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Insecticidal Effect of *Chrysanthemum coronarium* L. Flowers on the Pest *Spodoptera littoralis* Boisd and its Parasitoid *Microplitis rufiventris* Kok. with Identifying the Chemical Composition

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Abstract: The flower extract of *Chrysanthemum coronarium* L. and their fractions have shown insecticidal effect on the cotton leaf worm *Spodoptera littoralis*. The third instar larvae fed for two days on treated leaves were more susceptible to plant extracts and to their ethyl acetate and chloroform fractions. The active lowest concentration (5%) of the flower fractions showed no significant effect on the percent reduction of emerged adult parasitoids, *Microplitis rufiventris* Kok. GC/MS analysis revealed that the major constituents in ethyl acetate fraction were 3-dihydro-methylene-2- (3H) furanone (17.8%), jasmolin I (15.6%), carveol 1 (13.6%), phosphoric acid, tributyl ester (11.4%) and cinerin II (11.1%), while those of chloroform fraction were 5-hydroxy-3 methyl-1H-pyrazole (42.7%) and carveol 1(24.8%). The medicinal plant *C. coronarium* seems to be a promising plant for application in integrated pest management due to its safety to the surrounding environment.

Key words: Bioinsecticide, *Chrysanthemum coronarium*, Chemical composition, *Spodoptera littoralis*, Medicinal plant, *Microplitis rufiventris*

INTRODUCTION

There is a widespread opinion that the medicinal plant species have a strong biological activity on different insect pests (Jbilou *et al*., 2006). The potential use of plant extract and essential oil applications in biological control are recently increased because they are more rapidly degraded in the environment than synthetic compounds and some have increased specificity that favors beneficial insects (Moretti *et al*., 2002; Isman, 2006; Moreira *et al*., 2007; Shonouda *et al*., 2008). *Chrysanthemum* commonly known as the pyrethrum plant is an annual/seasonal herb available in tropical and sub-tropical zones (Prakash and Roa, 1997). The most widely used of botanical insecticides are extracts from the flowers of the pyrethrum daisy, *Chrysanthemum cinerariifolium*. Most of the world's pyrethrum crop is grown in Kenya (Trehane, 1995; Silva, 2004). Two species only of *Chrysanthemum* (*C. coronarium* L. and *C. segetum* L.) are widely distributed in the Mediterranean, western Africa and Asia. These species are occasionally used in the folk and traditional medicine and has been found to have biologically active substances. The *C. coronarium* is generally used against intestinal parasitic infections and flowers used as vermifuge and against itch (Boulos, 1983). The methylene chloride and methanol extracts of *C. coronarium* fresh flower heads have antibacterial activity (Urzua and Mendoza, 2003). Takenaka *et al*. (2000) proved that *C. coronarium* contains chlorogenic acid and its related compounds act as antioxidants and antimutagens. The plant *C. coronarium* has promising anti-inflammatory properties and this bioactivity is due to the high polyphenols contents (Strzelecka *et al*., 2005). Moreover, the plant *C. coronarium* has insecticidal, antifeedant and repellent properties in its flower, leaf and also in the whole plant extracts. The extracts or its dry powders have bioactivity against a wide range of the insect pests of agriculture (Tada and Chiba, 1984; Pandey *et al*., 1984; Prakash and Rao, 1997). It was found that the plant *Chrysanthemum* has toxic effect especially on the development of *Spodoptera* sp. pests (Bianchi *et al*., 2001).

From flower heads of *C. coronarium*, new sesquiterpene lactones (cumambrin and dihydrocumambrin) have been isolated and identified (ELMasry *et al*., 1984; Lee *et al*., 2002), while, polyacetylenic compounds have been isolated from the aerial parts and insect antijuvenile hormone activity has been detected for some of them (Bowers and Aregullin, 1987).

A little is known about the impact of botanical insecticides on the parasitoids especially the parasitoid

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Microplitis rufiventris. Hafez *et al.* (2003) reported that there were indirect effects of sorghum extract on the parasitoid, *M. rufiventris* regarding to egg, larval development, pupal period, parasitoid emergence percent and adult longevity.

