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Ovipositional Deterrence of Methanolic and Etherial Extracts of Five Plants to the Cowpea Bruchid, *Callosobruchus maculatus* (F.) (*Coleoptera: Bruchidae*)

E.A. Elhag, A.H. El Nadi and A.A. Zaitoon

College of Agriculture and Veterinary Medicine, King Saud University
Gassim Branch, Buraydah, P.O. Box 1482, Kingdom of Saudi Arabia

الأثر المانع لوضع البيض للمستخلصين الميثانولي والإثيري لخمس مواد نباتية عند خنفساء اللوبيا (*Callosobruchus maculatus*)

الملخص: تم تقييم الأثر المانع لوضع البيض للمستخلصين الميثانولي والإثيري لنبات الحرمل (*Rhazya stricta*) والرمرام (*Heliotropium bacciferum*) و بذور النيم (*Azadirachta indica*) و القرنفل(*Syzygeum aromaticum*) وقشور الحمضيات (*Citrus* sp.) على وضع البيض بواسطة خنفساء اللوبيا (*Callosobruchus maculatus*) في اختبار اختياري. أظهرت النتائج انخفاضا معنويا في معدل وضع البيض على البذور المعاملة بخلا المستخلصين لجميع المواد المختبرة. بلغت أعلى النسب لمنع وضع البيض ٩١,٨ و ٩٠,٩ و ٨٣,٩ و ٨٠,٠% في المستخلصات الميثانولي ٠,٥% للنيم والإثيري ٠,١% لقشور الحمضيات والميثانولي ٠,٥% للحرمل والإثيري ٠,١% للقرنفل على التوالي. نتج عن استخدام المستخلص الميثانولي للنيم والحرمل معدل منع أكبر لوضع البيض عن مستخلصهما الإثيري، بينما تساوت نسبة المنع لمستخلصي الرمرام. تبدو النتائج مشجعة لإدخال مانعات وضع البيض من المستخلصات النباتية في برامج الإدارة المتكاملة لآفات المواد المخزونة.

ABSTRACT: Methanol and diethyl ether extracts of Harmal, *Rhazya stricta* Decne.; neem seed kernels, *Azadirachta indica* A.Juss; cloves, Syzygeum *aromaticum* (L.); citrus peel and Ramram, *Heliotropium bacciferum* (Forssk.) were evaluated for their deterrence to oviposition by *Callosobruchus maculatus* (F.) on chickpeas in choice tests. Both extracts of all materials significantly reduced oviposition on treated seeds. Maximum deterrent effects (91.8%) were obtained in the neem seed methanol extract at 0.5% concentration, citrus peel 0.1% ether extract (90.9%), *R. stricta* 0.5% methanol extract (83.9%), and clove 0.1% ether extract (80.0%). Methanol extracts of neem seeds and *R. stricta* evoked higher deterrent effects than their etherial extracts, whereas the responses for cloves and citrus peel were more pronounced in their ether extracts. *H. bacciferum* % deterrency due to both types of extracts were practically identical. The results encourage future incorporation of such plant extracts as ovipositional deterrents in stored-product IPM programmes.

The cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is an economically important pest of a wide variety of stored legumes. The females lay eggs, which are deposited and glued to the seedcoat and after hatching the larvae feed inside the seed causing damage to the seeds. Up to 100% bruchid infestation can often take place in seeds stored for 3-5 months storage periods, (Singh, 1977), resulting in the damaged seeds having reduced weights, poor germination potential, and consequently rendered invaluable. Current control procedures rely on the use of synthetic insecticides such as pyrethroids, pirimiphos methyl; and fumigation with phosphine or methyl bromide, (Hole *et al.*, 1976; Price and Mills, 1988; Singh,1990). The widespread use of these pesticides has significant disadvantages which include development of resistant strains of insects (White, 1995), concerns about residues on grains and their threats to human health, and outbreaks of environmental hazards due to the contribution of fumigants such as methyl bromide on the stratospheric ozone loss,(Grahl, 1992; Noling and Becker, 1994; Taylor,1994). Buffin (1992) pointed to the calls to phase-out methyl bromide by the year 2001.

