THE REPELLENT EFFECT OF NEEM (*Azadirachta indica* A. Juss.) OIL AND ITS RESIDUAL EFFICACY AGAINST *Callosobruchus maculatus* (COLEOPTERA: BRUCHIDAE) ON COWPEA

Sarah H. DANIEL and R. H. SMITH

Department of Pure and Applied Zoology, University of Reading, P. O. Box 228, Whiteknights Reading RG6 2AJ, U.K.

ABSTRACT

Laboratory investigations on the repellent effect of crude neem oil and its efficacy against *C. maculatus* on cowpeas were made at 30°C and 70% r.h. Oviposition rate of adults on treated cowpeas was reduced more in two-way choice experimental jars than in a no-choice arena. This effect increased with concentration of neem oil applied in the order 1< 5< 10 ml/kg. Adult emergence was significantly reduced in all the treatments ($P<0.05$). In a two-way-choice arena there was high discrimination with more eggs laid on the untreated than on treated cowpeas. In jars containing layers of treated and untreated cowpeas, adults tended to move to the untreated layers. This tendency was more pronounced when the concentration of neem oil was increased.
Neem oil also showed high repellency when applied at 1, 5 and 10 ml/kg. Neem oil treatment gave good protection against *C. maculatus* and when applied at the rate of 5 or 10 ml/kg of cowpeas its residual efficacy lasted up to 6 months.

INTRODUCTION

The cowpea *Vigna unguiculata* (L.) Walp is an important pulse crop in West Africa and is a major source of protein (Stanton, 1966). The cowpea weevil, *Callosobruchus maculatus* (F.), the most important pest of stored cowpeas, causes severe losses (Caswell, 1968). This study evaluates neem oil as a residual repellent against *C. maculatus*.

MATERIALS AND METHODS

Insects and Neem

A Brazilian (Campinas) strain of *C. maculatus* was obtained from the Natural Resources Institute (formerly ODNRI, Slough, U.K.). Insects were cultured on cowpea and experiments undertaken at 30°C and 70% r.h. Crude neem oil was obtained from the Neem Mission, Puna, India.

Effects of neem oil on oviposition and progeny development (no choice)

50g batches of fresh cowpeas were each treated with 0, 1, 5 or 10 ml/kg (0.1, 0.5 or 1% w/v) neem oil. 20 adults (0-24 hrs old) of *C. maculatus* were added to each jar and left to lay their lifetime complement of eggs in about nine days (Dick and Credland, 1984). After two weeks, ten beans were randomly selected and the number of eggs per bean was recorded. The beans were isolated in tubes and the number of adults that emerged was recorded.

Two-way choice experiments with layers of cowpeas.

Cowpeas were treated as above and arranged in layers in jars. Layers were separated by plastic mesh with about 3.5mm apertures. Each jar contained two treated layers (both treated at the same neem concentration) sandwiched between a top and bottom layer of untreated cowpeas. Five pairs of newly-emerged adults were added (a) at the boundaries between layers (30 adults/jar) or (b) all 30 adults were released at the centre boundary layer (between both layers of treated beans). There were two replicates of (a) and two replicates of (b). The distribution of parents after a week was recorded. The number of adults that emerged over a period of ten weeks was also recorded. The result was taken as a mean of (a) and (b).

Two-way choice experiment without layers.

Ten treated beans were mixed with ten untreated marked beans in a vial. This was replicated three times. Two pairs of newly emerged adults were added to each vial and the number of eggs laid, hatched and adults produced was recorded.

Multiple choice experiment

Plastic petri dishes (9cm diameter) were prepared with 6 groups of 7 beans each; 5 groups were treated with either coconut, corn, groundnut, sesame or neem oil at the rate of 5 or 10ml/kg and the 6th group consisted of untreated cowpeas. Five pairs of newly emerged adults were introduced and left to oviposit. The total number of eggs laid and adults emerged was recorded. There were three replicates.

The residual efficacy of the neem oil

Two hundred undamaged cowpeas (about 50g) were mixed with neem oil at 0, 1, 5 or 10 ml/kg. Three replicates of each treatment were used. Initially, 20 adults (10 males, 10 females) of *C. maculatus* were introduced into each jar. Numbers of damaged seeds and insects were recorded monthly until there was a 100% seed damage. Whenever the insect population was found to be low, fresh batches of adult bruchids were added. This gave an indication of the residual efficacy of the neem oil for up to six months.

