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Full Length Research Paper

Impact of *Bacillus thuringiensis* β – exotoxin to some biochemical aspects of *Musca domestica* (Diptera: Muscidae)

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The present investigation aimed to evaluate the efficiency of *Bacillus thuringiensis* β – exotoxin as a biological agent to control *Musca domestica* and to study its impact on some biochemical parameters of 2nd larval instar. Laboratory studies are carried by applying topically the bacterium *B. thuringiensis* β – exotoxin at LC_{30} under laboratory conditions (27 ± 2°C and 60±5% RH). The total protein contents of the total body, the total lipids, total carbohydrate, enzymatic activities of carbohydrate hydrolyzing enzymes, alkaline and acid phosphatases and non-specific esterases were estimated. Our data revealed that, the total soluble protein contents in supernatant of the homogenated larvae post-treatment was generally decreased, as affected by the tested material at different time intervals as compared to control. As well as a significant reduction in the total lipid content was observed, this may be due to production of enzymes that utilize lipids to remove the invading agent. The total carbohydrate contents was significantly reduced and the reduction was (-14.989, -43.33, 63.93 and -51.69%) with respect to control at the four different time intervals. While the activities of α – and β – esterases were decreased in larvae treated with LC_{30} of *B. thuringiensis israelensis* and the reduction of enzymatic activity was highly significant. Alkaline and acid phosphatase showed a significant reduction in its activities. Bacterial treatments induced inhibition in the enzyme activity of invertase where the values of inhibition were (-27.85 , -5.92 , -19.46 and -39.96%) at different time intervals. Also, a similar reduction of trehalase enzyme activity of treated larvae was observed at different time intervals (-32.81 , 12. 95 , -18.20 and -5.16%). It may be possible in this instance to control flies by the use of this bacterium which incorporate spores and crystals of the appropriate strain of *B. thuringiensis israelensis*, by affecting the biochemical systems of the target insect. So, it is quite clear from our results that *B. thuringiensis israelensis* at concentration of LC_{30} significantly decreased the activities of all biochemical parameters, we investigated. The general disturbance in metabolism could originate primarily from inhibition of chitin synthesis.

Key words: Housefly, bacteria, biocides, sublethal effects, proteins, carbohydrates, lipids, enzymes.

INTRODUCTION

The house fly, *Musca domestica has* long been considered as a potential agent for disease transmission ever since its existence. The general truth of this assertion remains undisputed till the present day, inspite of increasing awareness toward an improved sanitation hygiene (Nazhi et al., 2005). The widespread use and massive application of hazardous insecticides, against insects of either agricultural or medical importance, frequently leads to serious problems such as the development of resistance and environmental pollution. Accordingly, attention has been given recently to safer insecticides. Therefore, they isolated and identified various naturally bioactive compounds to be used as pesticides. Many previous studies proved that these chemicals

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have a toxic action against insect species and dipterous in particular (Deb-Kirtaniya et al., 1980; Abbassy, 1981; Mesbah et al., 1985; Methela et al., 1989; El-Sherif et al., 1994; Soliman and El- Sherif, 1995; Wirth and Georghiou, 1996; Gheikh et al., 1998; Mulla et al., 2003; Mittal et al., 2004; Paul et al., 2005; Tikar et al., 2008).

There have been few studies of the effect of *Bacillus thuringiensis* toxins toward house flies. Yamvrias and Anagnou (1989) observed high levels of mortality of *Bactrocera* (Dacus) *oleae* (Gmelin) larvae, after application of an aqueous suspension of *B. thuringiensis;* although it is not known if β–endotoxin was present in their experimental preparation. In a different study, 55 strains of *B. thuringiensis* were tested for activity against larvae of *Anastrepha luden* with resulting mortality of 4 to 63%, although the β–exotoxin content of these preparations was not specified (Robacker et al., 1996; Roh et al., 2007). Cry toxins from *B. thuringiensis* are used for insect control.

