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TOXIC AND BIOCHEMICAL EFFECTS OF *Beauveria bassiana* AND SPINOSAD ON NURSE AND FIELD WORKERS OF HONEY BEE , *Apis mellifera*

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ABSTRACT

This study was conducted to evaluate the toxic and biochemical effects of multiple applications with Biofly (*Beauveria bassiana*) and SpinTor® (spinosad) on nurse and field honey bee (*Apis mellifera* L.) workers under laboratory conditions. The mortality percentages of workers treated with both biopesticides were determined after 24 h of one, two and three daily applications. The lethal concentrations of spinosad that caused 50% mortality (LC_{50}) were estimated. The effects of two biopesticides on the acetylcholinesterase (AChE) activities in heads, thoraces, and abdomens of surviving nurse and field workers were determined *in vivo* after 24 of single, two and three daily applications. The results indicated that spinosad was higher toxic than Biofly to both nurses and field worker. Also, the mortality percentages after 24 h of three daily applications of Biofly and spinosad were higher than that of two daily applications whatever worker type. In the same pattern, the mortality percentages after two daily applications were higher than that of single application. On the other hand, Biofly and spinosad were more toxic to foragers than to nurses after 24 h of one application, or two or three daily applications. Furthermore, our findings indicated that mean values of AChE activity in heads of nurse and field workers fed sugar syrup with 0, 187.5, 375, 750, 1500 and 3000 mg L^{-1} of Biofly, and 0, 2.5, 5, 10 and 20 mg L^{-1} of spinosad were higher than those in both thoraces and abdomens after 24 h of all treatments. Also, the average of AChE activities in heads was significantly ($p > 0.05$) higher than that obtained in both thoraces and abdomens whatever tested biopesticide, worker type or number of applications. In addition, tested concentrations caused various degrees of inhibition and activation in AChE activity in heads, thoraces and abdomens when compared with controls. Summed data revealed that Biofly and spinosad increased AChE activity of both worker types after 24 h of applications however it repeated or not.

Keywords: Honey bee, *Apis mellifera* L.*,* field worker, nurse, biopesticides, Biofly®, *Beauveria bassiana,* SpinTor®, spinosad*,* toxicity, acetylcholinesterase.

INTRODUCTION

Protecting honey bees, *Apis mellifera* L., from pesticides is vital and importance for agro system (Krupke *et al.,* 2012). New generations of pesticides, such as microbial biopesticides, are thought to be less harmful to human and the environment than conventional insecticides (Koul and Dhaliwal, 2002). Amounts of pesticide that considered 'safe' to use could affect the health of entire bee colonies. Since 2006, beekeepers in North America and Europe have lost about one-third of their managed bee colonies each year due to colony collapse disorder (CCD) which caused several billion dollars of direct economic losses by reduction in crop-yields (Murray *et al.*, 2009). While the exact cause is unknown, researchers believe pesticides have contributed to this decline (Cox-Foster and VanEngelsdorp 2009; VanEngelsdorp *et al.,* 2009; VanEngelsdorp and Meixner 2010; Eiri and Nieh,

2012). Sub-lethal effects of pesticides may have significant impacts on bees and pollination in addition to the more easily observable mortality, disrupting foraging and causing decreased pollination and/or bee reproduction. Some pesticides, even biopesticides, could affect bee learning and memory. Biopesticides such as Biofly® [(*Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales)] and SpinTor® (spinosad) are used in Egypt to control various agricultural pests. Spinosad is currently being registered in Egypt as a safer alternative to synthetic pesticides (Aboul-Enein *et al*., 2012). This stimulates interest in potential effects of them on honey bees.

The entomopathogenic fungus *B. bassiana* is a promising biological control agent of several greenhouse pests as well as outdoor crop pests. Also, a formulation containing its conidia has been considered promising alternative to chemical miticides (Chandler *et al.* 2000 and 2001) used for Varroa mites control (Meikle *et al.*, 2008 a, b) with no measurable negative impact on colony health or survivorship (Meikle *et al.,* 2008 a, b). But, the fungus should be avoided as a pest control option where pollinators are present (EPA, 1999) and has been listed in highly toxic category to honey bee (Mader, 2009). Furthermore, recent study indicated that Biofly is non-toxic to adult honey bee workers with LC_{50} of 49,766 mg L^{-1} but with detectable effects on the acetylcholinesterase (AChE) activity (Eid *et al.,* 2011).

Spinosad is a selective fast-acting, somewhat broad-spectrum biopesticide that acts on the insect through ingestion, or by direct contact with a spray droplet or a newly treated surface (Larson, 1997). It is a powerful neurotoxin against certain arthropods, especially lepidopterans larvae, coleopterans, some dipterans, Thysanoptera and Hymenoptera (Mayes *et al.,* 2003; Musser and Shelton, 2003). Also, it is an excellent natural, organic alternative to conventional synthetic insecticides like Sevin in many garden situations. Furthermore, spinosad does not significantly impact predatory beneficial insects, predatory mites, and spiders while controlling target pests. Surveys of non-target and beneficial insects showed no reduction of beneficial insect populations, and no acute toxicity or hazards to honey bees in citrus orchards receiving product sprays, containing a repellent to bees (Thomas and Mangan 2005; Mangani and Moreno, 2009). However, some reports suggested that some formulation containing spinosad adversely affected caged bees and other beneficial insects (Edwards *et al.*, 2003; Mader, 2009; Miles *et al.,* 2011). So, care must be taken when applying spinosad while honey bees are foraging; after residues dry (few hours), it is far less toxic to bees (Bret *et al.*, 1997). It must not be applied to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on the plants during this time period. Also, foragers may collect water, contaminated with spinosad, in which very little breakdown (hydrolysis) occurs, and it can be persistent. In addition, spinosad has the potential for bioaccumulation; it is photolabile, but resistant to hydrolysis (WHO, 2007). Furthermore, recent reports indicated that spinosad is highly toxic to adult honey bee workers with LC_{50} of 7.34 mg L^{-1} (Rabea *et al.,* 2010) and 11.60 mg L^{-1} with detectable effects on the AChE activity (Eid *et al.,* 2011).

Little studies has been carried out on the toxic effects of multiple application with biopesticides on honey bees and little information based on the enzymatic aspects of the host after exposure to biopesticides has been given in literature. So, more studies in this area are needed to evaluate their effects on the honey bee individuals. In a previous study, we investigated the toxic and biochemical effects of multiple applications with Biofly and spinosad on worker larvae. In order to further assess their effects, we conducted this study to determine the acute toxicities and the potential side effects of them, when applied multiply, on AChE activity of nurse and field honey bee workers.

