Toxic Effects of Some Desert Plant Exrtacts on the Red Flour Beetle, *Tribolium confusum* Duval (Coleoptera: Tenebrionidae)

A. G. Abdel-Rahman* , M. H. Belal , N. M. Ibrahim** and E. A. Ali***
* Plant Protection Dept., Desert Research Center, Mataria, Cairo, Egypt
**Economic Entomology & Pesticides Dept., Fac. Agric. Cairo Univ., Giza, Egypt.
(Received, November 14, 2003 ; Accepted, January 11, 2004)

ABSTRACTS

Of nine desert plants, three extracts (by petroleum ether, chloroform and acetone) from Aadar (*Artemisia monosperma*) leaves and chloroform and acetone extracts from Oshar (*Calotropis procera*) leaves showed promising insecticidal activity against the red flour beetle (*T. confusum*). LC_{50} and LC_{90} values of the three Aadar crude extracts were (8.06 and 26.15 mg/ml), (8.10 and 12.32 mg/ml) and (7.45 and 12.72 mg/ml), respectively 6 days after treatment. LC_{50} and LC_{90} values of chloroform and acetone Oshar leaf extracts were (8.28 and 38.10 mg/ml) and (5.58 and 28.96 mg/ml), respectively 6 days after treatment. Of ten TLC fractions of Aadar chloroform crude extract and twelve fractions in the acetone extract, fractions number 1 and 8 (R_f 0.17 and 0.70) of chloroform and fractions number 1, 4, 3 and 8 (R_f 0.09, 0.38, 0.33 and 0.59) of acetone extracts showed considerable insecticidal activity. Of ten TLC fractions of Oshar leaf chloroform crude extract, fractions number 5, 6 and 2 (R_f 0.33, 0.47 and 0.13) and of eleven TLC fractions of its acetone crude extracts only fractions 2, 8 and 4 (R_f 0.33, 0.77 and 0.48) exhibited the strongest toxic effects. Neemazal-F was more toxic than Neemazal –T and both of them were more toxic than any of Aadar or Oshar extracts

Key Words: *Tribolium confusum,* desert plants, extracts, fractions, toxicicty.

INTRODUCTION

The extensive use of synthetic chemical pesticides caused different hazards to man and his environment,*e,g,,* insect resistance, harms to beneficial biocontrol agents, soil, water and air contamination. Neem, pyrethrum, rotenone, and nicotine as natural products have been used to solve many agricultural problems. These natural products have biologically active chemicals exhibiting diverse effects on insects including toxic, anti-feedant action, repellent and attractant, and reproduction as well as retardation of growth and ovicidal effects. Su (1977) stated that black pepper and its extract were toxic to the rice weevil *Sitophilus oryzae* and cowpea weevil *Callosobruchus maculatus*. Mahgoub and Zewer (1989) used lemon, tegratol, neem and soybean oils to protect cowpea seeds against *C. maculatus* and wheat seeds against *S. granarius*. Jahan *et al.* (1991) found that incorporating powdered leaves of *Calotropis procera* into flour-yeast medium at concentrations ranging from 15000 to 75000 ppm were toxic to larvae of *Tribolium confusum* Duval. Talukder and Howse (1994) found that the crude extracts of *Aphanamixi polystachya* were toxic to *T. castaneum*. The ethanol extract was the most toxic of four extracts tested and showed the lowest LD_{50} value. Xie *et al.* (1995) investigated the toxicity of azadirachtin (98 % AZA) and 3 neem extracts (48, 23 and 7 % AZA) to three stored - product insects, the rusty grain beetle *Cryptolestes ferrugineus*, the rice weevil, *S. oryzae* and the red flour beetle, *T. castaneum*. The rusty grain beetle *C. ferrugineus* was the most susceptible. After six weeks, LC_{50} values for 48, 23, and 7 % AZA for *C. ferrugineus* were 18.8, 37.0, and 127.3 ppm, respectively.

This work aims to provide an overview for the toxicity of nine desert plant extracts against the red flour beetle (*T. confusum*).

MATERIALS AND METHODS

Tested Plants

Nine natural plants were collected from different areas in Sinai and along the Cairo - Suez desert road. Table 1 presents some information about these plants.

Extraction procedure:

One hundred grams of the ground plant material were successively extracted with three organic solvents of increasing polarities, petroleum ether (40-60C), chloroform and acetone (Belal *et. al* 2001). Crude extracts (10 mg/ml) were preliminarily tested against the red flour beetles (*T. confusum*) for their toxicity. The crude extracts, which gave promising results in these preliminary tests, were subjected to the same tests using a series of concentrations. Two commercial formulations (Neemazal F and T each contain 5 % AZA) of neem (*Azadirachta indica*) were also tested and used as comparison standards.