Their primary action is to lyse mid-gut epithelial cells. In this review, we will summarize recent findings on the Cry toxin receptor interaction and the role of receptor recognition in their mode of action. Cry toxins interact sequentially with multiple receptors. These toxins have a synergistic effect and CytIAa overcomes Cry toxin resistance. Recently, it was proposed that CytIAa synergizes or suppresses resistance to Cry toxins by functioning as a membrane-bound receptor for Cry toxin (Gomez et al., 2007; Pigott et al., 2008; Jorge et al., 2010). The objective of the current study was to determine the potential of *B. thuringiensis* β – endotoxin as an agent for control of *M. domestica*. This was achieved by a bioassay of the direct effects of a pure β–exotoxin product on insect mortality and sublethal effects of biochemical aspects in survivors.

MATERIALS AND METHODS

Test insects

Sources of colony

Adult susceptible strain of house fly *M. domestica vicina* used in the present study were obtained from a well established colony originated from the Biology Department, Faculty of Science for girls, King Abdul Aziz University. All experiments were carried out from March to December 2009.

Rearing technique

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27 ± 2°C and 60 ±5% R.H.) Each egg mass was placed in a clean Petri dish (10 cm diameter), previously constant technique described by Lelwallen (1954). Full grown larvae were allowed to pupate in clean glass Petri dishes. Following emergence, the adults were provided with a piece of cotton soaked in 10% sugar, 2% milk solution as a source of food.

Source of the bacterial pathogen

The bacterium *B. thuringiernsis israelensis* was chosen as a pathogen for this study because of its wide use in biological control. The powder was obtained from Valent Biosciences, U.S.A.

Biochemical studies

Estimation of the total proteins

The total protein content of the total body was estimated according to the method described by Bradford (1976). This method is based on the observation that Coomassie Brilliant Blue G-250 exists in two different colour forms, red and blue. The red colour is converted to the blue colour upon binding of the dye to protein; this binding causes a shift in maximum absorption of the dye from 465 to 595 nm. The intensity of the colour was measured at 595 nm.

Preparation of samples for the assay: The starved (for about 12 h) larvae were homogenized in distilled water (5 larvae / 5 ml) using a chilled glass Teflon tissue grinder for 3 min. Homogenates were centrifuged at 3500 r.p.m for 10 min at 2°C in a refrigerated centrifuge. The supernatant can be used directly or stored at 5°C till use (Max. 2 weeks).

Preparation of protein reagent: Commassie Brilliant Blue (CBB) G-250 (100 mg) was dissolved in 50 ml of 95% ethanol. To this solution 100 ml of 85% (w/v) phosphoric acid were added. The resulting solution was diluted to a final volume of 1 L.

Procedure: Sample solution (5 µl) for preparation of standard curve 50 µl of serial concentrations containing 10 to 100 µg bovine serum albumin were pipette into test tubes. The volume in the test tube was adjusted to 0.1 ml with phosphate buffer (pH= 6.6). 5 ml of protein reagent were added to the test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1 h against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) and 5 ml protein reagent. This procedure was repeated 3 times for each determination. The result is expressed as µg protein / larva.

Estimation of the total lipids

The total lipids were estimated by the method of Knight et al. (1972).

Preparation of samples of the assay: Homogenation was done as previously described in protein determination.

Phosphovanillin reagent: Pure vanillin (0.6 g) was dissolved in 10 ml ethyl alchohol and completed to 100 ml with distilled water. 400 ml of concentrated phosphoric acid was added, and the solution was stored in dark glass bottle at room temperature.

Procedure: 250 µl of samples solution was added to concentrated sulphuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the digest (500 µl) were added to phosphovanillin reagent (6.0 ml). After 45 min, the developed colour was measured at 525 nm against reagent blank prepared from 500 µl distilled water and 6.0 ml phosphovanillin reagent. The result is expressed as µg lipid / insect. For standard curve, serial concentrations of lipids (oleic and palmitic acids at a ratio of 7:3) from 1 to 5 µg were prepared in absolute ethanol (concentration of 1 to 5 µg/ml) were used treated in the same manner as the unknown. This procedure was repeated 3 times for

each determination. The result is expressed as µg lipid/larva.

Estimation of total carbohydrate

The total carbohydrates were estimated by the phenol sulphuric acid reaction of Dubois et al. (1956).

