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ACUTE TOXICITY OF SOME BIOPESTICIDES AND THEIR EFFECT ON ACETYLCHOLINESTERASE OF HONEY BEE (*Apis mellifera*) WORKERS

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ABSTRACT

Biopesticides are increasingly applied throughout Egypt. However, negative effects of these compounds on the honey bee (*Apis mellifera* L.), the most important pollinator for cultivated ecosystem, remained poorly investigated. The objective of our study was to evaluate the potential side effects of five biopesticides; bioarch (*Bacillus megaterium*), biofly (*Beauveria bassiana*), biozed *(Trichoderma album),* protikto BTK (*Bacillus thuringiensis kurstaki*), and spintor (spinosad) on mortality and acetylcholinesterase activity of honey bee workers. The mortality of treated workers was determined after 24 h of application, and the lethal concentrations that caused 50% mortalities (LC_{50}) were estimated. The impacts on acetylcholinesterase (AChE) activity were determined *in vivo* after 24 and 48 h in head, thorax and abdomen of surviving bees. Our results indicated that spinosad showed the most toxic action to adult honey bee workers with LC_{50} of 11.60 mg L^{-1} followed by biozed with LC_{50} of 114.12 mg L^{-1}, and lower degrees of toxicity were obtained with protikto (LC_{50} = 87,412 mg L^{-1}), biofly (LC_{50} = 49,766 mg L^{-1}) and bioarch (LC_{50} = 15,785 mg L^{-1}). In addition, all tested biopesticides caused various degrees of inhibition in AchE activity of adult honey bee workers (after 24 h of application) with the body region and with concentration. On the contrary, there were different degrees of activation in AChE of head, thorax and abdomen obtained after 48 h of application with tested biopesticides, except bioarch, biofly and protikto were inhibitions in AChE activities of the abdomen were obtained.

Keywords: Honey bee, *Apis mellifera* L.*,* biopesticides, Bioarch*, Bacillus megaterium,* Biofly, *Beauveria bassiana,* Biozed*, Trichoderma album,* Protikto*, Bacillus thuringiensis kurstaki,* Spintor, Spinosad*,* toxicity and Acetylcholinesterase.

INTRODUCTION

The widespread use and massive application of conventional pesticides, against agricultural pests and honey bee pests may lead to serious problems such as rapid development of resistance in addition to adverse effect on environment and health. Furthermore, the potential negative impact on biodiversity and non-target organisms (especially beneficial arthropods) still needs to be assessed carefully (Dale *et al.*, 2002). At least one-third of crops are pollinated by insects and other animals. Among which honey bees, *Apis mellifera L*. (Hymenoptera, Apidae), account for 80% of the total pollinating insects (Klein *et al*., 2007). It plays a major role in the agricultural productions, particularly in the crossing pollination of numerous plants and wild flowers (Celli and Maccagnani 2003; Chan *et al.,* 2006.). Furthermore, honey bees produce honey, pollen, royal jelly, propolis, wax (Kevan, 1999) and bee venom. Unfortunately, Colony Collapse Disorder (CCD) in honey bee colonies from Europe and USA since 2006 has caused

several billion dollars of direct economic losses by reduction in crop-yields (Murray *et al*., 2009). CCD appears to be a multifactorial syndrome and multiple causes have been proposed such as pests, pathogens, chemical pesticides, GM crops (Cox- Foster *et al.,* 2007; Desneux *et al.,* 2007; Oldroyd 2007; Cox-Foster and vanEngelsdorp 2009; vanEngelsdorp *et al.,* 2009; vanEngelsdorp and Meixner 2010). Therefore, alternatives to chemical control need to be developed.

Accordingly, attention has been given recently to develop biological or non-chemical pesticides such as biopesticides which may be safe at non-target organisms. It little has been reported on the side effects of biopesticides on honey bees and little information based on the enzymatic aspects of the host after exposure to biopesticides has been given in literature. Therefore, more studies in this area are needed to evaluate their effects on the honey bee individuals and colonies. Honey bees, as domesticated pollinators, may be constantly exposed to pesticides such as biopesticides whenever their colonies are sited in agricultural areas (Weick and Thorn 2002), specially when their pests are controlled inside bee hives.

The use of entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) has been considered a promising alternative to chemical miticides (Chandler *et al.,* 2000 and 2001) used for Varroa mites (the most virulent pest of honey bees) control (Meikle *et al.*, 2007 and 2008 a, b). Meikle *et al.,* (2008 a, b) found that single two successive applications of formulation containing *B. bassiana* conidia did not have the measurable negative impact on colony health or survivorship.

The entomopathogenic bacteria *Bacillus thuringiensis var kurstaki*, which could be formulated in Protikto BTK, produces a parasporal crystal δ-endotoxin protein which is lethal to many lepidopteran larvae (Adang *et al.,* 1985). *Bacillus megaterium*, which could be formulated in bioarch, has been frequently isolated from honeys (Gilliam and Valentine, 1976; Gilliam 1979; Alippi, 1995; Alippi *et al*., 2004). *B. megaterium* was frequently isolated from frass from feral honey bee colonies (Gilliam, 1985). *B. megaterium* is the gram-positive spore-forming bacterium that produces several enzymes and antibiotic-like compounds and has been found in diverse habitats, including honey (Pelletier and Sygusch, 1990; Padgham and Sikora, 2007; Vary *et al.*, 2007; López and Alippi, 2009). The biopesticide spinosad showed the highly toxic effect to honey bees in acute oral and contact toxicity studies (Mayes *et al.*, 2003 and Rabea *et al.,* 2010).

Biopesticides are increasingly produced and applied throughout Egypt to control various agricultural pests. However, negative effects of these compounds on the honey bee (*Apis mellifera* L.), the most important pollinator for cultivated ecosystem, remained poorly investigated. Therefore, the objective of our study was to evaluate the potential side effects of five biopesticides; bioarch (*Bacillus megaterium*), biofly (*Beauveria bassiana*), biozed (*Trichoderma album*), protikto BTK (*Bacillus thuringiensis kurstaki*) and spintor (spinosad) on mortality and acetylcholinesterase activity of honey bee workers. Thus, we could verify which of them could impact worker longevity and their activities, and consequently, could adversely affect colony strength and performance.

MATERIALS AND METHODS

Chemicals

The five biopesticides tested were Bioarch (*Bacillus megaterium*) 10%, Biozed (*Trichoderma album*) 2.5% and Protikto BTK (*Bacillus thuringiensis kurstaki*) 9.4% (supplied by Kafr El- Zayat Pesticides & Chemicals Co., Kafr El-Zayat, Egypt), Biofly (*Beauveria bassiana*) containing $30x10^6$ conidia/cm^3 (supplied by El-Nasr Co. for Fertilizaers and Biopesticides, El-Sadat city, Egypt), and Spintor 24% SC (Spinosad) (supplied by Dow Agro Sciences Co., England). Acetylthiocholine iodide (ATChI), 5,50- dithio-bis(2-nitrobenzoic) acid (DTNB) and Folin-Ciocalteu phenol regent were purchased from Sigma-Aldrich Chemical Co., USA.

