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Insecticidal and Repellent Properties of Subtropical Plant Extracts Against Pulse Beetle, *Callosobruchus chinensis*

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خواص مستخلصات النباتات شبه الاستوائية كمبيدات ومواد طاردة ضد خنفساء البقوليات (*Callosobruchus chinensis*)

حسن اللواتي وخاجه عزام ومايكل ديدمان

خلاصــة: تم تحضير ثمانية مستخلصات من نباتات عمانية (محلية) وهي القرط و المستعفل والنيم و اللبان و الخشخاش و زبروت و الياس و السويداء وذلك بنقع مسحوق الأوراق أو البذور المجففة في الظل في الماء و المذيب (ميثانول أو إيثانول). استُخدم المستخلص لدراسة الخاصية المبيدية والطاردة ضد حشرة الحبوب المخزونة. أدت المعاملة بمستخلص المستعفل إلى موت الحشرات بنسبة ١٠٠% خلال ٢٠ و ٤ ساعات من تعرضها لمستخلصات الميثانول و الإيثانول على التوالي، أما المستخلصات الأخرى فقد أدت إلى نسبة موت عالية أيضا مثل القرط و الخشخاش و الياس و السويداء في مذيب الميثانول، واللبان و زبروت و السويداء و النيم التجاري في مذيب الإيثانول. المستخلص من الياس في الميثانول أظهر نسبة عالية من الخاصية الطاردة مقارنة بالمستخلصات الأخرى في حين أن البذور المعاملة بمستخلص المستعفل أظهرت نسبة عالية من الخاصية الجاذبة .

ABSTRACT: Extracts of eight plants local to Oman, namely *Qarat (Acacia nilotica)*, *Mustafal (Annona squamosa)*, *Shereesh (Azadirachta indica)*, *Luban* (*Boswellia sacra)*, *Kheshkhash (Crotolaria juncea)*, *Zebrot* (*Jatropha dhofarica)* *Yas*, (*Myrtus communis)* and *Suwwad* (*Suaeda aegyptiaca*) were prepared by steeping shaded dried leaf/seed powder of each plant in water and solvent (methanol or ethanol). The extracts were tested for their insecticidal and repellent properties against the pulse beetles, *Callosobruchus chinensis*. The extracts from the seeds of *A. squamosa* recorded 100% mortality of beetles within twenty and four hours of their exposure to methanol and ethanol extracts, respectively. The other extracts that caused high mortality were from *A. nilotica, C. juncea, M. communis* and *S. aegyptiaca* in methanol and *B. sacra, J. dhofarica, S. aegyptiaca* and commercial neem in ethanol. Extracts of *M. communis* in methanol were highly repellent to the beetles compared to other extracts. Legume seeds treated with extracts of *A. squamosa* were not repellent, rather the beetles were attracted to them.

Keywords: insecticides, repellents, subtropical plant extracts, pulse beetle.

Synthetic organic insecticides have played a major role in pest control. However, their increasing use in recent years has created a range of ecological problems such as bio-magnification, resurgence and the development of insecticide tolerant strains of pest species. It has been reported in India, for example where insecticides have been used indiscriminately in cotton growing areas, that there has been a 164-300 fold increase in insect resistance to some insecticides (Dhingra *et al*., 1988).

The plant kingdom contains a huge array of chemical substances; many of these are used by plants for their defense against insect attack. Phytochemicals possess a wide spectrum of biological properties against insects. They may act as antifeedants, repellents, growth inhibitors, attractants, chemosterilants or as insecticides. Since these naturally occurring phytochemicals are usually biodegradable and non-toxic to plants, warm-blooded animals and the environment, they offer great potential as safer, more effective and economic pesticides

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for the control of insect pests attacking stored produce and agricultural crops. The use of plant derivatives for stored grain protection has gained popularity in recent years as replacements for synthetic insecticides (Singh and Srivastava, 1983; Malik and Myjtabe Naqvi, 1984; Saim and Meloan, 1986; Weaver *et al.*, 1991).

The use of plant products such as nicotine, pyrethrum and root extracts of rotenone date back to the 17th and 19th centuries respectively. Mixing dried neem (*Azadirachta indica*) leaves with grain in storage is a classic example of natural product use that has been practiced by farmers in many countries for many years. Neem is very effective against a wide rage of insect pests (Atwal and Pajni, 1964; Sachan and Pal, 1974; Chellapa and Chelliah, 1976; Rao and Prakash Rao, 1979). Pandey and Singh (1995) found that seeds of black gram could be effectively protected from damage by *Callosobruchus chinensis*, by mixing the seed with dried powder of Neem leaves at a rate of 100-400 mg/50 gm seed.