Preparation of samples of the assay: The starved (for about 12 h) larvae (five to ten) were homogenized in 0.3 N $HClO_4$ (5 ml) at 0°C for 1 min in a glass homogenizer with a Teflon pestle. The homogenate was kept in ice for a further 10 min. Insoluble matter was removed by centrifugation for 3 min at 2000 r.p.m., and washed twice in ice–cold $HClO_4$ (5 ml) by re–dispersion and centrifugation.

Procedure: Our hundred microliters sample or 100 µl of serial concentrations of glucose solution containing between 10 and 70 µg glucose (for preparation of total carbohydrate standard curve) were added into a colorimetric tube to 0.5 ml of phenol (20% w/v). Then 5 ml of concentrated sulphuric acid was added rapidly with shaking. The tubes were allowed to stand 10 min, then they were shaken and placed for 10 to 20 min in water bath at 25 to 30°C before readings. Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow – orange color is measured at 490 nm against blank. This procedure was repeated 3 times for each determination. The result is expressed as µg carbohydrates/larva.

Estimation of enzymatic activities

Preparation of samples of the assay: Homogenation was done as previously described in protein and lipid determination.

Carbohydrates hydrolyzing enzymes: The principle based on the digestion of trehalose, sucrose and starch by trehalase, invertase and amylase, respectively according to the method described by Ishaaya and Swirisk (1976). The free aldehydic group of glucose formed after trehalose, sucrose and starch digestion were determined using 3.5 dinitrosalicylic acid reagent. Trehalase reaction mixture consisted of 0.2 ml of 3% trehalose (substrate), 0.18 ml acetate buffer (pH 5.40 and 20 µl of larval homogenate). Invertase reaction mixture consisted of 0.2 ml of 4% sucrose (substrate) and 20 µl of larval homogenate. Amylase reaction mixture consisted of 0.2 ml of 2% starch (substrate), 0.16 ml of phosphate buffer (pH 6), and 40 µl larval homogenate.

The dinitrosalicilic acid reagent was prepared by dissolving one gram of 3.5 diniotrosalicylic acid in 20 ml of 2 N NaOH and 50 ml of distilled water with the aid of a magnetic stirrer. Potassium sodium tartarte (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. All test tubes were incubated at 37°C for 60 min, 0.8 ml of 3.5 dinitrosalicylic acid reagent were then added. The reaction mixture was heated for 5 min at 100°C in a boiling water bath followed by immediate cooling in an ice bath. The optical density (OD) of produced colour is measured at 550 nm using a spectrophotometer. The enzymatic activity was expressed as µg glucose/g body weight/min.

Preparation of standard curve of glucose: Serial concentrations of glucose solution containing 50, 100, 200, 300, 400 µg glucose / 0.4 ml distilled water were pippeted into test tubes, 0.8 ml of dinitrocalicylic acid solution was added to each tube .The mixture was heated for 5 min at 100°C in boiling water bath, and then cooled immediately in an ice bath. The resulting colour was measured spectrophotometrically at 550 nm. The optical densities was plotted against concentrations, thus a curve can be constructed.

Phosphatase enzymes (acid phosphatase and alkaline phosphatase): Acid phosphatase (Ac – pase) and alkaline phosphatase (Alk – pase) were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenyl-phosphate, reacts with 4 – aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown colour is produced. The reaction mixture consists of 1 ml carbonate buffer (pH 10.4) for Alk – pase, or 1 ml of citric buffer (pH 4.9) for Ac – pase , 1 ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml larval homogenate. Mix gently and incubate for exactly 30 min at 37°C . At the end of incubation period, 0.8 ml of 0.5 N NaOH was added to stop the reaction. Then added 1.2 ml of 0.5 N $NaHCO_3$, followed by 1 ml of 4 – aminoantipyrine solution and 1 ml of potassium ferricyanide. The produced colour was measured, immediately, by spectrophotometer at 510 nm. The enzymatic activity is expressed as µg phenol released / g body weight / min.