Honey bees, *Apis mellifera*

All experiments were carried out in the laboratory using honey bees *Apis mellifera* L. (Hymenoptera: Apidae). Local hybrid (derived from Italian bee *Apis mellifera ligustica* and Carniolan bee *Apis mellifera carnica* and other races existed in Egypt) adult honey bee workers were collected from one colony (free of obvious diseases) from an apiary located in Ezbet Haggag at El-Beheira Governorate on December. Worker bees *(Apis mellifera)* were caught by shacking combs covered with bees above an empty plastic container, then covered with a nylon mesh cover and transported to the laboratory. The collected bees were stored without feeding at room temperature in a dark, humid place for 24 h prior to application. The bees were anaesthetized by cooling in the deep freezer for 5 min. Immediately, the bees were kept during the experiment in experimental transparent plastic cups, covered with a nylon mesh. Each contained 20 adult workers at 25 ± 2°C and 65 ± 5% relative humidity. After recovering from the cooling, 3 cups were subjected to each concentration of each biopesticide and control.

Acute Toxicity Assessment

The acute toxicity of five biopesticides; Bioarch (*B. megaterium*), Biofly (*Beauveria bassiana*), Biozed (*Trichoderma album*), Protikto BTK (*Bacillus thuringiensis kurstaki*) and Spintor (Spinosad) were evaluated on adult honey bees (*A. mellifera L*.) workers by oral administration under laboratory conditions after 24 h (Weick and Thorn 2002). Preliminary screening tests were performed at the application rate in the field (25, 1500, 1.56, 4. 42 and 4.8 mg a.i./ L water for the above mentioned biopesticides, respectively). Stock solutions of biopesticides were prepared in sugar syrup 1:1 (w/v). Based on preliminary screening tests the application was made through feeding on sugar syrup 1:1 (w/v) containing different concentrations of the tested biopesticides. Tested concentrations were 125, 250, 500, 1000 and 2000 mg L^{-1} for Bioarch; 93.75, 187.5, 375, 750 and 1500 mg L^{-1} for Biofly; 31.25, 62.5, 125, 250 or 500 mg L^{-1} for biozed; 23.5, 47, 94,188 and 376 mg L^{-1} for Protikto; and 1.25, 2.5, 5, 10 and 20 mg L^{-1} for spinosad. Adult workers of each cup were fed for 24 h through the cotton bed attached to the nylon mesh and applied with the certain amount of sugar syrup. Three cups (20 worker /cup) were subjected to sugar syrup 1:1 (w/v) containing one of

the tested concentrations of every bio-pesticide. Other three cups were subjected to sugar syrup 1:1 (w/v) alone as controls. Worker bees were considered dead if they were unable to move. The numbers of dead bees were recorded after 24 h to calculate mortality percentages and the LC_{50} of each biopesticide according to Finney (1971). Then, all treated groups were fed as the control group on the sugar syrup alone for other 24 h to determine acetyl cholinesterase (AChE) activity in bees after 48 h of application.

Preparation of Bee Extract

All steps were carried out between 0 and 4 °C. Adult bee workers were anaesthetized by freezing in the deep freezer. All treatments were done in triplicates. The heads, thoraces, and abdomens of three survived anaesthetized workers (for each replicate from every concentration of tested biopesticides) were separated. Each group (containing 3 heads, thoraces, or abdomens) was immediately homogenized using hand glass Homogenizer under cooling with 0.1 M phosphate buffer (pH 7.0). The homogenates were then centrifuged at 5,000 rpm for 20 min. at 0°C. The supernatants were used as the enzyme source for assay of AChE activity.

Total Protein Assay

This assay was accomplished following the method of Lowry *et al.,* (1951)

Acetylcholinesterase (AChE) Activity Assay

The AChE activity assay *(in vivo)* was carried out following the method of Ellman *et al.,* (1961) using the spectrophotometric procedure. The activity, specifically attributable to acetyl cholinesterase in head, thorax, and abdomen of adult honey bee workers was determined by using DTNB (dithionitrobenzoic) after 24 and 48 h of feeding on the tested biopesticides. Absorbance measurements were conducted at a wavelength of 412 nm using the Jenway 6305 spectrophotometer. The specific activity of AChE was expressed as nmoles of acetylthiocholine iodide hydrolyzed/mg protein/min.

Statistical Analysis

The log dose–response curves allowed determination of the LC_{50} values for the insect bioassay according to probit analysis (Finney 1971). Data of effects of biopesticides on AChE activity experiments was subjected to two-way analysis of variance (ANOVA). However, the experiment of AChE activity was conducted in factorial (2x3x5). The experimental design was the completely randomized design with three replicates. Comparisons among means were made using the Least Significant Difference test (L.S.D.) at 5% level of probability with the aid of the SAS program (SAS Institute, 2000) version 8.0.

RESULTS

Acute Toxicity of Tested Biopesticides to Adult Honey Bees Workers

The results of the acute toxicity assay of Bioarch (*B. megaterium*), Biofly (*Beauveria bassiana*), Biozed (*Trichoderma album*), Protikto BTK (*Bacillus thuringiensis kurstaki*) and Spintor (Spinosad) on adult honey bees (*A. mellifera L*.) workers by oral administration under laboratory conditions after 24 h are summarized in Table (1). Based on LC_{50} values, spinosad showed the most toxic action to adult honey bee workers with LC_{50} of 11.60

mg L^{-1} followed by Biozed with LC_{50} of 114.12 mg L^{-1}. However, a lower toxicity was obtained with Protikto BTK, Biofly and Bioarch.

Table 1: Mortality of adult honey bee workers and acute toxicity of tested biopesticides.

Biopesticide	Concentration (mg L^{-1})	Mortality (%)	LC_{50} (mg L^{-1})	Slope ± SE	χ^2 *
Bioarch	125	1.67	15785	0.87	1.60
	250	8.33			
	500	8.33			
	1000	16.67			
	2000	20.00			
Biofly	93.75	1.67	49766	0.86±0.35	0.97
	187.5	1.67			
	375	1.67			
	750	6.67			
	1500	10.00			
Biozed	31.25	3.33	114.12	3.05±0.28	4.96
	62.5	16.67			
	125	65.00			
	250	85.00			
	500	95.00			
Protikto	23.5	0.00	87412	0.57±0.30	0.12
	47	0.00			
	94	3.33			
	188	6.67			
	376	10.00			
Spinosad	1.25	3.33	11.60	2.38±0.27	21.26
	2.5	8.33			
	5	16.67			
	10	23.33			
	20	86.67			

*** Chi square**

Impacts of Tested Biopesticides on Acetylcholinesterase (AChE) Activity after 24 h

The *in vivo* specific activity and inhibition of acetylcholinesterase (AChE) activity in different regions (head, thorax, and abdomen) of surviving adult honey bee workers after 24 of feeding on sugar syrup 1:1 (w/v) with different concentrations of biopesticides were calculated and presented in Tables 2 (bioarch), 3 (biofly), 4 (biozed), 5 (protikto), and 6 (spinosad). Data of specific activity are presented in units of nmoles of acetylthiocholine iodide hydrolyzed/mg protein/min., while those of inhibition are expressed as percentages.

