

Effect of some Insecticides on the Activity of Certain Enzymes on Cut Worm, *Agrotis ipsilon* and Red Palm Weevil, *Rhynchophorus ferrugineus*.

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Abstract

This study investigated the impact of some insecticides belonging to pyrethroids (cyper guard, lambadasetra, debemethrin), organophosphates (pestban, diazinon setra) and pyrazole (hatchi hatchi) on the biochemical aspects in two insects the 4th instar larvae of cut worm, *Agrotis ipsilon* and the 9th instar larvae of red palm weevil *Rhynchophorus ferrugineus*. The activities of three vital enzymes and total protein (TP) were laboratory tested at LC₅₀ concentration. These enzymes were amylase, chitinase and protease. Results showed that tested insecticides lead to decrease the activity of amylase and chitinase enzymes. On the other hand, the level of total protein and activity of protease enzyme were increased after treatment 4th instar larvae of *A. ipsilon* with the LC₅₀ for each compound. While, in 9th instar larvae of *R. ferrugineus* tested compounds caused increase in the activities of amylase, chitinase and protease enzymes. On contrary the tested compounds caused decrease in the total protein content. In general, tested insecticides were significantly affected on activities of enzymes and total protein compared with control.

Keywords: *Agrotis ipsilon*, *Rhynchophorus ferrugineus*, Amylase, Chitinase, Protease enzymes, total protein

Introduction

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is an insect pest with great agricultural importance worldwide. In Egypt, it infests cotton (*Gossypium barbadense*) seedlings and causes severe damage to many economic winter crops, for example, clover (*Trifolium alexandrinum*), wheat (*Triticum aestivum*), and bean (*Vicia faba*) (El-Kady *et al.* 1990 and Abo El-Ghar *et al.* 1994). It constitutes a major group of insect pests because of the damage they inflict to a large number of agricultural crops and their wide distribution (Vendramim *et al.*, 1982). It is one of the most dangerous species of underground pests and can feed on more than 100 host plants *viz.*, corn, wheat, cotton, soybean, vegetables and a variety of weeds (Liu *et al.*, 2015). It has habit of cutting off a seedling at ground level by chewing through the stem and got their name from this habit. One larva has the ability of cutting off several plant seedlings in a night. Sometimes they drag the cut plant parts beneath the soil and feed upon them during day time. When disturbed, the cut worms typically coil up tightly into 'C' shape. The cutworms usually remain hidden during the day and feed mostly at night. In particular, *A. ipsilon* larvae can cause serious damage at the fourth, sixth and/or higher instar stages (Showers, 1997). *A. ipsilon* and *A. segetum* (Denis & Schiffmuller) were found to be feeding on various crops (3–18% infestation) in Himachal Pradesh, India (Verma and Verma, 2002). Red palm weevil, *Rhynchophorus ferrugineus* (Olivier), is the most dangerous and important destructive borer to the palm trees cultivated in different Egyptian governorates and to palm trees in

many countries. The origin of this pest was South East of Asia (Pakistan, India, Burma, Pengladesh and Indonesia) and spread later in the other countries (Iran, Iraq, Saudi Arabia, Emirates) and recorded for the first time in Egypt by Saleh (1992). The weevils attack the top, bottom or middle of the palm tree in any stages of growth! Brand (1917), Batt and Girgis (1996) found this pest in Sharkyia, Ismailia and Qalubouia governorates (Egypt).

This research was planned to determine the biochemical effects of some insecticides on the cut worm *A. ipsilon* and red palm weevil *R. ferrugineus* on the activities of three vital enzymes amylase, chitinase and protease. Also total protein was measured to spot a light on the toxicity of such chemicals.

The two insect pests were controlled by the use of many methods. The important one of it is the use of pesticides. The mechanism and mode of action of these pesticides need to increase of study. Of these studies how pesticides effect in the activity of enzymes in order that the experiments were carried out to determine the effect of some insecticides in the activity of many enzymes and total protein contents on the cut worm *A. ipsilon* and red palm weevil *R. ferrugineus*

Materials and Methods

Insects

Rearing of the black cut worm, *Agrotis ipsilon* (Hufn):

The stock culture of susceptible black cutworm, *Agrotis ipsilon* (Hufn) was reared on castor bean leaves (*Ricinus communis*) for several generations at

laboratory conditions $27 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH. Egg masses were placed on castor bean leaves in cylindrical glass jars. The jars were covered with muslin cloth and fastened with rubber band. First instar larvae hatched within 2-3 days. The newly hatched larvae were transferred into rearing jars bottomed with sheet of towel paper to absorb excess humidity. Castor bean leaves were provided daily to the larvae in sufficient amounts. The accumulated faeces and debris were cleaned out daily. After pupation, pupae were collected and placed in wide clean jars until adults emergence. Then, the emerged adults were supplied with a piece of cotton wetted with 5-10% sugar solution a feeding source and branches of tafla (*Nerium oleander*) as a suitable site for oviposition. Newly laid egg masses were collected daily and transferred into the rearing jars.

