# Sub-lethal effects of spinetoram on the activities of some detoxifying enzymes in the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

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The present study was designed to investigate the activities of the detoxifying enzymes acetylcholinesterase (AChE), non-specific esterases ($\alpha$- and $\beta$-esterases), glutathione S-transferase (GST) and mixed-function oxidases (MFO) in spinetoram-treated *Agrotis ipsilon* (Hufnagel). For this purpose, fourth larval instars were exposed to three sub-lethal concentrations ($LC_{10}$, $LC_{20}$ and $LC_{50}$) of spinetoram for 24 h using the leaf dipping technique. The activities of detoxifying enzymes in the survivors after 2, 4 and 6 days of treatment were compared to non-treated larvae (control). AChE activity was significantly increased after 4 and 6 days of treatment with the $LC_{10}$. In contrast, this activity was significantly decreased after 2 days of treatment with the three sub-lethal concentrations applied, and after 4 and 6 days of treatment with the $LC_{20}$ and $LC_{50}$. While both $\alpha$- and $\beta$-esterase activities were significantly enhanced after 2 days of treatment with all the previous sub-lethal concentrations, with no constant pattern after 4 and 6 days of treatment. GST activity was significantly increased on the second day of treatment with the $LC_{10}$, and on the fourth day of treatment with the $LC_{10}$ and $LC_{50}$. Whereas the only significant decrease in GST activity was observed on the sixth day of treatment with the $LC_{10}$. No significant change was recorded on the second and sixth days of treatment with the $LC_{20}$ and $LC_{50}$, and on the fourth day of treatment with the $LC_{20}$. The activity of MFO was significantly enhanced up to the fourth day of treatment with all the concentrations investigated, whereas this activity was significantly decreased on the sixth day of treatment. Therefore, it appears that higher activities of detoxifying enzymes in *A. ipsilon* generally occurred in response to the intoxication by the lowest concentration of spinetoram, particularly after a relatively early time of treatment, and MFO may be considered the principal detoxifying enzymes.

**Key words**: insects, spinosyns, acetylcholinesterase, non-specific esterases, glutathione S-transferases, oxidases.

## INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hufnagel) is a serious worldwide insect pest. This species cuts off the seedlings at the soil surface. In Egypt, it causes damage to the seedlings of cotton, wheat, bean and clover.

The intensive use of broad-spectrum synthetic pesticides against insect pests has led to the development of resistance to many of the registered pesticides. The appearance of such problems has been accompanied by growing interest to use new safe bio-insecticides with new modes of action as an alternative technology for the integrated pest management (IPM) strategy. In this scenario, spinetoram is a second-generation spinosyn belonging to Insecticide Resistance Action Committee (IRAC) mode of action class 5 (IRAC 2009). Spinosyns are bio-insecticides based on the fermentation product of the soil actinomycete bacterium *Saccharopolyspora spinosa* (*Pseudonocardiaceae)* (Mertz & Yao 1990; Thompson *et al*. 2000). These compounds have two unique modes of action: acting primarily on the insect's nervous system at the nicotinic acetylcholine receptor, and exhibiting activity at the $\gamma$-aminobutyric acid (GABA) receptor (Salgado 1997; Watson 2001). They represent an important pest control option to IPM based on their low ecotoxicological profile, short persistence in the environment and low mammalian toxicity (Pineda *et al*. 2007). Nevertheless, any insecticide can develop resistance in target insects from the aspect of organic evolution. Recently, several insects have exhibited a rapid threatening ascending resistance to spinetoram in field populations (Sial & Brunner 2010; Sial *et al*. 2010).

Insect populations may survive the effect of toxic chemical compounds by different biochemical mechanisms. So far esterases, glutathione S-transferases and mixed-function oxidases (mono-oxygenases) are known to be involved in the de-

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toxification of the four major groups of insecticides (Feyereisen 2005; Li *et al*. 2007; Gacar & Taşkan 2009). Also acetylcholinesterase (AChE), responsible for neurotransmitter degradation at the cholinergic nerve synapse, is the target of both organophosphate and carbamate insecticides (Matsumura 1985). Selection of modified AChE less sensitive to these insecticides has been shown to be a common resistance mechanism and was observed in numerous insect pest species (Kranthi 2005).

As studies have revealed a positive correlation between levels of detoxifying enzymes and insecticide resistance, so quantification of these enzymes is used to monitor resistance against insecticides and will be helpful in future for the better management of insect pests (Yaqoob *et al*. 2013). Therefore, the current study aims to quantify the levels of the insecticide detoxifying enzymes acetylcholinesterase (AChE), non-specific esterases ($\alpha$- and $\beta$-esterases), glutathione S-transferase (GST) and mixed-function oxidases (MFO) in the fourth larval instars of *A. ipsilon* after 2, 4 and 6 days of treatment with the $LC_{10}$, $LC_{20}$ and $LC_{50}$ of spinetoram compared to non-treated ones (control).