Data of bioarch (after 24 h) are summarized and presented in Table 2. Mean values of acetylcholinesterase (AChE) activity in heads of workers fed on sugar syrup 1:1 (w/v) with 0, 125, 250, 500, 1000 and 2000 mg L^{-1} of bioarch were larger than those in thoraces, respectively. The lowest levels of the AchE activity were found in the bee abdomen. Furthermore, the average of AChE activities in the head (36.41 nmoles ATChI hydrolyzed/mg protein/min.) were larger than that in the thorax (19.72) compared with 14.28 in the abdomen. Statistical analysis revealed that the AChE activity in adult

worker heads was significantly ($p > 0.05$) higher than the activity in both of thoraces and abdomens. On the other hand, the activity in abdomens was significantly ($p > 0.05$) lower than the activity in thoraces. When data of head, thorax and abdomen were summed to estimate the effects of concentrations, all treatments resulted in various decreases in AChE activity compared with the control. The means of AChE activities in adult workers were 27.64, 20.19, 19.15, 17.75 and 14.20 nmoles ATChI hydrolyzed/mg protein/min. when treated with 125, 250, 500, 1000 or 2000 mg L^{-1} of bioarch, in respect, compared with 41.86 for untreated workers. Statistical analysis showed that there were significant differences ($p > 0.05$) among AChE activities in adult workers treated with different concentrations, except between 250 and 500, and between 500 and 1000 mg L^{-1}. Furthermore, the activity in control was significantly higher than any treatment. In regard to the inhibition of the enzyme activity, tested concentrations caused various degrees of inhibition of AChE activity in head, thorax and abdomen. The highest inhibition percentage means were recorded in the abdomen (ranged from 42.71 at the low concentration to 85.46% at the high concentration with an average of 57.86%), while the lowest inhibitions of enzyme activity were found in the head (ranged from 17.02 at the low concentration to 44.64% at the high concentration with an average of 27.89%. Furthermore, treatments resulted in inhibition percentage means in the thorax ranged from 47.22 at the low concentration to 76.44% at the high concentration with an average of 52.12%. Statistical analysis revealed that the inhibition of AChE activity in adult workers abdomen was significantly ($p > 0.05$) higher than that recorded in both of thoraces and heads. The inhibition in heads was significantly ($p > 0.05$) lower than that in thoraces. On the other hand, all treatments resulted in various degrees of inhibition in AChE activities in adult workers. They were 35.65, 54.14, 57.04, 60.06 and 68.85% when treated with 125, 250, 500, 1000 or 2000 mg L^{-1} of bioarch, in respect. Statistical analysis showed that all treatments resulted in significant inhibition ($p > 0.05$) in AChE activities in adult workers. Furthermore, highest significant inhibition was caused by the high concentration, while the lowest one was caused by the low concentration activity. Therefore, the biopesticide bioarch when found in sugar syrup at a concentration of 125, 250, 500, 1000 or 2000 mg L^{-1} has an inhibitory effect on AChE activity of adult honey bee workers after 24 h of application.

Data of biofly (after 24 h) are summarized and presented in Table 3. Mean values of acetylcholinesterase (AChE) activity in heads of workers fed on sugar syrup 1:1 (w/v) with 0, 93.75, 187.5, 375, 750 and 1500 mg L^{-1} of biofly were larger than those in thoraces, respectively. Furthermore, the average of AChE activities in the head (34.88 nmoles ATChI hydrolyzed/mg protein/min.) was larger than that in the thorax (22.11) compared with 22.61 in the abdomen. Statistical analysis revealed that the AChE activity in adult worker heads was significantly ($p > 0.05$) higher than the activity in both of thoraces and abdomens. On the other hand, the activity in thoraces did not differ significantly ($p > 0.05$) from the activity in abdomens.

Table 2: *In vivo* effects on acetylcholinesterase (AChE) activity in different regions of honey bee workers after 24 and 48 hours of feeding on sugar syrup with bioarch.

Concentration (mg L^{-1})	Bioarch-24 h				Bioarch-48 h			
	nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)		nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)	
	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average
Head								
0.0	50.50±0.26	**36.41 a***	0.00±0.00	**27.89 c**	63.34±6.14	**63.37 a**	0.00±0.00	**-0.05 b**
125	41.90±1.93		17.02±3.83		51.48±3.20		18.71±5.05	
250	32.61±4.18		35.42±8.28		71.32±4.39		-12.60±6.93	
500	33.97±4.31		32.72±8.54		40.32±2.47		36.34±3.91	
1000	31.54±3.11		37.55±6.17		58.09±3.02		8.28±4.76	
2000	27.95±3.23		44.64±6.40		95.66±2.46		-51.03±3.88	
Thorax								
0.0	41.19±1.92	**19.72 b**	0.00±0.00	**52.12 b**	24.20±1.23	**49.64 b**	0.00±0.00	**-105.14 c**
125	21.74±1.08		47.22±2.62		17.71±0.78		26.81±3.23	
250	18.23±0.71		55.74±1.73		36.55±3.63		-51.03±15.00	
500	14.67±1.13		64.37±2.74		66.24±0.98		-173.75±4.06	
1000	12.79±2.14		68.95±5.19		75.80±2.02		-213.26±8.36	
2000	9.70±3.55		76.44±8.61		77.34±3.80		-219.6±15.71	
Abdomen								
0.0	33.89±1.06	**14.28 c**	0.00±0.00	**57.86 a**	26.41±2.39	**23.68 c**	0.00±0.00	**10.35 a**
125	19.42±2.05		42.71±6.06		16.76±2.36		36.53±8.92	
250	9.74±1.17		71.26±3.46		15.79±1.51		40.23±5.73	
500	8.80±1.69		74.03±4.98		26.64±3.08		-0.87±11.65	
1000	8.92±1.38		73.67±4.08		23.75±1.82		10.09±6.89	
2000	4.93±0.63		85.46±1.87		32.72±3.05		-23.87±11.57	
Total	**Mean**		**Mean**		**Mean**		**Mean**	
0.0	41.86 a	**23.47**	0.00 e	**45.96**	37.98 e	**45.56**	0.00 b	**-31.61**
125	27.64 b		35.65 d		28.65 f		27.35 a	
250	20.19 c		54.14 c		41.22 d		-7.80 c	
500	19.15 cd		57.04 bc		44.40 c		-46.09 d	
1000	17.75 d		60.06 b		52.55 b		-64.96 e	
2000	14.20 e		68.85 a		68.57 a		-98.17 a	

*** Means in the same column followed by the same letter(s) are not significantly different according to L.S.D test at 0.05 level of probability.**

(-) before mean indicate that there is activation in AChE activity.

When data of head, thorax and abdomen were summed to estimate the effects of concentrations, all treatments resulted in various decreases in AChE activity compared with the control. The means of AChE activities in adult workers were 29.75, 21.89, 20.72, 25.52 and 19.48 nmoles ATChI hydrolyzed/mg protein/min. when treated with 93.75, 187.5, 375, 750 or 1500 mg L^{-1} of biofly, in respect, compared with 41.86 for untreated workers.

Statistical analysis showed that there were significant differences ($p > 0.05$) among the AChE activities in adult workers treated with different concentrations, except among activities at the concentrations of 187.5, 375 and 1500 mg L^{-1}. Unusual significant increase in the AChE activity was observed at the concentration of 750 mg L^{-1}. After that, the activity decreased as the concentration increased. Furthermore, the activity in control was significantly higher than any treatment.

Therefore, the biopesticide biofly when found in sugar solution at a concentration of 93.75, 187.5, 375, 750 or 1500 mg L^{-1} has the inhibitory effect on AChE activity of adult honey bee workers. In regard to the inhibition of the enzyme activity, tested concentrations caused various degrees of inhibition of AChE activity in the head, thorax and abdomen. The highest inhibition percentage means were recorded in the thorax (ranged from 44.01 at the low concentration to 66.57% at the concentration of 375 mg L^{-1} with an average of 46.33%), while lower inhibitions of enzyme activity were found in the head (30.92%) and abdomen (33.27%). Statistical analysis revealed that the inhibition of AChE activity in adult worker's thoraces was significantly ($p > 0.05$) higher than that recorded in both of heads and abdomens. On the other hand, all treatments resulted in various degrees of inhibition in AChE activities in adult workers. They were 28.28, 49.89, 50.41, 40.34 and 52.12 % when treated with 93.75, 187.5, 375, 750 or 1500 mg L^{-1} of biofly, in respect. Statistical analysis showed that all treatments resulted in significant inhibition ($p > 0.05$) in AChE activities in adult workers. Therefore, the biopesticide biofly when found in sugar syrup at a concentration of 93.75, 187.5, 375, 750 or 1500 mg L^{-1} has an inhibitory effect on AChE activity of adult honey bee workers after 24 h of application.

