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Toxicological Evaluation of the Desert Plant, *Artemisia monosperma* Delile Extracts and their Isolates on the Two-spotted Spider Mite, *Tetranychus urticae* Koch.

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ABSTRACT

Toxicity effects of four successive extracts from the plant *Artemisia monosperma* Delile (*i.e.* petroleum ether, chloroform, ethyl acetate and ethanol extracts) were evaluated against the two-spotted spider mite, *Tetranychus urticae* Koch. The toxic effects of the crude extracts according to their LC_{50} values of chloroform, ethanol, petroleum ether and ethyl acetate were 0.0360, 0.0414, 0.0471 and 0.0955 g/ml, respectively against eggs of *T. urticae*. On the other hand, LC_{50} values of petroleum ether, chloroform, ethyl acetate and ethanol were 0.008, 0.030, 0.046 and 0.135 g/ml, respectively against females. The toxicity effects of 11 fractions of petroleum ether and chloroform crude extracts of *A. monosperma* were also tested against eggs and females of *T. urticae*. The LC_{50} values of petroleum ether fraction extracts (using hexane/ethyl acetate 90: 10) were 0.000940 and 0.000195 g/ml exhibiting the most toxic effect on both eggs and females, respectively. The LC_{50} values of chloroform fraction extracts (using hexane / ethyl acetate 95:5 and 98: 2) were 0.00219 and 0.00144 g/ml exhibiting the most toxic effect on eggs and females of *T. urticae*, respectively.

Key Words: *Tetranychus urticae*, *Artemisia monosperma*, plant extracts, fractions/isolates and toxicity.

INTRODUCTION

Natural products are well known to have useful biological properties and have been used by man over thousands of years. The use of insecticidal plants is especially prevalent in the developing countries, where plants grown locally are cheaper for subsistence farmers to use than the synthetic chemical. Some pesticidal plants, *e.g. Azaderachta indica, Chrysanthemum cinerariefolium* and *Carum carvi* have been receiving global attention and their secondary metabolites have been formulated as botanical pesticides in plant protection (Varma and Dubey, 1999).

Species of wormwood plants belonging to the genus *Artemisia* (Compositae) are widely used in the world folk medicine. Different extracts and constituents derived from various parts of *Artemisia* spp. showed antimalarial (Acton and Klayman, 1985; Liu *et al.*, 1989; Cubukcu *et al.*, 1990 and Nkunya *et al.*, 1992), antibacterial (Yashpe *et al.* 1987), antioxidative (Ming *et al.* 1996; Kim *et al,* 1997 and 2004) and anti-inflammatory activities (Kim *et al.*, 1997 and Tigno *et al.*, 2000).

The Egyptian sand wormwood, *Artemisia monosperma* Delile is a wild important medicinal herb. Ethanol extract of *A. monosperma* exhibited antimicrobial (Zaki *et al.,* 1984 and Mohamed *et al.*, 2000) and cercaricidal activities (Shabana *et al.*, 1988). Different extracts and essential oil from whole or aerial parts of *A. monosperma* showed insecticidal activity (Saleh, 1984; Hifnawy *et al.*, 1990; Elgamal *et al.*, 1991; Anonymous, 1992; Assar and El-Sobdy, 2003). Adekenov *et al.* (1990) studied the repellent response of chloroform extract from aerial parts of wormwood, *Artemisia saissanika* (Krach) to the two-spotted spider mite, *Tetranychus urticae* Koch.

Constituents of extracts and oils from *Artemisia* spp. were isolated and identified by Acton and Klayman (1985), Ferrolino and Padolino (1985), Hikino (1985), Yashpe *et al.* (1987), Liu *et al.* (1989), Adekenov *et al.* (1990), Cubukcu *et al.* (1990), Rodriguez *et al.* (1990), Nkunya *et al.* (1992), Appendino *et al.* (1993), Ming *et al.* (1996), Misra *et al.* (1996), Kim, *et al.* (1997), Tigno *et al.* (2000), Vernin and Parkanyi (2001), Foglio *et al.* (2002) and Kim *et al* (2004). Phytochemical components of extracts and oils from *A. monosperma* were determined by Khafagy *et al.* (1979), Zaki *et al.* (1984), Hifnawy *et al.* (1990), Elgamal *et al.* (1991) and Mohamed *et al.* (2000). The insecticidal response for crude fractions of chloroform extract from leaves of *Artemisia vulgaris* L. was shown by Ferrolino and Padolino (1985). Chromatographic fractions of crude extract and essential oil from whole plants of *A. monosperma* exhibited insecticidal activity (Saleh, 1984).