Preparation of phenol standard curve: A stock of phenol was prepared by dissolving 1 g pure crystalline phenol in 1 L 0.1 N HCl, 10 ml of the stock solution (containing 10 mg) was diluted to 100 ml with distilled water. Aliquots of 0.05, 0.1, 0.2, 0.3 and 0.4 ml of the diluted phenol (equal to 5, 10, 20, 30, and 40 µg phenol) were pippeted into test tubes and the volume was completed to 1 ml with distilled water. 1.1 ml of buffer was added followed by 0.8 ml of NaOH, 1.2 ml of $NaHCOH_3$, 1 ml of aminoantipyrine and 1 ml of potassium fericyanide. Each tube was mixed well after each addition. The developed colour was measured at 510 nm.

Non–specific esterases: Alpha esterases (α – esterases) and beta esterases (β – esterases) were determined according to the method of Van Asperen (1962) using α–naphthyl acetate and β–naphthyl acetate as substrates, respectively. Naphthol produced as a result of hydrolysis of substrate can be prepared by the addition of diazoblue sodium laury sulphate solution which produce strong blue colour in case of α – naphthyl or strong red colour in the case of β–naphthyl, which can be measured spectrophotometrically. The reaction mixture consisted of 5 ml substrate solution ($3x10^{-4}$ M α or β–naphthyl acetate, 1% acetone and 0.04 M phosphate buffer pH 7) and 20 µl of larval homogenate. The mixture was incubated for exactly 15 min at 27°C then 1 ml. of diazoblue colour reagent (prepared by mixing 2 parts of 1% diazoblue and 5 part of 5% sodium lauryl sulphate) was added. The developed colour was read at 600 or 555 nm for α – and β – naphthol, respectively.

Preparation of standard curves of α – and β – naphthol: Stock solutions were prepared by dissolving 20 mg α – or β–naphthol in 100 ml of 0.04 M phosphate buffer (pH 7). Ten milliliter of stock solution was diluted up to 100 ml by the phosphate buffer. Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.6 ml of diluted solution (equal to 2, 4, 8, 16 and 32 µg naphthol) were pippeted into test tubes and completed to 5 ml by phosphate buffer. One milliliter of diazoblue reagent was added and the developed colour was measured as stated before.

Statistical analysis

Data were expressed as mean ± standard error. The statistical significance of differences between individual means was determined by student "t" test paired observations. The level of significance of each experiment was stated to be non-significant ($p>0.05$), significant ($p<0.05$) and highly significant ($p<0.05$).

RESULTS

The present investigation aimed to evaluate the efficiency

Table 1. Total body protein contents (µg/larva) of homogenate 2nd instar larvae of M. domestica determined at different time intervals post – treatment with LC_{30} of *B. thuringiensis.*

Hours post-treatment	Total body protein content (µg/larva) (Mean ± SE)		Decrease (%)
	Control	Treated	
6	257.33±0.048	183.56±0.052**	-28.67
12	285.33±0.041	196.89±0.052**	- 31.00
24	649.33±0.087	256.44±0.019**	- 60.51
48	979.11±0.183	208.00±0.110**	- 99.78

n = 3 replicates per test. ** Highly significant (P < 0.01).

Table 2. Total body lipid contents (µg/larva) of homogenate 2nd instar larvae of *M. domestica* determined at different time intervals post–treatment with LC_{30} of *B. thuringiensis israelensis.*

Hours post-treatment	Total body protein content (µg/larva) Mean ± SE		Decrease (%)
	Control	Treated	
6	60.44±0.032	40.89±0.018**	-32.35
12	144.89±0.062	44.44±0.032**	- 69.33
24	286.22±0.085	90.67±0.071**	- 68.32
48	264.00±0.055	128.00±0.041**	- 51.52

n = 3 replicates per test. **Highly significant (P < 0.01).