Toxicity test for the red flour bettle, *Tribolium confusum* Duval

Lab-strain of *T. confusum was* reared on flour and kept at 25 ± 2 °C, and 60 - 65 % R.H.

The residual film technique was used. Three ml of the desired tested concentration were evenly spread on a Petri-dish surface (9 cm in diameter). The solvent was allowed to evaporate leaving a thin film of the plant extract. Two days-old adult beetles were exposed to the thin film for six days. Each concentration was replicated ten times. Mortality counts were calculated daily along the six days exposure period. Mortality percentages were corrected according to Abbott's formula (Abbott, 1925). Concentration/mortality regression lines were drawn according to the method developed by Finney (1971) and LC_{50} and LC_{90} values were calculated as mg / ml.

Thin layer chromatographic analysis.

Silica gel thin layer plates were used to separate the fractions into bands. A mixture of hexane: acetone (3:2) was used as a developing solvent. Spots were visualized

Table (1): Scientific and Arabic names of the plants used in the present study.

No.	Family	Scientific Name	Arabic Name	Used Part
1	Compositae	*Artemisia monosperma*	Aadar	Leaves and stems
2	Asclepiadaceae	*Calotropis procera*	Oshar	leaves
3	Euphorbiaceae	*Chrozophora plicata*	Battikh El-Malaaeeka	leaves
4	Compositae	*Launaea spinosa*	Keddad	whole plant
5	Labiatae	*Lavandula stricta*	Natash	Leaves and stems
6	Euphorbiaceae	*Pergularia tomentosa*	Laban el –homaara	whole plant
7	Polygonaceae	*Polygonum equistiforme*	Gorthab	whole plant
8	Leguuminosae	*Retama ratm*	Rtm	whole plant
9	Zygophyllaceaeae	*Zygophyllum simplex*	Garmal	whole plant

Toxic effect of the crude extracts against the red flour beetle *Tribolium confusum*

Petroleum ether extracts at a concentration of 10 mg/ml presented in Table (2) show that the leaves of Aadar (*A. monosperma*) gave the most toxic effect which ranged between 60.0% and 65.3 % mortality during the six days exposure time. Other petroleum ether extracts were less effective and most of them gave mortalities below 20%. Concerning chloroform extracts, leaves of the Aadar plant were again the most toxic followed by leaves of Oshar (*C. procera*), Gorthab (*P. equisetiforme*) and the stems of Aadar. Their mortality percentages after six days were 75 - 78.9, 21.2 - 54.2, 20.0 - 38.8 and 25.0 - 33.7, respectively. The remaining extracts exhibited slight effects. The same trend was observed in the acetone extract where the leaves of Aadar were the most toxic followed by those of Oshar and Gorthab. Their induced mortality percentages were 70.0 - 78.7, 40.0 - 67.3 and 9.1 - 30.1, through the six days of treatment, respectively.

Results given in Table (3) show that Neemazal-T & F (5%) at 0.1 mg/ml were toxic against *T. confusum* but Neemazal-F was more toxic than Neemazal-T in the first five days. Both Neemazal-T & F gave 100 % mortality six days after treatment.

In a comparison between the tested plant extracts and Neemazal T or F, results prove that the three extracts of Aadar (*A. monosperma*), and acetone extract of Oshar (*C. procera*) were more toxic than Neemazal T during the first four days after treatment but as the time passed the toxic effect of Neemazal-T increased and complete mortality was obtained after 6 days as compared with 65.3 %, 78.9 % and 78.7 % mortality for the three extracts of Aadar. With Neemazal - F, the same trend was observed but with superior results for Aadar leaf extract during the first three days only. Compared with the other plant extracts, both Neemazal T & F were more toxic after any of the six exposure times.

From the previous data, it is clear that leaf extract of Aadar and Oshar plants gave the most toxic effect against the red flour beetle. So, these plants were chosen for detailed studies and a series of concentrations (10, 7.5, 5 and 2.5 mg/ml) were tested to calculate different toxicological parameters. Data of this study was represented in Table (4). These results take the same trend as those previously discussed in Table (2). It clear that the toxicity of the three Aadar extracts can be arranged descendingly according to their LC_{50} levels as follows; acetone (7.45 mg/ml), petroleum ether (8.06 mg/ml) and chloroform (8.10 mg/ml). Toxicity at LC_{90} level indicates that chloroform extract was the most toxic followed by acetone and petroleum ether with 12.32, 12.72 and 26.15 mg/ml, respectively.

