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Toxical Effect of *Peganum harmala* L. Leaves on the Cotton Leaf Worm, *Spodoptera littoralis* Boisd and its Parasitoids *Microplitis rufiventris* Kok

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Abstract: The leaf extract and its fractions of *Peganum harmala* L. have shown pronounced mortal effect, decreased percent pupation and adult emergence of the cotton leaf worm, *Spodoptera littoralis* Boisd. The third instar larvae fed for two days on treated leaves were more susceptible to plant extract and its ethyl acetate and chloroform fractions. The active lowest concentration (5%) of the leaf fractions of *P. harmala* showed significant effect on the percentage of emerged adult parasitoids, *Microplitis rufiventris* Kok. GC/MS analysis showed the major constituent in ethyl acetate fraction was (23S) ethylcholest-5-en-3 beta-ol (28.04%) while those of chloroform fraction were hydroxyfuranocoumarin (Bergaptol) (15.68%), piperidinone (12.08%), thymol (11.82%), phosphoric acid, tributyl ester (9.80%) and trimethyl-nonenol (9.66%). The medicinal plant *P. harmala* could be carefully applied in integrated pest management due to its strong effect on cotton leaf worm pest.

Key words: Bioinsecticide, *Peganum harmala*, *Spodoptera littoralis*, *Microplitis* parasitoid

INTRODUCTION

The plant *P. harmala* grows wild in semi arid areas in Egypt especially in the coastal region from Sallum to Rafa (Boulos, 1994). *Peganum harmala* L. was claimed to be an important medicinal plant. Various authors have undertaken studies on the antibacterial, antifungal, antiviral and antiprotozoal effects of *P. harmala* extracts (El-Rifaie, 1980; Lamchouri *et al*., 1999). Fresh plant used against rheumatism by rubbing; headache by smelling vapors of burnt plant and also against neurotic pains, while, dried powdered plant used for purulent conjunctivitis (Boulos, 1983). Also, alkaloids of *P. harmala* possess significant antitumour potential, which could be useful as a novel anticancer therapy (Lamchouri *et al*., 1999). However, its seeds were known to possess hypothermic and essentially hallucinogenic properties. Harmaline, the active principle of the plant seeds and its derivatives, cause visual troubles, loss of coordination and at high doses, it can produce paralysis (Lamchouri *et al*., 2002).

Concerning its toxicity against different insects, Jbilou *et al.* (2006) found recently that methanol extracts from different medicinal plants including *P. harmala* seeds have insecticidal effects on the larvae and adults of the stored grain pest *Tribolium castaneum* (Col., Tenebrionidae). Also, Abbassi *et al.,* (2003a) found the toxical effect of *P. harmala* on survival, feeding, behavior and reproduction of the desert locust, *Schistocerca gregaria* under laboratory conditions. There are few studies on the effect of the plant *P. harmala* against the cotton leaf worm, *S. littoralis*. The alcohol extracts of *P. harmala* seeds caused high mortality of *S. littoralis* larvae (El-Gengaihi *et al*., 1996). Nennah (1999) tested the activity of fourteen crude plant extracts against *S. littoralis* by using ethanol, petroleum ether, acetone and chloroform as different solvents. The susceptibility of *S. littoralis* to the extracts of *Achillea santolina* (L.), *Asphodelus microcarpus*, *Cephaelis imecacaunha* and *Peganum harmala* (L.) were more pronounced regarding their biological parameters and toxicity. The chemical composition of the *P. harmala* was investigated by Sharaf *et al.* (1997). They recorded that the aerial parts of *P. harmala* containing four new flavonoids: acacetin 7-O rhamnoside, 7-O-6-O glucosyl-2-O-(3-acetylrhamnosyl) glucoside, 7-O-2-O-rhamnosyl-2-0-glucosylglucoside and the glycoflavone-2-O-rhamnosyl-2-O-glucosylcytisoside. Also, the alkaloidal fraction of harmal seeds iodide derivatives contains three characteristic compounds: harmine, harmaline and harmalol. The biological activity of the iodide derivative and the three compounds were approved on *S. littoralis* (El-Gengaihi *et al*., 1997). Additionally, Kartal *et al.* (2003) separated and determined four compounds, harmol, harmalol, harmine and harmaline in seeds of *P. harmala* L. by using spectrophotometric detection. The present study aims to

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bioassay the toxicity of leaf extract and its fractions of the medicinal plant *Peganum harmala* L. on the development of the cotton leaf worm *S. littoralis* Boisd and its parasitoid *Microplitis rufiventris* Kok with identification of the most characteristic chemical compounds.