## MATERIAL AND METHODS

### *Insects*

A susceptible laboratory strain of *A. ipsilon* was obtained from a colony maintained in the Plant Protection Institute, Agricultural Research Centre, Dokki, Giza, Egypt, for several years without exposure to insecticides. Larvae were reared on the leaves of castor bean (*Ricinus communis* L.) at 25 ± 2 °C, 70 ± 5 % RH and 12:12 h (L:D) photoperiod. Adults were fed on 15 % honey solution. Filter paper was provided as an ovipositing substrate, and it was replaced periodically.

### *Insecticide*

The insecticide tested was spinetoram (Radiant® 12 % SC) obtained from Dow AgroSciences, Indianapolis, IN, U.S.A.

### *Bioassay*

Five serial aqueous concentrations of spinetoram (25, 12.5, 6.25, 3.125 and 1.5625 ppm) were prepared. The leaves of castor bean (*R. communis*) were dipped for 30 seconds in each concentration and air-dried for 1 h at room temperature. Newly-moulted fourth larval instars of *A. ipsilon*, pre-starved for 4 h, were allowed to feed on treated leaves for 24 h. Then they were fed on fresh untreated leaves until death or pupation. Larvae which were fed on castor bean leaves dipped in distilled water only were used as the control. Each concentration was repeated three times of 50 larvae each (total $n = 150$ larvae). The percentage mortality was determined and corrected using Abbott's formula (Abbott 1925). The $LC_{10}$, $LC_{20}$ and $LC_{50}$ were estimated as 3.33, 5.00 and 6.67 ppm, respectively, following probit analysis (Finney 1971).

### *Enzyme preparation*

Three groups of newly-moulted fourth larval instars of *A. ipsilon* (50 larvae each) were respectively treated with the $LC_{10}$, $LC_{20}$ and $LC_{50}$ of spinetoram, as mentioned above. After 2, 4 and 6 days of treatment, the surviving larvae were homogenized (1 g of tissue in 1 ml of distilled water) using a glass-Teflon homogenizer on an ice jacket. The crude homogenate was centrifuged at 14 000 rpm for 5 min at 4 °C. The supernatant was collected and used further as an enzyme solution for biochemical estimation of AChE, non-specific esterases ($\alpha$- and $\beta$-esterases), GST and MFO. A parallel control of non-treated larvae was also run. The experiment was replicated three times. The supernatants were kept at –20 °C till use.

### *AChE assay*

AChE activity was measured according to the methods described by Simpson *et al*. (1964) using acetylcholine bromide (ACh Br) as a substrate. The reaction mixture contained 20 $\mu$l enzyme solution, 0.5 ml 0.067 M phosphate buffer (pH 7) and 0.5 ml ACh Br (3 mM). The test tubes were incubated at 37 °C for exactly 30 min. One ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then, 0.5 ml of HCl (1 part of concentrated HCl and 2 parts of distilled water) was added. The mixture was shaken vigorously and allowed to stand for 2 min. Half ml of 0.9 M ferric chloride solution dissolved in 0.1 M HCl was added and mixed well. The decrease in ACh Br resulting from hydrolysis by AChE was read spectrophotometrically at 515 nm.

### *Non-specific esterases assay*

Non-specific esterases ($\alpha$- and $\beta$-esterases) activities were estimated according to the methods of

Van Asperen (1962) using $\alpha$-naphthyl and $\beta$-naphthyl acetate as substrates, respectively. Naphthol produced as a result of hydrolysis can be measured spectrophotometrically. The reaction mixture consisted of 5 ml substrate solution ($3 \times 10^{-4}$ M $\alpha$- or $\beta$-naphthyl acetate, 1 % acetone and 0.04 M phosphate buffer pH 7) and 2 $\mu$l of enzyme solution. The mixture was incubated for exactly 15 min at 27 °C, and then 1 ml of diazoblue colour reagent (prepared by mixing 2 parts of 1 % diazoblue and 5 parts of 5 % sodium lauryl sulphate) was added. The developed blue and red colours were read at 600 and 555 nm for $\alpha$- and $\beta$-naphthol, respectively.

The standard curves for $\alpha$- and $\beta$-naphthol can be prepared by dissolving 20 mg $\alpha$- or $\beta$-naphthol in 100 ml of 0.04 M phosphate buffer (pH 7) as stock solutions. Ten ml of the stock solution was diluted up to 100 ml by the phosphate buffer. Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.6 ml of diluted solution (equal to 2, 4, 8, 16 and 32 $\mu$g naphthol) were pipetted into test tubes and completed to 5 ml by phosphate buffer. One ml of diazoblue reagent was added and the developed colour was measured as stated above.