Concerning Oshar extracts, acetone extract was more effective than the chloroform and petroleum ether extracts. After six days exposure, respective LC_{50} and LC_{90} values for Oshar leaf extract were 5.58 and 28.96 mg/ml, as compared with 8.28 and 38.10 mg/ml, and 415.47 and 17092.00 mg/ml, for its chloroform and petroleum ether extracts, respectively.

Saleh *et al.* (1986) found that non-polar extracts of leaves of *A. monosperma* and seeds of *A. mexicana* had insecticidal activity against *T. confusum*, *C. maculates* and *S. oryzae*. Belal and El-Kabbany (1993) showed the relative toxicity of chloroform extract of *A. monosperma* and conventional insecticides against one-week old adults of *T. castaneum*.

Data presented in Table (5) are in accordance with that in Table (3). It proved that Neemazal-F was more toxic than Neemazal-T, at any of the six days exposure time. The LC_{50} and LC_{90} values for Neemazal-F were 0.04 and 0.09 mg/ml, respectively as compared with 0.05 and 0.11 mg/ml for Neemzal-T. Comparing with Aadar and Oshar extracts, results prove that both of the two tested neem formulations (Neemazal-T & F) were more toxic than any of Aadar or Oshar extracts. Xie *et al.* (1995) found that azadirachtin (98 %) and three neem extracts containing 48, 23 and 7 % AZA had toxic effects on the red flour beetle.

Toxic effect of TLC fractions of some effective crude plant extracts against the red flour beetle *T. confusum*

TLC studies showed ten fractions for Aadar leaves in chloroform crude extract, and twelve fractions in the acetone extract. Concerning Oshar leaf extracts, ten and eleven fractions were found in the chloroform and acetone crude extracts, respectively. Table (6) presents the R_f values of these fractions. The fractions obtained from TLC fractionation were tested for their toxicity against the red flour beetles (*T. confusum*). Fractions number 1 and 8 in chloroform extract and fractions number 1, 4, 3 and 8

Table 2: Toxicity of some desert plants crude extracts (10 mg/ml) against the adults of red flour beetles *T. confusum*.

Plant		Petroleum ether Exposure time (Days) % Mortality						Chloroform Exposure time (Days) % Mortality						Acetone Exposure time (Days) % Mortality					
Name	Used part	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Aadar	leaves	60.0	60.0	62.0	65.5	65.3	65.3	75.0	75.0	75.5	79.4	78.9	78.9	70.0	70.0	74.7	75.5	78.7	78.7
Aadar	stemes	6.0	11.0	17.3	17.3	21.4	30.6	25.0	30.0	28.6	34.7	33.7	33.7	16.2	16.2	19.4	19.4	22.4	22.4
Oshar	leaves	0.0	3.0	3.1	5.1	5.3	7.2	21.2	31.7	33.7	38.8	48.4	54.2	40.0	44.0	50.0	51.5	59.2	67.3
Battikh El-Malaaeeka	leaves	2.1	2.1	7.6	10.9	12.2	13.3	8.2	8.5	11.3	9.3	10.7	13.0	5.0	5.1	7.2	6.2	10.3	11.7
Keddad	whole plant	6.1	9.3	10.3	11.4	12.6	14.9	10.0	12.1	15.5	15.5	16.7	18.7	4.0	5.0	6.1	7.2	7.4	9.5
Natash	leaves	0.0	0.0	0.0	0.0	5.3	5.9	0.0	0.0	0.0	0.0	0.0	6.6	0.0	0.0	0.0	0.0	0.0	1.1
Natash	stems	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	5.0	5.0	10.0	15.0	19.2	19.2
Laban el homaara	whole plant	7.2	8.3	13.3	12.6	12.9	12.9	1.0	1.0	1.0	2.1	4.2	7.4	0.0	0.0	5.0	5.1	8.3	10.5
Gorthab	whole plant	0.0	0.0	5.0	2.1	5.1	5.1	20	25	28	28	30	38.8	9.1	11.8	23.6	23.6	26.9	30.1
Rtm	whole plant	0.0	3.1	4.2	5.4	5.3	7.6	3.1	7.4	7.4	9.4	9.7	14.0	5.2	9.6	9.7	9.7	10.5	10.9
Garmal	whole plant	0.0	0.0	1.0	1.0	3.1	5.3	0.0	0.0	1.0	3.1	7.2	9.5	0.0	3.1	4.2	7.5	7.6	8.6

Table 3: Toxicity of Neemazal T & F at 0.1 mg/ml against adults of the red flour beetles *T. confusum*

Exposure time (Days)	% Mortality	
	Neemazal-T	Neemazal-F
1	22.7	30.6
2	31.4	44.3
3	41.3	57.7
4	47.6	72.5
5	77.2	87.0
6	100.0	100.0

Table 4 : Cumulative LC_{50} and LC_{90} values (mg/ml) of Aadar and Oshar leaf extracts against the adults of *T. confusum*.

