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Antifeedant and Toxic Activity of Some Plant Extracts Against Larvae of Cotton Leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract: The biological activity of crude petroleum ether extracts of Oshar (*Calotropis procera*); Harmal (*Rhazya stricta*) and Hargal (*Solenostemma argel*) were assessed using the 4th larval instar of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). All extracts exhibited a significant antifeedant activity at the LC_{50} levels. Harmal extract deterred feeding potential of insect larvae by 52.96% but decreased to 26.76 and 18.00% for Hargal and Oshar, respectively. In nutritional assays, all plant extracts affected Growth Rate (GR mg) where Harmal caused the highest rate of efficiency and followed by Oshar against 4th larval instar fed for two days on castor leaves treated with botanical extracts (LC_{50}) and three days on un-treated leaves after that. Hargal didn't show a significant effect on growth rate compared with un-treated larvae. The differences between Consumption Index (CI mg) of larvae treated with Hramal and Hargal after five days of feeding in comparison with un-treated larvae were significant. Efficiency of Conversion of Ingested food (ECI%) to biomass reached 31.81±1.49, 26.06±1.89 and 48.67±1.54 after five days of treatment by Oshar, Harmal and Hargal, respectively. These values were 49.61±1.72, 39.12±0.54 and 53.20±0.96 for digested food utilization (ECD%) in larvae treated with the aforementioned extracts after five days, respectively. There was a remarkable inhibitory activity of plant extracts on the digestive carbohydrate enzymes, amylase and invertase *in vitro*. It was noticed that Harmal had a remarkable inhibitory action causing an average of 42.58% inhibition rate on amylase and 16.27% on invertase followed by Hargel and Oshar with inhibition rates of 33.27 and 19.58% against amylase, while these values averaged 11.19 and 5.97% of inhibition in case of invertase, respectively.

Key words: Toxic activity, antifeedants, plant extracts, Oshar, Harmal, Hargal, cotton leafworm

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd), is considered to be one of the most injurious and destructive polyphagous lepidopterous insect pests attacking crops, vegetables and fruit trees all over the world (Berlinger *et al*., 1997; Kandil *et al*., 2003). To reduce its tremendous crop losses, growers have used chemical insecticides for a long time which was the only control method. This control tactic is problematic not only because of resistance to insecticides, especially by 3rd-6th instars of this insect pest, but also the well known potential negative effects of pesticides on the environment. Other control tactics, such as botanical extracts offer desirable alternatives to using synthetic chemicals in agro-systems where protection of the environment and preservation of beneficial organisms are important (Weathersbee and Tang, 2002). The botanical compounds that can be effective against many pests, were phytochemically investigated to determine their biological activity. Several wild plant extracts or isolated active compounds have been shown to act as potent acute or chronic insecticides (Sammour, 1996; Emara *et al*., 2002; Talukader, 2006), antifeedant and insect growth regulator (Abo El-Ghar *et al*., 1996) against a variety of insect species including *S. littoralis*. Accordingly, the present investigation aims to find out some other alternative control methods against cotton leafworm. Therefore, the study evaluates the effects of sublethal concentration (LC_{50}) of three wild plant extracts, Oshar (*Calotropis procera*); Harmal (*Rhazya stricta*) and Hargal (*Solenostemma argel*) growing in Saudi Arabia on the consumption and utilization of food as well as growth rate of 4th instar of the cotton leafworm larvae *S. littoralis*. The changes in the midgut carbohydrate enzymatic activity were also assessed.

MATERIALS AND METHODS

Tested plants preparation and extraction: The fresh leaves of the three domestic wild plants Oshar (*Calotropis procera*, Asclepiadaceae); Harmal (*Rhazya*

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stricta, Apocynaleae) and Hargal (*Solenostemma argel*, Asclepiadaceae) were collected from Al-Qassim region, Saudi Arabia in 2004. Samples were left to dry by air under normal conditions in laboratory for two weeks. The dried leaves were subsequently ground and extracted according to the method adopted by Freedman *et al.* (1979). Each extract was evaporated under vacuum at 40-50°C where the obtained residue was weighted. LC_{50} of each extract was prepared in petroleum ether as a solvent.