MATERIALS AND METHODS

Chemicals

Biofly (*Beauveria bassiana*) containing $30x10^6$ conidia/cm^3 was supplied by El-Nasr Co. for Fertilizaers and Biopesticides, El-Sadat city, Egypt, SpinTor® 24% SC (spinosad) was supplied by Dow Agro Sciences Co., England. Acetylthiocholine iodide (ATChI), 5, 5- dithio-bis (2-nitrobenzoic acid) (DTNB) and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich Chemical Co., USA.

Honey bees, *Apis mellifera*

Local hybrid (derived from Italian bee *Apis mellifera ligustica* and Carniolan bee *Apis mellifera carnica* and other races existed in Egypt) honey bee colony (free of obvious diseases) from an apiary located in Ezbet Haggag at El-Beheira Governorate was used. Nurse and field honey bee workers were collected from the colony on April. Nurse bees, which work inside the hive tending brood, were caught by shacking open brood combs, covered with bees, above an empty plastic container. Meanwhile field bees, which collect food outside the hives, were caught by shacking honey combs, covered with bees, from the super above another empty plastic container. Then, they 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 kept during experiment in experimental transparent plastic cups, covered with a nylon mesh, each contain 20 adult workers at 25 ± 2°C and 65 ± 5% relative humidity. Three cups of nurse or field honey bee workers were subjected to each concentration of each biopesticide (one, or two or three daily applications) and control.

Acute Toxicity Bioassay

The acute toxicity of both biopesticides was evaluated on nurse and field honey bee workers by feeding under laboratory conditions after 24 h of one, two and three daily applications. A series of concentrations of each biopesticide were prepared in sugar syrup 1:1 (w/v). The application was made through feeding on sugar syrup 1:1 (w/v) containing different concentrations of the tested biopesticides. Biofly was tested at concentrations of 187.5, 375, 750, 1500 and 3000 mg L^{-1}, while spinosad was tested at 2.5, 5, 10, 20 and 40 mg L^{-1}. Adult workers of each cup were fed, in the beginning of application, through cotton bed attached to the nylon mesh and applied

with certain amount of sugar syrup. Three cups (20 worker /cup) were subjected to sugar syrup 1:1 (w/v) containing one of tested concentrations of every biopesticide. All treated workers were fed after 24 and 48 h of first feeding. 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 of one application, or two or three daily applications of both biopesticides to calculate mortality percentages and the LC_{50} of spinosad according to Finney (1971).

Preparation of Bee Extract

The heads, thoraces and abdomens of three nurse or field workers (for each replicate from every concentration of tested biopesticides) were separated without anaesthetizing. Then, 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 enzyme source for assay of AChE activity of nurse and field bee workers.

Total Protein Assay

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

Acetylcholinesterase (AChE) Activity Assay

The AChE activity assays (*in vivo*) of nurse and field bee workers were conducted according to the method of Ellman *et al.* (1961) using the spectrophotometric procedure. Biofly was tested at concentrations of 187.5, 375, 750, 1500 and 3000 mg L^{-1}, spinosad was tested at 2.5, 5, 10, 20 and 40 mg L^{-1}. Three cups of 20 nurse or field honey bee workers were subjected to each concentration of each biopesticide (one, two and three daily applications) and control. The activity of AChE in head, thorax, and abdomen of nurse or field honey bee workers was determined by using DTNB (dithionitrobenzoic) after 24 h of one feeding, two and three daily feedings on sugar syrup 1:1 (w/v) with one concentration of Biofly or spinosad. The supernatants were used as enzyme source for assay of AChE activity. Enzyme (150 µL), 100 µL DTNB (0.01 M), and 30 µL ATChI (0.075 M) were added to 2.8 mL 0.1 M phosphate buffer (pH 8.0). The mixture was incubated at 37 °C for 15 min. 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. Inhibition or activation percentages of the activities against control were considered in the enzymatic assay.

Statistical Analysis

The log dose-response curves allowed determination of the LC_{50} values, after 24 of one, two and three daily applications of spinosad were determined from the Ldp lines for the insect bioassay according to probit analysis (Finney 1971). Data of the effects of multiple applications with Biofly or spinosad on AChE acitivity experiment were subjected to two-way analysis of variance (ANOVA). The experiment of AChE acitivity was conducted in factorial (3x5). 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 Nurse and Field Honey Bee Workers

The results of acute toxicity assay of Biofly and spinosad on nurse and field honey bee workers by feeding under laboratory conditions after 24 h of one , two and three daily applications are summarized in Tables (1and 2, in respect). Spinosad caused higher mortality percentages of both nurse and field honey bee workers when compared with Biofly. Also, three daily applications of Biofly or spinosad caused mortality percentages higher than that caused by two daily applications whatever worker type. In the same pattern, the acute toxicity of two daily applications was higher than that of single application. On the other hand, Biofly and spinosad were more toxic to field workers than to nurses after 24 h of one, two and three daily applications.

Table 1: Mortality percentages of nurse and field honey bee workers after 24 h of one application (I), or two (II) or three daily applications (III) of Biofly.

Worker type	Concentration (mg L^{-1})	Mortality (%)		
		I	II	III
Nurse	187.5	3.39	5.09	6.78
	375	5.09	6.78	8.47
	750	10.17	11.86	13.56
	1500	15.25	16.95	18.64
	3000	16.95	23.73	27.12
Field	187.5	5.09	6.78	8.47
	375	6.78	8.47	10.17
	750	11.86	13.56	15.25
	1500	16.95	20.34	20.34
	3000	20.34	25.42	30.51

Impacts of Tested Biopesticides on AChE Activity of Nurse and Field Honey Bee Workers after 24 h of Single Feeding

The *in vivo* specific activity and inhibition of AChE in different regions (head, thorax, and abdomen) of surviving nurse and field honey bee workers after 24 of single feeding on sugar syrup 1:1 (w/v) with different concentrations of biopesticides were calculated and presented in Tables 3 (Biofly) and 4 (spinosad).

Table 2: Mortality percentages of nurse and field honey bee workers and acute toxicity after 24 h of one application (I), or two (II) or three daily applications (III) of spinosad.