Table 3. Total body carbohydrate contents (µg/larva) of homogenate 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *Bacillus thuringiensis israelensis.*

Hours post-treatment	Total body protein content (µg/larva) Mean ± SE		Decrease (%)
	Control	Treated	
6	125.88±0.020	107.03±0.012**	-14.98
12	200.47±0.029	113.60±0.011**	- 43.33
24	300.59±0.027	108.42±0.010**	- 63.93
48	299.84±0.029	1 144.85±0.038**	- 51.69

n = 3 replicates per test. ** Highly significant (P < 0.01).

of *B. thuringiensis israelensis* as a biological control agent on the 2nd instar larvae of *M. domestica.* Data in Table 1, indicate the changes in the level of total soluble protein in supernatant of the homogenated larvae post treatment which were generally decreased as affected by the tested material at different time intervals as compared to control, it recorded -28.67, -31.0, -60.51 and -99.78%, after 6, 12, 24, and 48 h post – treatment and this highly significant reduction was (p< 0.01). The total lipid content in the homogenate of 2nd instar larvae was significantly reduced when treatment was carried out with LC_{30} of the tested material at different time intervals post – treatment. The decrease in the haemolymph lipids may be due to produced enzymes that utilize lipids to remove the invading agent. The average of the decreasing percentage in the total lipid contents was -32.35, -69.33, -68.32 and – 51.52% at different time intervals (Table 2).

The total carbohydrate contents was significantly reduced and the reduction was (-14.9890, -43.33, -63.93 and -51.69%) with respect to control at the four different time intervals (Table 3). The activities of α and β – esterases were decreased in larvae treated with LC_{30} of Dipel 2X, and the reduction of enzymatic activity was highly significant (Figures 1 and 2). Alkaline phosphatase activity was highly significantly reduced (p < 0.01) at 6, 12 and 24 h post – treatment, whereas the reduction of enzymatic activities at these periods were (-18.04, -50.33, -51.77 and –20.07%), respectively (Figure 3). The same significance in the activity of acid phosphatase was also observed in Figure 4. Bacterial treatment induced inhibition in the enzyme activity of invertase where the values of inhibition were (-27.85, -5.92, -16.46 and -39.69%) at different time intervals of 6, 12, 24, and 48 h post – treatment, respectively (Figure 5).The percentages of reduction of trehalase enzyme activity of treated larvae was (-32.81, -12.59, -18.20, and -5.16%) at all inspected

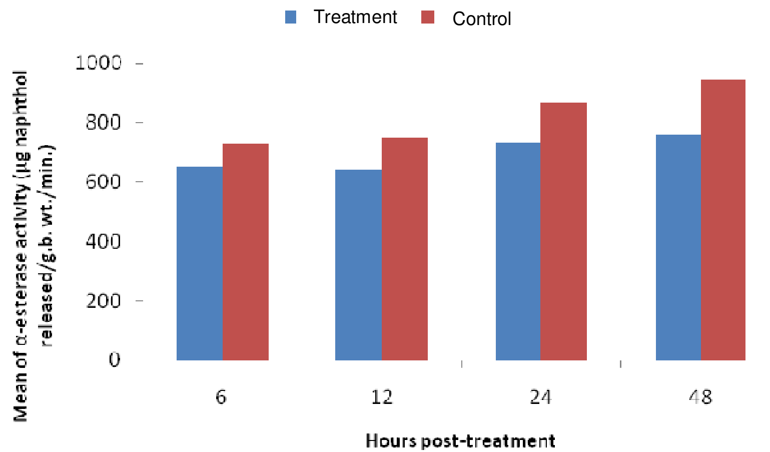

Figure 1. α-Esterase activity (µg naphthol released/g.b.wt./min) of homogenate of 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *B. thuringiensis israelensis*.

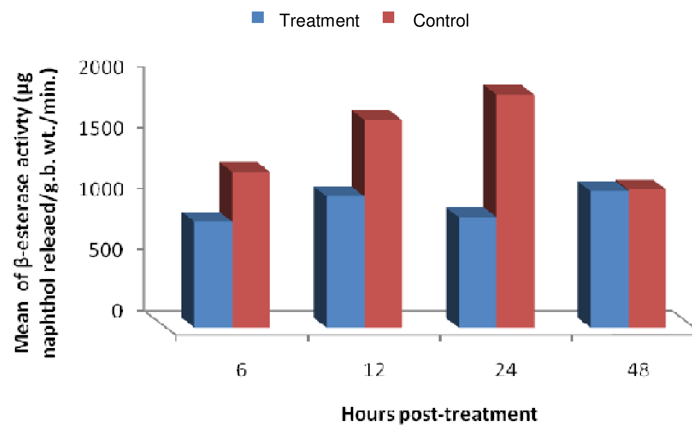

Figure 2. β-Esterase activity (µg naphthol released/g.b.wt./min.) of homogenate of 2nd instar larvae of *M. domestica.* determined at different time intervals post – treatment with LC_{30} of *B. thuringiensis israelensis*.

