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Insecticidal activity of *Francoeria crispa* (Forssk.) extract on the cotton leafworm, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The effect of different concentrations of *Francoeria crispa* (Fam.: Compositae) on the 2nd and 4th instar larvae of *Spodoptera littoralis* was studied in the laboratory. The crude extract was also identified using GC-MS. The study revealed that the ethyl acetate extract of *F. crispa* was highly toxic against the 2nd and 4th instar larvae of *S. littoralis*. The 2nd instar larvae were more susceptible (LC_{50} = 2.41 mg / ml) than the 4th instar larvae (LC_{50} = 9.73 mg / ml). Increasing the concentration caused an increase in mortalities among larva of both instars. The latent biological effect of the tested extract exhibited larval mortality and deformation in both pupae and adult stages. Results also showed that the crude extract was mainly characterized by high concentration of total terpene compounds (89.778 %).

INTRODUCTION

The search for natural products for crop protection serves the main objectives of the development of new pesticides and identification of new lead structures for chemical synthesis (Pillmoor *et al.*, 1993)

One source of potential new pesticides is natural products produced by plants. Not only might certain natural products be a source of new pesticides, but also botanical derivatives may be more environmentally benign than synthetic chemicals. Plants can produce a diverse range of secondary metabolites such as terpenoids (mono-di and sesqui-), alkaloids, polyacetylenes, flavonoids, fatty acids, fatty acids esters and sugars (Benner, 1993; Rice and Coats, 1994; Barakat, 2001 and Soliman, 2001).

Several plant extracts and/or their isolated active components were effective as potential acute or chronic insecticides, insect growth regulators,

or antifeedants against a variety of insect species (Sharma and Saxena, 1974; Beckage *et al.*, 1988; Jilani and Saxena, 1990; Watanable *et al.*, 1993; Shapiro *et al.*, 1994 and Blaske and Hertel, 2001)

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is a polyphagus insect, attacking a large number of host plants. The larval stage is the major enemy of several field crops, vegetables and deciduous fruits. Synthetic pesticides have been used for many years to control this pest. Recently, attempts have been made to replace conventional pesticides by natural ones, particularly plant-derived chemicals.

Francoeria crispa (Forssk.) (Family: Compositae) is widely grown plant in south and middle Sinai. Perennial bushy desert plant, often growing in cushion-shape, more branched near the lower part of the plant. The plant is used in folk medicine for various purposes.

The present study aims to test the insecticidal activity of the crude extract of Al-Kysoom plant, *Francoeria crispa*, on the larvae of cotton leafworm, *S. littoralis*. Identification and determination of the crude extract constituents was also investigated.

MATERIALS AND METHODS

1- Plant materials: *Francoeria crispa* (Forssk) (Fam: Compositae) was collected from different areas in Middle and South Sinai. Plant was air dried under natural laboratory conditions for one week. Dried plants were ground using an electric mill, sieved and kept for extraction.

2- Preparation of the crude extract: Plant extract was prepared according to the method adopted by Su and Horvat (1981) with some modifications. Among of 200 gm of powdered plant materials was extracted with organic solvent. The dried plant material was soaked in 1000 ml ethyl acetate at the rate of 5ml/gm and kept for 48 hrs under laboratory conditions. The mixture was mechanically shaken for 8 hrs. The extract was then filtered over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure using a rotary evaporator at 40 – 50 °C to dryness. The resulting crude extract was weighed and kept in the deep freezer until evaluation.