MATERIALS AND METHODS

Rearing of the cotton leaf worm *S. littoralis* Boisd: The 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 the 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 mesh 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 cups, provided with castor oil leaves and observed daily until the emergence of new parasitoids.

General method for extraction and fractionation of the plant leaves: The plant *Peganum harmala* L. (family: Zygophyllaceae) was collected from the western desert, 60 km west of Alexandria city, Egypt. 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 dried leaves of *P. harmala* was extracted with ethyl alcohol (3×1.0 L of 96%) by percolation at room temperature. The extract-ethyl alcohol was evaporated under vacuum at 45°C to obtain firstly the extract from the plant. Secondly, the extract was fractionated with different organic solvents (petroleum ether, chloroform and Ethyl acetate) by percolation at room temperature. Each individual fraction was also evaporated under vacuum at 45°C to obtain definite weight from each fraction. The obtained weight from the extract or from each fraction was formulated as emulsion by using Cremophore-EL. in 100 mL volumetric flask to form a stock solution. 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 *P. harmala* by diluting with 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 two hours before transmission into experimental plastic cups. The different concentrations of the extract were applied to bioassay the larval mortality of *S. littoralis*. Castor oil leaves were dipped in each concentration, allowed to dry and provided to the experimental cups. Five larvae were transferred to each plastic cup and allowed to feed on the treated leaves for one day 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 were recorded. 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.

Bioassay of leaf-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*. Two parasitoids of one-day age (1 female and 1 male) were introduced to each cup. The cups covered by cotton mesh and provided with honey drops. The parasitoids were left to work inside the cups for three hours in the first day and three hours in the second day. The parasitized larvae were fed on treated leaves with the mentioned concentration or fed on clean leaves as control. All larvae were fed on treated leaves for two days. Thereafter, the parasitized larvae were individually transferred into new cups provided with clean castor leaves and observed daily till the emerging of new parasitoids. Four replicates were done for each assayed fraction and control. The number of emerged parasitoids was counted in each cup and the percentage of reduction was calculated for each treatment according to Khazanie (1979).

Gas chromatography and mass spectrum analysis (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 80-100°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 is 67 min. Identifications were made by retention indices verified by mass spectrometry.

Statistical analysis: Percentages of larval mortality and total mortality were calculated and data were corrected using Abbott's formula (Abbott, 1925). All results 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 *P. harmala* leaf extract had mortal effect on the third larval instar fed for one day (Table 1). There was significant difference between the percent of larval mortality of all concentrations (20, 15, 10 and 5%) and the control ($F = 14.55$, $p<0.001$). The percentage of mortality continuously increased at the end of experiment. The total mortality ranged from 40-33.3% at the same range of concentrations. Also, there was significant difference between all concentrations and the control, ($F = 4.57$, $p<0.05$). The different concentrations of *P. harmala* extract also affected the percentage of pupation and adult emergence. The percent pupation was high (73.4%) at the lowest concentration (5%) and decreased (60%) at the highest concentration (20%), while, the percent of adult emergence was 66.7% and decreased to 60% at the same range of concentrations.

The third larval instar also showed strong response to different concentrations of leaf extract when fed for two days. There was significant difference between the percent larval mortality at different concentrations and the control ($F = 32.42$, $p<0.001$). At the end of experiment, the percentage of mortality did not increased except at the lowest concentration (5%), the total mortality increased to 40% and there was also significant difference between different concentrations and control ($F = 10.53$, $p<0.01$). The different concentrations of *P. harmala* extract affected the percentage of pupation and adult emergence. The percent pupation ranged from 66.7% at the lowest concentration (5%) to 46.6% at the highest concentration (20%), while the percent adult emergence was 60% and decreased to 46.6% at the same range of concentrations.