The present study was conducted to investigate:

1-Toxic effects of *A. monosperma* crude extracts to eggs and females of *T. urticae*
2-Fractionation of petroleum ether and chloroform crude extracts
3-Toxicity of the petroleum ether and chloroform fractions against eggs and females of *T. urticae*.

MATERIALS AND METHODS

Mite culture

The stock culture of *T. urticae* was maintained on Lima bean, *Phaseolus vulgaris* L. under laboratory conditions (25 ± 2 ºC and 60 ±5 % R.H.).

Plant material

The wild plant, *A. monosperma* was collected from different areas in South Sinai. Samples of the collected plants were left to dry under laboratory conditions. Dried plants were ground using an electric mill, sieved and kept for extraction.

Preparation of the crude extracts

Crude extracts were prepared according to the method described by Su and Horvat (1981) with some modifications. Samples of powdered plant material (200gm each) were successively extracted with organic solvents of increasing polarities, *i.e.* petroleum ether, chloroform, ethyl acetate and ethanol, respectively. Petroleum ether was used at first, where the dried plant materials were soaked in 1000 ml solvent at a rate of 5 ml/gm and kept for 48 h under laboratory conditions. The

mixtures were mechanically shacked for 6 h. The extracts were then filtered over anhydrous sodium sulfate and petroleum ether was evaporated using a rotary evaporator at 40-50 °C until dryness. The resulting crude extract was weighted and kept in a deep freezer until evaluation. The insoluble materials (residual powders) were allowed to dry before reextracted with the next solvent. The same procedure was carried out using each experimental solvent (Soliman 2001).

Fractionation of petroleum ether and chloroform crude extracts

Column was used to separate the isolated fractions of petroleum ether and chloroform crude extracts from *A. monosperma* (El-Naggar and Mosallam 1987). A column of 2.5 cm diameter X 50 cm length, with 20 cm height silica gel and a 2 cm anhydrous sodium sulphate was used for each fraction. The fractions were collected every 10 ml. A sample of petroleum ether and chloroform crude extracts was directly applied on the column top and then eluted with the following solvents: hexane/ethyl acetate 98:2, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60 and 30:70% and each of ethyl acetate and methanol 100%.

The eleven fractions were collected and the solvent was evaporated till dryness and then examined for toxicity against *T. urticae*.

Toxicity to the egg stage

To test the effect of crude extracts and their fractions of *A. monosperma* on the egg stage, twenty females of *T. urticae* were transferred to the lower surface of mulberry leaf discs, *Morus alba* L. and left for 24 h for oviposition and later removed. The accumulated eggs were sprayed with various concentrations of each extract and their fractions using a glass atomizer. Each test contained five concentrations, each with five replicates (20 eggs/replicate). Seven days after treatment, the number of unhatched eggs was counted.

Toxicity to the adult stage:

T. urticae females were sprayed with different concentrations of each extract and their fractions and placed on mulberry leaf discs. Each concentration was replicated five times (100 females/treatment). A control was included in each experiment. Mortality was recorded 48 h. after application, corrected according to Abbott's formula (1925) and submitted to probit analysis using Finney (1971). The toxicity index was determined according to Sun (1950) as the following equation

$$\text{Toxicity index} = \frac{LC_{50} \text{ of the effective insecticide}}{LC_{50} \text{ of the each insecticide used}} \times 100$$

RESULTS AND DISCUSSION

Toxic effects of crude extracts from *A. monosperma*

Data presented in Table (1) show the toxicity levels of crude extracts from *A. monosperma* to eggs and females of *T. urticae*. The toxic effect of tested extracts on eggs could be arranged descendingly according to their LC_{50} values as follows: chloroform, ethanol, petroleum ether and ethyl acetate. The corresponding LC_{50} values were 0.0360, 0.0414, 0.0471 and 0.0955 g/ml, respectively. Chloroform and ethanol extracts were considered the standard extracts in calculating the toxicity index at the LC_{50} and LC_{90} levels, respectively. It is obvious that chloroform extract proved to be the most toxic, while ethyl acetate extract was the least toxic to eggs of *T. urticae*.

