EFFECT OF SOME PLANT EXTRACTS ON BLACK CUT WORM *AGROTIS IPSILON* (HUFN). UNDER LABORATORY CONDITIONS

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Abstract

The effect of different concentrations for both of water flae-bane leaves and mango seed plant extracts were studied especially their biological and biochemical effects on the 4th larval instar of *Agrotis ipsilon* using toxic baits. The results clearly demonstrated that the tested extracts achieved significant increase in the pupal longevity and significant reduction in the larval longevity and pupal weight besides the percentage of pupation. Also, on the adult longevity for either male or female. There was remarkable significant increase in the percentage of malformation for both pupae and adults. The best treatment among all the tested extracts was water mango seed extract at 5% concentration followed by water flae-bane leaves at the same concentration. The biochemical studies proved that water mango seed extract caused increase in the secretion of both of glucsidase and Trehalse enzymes, and decrease in the secretion of acetyl choline esterase. While, water flae-bane leaves extract caused increase in the secretion of acetyl choline esterase. From this point of view, may be the extracts had an effect on the rate of glucose absorption in the blood.

INTRODUCTION

The growing awareness of hazards associated with the large scale of synthetic insecticides has evoked a world-wide interest of pest control agents from plant origin (Marin-Bettelo. 1977).

These natural products are mainly plant extracts which prove to have deleterious effects on target insects, without any problems can the environment suffering from contamination with harmful toxicants (Zhao *et. al.,* 1998). The use of natural product from plant origin is a new trend which may proof efficiency for pests' control. These efficacies are manifested in several ways, including direct toxicity (Hiremath *et. al.,* 1997) and suppression of calling behavior (Khan and Sexena, 1986).

The black cut worm, *A. ipsilon* (Lepidoptera: Noctuidae) is one of the most destructive insect pest attacking different field crops, such as cotton, soybean, corn, potatoes and tomatoes not only in Egypt but also in several countries of the throughout the year. Great losses occurred in yield due to *A. ipsilon* infestation especially at seedling stage.

The present work aims to study the effect of adding extracts to baits on some biological and biochemical aspects of 4th larval instar of *A. ipsilon* in laboratory.

MATERIALS AND METHODS

1. Insects

The tested insects in this study were obtained from the black cut worm department, plant protection research institute. ARC, Dokki, Giza, in which they were reared in the laboratory on castor leaves, *Ricinus communis* inside glass jars provided with saw dust layer in the bottom to absorb humidity according to Abdel-Rahim (2002) under conditions of 26 ± 1 °C and 65 – 70 % R.H. . Fourth instar larvae were chosen individually were fasted for one hours before experiment. The newly formed pupae were maintained inside clean jars until moths emergence.

2. Preparation of extracts

Two plants were chosen, washed by boiling water, air dried and grounded as powder in the laboratory. Extraction method described by Emara *et. al.,* (1994) and Yacoub (2006), in which extracts were prepared by soaking 25 gm of plant powder previously obtained from the chosen plants to 500 ml boiled water and were blended in a blender for 15 min., then filtered. The plants in this study – belonging to different plant families – were examined to evaluate their biological and biochemical effects on the 4th larval instar of *A. ipsilon*.

The scientific & English name and the used parts of the plants are shown in the following table:

Scientific name	Family	English name	Part used
Mangifera domestica	Anacacaridiaceae	Mango	Seed
Pulicaria dysenterica	compositae	Flea-bane	leaf

3. Preparation of baits

Three concentrations of each plant extract were prepared as 1.25, 2.5, 5%. Baits were prepared by mixing 25 gm of wheat bran, 25 cm^3 treacle solution as ratio 1 gm : 1 ml with a complete hand mixing and the mixture was left for 8 hours in room temperature.

4. Biological studies

Laboratory experiments were carried out to study the effect of the previous extracts when they were mixed with baits against 4th larval instar of *A. ipsilon.* Sixty larvae were divided into 4 replicates, kept individually in small plastic pots (3 x 6 cm) containing about 10 gm of the previously prepared baits after 3 days, the remaining alive larvae were allowed to feed on caster oil leaves to complete their life cycle.

Larvae mortality was daily inspected until pupation. Mortality percentages were calculated using the following formula:

$$\% \text{ Mortality} = \frac{\text{No. of dead larvae}}{\text{total No. of larvae}} \times 100$$

Larval, pupal duration, adult longevity for male & female (days), percentage of pupal and adult malformation and pupal weight, all were determined also, percentages of pupation and moth emergence were calculated.

5. Statistical analysis

The total percentage of larval mortality were recorded and corrected according to the check by using Abbot formula, 1925. The obtained data of the biological studies were statistically analyzed through Excel program for Windows 7 computer to determine the F value and least significant difference L.S.D. at 0.05% confidence degree.