### *GST assay*

GST activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, following the procedures of Habig *et al*. (1974). The reaction mixture comprised of 10 $\mu$l of 10 mM reduced glutathione (GSH) in 100 mM sodium phosphate buffer (pH 6.5) and 10 $\mu$l of enzyme solution. The reaction was initiated by adding 10 $\mu$l CDNB (6 mM in methanol), resulting in a final volume of 30 $\mu$l. The plates were immediately transferred to a microplate reader. The reactions were allowed to continue for 5 min and absorbance readings were taken at 340 nm automatically once per min against blanks (wells containing all reaction components except the enzyme solution). The increase in absorbance was linear throughout the 5-min reading interval. An extinction coefficient of 9.6 mM/cm was used to calculate the amount of CDNB conjugated.

### *MFO assay*

MFO activities were measured using p-nitroanisole O-demethylation, according to the methods of Hansen & Hodgson (1971) with slight modifications. The standard incubation mixture contained 1 ml of 0.1 M sodium phosphate buffer (pH 7.6), 1.5 ml enzyme solution, 0.2 ml nicotinamide adenine dinucleotide phosphate (NADPH, final concentration 1 mM), 0.2 ml glucose-6-phosphate (G-6 P, final concentration 1 mM) and 5 $\mu$g glucose-6-phosphate dehydrogenase (G-6 PD). The reaction was initiated by the addition of p-nitroanisole in 1 $\mu$l of acetone to give a final concentration of 0.8 mM and incubated for 30 min at 37 °C. The incubation period was terminated by the addition of 1 ml of 1 N HCl, and p-nitrophenol was extracted with $CHCl_3$ and 0.5 N NaOH. The absorbance of NaOH solution was measured at 405 nm. An extinction coefficient of 14.28 mM/cm was used to calculate 4-nitrophenol concentration.

### *Statistical analysis*

The data obtained were statistically analysed using one-way analysis of variance (ANOVA) within each post-treatment day (SAS Institute 2009). Where the difference was significant, the means were separated using Student's *t*-test. Significant difference was set at $P < 0.05$.

## RESULTS

The data shown (Fig. 1) revealed that the $LC_{10}$ of spinetoram significantly increased the activity of AChE in *A. ipsilon* fourth larval instars after 4 and 6 days of treatment compared to the control. This increase was most obvious on the fourth day, as it was about 45.66 % of the control. In contrast, AChE activity was significantly decreased after 2 days of treatment with the three sub-lethal concentrations applied ($LC_{10}$, $LC_{20}$ and $LC_{50}$), and after 4 and 6 days of treatment with the $LC_{20}$ and $LC_{50}$ compared to the control. The highest decrease was attained on the sixth day of treatment with the $LC_{20}$ followed by that reached on the second day of treatment with the $LC_{10}$, where it was about 65.11 and 56.58 % of the control, respectively.

$\alpha$-Esterase activity was significantly increased after 2 days of treatment with the three sub-lethal concentrations of spinetoram compared to the control (Fig. 2). This increase was about 114.04, 72.37 and 31.40 % of the control at the $LC_{10}$, $LC_{20}$ and $LC_{50}$, respectively. On the other hand, no constant pattern for $\alpha$-esterase activity was found after 4 and 6 days of treatment. In this context, no significant change was obtained on the fourth day of treatment with the $LC_{20}$ and $LC_{50}$, and on the sixth day of treatment with the $LC_{10}$ and $LC_{20}$

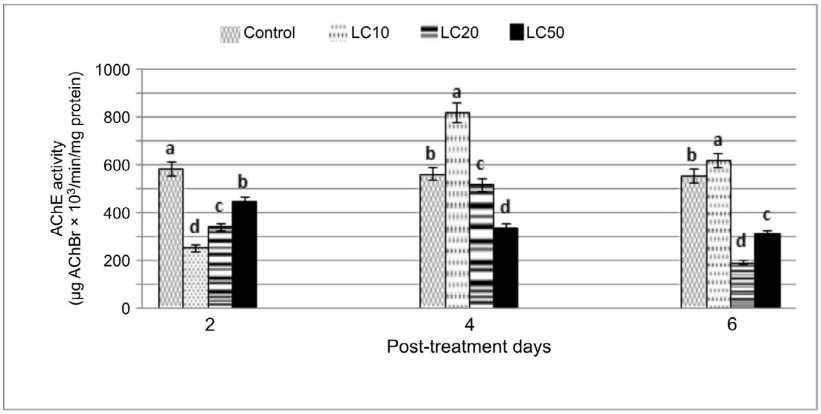


**Fig. 1**. Activity of acetylcholinesterase (AChE) in spinetoram-treated fourth larval instars of *Agrotis ipsilon*, using acetylcholine bromide (ACh Br) as a substrate.