Rearing of the Red palm weevil *Rhynchophorus ferrugineus*:

Various stages of RPW used in this study were originally obtained from infested palm trees at Al-Qassasin district (Ismailia Governorate), where those were collected by hand after the splitting the sites of injury. Each developmental stage was placed individually in rectangular plastic boxes with press-on tight-fitting lids (30 x 20 x 15 cm.). Portable wood saw was used to facilitate collecting weevils from heavily infested palm trees. The insects were cultured in a rearing room of the Insect Research Laboratory at the Plant Protection Department, Fac. of Agri., Benha University. The room was maintained at ($27 \pm 2^\circ\text{C}$, $70 \pm 5\%$ R.H.). The photoperiod was approximately 12:12. The room contained three large working benches, electrical outlets and sideboards. The room was also used as a media room for handling and preparing materials of natural diets. Larvae and adults of *R. ferrugineus* were provided with sugarcane stem pieces for feeding. *R. ferrugineus* rearing on sugarcane stem pieces was previously reported by **El-Zoghby and Abdel-Hameid, (2018)**. Natural diets were prepared for mass rearing of *R. ferrugineus* because of the availability of sugarcane in Egypt in culturing this insect. This diets were also used to avoid the use of expensive artificial diets for the culture of weevils. All plastic boxes were stored at room temperature until required. Larvae were placed on diets after total coolness.

Insecticides

hatchi hatchi 15%EC

Chemical name: 4-chloro-3-ethyl-1-methyl-N-[[4-(4-methylphenoxy) phenyl]methyl]-1H-pyrazole-5-Carboxamide

pestban 48% EC

Chemical name: O,O-dimethylO-3,5,6-trichloro-2-pyridinyl phosphorothioate

cyper gaurd 10% EC

Chemical name: (RS)-I-cyano-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-

(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (IUPAC) (RS)-I-cyano-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (Rothamsted IUPAC) cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (CA **lambada setra 5%EC**)

Chemical names:

IUPAC, A reaction product comprising equal quantities of (S)- α -cyano-3-phenoxybenzyl (Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (R)- α -cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate. CA, [1 α (S*), 3 α (Z)]-(±)- cyano (3-phenoxyphenyl)methyl3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate.

diazinon setra 60%EC

Chemical names:

0,0-diethyl-0-(2-isopropyl-6-methylpyrimidin-4-yl)phosphorothioate (IUPAC)- o.o-diethyl-0-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]phosphorothioate (CA).

debemethrin 5%EC

Chemical names:

IUPAC (S)- α -cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate CA[1R-[1 α (S*),3 α]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)- 2,2-dimethylcyclopropanecarboxylate

Toxicity tests

Black cut worm, *Agrotis ipsilon* (Hufn).

Castor bean leaves were dipped for 30 seconds in each concentration for each compound and left for natural dryness before administered to the tested fourth instar larvae. Three replicates of ten larvae/ each were made for each compound. Also, similar replicates of the same number of larvae were fed on untreated castor bean leaves to serve as check. Mortality counts were recorded after 24, 48, 72 and 96 hours.

Red palm weevil, *Rhynchophorus ferrugineus*.

The 9th newly moulted last instar of *R. ferrugineus* were fed on fresh internode piece of sugarcane stem after dipping it in different concentrations of each insecticide for 24h. Three replicates of 6 larvae/ each were made for each concentration. Also, similar replicates of the same number of larvae were fed on untreated piece of sugarcane stem to serve as check. Mortality counts were recorded after 1, 2, 3, 4, 5, 6, 7 and 8 days. Mortality percentages in the two cases were corrected for natural mortality according to **Abbott formula (1925)**. To estimate the LC₅₀ value, the corrected mortality percentages were subjected to probit analysis according to the method of **Finney (1971)**.

Biochemical studies:**Preparation of samples for biochemical assays:**

Larval samples used for biochemical assays were collected at 24hr post treatment of the 4th instar larvae of *Agrotis ipsilon* and 9th instar larvae of *Rhynophorus ferrugineus* with the LC₅₀ concentration for each compound. Untreated larvae were also used as control. For each trial, larvae were kept in clean jars. Samples were homogenized in distilled water using a Teflon homogenizer. The homogenates were centrifuged at 500 r.p.m for 10 minutes at 5°C then the supernatants were immediately assayed to determine the total soluble protein and the activities of amylase, protease and chitinase.