3- GC–MS analysis of the crude extract: The obtained crude extract was analyzed using GC-MS apparatus. GC-MS Finnigan mat SSQ 7000 Digital DEC 3000. Work station: Digital DEC 3000. Ionization mode Eleven 70.Column: DB-5 capillary column 30 m length, 0.32 mm i.d and 0.25 µm thickness. Carrier gas: Helium at 13 psi. Temperature-programming: initial column temp. was set at 50 °C for 3 min then the temp. increased at the rate of 7 °C / min to reach 250 °C, and hold for 10 min at 250 °C. Injector temperature was 200 °C and the injected volume was 1 µL. Transition-line and ion source temperatures were 250 °C and 150 °C, respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from *m/z* 40 to *m/z* 350. Ionization energy was set at 70 eV. Identification was based on the comparison with the MS computer library (NIST-Software Package, Finnigan) and on the respective retention indices. The separated components were identified by matching them with the National Institute of Standards and Technology (NIST) mass spectral library data with those of the published data by Adams (1995).

4- Test insect: A susceptible laboratory strain of cotton leafworm, *S. littoralis* larva were obtained from the Department of Pests and Plant Protection, National Research Centre, Egypt. The laboratory strain was reared for several years on castor bean leaves at 27±2°C and 65±5 % R.H. (El-Defrawi *et al.*, 1964).

5-Testing procedure: The tests were carried out using one-day-old second and fourth instar larvae. Different concentrations of the crude extract were prepared by dissolving a known weight (gm) in 1 ml ethyl acetate and five drops of the emulsifier Triton-X 100 were added, then completed to 100 ml water to obtain the stock emulsion solution. A series of dilutions were prepared using water. Castor bean leaves discs (7 cm in diameter) were dipped in the different concentrations for five seconds and left to dry. The treated discs were transferred to Petri dish containing ten (2^{nd} or 4^{th} instars) larvae of *S. littoralis*. Similar numbers of larvae were fed on castor bean discs dipped in water containing Triton-X 100 and served as control. Five replicates for each concentration were used. Mortality percentages were recorded after 48 hr. and corrected using Abbott's formula (1925). LC_{50}, LC_{90} and slope values were calculated according to Finney (1952). The percent of pupation was counted. Alive larva were maintained in Petri dishes under laboratory conditions and supplied with untreated castor leaves Mortality counts was recorded daily until pupation. The emergent

pupa was kept in Petri dishes until adult emergence. Percentage of pupation and adult emergency was calculated.

RESULTS AND DISCUSSION

1- Chemical constituents of the *F. crispa* crude extract: GC-MS analysis of the ethyl acetate extract of *F.crispa* is presented in Table (1) and Fig. (1). Forty-six compounds (comprising 96.517 % of the crude extract) were positively identified. The analysis showed that crude extract was mainly characterized by high concentration of total terpene compounds (89.778 %). Monoterpens accounted for 36.622 % from the total terpenes e.g. β-pinene (3.32 %), menthane (2.08 %), Pinene oxide (3.156 %), Thujene (3.44 %), Champhor (1.275 %), 2-cyclohexen-1-one, methyl-5-(3,4,4-trimethyl ethyl) (2.324 %) and Thujene-2-ol, stereoisomer (0.998 %). The total sesquiterpenes was found to be 36.622 % including α – Cubebene (0.254 %), Copaene (0.522 %), Lavandulyl isobutyrate (1.312 %), β-Gurjunene (1.126 %), (-)-Spathulenol (1.299 and Platambin (2.465 %).

Two fatty acids amounted for 3.364 % of the crude extract, which were identified as hexadecanoic acid (1.783 %) and octadecanoic acid (1.581 %). The percentage of fatty acid ethylester was found to be 1.79 % as hexadecanoic acid, ethylester only. The crude extract contains also 31.677 % hydrocarbons, the main of which is retinol (8.14 %), 4, 14-retro-retinol (5.614 %) and retinal, 9-cis (3.576 %).

Alejandro *et al.*, (1999) demonstrated that hexane extract of aerial part of *Santolina rosmarinifolia* subsp. *canescens* (Fam: Compositae) afforded eight new sesquiterpenes in addition to known compounds.

2- Effect of *F. crispa* extract on the 2nd and 4th instar larvae of *S. littoralis*: Data in Table (2) showed the mortality percentages of the second and fourth larval instars of *S. littoralis* at sequence periods after application with *F. crispa* extract.

