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Evaluation of Insecticidal Potentialities of Aqueous Extracts from *Calotropis procera* Ait. Against *Henosepilachna elaterii* Rossi

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Abstract: In an attempt to find natural and cheaper methods for the control of vegetable pest, locally available plant *C. procera* was used and two methods of the extraction were evaluated. Shaker aqueous extract of leaf, flower and roots of *C. procera* proved most effective in the control of *Henosepilachna elaterii* showed strong repellent activity and thus deterred the insects from feeding. Five percent shaker extract of different plant part gave 100% protection of cucurbit leaf and no larva survived after exposure to 5% extract, 1 and 2.5% concentrations of shaker extracts highly reduced the fecundity and longevity of the insect. Soxhlet extract had no anti-insect activity. The results show the potential of the aqueous shaker extract in the control of vegetable pests.

Key words: *Calotropis procera*, plant-based insecticides, extraction, *Henosepilachna elaterii*, pumpkin, *Cucurbita maxima*

INTRODUCTION

Growing vegetables is increasing in Sudan that is restricted to irrigated areas Dabrowski *et al*. (1994). Crops that have a good market are grown intensely in short rotations. Under tropical conditions, partly polyphagous insect pests (like *Henosepilachna elaterii* Rossi.) can attack several crops in one rotation, making intensive vegetable production unsustainable (Brader, 1979; Binyason, 1997). Frequent use of synthetic insecticides leads to a destabilization of the ecosystem and to enhanced resistance to insecticides in pests (Dittrich *et al*., 1990).

The African melon ladybird beetle, *Henosepilachna elaterii* Rossi (Coleoptera: Coccinellidae), is a key pest of the family cucurbitaceae in Sudan (Bashier and Abdalhadi, 1986). The exclusive use of chemical insecticides put a great strain on the national economy (Abdelrahman and Badar, 1989). The frequent use of chemical insecticides for several decades has disrupted biological control system by natural enemies and lead to outbreaks of insect pests, widespread development of resistance, undesirable effects on non-target organisms, environmental and human health concerns (Champ and Dyte, 1977; Subramanyam and Hagstrum, 1995; White and Leesch, 1995). Synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. These problems have highlighted the need for the development of new types of selective insect-control alternatives. One alternative to synthetic insecticides is insecticidal plants; Africans farmers are traditionally familiar with them.

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). Since these are often active against a limited number of species including specific target insects, are often biodegradable to non-toxic products and are potentially suitable for use in integrated pest management and they could lead to the development of new classes of safer insect-control agents. Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents.

Calotropis procera Ait. (Asclepiadaceae), with the common name Usher, is a xerophytic, erect shrub, growing widely through out the tropic of Africa and Asia and it is grown abundantly in arid and semi arid regions without irrigation, fertilization, pesticides, or other agronomic practices. This plant has been widely used in the Sudanese medicinal system (Ayoub and Kingston, 1981; Ayoub and Svendsen, 1981). The latex of the plant was reported having potential antiinflammatory, antidiarrhoeal, analgesic, antipyretic and schizonticidal activities (Dewan *et al*., 2000; Kumar and Basu, 1994; Kumar *et al*., 2001; Sharma and Sharma, 2000). Bioactivities of the

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plant such as insecticidal (Jacob and Sheila, 1993; Khan and Siddiqui, 1994; Moursy, 1997), acaricidal (Chungsamarnyart *et al*., 1994), nematocidal (Rakesh *et al*., 2001), molluscicidal (Hussein *et al*., 1994), had been reported. Water containing latex of the plants was able to avoid adult females of *Anopheles stephens* and *Culex fatigans* to oviposit in the water and the latex water could kill eggs and larvae of *A. stephens*, *C. fatigans* and *Aedes aegypti* (Girdhar *et al*., 1984).

Our study aimed at developing insecticides using plant materials that are economical, safe and locally available in East-West Africa with *H. elaterii* as a target. It is also essential that appropriate technology transfer system is develop to promote a direct preparation of traditional pesticides at the farm level for those resource-poor farmers who have no access to commercial pesticides or organic solvent to extract plant material or can not afford them. Nevertheless, no information about the activity of *C. procera* extracts on *H. elaterii* has been found in the literature.

MATERIALS AND METHODS

The study was conducted at the Faculty of Agriculture University of Khartoum Sudan during, 1997 and 1998.