6. Biochemical studies

6.1. Chemicals

Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA) Commasie brilliant blue G-250 was from sigma (sigma chemical co.) P- nicotina (purity 97%) was obtained from Ubichem Ltd. (Ham pshire), while nicotinamide ademine dinucleotide phosphate (reduced from, $NADP.H_2$) was from BDH chemicals Ltd. (Poole, England). The rest of chemicals were of high quality and purchased from commercial local companies.

6.2. Apparatus

Insects were homogenized for biochemical analysis in a chilled glass Telfon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at – 20 °C till use for biochemical assays. Double beam ultraviolet / visible spectrophotometer (spectronic 1201, Milton Roy Co. USA) was used to measure absorbance of colored substances or metabolic compounds.

6.3. Preparation of insects for analysis

The insects were homogenized in distilled water (50 mg / 1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5 °C in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use.

6.4. Determination of tested enzymes

6.4.1. β-glucsidase determination

β-glucsidase activity was measured by assaying glucose liberated by enzymatic hydrolysis of salicin as described by Lindorth, (1988).

One ml of the reaction mixture consisted 200 µl enzyme solution 0.1 M phosphate buffer (pH 6) and 50 µ mole salicin**.** Mixtures were incubated at 35 °C for 30 min, then boil for 2 min to stop the reaction.

Glucose that liberated by salicin hydrolysis was measured enzymatically by a glucose kit (Sigma kit, Sigma Co.) optical densities was measured against blank containing boiling enzyme. Enzyme activity was expressed as µg glucose liberated / min / mg protein.

6.4.2. Acetylcholinesterase determination

Acetylcholinesterase (Ache) activity was measured according to the method described by Simpson *et. al.,* (1964) using acetylcholinebromide (AchBr) as substrate. The reaction mixture contained 200 µl enzyme solution. 0.5 ml, 0.067 ml phosphate buffer pH = 7 and 0.5 ml AchBr (3 mM). The test tubes were incubated at 37 °C for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then 0.5 ml of HCl (1 part of conc. HCl and 2 parts of Δ H_2O) was added.

The mixture shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M $Fecl_3$ in 0.1M HCl) was added and mixed well. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

6.4.3. Trehalase activity determination

Digestive enzymes were determined according to the method described by Ishaaya and Swirski (1976) using trehalose, sucrose, and soluble starch as substrates for trehalase, invertase and α- analyses, respectively. Generally, 20 µl of diluted solution was incubated for 10 min at 30 °C with 250 µl 3% trehalose solution and 230 µl phosphate buffer (pH 5.4, 0.1M). The reaction was stopped by adding 250 µl DNS reagent to each tube in boiling water 5 min. Samples were cooled, diluted with 2.5 ml H_2O and was read at 550 nm on Spectronic 1201 (Beckman. USA).

Glucose was used as a standard. Appropriate dilutions of enzyme supernatant were used to obtain a linear production of glucose equivalents.

Generally, for each test, trehalase activity was determined from triplicate analyses of three groups of insects. The enzyme activity was expressed as µg glucose released / min / gm fresh weight.

7. Statistics

All experiments contained 3 – 4 replicates (insects homogenates) and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one – way of variance (ANOVA) using costat statistical software (cohort software, Berkeley). When the ANOVA statistics were significant ($p < 0.01$). means were compared by the Duncan's multiple range test.

RESULTS AND DISCUSSION

1-Effect of different plant extracts on certain biological aspects

1.a. Larval duration

Data presented in table (1), showed most of treatments caused significant shortage in the 4th *A. ipsilon* larval instar comparing control (23.5 days). The shortest larval duration was recorded in the treatment of water mango seed extract at 5% concentration being 15.25 days. Followed by water flea-bane leaf extract at same concentration achieving 16.5 days. On the contrary, the longest larval duration was obtained in the case of the treatment of water flea-bane leaf extract (1.25%) and mango seed extract being (20.5 and 19.25 days, respectively). The remaining treatments had intermediate effect on the larval duration achieving 17 & 18.5 days for water mango seed extract and flea-bane leaf extract both of them at 2.5% concentration, respectively.

1.b. Percentage of pupation

Data presented in Table (1), demonstrated that the 4*th* larval instar of *A. ipsilon* that were fed on castor oil leaves treated with the high concentration of water mango seed extract had the least percentage of pupation being 20% in comparison with the control which achieved 93.3%. On the other hand, flea-bane leaf water extract (1.25%) was the least in efficiency as it had 60% pupation. These results are in agree with that obtained from larval duration as incessantly.