Determination of amylase activity:

Amylase activity was determined according to **Ishaaya and Swirski (1970)** and **Ishaaya *et al.* (1971)** by using 3,5-dinitrosalicylic acid reagent for determining the free aldehydic groups of glucose formed after sucrose or starch digestion. This reaction is based on the reduction of dinitrosalicylic acid by aldehydic groups of glucose units in basic medium. The reduced dinitrosalicylic acid is measured spectrophotometrically at of 550 nm. The invertase reaction consisted of 0.2 ml 4% sucrose, 0.1 ml 0.2 M acetate buffer (pH 5.5), and 0.1 ml **haemgenate samples**; the amylase reaction consisted of 0.1 ml 2% starch, 0.1 ml 0.2 M phosphate buffer (pH 6.0), and 0.2 ml haemolymph sample. After 60 minutes of incubation at 37°C of invertase or amylase reaction, enzyme activity was terminated by adding 0.8 ml. 3.5-dinitro salicylic acid reagent. The reaction mixture was heated for 5 min at 100°C and followed by immediate cooling in an ice bath. The activity was measured spectrophotometrically at 550 nm.

Determination of total protein content:

The protein content of the haemgenate samples was determined using folin phenol reagent according to the method of **Lowry *et al.* (1951)** as follows:

Reagents:

- 1- Dissolve 2 gm of sodium carbonate in 0.1 sodium hydroxide, keep refrigerated.
- 2- 0.5 ml cupric sulphate solution: this was prepared by dissolving 0.5 gm of cupric sulphate in 1% potassium – sodium tartarate, keep in a refrigerator.
- 3- Buffer copper sulphate solution; this was prepared by mixing one part (ml) from reagent (1) with ten parts (ml) from reagent (2), prepare fresh.

4- Folin reagent 2N; this was prepared by mixing 1 ml from Folin ciocalteus with 2 ml of distilled water. This reagent was freshly prepared.

5- Stock standard of six series of bovine albumin solutions was prepared.

Procedure:

The haemgenate samples were added to 1 ml of 5% trichloroacetic acid, and the precipitated protein was dissolved by boiling for 5 min in 2 ml of 1 N NaOH solution. Then 0.2 ml from this alkaline protein solution was placed in clean test tubes, each contained one ml of reagent (3). After 10 minuets 0.2 ml from folin reagent was added to the mixture and the contents were heated for 2.5 minutes at 50°C (to dissolve the precipitate). The tubes were allowed to stand for 10 minutes to cool at room temperature. The blanks were similarly run using 0.2 ml NaOH instead of the haemolymph sample. Reading was measured spectrophotometrically at 750 nm.

Determination of chitinase activity:

Chitinase was assayed using 3, 5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to the method described by **Ishaaya and Casida (1974)**. (A). Preparation of 3, 5-dinitrosalicylic acid reagent (DNSA) The dinitrosalicylic acid reagent was prepared by dissolving one gram of 3, 5-dinitrosalicylic acid in 20 ml of 2N NaOH solution and 50 ml of distilled water with the aid of a magnetic stirrer. Potassium sodium tartarate (30 g) was added, and magnetic stirring was continued until a clear solution was obtained. Distilled water was then added to bring the final volume to 100 ml.

Assay of chitinase activity

The reaction mixture consisted of 0.12 ml (0.2M) phosphate buffer (pH 6.6); 0.3 ml 0.5% colloidal chitin; 0.18 ml sample homogenate. After 60 min. incubation at 37°C, enzyme activity was terminated by adding 1.2 ml 3,5-dinitrosalicylic acid reagent (DNSA). The reaction mixture was heated for 5 min. at 100 °C then cooled in an ice-bath, and diluted with 1.2 ml distilled water. Undigested chitin was sedimented by centrifugation for 15 min. at 6000 rpm and the absorbance of the supernatant was determined spectrophotomeically at 550 nm. Direct reaction of N-acetylglucosamine (NAGA) with DNSA reagent, under conditions similar to those of the enzyme reaction, gives a linear plot of absorbance value versus amount of NAGA, 1 mg of NAGA giving an absorbance value of 0.78.

Determination of protease activity:

The proteolytic enzyme activity was determined by the casein digestion method described by **Ishaaya *et al.* (1971)**. The reaction mixture consisted of 0.2 ml (0.2 M) glycine buffer (pH 10), 0.4 ml 1.5% casein solution, and 0.2 ml homogenate sample.

Enzymatic activity was terminated after 60 min. incubation at 37°C and adding 1.2 ml of 5% trichloroacetic acid solution. The reaction mixture was centrifuged at 6000 rpm for 15 min. and the supernatant was taken for enzymatic activity evaluation. The proteolytic activity was determined at 280 nm as O.D. unit x 10³.