Data of biozed (after 24 h) are summarized and presented in Table 4. Mean values of acetylcholinesterase (AChE) activity in heads of workers fed on sugar syrup 1:1 (w/v) with 0, 31.25, 62.5, 125, 250 and 500 mg L^{-1} of biozed were larger than those in thoraces, respectively. Furthermore, the average of AChE activities in the head (21.89 nmoles ATChI hydrolyzed/mg protein/min.) was larger than that in the thorax (20.11) compared with 19.34 in the abdomen. Statistical analysis revealed that the AChE activity in adult worker heads was significantly ($p > 0.05$) higher than the activity in both of thoraces and abdomens. On the other hand, the activity in thoraces did not differ significantly ($p > 0.05$) from the activity in abdomens. When data of head, thorax and abdomen were summed to estimate the effects of concentrations, all treatments resulted in various decreases in AChE activity compared with the control. The means of AChE activities in adult workers were 24.81, 18.31, 14.91, 13.21 and 9.58 nmoles ATChI hydrolyzed/mg protein/min. when treated with 31.25, 62.5, 125, 250 or 500 mg L^{-1} of biozed, in respect, compared with 41.86 for untreated workers. Statistical analysis showed that there were significant differences ($p > 0.05$) among the AChE activities in adult workers treated with different concentrations. Furthermore, the activity in control was significantly higher than any treatment. In addition, tested concentrations caused various degrees of inhibition of AChE activity in head, thorax and abdomen. The highest inhibition percentage means were recorded in the head (ranged from 39.12 at the low concentration to 88.43% at the high concentration with an average of 56.65%), while the lowest inhibition of enzyme activity were found in the abdomen (ranged from 48.09 at the low concentration to 56.26% at the high concentration with an average of 42.94%. Furthermore, treatments resulted in inhibition percentage means in thorax ranged from 36.61at the low concentration to 80.42% at the high concentration with an average of 51.17%.

Statistical analysis revealed that the inhibition of AChE activity in adult worker heads was significantly ($p > 0.05$) higher than that recorded in both of thoraces and abdomens. The inhibition in abdomens was significantly ($p > 0.05$) lower than that in thoraces. On the other hand, all treatments resulted in various degrees of inhibition in AChE activities in adult workers. They were 41.27, 55.27, 62.83, 67.11 and 75.04 % when treated with 31.25, 62.5, 125, 250 or 500 mg L^{-1} of biozed, in respect. Statistical analysis showed that all treatments resulted in significant inhibition ($p > 0.05$) in AChE activities in adult workers. Furthermore, highest significant inhibition was caused by the high concentration, while the lowest one was caused by the low concentration activity. Therefore, the biopesticide biozed when found in sugar syrup at a concentration of 31.25, 62.5, 125, 250 or 500 mg L^{-1} has an inhibitory effect on AChE activity of adult honey bee workers after 24 h of application.

Data of protikto (after 24 h) are summarized and presented in Table 5. Mean values of acetylcholinesterase (AChE) activity in the heads of workers fed on sugar syrup 1:1 (w/v) with 0, 23.5, 47, 94,188 and 376 mg L^{-1} of Protikto were larger than those in the thoraces, respectively. The lowest levels of the AChE activity were found in the bee abdomen. Furthermore, the average of AChE activities in the head (25.88 nmoles ATChI hydrolyzed/mg protein/min.) was larger than that in the thorax (16.87) compared with 12.10 in the abdomen. Statistical analysis revealed that the AChE activity in adult worker heads was significantly ($p > 0.05$) higher than the activity in both thoraces and abdomens. On the other hand, the activity in the abdomens was significantly ($p > 0.05$) lower than the activity in thoraces. When data of the head, thorax and abdomen were summed to estimate the effects of concentrations, all treatments resulted in various decreases in AChE activity compared with the control. The means of AChE activities in adult workers were 19.56, 19.46, 11.80, 9.78 and 7.25 nmoles ATChI hydrolyzed/mg protein/min. when treated with 23.5, 47, 94,188 and 376 mg L^{-1} of protikto, in respect, compared with 41.86 for untreated workers. Statistical analysis showed that there were significant differences ($p > 0.05$) among the AChE activities in adult workers treated with different concentrations, except between 23.5 and 47, and between 94 and 188 mg L^{-1}. Furthermore, the activity in control was significantly higher than any treatment. In addition, tested concentrations caused various degrees of inhibition of AChE activity in head, thorax and abdomen. The highest inhibition percentage means were recorded in abdomen (ranged from 69.05 at the concentration of 47 mg L^{-1} to 82.93% at the concentration of 188 mg L^{-1} with an average of 64.31%), while lower inhibition of enzyme activity was found in the head (48.75%) and in the thorax (59.04%). Statistical analysis revealed that the inhibition of AChE activity in the adult workers abdomens was significantly ($p > 0.05$) higher than that recorded in both thoraces and heads. The inhibition in the heads was significantly ($p > 0.05$) lower than that in the thoraces. On the other hand, all treatments resulted in various degrees of inhibition in AChE activities in adult workers. They were 55.30, 56.06, 73.21, 77.09 and 82.52 % when treated with 23.5, 47, 94,188 and 376 mg L^{-1} of protikto, in respect. Statistical analysis showed that all treatments resulted in significant inhibition ($p > 0.05$)

in AChE activities in adult workers. Furthermore, the highest significant inhibition was caused by the high concentration, while the lowest one was caused by the low concentration activity. Therefore, the biopesticide protikto when found in sugar syrup at a concentration of 23.5, 47, 94,188 or 376 mg L^{-1} has an inhibitory effect on AChE activity of adult honey bee workers after 24 h of application.

Data of spinosad (after 24 h) are summarized and presented in Table 6. Mean values of acetylcholinesterase (AChE) activity in heads of workers fed on sugar syrup 1:1 (w/v) with 0, 1.25, 2.5, 5, 10 and 20 mg L^{-1} of spinosad were larger than those in thoraces, respectively. The lowest levels of the AchE activity were found in the bee abdomen. Furthermore, the average of AChE activities in head (35.67 nmoles ATChI hydrolyzed/mg protein/min.) was larger than that in thorax (27.56) compared with 16.76 in abdomen. Statistical analysis revealed that the AChE activity in adult worker heads was significantly ($p > 0.05$) higher than the activity in both of thoraces and abdomens. On the other hand, the activity in abdomens was significantly ($p > 0.05$) lower than the activity in thoraces. When data of head, thorax and abdomen were summed to estimate the effects of concentrations, all treatments resulted in various decreases in AChE activity compared with the control. The means of AChE activities in adult workers were 26.38, 25.73, 22.04, 21.96 and 22.03 nmoles ATChI hydrolyzed/mg protein/min. when treated with 1.25, 2.5, 5, 10 or 20 mg L^{-1} of spinosad, in respect, compared with 41.86 for untreated workers. Statistical analysis illustrated that the activity in control was significantly ($p > 0.05$) higher than any treatment. On the other hand, the AChE activities in adult workers did not significantly ($p > 0.05$) differ when fed on sugar solution with 1.25 or 2.5 mg L^{-1}. Furthermore, the AChE activities did not significantly differ when the concentration increased from 5 to 10 mg L^{-1}. In addition, tested concentrations caused various degrees of inhibition of AChE activity in head, thorax and abdomen. The highest inhibition percentage means were recorded in abdomen (ranged from 51.65 at the concentration of 2.5 mg L^{-1} to 66.22% at the concentration of 10 mg L^{-1} with an average of 50.54%), while lower inhibition of enzyme activity was found in head (29.36%) and in thorax (33.08%). Statistical analysis revealed that the inhibition of AChE activity in adult workers abdomens was significantly ($p > 0.05$) higher than that recorded in both of thoraces and heads.