Worker type	Concentration (mg L^{-1})	Mortality (%)		
		I	II	III
Nurse	2.5	3.33	4.01	29.16
	5	10.00	12.00	62.50
	10	26.67	44.00	66.66
	20	76.67	95.19	95.83
	40	86.67	100.00	100.00
	LC_{50} (mg L^{-1})	13.99	9.44	4.38
	Slope ± SE	2.76±0.21	3.93±0.33	2.10±0.23
	χ^2 *	9.23	15.38	11.38
Field	2.5	6.67	10.00	35.97
	5	16.67	46.67	83.93
	10	23.33	60.00	88.01
	20	86.67	66.67	91.97
	40	90.00	96.67	100.00
	LC_{50} (mg L^{-1})	11.98	7.91	2.79
	Slope ± SE	2.66±0.20	1.39±0.15	2.04±0.25
	χ^2	27.71	18.38	17.85

*** Chi square**

Data of Biofly (single feeding) are summarized and presented in Table 3. Mean values of AChE activity in heads of nurse or field workers fed sugar syrup 1:1 (w/v) treated with 0, 187.5, 375, 750, 1500 and 3000 mg L^{-1} of Biofly were higher than those in thoraces and abdomens. Also, the averages of AChE activities in heads of nurse and field workers (116.81and 111.23 nmoles ATChI hydrolyzed/mg protein/min.) were significantly ($p > 0.05$) higher than those in both thoraces (9.97 and 9.79) and abdomens (17.88 and 15.93), respectively. On the other hand, the activities of AChE in abdomens were significantly higher than those obtained in thoraces. When data of heads, thoraces and abdomens were summed to estimate the total effects of pesticides significant increases in AChE activity of nurses (70.32 and 63.60 nmoles ATChI hydrolyzed/mg protein/min.) were obtained at concentrations (187.5 and 375 mg L^{-1}, respectively), while insignificant increase (47.20) was recorded at the concentration of 750 mg L^{-1}. Beside, these concentrations (187.5, 375, and 750 mg L^{-1}) caused significant increases in AChE activity of field workers (60.79, 59.69 and 48.28 nmoles ATChI hydrolyzed/mg protein/min., respectively). On the contrary, the higher concentrations (1500 and 3000 mg L^{-1}) caused significant decreases in AChE activity, 33.19 and 30.71 nmoles ATChI hydrolyzed/mg protein/min. in nurses, and 32.90 and 29.83 in foragers, respectively. In regard to the inhibition or activation of AChE activity compared with control, different activation percentage averages of 2.46, 28.73 and 60.48 were recorded in heads, thoraces and abdomens of nurses compared with 2.99, 12.08 and 51.68 in foragers, respectively.

The effect of spinosad (single feeding) on AchE activity in head, thorax, and abdomen of surviving nurse and field honey bee workers are summarized and presented in Table 4. Mean values of AChE activity in heads of nurse or field workers fed on sugar syrup 1:1 (w/v) mixed with 0, 2.5, 5, 10, 20 and 40 mg L^{-1} of spinosad were larger than those in both thoraces and abdomens. Also, the averages of AChE activities in heads of nurse and field workers (210.31and 203.38 nmoles ATChI hydrolyzed/mg

protein/min.) were significantly ($p > 0.05$) higher than those obtained in both thoraces (10.75 and 10.58) and abdomens (7.79 and 7.42), respectively. On the other hand, the difference between AChE activity of thoraces and abdomens in nurses was insignificant, while it was significant in foragers. Summed data revealed that all concentrations resulted in significant increases in AChE activity whatever worker type. In regard to the inhibition or activation of AChE activity compared with control, the averages of activations in heads of nurse and field workers (84.48 and 88.32 %) were significantly higher than those in thoraces (38.82 and 21.08 %), while the averages of inhibitions in abdomens were 30.06 and 29.32 %, respectively.

Impacts of Tested Biopesticides on AChE Activity of Nurse and Field Honey Bee Workers after 24 h of Two Daily Feedings

The *in vivo* specific activity and inhibition of AChE activity in different regions (head, thorax, and abdomen) of surviving nurse and field honey bee workers after 24 of two daily feedings on sugar syrup 1:1 (w/v) treated with different concentrations of biopesticides were calculated and presented in Tables 5 (Biofly) and 6 (spinosad).

The biochemical effects of double feeding on sugar syrup treated with Biofly on AChE activity is presented in Table 5. The data indicate that mean values of AChE activity in heads of nurse or field workers fed on sugar syrup 1:1 (w/v) with 0, 187.5, 375, 750, 1500 and 3000 mg L^{-1} of Biofly were higher than those in thoraces and abdomens. The averages of AChE activities in heads of nurses and foragers (223.38 and 210.49 nmoles ATChI hydrolyzed/mg protein/min.) were significantly ($p > 0.05$) higher than those in both thoraces (7.00 and 6.98) and abdomens (12.87 and 12.75), respectively. On the other hand, the activity of AChE in abdomens was significantly higher than that in thoraces whatever worker type. Statistical analysis revealed that there were significant differences among body regions of both worker types in inhibition or activation of AChE activity compared with control. Furthermore, the averages of activations in heads of nurses and foragers (102.99 and 94.45 %) were significantly higher than those in abdomens (17.11 and 20.91 %), while the averages of inhibitions in thoraces were 18.63 and 14.12 %, respectively.

The biochemical effects of double feeding on sugar syrup with spinosad on AChE activity is presented in Table 6. The data indicate that mean values of AChE activity in heads of nurse and field workers fed sugar syrup 1:1 (w/v) with 0, 2.5, 5, 10 and 20 mg L^{-1} of spinosad were higher than those in both thoraces and abdomens. Also, the averages of AChE activities in heads of nurses and foragers (393.53 and 353.36 nmoles ATChI hydrolyzed/mg protein/min.) significantly ($p > 0.05$) were higher than those recorded in both thoraces (17.81 and 17.81) and abdomens (14.22 and 13.59), respectively, which did not differ significantly. Summed data revealed that all concentrations resulted in significant increases in AChE activity of both worker types. Furthermore, the averages of activations in AChE activity in heads of nurse and field workers (257.61 and 226.44 %) were significantly higher than those obtained in both thoraces (106.98 and 119.24 %) and abdomens (29.38 and 28.88 %), respectively.

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Impacts of Tested Biopesticides on AChE Activity of Nurse and Field Honey Bee Workers after 24 h of Three Daily Feedings

The *in vivo* specific activity and inhibition of AChE activity in different regions (head, thorax, and abdomen) of surviving nurse and field workers after 24 of three daily feedings on sugar syrup 1:1 (w/v) with treated different concentrations of biopesticides were calculated and presented in Tables 7 (Biofly) and 8 (spinosad).

Table 7 presents data of triple feeding on sugar syrup treated with Biofly. As mentioned above, mean values of AChE activity in heads of nurses and field workers fed on sugar syrup 1:1 (w/v) mixed with 0, 187.5, 375, 750, 1500 and 3000 mg L^{-1} of Biofly were higher than those recorded in both thoraces and abdomens. Also, the averages of AChE activities in heads of nurses and foragers (180.16 and 169.78 nmoles ATChI hydrolyzed/mg protein/min.) were significantly ($p > 0.05$) higher than those found in both thoraces (15.26 and 14.63) and abdomens (9.05 and 8.34), respectively. On the other hand, the activity of AChE in thoraces did not differ significantly from that in abdomens of nurses, and was significantly higher in foragers. In addition, the averages of activations in AChE activity in thoraces of nurse and field workers (85.96 and 88.70 %) were significantly higher than those in heads (65.33 and 59.49 %), while the averages of inhibitions in abdomens were 19.06 and 22.30 %, respectively.

