

## THE INSECTICIDAL ACTIVITY OF TOOTH-PICK SEED (*AMMI VISNAGA L.*) EXTRACTS AGAINST COWPEA BEETLE (*CALLOSOBRUCHUS MACULATUS F.*)

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### Abstract

The effectiveness of treating cowpea seeds with petroleum ether, chloroform and acetone extracts of tooth-pick seed extracts against *Callosobruchus maculatus* (F.) was determined. LC<sub>50</sub> and LC<sub>95</sub> levels were also estimated. Results showed that *C. maculatus* (F.) adults was more sensitive to chloroform extract than other extracts.

Petroleum ether, chloroform and acetone extracts at 7000 ppm concentration gave 92, 100 and 70% kill, respectively after 3 days from treatment. All extracts at LC<sub>50</sub> and LC<sub>95</sub> gave (85.9 and 97.1), (100 and 100) and (98.1 and 100) % inhibition in F-progeny up to 45 days from treatment.

When used LC<sub>50</sub> and LC<sub>95</sub> for petroleum ether chloroform and acetone extracts of the number of eggs laid were (26 ± 9.7 and 22 ± 7), (12 ± 5.6 and 4 ± 1) and (19.5 ± 7.6 and 17 ± 7) respectively compared with untreated are 101.7 ± 23.

LC<sub>95</sub> for all extracts lead to complete protection of cowpea seeds up to 30 days from treatment.

The same concentrations had a determined reduction on germination of cowpea seeds after 75 days from treatment compared with non-treatment seeds.

### INTRODUCTION

Synthetic insecticides cause several problems, such as harmful residues in the food chain, risk of hazards and pollution of environment, thus disruption of biological balance and destruction of the natural enemies of certain insect pest. The use of natural products from plant origin is a new trend that preserve the environment from contamination with harmful toxicants. Several studies suggested the use of plant dusts and extracts (Petterson, 1975 ; Jaipal et al., 1984 ; Su, 1985; Mahgoub and Ahmed, 1996 and El-Lakwah and Mohamed, 1999; Mohamed, 1999 and Ahmed, 2001). The toxic effect of *Datura* leaf extracts in acetone and in mixtures with the LC<sub>25</sub> of Malathion was evaluated on mortalities and reduction in F<sub>1</sub>-progeny of the cowpea beetle *C. maculatus* (F.) by Mohamed (1997).

The present work was directed to evaluate the insecticidal properties of tooth-pick seeds extracts on adults of *C. maculatus*.

## MATERIALS AND METHODS

Test insects; *Callosobruchus maculatus* F. adults were taken from a laboratory culture reared on cowpea seeds, at  $27 \pm 1^\circ\text{C}$  and  $60 \pm 10\%$  R.H. Adults of 0-24 hours old were used to carry out the present work.

Preparation of extracts: 200 gm of dried tooth-pick seeds (*Ammi visnaga* L.) as ground by a high speed micromill. The powder was successively extracted with 400 ml petroleum ether (40-60) in glass flask and left for 48 hr. as described by Su (1985) and Afifi, *et al.* (1989). The extract was filtered and the solvent was evaporated at  $50^\circ\text{C}$  under reduced pressure by using a rotary evaporator. The powder was thoroughly dried before extraction with chloroform and again dried before extraction with acetone.

Primary evaluation of extract toxicity was carried out to determined different concentrations to be used.

Toxicity of each extract was determined by adding one ml of each crude extract to 10-ml solvent (chloroform, acetone and petroleum ether). Different dilutions were made for each extract using the appropriate solvent of extraction. One ml of different dilution of each extract was mixed thoroughly with 10 grams cowpea seeds in glass vials, and left for 2 hrs to evaporate the solvent. A group of 25 adults of the tested insect was introduced into the vials, then covered with muslin fixed with a robber band.

Each concentration was represented with three vials (replicates) and all vials were kept at  $27 \pm 1^\circ\text{C}$  and  $60 \pm 10\%$  R. H .

Three similar vials containing untreated cowpea seeds were used as a control and kept under the same conditions.

Mortality counts were recorded after 2, 3, 5, and 7 days and percentage of corrected mortality were calculated by Abbott's formula (1925). Three days after treatment percentages were statistically computed according to Finney (1971) to produce  $LC_{25}$ ,  $LC_{50}$ ,  $LC_{95}$  and toxicity slope for each extract.

Insecticide: (Malathion W. P. 1% produced by Kafer Elzayat Company) was used in preliminary experiments, 25 adults were confined with insecticide treated 10 grams of cowpea seeds in vials all treatments were replicated three time for each concentration. Mortality was assessed after 3 days from treatment.