On the other hand, all treatments resulted in various degrees of inhibition in AChE activities in adult workers. They were 38.86, 39.79, 49.07, 49.20 and 49.03% when treated with 1.25, 2.5, 5, 10 or 20 mg L^{-1} of spinosad, in respect. Statistical analysis showed that all treatments resulted in significant inhibition ($p > 0.05$) in AChE activities in adult workers. Therefore, the biopesticide spinosad when found in sugar syrup at concentration of 1.25, 2.5, 5, 10 or 20 mg L^{-1} has an inhibitory effect on AChE activity of adult honey bee workers after 24 h of application.

5

Comparing of the inhibitory effects of field application rates (250, 1500, 62.5, 47 and 20 mg L^{-1}) of bioarch, biofly, biozed, protikto and spinosad, in respect, on AChE activity of treated adult honey bee workers (after 24 h) indicated that all tested biopesticides (at field application rate) caused different inhibition percentages in AChE activity (of all body of workers). Significant inhibitions in AChE activity were observed by protikto (56.06%), biozed (55.27%), biofly (52.12%), bioarch (54.14%) or spinosad (49.03 %). The effects of field application rates differed with regions of workers. The high inhibitions of enzyme activity were recorded in workers abdomen when they fed on sugar syrup with bioarch (71.26%), protikto (69.05%) or spinosad (64.72%) compared with 55.74, 67.11 and 43.56% in thorax, respectively. The least inhibitory effects on abdomen AchE activity were caused by biozed (47.66%) and biofly (38.47%). Inhibitory effects (58.22 and 55.53%) on thorax AChE activity were obtained when bees were fed on sugar syrup with biofly or biozed, in respect. Lower inhibitions of enzyme activity were observed in workers head when they fed on sugar syrup with spinosad (38.81%) bioarch (35.42%) or protikto (32.01%) compared with those obtained in thorax. But, higher inhibitory effects on AChE activity in workers head were obtained by biozed (62.62%) and biofly (59.68%) compared with those obtained in thorax.

Impacts of Tested Biopesticides on Acetylcholinesterase (AChE) Activity after 48 h

Data of bioarch (after 48 h) are illustrated in Table 2. Variations in mean values of AChE activity, among the three regions of adult workers, followed the trend of those found after 24 h of application. But, obvious increases in AChE activities in all regions were found. The averages were 63.37, 49.64 and 23.68 in head, thorax and abdomen, in respect, compared with 36.41, 19.72 and 14.28 nmoles ATChI hydrolyzed/mg protein/min. after 24 h of application. Furthermore, data of protikto and spinosad (after 48 h) in Tables 5 and 6, in respect showed the same trend and increases in AChE activities in all regions as those caused by bioarch. The averages (in the case of protikto) were 100.59, 32.51 and 19.52 in head, thorax and abdomen, in respect, compared with 25.88, 16.87 and 12.10 nmoles ATChI hydrolyzed/mg protein/min. after 24 h of application. In regard to spinosad, the averages were 91.31, 34.15 and 28.58 in head, thorax and abdomen, in respect, compared with 35.67, 27.56 and 16.76 nmoles ATChI hydrolyzed/mg protein/min. after 24 h of application. As obtained after 24 h of application with bioarch, protikto or spinosad, statistical analysis of data after 48 h of application illustrated that the AChE activity in adult worker heads was significantly ($p > 0.05$) higher than the activity in both of thoraces and abdomens. Furthermore, the activity in abdomens was significantly ($p > 0.05$) lower than the activity in thoraces. In addition, data of biofly and biozed (after 48 h) illustrated in Table 3 and 4 followed a different trend. Obvious increases in AChE activities were obtained by biozed (in all regions) and biofly (in head and thorax), whereas there was a small decrease in AChE activity of abdomen by biofly. However, the highest increases in AChE activity (after 48 h) were obtained in heads of workers when treated with biofly, biozed,

protikto or spinosad, while the highest increase was obtained in thorax of workers when treated with bioarch.

On the contrary of inhibition in AChE activity obtained after 24 h, there were different degrees of activation in AChE activity of head, thorax and abdomen obtained after 48 h of application with tested biopesticides, except in the cases of bioarch, biofly and protikto where inhibition in AChE activities of abdomen were obtained. The highest activation percentage means were recorded in head of workers when treated with biozed (97.60%), protikto (58.82%) or spinosad (44.17%) whereas when treated with bioarch or biofly, the highest percentages of activation were obtained in thorax (105.14%) and in abdomen (53.91%), respectively.

The effects of field application rates (250, 1500, 62.5, 47 and 20 mg L^{-1}) of bioarch, biofly, biozed, protikto and spinosad, in respect, on AChE activity of treated adult honey bee workers (after 48 h) could be compared. The comparison indicated that all tested biopesticides (at field application rate) caused different activation percentages in AChE activity (of all body of workers), except biofly which caused a significant inhibition (36.01%). The activations in AChE activity were significant in the cases of biozed (66.45%), protikto (22.56%) and bioarch (7.80%), while spinosad resulted in insignificant activation (2.45%). The effects of field application rates differed with regions of workers. The high activations of enzyme activity were recorded in workers head when they fed on sugar syrup with biozed (124.18%), protikto (60.72%), spinosad (27.70%) or bioarch (12.60%). But, biofly at field application rate (1500 mg L^{-1}) had an inhibitory effect on workers head (17.74%). Lower activations of enzyme activity were observed in workers thorax when they fed on sugar syrup with biozed (78.77%), protikto (50.50%), spinosad (9.36%), while bioarch caused a higher activation (51.03%) compared with that obtained in head. On contrary, the field application rate of biofly inhibited enzyme activity in workers thorax with 16.84%. On the other hand, no activation of enzyme activity was observed in workers abdomen (after 48 h of application). But, all tested biopesticides, at field application rat, had inhibitory effects on abdomen AChE activity as found after 24 h of application. The highest inhibitory effect on abdomen AChE activity was caused by biofly (73.46%), while the least inhibition was found by biozed. However, the only biopesticide that did not cause any activation in AChE activity in all regions of workers was biofly.

DISCUSSION

Due to integrated pest programmed play implementation in the field of crop protection, biopesticides is one of these groups. Attention has been given recently to develop biological or non-chemical pesticides such as biopesticides which may be rather safe to non-target organisms as an alternative method to the broad use of conventional pesticides. Biopesticides are increasingly produced and applied throughout Egypt to control various agricultural pests. Little information has been given in literature on the negative effects of biopesticides on honey bees (*Apis mellifera* L.), the most important pollinator for cultivated ecosystem. Studies based on the enzymatic

aspects of adult honey bee workers after exposure to biopesticides remained poorly investigated. Therefore, the current study was carried out to evaluate the potential side effects of five members of this group on honey bee individuals. These biopesticides were; Bioarch (*Bacillus megaterium*), Biofly (*Beauveria bassiana*), Biozed (*Trichoderma album*), Protikto BTK (*Bacillus thuringiensis kurstaki*) and Spintor (Spinosad).