The present study aims at bioassay the biological activity of the *Chrysanthemum* flowers extract and their fractions on the mortality of the cotton leaf worm *Spodoptera littoralis* and how far such flower extract could be viewed as botanical insecticides. Also, evaluate their safety levels to one of its efficient larval parasitoid, *Microplitis rufiventris*. Additionally, the active compounds in the active fractions are determined by using Gas Chromatography and Mass-Spectrum (GC-MS). The control of insect pests by using natural products or botanical insecticides is an effective and environmentally friendly way to reduce the usage of pesticides. Subsequently, reducing the residue and hazards of chemical pesticides has additional benefits for pesticide resistance management and for preservation of beneficial insects.

MATERIALS AND METHODS

The rearing of insects and all biological experiments were conducted in laboratories of Faculty of Science, Alexandria University during the period of 2005-2006.

Rearing of the cotton leaf worm *S. littoralis* Boisd: A culture of the cotton leaf worm was reared under laboratory conditions of 26±2°C and 65±5% RH. The egg masses were allowed to hatch in clean jars provided with castor oil leaves. The larvae continued their development till pupation. The pupae were collected in separate jars until adult emergence. Moths were fed on 10% sugar solution. The colony was reared for several generations before using the larvae in the bioassay experiments.

Rearing of the larval parasitoid *Microplitis rufiventris* Kok.: For maintaining a culture of the parasitoid *Microplitis rufiventris*, two females and two males were placed in a transparent plastic cup (10×7 cm) containing 30 third instar larvae of *S. littoralis* with castor oil leaves for about three hours and then transferred to a new cup. The cups were covered with muslin mish bounded with gummy rubber. Drops of 10% honey were placed on the muslin mish as a source of food for the parasitoids. After parasitization, the parasitized larvae were transferred to new cup, provided with castor oil leaves and observed daily until the emergence of new parasitoids.

General method for extraction and fractionation of the plant flowers: The plant *Chrysanthemum coronarium* L. (family: Compositeae) was collected from the garden of Faculty of Science, Alexandria city. The methods of extraction and fractionation of the medicinal plant were done in laboratory of the Pharmaceutical Company (FARCO) as follow: 1.5 kg of ground dried flowers of *C. coronarium* was extracted with ethyl alcohol (3×1.0 L of 96%) by percolation at room temperature. The combined extracts were evaporated under vacuum at 45°C to obtain firstly the extract from the plant. Secondly, each extract was fractionated with different organic solvents (petroleum ether, chloroform and ethyl acetate) by percolation at room temperature. The individual fraction from each solvent was also evaporated under vacuum at 45°C to obtain definite percentage of weights of each fraction. The obtained weights of each extract or fraction were formulated as emulsion by using Cremophore-EL. The solvent Cremophore EL is an emulsifying and solubilizing agent, very safe and used as vehicle for various drugs and is very soluble in water (Reynolds, 1989). Different concentrations 20, 15, 10 and 5% were prepared for either extract or fraction of *C. coronarium* by addition of distilled water.

Bioassay of plant extracts and their fractions on *S. littoralis*: The investigations undertaken in this study were carried out on the third larval instars. The larvae were starved for 2 h before transmission into experimental plastic cups. The different mentioned concentrations of the extract were used to bioassay the larval mortality of *S. littoralis*. Castor oil leaves were dipped in each concentration, allowed to dry and provided to the experimental larvae. Five larvae were transferred to each plastic cup and allowed to feed on the treated leaves for one or two days. Nine replicates for each concentration were done. The control cups were prepared in the same manner except the leaves were treated with the vehicle solution. Every day, fresh castor oil leaves were supplied and the mortality counts recorded daily. Surviving larvae were transferred to clean cups containing sawdust and supplied with fresh leaves and observed daily until pupation. The percentages of pupation and adult emergence were calculated for each treatment. The percent total mortality was estimated at the end of experiments, which included mortality of larvae, pupae and adults. The experiment was repeated again in the same manner with the different fractions of the extract by means of three solvents (chloroform, ethyl acetate and petroleum ether).

