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INHIBITION OF GROWTH AND DEVELOPMENT OF COTTON LEAFWORM (LEPIDOPTERA : NOCTUIDAE) LARVAE BY β-CARBOLINE ALKALOIDS

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ABSTRACT

The feeding deterrence and growth retardant effect of petroleum ether extract and total β-carboline alkalods isolated from *Peganum harmala* L. seeds were assessed against the cotton leafworm *S. littoralis* larvae. Thin layer chromatography was used for separation of the compounds in the alkaloids mixture with R_f values of 0.24, 0.38, 0.60 and 0.68. Using the leaf disc technique, the total β-carboline alkaloids showed the highest feeding deterrence to the larvae (1000 ppm concentration caused 100% deterrence) followed in a descending order by petroleum ether extract. Acetone and methanol extracts exhibited slight antifeeding activity to the larvae. The larval average weight was relatively low by feeding on the alkaloid mixture-treated diet. Only 51.7% of the larvae pupated at the concentration of 0.2% (2000 μg/g diet). The petroleum ether extract also revealed antifeeding and growth retardant activity. A negative correlation between the concentration of total alkaloids in the diet and feeding response was found represented by larval growth and pupation. The results showed that the pre-pupal stage was highly affected by the petroleum ether extract and β-carboline alkaloid mixture.

INTRODUCTION

Alternative methods for controlling insects have increased greatly in recent years because of continuing problems with strict reliance on conventional insecticides. These problems include the development of insect resistance, secondary pests, effects on non-target organisms and environmental contamination. The use of behaviour-modifying compounds in pest control such as plant antifeedants or oviposition deterrents which reduce insect feeding or egg-laying without killing pests has intuitive appeal because such compounds should be safer to non-target organisms. Natural plant products that inhibit insect feeding offer considerable potential for crop protection because antifeedant activity is often highly species-dependent and phytochemicals are generally readily biodegraded. Insect damage to plants results from direct feeding or from indirect transmission of pathogens during feeding. Therefore, antifeedants which reduce or disturb feeding activities of insects by rendering plants unattractive or unpalatable offer a novel approach in pest and vector management.

Natural products derived from plants exhibited many of biological activities as biopesticides including the inhibitory activity to the feeding, growth and development of several phytophagous insects (McMikkian *et al*., 1969; Chapman, 1974; Isman and Rodriguez, 1983; El-Sebae *et al*., 1986 and Eldoksch *et al*., 1996, 1997).

The simple β-carboline alkaloids of *Peganum harmala* seeds have been shown to inhibit monoamine oxidase and induce tremor (Coates and Cox, 1972), to interact into DNA (Duportail and Lami, 1975), to interfere with UV-induced DNA damage repair systems (Chang *et al*., 1978), to inhibit sodium-potassium ATPase activity (Murumo *et al*., 1976) and also to be phototoxic against microorganisms (McKenna and Towers, 1981).

Because of the wide range of bio-activity of simple β-carboline alkaloids, the present study aimed to studying the antifeedant and larval growth inhibitory activity of *Peganum harmala* extracts and their β-carboline alkaloids against the cotton leafworm, *Spodoptera littoralis* (Boisd.) infesting many economic crops in Egypt. The latent effect of these natural extracts on some biological aspects of this insect was also investigated.

MATERIALS AND METHODS

Preparation of successive solvent extracts from *Peganum harmala* L.

Seeds of *Peganum harmala* were obtained from plants collected from the eastern desert near Suez governorate. Identification of plant samples was confirmed by the Department of Aromatic and Medicinal Plants, Horticultural Research Station, Sabahia, Alexandria. Dried powder of 200 g seeds of *P. harmala* was successively extracted by Soxhlet apparatus with petroleum ether, acetone and methanol. The extracts were filtered and evaporated (40-50^{o}C) to give the crude extract which was used for bioassay tests.

Isolation of β-carboline alkaloids

The β-carboline alkaloids were isolated from *P. harmala* seeds by the method described by Allen and Holmstedt (1980) with minor modification. Dried and ground seeds (150 g) were extracted with petroleum ether 40-60% for 8 h in a continuous extraction apparatus. The residue was dried and extracted with methanol, then it was evaporated and the residue was dissolved in 2% HCl. The acidic solution was basified with ammonia and extracted three times (3 x 200 ml) with $CHCl_3$. The chloroform extract was distilled off to give the basic fraction (Mayer's reagent test) which was used for insect antifeeding and growth inhibitory activity evaluation.