Statistical analysis:

The statistical analysis was carried out using two-way ANOVA using SPSS. ver. 22 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to Steel *et al.* (1997). Multiple comparisons were carried out applying Duncun tes.t The significance level was set at < 0.05.

Results and Discussion

Effect of some different insecticides on some biochemical aspects of the 4th instar larvae of *Agrotis ipsilon* in the laboratory:

Amylase activity:

Amylase enzyme hydrolyze the starch molecules into polymers composed of glucoses units.

Data in Table (1) and Fig.(1) Indicated that amylase activity was 49.49±1.29, 48.59±0.64, 54.75±0.54 and 57.17±0.67 µg glucose/ min/m after treatment with Lambada setra, Cypar gaurd ,Diazinon setra and debemethrin respectively, comparing with control (65.84±0.39 µg glucose/ min/m). Results showed reduction in the activity of amylase on the treated 4th instar larvae of cut worm *A.ipsilon* than the untreated ones.

Chitinase activity:

Chitinases, are hydrolytic enzymes that break down glycosidic bonds in chitin.Chitine is a component of the cell wall of insect and other animals.

Data in Table (1) and Fig. (2) indicated that, chitinase activity was 17.02±1.17, 20.26±0.28 ,18.26±0.13 and 17.12±0.17 µg NAGA/ min/ml after treatment with the LC₅₀ concentration for each compounds Lambada setra, Cypar gaurd, Diazinon setra and depmithren respectively, comparing with control (19.54±0.06 µg NAGA/ min/ml). Results showed that, only cyper gaurd caused increase on the activity of chitinase of the treated 4th instar larvae of *A.ipsilon* compared with the untreated ones. The tested compounds caused reduction in the values of chitinase activity in the supernatant homogenated larvae comparing with control (19.54±0.06 µg NAGA/ min/ml).

Protease activity:

Protease or proteinase is an enzyme which catalyze breaking down proteins.

Data in Table (1) and Fig .(3) in general indicated that, the tested compounds caused increase in the activity of protease of the treated 4th instar larvae of *A. ipsilon* comparing with the untreated ones (191.63 O.D. units x 10³/min/ml). The mean values of protease activities in the supernatant homogenated larvae reached to 224.33± 0.23, 221.07±0.77, 234.47±6.77and 235.70±4.10 O.D. units x 10³/min/ml when larvae treated at LC50 by Lambada setra, Cypar gaurd ,Diazinon Setra and Depemethrin , respectively .

Total protein:

Data in Table (1) and Fig. (4) Indicated that, the tested compounds caused increase in the mean of total protein of the treated 4th instar larvae of *A.ipsilon* comparing with the untreated ones. The values of total protein in the supernatant homogenated larvae to 326.74±3.3, 318.55±7.14, 344.78±14.44 and 341.64±5.61 ug protein/ min/ml when larvae were treated at lc₅₀ by Lambada setra, Cypar gaurd ,Diazinon setra and depemethrin , respectively comparing with 267.45± 15.24 ug protein/ min/ml of the control.

Table 1. Effect of some insecticides-treatment on certain biochemical aspects of 4th instar larvae of *Agrotis ipsilon*.

Compounds	Amylase (µg glucose/ min/ml)	Chitinase (µg NAGA/ min/ml)	Protease (O.D. units x 10 ³ /min/ml)	Total protein (ug protein/ min/ml)
lambada setra	49.49±1.29 ^b	17.02±1.17 ^b	224.33±0.23 ^{ab}	326.74±3.37 ^{ab}
cyper guard	48.59±0.64 ^b	20.26±0.28 ^a	221.07±0.77 ^b	318.55±7.14 ^b
diazenone setra	54.75±0.54 ^a	18.26±0.13 ^b	234.47±6.77 ^{ab}	349.78±14.94 ^a
Debemethrin	57.17±0.67 ^a	17.12±0.17 ^b	235.70±4.10 ^a	341.64±5.61 ^{ab}
Control	65.84±0.39 ^a	19.54±0.06 ^{ab}	191.63±4.57 ^a	267±15.24 ^a
LSD at 0.05 for	2.73	1.99	12.97	22.61