Bioassay of flower-extract fractions on the parasitoid *Microplitis rufiventris*: The lowest effective and promising concentration of plant fractions were only assessed on the adult parasitoid, *M. rufiventris*. Plastic

cups were prepared by providing each one by 15 third instar larvae of *S. littoralis*. Each cup was provided by a treated castor oil leaf and another cup was provided by clean leaf as control. Two parasitoids of one-day age (1 female and 1 male) were introduced to each cup; the cups covered with cotton mesh and provided with honey drops. The parasitoids were left to work inside the cups for 3 h on the first day and 3 h on the 2nd day. The parasitized larvae fed on treated or clean leaves for 2 days. Thereafter, the parasitized larvae were individually transferred into new cups provided with clean castor leaves and observed daily till the emergence of new parasitoids. Four replicates were done fore each assayed fraction. The number of emerged parasitoids was counted in each cup and the percentage of reduction was calculated for each treatment according to Khazanie (1979).

Analysis by Gas Chromatography and Mass Spectrum (GC-MS): Gas chromatography (GC) Agilent, model 6890 ~ series ~ with a mass selective detector (MSD) Agilent, model 6973 network and an 190195-433 capillary column were used to analyze the plant extract fractions. Fused silica capillary column (HP-5MS) was used with helium as carrier gas. The temperature was programmed from 80 to 100°C with rate 5°C min^{-1} and then to 280°C with rate 3°C min^{-1} (hold time 3 min), total run time was 67 min. Identifications were made by retention indices verified by mass spectrometry.

Statistical analysis: The obtained results of larval mortality and total mortality were subjected to analysis of variance (ANOVA) and means were separated by LSD at 0.05 levels (Steel and Torrie, 1980).

RESULTS

Effect of plant extract on third larval instar: The different concentrations of *C. coronarium* flower extract have mortal effect on the third larval instar fed for one day (Table 1). There was significant difference between the percent of larval mortality of the three higher concentrations 20, 15 and 10% and the control ($F = 5.21$, $p<0.05$), while there was no significant difference between the lowest concentration 5% and the control. The percent mortality continuously increased at the end of experiment. The total mortality ranged from 40 to 20% at the same range of concentrations. Also, there was significant difference between only the three higher concentrations and the control, ($F = 3.73$, $p<0.05$). The different concentrations of *C. coronarium* extract also being affected the percent pupation and adult emergence. The percent pupation was high 80% at the lowest concentration (5%) and decreased (66.7%) at the highest concentration (20%), while, the percent adult emergence was 80% and decreased to 60% at the same range of concentrations.

The third larval instar also showed strong response to different concentrations of flower extract when fed for two days. There was significant difference between the percent larval mortality at different concentrations and the control ($F = 36.37$, $p<0.001$). Moreover, the percent of mortality continuously increased at the end of experiment. The total mortality ranged from 53.4 to 33.3% at the same range of concentrations and the difference was significantly high ($F = 30.15$, $p<0.001$) in compare to control. The different concentrations of *C. coronarium* extract affected also the percent pupation and adult emergence. The percent pupation ranged from 80% at the lowest concentration 5 to 53.4% at the highest concentration (20%), while the percent adult emergence was 46.6% at the highest concentration and decreased to 66.7% at the lowest concentration.

Effect of different plant fractions on third larval instar: The different concentrations of ethyl acetate, chloroform and petroleum ether of *C. coronarium* flower have mortal effect on the third larval intsar fed for one day (Table 2-4). There was significant difference between the percent larval mortality at different concentrations of ethyl acetate fraction ($F = 54.89$, $p<0.001$), chloroform fraction ($F = 40.60$, $p<0.001$) and petroleum ether fraction ($F = 16.68$, $p<0.001$) as compared with control. At the end of experiment, the ethyl acetate fraction did not increase

Table 1: Effect of different concentrations of flower extract *C. coronarium* on the third larval instar of *S. littoralis* fed for one or two days

Conc.	Larval mortality (%)		Mean±SE		Pupation (%)		Adult emergence (%)		Total mortality (%)		Mean±SE	
(%)	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days
20	33.3	46.6	33.3±7.3a	46.6±6.6a	66.7	53.4	60.0	46.6	40.0	53.4	40.0±11.5a	53.4±6.6a
15	26.6	40.0	26.6±6.6a	40.0±0.0a	73.4	60.0	60.0	53.4	40.0	46.6	40.0±11.5a	46.6±6.6a
10	26.6	33.3	26.6±6.6a	33.3±6.6ab	73.4	66.7	66.7	60.0	33.3	40.0	33.3±6.6a	40.0±11.5a
5	20.0	20.0	20.0±11.5ab	20.0±3.3b	80.0	80.0	80.0	66.7	20.0	33.3	20.0±11.5ab	33.3±6.6a
Control	0.0	0.0	0.0b	0.0c	100.0	100.0	100.0	100.0	0.0	0.0	0.0b	0.0b
F-value			5.21	36.37							3.73	30.15
p-value			<0.05	<0.001							<0.05	<0.001