There is increasing interest in finding safer or alternative insecticides, and one such alternative, as pointed out by Arnason *et al.* (1989) and Isman (1994), is the use of biodegradable natural plant compounds as insecticides. The use of plant derivatives for stored grain protection has gained popularity in recent years as replacements for synthetic insecticides (Su, 1977, 1990; Singh and Srivastava, 1983; Malik and Naqvi, 1984; Saim and Meloan, 1986; Hassanali *et al.*, 1990; Weaver *et al.*, 1991; Xie *et al.*, 1995; Beckele *et al.*, 1996).

Most of the previous botanical research was focused on testing toxicity, antifeedant activity, repellency and effects on development of plant materials such as neem seeds and leaves (*Azadirachta indica* A.Juss), and locally recognized plant species. These included materials such as tumeric (*Curcuma longa* L.), bay leaves (*Laurus nobilis* L.), fenugreek (*Trigonella foenum-graecum* L.), citrus peels (*Citrus* sp.), *Ocimum suave* Willd, cloves, (*Syzygeum aromaticum* (L.) Merr. and Perry syn. *Eugenia caryophyllus* (Sprong.) Bullock and Harrison), etc. However, ovipositional deterrence of plant materials to stored product insects has received little attention despite its potential in suppressing population proliferation in stored grains. This has been investigated in other insects such as the cucurbit fruit fly, *Dacus cucurbitae* (Coq.) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel), (Singh and Srivastava, 1983; Chen *et al.*, 1996); the onion maggot, *Delia antiqua* (Meigen), (Javer *et al.*, 1987); and the cutworm, *Agrotis segetum* Schiff. (Anderson and Lofqvist, 1996). Elhag (1999), reported that crude extracts of four out of the nine plants tested, namely, Ramram, neem seeds, cloves and Harmal, produced ovipositional deterrence of between 56.8 and 82.0% to the cowpea bruchid, *C. maculatus*. Some of these plant materials were known to have biological activity against insects (Elhag *et al.*, 1996), leucopenic and cytotoxic effects on higher animals (Siddiqui and Bukhari, 1972; Hassan *et al.*, 1977), or of medicinal value used by public practitioners.

In this study, we have examined the deterrent activity of the diethyl ether and methanol extracts from five plant parts *viz.*, *R. stricta*; *A. indica*; S. *aromaticum*; citrus peels and *H. bacciferum* to oviposition by the cowpea bruchid.

Materials and Methods

INSECTS, EXTRACTS AND TREATMENTS: *Callosobruchus maculatus* adults were obtained from cultures maintained at 25±2°C, 65±5% RH, and 12:12 (L: D) h photoperiod on chickpea (*Cicer arietinum* L. cv. Cyperian), in a culture room. Extracts from five plants were tested: aerial parts of Harmal, *R. stricta* Decne., neem seed kernels (*A. indica*); cloves (*S. aromaticum*); citrus peels, and aerial parts of Ramram (*H. bacciferum*). Black pepper seeds (*Piper nigrum* L.) and seeds of Rashad, *Lepidium aucheri* Boiss. were used in an initial test. All materials were collected locally in the central region, Kingdom of Saudi Arabia. The plant parts were air dried in the laboratory, ground to a fine powder by using a ceramic mortar and pestle and extracted by the organic solvents, methanol and diethyl ether, at ambient temperatures. A gentle warming to 35-40°C was sometimes found necessary especially when the solvents were taken straight from the refrigerator. The powdered material was mechanically stirred for 2-3 hrs with the appropriate solvent and filtered. Solvents were very carefully removed by slow evaporation. All the solvents used in extraction processes were from Winlab Ltd, BDH or Merck products. Stock solutions were prepared by dissolving an accurately weighed dry extract in a known volume of warm distilled water in a volumetric flask. Test solutions of 1000 and 5000 ppm (0.1 and 0.5%) were prepared by diluting a definite volume of the stock solution to the required concentration. Chickpea seeds of about the same size were chosen from a refrigerated new crop stock. Seeds were allowed to reach room temperature, then dipped into the extract solution or water for 1 min and air dried before use.