Table 8 presents data of triple feeding on sugar syrup treated with spinosad. Mean values of AChE activity in heads of nurses and foragers fed sugar syrup 1:1 (w/v) with 0, 2.5, 5, 10 and 20 mg L^{-1} of spinosad were larger than those in both thoraces and abdomens. Also, the averages of AChE activities in heads of nurses and foragers (300.75 and 280.69 nmoles ATChI hydrolyzed/mg protein/min.) significantly ($p > 0.05$) higher than those obtained in both thoraces (17. 41 and 16.87) and abdomens (9.13 and 8.66), respectively. In addition, the averages of activations in AChE activity in heads of nurse and field workers (175.99 and 163.68 %) were significantly higher than those recorded in thoraces (112.24 and 117.58 %), while the averages of inhibitions in abdomens were 18.37 and 19.31 %, respectively.

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DISCUSSION

Attention has been given recently to develop biopesticides as an alternative to traditional pesticides in controlling of various agricultural pests. The rather recent phenomenon of colony collapse disorder (CCD) involving the sudden and massive disappearance of bee colonies around the world is worrisome (Stokstad, 2007). The explanation of CCD may be that honey bee workers collecting pollen and nectar in the field become unable to navigate back home. The severe loss of bee products and agricultural products caused by CCD (Swinton *et al.,* 2007) is of great concern to academics as well as to farmers. Exposure to new forms of pesticide that target insect acetylcholine receptors (AChRs) is emerging as a major contributing factor in bee population decline (Le Conte *et al.,* 2010; Maxim and van der Sluijs, 2010). Worker bees typically recruit their nestmates to good food with waggle dances, and it was discovered that the pesticide-treated honey bees danced less: honey bees that prefer only very sweet foods can dramatically reduce the amount of resources brought back to the colony (Eiri and Nieh, 2012). Accumulated stress in response to disease, combined with exposure to such toxins, could precipitate the rapid decline of the honey bee (Hawthorne and Dively, 2011; Wu et al., 2012). In addition, sub-lethal doses of neurotoxic pesticides that target cholinergic signaling can alter the behavior of insects such honey bees in subtle ways; their influence may not be readily apparent in simple mortality studies. Foragers may be exposed continuously along the flowering time of the crop/plant. Moreover, in the hive, pollen and honey stocks are likely to be contaminated too. As a consequence, food stocks consumption may lead to a prolonged and continuous contamination. The detection and uptake of nectar and pollen involves sophisticated nervous system activity which can be perturbed by the presence of pesticides, whether that behavior took place in the hive or outside (Desneux *et al.,* 2007). Khoury *et al*. (2011) showed that a decrease in workers lifespan may conduct to the collapse of the colony. Thus, investigating of the potential negative effects of biopesticides on honey bees is interest search area.

Our results indicated that the mortality percentage increases as the concentration increases whatever tested biopesticide, worker type or number of applications. So that, the acute toxicity increases as the application is repeated in the case of spinosad. Therefore, there was lethal cumulative effect of Biofly or spinosad on both nurses and foragers. Tested biopesticides were more toxic to foragers than to nurses. Our findings confirmed the highly toxic effect of spinosad showed to honey bee workers in acute oral and contact toxicity studies (Mayes *et al.*, 2003; Miles, 2003; Rabea *et al.,* 2010; Eid *et al.,* 2011).

According to Shapira *et al.* (2001), biochemical aspects of the AChE protein were similar in foragers and nurses. However, catalytic acetylcholinesterase (AChE) activity was significantly lower in foragers. The forager-related decrease in AChE activity was associated with decreased AChE mRNA levels. This can contribute to facilitated learning capabilities in forager bees. Also, AChE activity decreases in brains of adult workers with age (Loucif-Ayad *et al.,* 2008). Honey bees have two active forms of the

AChE (Belzunces *et al.,* 1988; Badiou *et al.,* 2007); one form, AChEm1, is expressed at a much higher level in the bee's head than the other, AChEm2, (Belzunces *et al.,* 1988). The inhibition of AChE leads to an excess of the ACh that results in prolonged activation of cholinergic receptors, followed by their desensitization (Pohanka, 2011). An interesting finding was that the AChE inhibiting activities of some pesticides were more potent on extracts from gut tissue than brain tissue (Williamson *et al.,* 2013). It may be suggested that levels of the different AChE enzymes differ between tissues. In addition, the AChE inhibitors had different ways of binding to the enzyme: some act at the active site of the enzyme, but the others bind at a peripheral anion site (Pohanka, 2011). Furthermore, the inhibition of AChE by some pesticides is readily reversible (Seltzer, 2005), while that by others is irreversible (Pohanka, 2011). On the other hand, acute exposure to AChE inhibitors improves learning and memory by enhancing cholinergic transmission in honey bees (Shapira *et al.,* 2001; Guez *et al.*, 2010; Williamson *et al*., 2012). Also Wu *et al*. (2012) related between the heavy infestations with the probable causative factor of CCD, gut parasite *Nosema ceranae*, of honey bees, reared in combs containing high levels of acaricides which disrupts AChE in bee gut. So, we searched for changes in AChE activity in heads, thoraces and abdomens of nurses and foragers after oral multiple applications with tested biopesticides.

Concerning the impacts of tested biopesticides on AChE activity of nurses and foragers after 24 h of single feeding, summed data revealed that the three lower concentrations of Biofly caused increases in AChE activity of both nurse and field workers. On the contrary, the higher concentrations caused decreases. However, all concentrations of spinosad resulted in significant increases in AChE activity whatever worker type. In regard to the inhibition or activation of AChE activity compared with control, tested concentrations of Biofly caused various degrees of inhibition and activation in heads, thoraces and abdomens. Tested concentrations of spinosad caused various degrees of activation in AChE activity in heads and thoraces, except in cases of the highest concentration in thoraces of nurses and the two higher concentrations in thoraces of foragers, while they caused various degrees of inhibition in abdomens. In addition, statistical analysis revealed that there were significant differences among body regions of both nurse and field workers in activation in AChE activity as a result of application with Biofly. But in the case of spinosad, the averages of activations in heads of nurse and field workers were significantly higher than those in thoraces, while in abdomens inhibition averages were recorded, in respect. Thus, the activator impact of spinosad on AChE activity isolated from heads or thoraces was clear compared with that of Biofly. Furthermore, summed data showed that the three lower concentrations of Biofly resulted in various degrees of significant activation in AChE activity whatever worker type, but the two higher concentrations caused insignificant and significant inhibitions in nurses, whereas the two degrees of inhibitions were significant in foragers. But, summed data of spinosad showed that all concentrations resulted in various degrees of significant activation in AChE activity, except the highest concentration which caused insignificant activation in nurses and inhibition in

foragers. Therefore, in general Biofly when found in sugar syrup at concentrations of 187.5, 375, 750, 1500 and 3000 mg L^{-1}, and spinosad when found in sugar syrup at concentrations of 2.5, 5, 10, 20 and 40 mg L^{-1} have activator impacts on AChE activity after 24 h of single feeding whatever worker type. AChE is an important enzyme responsible for the rapid hydrolyses of acetylcholine, a major neurotransmitter at the cholinergic synapses (Fahmy and Dahi, 2009), associated with learning in the insect brain. Our results confirmed the large distribution of it in the bee brain as stated by Belzunces *et al.* (1988), Kreissl and Bicker (1989), Huang and Knowles (1990), and Abdallah *et al.* (1991).