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

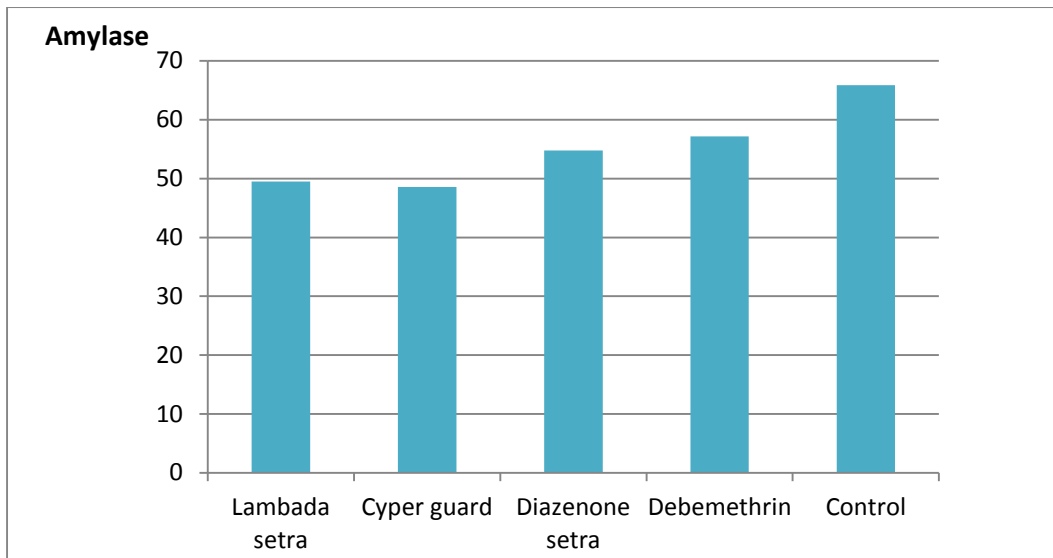


Fig.(1)

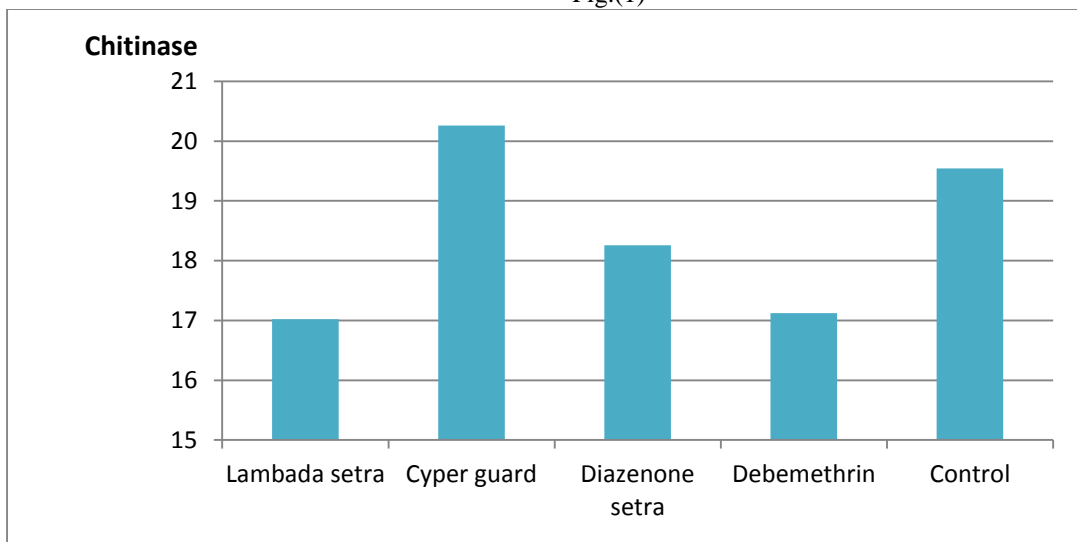


Fig. (2)

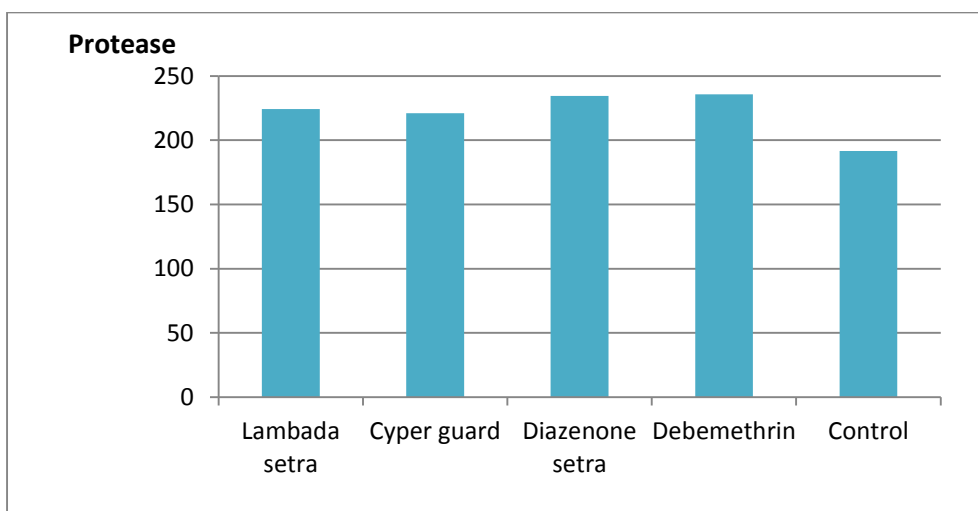


Fig. (3)