The results exhibited that the second instar larvae were more susceptible than the fourth instar larvae at all tested concentrations. The percent mortality of larvae increased with increasing concentration of plant extracts (Table 2)

Table (1) Chemical constituents and their relative abundance (%) of *F. crispa* crude extract

Peak No.	Constituent	%	Peak No.	Constituent	%
1	Hexen-2-one	0.137	24	Copaene Ylangene	1.518
2	2,3,3-Trimethylbutanol	1.453	25	Isomenthyl lactate	0.417
3	β-Pinene	3.316	26	Cuparene	1.659
4	Menthane	2.077	27	α- farnesene	1.271
5	Phellandrene	1.073	28	Cis-calamenene	1.686
6	Santolina alcohol	1.188	29	Agarospirol	2.135
7	Artemisia ketone	0.561	30	(-)-Spathulenol	1.299
8	Pinene oxide	3.159	31	Patchuli alcohol	2.290
9	Sabinene hydrate	0.253	32	Platambin	2.465
10	Thujene	3.439	33	Cedranol (5-Neo-)	0.168
11	Fanchol (exo-)	1.276	34	Guaiol acetate	2.049
12	Thujenol	3.688	35	Cedr-8-(1S)en-9-alpha-ol	0.542
13	Terpin-1-ol	5.134	36	Bisabolol oxide A	0.203
14	Camphor	1.275	37	β-Bisabolen-12-ol	0.635
15	Bicyclo[3.1.o]hexan-3-one,4 -methyl 1-1-(1-methylethyl).	3.771	38	Hexadecanoic acid,	1.783
16	Thujen-2-ol, stereoisomer	1.846	39	Hexadecanoic acid , ethyl ester	1.785
17	3,3,6-Trimethyl-1,5-heptadien -4-ol	1.172	40	Phytol	2.763
18	2-Cyclohexen-1-one, 6 – OH – 2 –methyl – 5 - (T methylethyl)	2.324	41	Thunbergol	8.952
19	Thujen-2-ol, stereoisomer	0.998	42	Octadecanoic acid	1.581
20	α-Cubebene	0.254	43	4,14 -Retro-retinol	5.614
21	Copaene	0.522	44	Retinol acetate	2.632
22	Lavandulyl isobutyrate	1.312	45	Retinol	8.14
23	β-Gurjunene	1.126	46	Retinal, 9-cis	3.576

The LC_{50} and LC_{90} values were presented in Table (3). Data obtained indicated that the 2nd instar larvae were more susceptible than the 4th instar larvae of *S. littoralis*. The corresponding LC_{50} values were 2.41 and 9.73 mg/ml, respectively While the LC_{90} values were 12.10 and 43.64 mg/ml The data are in agreement with that obtained by Badawy (2000) who found that the phytochemical compounds, neemix and nospod were more effective