Within the same column, data followed by the same letter(s) are not significantly different at $p>0.05$

Table 2: Effect of different concentrations of ethyl acetate fraction of *C. coronarium* flower extract on the third larval instar of *S. littoralis* fed for one or two days

Conc.	Larval mortality (%)		Mean±SE		Pupation (%)		Adult emergence (%)		Total mortality (%)		Mean±SE	
(%)	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days
20	80.0	100.0	80.0±12.2a	100.0±0.0a	20.0	0.0	20.0	0.0	80.0	100.0	80.0±12.2a	100.0±0.0a
15	66.7	80.0	66.7±6.6ab	80.0±9.3b	33.3	20.0	33.3	13.3	66.7	86.7	66.7±6.6ab	86.7±7.6a
10	53.4	53.4	53.4±6.6bc	53.4±6.6c	46.6	46.6	40.0	46.6	60.0	53.4	60.0±11.5ab	53.4±6.6b
5	46.6	46.6	46.6±6.6c	46.6±6.6c	53.4	53.4	53.4	53.4	46.6	46.6	46.6±6.6b	46.6±6.6b
Control	0.0	0.0	0.0d	0.0d	100.0	100.0	100.0	100.0	0.0	0.0	0.0c	0.0c
F-value			54.89	86.78							31.76	44.56
p-value			<0.001	<0.001							<0.001	<0.001

Within the same column, data followed by the same letter(s) are not significantly different at p>0.05

Table 3: Effect of different concentrations of chloroform fraction of *C. coronarium* flower extract on the third larval instar of *S. littoralis* fed for one or two days

Conc.	Larval mortality (%)		Mean±SE		Pupation (%)		Adult emergence (%)		Total mortality (%)		Mean±SE	
(%)	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days
20	53.4	100.0	53.4±6.6a	100.0±0.0a	46.6	0.0	40.0	0.0	60.0	100.0	60.0±11.6a	100.0±0.0a
15	46.6	66.7	46.6±6.6a	66.7±6.6b	53.4	33.3	46.6	6.6	53.4	93.4	53.4±6.6ab	93.4±6.6a
10	40.0	60.0	40.0±9.3ab	60.0±4.6b	60.0	40.0	60.0	33.3	40.0	66.7	40.0±9.3bc	66.7±6.6b
5	26.6	40.0	26.6±6.6b	40.0±3.3c	73.4	60.0	66.7	53.4	33.3	46.6	33.3±6.6c	46.6±6.6c
Control	0.0	0.0	0.0c	0.0d	100.0	100.0	100.0	100.0	0.0	0.0	0.0d	0.0d
F-value			40.6	97.78							55.69	74.36
p-value			<0.001	<0.001							<0.001	<0.001

Within the same column, data followed by the same letter(s) are not significantly different at p>0.05

Table 4: Effect of different concentrations of petroleum ether fraction of *C. coronarium* flower extract on the third larval instar of *S. littoralis* fed for one or two days

Conc.	Larval mortality (%)		Mean±SE		Pupation (%)		Adult emergence (%)		Total mortality (%)		Mean±SE	
(%)	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days
20	66.7	100.0	66.7±13.3a	100.0±0.0a	33.3	0.0	33.3	0.0	66.7	100.0	66.7±13.3a	100.0±0.0a
15	53.4	46.6	53.4±6.6a	46.6±6.6b	46.6	53.4	46.6	46.6	53.4	53.4	53.4±6.6ab	53.4±6.6b
10	26.6	40.0	26.6±6.6b	40.0±11.5b	73.4	60.0	66.7	60.0	33.3	40.0	33.3±6.6bc	40.0±11.5b
5	20.0	33.3	20.0±11.5bc	33.3±13.3b	80.0	66.7	80.0	66.7	20.0	33.3	20.0±11.5cd	33.3±13.3b
Control	0.0	0.0	0.0c	0.0c	100.0	100.0	100.0	100.0	0.0	0.0	0.0d	0.0c
F-value			16.68	66.49							12.52	61.02
p-value			<0.001	<0.001							<0.001	<0.001