Concerning the impacts of tested biopesticides on AChE activity of nurses and foragers after 24 h of double feeding, mean values of AChE activity in heads of both worker types were larger than those in thoraces and abdomens as found after single feeding, but the values in heads were much higher. Summed data revealed that all concentrations of Biofly resulted in significant increases, except the highest concentration which caused insignificant increase, in AChE activity of both nurse and field workers, but all concentrations of spinosad resulted in significant increases. In regard to the inhibition or activation of AChE activity in nurses and foragers compared with control, all concentrations of Biofly caused various degrees of activations in heads, whereas the two lower concentrations caused various degrees of activations in thoraces, while the higher concentrations caused inhibitions. Also, the three lower concentrations caused activations in AChE activity in abdomens, while the higher concentrations caused inhibitions. But, all concentrations of spinosad caused various degrees of activation in AChE activity in heads, thoraces and abdomens, except 10 mg L^{-1} concentration in thoraces which caused inhibitions whatever worker type. As found after single feeding, there were significant differences among body regions of both worker types. Furthermore, the averages of activations in heads of nurse and field workers were significantly higher than those in abdomens, while averages of inhibitions in thoraces were obtained as a result of Biofly application. But, spinosad caused activation averages in all regions: in heads of nurses and foragers were significantly higher than those obtained in both thoraces and abdomens. Thus, the activator impact of spinosad on AChE activity isolated from any region was clear compared with that of Biofly. On the other hand, the double feeding caused much higher degrees of activation in heads and thoraces than single feeding. Furthermore, summed data indicated that the three lower concentrations of Biofly resulted in various degrees of significant activation in AChE activity whatever worker type, but the concentration of 1500 mg L^{-1} caused insignificant inhibition in nurses and activation in foragers, and the concentration of 3000 mg L^{-1} caused significant inhibition in both worker types. But, summed data of spinosad illustrated that all concentrations resulted in various degrees of significant activation in AChE activity of both worker types. So, in general when found in sugar syrup at tested concentrations spinosad has an activator impact on AChE activity after 24 h of two daily feedings whatever worker type as obtained after single feeding. In general Biofly or spinosad when found in sugar syrup at tested

concentrations has an activator impact on AChE activity after 24 h of two daily feedings whatever worker type as obtained after single feeding, but with higher values especially in case of spinosad.

The results of triple application emphasized the above mentioned results of single and double applications. Summed data of Biofly illustrated that all concentrations resulted in significant increases, except 1500 and 3000 mg L^{-1} concentrations which caused insignificant increase and decrease, respectively, in AChE activity of nurse workers. Similar result was obtained in field workers. Summed data of spinosad illustrated that all concentrations resulted in significant increases in AChE activity of both worker types. On the other hand, the activity of AChE in thoraces did not differ significantly from that in abdomens of nurses, and was significantly higher in foragers after triple application with spinosad as found with Biofly. In regard to the inhibition or activation of AChE activity compared with control, all concentrations of Biofly in heads and thoraces caused various degrees of activations, except the highest concentration in thoraces, while in abdomens only the lowest concentration caused activation whatever worker type. Whereas, all concentrations of spinosad caused various degrees of activation in AChE activity in heads and thoraces whatever worker type. On contrary, various degrees of inhibition were obtained in abdomens as a result of spinosad application. In addition, the averages of activations in AChE activity caused by Biofly in thoraces of nurses and foragers were significantly higher than those in heads. Whereas, much higher activation averages in AChE activity had been caused by spinosad in heads of nurse and field workers which were significantly higher than those recorded in thoraces. But, inhibition averages were found in abdomens of nurses and foragers. Summed data indicated that only the highest concentration of Biofly caused significant inhibition in AChE activity of nurse workers, while the lower concentrations caused different degrees of significant activation. Whereas, summed data of spinosad indicated that all tested concentrations caused various degrees of significant activation in AChE activity of both worker types. So, in general spinosad as well as Biofly when found in sugar syrup at tested concentrations has an activator impact on AChE activity after 24 h of three daily feedings whatever worker type as obtained after single feeding or two daily feedings.

In regard to Biofly, it is considered safe to honey bees but had adverse biochemical impacts on AChE activities of both nurses and foragers according to our results of toxicity, which confirmed that of our previous studies on adult workers (Eid *et al.,* 2011). Spinosad is both a nerve poison and a stomach poison. It has a novel mode of action: it does not interact with known binding sites for other nicotinic or GABAergic insecticides such as neonicotinoids, fiproles, avermectins and cyclodienes (Salgado, 1998; Dow AgroSciences, 2001; Miles, 2003), and could 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 (Watson, 2001). Spinosad over stimulates nerve cells by prolonging electrical impulses across synapses by acting like acetylcholine (but attaching at some novel action site). Thus, AChE does not stop the impulse and nerve stimulation (Boucher, 1999). 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). Our findings confirmed the biochemical effect of spinosad on AChE activity of honey bees (Fahmy and Dahi, 2009; Rabea *et al.,* 2010; Eid *et al.,* 2011). Finally, although spinosad was more toxic than Biofly to both worker types, two biopesticides had adverse effects on AChE activities in different body regions of them. So, "when applications are to be made to crops in bloom these should be made at times which avoid the direct exposure of honey bees *i.e.* applications after sunset and before bees start to forage the following day" (Miles *et al*., 2011).