RESULTS

Effects of neem oil on oviposition and adult emergence (Table 1)

There were significant differences in the number of eggs laid, hatched and adults emerged from cowpeas treated with neem and the control ($P<0.005$). Though as many eggs were laid by females on the 1ml/kg treated cowpeas as the control, the number of eggs that hatched and adults that emerged were significantly lower ($P<0.05$). No eggs were laid on the 10ml/kg treated cowpeas and there were no adult progeny.

Table 1. Effect of neem oil on the oviposition and adult emergence of C. maculatus on cowpeas.

Treatment (ml/kg)	Mean eggs laid *	Mean eggs hatched	% egg hatch	Mean adult progeny	% survival, egg to adult
Control	32.60a	31.80a	97.77	27.00a	83.34a
Neem (1)	37.60a	26.20b	69.15	7.00b	18.69b
Neem (5)	20.00b	8.60c	41.15	0.20c	0.91c
Neem (10)	0.00c	0.00c	0.00	0.00c	0.00c

* Values followed vertically by different letters are significantly different ($P < 0.05$).

Two-way choice experiment with layers of cowpeas.

ANOVA showed that there was a significant difference in the way parent adults distribute themselves when introduced into jars containing layers of treated and untreated cowpeas ($F_{5,58} = 13.39$, $P<0.001$) and this distribution was similar whether the insects were introduced at one boundary layer or spread on all three boundaries within the jars. There were also no significant differences in the numbers of adults in the two similarly-treated levels in each jar ($P>0.05$). The repellent effect can thus be seen by comparing the mean number of adults in the treated layers with those in the two untreated layers. The repellent effect of the neem is seen in the distribution of parents one week after introduction (Table 2). The neem oil greatly repelled the insects and the effect was dose dependent. Similar total numbers of adults emerged from jars independent of the method of introduction ($F_{1,58} = 0.06$, $P>0.05$). However, the number of adults emerging from untreated and treated cowpeas differed significantly ($F_{1,58} = 102.13$, $P<0.001$).

Table 2. The repellent effect of the neem oil on adults of C. maculatus added to layers of treated and untreated cowpeas in the same jar. The data are the mean number of insects moving to the treated and untreated layers of cowpeas (four replicates).

Treatment (ml/kg)	Mean number of insects		Repellency class*
	Treated	Untreated	
0	14.25	15.75	---
1	8.75	21.25	III
5	3.50	26.50	IV
10	2.50	27.50	V

* Repellency classes calculated after Mac Donald et al, (1970).

Two-way choice experiment without layers.

When treated and untreated seeds were mixed in the same vial, the number of adults emerging from the untreated and treated cowpeas differed significantly and the F1 progeny decreased with increase in neem concentration (see Table 3). Neem oil deterred oviposition. Discrimination Quotient (Messina and Renwick, 1983) values ranging from 0.60 to 0.87 indicated that the effect was particularly strong. The neem oil affected the number of adults emerging from the untreated as well as the treated cowpeas. The presence of neem oil on cowpeas also affected the survival rate of adults emerging.

Multiple choice (Table 4)

Females laid more eggs on untreated than on treated cowpeas ($F_{11,24}$ = 77.12, $P<0.001$). Also, more eggs were laid on the untreated cowpeas in the presence of oils applied at 5 ml/kg than on the untreated cowpeas in the presence of cowpeas treated at 10ml/kg ($P<0.001$). The presence of neem oil treated cowpeas in the petri dish greatly reduced the oviposition rate of females of *C. maculatus*. When neem oil treated cowpeas were excluded from the petri dish, females laid as many eggs on the other vegetable oil-treated cowpeas as on the control, though adult emergence from these cowpeas was significantly low. Few or no adults emerged from cowpeas treated with groundnut, sesame and neem oils at 5 or 10 ml/kg. The repellency of the different oils can be classified thus:- neem > sesame ≥ groundnut >corn >coconut.

Table 3. Effect of neem oil on the oviposition and adult emergence of C. maculatus on treated and untreated cowpeas mixed in the same jar showing attractiveness and suitability of control (untreated) versus treated.