Mortality data corrected by Abbott's formula (1925) were subjected to probit analysis using a computer program according to Finney (1971) to produce  $LC_{25}$ ,  $LC_{50}$ ,  $LC_{95}$ , and toxicity slope for Malathion.

Assessment residual efficiency treatments of plant extracts:

1- On adults group of 30 glass vials, each containing 10 gm cowpea seeds treated with  $LC_{95}$  concentration of the petroleum ether extract was kept under insctary condition. Each week three vials were aimed 20 *Callosobruchus maculatus* (F.) adults

were introduced into each. The same technique was done for both chloroform acetone extracts and Malathion. Another 30 vials contained cowpea seeds; only treated with the solvent were dealt by the same way. Mortality counts were recorded after 72 hrs of introducing the adults.

2- On eggs, six vials (5 gm cowpea seeds in each) were used for each extract and Malathion there for LC<sub>50</sub> concentration and there for LC<sub>95</sub> one. Also, another three vials with untreated cowpea seeds were used as a control. Each vial received 2 couples of newly emerged adults and covered with musline. After one week, all dead insects were described and the seeds were examined for the number of eggs and hatchability % was indicated when they turned white showing that larvae had penetrated the seeds. Infested seeds were kept till emergence of offspring was counted.

Effect on percentage of germination and chlorophyll content:

Four replicates, (25 seeds each) were selected for the tested concentrations: 4080 and 7730 ppm of petroleum ether, 3100 and 7260 ppm of chloroform, 4700 and 1500 ppm of acetone extracts and 8 ppm of Malathion and 100 cowpea seeds kept untreated as a control. Seeds of each replicate were kept on moistened filter paper in of Petri-dishes (9 cm) for 7-10 days at the same condition, and the numbers of normal were counted, then the percentages of germination were calculated (Anonymous, 1966) chlorophyll content of the seedlings leaves was recorded after 10 days from planting the chlorophyll content of the seedlings leaves was estimated by chlorophyll meter SPAD-502.

## RESULTS AND DISCUSSION

The present work studied the effect of three different extracts of tooth-pick seeds and Malathion on *C. maculatus* adults as follows.

**Effect on adults.** Data shown in Table 1 proved that mortality percentages were concentration and exposure period depended one. Mortality values increased with increase of the concentration and exposure period. After three days from treatment, mortality ranged from 0.0 - 57, 26-73 and 0.0 - 46% for petroleum ether, chloroform and acetone extracts, compared with 93.3 %for Malathion, respectively, increased to 0.0-100, 68 - 100 and 67 - 100 % after 5 days post treatment at the various concentrations for all extracts compared with 100 % for Malathion, respectively. After 7 days the mortality percentages reached 53-100, 94-100 and 70 - 100 % for the same extracts, respectively.

As shown in Table 2, while LC<sub>25</sub> of Tooth-pick seed extracts ,after 72 hrs post-treatment ,was 4000, 2200 and 2900 ppm .But Malathion was 0.174 ppm ,LC<sub>50</sub> was 4080,3100 and 4700 ppm compared with 0.380 ppm for Malathion and LC<sub>95</sub> was

7730,7260 and 15000 ppm, Malathion was 1.82 ppm for the petroleum ether, chloroform and acetone extracts, respectively.

This result showed that *C. maculatus* adult is more sensitive to chloroform extracts than petroleum ether and acetone extracts .

**Effect on the number of eggs and F<sub>1</sub> -progeny.** Table 3 indicated that , the treatment of petroleum ether, chloroform ,acetone extracts and Malathion reduced the rate of oviposition compared with untreated seeds (control). Mean number of eggs per 2 females were in range from (26 ± 9.7) - (22 ± 7),(12 ± 5.6 -4 ± 1), ( 19.5 ± 7.6 – 17 ± 7) and (4.1 ± 1 -8 ± 1.6) for LC<sub>50</sub> and LC<sub>95</sub> for petroleum ether, chloroform, acetone extracts and Malathion, respectively. Also, no emerged adults were observed in case of LC<sub>50</sub> and LC<sub>95</sub> chloroform and Malathion and LC<sub>95</sub> only acetone extract and the reduction in F<sub>1</sub>-progeny was 100 % for chloroform and Malathion and LC<sub>95</sub> for acetone extract compared with untreated seeds.

The obtained results are in harmony with that obtained by (Heleri, 1989), (Mohamed, 1999 and 2001) and (EL-Lakwah and Mohamed, 1998).

**Residual effect.** Table 4 indicated that petroleum ether, chloroform , acetone extracts and Malathion at the concentration of LC<sub>95</sub> gave (3.6±0.3 ,83.3±1.3 ,95.3±0.3 and 100 %) kill against *C. maculatus* adults after 45 days from treatment ,respectively , and decreased highly till 75 days (0.0 , 0.0 , 10 ± 2.3 and 98.3±1 %) kill for petroleum ether, chloroform, acetone extract and Malathion, respectively.