Tested insect species: Laboratory strain of cotton leafworm *S. littoralis* was reared feeding on castor leaves under controlled conditions in laboratory (25±2°C and 65%±5 RH) according to El-Defrawi *et al.* (1964). The tests were carried out using 4th instar larvae of cotton leafworm.

Laboratory feeding tests: The LC_{50} of each plant extract diluted in petroleum ether was applied by pipette to both surfaces of castor bean leaf discs (7 cm in diameter). These discs were placed in small glass jar. A similar group of discs were treated with only petroleum ether and used as control. About fifty larvae were left without feeding for 24 h and their weights were recorded. After that, the starved larvae were divided into five replicates (10 larvae each) and provided with the treated leaf discs and left under the aforementioned conditions for 24 h. The larvae were then reweighed where starvation percentages of tested larvae were calculated according to the equation of Mostafa (1969) and Abdel-Mageed *et al.* (1975).

$$\text{Starvation (\%)} = C - E/C - S \times 100$$

Where:
C = Mean weight gain of untreated larvae after 24 h
E = Mean weight gain of treated larvae for each extract after 24 h
S = Mean weight gain of starved untreated larvae after 24 h

Metabolic parameters: The experiment was conducted to determine the effect of tested plant extracts LC_{50} on the nutritional indices of the newly moulted 4th instar latrvae of *S. littoralis*, which were previously starved for 3-4 h before treatment to ensure an empty intestine. Insect larvae were fed on treated castor leaves for 48 h and then fed on untreated leaves for three successive days. Untreated larvae in control were provided with leaves exposed to the solvent only for the same periods as mentioned. Each treatment was replicated five times 10 larvae each. On the other hand, fresh untreated leaves were kept in clean jars without larvae under the same conditions to determine the natural loss of moisture, which was used for calculating the corrected weight of consumed leaves. Weight of treated and untreated larvae were recorded daily before and after feeding. Faeces discharged by larvae were weighed daily.

The following parameters were calculated according to Waldbauer (1968) as follow:

Consumption Index (CI) = C/(T×A).
Growth Rate (GR) = G/(T×A).
Approximate Digestibility (AD) = (C-F/C)×100.
Efficiency of Conversion of Ingested food to biomass (ECI) = (G/C)×100.
Efficiency of Conversion of Digested food to biomass (ECD) = G/(C-F)×100.

Where:
C = Fresh weight of consumed leaves
T = Duration of feeding period
A = Mean fresh weight of larvae during feeding period
G = Fresh weight gain of larvae
F = Faeces weight during feeding period

Moreover, the mean fresh weight of treated leaves consumed by insect larvae was corrected by using the formula of Ghanema (2002):

Corrected weight of consumed treated leaves = (Cb/Ca)× Ta

Where:
Cb = Initial fresh weight of leaves without larvae.
Ca = Final weight (after exposure to natural dryness for 24 h) of leaves without larvae.
Ta = Final weight of treated leaves after feeding for 24 h.

Daily weight of consumed treated leaves/larva = (initial weight of treated leaves before feeding-corrected weight of treated leaves after feeding) /No. survived larvae.

Biochemical studies: The activity of carbohydrate digestive enzymes amylase and invertase were determined in larval homogenates according to method of Ishaaya and Swirski (1976).

Statistical analysis: All data were subjected to the Micro-Computer Program COSTAT, one way ANOVA using Student-Newman Keuls Test ($p<0.05$).

RESULTS AND DISCUSSION

The antifeedant activity of botanical extracts: Data presented in Table 1 showed that the tested botanical extracts clearly differed as antifeedants against the 4th larval instar of *S. littoralis*. It was noticed that starvation percentages averaged 18.00, 52.96 and 26.76% when larvae were treated with oshar, harmal and hargal extracts, respectively. Therefore, harmal proved to be the most effective extract as antifeedant and followed by hargal and oshar, respectively. These findings agree with previous researches who proved that harmal has alkaloids while hargal has steroids which may give both extracts their high potentiality as antifeedant agents against *S. littoralis* (Hassan *et al*., 1977; Hamed, 2001). Moreover, many authors found that the antifeedant effect may also due to the chemical constituents of plants such as alkaloids, flavonoids, terpenes, tannins and sterols (Salama *et al*., 1971; Salama and Sharaby, 1988). In general, the antifeeding effect of plant extracts depend mainly on insect species, however, the plant structure-activity relationship associated with its components on insect feeding is complex and no clear trends emerge (Bruno *et al*., 2002).