The pulse beetle attacks pulses prior to harvest in the field and the damage continues in storage after harvest. Among the pulses, pigeonpea (*Cajanus cajan* (L) Millsp.) is an important staple for a large number of people. To control the pulse beetle in storage, a number of synthetic organic insecticides such as malathion have been recommended. The admixture of synthetic insecticides with food grains has more recently been banned in many countries. There are also reports of the pulse beetle developing resistance to malathion (Singh, 1983).

With the growing awareness of the hazards associated with the use of synthetic organic insecticides, there is a need to explore suitable alternative methods of pest control; a number of plant-derived products are potential candidates. Nicotine, pyrethrum and rotenone are examples of plant-derived products that had been used as insecticides prior to the advent of the synthetic organic insecticides. The work presented in this paper relates to the insecticidal and repellent properties of eight plant extracts against the pulse beetle, *Callosobruchus chinensis* (Linn.). (Coleoptera: Bruchidae). Although most of the plants used in this study are indigenous to the Sultanate of Oman, little has been reported on their efficacy against insect pests, despite a large volume of work on other plants having been reported from other countries (Singh and Chua, 1977; Ohsawa *et al.*, 1990; Shivanna *et al.*, 1994 and Mansour, 1997).

Materials and Methods

Sub-tropical plants that are indigenous to Oman or introduced and cultivated were tested for their insecticidal and repellent properties against the beetles *C. chinensis*. Leaves of *B. sacra*, *J. dhofarica*, *M. communis* and *S. aegyptiaca*, and seeds of *A. nilotica*, *A. squamosa*, *A. indica* and *C. juncea* were collected from the plants and air-dried and powdered. 12.5 g of the powdered material of each plant was soaked separately, in the dark for 24 h in a glass jar in a solution of 12.5 ml water and 50 ml solvent (methanol or ethanol). Subsequently, the solutions were filtered through a Buchner filter under vacuum. The filtered solutions (extracts) were stored in the refrigerator (4°C) prior to use.

INSECTICIDAL PROPERTIES: One ml of each extract and a control (methanol and ethanol) was separately poured on the inner surface of a 9 cm plastic Petri dish in order to make a dry film of the extracts. The extract in the dish was allowed to dry for 2 h. Ten insects were released into each dish. There were 4 replications for each treatment.

The time of insect exposure varied for each experiment, i.e. 1, 2, 3, and 4 h after which the insects were placed in a clean Petri dish. The number of dead insects were recorded after releasing the insects in these dishes after 1, 2, 4, 20, 24, 48, and 72 h.

REPELLENCY PROPERTIES: An olfactometer was constructed from a large (19 cm diameter) plastic Petri dish. Ten small (5 cm diameter) Petri dishes were attached to the central dish around its circumference and a small hole was made to allow free passage between each small Petri dish and the large central dish. In the lid of the large dish a small hole was made to allow the release of insects into the chamber. This hole was closed during the experimental tests, preventing insects escape.

Twenty kidney bean seeds were thoroughly mixed with 2 ml of each plant extract. Residual extract was allowed to evaporate from the seeds. This experimental procedure was repeated for plant extract in methanol and ethanol. Each experimental test was replicated four times. For each replicate, fifty *C. chinensis* adults were released into the large dish and 20 grains of bean seeds, soaked in individual plant extracts were placed in the small, peripheral dishes. The direction of movement of beetles was recorded at 15 min, 30 min, 1hr, 2 h, and 24 h intervals.

Results and Discussion

INSECTICIDAL PROPERTIES: The cumulative mortality data of four exposure periods for both solvents, methanol and ethanol, are given in Tables 1, 2, 3 and 4 for 1, 2, 3 and 4 h, respectively. The methanol and ethanol extracts of *A. squamosa* caused mortality of beetles starting one hour (Table 1) after their exposure period (42.5% for methanol and 45% for ethanol).

TABLE 1

Cumulative mortality (%) of* C. chinensis *in methanol and ethanol extracts over 72 h, after exposing beetles for one hour.