Within the same column, data followed by the same letter(s) are not significantly different at p>0.05

the percent mortality except it was increased from 53.4 to 60% at concentration 10%. Also there was significant difference between the different concentrations and control ($F = 31.76$, $p<0.001$), while, the chloroform fraction increased the total mortality and ranged from 60 to 33.3% at the same range of concentrations (20-5%). There was significant difference between the different concentrations and control ($F = 55.69$, $p<0.001$). Concerning the petroleum ether fraction, the total mortality did not increase except at concentration 10%, which increased from 26.6 to 33.3% and there was also significant difference between different concentrations and control ($F = 12.52$, $p<0.001$). The different concentrations of flower fractions also affected the percent pupation and adult emergence. The percent pupations were: (53.4-20%) for ethyl acetate fraction, (73.4- 46.6%) for chloroform fraction and (80- 33.3%) for petroleum ether fraction at concentrations ranging from 5-20%. The percent adult emergence was (53.4-20%) for ethyl acetate fraction, (66.7-40%) for chloroform fraction and (80-33.3%) for petroleum ether fraction at the same range of concentrations. It is clear that the ethyl acetate fraction has generally strong mortal effect on larvae than the other fractions.

The third larval instar showed strong response to different concentrations of flower fractions when fed for two days (Table 2-4). There was significant difference between the percent larval mortality at different concentrations of ethyl acetate fraction ($F = 86.78$, $p<0.001$), chloroform fraction ($F = 97.78$, $p<0.001$) and petroleum ether fraction ($F = 66.49$, $p<0.001$) as compared with control. At the end of experiment, the ethyl acetate fraction increased only the percent mortality from 80 to 86.7% at concentration 15% and there was significant difference between the different concentrations and control ($F = 44.56$, $p<0.001$). The chloroform fraction increased the total mortality which ranged from 100 to 46.6% at concentrations ranging from 20 to 5% and there was significant difference between the different concentrations and control ($F = 74.36$, $p<0.001$).

Table 5: Total number, means (±SE), % of emerged parasitoid adults and % of reduction after two days feeding on effective concentrations of fractions of *C. coronarium* in addition to control

Treatment	Solvent	No. of emerged adults	Mean±SE	Emerged adult (%)	Reduction (%)
Chrysanthemum	Ethyl acetate	16	4.00±0.41a	26.7	36
	Chloroform	15	3.75±0.25a	25.0	40
Control	----	25	6.25±0.25a	41.7	0

Within the same column, data followed by the same letter(s) are not significantly different at p>0.05 by LSD test, (F = 2.10, p>0.05)

Concerning the petroleum ether fraction, the percent total mortality did not increase except it increased from 46.6 to 53.4% at concentration 15% and there was also significant difference (F = 61.02, p<0.001). The different concentrations of flower fractions affected also the percent pupation and adult emergence. The percent pupations were: (53.4-0%) for ethyl acetate fraction, (60-0%) for chloroform fraction and (66.7-0%) for petroleum ether fraction at concentrations ranging from 5 to 20%. The percent adult emergence ranged from 53.4 to 0% for both ethyl acetate and chloroform fractions, while, it was from 66.7 to 0% for petroleum ether fraction at the same range of concentrations. It is quite clear that the three fractions have strong mortal effect (100%) at the highest concentration (20%) but the fractions of ethyl acetate and chloroform have a strong effect than the petroleum ether fraction particularly at low concentrations (5, 10 and 15%).

Bioassay of plant fractions on the parasitoid *M. rufiventris*: The results showed that the plant fractions with solvents either ethyl acetate or chloroform had the higher mortal activity than the solvent petroleum ether. The concentration 5% of *C. coronarium* flower fractions was the lowest and significantly active against the third larval instars when fed on treated leaves for two days. This concentration was used to test their activity on the larval parasitoid *M. rufiventris*. The parasitized third instar larvae of *S. littoralis* were fed on treated leaves with the active mentioned concentrations for two days. The percent emerged parasitoid adults with ethyl acetate flower fraction and chloroform flower fraction were 26.7 and 25%, respectively (Table 5).