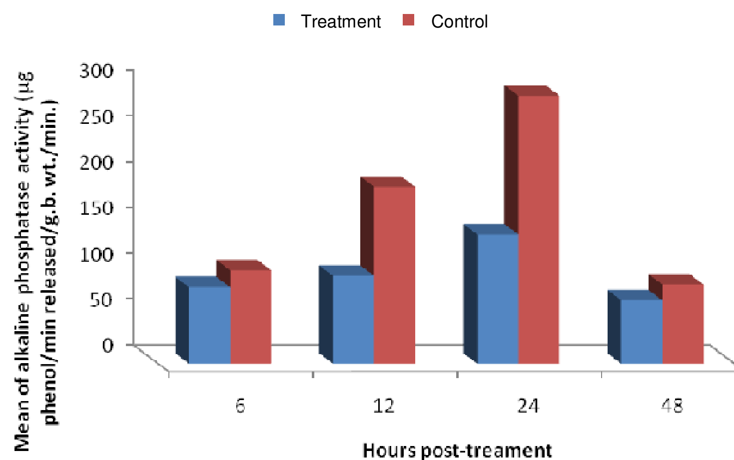

Figure 3. Alkaline phosphatase activity (µg phenol released/g.b.wt./min) of homogenate of 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *B. thuringiensis israelensis*.

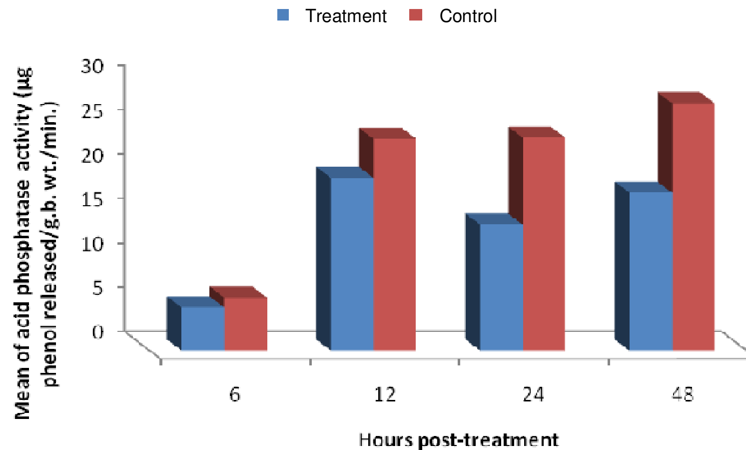

Figure 4. Acid phosphatase activity (µg phenol released/g. b. wt./min) of homogenate of 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *Bacillus thuringiensis israelensis*.

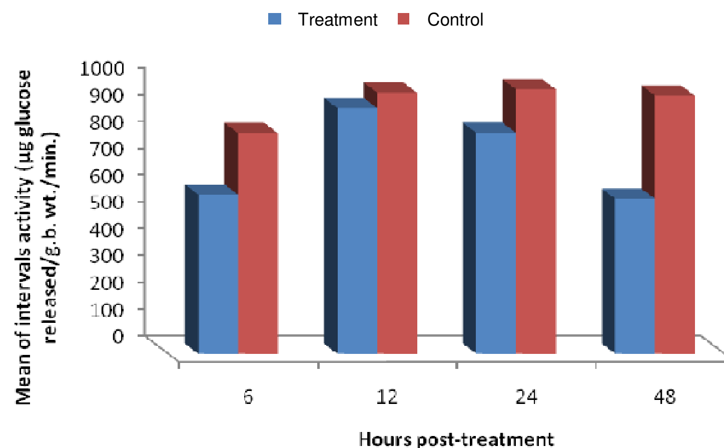

Figure 5. Invertase activity (µg glucose released/g.b.wt./min) of homogenate of 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *Bacillus thuringiensis israelensis*.