Treatments	Solv.	Methanol							Ethanol						
	Interv	1 h	2 h	4 h	20 h	24 h	48 h	72 h	1 h	2 h	4 h	20 h	24 h	48 h	72 h
Acacia	Ave	0	0	0	0	0.3	0.3	0.8	0	0	0	0.8	1.0	1.8	2.0
nilotica	%	0	0	0	0	2.5	2.5	7.5	0	0	0	7.5	10.0	17.5	20.0
Annona	Ave	0	0	0	0	0.3	0.3	0.8	4.5	6.0	10.0	10.0	10.0	10.0	10.0
squamosa	%	0	0	0	0	2.5	2.5	7.5	45.0	60.0	100.0	100.0	100.0	100.0	100.0
Azadirachta	Ave	0	0	0	0.8	1.0	1.8	2.3	0	0	0	0	0.3	0.8	1.5
indica	%	0	0	0	7.5	10.0	17.5	22.5	0	0	0	0	2.5	7.5	15.0
Boswella	Ave	0	0	0	0.3	0.3	1.0	1.8	0	0	0	0	0	1.0	2.0
sacra	%	0	0	0	2.5	2.5	10.0	17.5	0	0	0	0	0	10.0	20.0
Crotolaria	Ave	0	0	0	0.3	0.8	1.5	2.8	0	0	0	0	1.5	1.8	2.3
juncea	%	0	0	0	2.5	7.5	15.0	27.5	0	0	0	0	15.0	17.5	22.5
Jatropha	Ave	0	0	0	0.3	0.5	0.5	1.3	0	0	0	1.0	1.0	1.5	1.8
dhofarica	%	0	0	0	2.5	5.0	5.0	12.5	0	0	0	10.0	10.0	15.0	17.5
Myrtus	Ave	0	0	0	0.3	0.3	0.8	0.8	0	0	0	0.3	0.5	1.5	1.5
communis	%	0	0	0	2.5	2.5	7.5	7.5	0	0	0	2.5	5.0	15.0	15.0
Suaeda	Ave	0	0	0	1.0	1.0	2.0	2.5	0	0	0.8	1.3	1.8	2.0	3.0
egyptiaca	%	0	0	0	10.0	10.0	20.0	25.0	0	0	7.5	12.5	17.5	20.0	30.0
Commercial	Ave	0	0	0	0	0	0	0	1.0	1.0	1.0	1.5	2.0	2.0	2.5
neem	%	0	0	0	0	0	0	0	10.0	10.0	10.0	15.0	20.0	20.0	25.0
Control	Ave	0	0	0	0	0	1.3	2.0	0	0	0	0	0	0.3	1.3
	%	0	0	0	0	0	12.5	20.0	0	0	0	0	0	2.5	12.5

Average data presented is of 4 replications (each replication had 10 beetles).

TABLE 2

Cumulative mortality (%) of* C. chinensis *in methanol and ethanol extracts over 72 h, after exposing beetles for two hours.

Treatments	Solv.	Methanol							Ethanol						
	Interv	1 h	2 h	4 h	20 h	24 h	48 h	72 h	1 h	2 h	4 h	20 h	24 h	48 h	72 h
Acacia	Ave	0	0	0	1.3	2.0	2.5	3.0	0	0	0	2.0	2.3	3.8	4.5
nilotica	%	0	0	0	12.5	20.0	25.0	30.0	0	0	0	20.0	22.5	37.8	45.0
Annona	Ave	4.5	4.8	4.8	6.0	10.0	10.0	10.0	4.8	7.0	10.0	10.0	10.0	10.0	10.0
squamosa	%	45.0	47.5	47.5	60.0	100.0	100.0	100.0	47.5	70.0	100.0	100.0	100.0	100.0	100.0
Azadirachta	Ave	0	0	0	1.0	1.3	2.0	3.3	0	0	0	1.0	1.3	2.0	2.8
indica	%	0	0	0	10.0	12.5	20.0	32.5	0	0	0	10.0	12.5	20.0	27.5
Boswella	Ave	0	0	0	0.5	0.5	1.8	2.3	0	0	0	0.3	0.8	1.3	2.5
sacra	%	0	0	0	5.0	5.0	17.5	22.5	0	0	0	2.5	7.5	12.5	25.0
Crotolaria	Ave	0	0	0	0.8	1.0	2.0	3.8	0	0	0	1.3	1.8	2.5	3.0
juncea	%	0	0	0	7.5	10.0	20.0	37.5	0	0	0	12.5	17.5	25.0	30.0
Jatropha	Ave	1.3	1.3	1.3	1.3	1.3	1.5	2.5	0.3	0.3	0.8	1.0	1.5	1.8	2.0
dhofarica	%	12.5	12.5	12.5	12.5	12.5	15.0	25.0	2.5	2.5	7.5	10.0	15.0	17.5	20.0
Myrtus	Ave	0	0	0	0.3	0.5	1.3	2.8	0	0	0.3	0.5	0.8	1.5	2.8
communis	%	0	0	0	2.5	5.0	12.5	27.5	0	0	2.5	5.0	7.5	15.0	27.5
Suaeda	Ave	0	0	0	1.0	1.8	2.3	2.8	0.5	0.8	1.0	2.0	2.0	2.8	3.0
egyptiaca	%	0	0	0	10.0	17.5	22.5	27.5	5.0	7.5	10.0	20.0	20.0	27.5	30.0
Commercial	Ave	0	0	0	0.3	0.5	1.5	1.8	1.3	1.5	2.0	2.0	2.0	3.8	3.8
neem	%	0	0	0	2.5	5.0	15.0	17.5	12.5	15.0	20.0	20.0	20.0	37.5	37.5
Control	Ave	0	0	0	0	0.5	1.5	2.0	0	0	0	0.5	0.5	1.5	2.5
	%	0	0	0	0	5.0	15.0	20.0	0	0	0	5.0	5.0	15.0	25.0

Average data presented is of 4 replications (each replication had 10 beetles).