The toxic effect of tested extracts on females could be arranged desendingly according to their LC_{50} levels as follow: petroleum ether, chloroform, ethyl acetate and ethanol (Table 1). The corresponding LC_{50} values were 0.008, 0.030, 0.046 and 0.135 g/ml, respectively. Petroleum ether extract was considered the standard extract in calculating the toxicity index at both the LC_{50} and LC_{90} levels. It is clear that petroleum ether extract was the most toxic, whereas ethanol extract was the least toxic to females of *T. urticae*.

Table (1): Toxicity of *A. monosperma* crude extracts against eggs and females of *T. urticae*.

Extract	LC_{50} g/ml	LC_{90} g/ml	Slope	Toxicity index at: LC_{50}	Toxicity index at: LC_{90}
			Eggs		
Petroleum ether	0.0471	0.4866	1.26	76.4330	26.5105
Chloroform	0.0360	0.1641	1.94	100	78.6106
Ethyl acetate	0.0955	1.350	1.11	37.6960	09.5555
Ethanol	0.0414	0.1290	2.59	86.9565	100
			Females		
Petroleum ether	0.008	0.0374	1.96	100	100
Chloroform	0.030	0.0687	3.52	26.6667	54.4396
Ethyl acetate	0.046	0.1742	2.21	17.3913	21.4696
Ethanol	0.135	0.3217	3.41	5.9259	11.6257

The previous data indicate that chloroform and petroleum ether extracts proved to be the most potent on eggs and females of *T. urticae* as LC_{50} values were 0.036 and 0.008 g/ml, respectively.

Data obtained agree with those of Amer *et al.* (1991) who found that chloroform extracted from whole green marine algae, *Codium* spp. was the most efficient against eggs of *T. urticae*. However, Amer *et al.* (1989) reported that petroleum ether extract from seeds of red been vine, *Abrus precatorius* L. was the most toxic to eggs.

Regarding the toxicity of extracts to females of *T. urticae*, similar results were obtained by Schauer and Schmutter (1981) who found that petroleum ether extract from fruits of Labiatae, *Ajuga remota* L. was the most toxic to females of *T. urticae*. Amer *et al.*, (1991) concluded that petroleum ether extract from green marine algae, *Codium* spp. was highly toxic to females of *T. urticae*. Amer and Rasmy (1994) stated that petroleum ether extract from leaves of barnuf, *Conyza discoridis* L., and frenugreek, *Trigonella foenumgraecum* L. was the most potent extract on females. Dimetry *et al.*, (2000) found that petroleum ether extract from rhizomes of *Curcuma longa* L. and herb whole of *Nicandra physaloides* L. was the most potent to females of *T. urticae*. In contrast, Amer *et al.*, (1989) and Dimetry *et al.*, (2000) reported that ethanol extract from seeds of *A. precatorius* and leaves of *Dodonaea viscose* L. respectively was the most toxic to females of *T. urticae*. From the previous data it is clear

that chloroform and petroleum ether extracts of *A. monosperma* exhibited the most toxic effect on both eggs and females of *T. urticae*, respectively. So, the plant was conducted for detailed studies and column studies in crude extracts in the two previous mentioned solvent extracts.

Toxic effect of column fractions of petroleum ether and chloroform crude extracts against eggs and females of *T. urticae*.

The data presented in Table (2) show the LC_{50} values of isolates obtained from column fractions of petroleum ether crude extract. It is clear that the isolate number three (mixture of hexane: ethyl acetate 90:10) was the most toxic on eggs and females of *T. urticae*, respectively. The corresponding values of LC_{50} were 0.000940 and 0.000195 g/ml. The data presented in Table (3) show the LC_{50} values of isolates from chloroform crude extracts. The isolate number two (mixture of hexane: ethyl acetate 95:5%) and the isolate number one (mixture of hexane: ethyl acetate 98:2%) exhibited the most toxic effect to eggs and females of *T. urticae*, as the corresponding values of LC_{50} were 0.00219 and 0.00144g/ml, respectively.