times (6, 12, 24 and 48 h), respectively (Figure 6). The corresponding figures of amylase were (-7.19, -42.63, -51.04 and -30.64%) (Figure 7)

DISCUSSION

The biochemical response of *M. domestica* larvae was assessed after treatment of the 2nd larval instar with LC_{30} of *B. thuringiensis israelensis* the changes in activities of total soluble protein ,total lipids , total carbohydrates, non specific esterases (α– and β–esterases), alkaline and acid phosphatases and carbohydrate hydrolyzing enzymes (invertase, trehalase and amaylase). It is clear from the present study that the LC_{30} of the tested material induced highly significant reduction in the level of total soluble protein at different time intervals post – treatment as compared to control. However, this reduction in the protein content may be due to inhibition of DNA and RNA synthesis and this may reflect the decrease in enzymatic activities. Our results agree with that obtained by many authors (Baker et al., 1991; Zeenath and Nair, 1994; Latha et al., 1996; Abd El-Aziz, 2000; Tawfik et al., 2002). The changes in total lipid contents were conducted as a result of bacterial treatment, in general, the total lipid contents in the homogenated larvae was significantly reduced as compared to control at different time intervals. The reason for decrease in the total lipid contents may be due to its conversion to proteins to substitute the reduction in protein content or produce supplementary

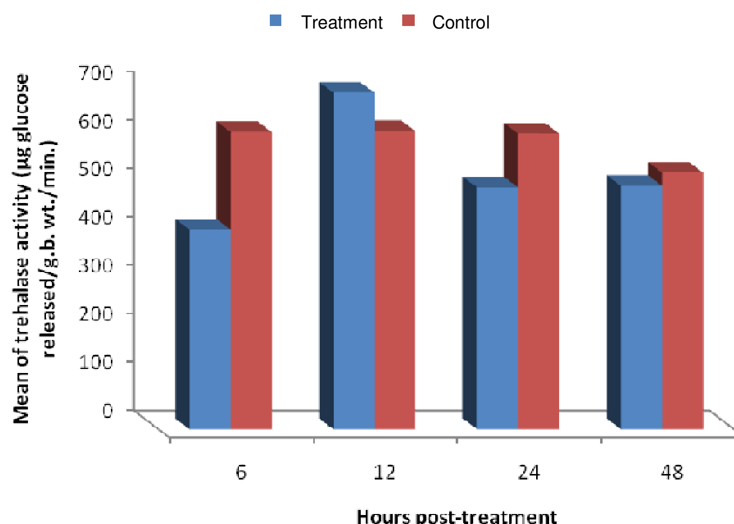

Figure 6. Trehalase activity (µg glucose released/g.b.wt./min.) of homogenate of 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *Bacillus thuringiensis israelensis.*

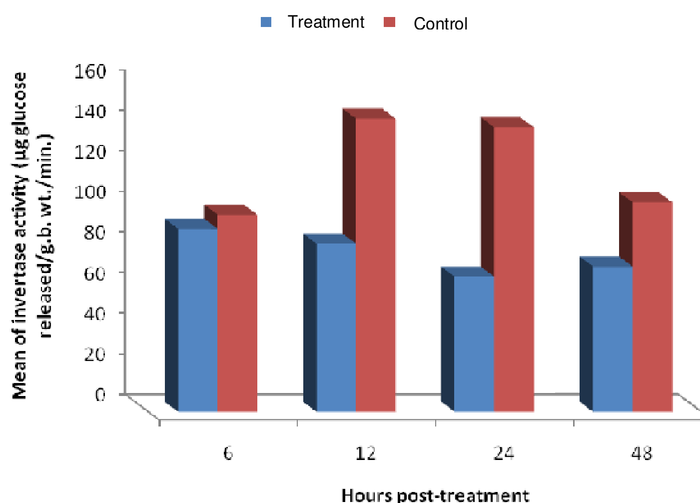

Figure 7. Amylase activity (µg glucose released/g.b.wt./min) of homogenate of 2nd instar larvae of *M. domestica* determined at different time intervals post – treatment with LC_{30} of *Bacillus thuringiensis israelensis.*