Test insects: Adults of the African melon ladybird beetle (*H. elaterii*) were collected from a cucurbit field at Shambat, North of Khartoum Sudan, maintained on fresh pumpkin *Cucurbita maxima* leaves in 60×30×30 cm glass boxes (aquaria). Their progeny at desired stage were used in this study.

Plant extracts: *Calotropis procera* (Asclepiadaceae) leaves and flowers and roots were collected in September, 1997 at Shambat, Sudan, air-dried and pulverized in an electric mill. The resulting powder of each plant parts was refrigerated in a glass jar with a lid until used. Two extraction methods were used in preparation of extract. Dried powder of leaves, flowers and roots were soxhlet-extracts with water using the procedure previously described by Valladares *et al*. (1997).

Shaker aqueous extracts of the different parts of the plant (50 g) were prepared by shaking the mixture of powdered plant material and distilled water for 24 h in an electric shaker. The mixture was filtered and then the extracts were evaporated.

The residues obtained by the two methods were diluted in water to obtain concentrations of 1, 2.5 and 5%.

Bioassays: Each of the extracts was assayed for anti-insect activity using the following tests except the test for repellency where only shaker extract were used.

Feeding repellence: The repellency activity of *C. procera* extracts prepared by the shaker method against the first instars larvae was investigated through choice bioassays using the same methods described by Valladares *et al.* (1997). The percent repellency (R) was calculated from the formula:

$$R = [(C- T)/ (T+C)] \times 100$$

Where T is the number of larvae found on the treated leaf-disc and C is the number of larvae found on the control leaf-disc. This experiment and the following experiments were carried out at room temperature range of 25-35°C.

Larval food consumption and survival: The effect of *C. procera* aqueous crude extracts on the food consumption and survival of the fourth instars larvae were investigated. The tests were carried as described by Tewari and Moorthy (1985) with some modification: simply the larvae were taken after the third molt (L4, less than 24 h after molt) placed individually in Petri dishes lined with moist filter paper. They were given known weigh (300 mg) of pumpkin leaf-discs containing 0, 1, 2.5 and 5% of the extract (n = 25 for each concentration). Each Petri dish with one larva constituted a replicate. After release of the larvae into petri dishes, respective leaf discs were inspected and weighted for each treatment on daily basis using a mettler balance, after that the treated leaf discs were renewed daily by treated ones until the end of the experiment.

Fecundity and longevity: The adults emerged from the aforementioned experiment were sexed and paired (6 pairs of one day old female and male). Each pair was transferred to a separate Petri dish lined with moist filter paper and fed with fresh pumpkin leaves, which were replaced daily. The number of eggs laid by each female and longevity of male and female was recorded to determine the effect of either extracts from each plant part on insect fecundity and longevity.

Data analysis: Effects of the extracts on larval feeding, survival, adult fecundity and longevity were analyzed by analysis of variance and means were separated with Duncan's multiple range tests when difference was detected at ANOVA using SAS software (SAS Institute, 2000. v.8.1).

RESULTS

Amounts of the extractive materials: The soxhlet technique yielded more extracts than the electric shaker, while the leaves gave more extracts than the other parts in both extractive methods (Table 1).

Table 1: The amount (mg) of the total extract of different parts of *C. procera* obtained by two extractive methods

Plant parts	Electric shaker	Soxhlet apparatus
Leaves	10.6±0.31^{a}	14.8±0.23^{a}
Flowers	7.2±0.12^{b}	12.5±0.29^{b}
Roots	5.4±0.12^{c}	10.2±0.31^{c}

Mean±SE followed by different small letters in same columns are different significantly at p<0.05 by Duncan's Multiple Range test

Table 2: Mean percentage repellent values for different parts of Calotropis procera shaker aqueous extracts on the first instar larvae of *Henosepilachna elaterii* exposed for the tested extracts for 24 h

	Shaker extract conc. (%)		
Plant parts	1	2.5	5
Leaf	66.0±2.5^{a}	62.0±2.2^{a}	100.0±0.0^{b}
Flower	60.0±2.4^{a}	66.0±2.9^{a}	100.0±0.0^{b}
Root	60.0±2.5^{a}	69.0±2.8^{a}	100.0±0.0^{b}
Untreated	0.0±0.0^{c}	0.0±0.0^{c}	0.0±0.0^{c}