1.c. Pupal duration

As shown in Table (1), all treatments caused prolongation and significant increase in pupal duration for water mango seed extract and water flea-bane leaf extract both of them at 5% concentration; being 15 and 14.25 days, respectively; compared with control 10.5 days. On the other hand, *A. ipsilon* larvae treated with flea-bane leaf extract at concentration 1.25 and 2.5% led to 11.5 and 11.8 days for pupal duraton, respectively. While, water mango seed extract at 1.25 & 2.5% concentrations caused 12.3 and 13.7 days, respectively.

1.d. Pupal weight

As shown in Table (1), it was remarkable that there was inverse proportion between the pupal weight and the concentration for both of the two water extracts; mango seed and flea-bane. All treatments caused variation in pupal weight ranged from 0.196 to 0.355 gm opposed to 0.438 gm for the control.

The highest effect on pupal weight was obtained from the water mango seed extract treatment at 5%, (0.196 gm / pupa) followed by flea-bane leaf extract at the same concentration; 0.204 gm / pupa. While, the least effect on pupal weight was

due to flea-bane leaf extract treatment at 1.25%, being 0.355 gm / pupa. The remaining treatments could be arranged according to their efficiency as; water flea-bane leaf extract 5% (0.204 gm / pupa), water mango seed extract 2.5%; (0.298 gm / pupa), water mango seed extract 1.25% (0.319 gm / pupa) and water flea-bane leaf extract 2.5% (0.260 gm / pupa), respectively.

1.e. Pupal malformation

Table (1), showed that there were morphological malformations which appeared due to the treatment with either one of the concentrations. The results demonstrated that all treatments caused high deformations in the developed pupae than control 2.5%.

Water mango seed extract at 2.5 and 5% concentration caused the highest malformation cases which ranged from 60 to 66.6%, respectively. While, the intermediate number of malformation caused by water flea bane leaf extract at both of the two concentrations 2.5 and 5% achieving the same percentage 50%. On the contrary, water extract of flea bane leaf and water mango at 1.25% caused the least number of malformed pupae 44.4 and 28.5%, respectively.

Pupal malformations appeared as pupa with indentation at ventral side between at antennal and wing regions and some destruction in the last abdominal regions, moulting integument remain with pupae and colored black. Also, moulting failare at last instar of larvae and emaciation of head region.

1.f. The percentage of moths emergency

Data presented in Table (1) showed that the 4th instar larvae of *A. ipsilon* which were treated by 5% concentration for water flea bane extract, and water mango seed extract at 2.5 & 5% concentration, caused the highest percentage reduction of adult emergence to 33.3% when compared with that of the control being 100%. While, the treatments at 2.5% for water flea-bane extract and at 1.25% for water mango seed extract caused decreasing in the percentage of adult emergence in which it reached 50%. From the pervious results it could be concluded that there was a significant reductions than control.

1.g. Adult longevity

Data presented in Table (1) indicated that all treatments decreased the adult longevity of *A. ipsilon* when 4th instar larvae were treated with either one of the plant extracts. Water mango seed extract at 5% ranked the first among all the treatments causing the shortest period in adult longevity being 7.5 and 8.25 days for male and female, respectively, followed by water flea bane leaf extract at 5% achieved 7.25 and 9.5 days for male and female, respectively, water mango seed extract and water flea bane leaf extract at 2.5% recorded (8.25 & 8.62 and 8.87 & 10 days for male and

female, respectively. On the other hand, concentration 1.25% of two extracts increased the adult longevity achieving 9.75 & 9.87 and 10.37 & 10.5 days for male and female, respectively.

1.h. Percentage of adult malformation

There were remarkable significant differences in percentages of adult malformation for all plant extracts. Treatments, at concentration 5% for water mango seed extract induced the highest percentage in adult malformation being 66% when compared to 0% in the control. The remaining treatments could be arranged descendingly as; water flea-bane extract at both concentrations of the extract (5% : 1.25%) achieved from 53.75 to 22.75%, respectively. The adult malformations showed zigzagged and twisted wings so those were unable to fly, broken antennae and deformed abdomen, sometimes pupal skin was remained and cover all moth body except head and its appendages also thoracic legs and throughout some observation it was the hind wings being shorter than normal

1.i. Effect on larval mortality percentages

Data presented in Table (2) clearly demonstrated that the larval mortality percentages after treating the 4th larval instar of *A. ipsilon* was influenced by the tested plant extracts. Mortality percentages were determined on the 1, 3, 5, 7 and 9 days. Water mango seed extract at 5% caused the highest mortality 80% when compared with the control 8.3%, followed by water flea bane leaf extract at 5% being 66.6%.

Generally, it was concluded that high concentration led to high mortality.

These results are in agreement with that obtained by Aboel-Ghar *et. al.,* (1994) who examined the zanzalakht extract and found that it caused malformations in the different stages of *A. ipsilon*. Also, Soad osman and El-Rawy (2008) found that lemon and orange plant extract caused a significant increase in larval and pupal duration, and induced a significant decrease in the pupation and adult longevity.