Thin layer chromatographic analysis of the basic fraction

The basic fraction was spotted on coated silica gel plastic plates as thin layer chromatogram which was developed with a mixture of $CHCl_3$: MeOH (7:3) and the alkaloid spots were revealed by spraying with Dragendorff's spray reagent.

Cultures of *Spodoptera littoralis* (Boisd.)

A laboratory strain of the Egyptian cotton leafworm *S. littoralis* was reared on castor bean leaves according to Eldefrawi *et al*.(1964). Another laboratory strain of the same insect was also reared on an artificial diet as described by Bakry *et al*.(1973).

Antifeeding leaf disc test

The leaf disc method which was reported by Kubo and Nakanishi (1977) with slight modification was used as a feeding technique to the insect. Leaf discs (each 4 cm^2) of the castor bean leaves were dipped in acetone solution of the treatment of plant extracts for five seconds and various concentrations were used for each plant material. Control discs were dipped in acetone and then left to dry by evaporation. A leaf disc coated by the treatment, a control disc and two 4^{th}-instar larvae of the cotton leafworm were placed together in a Petri dish (9 cm in diameter) and the temperature was kept at about 26°C. The discs were removed after 3 and 24 hr and weighed to determine the percentage eaten by the larvae. The percent average of 10 larvae per each concentration was calculated.

Bioassay of plant extracts and β-carboline alkaloids incorporated into an artificial diet

To evaluate the effect of plant extracts on insect feeding rate, survival and development of *S. littoralis* larvae, the method of McMillian *et al*.(1969) was applied. The various concentrations of each plant material were incorporated into an artificial diet of agar base in plastic vials by adding the desired quantity to a measured amount of the medium. Four replicates were used for each concentration and five 3^{rd}-instar larvae were placed in each vial. The vials were covered and maintained at 26°C. After 7 days of exposure, the larvae were removed and weighed and then they were transferred back to untreated diet. The rearing containers were checked daily for the presence of pupa. The percent mortality and percent pupation of larva were counted and corrected by using Abbott's formula (1925). The feeding inhibitory activity and acceptability of the treated diet was determined using the equation of Wada and Manukata (1968) for feeding ratio determination as follows:

$$\text{Feeding ratio} = \frac{B}{A} x\,100$$

where;
A = amount of diet consumed in control.
B = amount of diet consumed in treated diet.

RESULTS AND DISCUSSION

The antifeeding effect of petroleum ether, acetone, methanol extracts and β-carboline alkaloids isolated from *P. harmala* seeds was assessed against fourth instar larvae of *S. littoralis* using leaf disc technique. The data in Table (1) indicate that the castor bean leaf discs coated with petroleum ether extract at the concentration of 1000 ppm (0.1%) showed an antifeeding effect to the larvae. They did not eat the sample disc after 3 hr and ate only 32.7% after 24 hr, while they ate about 32.4 and 100% of the control leaves after 3 and 24 hr of treatment. Concentration of 500 ppm exhibited medium antifeeding activity. Acetone and methanol extracts revealed slight antifeeding activity to *Spodoptera* larvae. The crude petroleum ether extract

which exhibited promising antifeeding activity to the larvae gave positive test for alkaloids (Wagner's reagent test). Concerning total β-carboline alkaloid treatments, the results indicated that the potent antifeeding action was obtained by the concentration of 1000 and 500 ppm. The larvae did not eat the sample discs but ate about 33.3 and 37.8%, respectively after 3 h and ate 100% of the control leaves after 24 h. Concentration of 100 ppm revealed medium antifeeding activity and the percent of eaten weight was 64.3% after 24 hr. The thin layer chromatographic analysis of the β-carboline alkaloid mixture showed the presence of four spots with R_f values of 0.24, 0.38, 0.60 and 0.68. Therefore, it may be concluded that the total alkaloids exhibited the highest antifeeding activity against *Spodoptera* larvae followed in a descending order by petroleum ether extract while acetone and methanol extracts exhibited slight antifeeding activity.

Table (1): Antifeeding activity of plant extracts and β-carboline alkaloids of *Peganum harmala* against the 4th instar larvae of *Spodoptera littoralis* using leaf disc technique.