The effect of plant extracts on metabolic parameters

Consumption Index (CI): There were significant differences between the three plant extracts (LC_{50}) on the Consumption Index (CI mg) especially 24 h after treatment where the CI values averaged 3.3, 1.95 and 2.36 mg/larva treated with oshar, harmal and hargal, respectively. On the second day after treatment, there were no significant differences between consumption index values of the tested extracts but they significantly differed with untreated larvae as shown in Table 2. At the end of the experiments, data in Table 2 clearly showed that there were significant differences between consumption index values of harmal and hargal and between oshar and hargal where these values were 1.03, 0.7 and 1.95 mg/larva treated with oshar, harmal and hargal, respectively. Accordingly, the mean consumption index was 1.54, 1.14 and 1.83 mg for larvae treated with oshar, harmal and hargal extracts, respectively. The previous data cleared that harmal was the most effective extract in reducing consumption index of 4th larval instar of cotton leafworm five days after treatment and followed by oshar and hargal in comparison with untreated larvae. These results are in harmony with those of harmal effect as antifeedant agent.

Table 1: Starvation percentage (%) of the 4th larval instar of *Spodoptera littoralis* as affected by botanical extracts (LC_{50})

Treatments	Average weight at zero time (mg/larva)	Average weight after 24 h (mg/larva)	Difference* (mg/larva)	Starvation (%)
Calotropis procera	23.73	28.36	+ 4.63	18.00
Rhazya stricta	22.36	22.68	+ 0.32	52.96
Solenostemma argel	25.72	29.27	+ 3.55	26.76
Untreated check	24.34	31.19	+ 6.85	-----
Starved larvae	26.56	21.08	-5.48	-----

Average weight after 24 h-average weight at zero time

Growth rate (GR): Data in Table 3 clearly showed there were significant differences between the effect of oshar, harmal and hargal extracts (LC_{50}) on the growth rate of treated 4th larval instar of cotton leafworm and untreated larvae. Growth rate averaged 0.50, 0.35 and 0.66 mg and then averaged 0.67, 0.30 and 0.85 mg, 1 and 5 days after treatments with oshar, harmal and hargal, respectively. In other word, oshar, harmal and hargal reduced larval growth rate by a mean of 0.47, 0.29 and 0.61 mg. Therefore, it can be concluded that harmal gave the lowest growth rate of insect larvae and followed by oshar and hargal as shown in Table 3. These results agree with those of Sundaramurthy (1977) who indicated that the amount of growth reduction was proportional in general to food consumption reduced by the tested extracts. Similar results were previously obtained by Dahlman (1977) who suggested that reducing the conversion efficiency of ingested and assimilated food may give a depression of growth rate.

Table 2: Consumption index (CI mg) for the 4th larval instar of *Spodoptera littoralis* fed on castor leaves treated with botanical extracts (LC_{50}) for two days and on un-treated leaves for three days

	Consumption index (CI mg) after indicated days from treatment*					
Treatments	1st	2nd	3rd	4th	5th	Mean**
Calotropis procera	3.30±0.10c	0.96±0.07a	1.16±0.08a	1.24±0.19ab	1.03±0.07ab	1.54
Rhazya stricta	1.95±0.06a	0.95±0.02a	1.08±0.06a	1.00±0.21a	0.70±0.05a	1.14
Solenostemma argel	2.36±0.08b	1.16±0.10a	1.71±0.38b	1.95±0.30b	1.95±0.30c	1.83
Untreated check	3.96±0.18d	1.81±0.13b	2.84±0.08b	1.81±0.11b	1.50±0.13bc	2.38

*: Data are expressed as mean±SE (n = 5), **: Total means of each treatment at different time intervals, Values were analyzed by one-way ANOVA, where mean within each column followed by the same letter are not significantly different ($p<0.05$)

Table 3: Growth rate (GR mg) for the 4th larval instar of *Spodoptera littoralis* fed on castor leaves treated with botanical extracts (LC_{50}) for two days and on un-treated leaves for three days