TABLE 3

Cumulative mortality (%) of* C. chinensis *in methanol and ethanol extracts over 72 h, after exposing beetles for three hours.

Treatments	Solv.	Methanol							Ethanol						
	Interv	1 h	2 h	4 h	20 h	24 h	48 h	72 h	1 h	2 h	4 h	20 h	24 h	48 h	72 h
Acacia	Ave	0	0	0	2.0	2.0	3.5	4.3	0	0	0	2.3	2.3	4.0	5.5
nilotica	%	0	0	0	20.0	20.0	35.0	42.5	0	0	0	22.5	22.5	40.0	55.0
Annona	Ave	6.0	6.0	7.3	8.5	10.0	10.0	10.0	7.8	8.8	10.0	10.0	10.0	10.0	10.0
squamosa	%	60.0	60.0	72.5	85.0	100.0	100.0	100.0	77.5	87.5	100.0	100.0	100.0	100.0	100.0
Azadirachia	Ave	0	0	0	1.5	1.8	2.5	4.0	0	0	0	1.3	1.8	3.0	3.5
indica	%	0	0	0	15.0	17.5	25.0	40.0	0	0	0	12.5	17.5	30.0	35.0
Boswella	Ave	0	0	0	0.8	0.8	1.8	2.5	0	0	0.3	1.3	1.8	4.0	5.0
sacra	%	0	0	0	7.5	7.5	17.5	25.0	0	0	2.5	12.5	17.5	40.0	50.0
Crotolaria	Ave	0	0	0	1.3	2.0	3.8	4.8	0	0	0	1.3	2.0	4.0	5.0
juncea	%	0	0	0	12.5	20.0	37.5	47.5	0	0	0	12.5	20.0	40.0	50.0
Jatropha	Ave	1.3	1.3	1.3	1.3	1.8	3.3	3.8	0.5	0.5	0.8	2.0	2.0	2.8	3.3
dhofarica	%	12.5	12.5	12.5	12.5	17.5	32.5	37.5	5.0	5.0	7.5	20.0	20.0	27.5	32.5
Myrtus	Ave	0	0	0	1.3	1.5	3.3	3.8	0.3	0.3	0.3	0.5	1.0	3.8	5.0
communis	%	0	0	0	12.5	15.0	32.5	37.5	2.5	2.5	2.5	5.0	10.0	37.5	50.0
Suaeda	Ave	0	0	0	3.0	3.0	4.3	7.0	1.8	2.5	3.5	4.5	5.3	7.0	8.0
egyptiaca	%	0	0	0	30.0	30.0	42.5	70.0	17.5	25.0	35.0	45.0	52.5	70.0	80.0
Commercial	Ave	2.0	2.0	2.8	2.3	3.3	5.5	7.0	1.8	1.8	3.0	3.5	4.3	6.5	8.5
neem	%	20.0	20.0	27.5	27.5	32.5	55.0	70.0	17.5	17.5	30.0	35.0	42.5	65.0	85.0
Control	Ave	0	0	0	0.8	1.5	3.8	5.0	0	0	0	1.5	2.0	3.3	7.0
	%	0	0	0	7.5	15.0	37.5	50.0	0	0	0	15.0	20.0	32.5	70.0

Average data presented is of 4 replications (each replication had 10 beetles).

TABLE 4

Cumulative mortality (%) of* C. chinensis *in methanol and ethanol extracts over 72 h, after exposing beetles for four hours.