energy . Our results agree with those obtained by many investigators (Abo-Ela et al., 1998; Abde El-Aziz, 2000; Omar et al., 2005b). Nonspecific esterases in different insect species have been implicated in reproductive behavior, phormone and hormone metabolism digestion, neurotransmission and resistance to insecticides particularly organo phosphorus compounds (Georghiou and Pasteur, 1980). Esterases may contribute to resistance by hydrolyzing the pesticide (Devonshire and Moores, 1982) or by temporary binding to the pesticide when the catalytic activity is relatively low (Devonshire, 1989). It is quite clear from our results that the LC_{30} applied of *B. thuringiensis israelensis* induced decrease in the activity of both α– and β–esterases in the supernatant of the homogenate larnae of *M. domestica.* These results are in accordance with that demonstrated by El- Ghar et al. (1995b), Salem et al. (1995) and Hanafy et al. (2005).

Acid and alkaline phosphatases have been shown to be associated with insect development, especially in

relation to nutrition, egg maturation (Tsumuki and Kanehisa, 1984). Our results clearly indicated that the alkaline and acid phosphatases activities were highly significantly reduced at all time interval post – treatment ($p < 0.01$). These results are in conformity with the findings of Abdel Hafez et al. (1988), Ayyangar and Rao (1990) and Sokar (1995). It is quite clear from our results that *B. thuringiensis israelensis* at concentration of LC_{30}, significantly decreased the activities of the carbohydrate hydrolyzing enzymes (invertase, trehalase and amaylase) as compared to control groups. The general disturbance in carbohydrate metabolism as expressed by reduction of carbohydrate hydrolyzing enzyme activities could be as a result from chain effect originating primarily from inhibition of chitin synthesis (Salem et al., 1995; Auda and Hedaya, 1997; Shakoori et al., 1998; Khedr, 2002; El-Mageed and Elgohary, 2006). Also, Bravo et al. (2007) stated that *B. thuringiensis* Crystal (Cry) and Cytolitic (Cyt) protein families are a diverse group of proteins with activity against insects of different orders, Lepidoptera, Coleoptera, Diptera and also against other invertebrates such as nematodes.

Their primary action is to lyse midgut epithelial cells by inserting into the target membrane and forming pores. Tilquin et al. (2008) concluded that they raise the issue of the persistence, potential proliferation and environmental accumulation of human-spread *B. thuringiensis israelensis* in natural mosquito habitats. Such *B. thuringiensis israelensis* environmental persistence may lengthen the exposure time of insects to this bio-insecticide, thereby increasing the risk of resistance acquisition in target insects, and of a negative impact on non-target insects. Mwamburi et al. (2009) suggested that the control of house fly larvae and the efficacy of *B. thuringiensis israelensis* applied as a larvicide, may be improved with frequent spray applications to chicken manure. Lysyk et al. (2010) was in agreement with our results, also Wirth et al. (2010) who suggest that recombinant bacterial strains have improved mosquito and vector management properties compared with the wild type strains used in current commercial formulations, and should prove useful in controlling important human diseases such as malaria and filariasis on a long term basis, even when used intensively under field conditions. Many authors investigate the role of an alpha-amylase receptor for *B. thuringiensis israelensis* Cry4Ba and Cry11Aa toxins in the malaria vector mosquito *Anopheles albimanus,* (Fernandez et al., 2010).

Generally, our results in this investigation concluded that there was a clear significant effect on all biochemical parameters discussed, as a result of treatment with the bacterium *B.* thuringiensis at the dose of LC_{30}. The total protein, lipid, carbohydrate, non-specific esterases, α- and β- phosphatases and carbohydrate hydrolysing enzymes in the supernatant homogenated 2nd larval instar of *M. domestica* was highly significantly decreased as compared to control groups at all different time intervals, the reduction was continual during all the tested periods.

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