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Repellent, Antifeedent and Toxic Effects of Certain Plant Extracts on Cotton Leafworm, *Spodoptera littoralis* Boisd.

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Two plants, i.e. *Anthemis melanopodia* and *Artemesia monosperma* were tested against the 4th instars larvae of S*podoptera littoralis* (Boisd.) under laboratory conditions. Four crude extracts (Hexane, Chloroform, Ethyl acetate and Ethanol) from each of the two plants used were evaluated by two different technique, i.e. residual films and dipping. Results indicated different percentage of mortality in larval depending on the efficiency of organic solvent extraction of the active materials from tested plants and method of application. The residual film assay was higher effective technique than the leaf-dipping. Considering the effect of four crude extracts on egg laid by the females resulting from survived larvae. Data revealed that all the tested extracts caused high reduction in egg laying rate compared with the untreated control. As for the effect of tested extracts on hatchability, data indicated that the four extracts reduced egg hatchability percentages and this effect was clearly reflected in the obtained sterility percentages. The effects were more pronounced in residual film than leaf-dipping technique particularly with the higher concentration used.

Keywords: repellent, antifeedent, plant extracts and *Spodoptera littoralis* Boisd.

During the last decade the strategy of the Ministry of Agriculture is to minimize the usage of chemical pesticides in pest control, for reason has created important problems such as the environmental pollution and pest resistance. Therefore, it has become necessity to looking for new insecticides with different mode of action and improved effectiveness and safety. Botanical extractive, as non toxic bio-rational substances are capable of bringing soil-plant pest system into balance. This sequence leads to good crop health. Recently, attention has been given to the isolation and identification from plant source for various botanical compounds, possessing insecticidal properties (attracting, repelling, feeding, deterring, growth inhibiting and reproduction sterilizing effects). Plants attracted to the attention of entomologists because most of botanical extracts are not toxic to warm-blooded animals and show no/or moderate side-effect on natural enemies (Schmulterer, 1990).

Several attempts have been made to monitor the insecticidal activity and other insecticides effects in extracts of different plant species against various insects (Ahmed, 1983; Barakat et al., 1985; El-Sisi and Badr, 1995; Farrag, 2000; Soliman et al., 2003).

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The plant crude extracts were utilized in pest control (Blask and Hertel, 2001; Soliman et al., 2003). Intensive work has been carried out to develop insect feeding deterrent or botanical insecticides known to occur naturally in many plants grown as weeds or crops (Regnoult and Hamraoui 1995; El-Baroty and Abdel-Lattif, 1997).

The cotton leaf worm, *S. littoralis* (Boisd.), is important polyphagous pest of cultivated crops primarily in tropical and subtropical regions (Brown and Dewhurt, 1975). Synthetic pesticides have been used for many years to control this pest. Due to high cost of protecting crops from this pest with chemical pesticides and the increasing resistance and resurgence to many chemical pesticides (Armes et al., 1992; Brewer and Trumble, 1994) there is growing interest in the use of biological products such as bacterial and viral-based insecticides, and botanical pesticides (Rao et al., 1990; Aggarwal et al., 2006). These groups have different mode of action from conventional products (Thomson et al., 1999) and their properties, may differ considerably from the conventional chemicals with which growers are familiar. It is therefore important to generate information on the likely differences in the performance of these products to educate growers and facilitate adoption. Recently, attempts have been made to replace conventional pesticides by natural ones, particularly plant-derived chemicals. The objective of this study was to evaluate biological and botanical pesticides effect of the four crude extracts of two plants, i.e. *Anthemis melanpodia* and *Artemisia monosperma* on the 4th instars larva of *S. littoralis* under laboratory conditions.

Materials and Methods

Plant materials

The plants were used in the present study are *Anthemis melanpodia* and *Artemisia monosperma* were collected from different areas in middle and south Sinai and also, from some parts of west desert from March to April every year. The whole plants were air dried under natural laboratory conditions for one week. Dried plants were ground using an electric mill sieved and kept for extraction.