Effect of different plant fractions on third larval instar: The different concentrations of ethyl acetate, chloroform and petroleum ether fractions of *P. harmala* leaves had 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 ($F = 2.90$, $p<0.05$), chloroform fraction ($F = 11.64$, $p<0.01$) and only at high concentration (20%) of petroleum ether fraction ($F = 2.99$, $p<0.05$) in compare to control. At the end of experiment, the ethyl acetate fraction and chloroform fraction did not increase the percentage of mortality, while, the petroleum ether fraction increased the total mortality only at high concentration 20% from 33.3-40% and there was also significant difference between high concentration and control ($F = 2.69$, $p<0.05$). The different concentrations of leaf fractions also affected the percentage of pupation and adult emergence. The percent pupation and adult emergence had the same range for ethyl acetate fraction (73.4-60%) and for chloroform fraction (73.4-13.3%) while, for petroleum ether fraction, the percent pupation was (86.7-66.7%) and the percent adult emergence was (86.7-60%) at concentrations ranged from 5-20%. It is clear the chloroform fraction has generally strong mortal effect on larvae than the other fractions especially at the high concentrations.

The third larval instar showed strong response to different concentrations of leaf fractions when fed for 2 days (Table 2-4). There was significant difference between the percent larval mortality at different concentrations of ethyl acetate fraction ($F = 9.34$, $p<0.01$), chloroform fraction ($F = 15.48$, $p<0.001$) and only at high

Table 1: Effect of different concentrations of plant extract *P. harmala* 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	40.0	53.4	40.0±11.5^{a}	53.4±6.6^{a}	60.0	46.6	60.0	46.6	40.0	53.4	35.7±11.5^{a}	50.1±6.6^{a}
15	40.0	46.6	40.0±11.5^{a}	46.6±6.6^{ab}	60.0	53.4	60.0	53.4	40.0	46.6	35.7±11.5^{a}	42.9±6.6^{a}
10	33.3	40.0	33.3±6.6^{a}	40.0±0.0^{ab}	66.7	60.0	66.7	60.0	33.3	40.0	28.6±6.60^{a}	35.7±6.6^{a}
5	26.6	33.3	26.6±6.6^{a}	33.3±6.6^{b}	73.4	66.7	66.7	60.0	33.3	40.0	28.6±6.60^{a}	35.7±6.6^{a}
Control	0.0	0.0	0.0^{b}	0.0^{c}	100.0	100.0	93.4	93.3	6.6	6.6	6.6±6.60^{b}	6.6±6.6^{b}
F-value			14.55	32.42							4.57	10.53
p-value			<0.001	<0.001							<0.05	<0.01