Solvent extract (ppm)	% of eaten weight at indicated hr post-treatment			
	3 hr		24 hr	
	Control	Test sample	Control	Test sample
Petroleum ether				
1000	32.4	0.0	100	32.7
500	35.8	10.6	100	66.3
100	37.0	33.0	100	100
Acetone				
1000	29.8	26.1	100	100
500	33.2	31.5	100	100
100	35.6	33.3	100	100
Methanol				
1000	28.3	33.6	100	100
500	41.6	26.3	100	100
100	36.4	35.8	100	100
β-carboline alkaloids				
1000	33.3	0.0	100	0.0
500	37.8	0.0	100	15.8
100	31.6	37.2	100	64.3

The present data are in agreement with those reported by Schroeder (1976), who indicated that the insects whose diet includes such compounds may suffer some shortage in their nutritional requirements leading to some physiological changes in the normal consumption and conversion of the food to the larval tissue.

The data in Table (2) indicate that petroleum ether extract and β-carboline alkaloids caused concentration-dependent feeding reduction against *S. littoralis* larvae after 7 days of exposure using an artificial diet mixed with tested plant materials. In case of petroleum ether extract treatments, feeding was significantly reduced at concentrations ranged between 1000 and 2000 μg/g diet (ppm) with percent feeding inhibition ranged between 34.27 and 48.17%.

Table (2): Antifeeding activity of solvent extracts and β-carboline alkaloids mixed with semi-artificial diet against the 3rd instar larvae of *Spodoptera littoralis*.

Treatment (μg/g diet) (ppm)	Average wt. of diet consumed / 5 larvae during 7 days (g±SE)	Feeding** ratio (B/Ax100)	Feeding inhibition (%)	Larval growth % of control
Petroleum ether extract				
2000	5.37 ± 0.31*	51.83	48.17	59.38
1000	6.81 ± 0.63*	65.73	34.27	64.18
500	6.97 ± 0.58*	67.27	32.73	90.30
0 (control)	10.36 ± 0.46	100	0.0	100
Methanol extract				
2000	8.77 ± 0.38	85.81	14.19	87.48
1000	8.98 ± 0.71	87.86	12.14	91.35
500	9.47 ± 0.26	92.66	7.34	84.89
0 (control)	10.22 ± 0.34	100	0.0	100
β-carboline alkaloids				
2000	2.82 ± 0.21*	26.83	73.17	49.81
1000	3.63 ± 0.55*	34.53	65.47	65.48
500	7.33 ± 0.61*	69.74	30.26	83.18
0 (control)	10.51 ± 0.66	100	0.0	100

***: Significantly different from untreated diet (control) at the 0.05 level of probability.**
****: A = Amount of diet consumed in control., B = Amount of diet consumed in treated diet.**

Concerning the β-carboline alkaloid treatments, larval feeding inhibition percentages ranged between 30.26 and 73.2% at the concentrations tested and larval growth percentages ranged between 49.8 and 83.2% in

comparison with the control. The data also revealed that alkaloid mixture exhibited insect antifeeding activity more potent than petroleum ether extract, while methanol extract was relatively the least effective in this respect.

The data in Table (3) indicated that the alkaloid mixture was the most effective on the larval growth and pupation rate compared with the control and other treatments. The results showed significant growth inhibitory activity of the larvae due to exposure to β-carboline alkaloids of 1 and 2 mg/g diet. The percentages reduction in larval weight were 34.52 and 50.19%, respectively.

Table (3): Effect of plant extracts and β-carboline alkaloids on survival, growth and pupation of *S. littoralis* larvae.

Treatment (µg/g diet) (ppm)	Average weight (mg±SE) of larvae at the initial of experiment	Survival (%) and average weight after 7 days of exposure		Reduction in larval weight (% of control)	Mortality* of larval stage (%)	Pupated larvae (%)
		Survival	Average weight (mg±SE)			
Petroleum ether extract						
2000	17.7 ± 0.5	87.3	215.3 ± 9	40.62	36.4	63.6
1000	16.1 ± 0.9	85.8	232.7 ± 6	35.82	31.7	68.3
500	16.8 ± 1.1	100	327.4 ± 8	9.70	16.3	83.7
(0 (control)	16.9 ± 1.1	100	349.2 ± 6	0.0	0.0	100
Methanol extract						
2000	16.8 ± 0.5	100	317.2 ± 11	12.52	18.4	81.6
1000	17.1 ± 0.9	100	331.2 ± 6	8.65	10.6	89.4
500	18.0 ± 0.7	100	307.8 ± 5	15.11	10.6	89.4
(0 (control)	17.2 ± 1.3	100	362.5 ± 8	0.0	0.0	100
β-carboline alkaloids						
2000	16.7 ± 0.9	70.8	180.5 ± 6	50.19	48.3	51.7
1000	16.9 ± 1.1	76.2	237.4 ± 8	34.52	37.7	62.3
500	16.3 ± 0.8	100	301.6 ± 4	16.82	20.3	79.7
(0 (control)	16.8 ± 1.2	100	355.3 ± 6	0.0	0.0	100