Preparation of the crude extract

Plant extracts were prepared according to the method adopted by Freedman et al. (1979) and Su and Horvat (1981) with some modifications. Samples (1 kg) of each plant materials were soaked in 3 liters of chloroform : methanol mixture (1:1 w/v) and kept for 24 hours in brown color bottles provided with tight stoppers with continuous shaking for overnight. The solvent was separated from the insoluble plant materials and the later was re-extracted with another 2 liters from the same mixtures for 8 hours and the solvent was separated. The combined extracts (5 liters) were filtered over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure using a Rotary evaporator. The remaining residue was subjected to successive extraction with four different solvents, firstly with *n*-hexane (250 ml) then with chloroform (250 ml), ethyl acetate (250 ml) and ethanol (250

ml). The crude extract of each solvent was filtered over anhydrous sodium sulphate and the solvent was evaporated using a Rotary evaporator at 40–50 °C to dryness. The resulting crude extract of each solvent was weighted and kept in the deep freezer until evaluation.

Test insect

A susceptible laboratory strain of cotton leaf worm, *S. littoralis* was reared for several years in the laboratory on castor bean leaves away from any residual contamination at 27 ± 2 °C and 65 ± 5% R.H. (El-Defrawi et al., 1964).

Bioassay methods

LEAF DIPPING TECHNIQUE

The tests were carried out using newly 4th instars larvae. Different concentrations of the crude extract were prepared using acetone. Castor bean leaves discs (7 cm diameters) were dipped in the different concentrations for five second and left to dry. The treated discs were transferred to Petri dishes contain ten larvae of *S. littoralis*. Similar numbers of larvae were fed on castor bean discs dipped in acetone and served as control. Four replicates for each concentration were used. Live larvae were maintained in Petri dishes under laboratory conditions and supplied with untreated castor bean leaves after 24 hours. The insect were observed and examined daily. Mortality percentages were recorded daily until pupation. The percent of pupation was counted. The pupae were kept in glass container until adult emergence. Percentages of adult emergence were recorded.

The residual film technique

This technique is similar to that used by (Brady, 1966) on the house fly with some modifications. The crude extracts were dissolved in acetone and prepared several concentrations from each extract, and one ml of each was spread evenly on the inner surface of Petri dish (9 cm diameter) by gently moving the dish. The solvent was evaporated leaving a thin film of extract on the surface of the Petri dish after then; ten larvae were transferred and exposure to extract for 6 hours. Each concentration was replicated four times. After that, the larvae were transferred into clean glass containers and fed on castor bean leaves. Mortality counts were recorded daily until pupation and adult emergence. Corrected mortality counts according to Abbott's formula (1925) and was statistically analyzed by Finney (1971). The possible biological aspects were studied. The equations were used to calculate the fecundity and egg-hatchability as follow.

$$\% \text{ Fecundity} = \frac{\text{No. of deposite eggs (Check)}}{\text{Check}}$$

$$\% \text{ Hatchability} = \frac{\text{No. of hatched eggs}}{\text{No. of deposited eggs}} \times 100$$

The percent sterility of the mated females was calculated according to the equation of Toppozada et al. (1966):

$$\% \text{ of sterility} = \left[100 - \frac{(a \times b)}{(A \times B)} \right] \times 100$$

Where:
a = Total No. of eggs laid/female in treatment
b = % of egg-hatch in treatment
A = Total No. of deposited eggs in control
B = % of egg-hatch in control

Results and Discussions

Table 1 represented the percentage of mortality in larval, pupae stages and percent of adult emergence resulting from feeding the 4th instars larvae of *S. littoralis* (Boisd.) on castor bean leaves pretreated with the tested plant extracts *A. melanpodia* which extracted as two levels of concentrations (the concentration and its twice) for each tested extract by leaf-dipping technique. Results indicated different percentage of mortality in larval depending on the efficiency of solvent extracts of the active materials from tested plants. The percentages of died larvae before the pre-pupal stage due to the treatment with the hexane crude extract for concentrations 140 and 280 mg/cm^2 were 50 and 70%; 50 – 60% with the concentrations 42.5 and 85 mg/cm^2 for chloroform extract, 30 and 55% with the concentrations 16.7 and 33.5 mg/cm^2 of ethyl acetate extract and 50 and 65% with the concentrations 38 and 76 mg/cm^2 from the ethanol extract, respectively. As for treating the 4th larval instars by residual film technique, the respective values were 72.5 and 97.5% hexane; 70 and 77% chloroform; 65 and 77.5% ethyl acetate and 90 and 100% ethanol, respectively *(Table 2).*