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 *P. harmala* 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	40.0	73.4	35.7±11.5^{a}	71.5±6.60^{a}	60.0	26.6	60.0	20.0	40.0	80.0	35.7±11.5^{a}	76.5±11.5^{a}
15	33.3	60.0	28.6±6.60^{a}	57.1±11.5^{a}	66.7	40.0	66.7	33.3	33.3	66.7	28.6±6.60^{a}	64.3±6.60^{a}
10	26.6	33.3	21.4±6.60^{a}	28.6±6.60^{a}	73.4	66.7	73.4	66.7	26.6	33.3	21.4±6.60^{a}	28.6±6.60^{a}
5	26.6	33.3	21.4±6.60^{a}	28.6±6.60^{a}	73.4	66.7	73.4	66.7	26.6	33.3	21.4±6.60^{a}	28.6±6.60^{a}
Control	6.6	6.6	6.6±6.60^{b}	6.6±6.60^{b}	93.4	93.4	93.4	93.4	6.6	6.6	6.6±6.60^{b}	6.6^{b}
F-value			2.90	9.34							2.90	21.79
p-value			<0.05	<0.01							<0.05	<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 *P. harmala* 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	86.7	100.0	85.7±6.60^{a}	100.0±0.00^{a}	13.3	0.0	13.3	0.0	86.7	100.0	85.7±6.60^{a}	100.0±0.00^{a}
15	73.4	100.0	71.5±6.60^{a}	100.0±0.00^{a}	26.6	0.0	26.6	0.0	73.4	100.0	71.5±6.60^{a}	100.0±0.00^{a}
10	60.0	80.0	57.1±11.5^{a}	78.6±11.5^{a}	40.0	20.0	40.0	20.0	60.0	80.0	57.1±11.5^{a}	78.6±11.5^{a}
5	26.6	40.0	21.4±6.60^{a}	35.7±0.00^{a}	73.4	60.0	73.4	60.0	26.6	40.0	21.4±6.60^{a}	35.7±0.00^{a}
Control	6.6	6.6	6.6±6.60^{b}	6.6±6.60^{b}	93.4	93.4	93.4	93.4	6.6	6.6	6.6±6.60^{b}	6.6±6.60^{b}
F-value			11.64	15.48							11.64	15.48
p-value			<0.01	<0.001							<0.01	<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 *P. harmala* 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	33.3	46.6	28.6±6.6^{a}	42.9±6.6^{a}	66.7	53.4	60.0	53.4	40.0	46.6	35.7±11.5^{a}	42.9±6.60^{a}
15	20.0	33.3	14.3±6.6^{ab}	28.6±0.0^{a}	80.0	66.7	80.0	66.7	20.0	33.3	14.3±11.5^{ab}	28.6±13.3^{a}
10	13.3	13.3	7.9±6.6^{b}	7.9±6.6^{b}	86.7	86.7	86.7	86.7	13.3	13.3	7.9±6.60^{b}	7.9±6.60^{b}
5	13.3	13.3	7.9±6.6^{b}	7.9±6.6^{b}	86.7	86.7	86.7	86.7	13.3	13.3	7.9±6.60^{b}	7.9±6.60^{b}
Control	6.6	6.6	6.6±6.6^{b}	6.6±6.6^{b}	93.4	93.4	93.4	93.4	6.6	6.6	6.6±6.60^{b}	6.6±6.60^{b}
F-value			2.99	5.30							2.69	5.30
p-value			<0.05	<0.05							<0.05	<0.05

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

concentrations (20 and 15%) of petroleum ether fraction (F = 5.30, p<0.05) in compare to control. At the end of experiment, the ethyl acetate fraction increased the percentage of mortality from 73.4-80% at concentration 20% and from 60- 66.7% at concentration 15%. There was significant difference between the different concentrations and control (F = 21.79, p<0.001). The chloroform fraction kept the total mortality high and ranged from 100-40% at concentrations ranged from 20-5% and there was significant difference between the different concentrations and control (F = 15.48, p<0.001). Concerning the petroleum ether fraction, the percentage of total mortality did not increase and still ranged from 46.6-13.3% at the same rang of concentrations. The different concentrations of leaf fractions affected also the percentage of pupation and adult emergence. The percent pupation ranged from 66.7-26.6% and adult emergence from 66.7-20% for ethyl acetate fraction while they had the same range (60-0%) for chloroform fraction and (86.7-53.4%) for petroleum ether fraction at the concentrations ranged from 5-20%. It is obviously clear that the chloroform fraction has strong mortal effect (100%) at high concentrations (20 and 15%) and the fractions of ethyl acetate and chloroform have a comparable strong total mortal effect (33.3 and 40%) than the petroleum ether fraction (13.3%) certainly at the lowest concentration (5%).

Bioassay of plant fractions on the parasitoid *M. rufiventris*: Ethyl acetate or chloroform fractions had the higher mortal activity than the petroleum ether fraction. The concentration 5% of *P. harmala* of both leaf fractions was the lowest and significantly active against the third larval instars fed on treated leaves for two days. This concentration was mentioned to test its activity on the emerged adult parasitoid *M. rufiventris*. The parasitized third instar larvae of *S. littoralis* were fed on treated leaves with the active mentioned concentration for two days. The percentage of emerged adult parasitoids with ethyl acetate flower fraction and chloroform flower fraction were 25 and 23.3%, respectively, (Table 5). It is clear there was significant difference between the means of emerged parasitoids with both leaf fractions of *P. harmala* (F = 4.69, p<0.05) and control. The percent