***: Mortality was based on % of larvae that did not pupate.**
****: Data were corrected by using Abbott's formula (1925).**

Increasing the concentration of alkaloid mixture in the diet reduced larval weight and raised larval mortality. About 51.7% of the larvae could not pupate and died at 2000 ppm (2 mg/g diet) concentration. The incorporation of petroleum ether extract into an artificial diet induced a strong antibiotic effect on the larval growth. The larvae fed on diet containing 2 mg/g diet of crude petroleum ether extract of *P. harmala* were relatively small after 7 days of exposure compared with the control. About 36.4% of the larvae died before pupation. At all tested concentrations, the pupation rate and pupal weight were less than those of the control. The data also revealed that increasing

the concentration of petroleum ether extract in the diet reduced larval growth and consequently affected larval development. Examination of the dead individuals showed that the pre-pupal stage was highly affected by petroleum ether extract and β-carboline alkaloid mixture. The present results are in agreement with those reported by Bentley *et al*.(1984), who found that pyrrolizidine alkaloids caused significant feeding deterrence toward the spruce budworm, *Choristoneura fumiferana* larvae, and also Harley and Thorsteinson (1967), who indicated that the alkaloid α-tomatin of *Solanum* and *Lycopersicon* interfered with growth and development of nymphs of the two-striped grasshopper, *Melanoplus bitvittatus* (Say). Also, Eldoksch *et al*.(1996) indicated that pyrrolizidin alkaloids isolated from *Senecio vulgaris* L. exhibited larval feeding deterrence and growth retardant effects to the cotton leafworm *S. littoralis* (Boisd.). It could be concluded that total β-carboline alkaloids exhibited the most potent feeding deterrent and growth inhibitory activity against *S. littoralis* larvae followed by petroleum ether extract which subsequently affected growth and development of insect larvae.

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تثبيط النمو والتطور ليرقات دودة ورق القطن باستخدام المواد القلويدية بيتاكاربولين

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تم دراسة تأثير بعض المواد النباتية المستخلصة من نبات الحرمل وهى مستخلصات البتروليم ايثير والأسيتون والميثانول وأيضاً المواد القلويدية الكلية (بيتاكاربولين الكالويد) كمواد مانعة ومعوقة للتغذية ومثبطة لنمو وتطور دودة ورق القطن.

مستخلص البتروليم ايثير ومخلوط المواد القلويدية أظهروا تأثيراً عالياً كمواد مانعة للتغذية لليرقات ومثبطة للنمو والتطور. وقد استخدم التحليل الكروماتوجرافى بالطبقة الرقيقة لفصل المواد القلويدية فى المخلوط وقدرت قيمة الـ R_f لكل منها وهى ٠,٢٤، ٠,٣٨، ٠,٦٠، ٠,٦٨.

وقد اتضح من النتائج أنه باستخدام طريقة التعريض للقطع المعاملة أن القلويدات الكلية (كاربولين الكالويد) قد أعطت التأثير المضاد للتغذية الأعلى ضد اليرقات وذلك عند تركيز ١٠٠٠ جزء فى المليون والذى أعطى ١٠٠% إعاقة لعملية التغذية. وأعطى مستخلص البتروليم ايثير تأثيراً مضاداً للتغذية أقل نسبياً بينما أظهرت مستخلصات الأسيتون والميثانول نشاط بيولوجى ضعيف.

وعند خلط المواد النباتية مع غذاء صناعى لدودة ورق القطن وجد أن هناك خفض حيوى معنوى فى وزن اليرقات والتى تناولت غذاء به تركيزات من مخلوط المواد القلويدية. وقد وجد أن ٥١,٧% فقط من اليرقات حدث لها تحول إلى طور العذراء وذلك عند التركيز المختبر ٠,٢% (٢ مليجرام/جرام غذاء). أيضاً أظهر مستخلص البتروليم ايثير نشاطاً معنوياً مضاداً للتغذية ومعوقاً للنمو. ولقد تبين من النتائج أيضاً أن مرحلة قبل تكون العذارى كانت عالية التأثر بواسطة معاملات كلاً من مستخلص البتروليم ايثير ومخلوط المواد القلويدية لنبات الحرمل مما سبب انخفاضاً معنوياً فى وزن اليرقات ومعدل تكوين العذارى.