The acute toxicities of these compounds to caged adult honey bee workers under laboratory conditions were compared. Spinosad showed the most toxic action to adult honey bee workers with LC_{50} of 11.60 mg L^{-1} followed by biozed with LC_{50} of 114.12 mg L^{-1}, compared with the application rates in the field (20 and 62.5 mg L^{-1}, respectively). However, lower toxicities were obtained with protikto BTK (LC_{50} = 87,412 mg L^{-1}), biofly (LC_{50} = 49,766 mg L^{-1}) and bioarch (LC_{50} = 15,785 mg L^{-1}). Spinosad is a novel biopesticide derived by fermentation of the naturally occurring soil actinomycete bacterium, *Saccharopolyspora spinosa* (Sparks *et al.,* 1998), and found to be effective in controlling many pests in some fruit, vegetables and ornamental cultivation. The active ingredient is a mixture of spinosyns A and D (Thompson *et al.,* 1997). The biopesticide spinosad showed highly toxic effect to honey bees in acute oral and contact toxicity studies (Mayes *et al.*, 2003; Miles, 2003; Rabea *et al.,* 2010). Our findings indicate that feeding adult honey bee workers on sugar syrup 1:1 (w/v) containing 1.25-20 mg L^{-1} (LC_{50} = 11.60 mg L^{-1}) of spinosad for 24 h resulted in high mortality of treated bees. This result confirmed the findings of Miles (2003) and Rabea *et al.* (2010) who found that exposure to spinosad for 24 h under laboratory conditions caused high mortality of honey bees (LC_{50} = 7.34 mg L^{-1}). Furthermore, our results supported the topical acute activity of spinosad against honey bees (less than 1 µg/bee) obtained by the Environmental Protection Agency (EPA), i.e., thus it has placed in the highly toxic category to bees. On contrary, field studies indicated that dry residues of spinosad were safe to foraging worker honey bees, with no adverse effects seen on mortality, foraging behavior, brood or queen (Mayes *et al.*, 2003; Miles, 2003). So, spinosad must be applied allowing drying time before forager bees are exposure to it (Rabea *et al.,* 2010). Because of its unique action mechanism, as it has strong insecticidal activity especially against Lepidoptera larvae with low levels of mammalian toxicity and relatively little toxicity to non-target insects (Bret *et al.,*1997), it could be used for controlling wax moth (one of the serious bee pests) in stored combs. In regard to biofly, it is considered safe to honey bees according to our results of toxicity (LC_{50} = 49,766 mg L^{-1}). Meikle *et al.* (2008 a, b) found that a single two successive applications of formulation containing *B. bassiana* conidia did not have measurable negative impact on colony health or survivorship. However, the use of an entomopathogenic fungus inside hives involves some risk. In addition, the entomopathogenic bacteria *B. thuringiensis var kurstaki* (protikto BTK) produces a parasporal crystal δ-endotoxin protein which is lethal to many lepidopteran larvae (Adang *et al.,* 1985) such as wax moths. Because of its very low toxicity (LC_{50} = 87,412 mg L^{-1}) against honey bees, it could be used for controlling wax moths. Concerning bioarch, it was found to

be low toxic (LC_{50} = 15,785 mg L^{-1}) against honey bees. In *Bacillus megaterium,* isolated from honeys, there was a high positive correlation between coagulase (bound and free) and the haemolytic activity (López and Alippi, 2010). Hemolytic and coagulase activities were observed in 77% and 74% of *B. megaterium* isolates from honeys (López and Alippi, 2009). Coagulase may be correlated with virulence of certain organisms (Dinges and Orwin, 2003). *Bacillus megaterium* (m435) showed an antagonistic effect to the fungus *Ascosphaera apis*, the causative agent of chalkbrood disease in honey bee larvae (Reynaldi *et al.,* 2004).

Numerous studies have demonstrated that esterases play an important role in conferring or contributing to insecticide detoxifications in insect and other arthropod species (Mouches *et al.,* 1986). Acetylcholinesterase (AChE) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. In insects, AChE is the only cholinesterase (Salgado *et al.,* 1998). AChE represents a biomarker of neurotoxicity widely used for identifying exposure to chemicals such as organophosphorous, carbamate insecticides (Bandyopadhyay, 1982; Guilhermino *et al*., 1998), and some pyrethroids (Bendahou *et al*., 1999; Badiou *et al*., 2008). Other classes of environmental contaminants are Furthermore involved in AChE reduction (Frasco *et al.,* 2005). High exposure levels can kill forager bees, but sublethal exposures may Furthermore adversely affect colony function (Currie, 1999). So, any pesticide has adverse effect on longevity or/and has sublethal effects such as inhibiting of AChE activity of honey bee workers, may Furthermore adversely affects colony strength and performance. Therefore, the current study investigates the possibility of using AChE activity as a biomarker of honey bee exposure to tested biopesticides in order to determine the probable cause of lethal and sublethal effects. AChE is an important enzyme responsible for rapid hydrolysis of acetylcholine at the cholinergic synapses (Fahmy and Dahi, 2009), thus allowing precise control and modulation of neural transmission (Rabea *et al.,* 2010). It is largely distributed in the bee brain (Huang and Knowles, 1990).

Our results indicated that all tested biopesticides caused various degrees of inhibition in AChE activity of adult honey bee workers (after 24 h of application) differed with body region and with concentration. On contrary, there were different degrees of activation in AChE activity of head, thorax and abdomen obtained after 48 h of application with tested biopesticides, except in the cases of bioarch, biofly and protikto where inhibition in AChE activities of abdomen were obtained. The activation in AChE activities may be due to replacement of cotton bed applied with sugar syrup plus biopesticde with another one free of biopesticide after 24 of application. It is obvious that bees could recovery of inhibitory effects caused by most biopesticdes if exposure stopped before reaching to permanent adverse effect. Concerning spinosad, our findings confirmed those obtained by Fahmy and Dahi (2009) and Rabea *et al.,* (2010) who found that exposure to spinosad significantly inhibited AChE activity in honey bee workers after 24 h. Because of the prolonged hyperexcitation, insects eventually became paralyzed, apparently due to neuromuscular fatigue (Salgado *et al.,* 1998). Spinosad seems to work via

mimicking ACh, thus it enhances the overproduction of AchE but it doesn't combat Ach responsible causing symptoms due to its toxicity (Fahmy and Dahi, 2009). This hyperactivity of AChE and the overproduction of Ach which is found in this study may be explained according to Salgado *et al.,* (1998) who demonstrated that spinosad could attack the nicotinic acetylcholine receptor (nAChR) with Ach simultaneously, as well as acting on a new site differing from the site on which ACh acts. Their hypothesis may explain the actions of spinosad and other biopesticdes as there were two special sites on nAChR for spinosad and Ach individually. When both spinosad and Ach are absent, the receptor channel will keep closed. When either of them is present or both of them are present, the channel will open up and subsequently the receptor will be activated. The mode of action of spinosad is characterized by excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and paralysis (Miles and Dutton, 2000; Rabea *et al.,* 2010). Furthermore, Watson (2001) indicated that spinosad could Furthermore act on γ-aminobutyric acid (GABA) receptor and increase neural activity of pest in excess and subsequently make the pest fall into a decline and be dead eventually.

