

BIOLOGICAL AND BIOCHEMICAL EFFECTS OF TWO PLANT EXTRACTS ON THE BLACK CUTWORM *AGROTIS IPSILON* (HÜFN.)

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(Manuscript received 2 October 2007)

Abstract

The biological and biochemical effects of the two plant extracts i.e. lemon and orange peels were studied on the Black Cut Worm (BCW) *Agrotis ipsilon*. For the biological studies, of the second instar larvae were treated by series concentrations of each extract (0.25, 0.50, 1, 2 and 4%). To determine the aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and total lipid contents in 4th instar larvae, the 2nd instar larvae were treated with the LC₅₀ concentration of each extract. Results showed that the two tested extracts caused a significant increase in duration of larval and pupal stages. While, induced a significant decrease in the pupation, adult longevity and fertility. Also, the two tested extracts reduced the activity of AST and ALT enzymes and decreased in content both of total protein and total lipid for 4th larval instar.

INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hüfn.) has been the most destructive insect pest, attacking many valuable field crops in Egypt. Control of this pest in Egypt, has usually depended exclusively on conventional insecticides which are costly and cause problems of resistance, residues, pollution and health hazards. Several plant extracts or isolated active compounds have been shown to act as repellents or attractants (El-Shershby *et al.*, 2004) or as potential acute or chronic insecticides (Abdalla and Sammour, 1991-1992) or as antifeedants (Khalil *et al.*, 1994) against a variety of insect species including *A. ipsilon*.

This work was designed to evaluate some of the biological and biochemical activities of two tested plant extracts, i.e. lemon and sweet orange treated as 2nd larval instar of *A. ipsilon*.

MATERIALS AND METHODS

1- Tested Plants :

Citrus aurantifolia (lemon) and *Citrus sinensis* (sweet orange) were obtained from the local market.

2- Tested Insect :

Larvae of the black cutworm *Agrotis ipsilon* (Hüfn.) were obtained from the laboratory culture of Plant Protection Research Institute, ARC. This strain was reared in the laboratory under constant conditions of $25 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ R.H. as mentioned by (El-Shershby *et al.*, 2004).

3- Extraction Technique :

Lemon and orange peels were prepared according to the method described by El-Sayed and Abdel-Razik (1984-1985). The crude extract of the two tested plants were weighted and dissolved in the ethyl alcohol to get the appropriate concentrations (0.25, 0.50, 1, 2 and 4%).

4- Biological Studies:

The experiments were carried out on the 2nd larval instar that starved for 8hrs. Then, castor bean oil leaves were dipped in different conc. (0.25, 0.50, 1, 2 and 4%) of tested plant extracts and left in the air for 1hr. to insure that the complete evaporation of solvents, and then introduced to the larvae for feeding for 24hrs. New untreated castor bean oil leaves were replaced for feeding till pupation. Four replicates contained 10 larvae/glass jar (1L-volume) were used for each treatment till the 4th larval instar and also for the control experiments, which carried out, by solvent only. The 4th instar larvae were reared individually in small plastic pot (4x7cm) to avoid cannibalistic behavior until pupation. The pupae were placed individually in a glass vial. Adults were paired in glass jars (2L-volume) with a sex ratio of (1:1) and fed on (2%) sugar solution. These tests were carried out to determine the larval mortality, larval and pupal durations, percentage of pupation, incubation period, no. of egg hatching, adult longevity and life span.

5- Biochemical Studies:

To study the physiological effects of the two tested extracts, the responses on the 4th instar larvae were determined. The second instar larvae were treated with the LC₅₀ concentration of each extract. Larvae were fed on castor bean oil leaves treated with the LC₅₀ concentrations for 24 hrs. Clean untreated castor bean oil leaves were replaced for feeding till the 4th larval instar. Four replicates contained 10 leaves/glass jar (1L-volume) were used for each extract and also for the control without treatment. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and total lipid contents were determined on the 4th larval instar.

5.1- Determination of AST and ALT Enzymes:

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined according to the method of Reitman and Frankel (1957) using commercial reagents. This method depends on photometric determination of the

concentration of oxaloacetate hydrazone in case of AST or pyruvate hydrazone in case of ALT formed with 2, 4-dinitrophenyl hydrazine.

5.2- Determination of Total Protein:

The total protein content was determined by using the Biuret method in which biuret reagent was added to larvae homogenate and left to cool (Wooten, 1994), then the optical density (O.D.) was measured. The total protein concentration was calculated according to the equation:

$$\text{Total protein} = \frac{\text{Reading of test} - \text{Reading of blank}}{\text{Reading of standard} - \text{Reading of blank}} \times 100$$

Where:

Blank is biuret reagent.