Enzyme activity was measured after 2, 4 and 6 days of treatment with different concentrations of spinetoram ($LC_{10}$, $LC_{20}$, $LC_{50}$ = 3.33, 5.00, 6.67 ppm spinetoram, respectively). The experiment was repeated three times, each point represents the mean ± S.D. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way analysis of variance (ANOVA).

compared to the control. A significant decrease was reached after 4 days of treatment with the $LC_{10}$ (33.39 %), whereas a significant increase was observed after 6 days of treatment with the $LC_{50}$ (55.18 %) compared to the control.

$\beta$-Esterase activity in spinetoram-treated *A. ipsilon* larvae displayed a pattern comparable to a large extent to that found in $\alpha$-esterase activity, where this activity was also significantly increased on the second day of treatment with the three studied sub-lethal concentrations compared to the control (Fig. 3). This increase was about 72.22, 49.38 and 43.21 % of the control after treatment with the $LC_{10}$, $LC_{20}$ and $LC_{50}$, respectively. As in the case of $\alpha$-esterase activity, no constant pattern was also noticed for $\beta$-esterase activity on the fourth and sixth days of treatment. In this context, a significant increase was found on the fourth day of treatment with the $LC_{10}$ (20.35 %) and $LC_{50}$ (57.08 %), whereas a significant decrease was recorded on the sixth day of treatment with the $LC_{10}$ (25.38 %) compared to the control. However, no significant change was obtained on the fourth day of treatment with the $LC_{20}$, and on the sixth day of treat-

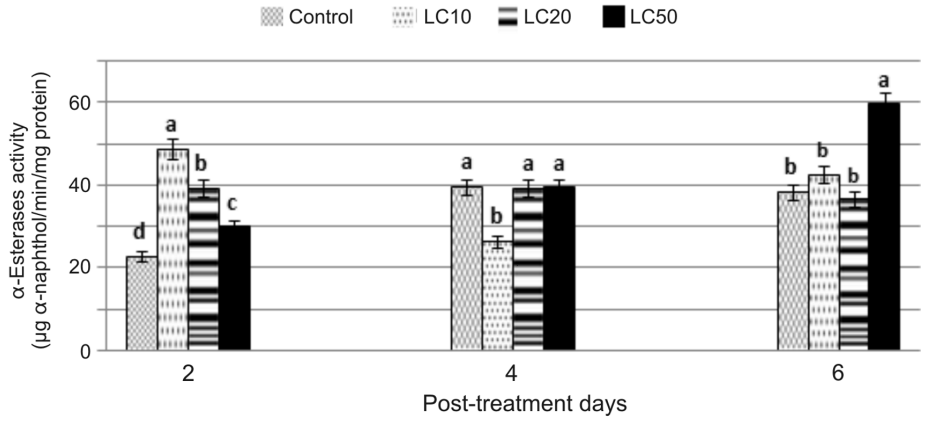


**Fig. 2**. Activity of α-esterase in spinetoram-treated fourth larval instars of *Agrotis ipsilon*, using α-naphthol as a substrate.

Enzyme activity was measured after 2, 4 and 6 days of treatment with different concentrations of spinetoram ($LC_{10}$, $LC_{20}$, $LC_{50}$ = 3.33, 5.00, 6.67 ppm spinetoram, respectively). The experiment was repeated three times, each point represents the mean ± S.D. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way analysis of variance (ANOVA).

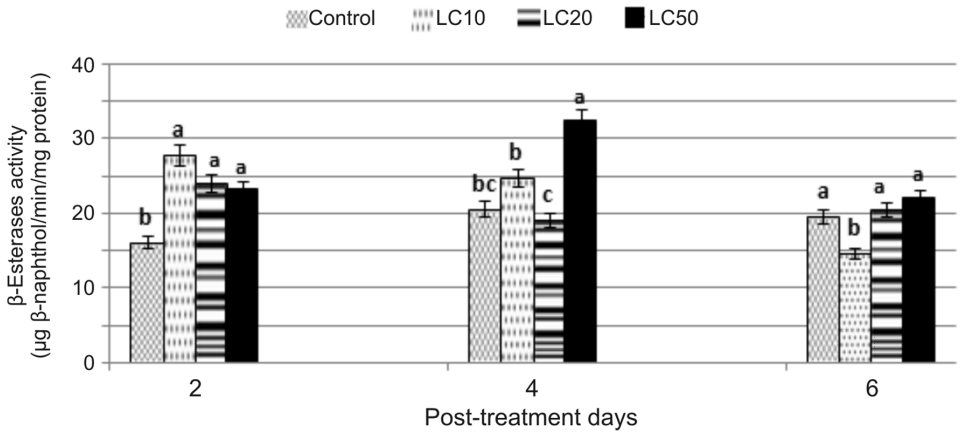


**Fig. 3**. Activity of β-esterase in spinetoram-treated fourth larval instars of *Agrotis ipsilon*, using β-naphthol as a substrate.