Exposure time (Days)	Petroleum ether			Chloroform			Acetone		
Aadar leaves	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope
1	10.15	13.95	9.3	9.25	13.29	8.1	9.33	16.13	5.4
2	10.17	14.57	8.47	9.02	14.22	6.5	8.96	14.95	5.76
3	9.91	13.00	10.9	8.52	11.90	8.7	8.71	14.72	5.70
4	9.75	16.07	5.9	8.34	12.25	7.7	8.7	15.10	5.36
5	9.12	21.01	3.5	8.37	12.14	7.9	7.99	13.11	5.9
6	8.06	26.15	2.5	8.10	12.32	7.0	7.45	12.72	5.5
Oshar leaves									
1	No effect	No effect	----	106.17	6994.6	0.7	12.24	115.04	1.3
2	No effect	No effect	----	41.80	1187.1	0.9	9.27	68.01	1.5
3	No effect	No effect	----	19.03	208.38	1.2	8.19	56.96	1.5
4	10.60	11.31	53.0	14.11	137.56	1.3	6.86	41.26	1.6
5	95.63	824.44	1.4	9.64	48.45	1.8	6.05	29.44	1.9
6	415.47	17092	0.8	8.28	38.10	1.9	5.58	28.96	1.8

Table 5: Cumulative LC_{50} and LC_{90} values (mg/ml) of Neemazal -T and Neemazal - F against (*T. confusum*) adults.

Exposure time (Days)	Neemazal -T			Neemazal -F		
	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope
1	0.22	0.85	2.2	0.43	3.91	1.3
2	0.11	0.42	2.2	0.09	0.33	2.2
3	0.09	0.37	2.1	0.07	0.26	1.8
4	0.06	0.15	3.4	0.04	0.16	2.0
5	0.06	0.14	3.5	0.05	0.22	1.9
6	0.05	0.11	3.9	0.04	0.09	3.1

Table 6 : Toxicity of TLC fractions of chloroform and acetone extracts of Aadar and Oshar leaves against the red flour beetles *T. confusum*

No. of Fraction	% Mortality																				
	Chloroform										Acetone										
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	7	8	9	10	11	12
A. monosperma (Aadar leaves)																					
R_f values	0.17	0.36	0.45	0.49	0.54	0.61	0.64	0.70	0.76	0.85	0.09	.21	0.33	0.38	0.44	0.52	0.54	0.59	0.62	0.65	0.72	0.83
Exp. Time																					
1 Day	20.0	0.0	3.0	1.0	4.0	1.0	3.0	9.1	2.0	4.0	25.0	5.0	8.0	0.0	0.0	4.0	4.1	10.0	4.0	0.0	0.0	10.0
2 Days	30.0	0.0	5.0	3.0	5.0	1.0	4.0	12.1	2.0	5.1	30.0	5.0	17.0	0.0	0.0	7.2	5.0	15.0	9.1	6.1	1.0	13.1
3 Days	27.1	0.0	3.1	3.1	5.0	2.0	7.1	14.1	2.0	6.1	31.0	6.0	20.0	10.0	5.1	8.0	5.1	15.5	8.2	7.2	2.0	16.3
4 Days	37.5	4.2	7.1	4.1	7.1	2.0	7.1	14.3	3.2	9.3	32.3	7.1	24.2	19.2	12.4	8.2	6.0	17.5	8.2	8.2	3.1	17.5
5 Days	52.2	4.3	9.4	4.1	7.2	2.1	7.2	17.5	6.2	10.4	34.7	8.2	23.5	24.2	12.8	8.5	9.6	20.2	14.6	10.4	4.2	18.7
6 Days	64.4	11.1	12.6	5.3	6.3	2.1	7.4	29.5	9.4	10.5	36.1	9.3	29.9	31.9	12.9	8.6	11.8	24.7	14.9	10.6	8.3	18.1
C. procera (Oshar leaves)																					
R_f values	0.03	0.13	0.17	0.27	0.33	0.47	0.49	0.53	0.55	0.71	0.11	0.33	0.41	0.48	0.60	0.67	0.69	0.77	0.85	0.88	0.96	-
Exp. Time																					
1 Day	0.0	4.0	6.0	0.0	10.0	16.0	0.0	0.0	0.0	0.0	2.0	20.0	0.0	10.0	8.0	0.0	0.0	16.0	0.0	0.0	4.0	
2 Days	0.0	8.0	10.0	6.1	28.5	18.4	0.0	0.0	0.0	0.0	2.0	20.0	0.0	20.0	12.0	0.0	2.0	20.0	0.0	0.0	4.0	
3 Days	6.2	8.3	12.5	8.2	29.0	22.9	0.0	0.0	0.0	0.0	4.0	22.0	0.0	24.0	12.0	4.0	4.0	30.0	0.0	0.0	8.0	
4 Days	12.5	29.2	16.7	8.3	37.5	28.6	2.1	0.0	6.1	4.1	10.6	25.5	4.2	28.7	13.8	4.2	8.0	30.0	10.0	4.0	8.0	
5 Days	14.9	31.9	19.1	12.5	47.0	39.5	4.2	0.0	12.5	6.2	13.0	39.1	8.7	23.9	13.0	4.3	14.9	25.5	14.9	6.0	10.0	
6 Days	20.0	33.3	22.2	20.8	72.9	39.5	4.2	4.2	16.7	12.5	22.2	44.4	11.1	33.3	17.7	6.7	23.9	43.0	17.4	8.0	16.0	