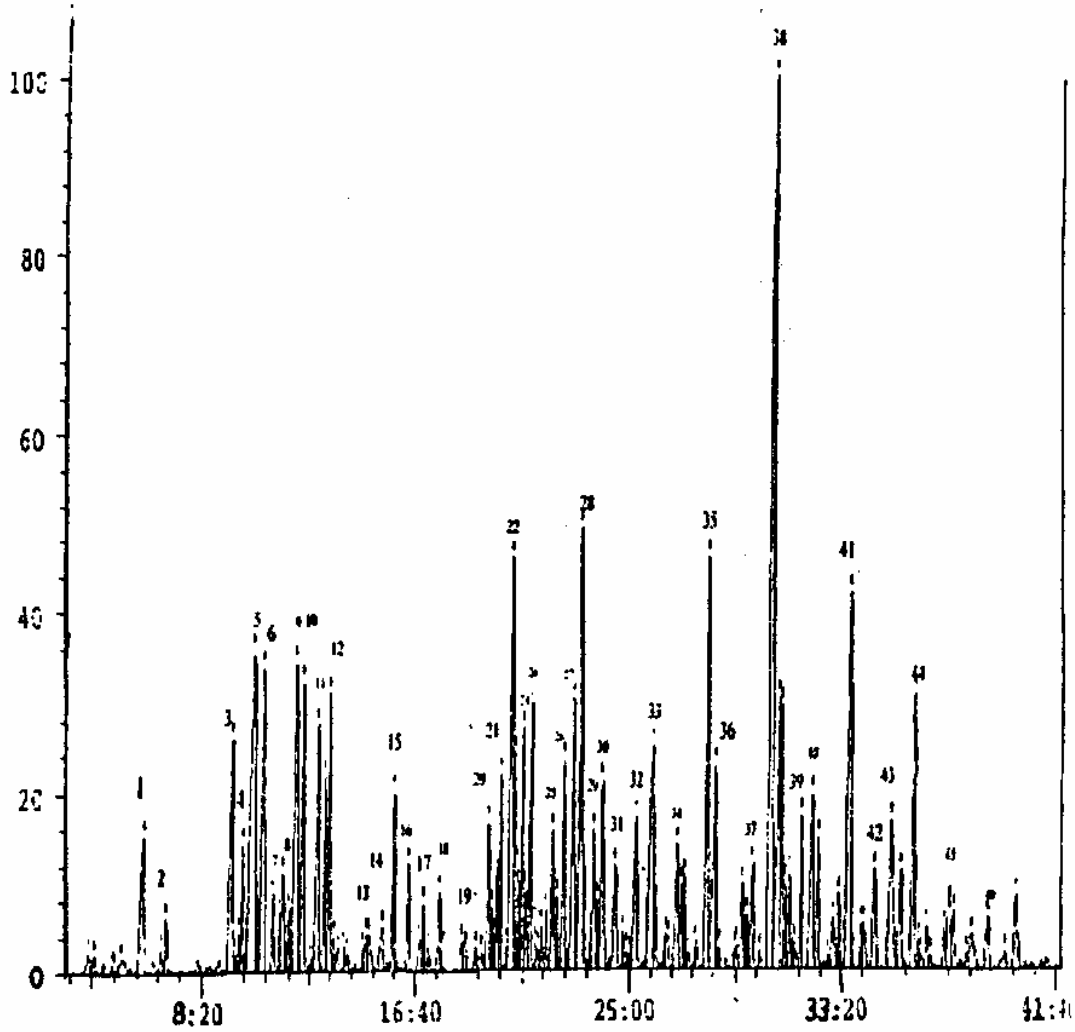

Fig (1): GC-Chromatogram of ethyl acetate crude extract of *Francoeria crispa* plant.

against the second than the fourth larval instar of *S. littoralis*. Mogahed *et al.* (1997) showed that the third and fourth instar larvae were less susceptible to *Callistemon lanceolatus* leaves, Schinus leaves and Schinus fruit extracts.

Table (2): Percent mortality and latent effects of *F. crispa* on the 2nd and 4th instar larvae of *S. littoralis*.

Conc. mg/ml	Accumulated Mortality percentage after application (days)				Latent effect			
	2	3	4	7	Pupation %	Adult Emergence %	Malformed %	
							Pupa	Adult
Second instar larvae								
22.25	92.31	95.54	97.62	100.00	Zero	-	-	-
11.25	88.24	91.16	95.69	100.00	Zero	-	-	-
7.813	70.27	82.91	92.28	97.78	2.22	Zero	2.22	-
1.391	37.74	59.16	71.78	84.97	15.03	9.34	5.69	9.34
0.445	17.65	28.49	60.87	65.97	34.03	20.49	13.54	16.45
Fourth instar larvae								
44.5	83.26	92.68	97.67	100.00	Zero	-	-	-
22.25	72.58	81.58	91.18	98.04	1.96	Zero	1.96	-
11.13	54.29	67.86	84.21	94.34	5.66	Zero	5.66	-
5.56	29.30	35.29	57.90	70.46	29.54	10.43	19.02	10.43
0.89	11.50	16.22	35.30	58.50	41.50	18.36	23.14	11.64