Enzyme activity was measured after 2, 4 and 6 days of treatment with different concentrations of spinetoram ($LC_{10}$, $LC_{20}$, $LC_{50}$ = 3.33, 5.00, 6.67 ppm spinetoram, respectively). The experiment was repeated three times, each point represents the mean ± S.D. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way analysis of variance (ANOVA).

ment with the $LC_{20}$ and $LC_{50}$ compared to the control.

GST activity in *A. ipsilon* larvae was significantly increased after 2 days of treatment with the $LC_{10}$ (65.84 %), and after 4 days of treatment with the $LC_{10}$ (30.21 %) and $LC_{50}$ (34.38 %) of spinetoram compared to the control (Fig. 4). In contrast, a significant decrease was observed on the sixth day of treatment with the $LC_{10}$ (17.45 %), while no significant change was found after 2 and 6 days of treatment with the $LC_{20}$ and $LC_{50}$, and after 4 days of treatment with the $LC_{20}$ compared to the control.

Comparable to the activities of $\alpha$-and $\beta$-esterases, MFO activity in *A. ipsilon* larvae was also significantly increased after 2 and 4 days of treatment with the three sub-lethal concentrations of spinetoram compared to the control. On the contrary, a significant decrease in the activity was observed on the sixth day of treatment with all the sub-lethal concentrations tested compared to the control (Fig. 5).

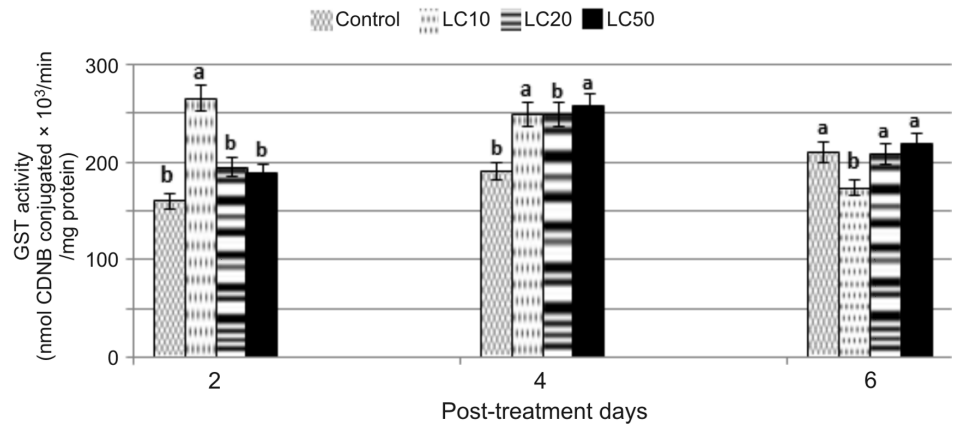


**Fig. 4**. Activity of glutathione S-transferase (GST) in spinetoram-treated fourth larval instars of *Agrotis ipsilon*, using 1-chloro-2,4 dinitrobenzene (CDNB) as a substrate.

Enzyme activity was measured after 2, 4 and 6 days of treatment with different concentrations of spinetoram ($LC_{10}$, $LC_{20}$, $LC_{50}$ = 3.33, 5.00, 6.67 ppm spinetoram, respectively). The experiment was repeated three times, each point represents the mean ± S.D. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way analysis of variance (ANOVA).

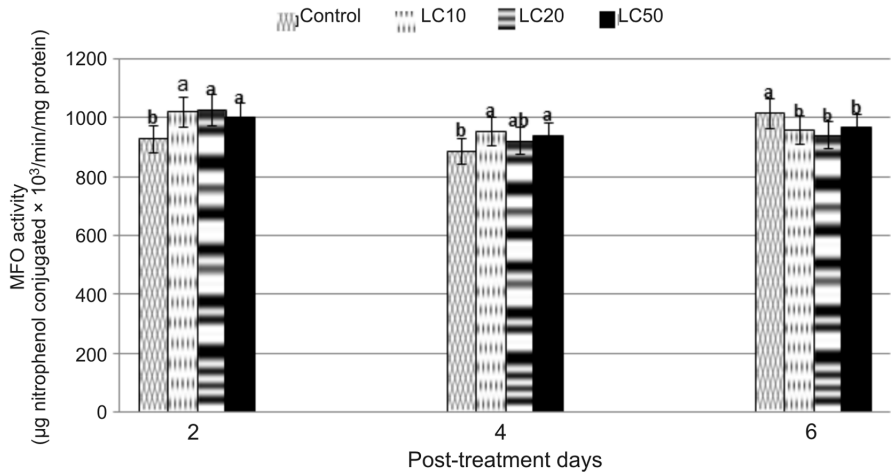


**Fig. 5**. Activity of mixed-function oxidases (MFO) in spinetoram-treated fourth larval instars of *Agrotis ipsilon*, using p-nitroanisole O-demethylation as a substrate.