The equipment used for evaluating the ovipositional deterrence response of *C. maculatus* to the test materials was a modified version of the choice test apparatus of Laudani and Swank (1954), shown in Figure1. It consists of a circular platform, 50cm in diameter, and 15cm high, with 12 holes, cut to fit Petri dishes 9 cm in diameter and 1.5 cm deep equidistantly spaced around the periphery of the platform. A hole was cut into the centre of the apparatus lid to allow insertion of a 0.5cm diameter rubber tube through which the insects were introduced.

TEST PROCEDURES: Methanol extracts of six materials, *P. nigrum, L. aucheri,* neem leaves, neem seeds, citrus peels, and *R. stricta,* were first tested. Three types of tests were carried out:

(a)Apparatus multi-choice test: Seven treated seeds with 0.1% methanol extracts of each of the six materials and a control were placed in a Petri dish, 9 cm in diameter and 1.5 cm deep, making 6 Petri dishes (each containing one seed from each material and a control). Petri dishes were placed into holes in the apparatus spaced uniformly around the centre. About 250 unsexed 3-5 days old *C. maculatus* adults were poured down the centre tube. After a few minutes the restraining dish was raised to allow insects free movement inside the

OVIPOSITIONAL DETERRENCE OF METHANOLIC AND ETHERIAL EXTRACTS OF FIVE PLANTS TO THE COWPEA BRUCHID, *CALLOSOBRUCHUS MACULATUS* (F.) (*COLEOPTERA: BRUCHIDAE*)

Figure 1. The apparatus used for testing C. maculatus ovipositional deterrence.

apparatus and female oviposition on the desired seeds. Three days later the dishes were removed and the eggs glued on each chickpea seed were counted under a stereo-microscope and recorded.

(b) Petri-dish choice test: In this test the 0.1% methanol extract treated seeds and the control , as in test (a), were not placed into the apparatus but each Petri dish constituted a separate ovipositional unit, i.e. one replication. Twenty unsexed 3-5 days old *C. maculatus* adults were introduced into each unit, and the same procedure as in (a) was followed.

(c)Apparatus single-material choice test: Four chickpea seeds treated with 0, 0.1, and 0.5% ether or methanol extracts were placed in Petri dishes and inserted into the apparatus holes, with 4 replications (i.e. 4 Petri dishes) for each concentration, using 250 *C. maculatus* unsexed adults. This test was run twice for each of the ether or methanol extracts of five plant materials: *R.stricta, S.aromaticum, H.bacciferum,* neem seed, and citrus peels. *P.nigrum* and *L.aucheri* were omitted from this test because they showed marked ovipositional attraction in tests (a) and (b). The eggs laid on seeds were determined as in previous tests.

DATA ANALYSIS: Data were analyzed using ANOVA and the treatment means were separated using the Duncan's Multiple Range Test. The performance of each plant material was assessed by its ability to deter female cowpea bruchids from ovipositing on treated seeds compared with the control. Percent ovipositional deterrency values (POD) were computed as: POD={(NC-NT),(NC+NT)}C 100, where NC= number of eggs oviposited on control seeds and NT= number of eggs oviposited on treatment seeds.

Results

Except for *P. nigrum and L. aucheri,* the methanol extracts of four materials (*R. stricta*; *A. indica* seeds; S. *aromaticum*; citrus peels and *H. bacciferum*) showed positive ovipositional deterrency in both multiple and Petri dish choice tests, Table 1. However, no significant differences were observed except for *R. stricta* a maximum deterrency (22.2 and 27.6%, respectively) in both (a) and (b) tests. In fact *P. nigrum and L. aucheri* attracted more laying females than the controls, thus exhibiting negative deterrency values (-33.2 and -16.8%, respectively). Neem leaves, neem seeds and citrus peels produced higher deterrency in the Petri dish choice tests than in the multi-choice test.