Treatment	Mean eggs laid	Mean adults emerged ±SE	Mean %adult survival±SE*	DQ**	P***
Untreated vs untreated	50.00 46.00	41.33±11.06 38.67± 9.61	83.87± 5.42a 83.87± 2.54a	--	n.s.
Untreated vs 1 ml/kg	46.33 11.67	35.33± 1.53 2.00± 1.00	76.32± 2.91a 10.00±10.00d	0.61	0.001
Untreated vs 5 ml/kg	49.67 5.33	29.33± 4.93 0.33± 0.58	58.99± 3.40b 6.67±11.55d	0.81	0.001
Untreated vs 10 ml/kg	41.33 3.00	25.00± 2.65 0.00± 0.00	60.75± 7.35b 0.00± 0.00d	0.87	0.001
1 ml/kg vs 1 ml/kg	29.67 30.67	10.00± 1.73 11.67± 2.08	33.81± 6.63c 37.87± 3.94c	--	n.s.
5 ml/kg vs 5 ml/kg	9.33 8.67	0.00± 0.00 0.00± 0.00	0.00± 0.00d 0.00± 0.00d	--	n.s.
10 ml/kg vs 10 ml/kg	0.00 0.00	0.00± 0.00 0.00± 0.00	0.00± 0.00d 0.00± 0.00d	--	n.s.

* Values followed by same letters vertically are not significantly different (P> 0.05).

** Discrimination quotient =
$$\frac{\text{(No. of eggs on untreated - No. of eggs on treated)}}{\text{(No. of eggs on untreated + No. of eggs on treated)}}$$

*** Chi-square, compares number of eggs on untreated and treated.

Table 4. The repellent and inhibitory effect of neem oil on the oviposition and survival of C. maculatus in multiple-choice oviposition dishes in the presence of cowpeas treated with other vegetable oils at 5 and 10 ml/kg.

Treatment	Oil applied	Mean eggs* laid ± SE	Mean % adult survival ± SE
5 ml/kg	untreated	58.33 ± 1.16a	82.35 ± 12.47a
	coconut	29.33 ± 2.08b	42.78 ± 15.59b
	corn	24.00 ± 5.20c	14.82 ± 7.41c
	groundnut	23.33 ± 1.16c	2.91 ± 2.52e
	sesame	23.33 ± 6.11c	2.63 ± 0.00e
	neem	1.00 ± 1.00e	0.00 ± 0.00e
10 ml/kg	untreated	42.00 ± 4.36a	88.24 ± 5.90a
	coconut	22.67 ± 3.79b	16.29 ± 2.39c
	corn	17.33 ± 3.06c	5.19 ± 5.01d
	groundnut	10.00 ± 1.00d	0.00 ± 0.00e
	sesame	10.00 ± 3.46d	0.00 ± 0.00e
	neem	0.00 ± 0.00e	0.00 ± 0.00e

* Values followed vertically by different letters are significantly different ($P < 0.05$).

Table 5. Average number of adults emerged from treated cowpeas and percent seed damage by C. maculatus over a six month period.

Neem oil	% seed damage			adult insects emerged		
(ml/kg)	1 month	2 months	6 months	1 month	2 months	6 months
0	61	100	100	122	650	650
1	42	100	100	122	634	634
5	0	19	100	0	65	470
10	0	0	0	0	0	0

Residual efficacy of neem oil (Table 5)

Neem oil at 10ml/kg cowpeas offered protection for up to six months. There was no adult progeny from these treated seeds for up to six months even when fresh adults were intermittently introduced. Also, there were no seeds holed. When the cowpeas were treated at 1ml/kg, as many adults emerged as in the control. There was a 100% seed damage in both cases. However, in 5ml/kg treated seeds, it took up to six months before the seeds were 100% damaged, and up to 40 fresh adults had to be subsequently introduced into the jars. A positive correlation ($r = 0.987$) was observed between emergence of weevil offspring and damage to cowpea grains.

DISCUSSION

Our previous work showed that *C. maculatus* lays as many eggs on untreated as on groundnut, sesame and corn oil treated cowpeas in a no-choice arena. With neem oil treatment, however, fewer eggs were laid than the control (Table 1). Also, when parents were introduced into jars containing treated and untreated cowpeas they moved to the untreated sections and laid more eggs on them. Messina and Renwick (1983) reported the DQ of peanut oil applied at 5 ml/kg to be 0.16 and showed no significant oviposition preference to untreated in the presence of 1 ml/kg treated cowpeas. In contrast, neem oil applied at 1 or 5 ml/kg had DQ values of 0.60 and 0.81 respectively, showing a high deterring factor. Adults of *C. maculatus* showed a significant oviposition preference to untreated cowpeas. Not only did the neem oil repel adults and reduce oviposition (on both treated and untreated cowpeas) but it also affected hatching of the eggs laid. The lack of hatching eggs in 1 ml/kg neem oil treated seeds was due to physical mode of action of this oil. It is ovicidal by blocking egg-pores causing anoxia with a lethal critical period of only 5 seconds at about 30°C. Since ovicidal efficiency increases with increase in molecular weight and viscosity (Fiory *et al*, 1963) neem oil, being the most viscous (104.50 cp at 30°C) of 9 oils tested, was the best ovicide (S. H. Daniel, unpublished data).