CONCLUSIONS

The current study was conducted to evaluate the toxic and biochemical effects of multiple applications with Biofly (*Beauveria bassiana*) and SpinTor® (spinosad) on nurse and field honey bee (*Apis mellifera* L.) workers under laboratory conditions. Caged groups of nurse and field workers were fed once, twice or three times at 1-day intervals on sugar syrup containing different concentrations of the two biopesticides. Mortality percentages of workers treated with both biopesticides and the lethal concentration of spinosad that caused 50% mortality (LC_{50}) were estimated to investigate the toxic effects, and the acetylcholinesterase (AChE) activities in heads, thoraces, and abdomens of surviving workers were determined *in vivo* to investigate the biochemical effects. Results indicated that spinosad was higher toxic than Biofly to both nurse and field workers. Also, the mortality percentages after three daily applications of Biofly and spinosad were higher than that of two daily applications whatever worker type. In the same pattern, the mortality percentages after two daily applications were higher than that of single application. Therefore, there was lethal cumulative effect of Biofly or spinosad on both worker types. On the other hand, Biofly and spinosad were more toxic to foragers than to nurses whatever number of applications. Furthermore, findings revealed that mean values of AChE activity in heads were larger than those in both of thoraces and abdomens, and the average of AChE activities in heads was significantly ($p > 0.05$) higher than that obtained in both of thoraces and abdomens whatever tested biopesticide, worker type or number of applications. In addition, tested concentrations caused various degrees of inhibition and activation in AChE activity in heads, thoraces and abdomens when compared with controls. But, in general summed data revealed that Biofly and spinosad showed activating effects on AChE activity of both worker types after 24 h of application however it repeated or not.

This study illustrates that safe biopesticides such as Biofly, in simple toxicity studies, have biochemical effects on AchE activity of honey bee workers as well as toxic ones such as spinosad. So, we confirmed our previous studies and suggested that repetitive exposure of field worker bees (in the field) or/ and nurse bees (inside hives) to sub lethal concentrations of safe or toxic biopesticide residues may adversely affect their olfactory and behavior. Our results may explain the failure of workers in returning to their

hives which may participate in the multifactorial syndrome Colony Collapse Disorder (CCD) in stressed honey bee colonies. Further studies are needed to investigate the biochemical effects of chronic exposure to such compounds on foraging and navigation of honey bees especially in stressed colonies with other probable causative factors of CCD.

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التـأثيرات السـامة والكيموحيويـة لفطـر *Beauveria bassiana* ومبيـد الاسبينوساد على شغالات نحل العسل الحاضنة والحقلية

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أجريت الدراسة لتقييم التأثيرات السامة والكيموحيوية للمعاملات المتعددة بفطر (*Beauveria bassiana*)، وسبينتور (Spinosad) على شغالات نحل العسل (*Apis mellifera* L.) الحاضنة والحقلية تحت الظروف المعملية. وتم تحديد النسب المئوية لموت الشغالات المعاملة بكل من المبيدين الحيوين بعد 24 ساعة من معاملة واحدة، أو اثنين أو ثلاث معاملات يومية. وتم تقدير تركيزات الاسبينوساد القاتلة لـ 50% من الشغالات (LC_{50}). وتم تحديد تأثير المبيدين الحيويين على نشاط إنزيم الأسيتيل كولين إستيريز (AChE) برؤوس وصدور وبطون الشغالات الحاضنة والحقلية الحية حيويا بعد 24 ساعة من المعاملة الفردية أو المزدوجة أو الثلاثية. وأشارت النتائج أن سبينوساد أظهر تأثيرات سامة أعلى من البيوفلاى لكلا من الشغالات الحاضنة والحقلية. وكذلك فإن النسب المئوية للموت بعد 24 ساعة من المعاملة الثلاثية بالبيوفلاى أو بالسبينوساد كانت أعلى من القيمة المناظرة للمعاملة المزدوجة أيا كان نوع الشغالات. وعلى نفس المنوال فإن النسب المئوية للموت نتيجة للمعاملة المزدوجة كانت أعلى من تلك الخاصة بالمعاملة الفردية. ومن جهة أخرى فإن سمية كلا من البيوفلاى و الاسبينوساد كانت أعلى للشغالات الحقلية من سميته للشغالات الحاضنة بعد 24 ساعة من المعاملة الفردية أو المزدوجة أو الثلاثية. وعلاوة على ذلك فقد أشارت النتائج إلى أن قيم متوسطات نشاط AChE فى رؤوس الشغالات الحاضنة أو الحقلية المغذاة على المحلول السكرى المحتوى على 0، 187.5، 375، 750، 1500، 3000 مجم/ لتر من البيوفلاى أو 0، 2.5، 5، 10، 20 مجم/ لتر من الاسبينوساد كانت أعلى من قيم المتوسطات في كل من الصدر والبطن بعد 24 ساعة فى كل المعاملات. كما وجد أن متوسط نشاطات AChE فى رؤوس الشغالات أعلى معنويا ($p > 0.05$) منه فى كل من الصدور والبطون أيا كان المبيد أو نوع الشغالات أو عدد مرات المعاملة. وعلاوة على ذلك فإن التركيزات المختبرة سببت درجات متفاوتة من التثبيط والتنشيط فى نشاط AChE فى الراس والصدر والبطن مقارنة بالنشاط فى المقارنات. وأوضحت البيانات التجميعية أن البيوفلاى والاسبينوساد يزيد نشاط AChE بكلا النوعين من الشغالات بعد 24 ساعة من المعاملة سواء تم تكرارها أم لا.

Keywords: نحل العسل، الشغالات، الحاضنة، الحقلية، المبيدات الحيوية للآفات، *Beauveria bassiana*، البيوفلاى، سبينتور، سبينوساد، السمية، إنزيم الأسيتيل كولين.

قام بتحكيم البحث

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Table 3: *In vivo* effects of Biofly on AChE activity in different regions of nurse and field honey bee workers after 24 hours of single feeding on treated sugar syrup.

Concentration (mg L^{-1})	Nurse				Field			
	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	114.00±2.67	116.81 a	0.00±0.00	-2.46 a	108.00±2.49	111.23 a	0.00±0.00	-2.99 a
187.5	169.78±16.86		-48.93±14.79		145.85±7.52		-35.05±6.96	
375	143.06±3.65		-25.49±3.20		136.23±1.16		-26.14±1.08	
750	115.05±15.97		-0.92±14.01		120.44±7.30		-11.52±6.76	
1500	80.88±1.38		29.05±1.21		81.24±3.91		24.78±3.62	
3000	78.08±1.00		31.51±0.88		75.64±2.75		29.96±2.55	
Thorax								
0.0	7.75±1.48	9.97 c	0.00±0.00	-28.73 b	8.73±1.06	9.79 c	0.00±0.00	-12.08 b
187.5	20.34±1.64		-162.58±21.19		18.78±1.21		-114.99±13.89	
375	11.29±0.76		-45.76±9.75		11.04±0.78		-26.40±8.91	
750	7.24±0.20		6.51±2.52		6.83±0.20		21.84±2.24	
1500	8.91±1.71		-15.02±22.04		7.96±0.90		8.85±10.26	
3000	4.30±0.49		44.48±6.38		5.40±0.37		38.20±4.26	
Abdomen								
0.0	11.14±1.95	17.88 b	0.00±0.00	-60.48 c	10.50±0.68	15.93 b	0.00±0.00	-51.68 c
187.5	20.82±0.72		-86.93±6.49		17.75±1.43		-69.01±13.59	
375	36.46±1.40		-227.29±12.57		31.81±0.82		-202.83±7.79	
750	19.31±1.48		-73.35±13.30		17.57±2.14		-67.25±20.34	
1500	9.79±0.50		12.08±4.50		9.50±0.68		9.56±6.47	
3000	9.74±0.57		12.59±5.15		8.46±0.14		19.44±1.31	
Total	**Mean**		**Mean**		**Mean**		**Mean**	
0.0	44.30 c	48.22	0.00 b	-30.56	42.41 c	45.65	0.00 c	-22.03
187.5	70.32 a		-99.48 d		60.79 a		-73.02 e	
375	63.60 b		-99.52 d		59.69 a		-85.12 f	
750	47.20 c		-22.59 c		48.28 b		-18.98 d	
1500	33.19 d		8.71 b		32.90 d		14.40 b	
3000	30.71 d		29.53 a		29.83 e		29.20 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.