Standard is bovine serum albumin.

5.3- Determination of Total Lipid:

The total lipid content was determined according to Knight *et al.*, 1972. The total lipid concentration was calculated according to the equation:

$$\text{Total lipid} = \frac{\text{Reading of test}}{\text{Reading of standard}} \times \text{conc. of standard}$$

6- Calculations and Data Analysis :

The percentage of larvae mortality was corrected according to Abbott's formula (Abbott, 1925) and differences between means were analyzed by using analysis of variance (F-test and least significant difference at 5% level of probability).

RESULTS AND DISCUSSION

The present study was carried out to clarify the effect of the two tested plant extracts (lemon and orange) on the BCW on some biological aspects of different stages. While, treated the 2nd larval instar with the LC₅₀ concentration of each tested extract to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and total lipid contents on the 4th larval instar.

1- Biological Studies:

1.1- Effect on Larval Stage:

Data in Table 1 revealed that, the lemon extract was more effective than orange extract, while the first extract gave 85.40% larval mortality after 2 days at the concentration 4%, orange extract gave 74.97% at the same concentration. The two extracts (lemon and orange) caused significant increase in larval duration of *A. ipsilon* showing 27±0.81 and 26±0.81 days, respectively at 4% concentration compared to 23 ± 0.81 days in the control. The plant extract may be delaying the moulting process (Khalil *et al.*, 1994). These aforesaid results similar to that recorded by Emará *et al.*

(2002) reported that lemon extract was more toxic against larvae of *Spodoptera littoralis* than orange extract and also, Osman (2006) who found that lemon oil was more toxic and effective larvae of *A. ipsilon* and *S. littoralis* than orange oil and these oils caused increases in larval duration of these insects.

1.2- Effect on Pupal Stage:

The percentage of pupation was decreased with an increase of the concentrations of the two tested extracts. While, the pupal duration was increased with the increase of the concentrations of two plant extracts, which was recorded 12.25 ± 0.50 and 11.75 ± 0.50 days at concentration 4% for lemon and orange, respectively, compared to 9.75 ± 0.50 days in the control. The decrease of percentage of pupation and increase of pupal duration may reflect metamorphosis disruption by plant extract (Khalil *et al.*, 1994). Similar findings were also reported by many authors using the extract of *Abrus precatorious* (Indian liquorice) for *S. littoralis* (Dimetry & Abdalla, 1991-1992) and Lemon and orange oils for *A. ipsilon* and *S. littoralis* (Osman, 2006).

1.3- Effect on Fertility:

Data in Table (1) indicate that, the percentage of egg hatching (fertility) was decreased gradually with the increase of concentration used. The lemon extract has relatively higher effect on the fertility ($44.75 \pm 3.56\%$) than orange extract ($49.22 \pm 0.77\%$) at 4% concentration compared to $89.25 \pm 4.55\%$ in the control. In the present investigation, the suppression of egg production pretreated female may be due to interference of the extracts with the oogenesis and vitellogenesis processes. However, in lepidopterous insects, the corpus allatum hormone stimulated ovarian development and formation of mature eggs in adult female. Consequently, it seems likely that the plant oils interfere with these processes and in turn may lead to reduction of the reproductive capacity of treated adult females. This may be also attributed to sterilization of either females and/or males, or may be due to inhibiting of the sperms to be transferred to the female during copulation (Ismail, 1980). This result was declared earlier by many investigators who used lemon extract for the cotton leaf hopper, *Amrasca devastans* (Saxena and Basit, 1982); lemon and orange extracts for *S. littoralis* (Emara *et al.*, 2002) and lemon and orange oils for *A. ipsilon* and *S. littoralis* (Osman, 2006).

1.4- Effect on Adult Longevity:

Data in Table 1 showed that the adult longevity decreased with the increase of the used concentrations reaching 3.25 ± 0.05 and 6.00 ± 0.95 days at 4% concentration in lemon and orange extracts, respectively, compared to 12 ± 0.81 days in the control. This decrease returned to the oils in these extracts, were affected antifeedant and repellent against the larvae and resulted weakness larvae that were caused an inhibit in all biological activities. Lu and Metcalf (1978) who suggested that such decreases due to the accumulation of toxic substances in any organism and

affected on longevity of *Musca domestica* and *Phormia regina*. Similar results were also reported against the same insect species using different extracts and oils against *A. ipsilon* and *S. littoralis* (Dimetry and Abdalla, 1991-1992; Emara *et al.*, 2002 and Osman, 2006).