Neem has been shown to contain compounds like azadirachtin, meliantriol and salanin (Butterworth and Morgan,1968) which are said to be repellent, antifeedant and have growth disrupting effects (Schmutterer, 1990). Neem oil applied at the rate of 5 ml and 10 ml/kg could effectively protect cowpeas up to six months. Unless the cowpea testa is removed neem oil treatments may affect taste after cooking. However, neem oil does not impair germination (Mummigatti *et al*, 1977 and Chander and Ahmed, 1982). This study has shown that neem oil can protect stored seeds for six months, which is the normal period seeds are stored before planting in the rainy season.

REFERENCES

Butterworth, J.H. and Morgan, E.D. (1968) Isolation of a substance that suppresses feeding in locusts. *Chemical Communication*, **1**, 23-24.

Caswell, G.H. (1968) The storage of cowpea in the Northern States of Nigeria. *Proceeding of Agricultural Society of Nigeria*, **5**, 4-6.

Chander, H., Ahmed, S.M. (1982) Extractives of medicinal plants as protectants against *Callosobruchus chinensis* L. infestation. *Journal of Food Science and Technology, India*, **19**, 50-52.

Dick, M. and Credland, P.F. (1984) Egg production and development of three strains of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) *Journal of Stored Products Research*, **20**, 221-227.

Fiory, B. J., Smith, E. H. and Chapman, P. J. (1963) Some factors influencing the ovicidal effectiveness of saturated petroleum oils and synthetic isoparaffins. *Journal of Economic Entomology*, **56**, 885-888.

MacDonald, L.L., Guy, R.H. and Speirs, R.D. (1970) Preliminary evaluation of new candidate materials as toxicant repellents and attractants against stored product insects. *USDA Marketing Research Reports* No. 882.

Messina, F.J. and Renwick, J.A.A. (1983) Effectiveness of oils in protecting stored cowpeas from the cowpea weevil (Coleoptera: Bruchidae). *Journal of Economic Entomology*, **76**, 634-636.

Mummigatti, S.G. and Ragunathan, A.N. (1977) Inhibition of the multiplication of *Callosobruchus chinensis* by vegetable oils. *Journal of Food Science and Technology*, **4**, 184-185.

Schmutterer H. (1990) Properties and potential of natural pesticides from the Neem tree, *Azadirachta indica*. *Annual Review of Entomology*, **35**, 271-297.

Stanton, W.R. (1966) *Grain legumes in Africa* 183pp. FAO, Rome.

EFFET REPULSIF DE L'HUILE DE NEEM SUR *CALLOSOBRUCHUS MACULATUS* SUR NIEBE

Sarah H. DANIEL and R.H. SMITH

University of Reading
Dept. of Pure & Applied Zoology, P.O. Box 228
Reading RG6 2AJ, U.K.

Résumé

Des études de laboratoire portant sur l'effet répulsif de l'huile de neem brute et sur son efficacité sur *C. maculatus* vivant sur le niébé ont été entreprises à 30°C et 80 % HR. Les résultats montrent que le taux de ponte des adultes sur les pois traités s'est trouvé plus grandement affecté au cours d'une expérience en arène à double choix qu'il ne l'était au cours d'une expérience en arène sans choix et que cet effet s'est accru avec l'augmentation de la concentration d'huile de margousier appliquée à raison de 1,5 ou 10 ml/kg de pois.

Le taux d'émergence des adultes a fortement diminué pour tous les traitements (P 0,05). Dans l'expérience à choix unique, on a noté une forte inhibition de la ponte sur les pois traités ayant abouti à une augmentation de la ponte sur les pois non traités. La distribution des populations d'adultes montrait que ceux-ci se retrouvaient aussi préférentiellement sur les pois non traités, et ceci en fonction de la dose. On a noté une corelation positive (r = 0,98) entre le nombre moyen d'insectes émergés et celui de graines détériorées. Le traitement à l'huile de neem a conféré une bonne protection contre *C. maculatus* et ses effets résiduels ont duré jusqu'à six mois pour une application au taux de 5 ou 10 ml/kg de pois.