Enzyme activity was measured after 2, 4 and 6 days of treatment with different concentrations of spinetoram ($LC_{10}$, $LC_{20}$, $LC_{50}$ = 3.33, 5.00, 6.67 ppm spinetoram, respectively). The experiment was repeated three times, each point represents the mean ± S.D. Columns with different letters within each post-treatment day are significantly different ($P < 0.05$) using one-way analysis of variance (ANOVA).

## DISCUSSION

The present study represents the first report on the quantification of detoxifying enzymes in spinetoram-treated *A. ipsilon*. It will be helpful in monitoring resistance against spinetoram, and consequently better management of *A. ipsilon* in future. Insecticide resistance presents a major risk to the sustainability of IPM programmes. Resistance management strategies could slow the development of resistance only if implemented in a timely manner. However, the effectiveness of resistance management strategies may be reduced without the knowledge of biochemical mechanisms conferring resistance to insecticides used in IPM programmes (Sial *et al*. 2011).

AChE activity in older *A. ipsilon* fourth larval instars was higher than that in younger ones using the lowest concentration of spinetoram ($LC_{10}$). In agreement with these results, spinetoram significantly increased the activity of AChE in *Spodoptera littoralis* (Boisd.) (El-Bakry *et al.* 2008; Fahmy & Dahi 2009). According to Matsumura (1985), AChE has a key role in neurotransmission by hydrolysing the neurotransmitter acetylcholine (ACh) in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides. Salgado *et al*. (1998) demonstrated that spinosad could attack the nicotinc acetylcholine receptor (nAChR) with ACh simultaneously, as well as acting on a new site differing from the site on which ACh acts. They postulated that 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. But when they are present, the channel will open up and subsequently the receptor will be activated.

In contrast to the results of AChE, the activities of $\alpha$- and $\beta$-esterases in younger larvae treated with spinetoram were higher than those in older ones regardless of the concentration applied. Similarly, the activity of $\beta$-esterase in *S. littoralis* fourth larval instars was significantly increased after 4 days of treatment with the $LC_{50}$ of spinosad (Abd El-Mageed & El-Gohary 2006). In contrast, exposure of *Culex pipiens* L. and *Anopheles multicolor* Cambouliu to the $LC_{50}$ of spinetoram for 24 h significantly decreased the activities of both $\alpha$- and $\beta$-esterases (El-Kady *et al*. 2008). Fahmy & Dahi (2009) attained variable $\alpha$- and $\beta$-esterases activities in a field population of *S. littoralis* sprayed with spinetoram in two different governorates (Behira and Qalyobia) in Egypt. They attributed these results to the variations in the environmental conditions in each tested governorate, or to the specific characteristics of each strain. Esterases either produce broad-spectrum insecticide resistance through rapid-binding and slow turnover of insecticides, *i.e.* sequestration, or narrow-spectrum resistance through metabolism of a very restricted

range of insecticides containing a common ester bond (Herath *et al*. 1987). The majority of esterases which function by sequestration are elevated through gene amplification (Vaughan & Hemingway 1995). Members of the esterase cluster probably play a role in the detoxification of xenobiotic esters (Mouches *et al*. 1986; Gacar & Taşkan 2009).

The present results, which indicate that GST activity in *A. ipsilon* was significantly increased after 4 days of treatment with the $LC_{50}$ of spinetoram, are similar to those which indicated an increase in GST activity in *Oryzaephilus surinamensis* (L.) after exposure to the $LC_{50}$ of spinosad (Al-Dhaheri & Al-Deeb 2012). However, no significant change in GST activity was observed in *Helicoverpa armigera* (Hübner) treated with spinosad (Wang *et al*. 2009a) and *S. littoralis* (Fahmy & Dahi 2009) and *Choristoneura rosaceana* (Harris) treated with spinetoram (Sial *et al*. 2011). Glutathione is implied in the phase II metabolism of xenobiotics including insecticides by the activity of GST, and in the antioxidant defence by the activity of glutathione peroxidase and glutathione reductase (Sies 1999). Hemingway *et al*. (2004) explained that GST carries out O-dearylation and O-dealkylation processes in order to detoxify organophosphate based insecticides.