	Growth rate (GR mg) after indicated days from treatment*					
Treatments	1st	2nd	3rd	4th	5th	Mean**
Calotropis procera	0.50±0.03B	0.26±0.01a	0.53±0.03B	0.37±0.02ab	0.67±0.04b	0.47
Rhazya stricta	0.35±0.03A	0.26±0.02a	0.25±0.03A	0.30±0.03a	0.30±0.03A	0.29
Solenostemma argel	0.66±0.06C	0.30±0.01a	0.75±0.05C	0.47±0.07b	0.85±0.04C	0.61
Untreated check	0.91±0.04D	0.46±0.02b	0.80±0.04C	0.69±0.03c	0.83±0.05C	0.74

*: Data are expressed as mean±SE (n = 5), **: Total means of each treatment at different time intervals, Values were analyzed by one-way ANOVA, where mean within each column followed by the same letter are not significantly different ($p<0.05$)

Table 4: Efficiency of conversion of ingested food (ECI%) for the 4th larval instar of *Spodoptera littoralis* fed on castor leaves treated with botanical extracts (LC_{50}) for two days and on un-treated leaves for three days

	ECI values (%) after different time intervals*					
Treatments	1st	2nd	3rd	4th	5th	Mean**
Calotropis procera	29.95±0.79b	19.36±0.71a	22.83±1.25b	29.40±1.74b	31.81±1.49b	26.67
Rhazya stricta	11.79±0.70a	30.12±1.01b	18.33±0.63a	9.93±0.83a	26.06±1.89a	19.25
Solenostemma argel	33.49±1.68b	29.54±1.04b	38.12±1.40c	39.55±1.06c	48.67±1.54c	37.87
Untreated check	32.42±1.59b	36.38±1.42c	52.35±1.54d	47.97±1.16d	50.58±0.97c	43.94

*: Data are expressed as mean±SE (n = 5), **: Total mean of different treatments after different time interval, Values were analyzed by one-way ANOVA, where mean within each column followed by the same letter are not significantly different ($p<0.05$)

Table 5: Efficiency of conversion of digested food (ECD%) for the 4th larval instar of *Spodoptera littoralis* fed on castor leaves treated with botanical extracts (LC_{50}) for two days and on un-treated leaves for three days

	ECD% values after different time intervals*					
Treatments	1st	2nd	3rd	4th	5th	Mean**
Calotropis procera	32.97±1.17b	25.23±1.29a	42.20±1.00c	36.36±0.84b	49.61±1.72b	37.27
Rhazya stricta	16.30±0.83a	43.34±1.19b	16.62±0.80a	28.95±0.96a	39.12±0.54a	28.87
Solenostemma argel	18.49±0.91a	52.60±2.51c	26.47±1.36b	45.10±1.25c	53.20±0.96bc	39.17
Untreated check	43.92±0.87c	29.03±0.94a	62.42±0.85d	51.60±0.76c	56.99±1.62c	48.79

*: Data are expressed as mean±SE (n = 5), **: Total mean of different treatments at each time interval, Values were analyzed by one-way ANOVA, where mean within each column followed by the same letter are not significantly different ($p<0.05$)

Efficiency of conversion of ingested food (ECI%): Data in Table 4 indicated that there were significant differences between the effect of each of plant extract on conversion of ingested food and untreated larvae, where the mean values of ECI% averaged 26.76, 19.25 and 37.87% for larvae treated with oshar, harmal and hargal, in comparison with untreated larvae 43.94% five days after treatments, respectively. Accordingly, harmal again proved to be the most effective extract against cotton leafworm *S. littoralis*. In other word, ECI% may vary with the digestibility of food and proportional amount of the digestible portion of food which is converted to body mass and metabolized for energy needed for vital activity. These findings agree with those of El-Shazly (1993).

Efficiency of conversion of digested food (ECD%): After 24 h, there were no significant differences between the effect of harmal and hargal on ECD values. These results obviously changed three days after treatments where there were significant differences between the effect of all tested extracts. The conversion of digested food % to untreated larvae and that may due to the disruption in metabolism rate. Harmal showed the highest potentiality in reducing the approximate digestibility (53.05%) and followed by oshar (64.28%) and hargal (70.05%) compared with untreated larvae (74.86%), respectively. biomass were 42.20, 16.62 and 26.47% for larvae treated with oshar, harmal and hargal three days after treatment, respectively (Table 5).