Conclusions

Our results indicated that spinosad showed the highest adverse effect against adult honey bee workers with LC_{50} of 11.60 mg L^{-1} followed by Biozed with LC_{50} of 114.12 mg L^{-1} whereas Protikto, Biofly and Bioarch were safe. This study demonstrates that all tested biopesticides caused various degrees of inhibition in AchE activity of adult honey bee workers (after 24 h of application) according to on body region and concentration. On contrary, there were different degrees of activation in AchE activity of head, thorax and abdomen obtained after 48 h of application with tested biopesticides, except in the cases of bioarch, biofly and protikto where inhibition in AchE activities of abdomen were obtained. The activation in AchE activities may be due to replacement of cotton bed applied with sugar syrup plus biopesticde with another one free of biopesticide after 24 of application allowing a chance to recovery. So, exposure of forager bees (in the field) or/ and house bees inside hives to sublethal concentrations of some biopesticide residues may affect olfactory and behavior resulting in unsuccessful in returning to their hives which may participate in the multifactorial syndrome Colony Collapse Disorder (CCD) in honey bee colonies. Further studies are needed to investigate the interactions of these compounds with other probable causative factors of CCD.

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تـأثيرات بعض مبيـدات حيويـة للآفـات علـى نشـاط إنـزيم الأسـيتيل كـولين إسـتيريز (AChE) وسميتها الحادة لشغالات نحل العسل

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هناك زيادة مضطردة في تطبيق المبيدات الحيوية للآفات في مصر. ولم يتم دراسة التأثيرات السلبية لهذه المركبات عل نحل العسل (الملقح الأكثر أهمية للنظام البيئي الزراعي) بشكل جيد. وكان هدف هذه الدراسة هو تقييم التأثيرات الجانبية المحتملة لخمسة مبيدات حيوية للآفات: & biozed & biofly (*Beauveria bassiana*) bioarch (*Bacillus megaterium*) & protikto BTK (*Bacillus thuringiensis kurstaki*) & *(Trichoderma album)* spintor (spinosad) على نسب الموت ونشاط إنزيم الأسيتيل كولين إستيريز لشغالات نحل العسل. تم تحديد نسب موت الشغالات بعد 24 ساعة من التطبيق وتقدير التركيزات القاتلة ل 50% من الشغالات (LC_{50}). وكان يتم تقييم التأثيرات على نشاط إنزيم الأسيتيل كولين إستيريز (AChE) حيويا بعد 24 و 48 ساعة في رؤوس وصدور وبطون النحل الحي. وأظهرت النتائج أن spinosad كان الأعلى سمية لشغالات نحل العسل بقيمة LC_{50} تقدر ب 11.6 مجم/ لتر وكان biozed التالى فى السمية بقيمة LC_{50} تقدر ب 114.12 ملليجرام/ لتر. ووجدت درجات سمية أقل عند المعاملة ب protikto (LC_{50} = 87412 ملليجرام / لتر) و biofly (LC_{50} = 49766 ملليجرام / لتر) و bioarch (LC_{50} = 15785 ملليجرام / لتر). وعلاوة على ذلك فإن كل المبيدات الحيوية تحت الدراسة سببت درجات مختلفة من تثبيط نشاط إنزيم الأسيتيل كولين إستيريز لشغالات نحل العسل (بعد 24 و 48 ساعة من المعاملة) اختلفت مع اختلاف منطقة الجسم ومع اختلاف التركيز. وعلى النقيض حدثت درجات مختلفة من تنشيط نشاط إنزيم الأسيتيل كولين إستيريز برؤوس وصدور وبطون شغالات نحل العسل بعد 48 ساعة من المعاملة بالمبيد الحيوى فيما عدا عند المعاملة ب bioarch و biofly و protikto والتي سببت تثبيط نشاط إنزيم الأسيتيل كولين إستيريز في منطقة البطن.

كلمات مفتاحية : نحل العسل ، المبيدات الحيوية للآفات ، السمية ، إنزيم الأسيتيل كولين إستيريز و Biozed و *Beauveria bassiana* و Biofly و *Bacillus megaterium,* و Bioarch و *Bacillus thuringiensis kurstaki* و Protikto و *Trichoderma album* و Spintor وSpinosad,.

قام بتحكيم البحث

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Table 3: *In vivo* effects on acetylcholinesterase (AChE) activity in different regions of honey bee workers after 24 and 48 hours of feeding on sugar syrup with biofly.

Concentration (mg L-1)	Biofly-24 h				Biofly-48 h			
	nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)		nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)	
	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average
Head								
0.0	50.50±0.26	34.88 a	0.00±0.00	30.92 b	63.34±6.14	97.48 a	0.00±0.00	-53.91 c
93.75	37.27±1.05		26.20±2.08		135.33±4.58		-113.68±7.24 @	
187.5	36.25±1.17		28.22±2.31		131.76±2.36		-108.03±3.72	
375	28.27±5.24		44.01±10.37		120.55±8.41		-90.33±13.28	
750	36.65±2.00		27.42±3.97		81.82±13.16		-29.18±20.78	
1500	20.36±0.64		59.68±1.27		52.10±2.72		17.74±4.29	
Thorax								
0.0	41.19±1.92	22.11 b	0.00±0.00	46.33 a	24.20±1.23	25.32 b	0.00±0.00	-4.63 b
93.75	23.06±2.36		44.01±5.73		30.66±0.58		-26.69±2.41	
187.5	15.80±3.26		61.65±7.92		24.50±0.96		-1.25±3.98	
375	13.77±3.03		66.57±7.34		26.41±1.10		-9.15±4.54	
750	21.62±0.90		47.50±2.18		26.02±1.50		-7.52±6.18	
1500	17.21±1.33		58.22±3.24		20.12±2.11		16.84±8.72	
Abdomen								
0.0	33.89±1.06	22.61 b	0.00±0.00	33.27 b	26.41±2.39	18.77 c	0.00±0.00	28.94 a
93.75	28.93±2.91		14.63±8.58		21.17±2.73		19.84±10.34	
187.5	13.62±2.66		59.80±7.84		23.10±1.53		12.53±5.79	
375	20.11±2.41		40.66±7.12		21.32±0.50		19.27±1.90	
750	18.27±5.04		46.08±14.87		13.60±3.53		48.51±13.37	
1500	20.85±1.15		38.47±3.39		7.01±1.99		73.46±7.54	
Total	Mean		Mean		Mean		Mean	
0.0	41.86 a	26.53	0.00 d	36.84	37.98 c	47.19	0.00 b	-9.87
93.75	29.75 b		28.28 c		62.39 a		-40.17 d	
187.5	21.89 d		49.89 a		59.79 ab		-32.25 c	
375	20.72 d		50.41 a		56.09 b		-26.73 c	
750	25.52 c		40.34 b		40.48 c		3.94 b	
1500	19.48 d		52.12 a		26.41 d		36.01 a	

*** Means in the same column followed by the same letter(s) are not significantly different according to L.S.D test at 0.05 level of probability.**

(-) before mean indicate that there is activation in AChE activity.

Table 4: *In vivo* effects on acetylcholinesterase (AChE) activity in different regions of honey bee workers after 24 and 48 hours of feeding on sugar syrup with biozed.