3-Latent biological effects: Results in Table (2) and Fig. (2) showed toxic effect on the developmental stages of *S. littoralis* at low concentrations of *F.crispa* extract. The percentages of adult emergence were less than that in the pupal stage. The adult emergence percentages were 9.34 and 20.49 % following plant extract treatment to the second instar larvae. However, they were 10.43 and 18.36 % following treatment to the fourth instar larvae.

The malformed pupae and adults accounted for 13.54 and 16.45 % following treatment with 0.445 mg/ml of the plant extract to the second instar larvae. However, they were 23.14 and 11.64 % following treatment with 0.89 mg/ml of the plant extract to the fourth instar larvae.

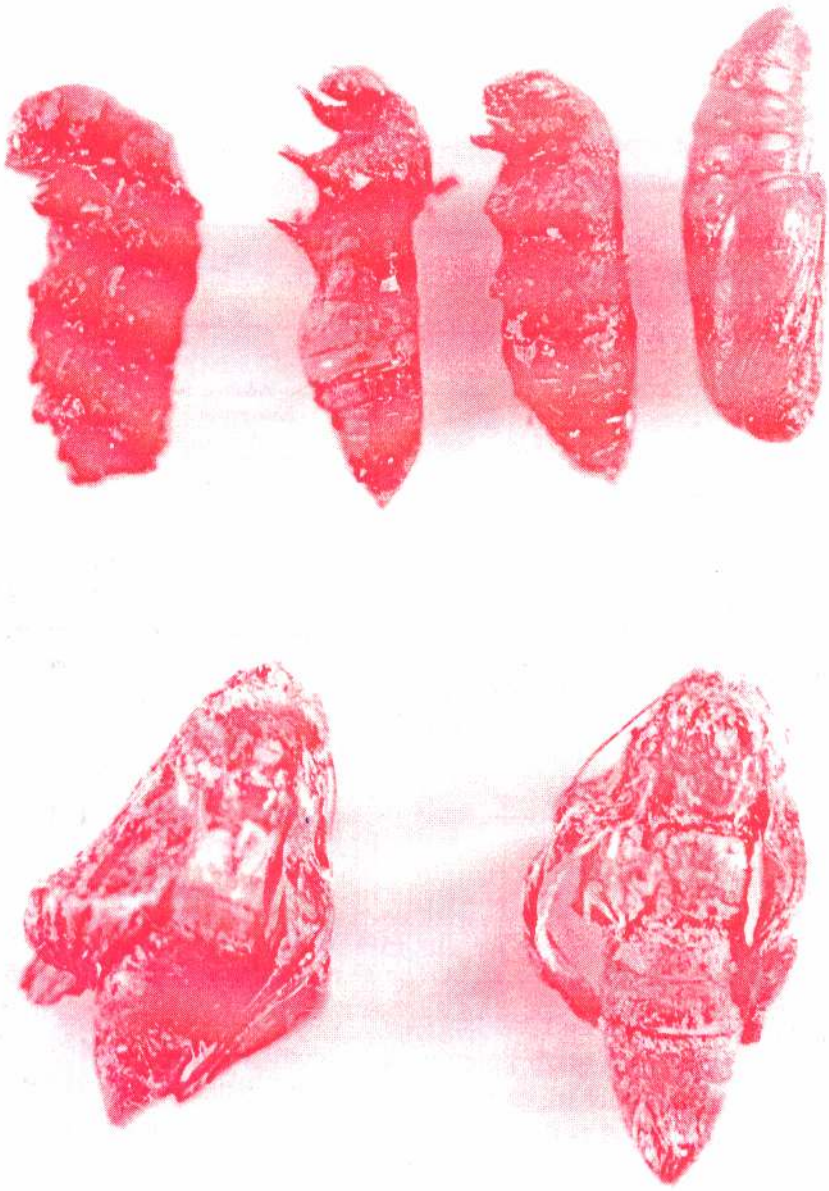

Fig.(2) : Abnormal and deformed larvae, pre-pupa, pupae and adult stages of *Spodoptera littoralis*