2- Biochemical Studies:

2.1- Effect on AST and ALT Enzymes:

Data in Table 2 indicated that, the two tested plant extracts caused reduction in AST and ALT enzymes showing 19.46 and 29.18% for lemon extract and 14.85 and 16.24% for orange extract compared with untreated larvae. These results are in agreement with Osman (2004), who found that the AST and ALT enzymes decreased activity when 4th larval instar treated with plant extracts. The transaminases (AST and ALT) are key enzymes in the formation of non-essential amino acid, in metabolism of nitrogen waste and gluconeogenesis (Mordue and Goldworthy, 1973). The same authors stated that the changes in transaminases levels have been correlated with protein anabolism or catabolism. The decrease or increase in the transaminases activity are may due to usage of botanical extract and its effects on neurosecretory hormonal effect (Bakr *et al.*, 2002).

2.2- Effect on Total Protein:

Lemon and orange peel extracts caused reduction in protein content of 4th larval instar of *A. ipsilon* indicating 57.47 and 55.27%, respectively compared with untreated larvae. These findings are coincide with those of Chitra and Reddy (2000) and Bakr *et al.* (2002). In general, proteins are the most complex and at the same time the most characteristic of living matter. They are present in all viable cells; they are the compounds which, as nucleoproteins, are essential to the process of cell division and, as enzymes and hormones, control many chemical reactions in the metabolism of cells. Thus, the inhibition of protein can explain some other experimental results such as reduction in weight, slow development, tissue degeneration and preventing adult emergence.

2.3- Effect on Total Lipid:

Data in Table 2 indicated that, the two tested extracts lemon and orange caused reduction in lipid content of 4th larval instar of *A. ipsilon* reaching 41.46 and 29.26%, respectively compared with untreated larvae. These results are in agreement with that obtained by Bakr *et al.* (2002) who reported that lupine, zanzalakht and lemon extracts have significant inhibition in lipid content (-80%) in case of 4th larval instar of *S. littoralis* after treatment of the 2nd larval instar with sublethal conc. of this extracts. In general lipids are essential structural components of the cell membrane and cuticle. They provide a rich source of metabolic energy for periods of sustained energy demand. Also, they facilitate water conservation both by the formation of an impermeable cuticular barrier, by yielding metabolic water upon oxidation, and they include important hormones and pheromones.

Table 1. Effect of lemon and orange peel extracts on some biological aspects of different stages of *Agrotis ipsilon* after treated as 2nd larval instar.

Conc. %	Lemon extract						Orange extract							
	Corrected larval mortality % (after 2 days of treatment)	Larval duration (days)	Pupation %	Pupal duration (days)	Egg hatching % (fertility)	Adult longevity (days)	Life span	Corrected larval mortality % (after 2 days of treatment)	Larval duration (days)	Pupation %	Pupal duration (days)	Egg hatching % (fertility)	Adult longevity (days)	Life span
0.25	54.15	25.25±0.50	84.40±0.74	10.75±0.95	81.48±1.69	8.25±0.95	48.75±1.26	43.70	24.75±0.50	87.27±0.82	10.25±0.50	83.37±1.30	9.00±0.81	48.50±1.00
0.50	60.38	25.75±0.50	79.73±1.44	11.00±0.81	66.18±4.96	7.50±0.57	48.75±1.50	52.05	25.25±0.50	82.95±0.44	10.75±0.50	74.23±4.09	7.75±0.50	48.25±0.50
1.00	62.45	26.25±0.50	61.33±1.35	11.50±0.57	58.90±1.45	5.25±0.50	48.25±0.96	54.20	25.50±0.57	68.62±1.61	11.00±0.81	65.79±2.14	6.50±0.57	47.75±1.26
2.00	75.00	26.50±1.00	53.63±1.04	11.75±0.50	49.78±1.04	4.75±0.50	48.50±1.29	64.55	25.50±0.57	59.60±1.80	11.25±0.50	64.90±3.62	6.25±0.57	48.00±0.82
4.00	85.40	27.00±0.81	44.38±1.78	12.25±0.50	44.75±3.56	3.25±0.50	48.50±1.73	74.97	26.00±0.81	50.35±2.96	11.75±0.50	49.22±0.77	6.00±0.95	48.75±1.50
Unreared check	0	23.00±0.81	90.83±1.00	9.75±0.50	89.25±4.95	12.00±0.81	49.00±1.41	0	23.00±0.81	90.83±1.00	9.75±0.50	89.25±4.55	12.00±0.81	49.00±1.41
LSD at 5%		1.06	1.89	0.96	4.02	0.99	NS		0.96	2.83	0.84	3.71	1.13	NS
				0.228							0.482			

LSD: Least significant differences between the means.