It is clear that there was no significant difference between the means of emerged parasitoids with fractions of *C. coronarium* flowers (F = 2.10, p≥0.05) and control. The percent reduction with ethyl acetate fraction and chloroform fraction were 36 and 40% with *C. coronarium* flowers indicating that there was no adverse effect on the parasitoids.

GC/MS analysis of the active flower-extract fractions: Chemical composition of the active fractions (chloroform and ethyl acetate) of the medicinal plant, *C. coronarium*; was determined by GC/MS analysis. The peaks were identified according to their retention time and verified by mass spectrometry. The results of identified peaks were summarized and arranged referring to the percentage of existence (Table 6, 7).

Table 6: Chemical composition of ethyl acetate fraction of *C. coronarium*

Retention time (RT)	Compound	Area (%)
4.15	Phenylethyl alcohol	1.43
12.17	2H-Benzopyran-2-one	2.43
31.08	Carveol 1*	13.63
32.69	3-dihydro-methylene-2- (3H) furanone*	17.87
35.11	7-methoxy-8-ethoxy dimethylchromanone	0.87
37.39	Methyl ester- 9,12-octadecadienoic acid	1.30
40.37	Dioxaspiro-decan-8-one	8.36
41.33	Hexylcyclopentanone	5.73
42.01	Diisooctyl ester phthalate	7.27
47.19	Tributyl ester, phosphoric acid*	11.43
53.62	Jasmolin I*	15.62
59.46	Cinerin II*	11.18

*Represent the compounds with highest area (%)

Table 7: Chemical composition of chloroform fraction of *C. coronarium*

Retention time (RT)	Compound	Area (%)
24.86	Trans-chrysanthemol	0.72
29.32	Trans- (+) carveol	1.17
29.54	Methyl ester, chrysanthemic acid	0.62
31.34	Carveol 1*	24.87
32.77	5-hydroxy-3-dimethyl-1, 4- naphthoquinone	5.89
33.23	Tricyclo-5-3-decan	1.12
33.94	3-methanol-5-diethyl, 2-dioxide 2-oxathiane	2.27
34.45	Ethoxy-6-methoxy-2, 2-dimethyl chromanone	1.20
35.22	4-hydroxy-5-trimethoxybut-2-ynophenone	2.86
36.21	Trans-carveol	1.41
36.64	Camphor	1.55
37.74	Dimethyl-1H-carbazol	1.68
38.23	Umbellulone	2.72
40.36	Phosphoric acid, tributyl ester	1.19
42.32	5-hydroxy-3 methyl-1H-pyrazole *	42.73

*Represent the compounds with highest area (%)

DISCUSSION

The comparison of the percent total mortality of the present results gives a good vision about the bioaction of the flower extract and their fractions on the cotton leaf worm.

Firstly, the flower extract of *C. coronarium* affected larval development and increased larval mortality when larvae fed on treated leaves with different concentrations for one or two days. At the end of experiment, the percent total mortality of third larval instar fed for two days (53.4 to 33.3%) was higher than larvae fed for one day (40 to 20%) at concentrations ranging from 20 to 5%, respectively.

The flower fractions of *C. coronarium* with different solvents also showed high percent total mortality with third larval instar. The percent total mortality of third larval instar fed for two days is significantly high at all concentrations with the three solvents in compare with control and even with larvae fed for one day. The results also showed that the solvents ethyl acetate and chloroform were mostly more effective and lead to high percent total mortality than the solvent petroleum ether especially with the lowest concentration. At lowest concentration (5%), the high percent total mortality was 46.6% with either ethyl acetate or chloroform while, the percent total mortality decreased to 33.3% with petroleum ether.

The toxic effect of *C. coronarium* flower was also showed by Prakash and Rao (1997). They reported that ten species of chrysanthemum were known for possessing insecticidal, antifeedant and repellent properties in its flower, leaf and also whole plant extracts against a wide range of insect pests. Pyrethrum, mostly a mixture of certain chrysanthemum species, was known to be an insecticide and was being used for a long time in plant protection. The efficacy of pyrethrum was high when extracted with ethanol; however, it almost totally looses its potency when prepared as an aqueous extract (Stein and Klingauf, 1990). They added that the highest mortality rates of *Plutella xylostella* (Lep. Plutellidae) were obtained with extracts from pyrethrum flowers *Chrysanthemum cinerariaefolium* and the high mortality rates were obtained with third and fourth larval instars. Moreover, the toxic effect was also observed when developmental rates of *Spodoptera exigua* larvae on greenhouse chrysanthemum were 36% lower than on an artificial diet (Bianchi *et al*., 2001).

