seed (Don-Pedro 1989). Copping and Menn (2000) proposed that application of oil to *C. maculatus* eggs might occlude the funnel, and thus lead to the death of the developing insect by asphyxiation.

The results of germination tests are presented in Table 3. Both seed oil and Pirimiphos methyl E.C. at all concentrations significantly ($P < 0.05$) reduced germination. The seed oil and Pirimiphos methyl E.C. significantly ($P < 0.05$) reduced the damage and weight loss of infested cowpea grains. Thus, seed oil of *K. senegalensis* may be more suitable for protecting grains for consumption, less so for seeds for planting as it appears to affect the viability of seed.

In medicinal purposes, the leaves and bark of *K. senegalensis* are used for treatment of stomach upset in both humans and livestock (Datziel 1948). Its use in crop protection dates back as early as 1900 when it was used for preserving seeds for next year's planting (Thomson 1910;

Table 1. Adult mortality of and oviposition by *C. maculatus* after treatment of cowpea grain with *K. senegalensis* seed oil

Sample No.	Treatments	24 hours	48 hours	72 hours	Oviposition
1	seed oil – 1 ml	$10.00^{a} \pm 0.58$	0.00	0.00	$1.74^{ab} \pm 0.02$
2	– 2 ml	$10.00^{a} \pm 0.76$	0.00	0.00	$0.97^{c} \pm 0.02$
3	– 3 ml	$9.33^{a} \pm 0.33$	0.40	0.27	$0.83^{c} \pm 0.01$
4	Pirimiphos methyl E.C. – 1 ml	$10.00^{a} \pm 0.58$	0.00	0.00	$1.74^{abc} \pm 0.05$
5	– 2 ml	$10.00^{a} \pm 0.58$	0.00	0.00	$1.74^{abc} \pm 0.05$
6	– 3 ml	$10.00^{a} \pm 0.58$	0.00	0.00	$1.17^{abc} \pm 0.01$
7	control	$3.59^{d} \pm 0.33$	3.33	3.08	$1.50^{abc} \pm 0.04$

Oviposition was counted 14 days after treatment
Means followed by the same letter are not significantly different at 5% ($P < 0.05$)

Table 2. Effect of *K. senegalensis* seed oil on the number of emerged progeny of *C. maculatus*

Sample No.	Treatments	28 DAT	56 DAT
1	seed oil – 1 ml	$13.67^{b} \pm 0.01$	$1.52^{d} \pm 0.03$
2	– 2 ml	$10.00^{bc} \pm 0.08$	$0.83^{d} \pm 0.01$
3	– 3 ml	$5.67^{bc} \pm 0.01$	$0.33^{d} \pm 0.00$
4	Pirimiphos methyl E.C. – 1 ml	$11.33^{bcd} \pm 0.07$	$2.11^{d} \pm 0.66$
5	– 2 ml	$11.33^{bc} \pm 0.04$	$7.05^{d} \pm 0.03$
6	– 3 ml	$8.33^{bc} \pm 0.01$	$1.64^{d} \pm 0.01$
7	control	$48.00^{a} \pm 0.09$	$22.70^{a} \pm 0.00$

Means followed by the same letter are not significantly different at 5% ($P < 0.05$)
DAT= days after treatment

Table 3. Effect of *K. senegalensis* seed oil on percent damage and weight loss of cowpea grains

Sample No.	Treatments	Damage (%)	Weight loss (%)
1	seed oil – 1 ml	0.80 ± 0.06	0.41 ± 0.01
2	– 2 ml	0.70 ± 0.07	0.40 ± 0.00
3	– 3 ml	0.40 ± 0.06	0.36 ± 0.01
4	Pirimiphos methyl E.C. – 1 ml	0.10 ± 0.03	0.14 ± 0.00
5	– 2 ml	0.10 ± 0.03	0.12 ± 0.00
6	– 3 ml	0.10 ± 0.03	0.01 ± 0.00
7	control	30.00 ± 9.21	2.00 ± 0.00

Means followed by the same letter are not significantly different at 5% ($P < 0.05$)

Meek 1931). The oil is used as a dry skin lotion in the Middle Belt of Nigeria (personal communication). Therefore, *K. senegalensis* products are less harmful to humans than most conventional insecticides. Studies have reported that plant oils are readily biodegradable and less detrimental to non-target organisms than synthetic pesticides (Tunc *et al.* 2000; Lale 2002).

Shaaya *et al.* (1997) have indicated that there is absorption of plant oils by the treated commodity. They suggested however that higher concentrations and longer exposure periods were needed to achieve a similar level of mortality for a given species than required when the oils were applied in space fumigation.

Table 4. Effect of *K. senegalensis* seed oil on germination of cowpea grains

Sample No.	Treatments	Germination count (%) ± S.E.
1	seed oil – 1 ml/100 g	90.00^{b} ± 0.00
2	– 2 ml/100 g	90.67^{b} ± 0.02
3	– 3 ml/100 g	100.00^{b} ± 0.02
4	Actellic EC – 1 ml/100 g	80.00^{b} ± 0.03
5	– 2 ml/100 g	96.67^{b} ± 0.02
6	Pirimiphos methyl E.C. – 3 ml/100 g	96.67^{b} ± 0.06
7	control	70.00^{b} ± 0.06

Means followed by the same letter are not significantly different at 5% ($P < 0.05$)

Large quantities of plant materials have to be processed in order to obtain oils in quantities sufficient for commercial scale tests. Lale (2002) has suggested the use of ethanol as a carrier when applying most plant oils. The carrier ensures the proper dissipation and spread of the oil all over the stored produce.

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