The corresponding figures with the crude extracts of *A. monosperma* on the 4th instar larvae treated by leaf-dipping technique were illustrated in *(Table 3).* The mortality percentages on larval instars were 60 and 75% for the hexane concentrations 52.5 and 105; 65 and 75% mortality for the chloroform concentrations 67.5 and 135; 20 and 65% mortality for the ethyl acetate concentrations 80 and 160; 60 and 85% mortality for the ethanol concentrations 92.5 and 185 mg/cm^2, respectively.

Sadek (2003) found that the leaf extract of *Adhatoda vasica* exhibited strong antifeedant and toxic activity against the larvae of *S. littoralis* when applied either on leaf discs or incorporated into artificial diet.

Considering the effect of treating technique used, the respective values in larval stages for residual film technique with the same concentrations were (70 and 92.5%); (72.5 and 75%); (55 and 70%) and (75 and 100%) mortality, respectively *(Table 4).* In all the tested plant extracts used, most of the mortality in larvae could be described to the molt-disturbing effect. A similar reduction in normal pupation was also produced. The percentages of mortality obtained from the different concentrations used were clearly reflected on

Table 1

Insecticidal and productive potential of *Anthims melanpodia* extracts on the 4th instar larvae of *S. littoralis* by leaf dipping technique

Solvent extract	Conc. (mg/cm^2)	% Mortality in larvae (days)						% Pupation	% Mortality in pupal stage	% Total mortality	Mean No. of eggs/female	% Hatchability	% Sterility
		1	2	4	7	9	11						
Hexane	140	5	0	10	35	45	50	75	0	60	1249	0	55
	280	35	0	50	55	60	70	70	10	70	0	0	100
Chloroform	42.5	5	0	10	20	30	50	66.7	0	50	1093	29.7	73
	85	10	0	13	40	55	60	50	0	60	100	0	100
Ethyl acetate	16.75	0	0	10	20	25	30	66.7	0	30	763	10.6	93.4
	33.5	5	0	25	30	35	55	61.5	0	55	268	0	100
Ethanol	38	10	0	10	25	25	50	66	0	50	628	31.85	84
	76	10	0	25	50	55	65	46.7	0	65	0	0	100
Control		0	0	0	0	0	0	98	0	10	1250	97	0

Table 2

Insecticidal and productive potential of *Anthims melanpodia* extracts on the 4th instar larvae of *S. littoralis* by residual film technique

Solvent extract	Conc. (mg/cm^2)	% Mortality in larvae (days)						% Pupation	% Mortality in pupal stage	% Total mortality	Mean No. of eggs/female	% Hatchability	% Sterility
		1	2	4	7	9	11						
Hexane	140	7.5	0	0	45	50	72.5	55	0	72.5	971	0	100
	280	90	0	0	0	95	97.5	0	0	97.5	0	0	100
Chloroform	42.5	10	0	0	0	0	70	62.5	0	70	0	0	100
	85	22.5	0	0	45	60	77.5	18	0	77.5	0	0	100
Ethyl acetate	16.75	10	0	0	27.5	50	65	56.3	0	65.5	604	27.9	82.7
	33.5	27.5	0	0	45.5	60	77.5	55	0	77.5	0	0	100
Ethanol	38	72.5	0	0	82.5	87.5	90	56	0	90	0	0	100
	76	100	0	0	0	0	0	0	0	100	0	0	100
Control		0	0	0	10	10	10	95	0	10	1290	98	0