Table4: *In vivo* effects of spinosad on AChE activity in different regions of nurse and field honey bee workers after 24 hours of single feeding on treated sugar syrup.

Concentration (mg L^{-1})	Nurse				Field			
	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	114.00±2.67	210.31 a	0.00±0.00	-84.48 c	108.00±2.49	203.38 a	0.00±0.00	-88.32 c
2.5	242.71±9.95		-112.90±8.73		235.74±10.76		-118.28±9.96	
5	231.96±1.24		-103.48±1.08		221.43±3.30		-105.03±3.05	
10	223.63±2.40		-96.17±2.11		211.66±5.61		-95.98±5.19	
20	269.45±23.70		-136.36±20.8		262.25±9.81		-142.83±9.09	
40	180.08±9.76		-57.97±8.56		181.22±6.10		-67.80±5.65	
Thorax								
0.0	7.75±1.48	10.75 b	0.00±0.00	-38.82 b	8.73±1.06	10.58 b	0.00±0.00	-21.08 b
2.5	16.42±0.74		-111.98±9.59		15.85±0.54		-81.45±6.14	
5	13.06±0.55		-68.63±7.15		12.69±0.46		-45.34±5.32	
10	12.03±0.58		-55.28±7.45		11.00±0.39		-25.91±4.50	
20	8.13±0.52		-4.99±6.70		7.79±0.61		10.83±6.94	
40	7.13±0.41		7.97±5.35		7.39±0.39		15.40±4.52	
Abdomen								
0.0	11.14±1.95	7.79 b	0.00±0.00	30.06 a	10.50±0.68	7.42 c	0.00±0.00	29.32 a
2.5	8.07±0.39		27.52±3.50		7.86±0.46		25.16±4.38	
5	8.24±0.34		26.05±3.05		8.03±0.33		23.57±3.12	
10	7.76±0.84		30.38±7.52		7.58±0.74		27.82±7.06	
20	5.92±0.65		46.88±5.85		5.65±0.07		46.20±0.70	
40	5.62±0.27		49.55±2.41		4.92±0.24		53.18±2.26	
Total	Mean		Mean		Mean		Mean	
0.0	44.30 e	76.28	0.00 a	-31.08	42.41 f	73.79	0.00 a	-26.69
2.5	89.07 ab		-65.79 e		86.48 b		-58.19 d	
5	84.42 bc		-48.69 d		80.72 c		-42.27 c	
10	81.14 c		-40.39 c		76.75 d		-31.36 b	
20	94.50 a		-31.49 b		91.90 a		-28.60 b	
40	64.28 d		-0.15 a		64.51 e		0.26 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 5: *In vivo* effects of Biofly on AChE activity in different regions of nurse and field honey bee workers after 24 hours of two daily feedings on treated sugar syrup.

Concentration (mg L^{-1})	Nurse				Field			
	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	110.05±3.79	223.38 a	0.00±0.00	-102.99 c	108.25±1.79	210.49 a	0.00±0.00	-94.45 c
187.5	393.95±5.33		-257.98±4.84		349.92±7.16		-223.26±6.61	
375	266.97±16.90		-142.60±15.36		259.90±11.56		-140.10±10.68	
750	246.44±18.50		-123.95±16.81		231.33±18.03		-113.70±16.66	
1500	196.32±5.56		-78.40±5.05		191.96±3.98		-77.33±3.68	
3000	126.55±5.20		-15.00±4.72		121.59±2.44		-12.33±2.26	
Thorax								
0.0	8.60±1.18	7.00 c	0.00±0.00	18.63 a	8.12±0.31	6.98 c	0.00±0.00	14.12 a
187.5	14.65±1.18		-70.26±13.76		14.36±0.86		-76.71±10.63	
375	9.93±0.98		-15.37±11.35		10.24±0.56		-26.01±6.84	
750	5.41±0.08		37.13±0.93		5.25±0.24		35.36±2.98	
1500	2.27±0.33		73.66±3.86		2.57±0.53		68.39±6.51	
3000	1.15±0.26		86.60±2.98		1.32±0.35		83.71±4.27	
Abdomen								
0.0	10.99±1.64	12.87 b	0.00±0.00	-17.11 b	10.54±0.79	12.75 b	0.00±0.00	-20.91 b
187.5	21.31±0.21		-93.89±1.93		21.36±0.97		-102.62±9.22	
375	15.36±1.50		-39.82±13.63		15.15±0.58		-43.73±5.48	
750	11.82±0.27		-7.52±2.486		11.61±0.57		-10.18±5.41	
1500	10.34±0.18		5.92±1.62		10.30±0.56		2.26±5.34	
3000	7.40±0.51		32.65±4.62		7.50±0.737		28.81±6.99	
Total	Mean		Mean		Mean		Mean	
0.0	43.21 e	81.08	0.00 b	-33.82	42.30 e	76.74	0.00 b	-33.75
187.5	143.30 a		-140.71 e		128.55 a		-134.20 e	
375	97.42 b		-65.93 d		95.10 b		-69.95 d	
750	87.89 c		-31.45 c		82.73 c		-29.51 c	
1500	69.64 d		0.39 b		68.28 d		-2.23 b	
3000	45.04 e		34.75 a		43.47 e		33.40 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 of spinosad on AChE activity in different regions of nurse and field honey bee workers after 24 hours of two daily feedings on treated sugar syrup.