The second aim is to evaluate the effect of the different active plant fractions on the braconid parasitoid *Microplitis rufiventris*, the most natural enemy associated with the cotton pest *S. littoralis*. The lowest effective concentration (5%) of the ethyl acetate and chloroform flower extract fraction of *C. coronarium* was tested on the parasitized larvae of *S. littoralis* fed for two days on the treated leaves. Both fractions of *Chrysanthemum* flowers had no significant effect on the percent emerged adult parasitoids as compared with control ($p>0.05$). The results indicated that the percent reduction of emerged parasitoid with fractions of *Chrysanthemum* was not lower significantly than that with control and the flowers of plant could be used in control of cotton leaf worm *S. littoralis* without deleterious effect on its associated parasitoid *M. rufiventris* (Table 5). Hafez *et al.* (2003) reported that *Sorghum* extract appears to be more effective in controlling the cotton leaf worm *S. littoralis*. However, it may be incompatible with biological control in the presence of the parasitoid *M. rufiventris* due to the high mortality of their immature parasitoids especially when *Sorghum*-treatment followed parasitization.

The last aim of the present study is to identify the chemical ingredients in each active plant fractions. Gas chromatography (GC) analysis of the ethyl acetate and chloroform fractions of *Chrysanthemum* flowers revealed the presence of several peaks. The high peaks of ethyl acetate fraction of *C. coronarium* are: 3-dihydro-methylene-2-(3H) furanone (17.87%); jasmolin I (15.62%); carveol 1 (13.63%); Phosphoric acid, tributyl ester (11.43%) and cinerin II (11.18%), while, the high peaks of chloroform fraction are: 5-hydroxy-3 methyl-1H-pyrazole (42.73%) and carveol 1 (24.87%). It is clear that the carveol 1 exists in high percent in both fractions as compared with other chemical compounds indicating that the carveol 1 was a dominant compound in *C. coronarium* flowers in the present study. Meisner *et al.* (1982) reported that the carvone has insecticidal effect on the larvae of *S. littoralis*, only 2.5% of the larvae pupated and no moths emerged. The ethyl acetate fraction of chrysanthemum flower contains additional characteristic compounds such as jasmolin I, cinerin II and camphor, while, the chloroform fraction contains trans-chrysanthemol and chrysanthemic acid methyl ester. Both compounds of chloroform fraction exist in low percent comparing to other characteristic compounds. The identified compounds of *C. coronarium* flowers in the present study are more-or-less coincidental with those mentioned by Prakash and Rao (1997). They reported that extracts from pyrethrum flowers contain different insecticidal constituents differed greatly with different clones. The main constituents are cinerin, jasmolin and pyrethrin. Alvarez-Castellanos *et al.* (2000) identified the main active compounds in the flower head oil of *C. coronarium* as camphor (29.2%), alpha pinene (14.8%), Beta pinene (9.5%) and lyratyl acetate (9.8%). Also, Flamini *et al.* (2003) found that the camphor and cis-chrysanthenyl acetate were the main constitutes of the oil from *chrysanthemum* flowers. Moreover, Urzua and Mendoza (2003) reported cumambrin A in addition to camphor, trans-chrysanthenyl acetate are the main compounds of flower head of *C. coronarium*. They added the chloroform and ethyl acetate fractions contain the previous compounds in addition to quercetin.

It could be concluded that the flower extracts of *C. coronarium* and its fractions have insecticidal effect on the cotton leaf worm, *S. littoralis*. The lowest active concentration of the ethyl acetate and chloroform fractions of *C. coronarium* showed no significant effect on the percent reduction of emerged adult parasitoids.

The flower fractions of *C. coronarium* have many active chemical compounds and each plant fraction contains characteristic compounds may be magnified the bioactive action. Finally, *C. coronarium* plant seems to be a promising plant for application in pest control because it is safe to beneficial natural enemies, human and environment. More studies on the effect of each individual compound in the active fraction(s) and their mixtures should be conducted to determine the most effective ingredients.

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