Treatments	Solv.	Methanol							Ethanol						
	Interv	1 h	2 h	4 h	20 h	24 h	48 h	72 h	1 h	2 h	4 h	20 h	24 h	48 h	72 h
Acacia	Ave	0	0	0	2.0	2.8	5.3	7.0	0	0	0	3.5	3.5	4.5	5.8
nilotica	%	0	0	0	20.0	27.5	52.5	70.0	0	0	0	35.0	35.0	45.0	57.5
Annona	Ave	7.8	7.8	9.0	10.0	10.0	10.0	10.0	8.5	9.0	10.0	10.0	10.0	10.0	10.0
squamosa	%	77.5	77.5	90.0	100.0	100.0	100.0	100.0	85.0	90.0	100.0	100.0	100.0	100.0	100.0
Azadirachia	Ave	0	0	0	1.5	2.3	2.8	4.8	0	0	0	1.3	2.5	4.8	6.3
indica	%	0	0	0	15.0	22.5	27.5	47.5	0	0	0	12.5	25.0	47.5	62.5
Boswella	Ave	0	0	0	1.0	2.5	4.0	6.5	0	0	0.5	1.8	2.5	4.8	7.3
sacra	%	0	0	0	10.0	25.0	40.0	65.0	0	0	5.0	17.5	25.0	47.5	72.5
Crotolaria	Ave	0	0	0	2.3	3.3	6.5	7.5	0	0	0	1.3	2.5	4.5	6.5
juncea	%	0	0	0	22.5	32.5	65.0	75.0	0	0	0	12.5	25.0	45.0	65.0
Jatropha	Ave	1.3	1.3	1.5	1.8	1.8	3.3	5.0	0.8	0.8	1.0	5.3	5.5	7.0	7.0
dhofarica	%	12.5	12.5	15.0	17.5	17.5	32.5	50.0	7.5	7.5	10.0	52.5	55.0	70.0	70.0
Myrtus	Ave	0	0	0	1.3	2.5	4.0	6.8	0.3	0.8	0.8	2.0	2.5	4.5	5.8
communis	%	0	0	0	12.5	25.0	40.0	67.5	2.5	7.5	7.5	20.0	25.0	45.0	57.5
Suaeda	Ave	0	0	0	3.0	3.0	4.3	7.0	1.8	2.5	3.5	4.5	5.25	7.0	8.0
egyptiaca	%	0	0	0	30.0	30.0	42.5	70.0	17.5	25.0	35.0	45.0	52.5	70.0	80.0
Commercial	Ave	2.0	2.0	2.8	2.8	3.3	5.5	7.0	1.8	1.8	3.0	3.5	4.25	6.5	8.5
neem	%	20.0	20.0	27.5	27.5	32.5	55.0	70.0	17.5	17.5	30.0	35.0	42.5	65.0	85.0
Control	Ave	0	0	0	0.8	1.5	3.8	5.0	0	0	0	1.5	2.0	3.3	7.0
	%	0	0	0	7.5	15.0	37.5	50.0	0	0	0	15.0	20.0	32.5	70.0

Average data presented is of 4 replications (each replication had 10 beetles).

TABLE 5

Mortality after one hour of exposure of beetles in methanol and ethanol solvents in different treatments (a: more, b: less, c: lowest).

Treatments	Intervals in	Methanol	Intervals in	Ethanol
Acacia nilotica	1, 2, 4, 20 24, 48, 72	**	48, 72	c
Annona squamosa	1, 2, 4, 20 24, 48, 72	b a	1, 2 4, 20, 24, 48, 72	b a
Azadirachia indica	48, 72	c	72	c
Boswella sacra	72	c	1, 2, 4, 20 24, 48, 72	**
Crotolaria juncea	48, 72	c	24, 48, 72	c
Jatropha dhofarica	72	c	48, 72	c
Myrtus communis	1, 2, 4, 20 24, 48, 72	**	48, 72	c
Suaeda egyptiaca	48, 72	c	20, 24, 48, 72	c
Commercial neem	1, 2, 4, 20 24, 48, 72	**	20, 24, 48, 72	c
Control	48, 72	c	72	c

Within the solvent, cumulative mortality with different letters are significantly different ($P < 0.05$)
** The mortality was not significantly different among the time intervals.

TABLE 6

Mortality after two hours of exposure of beetles in methanol and ethanol solvents in different treatments (a: more, b: less, c: lowest).

Treatments	Intervals in	Methanol	Intervals in	Ethanol
Acacia nilotica	24, 48, 72	d c	20, 24 48, 72	d c
Annona squamosa	1, 2, 4, 20, 24, 48, 72	c b a	1, 2 4, 20, 24, 48, 72	c b a
Azadirachia indica	48 72	d c	48, 72	d
Boswella sacra	72	c	72	d
Crotolaria juncea	48 72	d c	48 72	d c
Jatropha dhofarica	72	c	72	d
Myrtus communis	72	c	72	d
Suaeda egyptiaca	48, 72	d	20, 24, 48, 72	d c
Commercial neem	1, 2, 4, 20 24, 48, 72	**	4, 20, 24 48, 72	d c
Control	72	d	72	d

Within the solvent, cumulative mortality with different letters are significantly different ($P < 0.05$)
** The mortality was not significantly different among the time intervals.

Mortality reached 100% within 24 h (methanol) and 4 h (ethanol), respectively. In other treatments mortality occurred either after 20 or 24 h in both the solvent extracts but did not exceed more than 27.5% in *C. juncea* in methanol and 30% in *S. aegyptiaca* in ethanol after 72 h. After two hours (Table 2) exposure of beetles, extracts of *A. squamosa* caused a similar trend to that for the 1 h exposure. However, high mortality was observed for methanol extracts of *A. nilotica*, *A. indica*, *M. communis*, *S. aegyptiaca* and *J. dhofarica* and ethanol extracts of *A. nilotica*, *A. indica*, *C. juncea*, *S. aegyptiaca* and commercial neem.