Table 3

Insecticidal and productive potential of *S. littoralis* moths emerged from previously treated 4th instar larvae with *Artemisia monosperma* extracts by leaf dipping technique

Solvent extract	Conc. (mg/cm^2)	% Mortality in larvae (days)						% Pupation	% Mortality in pupal stage	% Total mortality	Mean No. of eggs/female	% Hatchability	% Sterility
		1	2	4	7	9	11						
Hexane	52.5	15	0	25	30	30	60	40	0	60	430	11	96
	105	20	0	50	50	55	75	21.4	0	75	0	0	100
Chloroform	67.5	30	0	40	45	45	0	75	0	65	621	0	100
	135	50	0	55	65	65	0	54.5	5	80	0	0	100
Ethyl acetate	80	5	0	10	15	35	20	75	0	0	624	0	92
	160	10	0	20	30	40	65	46.2	0	0	0	0	100
Ethanol	92.5	10	0	25	35	55	60	70	0	60	0	0	100
	185	45	0	60	65	75	85	40	5	90	882	0	100
Control		0	0	0	0	0	7.5	98	0	7.5	1250	95	0

Table 4

Insecticidal and productive potential of *Anthims melanpodia* extracts on the 4th instar larvae of S. littoralis by residual film technique

Solvent extract	Conc. (mg/cm^2)	% Mortality in larvae (days)						% Pupation	% Mortality in pupal stage	% Total mortality	Mean No. of eggs/female	% Hatchability	% Sterility
		1	2	4	7	9	11						
Hexane	52.5	45	0	0	60	62.5	70	42.5	0	70	0	0	100
	105	72.5	0	0	82.5	87.5	92.5	40	0	92.5	0	0	100
Chloroform	67.5	32.5	0	0	47.5	62.5	72.5	44	0	72.5	0	0	100
	135	42.5	0	0	65	65	75	13	0	75	0	0	100
Ethyl acetate	80	7.5	0	0	32	52	55	58.8	0	55	253	0	100
	160	22.5	0	0	45	57	70	42.5	0	70	241	0	100
Ethanol	92.5	57	0	0	60	70	75	41.6	0	75	0	0	100
	185	100	0	0	0	100	100	0	0	100	0	0	100
Control	0	0	0	10	10	10	10	95	0	10	1124	98	0

the adult emergence. It is obvious that the increase in the tested plant extract concentrations was proportional with the reduction in pupation percentages and adult emergence; similar results was obtained by Shaaban and Mostafa (1993) using Neemark plant extract on the 4th instars larvae of *S. littoralis*. They indicated that the compound was highly active against the instar larvae and the pattern of inhibition in normal pupation and adult emergence produced by different concentrations was clearly reflected in percentages of total mortality.

According to the total mortality percentages obtained on the four crude extracts of two wild plants used results indicated that the residual film method was the higher effective technique than leaf dipping. These results indicated that the method of application plays an important role in the comparative toxicity of any chemicals against the cotton leafworm *S. littoralis* larvae. Different application methods of contact and stomach toxicity must be taken on consideration because some compounds have same toxic action by stomach poison and vice versa are both of two reactions. The differences in the results obtained with the two methods used may be due to the route of entry in each method.

The effect of the crude extracts (hexane, chloroform, ethyl acetate and ethanol) of *A. melanpodia* against the 4th instar larvae of *S. littoralis* was illustrated in *Table 5*. Ethanol crude extract was the most effective against 4th instar larvae, LC_{50} value was 30.55 mg/cm^2. Also, ethyl acetate crude extract gave high potent effect with LC_{50} values 36.6 mg/cm^2, where *A. melanpodia* hexane was more toxic than chloroform extract with LC_{50} values 82.10 and 178.21 mg/cm^2, and the toxicity index were 100, 83, 37 and 17%, respectively.