Concentration (mg L-1)	Biozed-24 h				Biozed-48 h			
	nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)		nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)	
	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average
Head								
0.0	50.50±0.26	21.89 a	0.00±0.00	56.65 a	63.34±6.14	125.15 a	0.00±0.00	-97.60 c
31.25	30.74±0.65		39.12±1.29		155.57±6.19		-145.63±9.77	
62.5	18.88±2.67		62.62±5.29		141.99±6.32		-124.18±9.98	
125	13.48±1.96		73.31±3.88		95.10±5.13		-50.14±8.09	
250	11.89±0.77		76.45±1.52		162.95±6.22		-157.28±9.81	
500	5.84±1.12		88.43±2.22		131.96±5.85		-108.35±9.24	
Thorax								
0.0	41.19±1.92	20.11 b	0.00±0.00	51.17 b	24.20±1.23	39.67 b	0.00±0.00	-63.93 b
31.25	26.11±1.53		36.61±3.71		30.83±2.09		-27.42±8.65	
62.5	18.32±1.43		55.53±3.47		43.26±2.72		-78.77±11.25	
125	14.18±3.04		65.57±7.37		53.06±0.65		-119.26±2.67	
250	12.82±1.38		68.88±3.35		64.30±0.62		-165.73±2.56	
500	8.07±0.71		80.42±1.73		22.35±2.33		7.62±9.62	
Abdomen								
0.0	33.89±1.06	19.34 b	0.00±0.00	42.94 c	26.41±2.39	26.66 c	0.00±0.00	-0.93 a
31.25	17.59±2.67		48.09±7.89		31.36±3.39		-18.73±12.82	
62.5	17.74±1.04		47.66±3.07		25.46±1.50		3.59±5.66	
125	17.08±1.49		49.61±4.39		26.01±1.59		1.52±6.01	
250	14.91±1.30		56.00±3.83		19.67±1.15		25.54±4.36	
500	14.82±1.88		56.26±5.53		31.03±2.14		-17.49±8.12	
Total	Mean		Mean		Mean		Mean	
0.0	41.86 a	20.45	0.00 f	50.25	37.98 e	63.82	0.00 a	-54.15
31.25	24.81 b		41.27 e		72.59 b		-63.93 d	
62.5	18.31 c		55.27 d		70.24 b		-66.45 d	
125	14.91 d		62.83 c		58.05 d		-55.96 c	
250	13.21 e		67.11 b		82.31 a		-99.16 e	
500	9.58 f		75.04 a		61.78 c		-39.41 b	

*** Means in the same column followed by the same letter(s) are not significantly different according to L.S.D test at 0.05 level of probability.**

(-) before mean indicate that there is activation in AChE activity.

Table 5: *In vivo* effects on acetylcholinesterase (AChE) activity in different regions of honey bee workers after 24 and 48 hours of feeding on sugar syrup with Protikto.

Concentration (mg L-1)	Protikto-24 h				Protikto-48 h			
	nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)		nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)	
	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average
Head								
0.0	50.50±0.26	25.88 a	0.00±0.00	48.75 c	63.34±6.14	100.59 a	0.00±0.00	-58.82 c
23.5	30.70±2.93		39.21±5.80		120.76±12.30		-90.66±19.42	
47	34.33±2.59		32.01±5.14		101.79±12.78		-60.72±20.18	
94	19.57±2.23		61.26±4.42		156.95±20.77		-147.8±32.79	
188	12.33±3.19		75.58±6.31		82.00±2.39		-29.47±3.77	
376	7.86±3.78		84.43±7.48		78.72±6.45		-24.29±10.18	
Thorax								
0.0	41.19±1.92	16.87 b	0.00±0.00	59.04 b	24.20±1.23	32.51 b	0.00±0.00	-34.35 b
23.5	17.76±3.79		56.89±9.21		42.87±6.11		-77.18±25.26	
47	13.55±2.40		67.11±5.82		36.42±6.96		-50.50±28.75	
94	9.72±0.75		76.41±1.83		37.98±2.19		-56.97±9.07	
188	11.22±1.15		72.77±2.79		25.96±2.62		-7.27±10.83	
376	7.81±2.02		81.04±4.91		27.63±2.28		-14.17±9.44	
Abdomen								
0.0	33.89±1.06	12.10 c	0.00±0.00	64.31 a	26.41±2.39	19.52 c	0.00±0.00	26.11 a
23.5	10.23±2.06		69.80±6.07		25.68±3.29		2.75±12.44	
47	10.41±2.86		69.05±8.44		14.91±0.51		43.53±1.93	
94	6.11±0.94		81.97±2.79		27.19±0.85		-2.94±3.20	
188	5.79±1.34		82.93±3.95		15.91±3.54		39.75±13.42	
376	6.07±1.36		82.09±4.01		6.98±1.63		73.56±6.17	
Total	Mean		Mean		Mean		Mean	
0.0	41.86 a	18.28	0.00 d	57.36	37.98 d	50.87	0.00 a	-22.35
23.5	19.56 b		55.30 c		63.10 b		-55.03 c	
47	19.46 b		56.06 c		51.04 c		-22.56 b	
94	11.80 c		73.21 b		74.04 a		-69.24 b	
188	9.78 c		77.09 b		41.29 d		1.01 a	
376	7.25 d		82.52 a		37.78 d		11.70 a	

*** Means in the same column followed by the same letter(s) are not significantly different according to L.S.D test at 0.05 level of probability.**

(-) before mean indicate that there is activation in AChE activity.

Table.6: *In vivo* effects on acetylcholinesterase (AChE) activity in different regions of honey bee workers after 24 and 48 hours of feeding on sugar syrup with spinosad.

Concentration (mg L-1)	Spinosad-24 h				Spinosad-48 h			
	nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)		nmoles ATChI hydrolyzed /mg protein/min.		Inhibition (%)	
	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average	Mean±SD	Average
Head								
0.0	50.50±0.26	35.67 a	0.00±0.00	29.36 b	63.34±6.14	91.31 a	0.00±0.00	-44.17 b
1.25	36.71±3.27		27.30±6.47		114.87±4.53		-81.37±7.15	
2.5	34.23±1.48		32.21±2.92		107.90±7.21		-70.36±11.39	
5	31.51±2.28		37.60±4.52		90.04±15.74		-42.16±24.84	
10	30.18±3.87		40.24±7.66		90.85±7.32		-43.45±11.56	
20	30.90±1.58		38.81±3.14		80.88±4.26		-27.70±6.73	
Thorax								
0.0	41.19±1.92	27.56 b	0.00±0.00	33.08 b	24.20±1.23	34.15 b	0.00±0.00	-41.11 b
1.25	27.69±1.39		32.77±3.36		35.98±4.26		-48.71±17.59	
2.5	26.57±2.38		35.50±5.77		31.05±2.71		-28.33±11.18	
5	22.45±5.49		45.50±13.33		45.21±6.65		-86.85±27.46	
10	24.24±0.62		41.15±1.50		41.96±2.04		-73.41±8.41	
20	23.25±1.42		43.56±3.45		26.46±2.10		-9.36±8.67	
Abdomen								
0.0	33.89±1.06	16.76 c	0.00±0.00	50.54 a	26.41±2.39	28.58 c	0.00±0.00	-8.21 a
1.25	14.74±1.37		56.51±4.05		33.70±2.93		-27.58±1.11	
2.5	16.38±4.36		51.65±12.87		27.36±1.27		-3.60±4.82	
5	12.16±3.42		64.10±10.08		36.49±1.80		-38.17±6.81	
10	11.45±2.33		66.22±6.88		28.94±2.39		-9.59±9.04	
20	11.95±2.19		64.72±6.45		18.56±2.76		29.72±10.46	
Total	Mean		Mean		Mean		Mean	
0.0	41.86 a	26.67	0.00 c	37.66	37.98 c	51.35	0.00 a	-31.16
1.25	26.38 b		38.86 b		61.52 a		-52.55 cd	
2.5	25.73 b		39.79 b		55.44 b		-34.10 b	
5	22.04 c		49.07 a		57.25 ab		-55.73 d	
10	21.96 c		49.20 a		53.92 b		-42.15 bc	
20	22.03 c		49.03 a		41.97 c		-2.45 a	

*** Means in the same column followed by the same letter(s) are not significantly different according to L.S.D test at 0.05 level of probability.**
(-) before mean indicate that there is activation in AChE activity.