Mean±SE followed by the same letter in the same column are not significantly different (p>0.05) by Duncan's Multiple Range Test

Table 3: Food consumption and survival of the 4th instar larvae of *Henosepilachna elaterii* exposed continuously to pumpkin leaves coated with aqueous extracts of *Calotropis procera*

Extraction method	Plant parts	Extract Conc. (%)	% Leaf area eaten (mg)	Larval mortality (%)	Die at (day)
Shaker	Leaf	1	36.83±6.6^{b}	0.0	
		2.5	29.17±5.2^{b}	0.0	
		5	0.00±0.0^{c}	100	6.4±0.5
	Flower	1	38.57±5.8^{b}	0.0	
		2.5	30.9±4.9^{b}	0.0	
		5	0.00±0.0^{c}	100	6.8±0.9
	Root	1	42.2±5.1^{b}	0.0	
		2.5	30.33±4.2^{b}	0.0	
		5	0.00±0.0c	100	7.2±0.7
Soxhlet	Leaf	1	56.00±5.5^{a}	0.0	
		2.5	60.00±6.00^{a}	0.0	
		5	58.20±4.9^{a}	0.0	
	Flower	1	62.67±4.2^{a}	0.0	
		2.5	62.8±5.32^{a}	0.0	
		5	61.87±4.80^{a}	0.0	
	Root	1	61.88±4.45^{a}	0.0	
		2.5	60.87±4.9^{a}	0.0	
		5	58.93±5.1^{a}	0.0	
Control			64.76±4.19^{a}	0.0	

Mean±SE. Means followed by the same letter in the same column are not significantly different (p>0.05) by Duncan's Multiple Range Test

Feeding repellency: Table 2 shows the mean repellency values for each of the extracted plant parts at different concentrations. In choice tests between the control and treated leaf-discs with *C. procera* shaker extracts, a strong repellent efficacy of the crude extracts against neonate larvae was observed. Analysis of variance indicated significant differences (p<0.01) between the repellent effect of the all concentrations of each plant parts and the control. At the highest concentrations of the all plant part extracts, the repellent rates went up to 100% without any exception.

Effects on larval food consumption and survival: Table 3 shows the effect of two methods of extraction of different plant part of *C. procera* on feeding and survival of *H. elaterii*. Extracts obtained from different plant parts of *C. procera* by both extractive methods were compared. Shaker extract from different plant parts were significantly reduced the larval food consumption. Although the reduction depended on the concentrations of the extracts applied to the leaf-discs, at the lowest concentrations (1%) the L4 consumed 36.8 to 42.2% of the available treated leaf area whereas the control larvae was consumed 64.76%. Higher concentration of the shaker extract (5%) protected the pumpkin leaf-disk by 100%. Soxhlet extract had no significant effect on the larval feeding at all the tested concentrations. The larvae consumed statistically same amount of the treated and untreated leaves.

All larvae exposed to the treated leaves with 5% of shaker extract were died after 7.2 days, but all larvae exposed to lower concentrations (1 and 2.5%) and control survived to adult.

Table 4: Effect of shaker and soxhlet extracts of Ushar leaf, flower and root on fecundity and longevity of adults emerged from fourth instars *Henosepilachna elaterii* exposed to treated pumpkin leaves for 2 days

Extraction method	Plant parts	Extract conc. (%)	Fecundity	Longevity (days)
Shaker	Leaf	1	108±4.4^{b}	24.6±2.7^{b}
		2.5	90±3.4^{b}	20.2±2.6^{b}
	Flower	1	105±3.8^{b}	19.6±3.8^{b}
		2.5	99±3.5^{b}	22.5±4.3^{b}
	Root	1	110±4.1^{b}	20.3±3.3^{b}
		2.5	102±3.6^{b}	24.2±3.5^{b}
Soxhlet	Leaf	1	378±4.4^{a}	39.6±3.5a
		2.5	383±3.3^{a}	42.8±3.9^{a}
		5	388±4.7^{a}	42.2±3.9^{a}
	Flower	1	4250±4.2^{a}	41.2±2.6^{a}
		2.5	435±3.4^{a}	45.8±3.6^{a}
		5	385±3.3^{a}	42.6±3.2^{a}
	Root	1	385±3.4^{a}	46.8±3.4^{a}
		2.5	378±3.5^{a}	44.6±3.5^{a}
		5	390±3.7^{a}	44.2±3.6^{a}
Control			420±1.7^{a}	45.2±2.8^{a}

Mean±SE followed by the same letter in the same column are not significantly different at (p>0.05) by Duncan's Multiple Range Test

Effect on fecundity and longevity: The fecundity and longevity of the adults emerged from the larvae fed on the aqueous shaker extract treated pumpkin leave-discs were significantly reduced (Table 4) when compared with control adults. Aqueous, soxhlet extracts showed no significant difference among different plant part extracts and between the control in respect to the fecundity and longevity.