TABLE 1

Ovipositional deterrency of methanolic plant extracts to Callosobruchus maculatus.

Methanol extract 0.1% of material	Mean eggs deposited/seed ± S.E.* Apparatus multiple choice (a)	% ** Deterrency	Petri-dishes Choice test (b)	% ** Deterrency
Control	27.2 ± 10.8^{c}	-	13.4 ± 3.5^{bc}	-
Piper nigrum	54.3 ± 8.7^{a}	-32.2	20.4 ± 9.1^{a}	-20.7
Neem leaves	22.3 ± 5.9^{de}	9.9	7.9 ± 4.9^{d}	25.8
Citrus peel	25.0 ± 6.3^{de}	8.1	8.9 ± 3.8^{bcd}	20.2
Lepidium aucheri	38.2 ± 9.4^{b}	7.9	9.0 ± 4.1^{bcd}	19.6
Neem seeds	23.2 ± 6.6^{de}	7.9	9.0 ± 4.1^{bcd}	19.6
Rhazya stricta	17.3 ± 4.5^{d}	22.2	7.6 ± 5.0^{d}	27.6

* Means followed by the same letter within a column are not significantly different at 5% level by DMRT.

** Percent deterrency (PD) = [Nc-Nt)/(Nc+Nt] x 100

TABLE 2

Ovipositional deterrency of diethylether and methanol extracts of 5 plant materials to Callosobruchus maculatus.

Material and Concentration	(ppm)	Ether Extract X eggs* deposited/seed	Ether Extract % ** Deterrency	Methanol Extract X eggs* deposited/seed	Methanol Extract % ** Deterrency
Rhazya stricta	0	7.70ᵃ	-	25.33ᵃ	-
	1000	5.80ᵇ	14.1	7.80ᵇ	52.9
	5000	3.95ᵇ	32.2	4.08ᵇ	83.9
	LSD	1.85	-	3.75ᵇ	-
Neem seeds	0	21.50ᵃ	-	12.88ᵃ	-
	1000	7.73ᵇ	47.2	1.18ᵇ	83.2
	5000	5.75ᵇ	57.8	0.55ᵇ	91.8
	LSD	3.89	-	1.38	-
Cloves	0	22.50ᵃ	-	22.65ᵃ	-
	1000	3.50ᵇ	73.1	7.98ᵇ	47.9
	5000	2.50ᵇ	80	8.68ᵇ	44.6
	LSD	7.13	-	2.31	-
Citrus peel	0	3.80ᵃ	-	28.82ᵃ	-
	1000	0.96ᵇ	59.7	19.45ᵇ	19.4
	5000	0.18ᵇ	90.9	21.20ᵇ	26.4
	LSD	0.91	-	5.83	-
Heliotropium bacciferum	0	28.00ᵃ	-	24.43ᵃ	-
	1000	9.78ᵇ	48.2	9.28ᵇ	44.9
	5000	5.40ᶜ	67.7	5.73ᶜ	62
	LSD	2.29	-	2.09	-

* Means followed by the same letter within a column are not significantly different at 5% level by DMRT.

** Percent deterrency (PD) = [Nc-Nt)/(Nc+Nt] x 100

The methanol extracts of all five materials tested in test (c) significantly (P=0.05) reduced oviposition by *C. maculatus* females (Table 2). Maximum deterrency was observed in the neem seed extract at both 0.1 and 0.5% treatments (83.2 and 91.8%, respectively); followed by *R. stricta* (52.9 and 83.9%, respectively); and *H. bacciferum* at the 0.5% treatment (62.0%). Methanol extract of citrus peels exhibited the lowest values, 19.4 and 26.4% at 0.1 and 0.5% treatments, respectively. No significant differences were observed between the 0.1 and 0.5% treatments, in all extracts, except for *H. bacciferum*.