**Effect on cowpea seeds germination.** Table 5 showed that the extracts tested had detrimental effect on the germination of cowpea seeds. This effect was much more obvious in case of the higher concentration (LC<sub>95</sub>) and the longer period of storage (75 days).

The germination percentages it were (50 ± 4.6, 75.9 ± 6.7 and 54.3 ± 4.3) % for petroleum ether, chloroform and acetone extracts, respectively. After 75 days from treatment compared with untreated cowpea seeds it was 95.7 ± 3.3 %germination at the same storage period. This result is in harmony with that obtained by (Ahmed, 2001)

**Effect on total chlorophyll of cowpea seed plant.** Table 6 showed that the effect of the extracts effect was much more obvious in case of the higher concentration (LC<sub>95</sub>) and the longer period of storage (75 days).

The total chlorophyll percentages it were (32.7 ± 4.3 ,22.8 ± 1.9 and 21.1 ± 3.9 %) for petroleum ether, chloroform and acetone extracts, respectively after 75 days form treatment compared with untreated cowpea seeds which was 45.6 ± 4.7 % at the same storage period.

Table 1. Effect of Tooth-pick seed extracts and Malathion against adults of *Callosobuchus maculatus* (F.) .

Solvent	Concentration ppm	% Mortality after indicated days			
		2	3	5	7
Petroleum ether	2000	0.0	0.0	0.0	53
	3000	0.0	4.0	10	70
	4000	4	26	38	86
	5000	10	48	84	100
	6000	24	72	100	100
	7000	34	92	100	100
	8000	45	100	100	100
	9000	57	100	100	100
Chloroform	2000	26	25	68	94
	3000	24	39	78	98
	4000	38	60	80	100
	5000	48	79	94	100
	6000	50	94	100	100
	7000	53	100	100	100
	8000	60	100	100	100
	9000	73	100	100	100
Acetone	2000	0.0	0.0	67	70
	3000	0.0	0.0	75	81
	4000	0.0	26	82	90
	5000	14	54	88	98
	6000	24	60	90	100
	7000	30	70	96	100
	8000	30	81	100	100
	9000	46	92	100	100
Malathion	8	93.3	98.9	100	100

Table 2. LC<sub>25</sub>, LC<sub>50</sub> and LC<sub>95</sub> values and slopes of regression lines for petroleum ether, Chloroform, Acetone extracts of tooth-pick seeds and Malathion against *Callosobruchus maculatus* (F.) adults, 72 hrs. after treatment.

Treatment	Petroleum Ether				Chloroform				Acetone				Malathion			
	LC <sub>25</sub>	LC <sub>50</sub>	LC <sub>95</sub>	Slope	LC <sub>25</sub>	LC <sub>50</sub>	LC <sub>95</sub>	Slope	LC <sub>25</sub>	LC <sub>50</sub>	LC <sub>95</sub>	Slope	LC <sub>25</sub>	LC <sub>50</sub>	LC <sub>95</sub>	Slope
<i>C. maculatus</i>	4000	4080	7730	8.27	2200	3100	7260	4.50	2900	4700	15000	3.25	0.174	0.380	1.82	1.94

Table 3. LC<sub>50</sub> and LC<sub>95</sub> of the extracts and Malathion in relation to certain biological aspects of *C. maculatus* (F.) adults at 27 ± 1° C and 55 ± 5% R.H.

Treatment	Pet. ether				Chloroform				Acetone				Malathion			
	No. of eggs/ 2 pairs	Hatchability %	No. of F <sub>1</sub> - progeny	Reduction in F <sub>1</sub> - progeny%	No. of eggs/ 2 pairs	Hatchability %	No. of F <sub>1</sub> - progeny	Reduction in F <sub>1</sub> - progeny%	No. of eggs/ 2 pairs	Hatchability %	No. of F <sub>1</sub> - progeny	Reduction in F <sub>1</sub> - progeny%	No. of eggs/ 2 pairs	Hatchability %	No. of F <sub>1</sub> - progeny	Reduction in F <sub>1</sub> - progeny%
LC <sub>50</sub>	26±9.7	78.1±9	9.7±1	85.9	12±5.6	74.1±8.3	0.0	100	19.5±7.9	66.4±7.9	1.3±0.3	98.1	4.1±1	6±2	0.0	100
LC <sub>95</sub>	22±7	66.7±3.3	2±0.3	97.1	4±1	55±2.3	0.0	100	17±7	58.5±2.6	0.0	100	8±1.6	4±2	0.0	100
control	101.7±23	88.3±7	68.6±3	-	101.7±23	88.3±7	68.6±3	-	101.7±23	88.3±7	68.6±3	-	101.7±7	88.3±7	68.6±3	-

Table 4. Corrected mortality percentages of *C. maculatus* adults after 3 days of exposure to cowpea seeds treated with  $LC_{95}$  of extracted and kept for different periods under insectary conditions.