MFO activity in spinetoram-treated *A. ipsilon* larvae increased up to 4 days of treatment with the three sub-lethal concentrations used compared to the other examined detoxifying enzymes. Thus, MFO may be considered the principal detoxifying enzymes in *A. ipsilon*. The involvement of oxidases in the mechanism of resistance to spinetoram (Sial *et al*. 2011) and spinosad (Scot 1998; Wang *et al*. 2006; Wang *et al*. 2009b) has been reported. MFO are phase I metabolic enzymes (Li *et al*. 2007), where they metabolise a wide range of insecticides by hydroxylation, epoxidation and oxidation (Feyereisen 2005). Yaqoob *et al*. (2013) believed that MFO and esterases are involved essentially and equally in the detoxification of insecticides. However, the latter speculation does not extend to the results obtained in the present study, as the activities of MFO and esterases were not equally involved in the detoxification of spinetoram.

The general picture of the detoxifying enzymes in *A. ipsilon* larvae to spinetoram indicates that their activities were generally enhanced in response to the intoxication by the lowest sub-lethal concentration ($LC_{10}$), particularly after a relatively early time of treatment. In accordance with these findings, the insecticide hormoligosis hypothesis (Luckey 1968) predicts that sub-harmful quantities of any stressing agent will be stimulatory to the organism by making it more sensitive to changes in its environment and more able to develop new systems to fit a sub-optimum environment.

## CONCLUSION

The information reached in the present study indicates that not all the studied enzymes were equally involved in the detoxification of spinetoram in *A. ipsilon*, with the highest magnitude for oxidases. This information will enable growers to incorporate this reduced-risk insecticide into resistance management programs based on scientific knowledge leading to successful control of *A. ipsilon* on a sustainable basis.

## REFERENCES

ABBOTT, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265–267.

ABD EL-MAGEED, A.E.M. & EL-GOHARY, L.R.A. 2006. Impact of spinosad on some enzymatic activities of the cotton leafworm. *Pakistan Journal of Biological Sciences* **9**: 713–716.

AL-DHAHERI, H.A. & AL-DEEB, M.A. 2012. Mortality and GST enzyme response of saw-toothed grain beetles, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) exposed to low insecticide concentrations. *Journal of Entomology* **9**: 396–402.

EL-BAKRY, N.M., DAHI, H.F. & EL-SAYED, Y.A. 2008. Toxicological evaluation and biochemical impacts for radiant as a new generation of spinosyn on *Spodoptera littoralis* (Boisd.) larvae. *Egyptian Academic Journal of Biological Sciences* **1**: 85–97.

EL-KADY, G.A., KAMEL, N.H., MOSLEH, Y.Y. & BAHGAT, I.M. 2008. Comparative toxicity of two bio-insecticides (spinetoram and vertemic) compared with methomyl against *Culex pipiens* and *Anopheles multicolor*. *World Journal of Agricultural Sciences* **4**: 198–205.

FAHMY, N.M. & DAHI, H.F. 2009. Changes in detoxifying enzymes and carbohydrate metabolism associated with spinetoram in two field-collected strains of *Spodoptera littoralis* (Boisd.). *Egyptian Academic Journal of Biological Sciences* **1**: 15–26.

FEYEREISEN, R. 2005. Insect cytochrome P450. In: Gilbert, L.I., Iatrou, K. & Gill, S.S. (Eds) *Comprehensive Molecular Insect Science*. Volume 4. 1–77. Elsevier, Oxford, U.K.

FINNEY, D.J. 1971. *Probit Analysis*. 3rd Edition. Cambridge University Press, London, U.K.

GACAR, F. & TAŞKAN, V. 2009. Partial base sequence analysis of Md$\alpha$E7 gene and ali-esterase enzyme

activities in field collected populations of housefly (*Musca domestica* L.) from Mediterranean and Aegean Regions of Turkey. *Pesticide Biochemistry and Physiology* **94**: 86–92.

HABIG, W.H., PABST, M.J. & JACOBY, W.B. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* **249**: 7130–7139.

HANSEN, I.G. & HODGSON, E. 1971. Biochemical characteristics of insect microsomes N-and O-demethylation. *Biochemical Pharmacology* **20**: 1569–1578.

HEMINGWAY, J., HAWKES, N.J., McCARROLL, L. & RANSON, H. 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology* **34**: 653–665.

HERATH, P.R., HEMINGWAY, J., & JAYAWARDENA, K.G.I. 1987. The detection and characterization of malathion resistance in field populations of *Anopheles culicifacies* B in Sri Lanka. *Pesticide Biochemistry and Physiology* **29**: 157–162.

IRAC. 2009. Insecticide Resistance Action Committee, IRAC Mode of Action Classification, vol. 6.3, IRAC Mode of Action Working Group. Online at: http://www.irac-online.org/documents/MoA%20classification_v6.3.3_28july09.pdf

KRANTHI, K.R. 2005. *Insecticide Resistance: Monitoring, Mechanisms and Management Manual*. CICR, Nagpur, India, and ICAC, Washington, U.S.A.

LI, X., SCHULER, M.A. & BERENBAUM, M.R. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology* **52**: 231–253.