The diethyl ether extracts of all five materials significantly reduced oviposition on treated seeds, however, the deterrence was at a lower rate for *R. stricta* and neem seeds than that obtained in the methanol extracts. The maximum effect observed in the diethyl ether 0.1% extracts was obtained in the *S. aromaticum* treatment (73.1%), followed by citrus peels (59.7%), *H. bacciferum* (48.2%) and neem seeds (47.2%). Citrus peels ether extract at 0.5% concentration almost completely suppressed oviposition where 90.9% ovipositional deterrency was obtained. *S. aromaticum* gave 80.0%, *H. bacciferum* 67.7%, neem seeds 57.8% and *R. stricta* gave only 32.2%.

Discussion

Females of *C. maculatus* were deterred, at varying degrees, by the diethyl ether and methanol extracts of the five tested plant materials. A striking feature of the data contained in Table 2, is that biological activity performed by different allelopathic classes of chemicals has been detected. Although this has been detected to varying extents, depending on the type of solvent employed for extraction and type of plant material, the biological activity recorded is considered to be sufficiently remarkable in all the five samples investigated.

Previous studies on crude extracts of nine plant materials singled out these five materials as having, relatively, the strongest ovipositional deterrent effect, (Elhag, 1998), of which *R. stricta* significantly reduced oviposition and F1 offspring. In this study the effect of *R. stricta* was more pronounced in the methanolic fraction, where it evoked the highest significant ovipositional deterrency in both apparatus multi-choice and Petri dish methanol extract tests. Toxic action of *R. stricta* against insects has been confirmed in *Culex pipiens* L. where its lyophilized aqueous extract impaired egg hatching, caused larval mortality and decreased pupation,(Elhag *et al.*, 1996). *R. stricta* is known to be rich in alkaloids of different classes, (Hassan *et al.*, 1977; Ahmad *et al.*, 1983). Methods of isolation and structural elucidation of alkaloidal constituents in *R.stricta*, such as sewarine, strictalamine, rhazimal, rhazimol and others, have been quite successfully demonstrated by utilization of modern physical methods of both isolation and structural elucidation,(Hassan *et al.*, 1977; Ahmad *et al.*, 1983).

Steroidal alkaloids are widely reported for their toxicity to fungi and insects and correlation have been established between the alkaloid levels in plants and their resistance to infestations (Roddick, 1986). Different classes of alkaloids have been shown to affect behavior in noctuid moths (Ramaswamy *et al.*, 1992). In this investigation the data in Table 2 show that % deterrency due to alkaloidal methanolic extract is on average more than three fold % deterrency due to other chemical classes present in ether extracts. This finding is in harmony with that obtained by both Roddick (1986) and Ramaswamy *et al.* (1992).

Neem seed extracts evoked a significantly higher oviposition deterring activity in the methanol single-material test, compared to the control, nearly twice that obtained in the ether extract. Various neem extracts have been shown to exhibit a wide range of biological activities mostly referring to insecticidal, antifeedant, growth inhibiting, oviposition deterring against a broad spectrum of insects, including stored-product insects, (Saxeena *et al.*, 1988; Schmutterer, 1990; Murdue and Blackwell, 1993; Xie *et al.*, 1995). Reference has been made to the liminoid type of terpenoid compounds, azadirachtin, as its major active constituent. The ovipositional deterrency of neem on stored-product insects has not been studied previously, however, Jilani and Malik (1973) demonstrated the repellency of water and ethanol extracts of neem leaves and seeds against adults and larvae of three stored-product pests. Xie *et al.* (1995) reported on the repellency, toxicity, and reduction in offspring of three stored-product beetles by azadirachtin and neem extracts. The results of this study on neem seeds and leaves do not deviate from these findings. They are in fact in full accord with these findings especially those of Chen *et al.* (1996) who obtained reduction in oviposition upto 99.2%, using 4% diethyl ether neem seed extract on the oriental fruit fly, *Bactrocera dorsalis* (Hendel), and, Singh and Srivastava (1983) who found that a 20% concentration of ethanolic neem seed extract deterred oviposition of *Dacus dorsalis* Coq. on guava fruits.