The three hours (Table 3) of exposure to *A. squamosa* caused higher mortality of beetles after 4 hours in methanol extract (72.5%) and 2 h in ethanol extract (87.5%). Mortality reached 100% by 24 h and 4 h for the methanol and ethanol extracts, respectively. In other extracts, though none caused 100 % mortality by 72 hours, the cumulative mortality was comparatively higher than for the one and two hour exposure periods. Mortality reached 42.5% in *A. nilotica*, 40% in *A. indica*, 47.5% in *C. juncea*, 37.5% in *J. dhofarica* and *M. communis*, 47.5% in *S. aegyptiaca*, 40% in commercial neem and 45% in the control methanol extracts. The cumulative mortality following exposure of the beetles for 4 h (Table 4) in different extracts resulted in 100% mortality in *A. squamosa* within 20 h and 4 h in methanol and ethanol extracts, respectively. The other extracts also caused higher mortality, reaching 70% and above in *A. nilotica*, *C. juncea*, *M. communis* and *S. aegyptiaca* in methanol extracts and *B. sacra*, *J. dhofarica*, *S. aegyptiaca*, commercial neem and control ethanol extracts.

Data were analyzed using Statistical Analysis System (SAS, 1985) by the General Linear Models Procedures (GLM), and LSD values were used to test treatment difference (Tables 5,6,7,8). After one hour of exposure (Table 5), cumulative mortality in *A. squamosa* at 1, 2, 4 and 20 h were significantly less ($P < 0.05$) than 24, 48 and 72 h in methanol extracts. However, the mortality in other treatments were lower ($P < 0.05$) than *A. squamosa*. In ethanol extracts after 1 and 2 hours of exposure the mortality was lower than those at 4, 20, 24, 48 and 72 h ($P < 0.05$) in *A. squamosa*. Other plant extracts showed lower mortality than *A. squamosa* ($P < 0.05$) and there was no significant mortality differences among them.

After two hours of exposure (Table 6), cumulative mortality in *A. squamosa* extracts in methanol were significantly different ($P < 0.05$) at 24, 48 and 72 h. However, the mortality of *A. squamosa* at 20 hours was lower than 24, 48 and 72 h. Mortalities at 1, 2 and 4 h in *A. squamosa* were not significantly different ($P > 0.05$) from *A. nilotica* at 48 and 72 h, and from *A. indica*, *B. sacra*, *C. juncea*, *J. dhofarica* and *M. communis* at 72 h. At the one hour exposure period for *A. squamosa* extracts in ethanol, the mortality was lower ($P < 0.05$) than 2 h and was also lower ($P < 0.05$) than 4, 20, 24, 48 and 72 h. In addition, one hour exposure in *A. squamosa* was not significantly different

TABLE 7

Mortality after three hours of exposure of beetles in methanol and ethanol solvents in different treatments (a: more, b: less, c: lowest).

Treatments	Intervals in	Methanol	Intervals in	Ethanol
Acacia nilotica	20, 24, 48	f	20, 24	f
	72	e	48	e
			72	d
Annona squamosa	1, 2	d	1	c
	4	c	2	b
	20	b	4, 20, 24, 84, 72	a
	24, 48, 72	a		
Azadirachia indica	48,72	f	20, 24	f
			48, 72	e
Boswella sacra	72	f	20, 24	f
			48	e
			72	d
Crotolaria juncea	24, 48	f	20, 24	f
	72	e	48	e
			72	d
Jatropha dhofarica	48,72	f	20, 24, 48	f
			72	e
Myrtus communis	48, 72	f	24	f
			48	e
			72	d
Suaeda egyptiaca	48, 72	f	1, 2, 4	f
			20, 24	e
			48, 72	d
Commercial neem	20, 24, 48	f	1,2, 4	f
	72	e	20, 24, 48	e
			72	d
Control	48	f	20, 24	f
	72	e	48, 72	e

Within the solvent, cumulative mortality with different letters are significantly different ($P < 0.05$).

TABLE 8

Mortality after four hours of exposure of beetles in methanol and ethanol solvents in different treatments (a: more, b: less, c: lowest).

Treatments	Intervals in	Methanol	Intervals in	Ethanol
Acacia nilotica	20, 24	c	20, 24, 48	c
	48	b	72	b
	72	a		
Annona squamosa	1, 2, 4, 20, 24, 48, 72	a	1, 2, 4, 20, 24, 48, 72	a
Azadirachia indica	20, 24, 48	c	20, 24, 48	c
	72	b	72	a
Boswella sacra	20, 24	c	20, 24, 48	c
	48	b	72	b
	72	a		
Crotolaria juncea	20	c	20, 24, 48	c
	24	b	72	b
	48, 72	a		
Jatropha dhofarica	1, 2, 4,	c	20, 24, 48, 72	b
	20, 24	b		
	48, 72			
Myrtus communis	48	b	20, 24, 48	c
	72	a	72	b
Suaeda egyptiaca	20, 24, 48	b	1, 2, 4, 20	c
	72	a	24, 48	b
			72	a
Commercial neem	1, 2, 4, 20	c	1, 2, 4,	c
	24, 48	b	20, 24	b
	72	a	48	a
			72	
Control	24	c	20, 24, 48	c
	48, 72	b	72	b

Within the solvent, cumulative mortality with different letters are significantly different ($P < 0.05$).