Concentration (mg L^{-1})	Nurse				Field			
	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	110.05±3.79	393.53 a	0.00±0.00	-257.61 c	108.25±1.79	353.36 a	0.00±0.00	-226.44 c
2.5	486.42±46.78		-342.01±42.51		431.69±5.60		-298.80±5.18	
5	522.88±81.81		-375.14±74.35		441.56±10.21		-307.92±9.43	
10	482.87±62.39		-338.79±56.70		432.56±22.50		-299.61±20.79	
20	365.46±35.69		-232.10±32.43		352.74±28.76		-225.86±26.57	
Thorax								
0.0	8.61±1.18	17.81 b	0.00±0.00	-106.98 b	8.12±0.31	17.81 b	0.00±0.00	-119.24 b
2.5	43.59±3.01		-406.51±34.94		44.05±2.10		-442.11±25.87	
5	18.43±1.37		-114.12±15.95		18.38±1.18		-126.20±14.47	
10	7.34±0.32		14.76±3.67		7.41±0.26		8.84±3.19	
20	11.11±2.11		-29.04±24.48		11.11±0.68		-36.70±8.42	
Abdomen								
0.0	10.99±1.64	14.22 b	0.00±0.00	-29.38 a	10.54±0.79	13.59 b	0.00±0.00	-28.88 a
2.5	13.01±0.52		-18.39±4.74		12.91±0.78		-22.48±7.43	
5	11.30±1.12		-2.83±10.15		11.28±0.26		-7.03±2.46	
10	16.70±1.85		-51.97±16.88		16.18±0.79		-53.47±7.48	
20	19.09±2.53		-73.72±23.00		17.02±1.56		-61.43±14.79	
Total	Mean		Mean		Mean		Mean	
0.0	43.21 c	141.86	0.00 a	-131.32	42.30 d	128.25	0.00 a	-124.85
2.5	181.01 a		-255.64 d		162.88 a		-254.46 d	
5	184.20 a		-164.03 c		157.07 ab		-147.05 c	
10	168.97 a		-125.33 b		152.05 b		-114.75 b	
20	131.88 b		-111.62 b		126.95 c		-108.00 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 7: *In vivo* effects of Biofly on AChE activity in different regions of nurse and field honey bee workers after 24 hours of three daily feedings on treated sugar syrup.

Concentration (mg L^{-1})	Nurse				Field			
	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	108.97±4.09	180.16 a	0.00±0.00	-65.33 b	106.45±3.30	169.78 a	0.00±0.00	-59.49 b
187.5	356.61±26.90		-227.25±24.69		336.82±12.34		-216.41±11.59	
375	249.68±5.98		-129.12±5.49		225.64±15.73		-111.96±14.78	
750	139.19±7.64		-27.71±7.01		130.63±6.60		-22.71±6.20	
1500	114.02±7.42		-4.63±6.81		107.56±4.80		-1.04±4.51	
3000	112.55±8.56		-3.28±7.85		111.59±7.98		-4.82±7.50	
Thorax								
0.0	8.20±0.92	15.26 b	0.00±0.00	-85.96 c	7.75±0.34	14.63 b	0.00±0.00	-88.70 c
187.5	16.12±1.00		-96.46±12.23		15.54±0.93		-100.41±11.99	
375	23.24±1.66		-183.26±20.24		21.47±1.12		-176.92±14.50	
750	20.72±1.53		-152.60±18.64		19.88±0.47		-156.48±6.10	
1500	17.96±1.38		-118.87±16.84		17.30±1.08		-123.11±13.97	
3000	5.30±0.81		35.45±9.87		5.84±0.62		24.69±7.94	
Abdomen								
0.0	11.19±1.16	9.05 b	0.00±0.00	19.06 a	10.73±0.45	8.34 c	0.00±0.00	22.30 a
187.5	15.00±1.07		-34.05±9.53		13.92±1.38		-29.75±12.84	
375	6.53±0.80		41.67±7.12		6.18±0.64		42.44±6.00	
750	8.70±0.52		22.23±4.65		8.07±0.29		24.79±2.68	
1500	6.80±0.64		39.18±5.75		6.01±0.91		43.99±8.51	
3000	6.11±0.83		45.36±7.43		5.12±0.80		52.33±7.43	
Total	**Mean**		**Mean**		**Mean**		**Mean**	
0.0	42.79 d	68.16	0.00 b	-44.07	41.65 d	64.25	0.00 b	-41.96
187.5	129.24 a		-119.25 f		122.09 a		-115.52 f	
375	93.15 b		-90.24 e		84.43 b		-82.15 e	
750	56.20 c		-52.69 d		52.86 c		-51.47 d	
1500	46.26 d		-28.11 c		43.62 d		-26.72 c	
3000	41.32 d		25.84 a		40.85 d		24.07 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 8: *In vivo* effects of spinosad on AChE activity in different regions of nurse and field honey bee workers after 24 hours of three daily feedings on treated sugar syrup.

Concentration (mg L^{-1})	Nurse				Field			
	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	108.97±4.09	300.75 a	0.00±0.00	-175.99 c	106.45±3.30	280.69 a	0.00±0.00	-163.68 c
2.5	279.92 ±13.12		-156.87 ±12.04		262.62±11.21		-146.71±10.53	
5	296.92 ±1.58		-172.48±1.45		285.02±4.96		-167.74±4.66	
10	374.28 ±21.87		-243.47±20.07		341.23±13.35		-220.55±12.55	
20	443.64±45.58		-307.11±41.83		408.10±27.74		-283.37±26.06	
Thorax								
0.0	8.20±0.92	17.41b	0.00±0.00	-112.24 b	7.75±0.34	16.87 b	0.00±0.00	-117.58 b
2.5	26.52±0.59		-223.24±7.21		24.69±1.10		-218.53±14.26	
5	19.04±0.65		-132.04±7.97		19.13±0.67		-146.74±8.62	
10	15.83 ±0.98		-92.98±11.95		16.08±0.91		-107.47±11.76	
20	17.47 ±1.24		-112.95±15.06		16.68±0.51		-115.16±6.59	
Abdomen								
0.0	11.19±1.16	9.13 b	0.00±0.00	18.37 a	10.73±0.45	8.66 c	0.00±0.00	19.31 a
2.5	6.97±0.23		37.71±2.10		6.89±0.32		35.83±2.94	
5	7.08±0.35		36.72±3.12		6.60±0.20		38.54±1.84	
10	9.83±0.96		12.17±8.57		9.06±0.65		15.57±6.08	
20	10.60±0.20		5.26±1.83		10.02±0.39		6.60±3.60	
Total	**Mean**		**Mean**		**Mean**		**Mean**	
0.0	42.79 d	109.10	0.00 a	-89.95	41.65 d	102.07	0.00 a	-87.31
2.5	104.47 c		-114.14 c		98.07 c		-109.80 c	
5	107.68 c		-89.27 b		103.58 c		-91.98 b	
10	133.31 b		-108.10 c		122.13 b		-104.15 c	
20	157.24 a		-138.27 d		144.94 a		-130.64 d	

*** 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.