S. aromaticum, cloves and citrus peels, were unlike *R.stricta* and neem seeds, in that their allelopathic response was more pronounced for ether extract than for methanol extract, indicating that the proportion of alkaloidal chemicals present are relatively lesser than the other biologically active chemicals, among which terpenoids may be dominant. The very clear difference between these two plants is that, on average the ratio of % deterrency due to ether/methanol extracts is >3:1 and 2:1 in citrus peels and *S. aromaticum* cloves, respectively. The use of cloves has not been widely investigated, however, Hassanali *et al.* (1990) reported that weevil repellent constituents in *E. caryophyllata* cloves justify their use as grain protectants; and Caledrone *et al.* (1991) reported significant acaricidal properties in clove oil. Elhag (1998) obtained up to 87.9% reduction in oviposition of *C. maculatus* on chickpea seeds dipped into 0.1% cloves crude extract. Citrus peels components are known to have bioactivity against insects, and as Don-Pedro (1985) reported, they have been found toxic to *Dermestes maculatus* (L.) and *C. maculatus*. In this study a more quantitative determination of bioactivity of citrus peels ether extract revealed % deterrency of 59.7 and 90.9% in the 0.1 and 0.5% concentration levels, respectively. In 0.1% crude extract multiple-choice test (Elhag, 1998) citrus peels reduced oviposition by 59%.

H. bacciferum is a common herb in the semi-desert central parts of Saudi Arabia, known in folk traditional medicine to cure scorpion bites when applied topically or orally as a drink. Its lyophilized aqueous extracts had little effect on *C.pipiens* egg hatchability (Elhag *et al.*, 1996), however, its 0.1% crude extracts deterred *C. maculatus* oviposition by 59.2%, (Elhag, 1998). In this study the % deterrency due to both types of extracts is practically identical (Table 2) indicating that alkaloidal chemicals as well as other biologically active chemicals are present in nearly balanced proportions in this plant. *H. bacciferum* was also the only plant material that exhibited its action in a dose dependent manner, where in both extracts the number of eggs deposited on 0.1 and 0.5% treated seeds varied significantly,($P=0.05$).

It is rather uncertain yet that the reduced oviposition rates on treated seeds have resulted from the repellent action of the plant chemicals or from non-volatile components detected by the insect's ovipositor as a stimulus to reduce egg laying. Chen *et al.* (1996) defined ovipositional deterrence as "reducing oviposition on hosts", and repellence as "reducing insects landing on hosts". They found that a concentration as low as 0.2% diethyl ether neem extract effectively deterred oviposition by *D. dorsalis* in treated guava fruits. In our study, it was quite evident in *R. stricta,* neem seeds, *S. aromaticum* cloves and citrus peels tests that increasing the dose did not significantly increase the magnitude of ovipositional deterrence. This may imply that *C.maculatus* females are not oriented by olfactory cues that prevent their landing on treated seeds, but the plant component is rather detected by the ovipositor. This is supported by our observation that bruchids are not actually repelled from treated seeds but were always found in all treatments.

Further investigations are needed to reveal the identity of the bioactive components in the diethyl ether and/or methanol fractions of some of the plant materials. However, Xie *et al.* (1995) pointed out that the use of neem extracts as an insecticide is advantageous because the isolation of azadirachtin is difficult. In addition, there are several other compounds in neem extracts that have been shown toxic to insects and could cause additional mortality (Ley *et al.*, 1993; Murdue and Blackwell, 1993).

It is therefore quite justifiable to conclude that the results obtained from this investigation suggest a promising potential for the use of *R. stricta,* neem seeds, *S. aromaticum* cloves, *H. bacciferum* and citrus peels as ovipositional deterrents, in stored-product pest management programmes, that could lower insect populations in stored grains. Moreover, these materials are cheap, safe, act on a broad spectrum of insects, and much more easy to handle than synthetic insecticides.

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