Table (3): Toxicity of *F. crispa* ethyl acetate extract against the 2nd and 4th instar larvae of *S. littoralis*

Larval instar	LC_{50} (mg/ml)	LC_{90} (mg/ml)	Slope	Confidence limit at LC_{50}	
				Lower	Upper
Second	2.41	12.10	1.36	1.63	3.52
Fourth	9.73	43.64	1.37	6.54	15.33

Results indicated obvious latent effects that took place later in larval, pupal and adult stages. There were symptoms of failure of transformation of larval instar to another, i.e. antimoulting action, the exuvia were attached to the new cuticle. The last larval instar as well as the pupal stage failed to moult and thus larval-pupal and pupal-adult intermediates took places; such individuals sonly die with few hours. Similar observations were found by several investigators; Harwood *et al.*, (1990) demonstrated that reduced growth in larvae of the noctuid *Peridroma samcia* was the result of feeding inhibition in larvae fed menthone and pulegone and moulting abnormalities in larvae receiving menthol as well as completely inhibited pupation. Mostafa (1993) showed that petroleum ether extract of *Melia azedarachta* caused 80 % mortality, and malformations of the cotton leafworm, *S. littoralis* were recorded. Sabbour and Abd El-Aziz (2001 / 2002) found that eucalyptus and clove oils retarded larval development, increased larval mortality and caused deformation in both pupae and adult of *S. littoralis*. They also found that fennel oil caused high percentage of adult malformation and the highest reduction in egg hatchability compared to the control.

The insecticidal activity of the ethyl acetate extract of *F. crispa* on the larval instars and on the developmental stages of cotton leafworms may be due to the inactivation of juvenile hormone (JH) by altering the microsomal cytochrome P-450 oxidase system and or by mimic JH action that induces malformations (Soderlund *et al*, 1981). Also, the toxic action of the tested plant extract against, *S. littoralis* may be attributed to the toxic constitueuts

(terpenes) present in such plants. These may act individually or together in synergistic role with other active components. Therefore, utilization of such material as alternative insecticides in insect control programmes should be considered.

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النشاط الابادى لمستخلص نبات القيصوم *Francoeria crispa* على دودة ورق القطن *Spodoptera littoralis*

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تم دراسة تأثير النشاط الابادى لمستخلص الايثيل اسيتات لنبات القيصوم التابع للعائلة المركبة على العمر الثاني والرابع للطور اليرقى لحشرة دودة ورق القطن وكذلك تم تحليل وتعريف المركبات المكونة لهذا المستخلص. أوضحت النتائج ان هذا المستخلص اظهر سمية عالية تجاه العمر اليرقى الثاني والرابع لدودة ورق القطن وكانت يرقات العمر الثاني اكثر حساسية للمستخلص حيث بلغت قيمة التركيز السام النصفي (LC_{50}) 2.41 mg/ml بينما كانت فاعلية المستخلص على يرقات العمر الرابع اقل (LC_{50} = 9.73 mg/ml) ومن النتائج أيضا يتضح ان للمستخلص تأثير على تحول الطور اليرقى لطور ما قبل العذراء حيث سببت بعض التركيزات تشوهات في طور ما قبل العذراء وكذلك تشوهات في طور العذراء وكذلك للطور الحشري الكامل الناتج عن المعاملة بالمستخلص. ومن نتيجة تحليل وتعريف المكونات لهذا المستخلص وجد انه يحتوى على مكونات التربينات حيث تمثل اكثر من ٨٩,٧٧٨ % في المستخلص.