DISCUSSION

It was observed from the results presented that leaves, flowers and root aqueous shaker extracts from *C. procera* showed biological effects on several targets. Exposure of the fourth larval instars of *H. elaterii* to the treated pumpkin leaves with highest extracts concentration (5%) tested induced 100% larval mortality within 7 days delayed effect and also gave complete protection of pumpkin leaves. Also reduction in oviposition and decreased longevity of the adults which emerged from the larvae fed on the leaves treated with 1 and 2.5% concentrations of the extract were observed in this study. Choice test of the plant extracts also produced 100% repellency of the neonate larvae. Soxhlet extract of leaf, flower and root tested concentrations, did not have effect on larval feeding and survival and also had no effect on fecundity and longevity of the insect. This may be due to the relatively high temperature during the soxhlet extraction, which might have destroyed the natural product.

A comparative study was carried out on *C. procera* originating from India by Girdhar *et al.* (1984). The authors showed that latex water was 100% ovicidal and larvicidal at 10 000 ppm against *A. stephens*, *Culex fatigan* and *Aedes aegypti*. Other studies also showed that extracts from different plant parts of *C. procera* have insecticidal effects. Abbassi *et al.* (2003) reported that alkaloid extracted from the leaves of *C. procera* was able to cause a considerable mortality of *Schistocerca gregaria*. Also Jahan *et al.* (1991) showed the toxicity of leaf powder of *C. procera* against larvae of *Tribolium confusum*. Aqueous and methanolic extracts of *C. procera* showed toxic effect against nematode *Meloidogyne javanica* (Verma *et al.*, 1989). Ahmed (1993) reported that usher plant parts (leaves, flowers and roots) powders and aqueous and alcoholic extracts reduced damage on wheat cause by khapra beetle *Trogoderma granarium granarium* Everts as well as the reduction of the beetle emergence. The identities of insecticidal compounds have not yet been determined but various researchers have undertaken the chemical analysis of *C. procera* latex and various compounds have been identified such as cardenolides, proteolytic enzymes, alkaloids and carbohydrates (Dhar and Singh, 1973; Seiber *et al.*, 1982).

The neonate larvae were exposed to short term choice tests, in which control and treated foods were available and undisturbed for 24 h. most of the larvae had chosen the control food as early as 24 h after the onset of the test. The quick rejection of the treated leaf might have been due to instantaneous suppression or rapid post-ingestive feed backs (Bernays *et al.*, 2000).

The suppression of reproductive activity and adults longevity showed by the plant extracts may be due to malnutrition effect because the larvae exposed to the treated leave consumed considerably significant fewer amounts than the control. Other workers have previously reported that botanical insecticide reduce the oviposition and longevity under laboratory conditions. The plants involved include: Some African plants traditionally used for the Protection of Stored Cowpea with *Callosobruchus maculates* (Boekea *et al.*, 2004) and four vegetable oils and ten botanical powders with *Callosobruchus maculatus*, *C. chinesis* and *C. rhodesianus* (Rajapakse and Vanemden, 1997).

It is noteworthy that *C. procera* aqueous shaker extract is very promising and the concentration of aqueous phase needed for the suppression of larval feeding, repellant, adult longevity and reproduction ability is very low. Furthermore, this plant grows wild in uncultivated dry zones in a wide area of Sudan and its leaves which are available throughout the year could be easily collected without any additional cost. Therefore, leaves which gave a highest extractive amount among all three parts tested could be used as insecticidal agent in an integrated vegetable pests control programmed. The use of the plant materials in the pest control could become important supplements to imported synthetic pesticides, especially in developing countries like Sudan. Developing more appropriate and handy methods for direct preparation of the botanical extracts at the farm level are necessary for those resource-poor farmers who have no access to commercial pesticides or cannot afford them. Separation of the active principles, research into their mode of action, effect on non target organisms and field evaluation are presently under investigation.

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