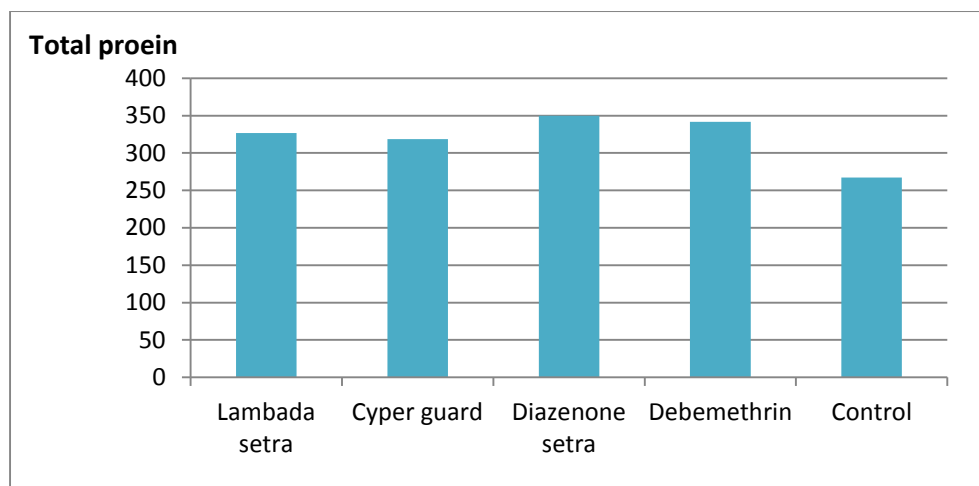


Fig. (4)

Effect of the pestban and hatchi hatchi on some biochemical aspects of the 9th instar larvae of *Rhynchophorus ferrugineus* in the laboratory.

Amylase activity:

After treatment the 9th instar larvae of *Rhynchophorus ferrugineus* with the two insecticides pestban and hatchi hatchi, Data in Table(2) and Fig.(5) indicated that the Amylase activity was 188.39 ± 0.55 and 118 ± 0.38 after treatment with pestban and hatchi hatchi, respectively, comparing with the control (128.96 ± 0.68). The results indicated that there were significant differences between the treatments of pestban and hatchi hatchi.

chitinase activity:

Data in Table (2) and Fig.(5) showed that the chitinase activity was 77.86 ± 0.25 and 67.00 ± 0.56 . When larvae treated with LC₅₀ by pestban and hatchi hatchi. Results showed increase in the activity of chitinase comparing with untreated control (50.07 ± 0.53). The results indicated that there were significant differences between treatments pestban and hatchi Hhatchi.

Protease activity:

Data in table (2) and fig.(7) revealed that, the insecticide pestban caused significant increase in the activity of protease enzymes than the control. The mean values of protease activities in the supernatant homogenated larvae reached 525.04 ± 0.56 and 346.62 ± 1.13 when larvae treated with LC₅₀ by pestban and hatchi hatchi compared with 432.37 ± 0.56 in control. There were significant differences between the two treatments of pestban and hatchi hatchi. The protease activity increased comparing with the control (432.37 ± 0.56) with the treatment of pestban and decreased by hatchi hatchi.

Total protein:

Data in Table (2) and fig. (8) showed that, tested insecticides caused significant reduction of total protein of the treated 9th instar larvae of *R. ferrigenous* than the untreated ones. The mean values

of total protein reached 443.93 ± 1.98 and 396.87 ± 119 when larvae treated with LC₅₀ of the tested insecticides compared with 681.99 ± 1.58 of the control. In general, the results showed that there was decrease in the total protein contents comparing with the control.