The previous results indicated that the third fraction in the petroleum ether extract was the most effective against both eggs and adults of *T. urticae*. On the other hand, the second and the first fractions of the chloroform extract were the most active on both eggs and adults, respectively. Ferrolino and Padolino (1985) separated different components with insecticidal activities from the chloroform extract of the leaves of *A. vulgaries* using two procedures. Adekenov *et al.* (1990) isolated 5 components of chloroform extract of buds, leaves and twigs of budding zaisan wormwood, *A. saissanica*, with repelling effect to *T. urticae*. Belal and El-Kabbany (1993) found that of nine fractions in chloroform extracts from *A. monosperma*, the fraction number 1, 2, 3 and 4 (R_f 0.034, 0.65, 0.94 and 0.25) exhibited considerable insecticidal activity. Abdel-Rahman *et al.*, (2004) found that the first fraction in the chloroform *A. monosperma* leaf extract and the fifth fraction in the chloroform Oshar, *Calotropis procera* leaf extract and eighth fractions in the acetone Oshar leaf extract exhibited the most insecticidal activity. Tanaka *et al.*, (1985) isolated two active compounds from methanol extract of the leaves of *Skimica repens* which exhibited high toxicity to eggs of *T. urticae*. Dimetry *et al.*, (1990) isolated active compounds from the non-saponified fraction of a crude petroleum ether extract of *Abrus precatorius* seeds which exhibited a significant reduction in fecundity and the viability of resulting eggs. Reichling *et al.* (1991) isolated 3 compounds of acaricidal activity to *T. urticae* from plants of the genus *Pimpinell.*

It can be concluded that chloroform and petroleum ether extracts were the most potent on eggs and females of *T. urticae*. From petroleum ether crude extract and their isolates, the isolate number three was the most toxic on eggs and females, while the isolates number two and one of the chloroform crude extract were the most toxic effect to eggs and females of *T. urticae*, respectively.

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Table (2): Toxicity of petroleum ether extract fraction from *A. monosperma* on eggs and females of *T. urticae*.

No. of isolate	LC_{50} g/ml	LC_{90} g/ml	Slope	Toxicity index at:	
				LC_{50}	LC_{90}
			Eggs		
1	0.01511	0.35931	0.93	6.22	0.72
2	0.00834	0.18974	0.94	11.27	1.36
3	0.000940	0.00258	2.91	100	100
4	0.07284	0.74386	1.27	1.29	0.35
5	0.00256	0.01436	1.71	36.72	17.97
6	0.03535	0.30289	1.37	2.66	0.85
7	0.00490	0.02363	1.87	19.18	10.92
8	0.0010	0.00298	2.71	94.0	86.58
9	0.00979	0.02846	2.76	9.60	9.07
10	0.00184	0.00465	3.19	51.09	55.48
11	0.00730	0.02366	2.51	12.88	10.90
			Females		
1	0.00252	0.00527	3.99	7.74	14.61
2	0.00726	0.01622	3.78	2.69	4.75
3	0.000195	0.00122	1.61	100	63.11
4	0.00610	0.02213	2.29	3.20	3.48
5	0.000725	0.00217	2.69	26.90	35.48
6	0.00348	0.00958	2.91	5.60	8.04
7	0.00036	0.00083	3.53	54.17	92.77
8	0.00020	0.00077	2.19	97.50	100
9	0.00149	0.00343	3.54	13.09	22.45
10	0.00052	0.00134	3.11	37.5	57.46
11	0.0016	0.0037	3.58	12.19	20.81

Table (3): Toxicity of chloroform extract fraction from *A. monosperma* on eggs and females of *T. urticae*.

No. of isolate	LC_{50} g/ml	LC_{90} g/ml	Slope	Toxicity index at:	
				LC_{50}	LC_{90}
			Eggs		
1	0.00833	0.18739	0.95	26.29	3.68
2	0.00219	0.00690	2.57	100	100
3	0.00367	0.01122	2.64	59.67	61.50
4	0.02510	0.14048	1.71	8.73	4.91
5	0.00863	0.02469	2.80	25.38	27.95
6	0.02309	0.14631	1.60	9.48	4.72
7	0.00362	0.03890	1.24	60.50	17.74
8	0.01383	0.03332	3.35	15.84	20.71
9	0.01071	0.03737	2.36	20.45	18.46
10	0.02015	0.04895	3.32	10.87	14.50
11	0.03076	0.08526	2.89	7.12	8.09
			Females		
1	0.00144	0.00918	1.59	100	83.99
2	0.00197	0.00771	2.16	73.10	100
3	0.00285	0.01054	2.25	50.53	73.15
4	0.01039	0.01653	6.35	13.86	46.64
5	0.00519	0.01279	3.27	27.75	60.28
6	0.00805	0.03870	1.88	17.89	19.92
7	0.00409	0.01990	1.86	35.21	38.74
8	0.00970	0.02945	2.65	14.85	26.18
9	0.01716	0.07884	1.93	8.39	9.78
10	0.02591	0.06307	3.31	5.56	12.22
11	0.02076	0.06292	2.66	6.94	12.25

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