Periods ( days )	% Mortality of exposed insects			
	Petroleum Ether	Chloroform	Acetone	Malathion
4 (hrs )	100	100	100	100
15	67.3±1.3	100	100	100
21	12.3±1	100	100	100
30	3.3±0	100	100	100
37	3.3±0.3	93.3±1.6	100	100
45	3.5±0.3	83.3±1.3	95.3 ±0.3	100
52	3.6±0.7	50±2.3	37.7 ±3.6	100
60	0	5±0	17.6 ±0.3	100
67	0	4±0	10±1.8	100
75	0	0	10±2.3	98.3 ±1

Table 5. Effect of Tooth-pick seed extracts on the germination of cowpea seeds after different periods of storage under insectary conditions

Treatment	Concentration ppm	% germination after	
		4hrs	75 days
Pet.ether	4080	62 ± 7.2	58.6 ± 3.1
	7730	58 ± 3.8	50 ± 4.6
Chloroform	3100	81.4 ± 4.7	77.1 ± 2.1
	7260	78.7 ± 6.3	75.9 ± 6.7
Acetone	4700	70.9 ± 2.3	61.7 ± 2.3
	15000	57.3 ± 4.9	54.3 ± 4.3
Malathion	8	93.8 ± 1	93.6 ± 2
control	-	94.3 ± 2	95.7 ± 3.3

Table 6. Effect of Tooth-pick seed extracts on the total chlorophyll of cowpea seeds plant germinate stored for 75 days

Tested extracts	Concentration ppm	Total chlorophyll %	
Pet.ether	480	44.7±4.8	37.3±1.7
	773	41.7±1.2	32.78±4.3
Chloroform	314	43.3±4.1	29.9±3.7
	726	35.6±6.2	22.8±1.9
Acetone	486	44.3±1.5	36.3±4.6
	1497	38.6±0.8	21.1±3.9
Malathion	8	43.8±1.3	44.6±2.3
Control		44.3±3.4	45.6±4.7

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## النشاط الإبادى لمستخلصات بذور نبات الخلة البلدى ضد حشرة خنفساء اللوبيا

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أجريت دراسات معملية لتقييم فاعلية مستخلصات الاثير البترولى والكلوروفورم والاسيتون لبذور الخلة البلدى فى وقاية بذور اللوبيا ضد حشرة خنفساء اللوبيا .  
أوضحت النتائج أن خنفساء اللوبيا أكثر حساسية لمستخلص الكلوروفورم مقارنة بمستخلص الاثير البترولى والاسيتون . حيث أنه عند استعمال ٧٠٠٠ جزء فى المليون من مستخلص الاثير البترولى والكلوروفورم والاسيتون أعطت نسب موت مقدارها ٩٢ ، ١٠٠ ، ٧٠ % للمستخلصات على التوالي بعد ثلاثة أيام من المعاملة . وقد أدى استعمال المستخلصات الثلاثة بتركيزات  $LC_{50}$  ،  $LC_{95}$  لتقليل الخلفة الناتجة بنسب ( ٨٥,٩ و ٩٧,١ ) و ( ١٠٠ و ١٠٠ ) و ( ٩٨,١ و ١٠٠ % ) للمستخلصات الثلاث على التوالي بعد ٤٥ من المعاملة مقارنة بالكنترول بينما أدى استعمال تركيز  $LC_{50}$  ،  $LC_{95}$  للمستخلصات الثلاثة الى انخفاض عدد البيض الموضوع كما يلى (  $1.7 \pm 26$  ) و (  $7 \pm 22$  ) و (  $5,6 \pm 12$  ) و (  $1 \pm 4$  ) و (  $7,6 \pm 19,5$  ) و (  $7 \pm 17$  ) و (  $7,6 \pm 19,5$  ) مقارنة بالكنترول (  $23 \pm 101,7$  ) .

وعند استعمال تركيز  $LC_{95}$  للمستخلصات الثلاثة كانت هناك وقاية كاملة لبذور اللوبيا من خنفساء اللوبيا حتى ثلاثين يوما من المعاملة بينما أدى هذا التركيز الى انخفاض فى نسبة انبات البذور بعد ٧٥ يوم من المعاملة مقارنة بالبذور الغير معاملة.