Sush finding are in agreement with those obtained by **Taha and Al-Hadek (2017)** who studied the effect of chlorfluazuron and diflubenzuron on biochemically of early 2nd instar larvae of *Agrotis ipsilon* to show changes in chitinase and phenoloxidase activities at three time intervals. Results showed increase in the chitinase activity at 48h compared with 24h and 72h. Also, **Zibae, et al. (2008)** evaluated the effect of diazinon and activity levels of a-amylase on *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). Results indicated that diazinon decreased activity levels of several enzymes, ATPases, LDH and a-amylase, that had a significant effect on metabolism of nutrients in insects body. **Saleem & Shakoori (1987)** found that pyrethroids sublethal concentrations decrease gut amylase activity in larvae of the beetle *Tribolium castaneum*. **Mohamady (2000)**, who investigated the effect of treatment of the 4th instar larvae of *S.littoralis* with LC₂₅ and LC₅₀ of fenvalrate on the activity of amylase enzyme at different time intervals (24, 48, 72hr). The results indicated that, there was great reduction in the activity of amylase after treatment. On the other hand, **El-Sheakh, et al(1990)** found that the treatment of 4th larvae instar of *S.littoralis* with sumithion increased the total soluble protein. It increased after 2 days of sumithion treatment. **Mohamady (2000)**, investigated the effect of treatment of the 4th instar larvae of *S.littoralis* with the with LC₂₅ and LC₅₀ of fenvalerate on the total protein at different time intervals (24,48 and 72hrs). The results indicated that there was high reduction in the level of total protein due to the treatment.

Table 2. Activity of amylase, chitinase, protease and total protein content of 9th instar larvae of Red palm weevil, *Rhynchophorus ferrugineus* as affected by different insecticides

Compounds	Amylase (μg glucose/ min/ml)	Chitinase (μg NAGA/ min/ml)	Protease (O.D. units x 10^3 /min/ml)	Total protein (μg protein/ min/ml)
Control	128.96 \pm 0.68 ^b	50.07 \pm 0.53 ^c	432.37 \pm 0.56 ^b	681.99 \pm 1.58 ^a
pestban	188.39 \pm 0.55 ^a	77.86 \pm 0.25 ^a	525.04 \pm 0.56 ^a	443.93 \pm 1.98 ^b
Hatchi hatchi	118.95 \pm 0.38 ^c	67.00 \pm 0.56 ^b	346.62 \pm 1.13 ^c	396.87 \pm 1.19 ^c
LSD at 0.05 for	1.89	1.61	2.76	5.60

a, b & c: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter.

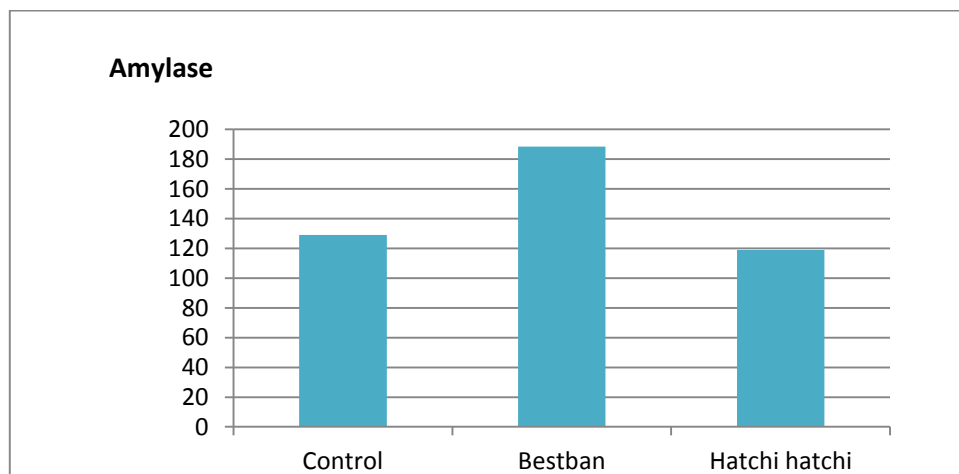


Fig. (5)

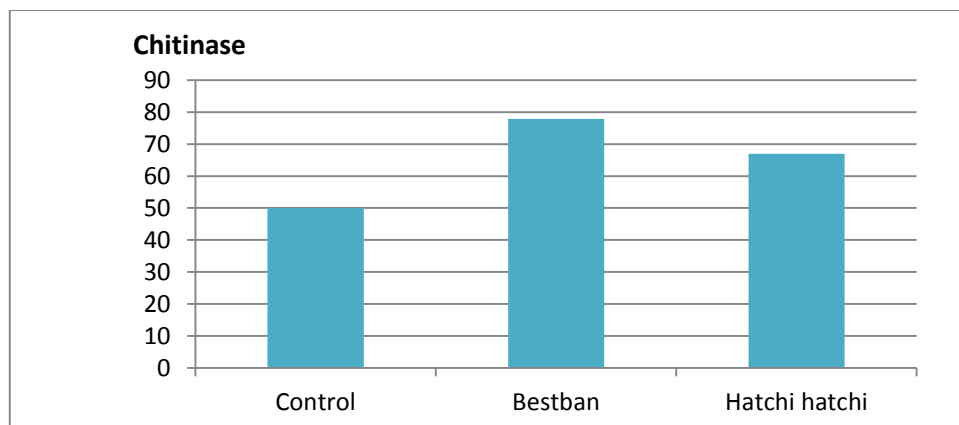


Fig.(6)