Means followed by same letters in each column are not significantly different at $P = 0.05$.

Table 2. Changes in amino acid transferase (AST and ALT) activities and total protein and total lipid contents of 4th larval instar of *Agrotis ipsilon* after treated as 2nd larval instar with LC₅₀ conc. of lemon and orange peel extracts.

Plant extract	AST (u/mg)		ALT (u/mg)		Total protein (u/mg)		Total lipid (u/mg)	
	M±S.E	Change %	M±S.E	Change %	M±S.E	Change %	M±S.E	Change %
Lemon	767.12±36.85 c	-19.46	97.32±3.54 c	-29.18	0.854±0.05 b	-57.47	0.048±0.01 b	-41.46
Orange	811.05±18.32 b	-14.85	115.10±5.19 b	-16.24	0.898±0.12 b	-55.27	0.058±0.02 b	-29.26
Untreated check	952.52±8.76 a	-	137.42±3.89 a	-	2.008±0.03 a	-	0.082±0.00 a	-
LSD at 5%	15.39	-	8.47	-	0.123	-	0.015	-

LSD: Least significant differences between the means.

Means followed by different letters in each column are significantly different at $P = 0.05$.

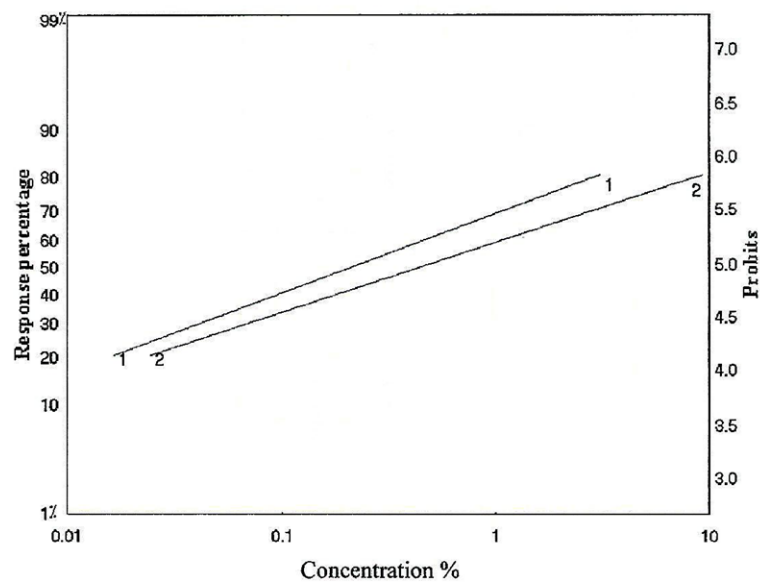


Fig. 1. Toxicity regression lines two days after feeding the 2nd instar *A. ipsilon* larvae on castor bean oil leaves treated with the tested extracts.

- 1- Lemon peel extract.
- 2- Orange peel extract.

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التأثيرات البيولوجية والبيوكيميائية لمستخلصين نباتيين على الدودة القارضة السوداء

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تم دراسة التأثيرات البيولوجية والبيوكيميائية لمستخلصين نباتيين هما قشور كل من الليمون والبرتقال السكري على الدودة القارضة السوداء، حيث تم معاملة يرقات العمر الثاني بتركيزات مختلفة من المستخلصين مجال الإختبار (٠،٢٥، ٠،٥٠، ١، ٢، ٤%) للدراسات البيولوجية ومعاملة يرقات العمر الثاني بالجرعات النصف مميتة لهذين المستخلصين لتقدير نشاط أنزيمي ALT، AST وكذلك المحتوى البروتيني الكلي والليبيدات الكلية للعمر اليرقي الرابع. أظهرت النتائج أن كلاً من المستخلصين المختبرين أحدثا زيادة معنوية في طول فترة كل من طوري اليرقة والعزراء وانخفاضاً معنوياً في النسبة المئوية للتخدير وفترة حياة الفراشات والنسبة المئوية لفقس البيض الناتج وكذلك أحدثا خفضاً معنوياً في نشاط أنزيمي ALT، AST مع إنخفاض المحتوى البروتيني الكلي والليبيدات الكلية للعمر اليرقي الرابع.