It is well known that the degree of food utilization depends upon the digestibility of food and the efficiency with which digested food is converted into biomass. Many authors suggested that the mode of action of the antifeedant agents may be due to the disrupt membrane integrity and metabolism in gut epithelium. Digestion inhibition may also be as result from the formation of covalent bonds with dietary proteins or digestive enzymes. Therefore, some chemical plant constituents may have direct negative effects on herbivorous as feeding deterrents and digestion inhibitors (Batista Pereira *et al*., 2002).

Approximate Digestibility (AD%): The tested extracts significantly affected the Approximate Digestibility (AD%) in treated larvae as shown in Table 6 where AD% values have obviously fluctuated within five day experiment. These values had a similar trend in case of

Table 6: Approximate digestibility (AD%) of the 4th larval instar of *Spodoptera littoralis* fed on castor leaves treated with different botanical extracts (LC_{50}) for two days and on un-treated leaves for three days

	Approximate digestibility (AD%)*					
Treatments	1st	2nd	3rd	4th	5th	Mean**
Calotropis procera	59.32±2.97b	82.76±3.26c	67.65±2.40b	49.62±3.05a	62.03±1.81b	64.28
Rhazya stricta	46.74±2.18a	41.28±1.12a	53.48±2.54a	69.94±4.00b	53.81±3.71a	53.05
Solenostemma argel	79.74±2.59c	67.81±1.70b	64.36±2.29b	57.85±2.15a	80.48±2.15d	70.05
Untreated check`	78.13±0.37c	80.01±0.38c	72.49±4.67b	72.86±3.46b	70.81±0.78c	74.86

*: Data are expressed as mean±SE (n = 5), **: Total mean of different treatments after different time interval, Values were analyzed by one-way ANOVA, where mean within each column followed by the same letter are not significantly different ($p<0.05$)

Table 7: Effect of different botanical extracts (LC_{50}) on the digestive enzymes of the 4th larval instar of *Spodoptera littoralis*

	Digestive enzymes activity (µmol) glucose/min/g tissue*			
	Amylase		Invertase	
Treatment	Enzyme activity±SE	Difference (%)	Enzyme activity±SE	Difference (%)
Calotropis procera	40.46±0.46c	19.58	81.96±0.86c	5.97
Rhazya stricta	28.89±0.56a	42.58	72.98±1.02a	16.27
Solenostemma argel	33.57±0.94b	33.27	77.41±0.84b	11.19
Untreated check	50.31±0.71d	------	87.16±0.72d	------

*: Data are expressed as means±SE (n=5). Values were analyzed by one-way ANOVA, where means within each column followed by the same letter are not significantly different ($p<0.05$)

untreated larvae and that may due to the disruption in metabolism rate. Harmel showed the highest potentiality in reducing the approximate digestibility (53.05%) and followed by oshar (64.28%) and hargel (70.05%) compared with untreated larvae (74.86%), respectively.

From the previous results, it can be concluded that plant extracts may offer a source of compounds that have a high potentiality as pest control agents.

The effect of plant extracts on digestive enzymes: Data showed that the tested plant extracts clearly reduced invertase enzyme activity of treated larvae compared with untreated ones. Table 7 indicated that reduction percentages of invertase enzyme were significantly affected by extracts used where harmal highly reduced its activity and followed by hargal and oshar. Reduction percentages averaged 72.98, 77.41 and 81.96 unit when larvae were treated with the aforementioned plant extracts, respectively. The influence of these extracts were significantly higher on amylase enzyme activity where the reduction averaged 19.58, 42.58 and 33.27% which were represented by enzyme activity of 40.46, 28.89 and 33.57 unit when larvae were treated with oshar, harmal and hargal, respectively. Harmal repeatedly proved to be the most effective extract influencing amylase and invertase enzymes and that may be due to its antifeeding effect which may as a result of reduction in digestive enzyme excretion. These results agree with those obtained by El-Naggar (1999) and Salem *et al.* (2003). In conclusion, further studies are required to isolate and identify the chemical components in these plants which are responsible as antifeedant agents.

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