($P > 0.05$) from *A. nilotica* and commercial neem at 48 and 72 h and *C. juncea*, *S. aegyptiaca* at 72 h.

Cumulative mortality after three hours of exposure of the beetles to plant extracts showed (Table 7) that *A. squamosa* had a wider range of significance than all other extracts. At 24, 48 and 72 h in methanol extracts, *A. squamosa* caused more significant mortality than 20 h ($P < 0.05$) and was better than 4 h ($P < 0.05$); the lowest was at 1 and 2 h. In ethanol, *A. squamosa* showed significantly higher mortality for the 4 to 72 h exposure period than for the 1 and 2 h exposures ($P < 0.05$). The other plant extract treatments, in both methanol and ethanol, were significantly lower ($P < 0.05$) than *A. squamosa*.

After four hours of exposure (Table 8), cumulative mortality of *A. squamosa* at all time intervals in both methanol and ethanol solvents was significantly higher ($P < 0.05$) than for all other plant extracts. However, other plant extracts (*A. nilotica*, *B. sacra*, *M. communis*, *S. aegyptiaca* and commercial neem) showed a similar mortality level to *A. squamosa* at 72 h. In ethanol, *A. indica*, *S. aegyptiaca* and commercial neem showed similar mortality levels at 72 h to those of *A. squamosa*.

Overall, the statistical analysis of the data revealed that ethanol was a superior solvent for the plant extracts to methanol in terms of causing insect mortality. The longer the exposure of the insects to the extracts the higher the mortality. Overall, *A. squamosa* was the most effective of all the plant extracts.

The results of present study are in agreement with some earlier studies. Evaluation of the extracts from 10 plant species of family Annonaceae against *C. chinensis* by Ohsawa *et al*. (1990) showed that extracts of *A. squamosa* were particularly toxic to this beetle. Studies on the residual effect of neem (*Azadirachta indica*) against *C. chinensis* conducted by Choudhary (1990) showed that the damage by this beetle was reduced on chickpea. Jacob and Sheila (1990) reported that the effectiveness of neem oil against *C. chinensis* on green gram resulted in >60% mortality of the bruchid after 3 days. Studies conducted by Pandey and Singh (1977, 1995) showed that neem leaf powder could effectively protect black gram seed from damage of *C. chinensis*. They also found that neem bark powder was effective in reducing the damage. Mansour (1997) tested NeemAzal-S against *C.*

chinensis in the laboratory and found that 0.5% NeemAzal-S gave 100% mortality of different stages of the pest up to three months. Further work is now in progress to identify and purify the active fractions of the plant extracts. This will allow mode of action studies to be made.

REPELLENCY PROPERTIES: The repellent properties of nine plant extracts in each of the two solvents methanol and ethanol were studied along with a solvent only control.

MOVEMENT IN RELATION TO SEEDS TREATED WITH PLANT PRODUCTS EXTRACTED WITH METHANOL: The movement of insects immediately after release and for approximately 30 minutes after release was random (Table 9), with movements into and out of the different extract chambers. Thereafter, individual movements were more limited. After 2 h of release, the grains treated with plant extracts of *B. sacra* and *M. communis* showed a significant repellency effect (Table 9). Extracts of *A. squamosa*, *squamosa*, *A. indica* and *S. aegyptiaca* exhibited less repellency. For most of the remaining extracts and the methanol treated seeds, the movement of beetles was not significantly different from the methanol control. In the case of the commercial neem extract, there was a significant movement towards that treatment (Table 9, 11).

Twenty-four hours after release *B. sacra* and *M. communis* treated seeds had attracted the least number of beetles, suggesting that these methanol extracts of these two plants were repelling the beetles. Extracts of *A. indica*, *J. dhofarica* and the control showed some repellency after 24 h. Extracts of *A. squamosa* had attracted significant numbers of beetles after 24 h (Table 9).

MOVEMENT IN RELATION TO SEEDS TREATED WITH PLANT PRODUCTS EXTRACTED WITH ETHANOL: For the ethanol-based extracts, there was also a random movement of beetles for approximately 30 min after release. The repellency effects of extracts of B. sacra, M. communis, S. aegyptiaca, commercial neem and the ethanol control was similar after 2 hours but was significantly greater than for the remaining plant extracts (Table 9,10,11). After 24 hours this trend was not retained, except in the case of B. sacra where some repellency was still evident. The ethanol-based A. squamosa extracts showed the same effect as the methanol-based extract, where significant numbers of insects were attracted. For both solvent-based extracts few insects remained in the central chamber of the olfactrometer.