in the acetone extract were the most toxic fractions in the Aadar leaf extracts. They killed 64.4, 29.5, 36.1, 31.91, 29.93 and 24.7 % of the treated beetles six days after treatment, respectively. In the same way, fractions number 5, 6, and 2 in the chloroform extract and fractions number 2, 8 and 4 in the acetone extract were the most effective fractions in Oshar leaf extracts inducing 72.9, 39.5, 33.3, 44.4, 43.0 and 33.3 percent mortality by the sixth day after treatment, respectively.

The previous results indicate that the first fraction in the chloroform Aadar leaf extract and the fifth fraction in the chloroform Oshar leaf extract and the second and eighth fractions in the acetone Oshar leaf extract were the most active. Their induced mortalities ranged between 43 % and 72.9 %. More detailed studies should be done on these plants and their extracts. Belal and El-Kabbany (1993) found that of nine fractions in chloroform extracts from *A. monosperma,* the fractions number 1, 2, 3 and 4 (R_f 0.34, 0.65, 0.94 and 0.25) exhibited considerable insecticidal activity.

REFRENCES

Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18 : 265 – 267.

Belal M. H. and M. S. El-Kabbany (1993): Separation and identification of biological active components of *Artemisia monosperma* against some insects. 5 th. Nat. Conf. of pests & Dis. of Veg. & Fruits in Egypt. Ismalia. 273 - 286.

Belal, M. H.; A. G. Abdel-Rahman; N. M. Ibrahim and E. A. Ali (2001): Observations on repellent effect of some desert plant extracts on the red flour beetle *Tribolium confusum*. Proceeding of the 1st. Congress "Integrated Pest Management" Cairo Univ., Dept. Econom. Entomol. & Pesticides, 22-23 April 2001.

Finney, C. E. (1971): Probit Analysis, A statistical treatment of the sigmoid response curve. 7th Ed. Cambridge Univ. Press, Cambridge, England

Jahan, S.; A. Mannan; A.R. Khan and P. Karmaker (1991): Insecticidal effect of akanada *Calotopis procera* on *Tribolium confusum* Duval (Cleoptera : Tenebionide). Bangladesh J. Zoology. 19(2): 261 - 262.

Mahgoub, M. S. and M. M. Zewer (1989): Studies on the efficancy of oils as protectants against the coepea weevil, *Callosbruchus maculatus* F., and the granaria weevil *Sitophilus granarius*. Proc. 1st. Int. Conf. Econ. Ent. 1: 427 - 438.

Saleh, M. A.; N.A. Ibrahim; M.M. El-Bolok and K.A. Abdel-Salam. (1986): Insecticidal activity of selected Egyptian wild plants. Bull. Agric., Univ. of Cairo, 37(1): 517 – 526.

Su, H. C. F. (1977): Insecticidal properties of black pepper to rice weevils and cowpea weevil. J. Econ. Entomol., 70(1): 19 – 21.

Talukder F. A. and P. E. Howse (1994): Evaluation of *Aphananixis polystachya* as a source of repellents, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* Herbst. J. Stored Prod. Res. 31 (1): 55 - 61.

Xie Y. S. P. G. Fields, and M. B. Isman (1995). Repellency and toxicity of Azadirachtin and neem concentrates to three stored-product beetles. J. Econ. Entomol. 88 (4): 1024 - 1031.