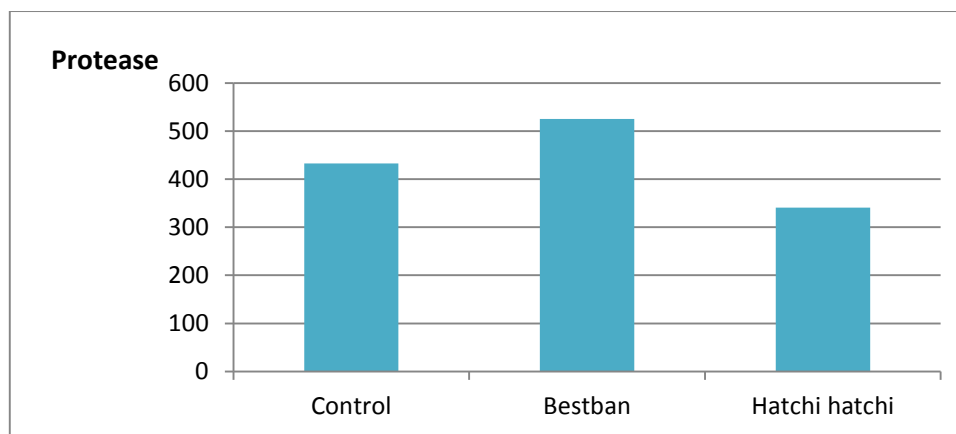


Fig.(7)

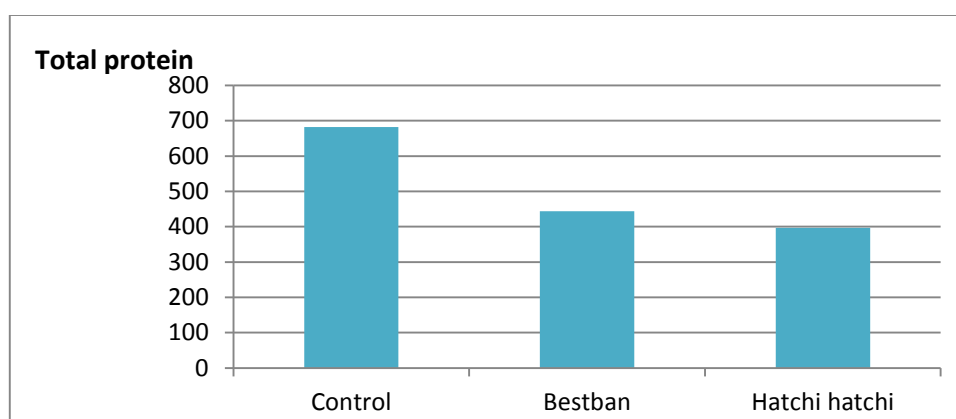


Fig. (8)

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تأثير بعض المبيدات الحشرية على بعض الأنزيمات في حشرتي الدودة القارضة وسوسة النخيل الحمراء
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تم دراسة تأثير بعض المبيدات الحشرية المختلفة مثل:

(Lambada setra, Cyper gaurd) Diazinon setra, Debeme thrin, Hatchi hatchi and pestban

على التأثيرات البيوكيميائية بإستخدام تركيز (LC₅₀) علي يرقات كلاً من الدودة القارضة وسوسة النخيل الحمراء تحت ظروف المعمل من درجة حرارة 1±27 درجة مئوية ورطوبة نسبية 75-80% وقد اوضحت نتائج الدراسة المتحصل عليها ما يلي :

1- بالنسبة لحشرة الدودة القارضة

اوضحت النتائج بأن هناك إنخفاض في مستوى إنزيمي الأميليز والكتينيز مقارنة بالكنترول . بينما علي الجانب الأخر ادت المعاملة بالمبيدات المختلفة الي وجود زيادة كبيرة في مستوى البروتين الكلي ونشاط إنزيم البروتيز مقارنة بالكنترول .

2- حشرة سوسة النخيل الحمراء

أوضحت النتائج المتحصل عليها بأن مبيد pestban تسبب في حدوث زيادة معنوية كبيرة في نشاط كلاً من إنزيمات الأميليز والكتينيز والبروتيز وكذلك مستوى البروتين الكلي علي النقيض من المبيد الحشري hatchi hatchi الذي تسبب في حدوث إنخفاض معنوي في نشاط تلك الإنزيمات وكذلك مستوى البروتين الكلي مقارنة بالحشرات الغير معاملة.