LUCKEY, T.D. 1968. Insecticide hormoligosis. *Journal of Economic Entomology* **61**: 7–12.

MATSUMURA, F. 1985. *Toxicology of Insecticides*. 2nd Edition. Plenum Press, New York, U.S.A.

MERTZ, F.P. & YAO, R.C. 1990. *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar mill rum still. *International Journal of Systematic Bacteriology* **40**: 34–39.

MOUCHES, C., PASTEUR, N., BERGE, J.B., HYRIEN, O., RAYMOND, M., DE SAINT VINCENT, B.R., DE SILVESTRI, M. & GEORGHIOU, G.P. 1986. Amplification of an esterase gene is responsible for insecticide resistance in a Californian *Culex* mosquito. *Science* **233**: 778–780.

PINEDA, S., SCHNEIDER, M., SMAGGHE, G., MARTIN, A., ESTAL, P. DEL, VIÑUELA, E., VALLE, J. & BUDIA, F. 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **100**: 773–780.

SALGADO, V. L. 1997. The modes of action of spinosad and other insect control products. *Down to Earth* **52**: 35–43.

SALGADO, V.L., SHEETS, J.J., WATSON, G.B. & SCHMIDT, A.L. 1998. Studies on the mode of action of spinosad: the internal effective concentration and the concentration dependence of neural excitation. *Pesticide Biochemistry and Physiology* **60**: 103–110.

SAS INSTITUTE. 2009. *JMP 8.0.2*. SAS Institute Inc., Cary, NC, U.S.A.

SCOTT, J.G. 1998. Toxicity of spinosad to susceptible and resistant strains of houseflies, *Musca domestica*. *Pesticide Science* **54**: 131–133.

SIAL, A.A. & BRUNNER, J.F. 2010. Assessment of resistance risk in obliquebanded leafroller (Lepidoptera: Totricidae) to the reduced-risk insecticides chlorantraniliprole and spinetoram. *Journal of Economic Entomology* **103**: 1378–1385.

SIAL, A.A., BRUNNER, J.F. & DOERR, M.D. 2010. Susceptibility of obliquebanded leafroller (Lepidoptera: Totricidae) to two new reduced-risk insecticides. *Journal of Economic Entomology* **103**: 140–146.

SIAL, A.A., BRUNNER, J.F. & GARCZYNSKI, S.F. 2011. Biochemical characterization of chlorantraniliprole and spinetoram resistance in laboratory-selected obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Totricidae). *Pesticide Biochemistry and Physiology* **99**: 274–279.

SIES, H. 1999. Glutathione and its role in cellular functions. *Free Radical Biology & Medicine* **27**: 916–921.

SIMPSON, D.R., BULLAND, D.A. & LINDQUIST, D.A. 1964. A semi-microtechnique for estimation of cholinesterase activity in boll weevils. *Annals of Entomological Society of America* **57**: 367–371.

THOMPSON, G.D., DUTTON, R. & SPARKS, T.C. 2000. Spinosad – a case study: an example from a natural products discovery programme. *Pest Management Science* **56**: 696–702.

VAN ASPEREN, R. 1962. A study of housefly esterase by means of sensitive colourimetric method. *Journal of Insect Physiology* **8**: 401–416.

VAUGHAN, A. & HEMINGWAY, J. 1995. Mosquito carboxylesterase Est$\alpha 2^1$ ($A_2$). Cloning and sequence of the full length cDNA for a major insecticide resistance gene worldwide in the mosquito *Culex quinquefasciatus*. *Journal of Biological Chemistry* **270**: 17044–17049.

WANG, D., QIU, X., REN, X., ZHUANG, W. & WANG, K. 2009a. Effects of spinosad on *Helicoverpa armigera* (Lepidoptera: Noctuidae) from China: tolerance status, synergism and enzymatic responses. *Pest Management Science* **65**: 1040–1046.

WANG, D., QIU, X., REN, X., NIU, K. & WANG, K. 2009b. Resistance selection and biochemical characterization of spinosad resistance in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* **95**: 90–94.

WANG, W., MO, J., CHENG, P., ZHUANG, P. & TANG, Z. 2006. Selection and characterization of spinosad in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* **84**: 180–187.

WATSON, G.B. 2001. Actions of insecticidal spinosyns on $\gamma$-aminobutyric acid receptors from small-diameter cockroach neurons. *Pesticide Biochemistry and Physiology* **71**: 20–28.

YAQOOB, R., TAHIR, H.M., KHAN, S.Y. & NASEEM, S. 2013. Insecticide resistance in *Bactocera zonata* (Diptera: Tephritidae) in district, Sargodha, Pakistan. *Biochemistry & Pharmacology* **2**: 114. doi:10.4172/2167-0501.1000114

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