There have been few many studies reported on the repellent action of plant extracts against the pulse beetle. The few that have been published show the repellent action of neem against this pest. Khaire *et al.* (1993) reported that seeds treated with neem oil had a repellent action against egg laying activities of adult beetles. Pandey *et al.* (1986) also found that plant extracts of neem leaves and twigs gave a high repellent action against *C. chinensis*. Khaire *et al.* (1993) reported that treating pigeon pea seeds with neem oil showed significant repellent action against egg laying by adult *C. chinensis* beetles for up to 100 days after treatment.

Among the plant extracts in methanol tested for repellency, *Myrtus communis* showed high repellency at all time intervals followed by *Azadirachta indica* at 24 h.

TABLE 9

Repellent effects of plant derived natural products in methanol.

Treatments	Number of Beetles Present After: 15 min		30 min		1 hr		2 hrs		24 hrs	
	Total [1]	Mean [2]	Total	Mean	Total	Mean	Total	Mean	Total	Mean
Acacia nilotica	19	4.8	17	4.3	19	4.8	21	5.3	21	5.3
Annona squamosa	10	2.5	10	2.5	7	1.8	15	3.8	23	5.8
Azadirachia indica	6	1.5	15	3.8	13	3.3	14	3.5	9	2.3
Boswella sacra	8	2.0	10	2.5	7	1.8	4	1.0	8	2.0
Crotolaria juncea	14	3.5	23	5.8	23	5.8	23	5.8	22	5.5
Jatropha dhofarica	18	4.5	18	4.5	25	6.3	20	5.0	10	2.5
Myrtus communis	7	1.8	8	2.0	9	2.3	6	1.5	6	1.5
Suaeda egyptiaca	15	3.8	19	4.8	18	4.5	14	3.5	19	4.8
Commercial neem	18	4.5	18	4.5	19	4.8	26	6.5	40	10.0
Control Methanol	14	3.5	19	4.8	24	6.0	25	6.3	12	3.0

[1]Total represents number of beetles of 4 replications.
[2]Mean represents number of beetles of 4 replications.

TABLE 10

Repellent effects of plant derived natural products in ethanol.

Treatments	Number of Beetles Present After									
	15 min		30 min		1 hr		2 hrs		24 hrs	
	Total [1]	Mean [2]	Total	Mean	Total	Mean	Total	Mean	Total	Mean
Acacia nilotica	20	5.0	21	5.3	19	4.8	17	4.3	15	3.8
Annona squamosa	11	2.8	16	4.0	13	3.3	17	4.3	33	8.3
Azadirachia indica	11	2.8	15	3.8	18	4.5	17	4.3	19	4.8
Boswella sacra	15	3.8	15	3.8	13	3.3	13	3.3	12	3.0
Crotolaria juncea	14	3.5	20	5.0	23	5.8	19	4.8	10	2.5
Jatropha dhofarica	21	5.3	18	4.5	21	5.3	20	5.0	13	3.3
Myrtus communis	14	3.5	13	3.3	13	3.3	13	3.3	20	5.0
Suaeda egyptiaca	11	2.8	10	2.5	11	2.8	12	3.0	16	4.0
Commercial neem	10	2.5	13	3.3	14	3.5	13	3.3	22	5.5
Control Methanol	13	3.3	11	2.8	10	2.5	11	2.8	4	1.0

[1]Total represents number of beetles of 4 replications.
[2]Mean represents number of beetles of 4 replications.

TABLE 11

Analysis of variance of repellency effects of methanol and ethanol-based plant extracts.

Treatments	Intervals in methanol (hr)[1,2]	Intervals in ethanol (hr)[1,2]
Acacia nilotica	1, 2 hrs, 24 hrs*[b]	1, 2 hrs, 24 hrs[b]
Annona squamosa	2 hrs, 24 hrs[b], 1 hr[c]	24 hrs[a], 2 hrs[b]
Azadirachia indica	2 hrs[b] 24 hrs[c]	1, 2 hrs, 24 hrs*[b]
Boswella sacra	1, 2 hrs, 24 hrs*[b]	1, 2 hrs, 24 hrs*[b]
Crotolaria juncea	1, 2 hrs, 24 hrs*[b]	1 hr[a], 2 hr, 24 hrs[b]
Jatropha dhofarica	1, 2 hrs, 24 hrs*[b]	1, 2 hrs[a] 24 hrs[b]
Myrtus communis	1, 2 hrs, 24 hrs*[c]	24 hrs[a] 2 hrs[b]
Suaeda egyptiaca	1, 2 hrs, 24 hrs*[b]	1, 2 hrs, 24 hrs*[b]
Commercial neem	24 hrs[a] 2 hrs[b]	24 hrs[a] 2 hrs[b]
Control	1, 2 hrs, 24 hrs*[b]	1, 2 hrs, 24 hrs*[b]

[1]a - low repellency, b – medium repellency, c – high repellency
[2]Within solvents, different letters are significantly different ($P < 0.05$).
*Regardless to the time intervals the repellency was not significant.

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