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

Treatments	Solvent	No. of emerged adults	Mean±SE	Emerged adult (%)	Reduction (%)
Peganum	Ethyl acetate	15	3.75±0.25[a]	25.0	40
	Chloroform	14	3.50±0.29[a]	23.3	44
Control	----	25	6.25±0.25[b]	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, ($F = 4.69$, $p<0.05$)

Table 6: Chemical composition of ethyl acetate fraction of *P. harmala*

Compounds	Retention time (RT)	Area (%)
Hexadecanoic acid	30.08	6.57
4-phenylisoquinoline	30.96	4.69
Flavoxate (Urispas)	32.70	4.84
Phytol	34.49	2.35
9,12,15-octadecatrienoic acid	35.34	5.91
1,4-dioxaspiro (4,5) decane-8-one	40.37	1.80
Benzenedicarboxylic acid, diisooctyl ester	46.23	5.18
Thymol	47.19	3.14
Piperidinone	53.52	3.15
Phosphoric acid, tributyl ester	59.46	2.30
4-methyl-2-phenylindole	60.71	8.80
Cyclotrisiloxane, hexamethyl	61.41	7.69
(23S) Ethylcholest-5-en-3 beta-ol*	62.56	28.04
1,3-dimethyl-4-azaphenanthrene	64.84	2.51
Tribromochloromethane	65.17	3.47
Hexestrol	66.64	5.41

*: Represent the compounds with highest area (%)

Table 7: Chemical composition of chloroform fraction of *P. harmala*

Compounds	Retention time (RT)	Area (%)
Quinoline	6.55	0.86
2-ethoxycarbonyl-pyrrolidine	12.31	1.55
4(1H)-quinazolinone	20.97	2.68
Malononitrile	28.86	3.90
5-phenylisoquinoline	30.96	2.20
Hydroxyfuranocoumarin (Bergaptol)*	31.28	15.68
Trimethyl-nonenol*	32.68	9.66
Phytol	34.46	2.41
1,4-dioxaspiro (4,5) decane-8-one	40.35	8.68
Thymol *	47.19	11.82
Piperidinone*	53.63	12.08
Phosphoric acid, tributyl ester*	59.46	9.80
(23S) Ethylcholest-5-en-3 beta-ol	62.56	6.81
1,3-dimethyl-4-azaphenanthrene	64.82	5.47

*: Represent the compounds with highest area (%)

reduction with ethyl acetate and chloroform leaf fractions were 40 and 44% indicating that there were moderate 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, *P. harmala*; was determined by GC/MS analysis, Gas chromatography analysis of the active fraction of *P. harmala* revealed the presence of several peaks. There were 16 chemical compounds in the ethyl acetate fraction represent 95.85 % of the total area, while, there were 14 chemical compounds in chloroform fraction represent 93.6% of the total area. There were 8 compounds representing in both fractions but with different percentages. The results of identified compounds were summarized and arranged referring to the percentage of existence (Table 6, 7).

DISCUSSION

The comparison between the percent total mortality of different treatments gives a good vision about the bioactivity of the leaf extract and their fractions on the cotton leaf worm. The first concept of our study is to show the toxicity of the leaf extract and its different fractions on the development of cotton leaf worm. The different concentrations of *P. harmala* leaf extract affected the larval development and increased the larval mortality of *S. littoralis* when fed for one or two days. At the end of the experiment, the percent total mortality of larvae fed for two days (53.4-40%) was higher than larvae fed for one day (40-33.3%) at concentrations ranged from 20-5%, respectively. Also, the different leaf fractions of *P. harmala* showed strong effect on the third larval instar. The percent total mortality of third larval instar fed for two days is significantly high at all concentrations with ethyl acetate and chloroform fractions in compare with control and even high if compared with the petroleum ether fraction. The high percent total mortality ranged from 80-33.3% with ethyl acetate fraction and ranged from 100-40% with chloroform fraction, while, the percent total mortality was low with petroleum ether fraction and ranged from 46.6-13.3%. The bioactivity of harmal plant was shown by El-Ansary *et al.* (2001). They reported that the dry powdered *P. harmala* plant had molluscicidal activity against *Biomphlaria alexandrina*, specific intermediate hosts of *Schistosoma mansoni.* Concerning insect pests, Abbassi *et al.* (2003b) found that the alkaloids extracted by ethanol from *P. harmala* leaves caused a significant mortality of the desert locust, *Schistocerca gregaria* and reduced the female fecundity as well as hatching rate compared to untreated control. Also, the methanol extract of *P. harmala* seeds had insecticidal effect on the stored grain pest *Tribolium castaneum* (Herbst). Larval growth was significantly inhibited when they were fed on extracts mixed with their diets (Jbilou *et al.*, 2006).