2. Biochemical studies

2.a. Glucosidase assessment

Table (3) showed changes in glucosidase enzymes levels in the 4th larval instar of *A. ipsilon* as a result of treatment with different plant extracts – water mango seed and flea bane leaf extracts – in which the amount of enzyme differs increasing or decreasing – as a mean expressed by µg / mg protein – according to the response of larvae against the two plant extracts. Water flea bane leaf extract caused the highest decrease 0.835.3 µg / mg; 32.8% decreasing than control which achieved 1242.7 µg / mg. On the contrary, Water mango seed extract caused high increase being 0.1530 µg glucosidase / mg; 23.1% than control.

2.b. Trehalse assessment

Table (4), showed variation in the rate of trehalse enzyme after determining it in the treated *A. ipsilon* 4th larval instar with the different plant extracts; in which results obtained showed similarity with that obtained previously in the glucosidase enzyme assessment. Water flea bane leaf extract caused mean 83.0 µg / mg protein as 25% reduction than control that recorded 110.7 µg glucose / mg protein. Also, Water mango seed extract caused increasing in the rate of trehalse enzyme being 127.3 µg / mg protein as 15% increasing than control.

2.c. Acetyl choline esterase (Ache) assessment

From Table (5), the results obtained were different than the one obtained from the previous two enzymes. The results clearly demonstrated that the Acetyl choline esterase (Ache) activity recorded in the treated 4th larval instar of *A. ipsilon* decreased after the treatment with water mango seed extract (22.9% reduction than control; 1.75 µg / mg protein). While the activity of the same enzyme (Ache) increased as result of treating the larvae with water flea bane leaf extract achieving 2.64 µg Ache / mg protein; 50.9% increasing than control.

Table 3. Effect of plant extracts on glucosidase enzyme; determined in the 4th instar larvae of *A. ipsilon.*

Treatments	µg glucosidase 10^3/ min / mg protein				% reduction / increasing than control
	R1	R2	R3	Mean ±SD	
water flea-bane leaf extract	802	833	871	835.3 ± 28.2	32.8
water mango seed extract	1575	1574	1441	1530 ± 62.9	23.1
Control	1227	1200	1301	1242.7 ± 42.6	

Table 4. Effect of plant extracts on Trehalse enzyme; determined in the 4th instar larvae of A. ipsilon.

Treatments	µg glucose / min / mg protein				% reduction / increasing than control
	R1	R2	R3	Mean ± SD	
water flea-bane leaf extract	86	84	79	83 ± 2.9	25
water mango seed extract	129	131	122	127.3 ± 3.8	15
Control	111	118	103	110.7 ± 6.1	–

Table 5. Effect of plant extracts on AchE; determined in the 4th instar larvae of A. ipsilon.

Treatments	µg AchBr / min / mg protein				% reduction / increasing than control
	R1	R2	R3	Mean ± SD	
water flea-bane leaf extract	2.50	2.61	2.80	2.64 ± 0.63	50.9
water mango seed extract	1.38	1.37	1.29	1.35 ± 0.04	22.9
Control	1.63	1.71	1.90	1.75 ± 0.11	

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دراسات معملية لبعض المستخلصات النباتية على دودة القارضة

رشا على الحصرى[1] ، شنـــوده سيـــد يعقـــوب[2] ، أمـــاني ســـامي الحفنى[2]

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تمت دراسة تأثير عدد من التركيزات المختلفة لكلا من المستخلص المائى لاوراق نبات رعرع ايوب ومسحوق بذور المانجو خاصة من حيث على بعض الخصائص البيولوجية والبيوكيمائية للعمر اليرقى الرابع للدودة القارضة باستخدام الطعوم السامة. وأظهرت نتائج الدراسات البيولوجية ان كلا المستخلصين محل الدراسة احدثا زيادة معنوية فى كل من طول العمر العذرى وانخفاضا معنويا فى طور العمر اليرقى ووزن العذارى اضافة لنسبة التعذير وكذلك على عمر الفراشات الذكور والاناث على حد سواء. كما احدثا زيادة معنوية فى النسبة المئوية لتشوهات العذارى والفراشات. وكان أفضل المواد المختبرة هو مستخلص بذور المانجو المائى تركيز 5% يليه بنفس التركيز مستخلص نبات الرعرع .

وأثبتت الدراسات البيوكيمائية ان مستخلص بذور المانجو المائى ادى الى زيادة فى افراز انزيمى جلوكوسيديز والتريهاليز، ونقصا فى افراز انزيم الاستيل كولين اسريز، بينما كان المستخلص المائى لنبات رعرع ايوب أدى الى زيادة افراز انزيم الاستيل كولين اسريز. ومن هنا نجد ان هذه المستخلصات ربما تؤثر على معدل امتصاص الجلوكوز فى الدم.