Table 5

Toxic effects of plant crude extracts, *Anthims melanopodia* and *Artemisia monosperma* against 4th instar larvae of *S. littoralis* by residual film technique

Crude type	LC_{50} mg/cm^2	LC_{90} mg/cm^2	Slope	Toxicity index at LC_{50}	Relative toxicity at LC_{50}
Anthims melanopodia					
Hexane	82.10	385.69	1.90	37.0	2.17
Chloroform	178.21	603.12	1.11	17.0	1.00
Ethyl acetate	36.60	71.28	4.40	83.4	4.87
Ethanol	30.55	57.03	4.72	100	5.80

Crude type	LC_{50} mg/cm^2	LC_{90} mg/cm^2	Slope	Toxicity index at LC_{50}	Relative toxicity at LC_{50}
Artemisia monosperma					
Hexane	29.28	133.6	1.97	100	7.21
Chloroform	92.29	400.97	1.22	31.70	2.29
Ethyl acetate	211.30	362.78	5.00	13.68	1.00
Ethanol	63.65	273.68	2.00	46.00	3.32

As for the crude extracts of *A. monosperma* according to the LC_{50} and LC_{90} and toxicity index values, the extracts can be arranged as descending order, where hexane extract was the most effective extract in this respect followed by ethanol extract, chloroform extract and ethyl acetate extract. The LC_{50} values were 29.3, 63.5, 92.3 and 211.3 mg/cm^2, the toxicity index were 100, 46.0, 31.7 and 13.9%. These results revealed that solvent type used in extraction play an important role in the efficiency of the plant extract. Das et al. (2000) tested a solvent based neem seed kernel extract (1500 ppm) against *H. armigera* in pigeon pea and observed the best results in the first and second instars, respectively.

Considering the effect of the four crude extractions for each of the two plants on egg deposition, data revealed that all the tested extraction caused high reduction in egg laying rate compared with untreated control. Mogahed et al. (1997) found that both alcoholic extracts of c*allistemon lancesiatus* (leaves and stems) and the flavenoid groups isolated from this extract caused clear reduction in the rate of egg laying per female of *S. littoralis*.

As the effect of the crude extracts on eggs hatchability, results indicated that the crude extracts reduced eggs hatchability percentages. The effect was more pronounced in residual film than leaf dipping technique particularly with the higher concentration. These results are in agreement with Abd El-Aziz and Ezz El-Din (2007) who observed the lowest number of eggs of *S. littoralis* and did not hatch, as result treated with *Anabasis setifera* extract.

Soliman (2006) tested the effects of *Artemisia herba-alba* (Asso) and *Artemisia monosperma* (Delile) essential oils against *Bemisia tabaci* (Gennadius), *Aphis gossypii* (Glover) and *Thrips tabaci* (Lindman) under laboratory and greenhouse conditions. Laboratory results showed that the LC_{50} of *A. herba-alba* and *A. monosperma* were 0.042, 0.186% for eggs and 0.074, 0.075% for immature stages of *B. tabaci*. Also, both oils gave a high toxicity on *A. gossypii* with LC_{50} 0.023 and 0.085%. *Artemisia herba-alba* and *A. monosperma* were more toxic on *T. tabaci* and *A. gossypii* than *B. tabaci* in the laboratory test. In contrast *T. tabaci* was sensitive for both oils (LC_{50} 0.011 and 0.038%). These oils were efficient for controlling tested insects on cucumber plants at greenhouses. This treatment caused 85.41, 83.57% reduction in the population of *B. tabaci*, 90.44, 88.00% for *Aphis gossypii* and 87.45, 84.45% for *T. tabaci*. Chemical analysis of *A. herba-alba* and *A. monosperma* oils detected the presence of hydrocarbon terpenes, oxygenated terpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes represented about 16.38%, 58.91%, 21.61%, 2.74% and 21.53%, 57.17%, 19.32%, 1.70%, of the oil content, respectively. Moriarty (1969) mentioned that the sub-lethal effects of insecticides can influence on the reproductive potential by increasing or decreasing the number of eggs produced or fertility could be caused either directly by inhibition or distortion of ovary development or indirectly by reduced feeding. This hypothesis may explain most of the obtained results.

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