The second concept is to evaluate the toxicity of the active plant fractions on the female 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 leaf fractions of *P. harmala* was tested on the parasitized larvae of *S. littoralis*. Both fractions of leaf extract had significant effect on the percentage of emerged adult parasitoids comparing to control ($p<0.05$). The results indicated that the percent reduction of emerged parasitoid with fractions of *Peganum* plant were lower than that with control and the leaves of *Peganum* must be carefully used in control of cotton leaf worm *S. littoralis* because they have strong effect on its associated parasitoid *M. rufiventris* (Table 5). Other plant extracts may have deleterious effect on the biological agents. 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 compounds in each active leaf fraction. GC/MS analysis showed only one high peak in ethyl acetate fraction. It is identified as (23S) ethylcholest-5-en-3 beta-ol (28.04%); while the high peaks of chloroform fraction are: hydroxyfuranocoumarin (15.68%); piperidinone (12.08%); thymol (11.82%); phosphoric acid, tributyl ester (9.80%) and trimethyl-nonenol (9.66%). There are many biological importance for the identified compounds. The most abundant compound in ethyl acetate fraction is also known as stigmast-5-en-3 ol. This phytosterol compound is available as a cholesterol-lowering drug and in some cases has adverse reactions include gastrointestinal problems (Rahway, 1989). In chloroform fraction; it was found the furanocoumarin derivatives such as bergaptol which present in different plants. These phytochemicals are shown their insecticidal activities against a wide range of herbivores (Zangerl, 1990) and have antimicrobial activities against bacteria and fungi (Kuete *et al*., 2007). The monoterpenoid thymol commonly found in plant essential oils. It found in both fractions of *P. harmal* leaves but in high concentration in chloroform fraction. It has acute toxicity on the cutworms *Spodoptera litura* (Hummelbrunner and Isman, 2001). The phosphoric acid, tributyl ester, has synonym tributyl phosphate (TBP) and it is used as a good solvent for cellulose esters (plasticizer). Additionally, it is used as a solvent for fungicides and herbicides and it is used as rodenticides with major effect on nervous system (Morales-Rojas and Moss, 2002). Also, the good organic solvent piperidinone is present in high concentration in the chloroform fraction and is used in many pharmaceutical and agrochemical industries (Lierop van *et al*., 1998). Many authors showed the biological activity of the characteristic compounds of *P. harmala*. Abbassi *et al.* (2003a) found that the ethanolic extract of *P. harmala* had a majority of the indolic alkaloids and these compounds are responsible for the toxicity of the plant. Moreover, Kartal *et al.* (2003) separated and identified the bioactive compounds from the seeds of *P. harmala* as harmol, harmalol, harmine and harmaline. Also, Lala *et al.* (2004) found that harmine (a β-carboline amine alkaloids) isolated from *P. harmala* have antileishmanial properties. It could be concluded that the leaf extract and its fractions of *P. harmala* have insecticidal effect on the development of the cotton leaf worm *Spodoptera littoralis*. The leaf fractions of *P. harmala* have many active chemical compounds. The chemical constituents and their percentage of existence in the plant may differ from one region to another according to the environmental conditions. GC/MS analysis showed that each plant fraction contains characteristic compounds may be magnified the bioactive action. These naturally occurring plant extract, its fractions and/or its characteristic compounds could be useful for pest management of leaf cotton worm. More studies are needed to bioassay the activity of each identified compound against cotton leaf worm and other different pests.

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