Sublethal Effects of Spinosad on Some Biochemical and Biological Parameters of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

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Abstract

Piri F., Sahragard A., Ghadamyari M. (2014): **Sublethal effects of spinosad on some biochemical and biological parameters of** *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). Plant Protect. Sci., **50**: 135–144.

The susceptibility of *G. pyloalis* larvae to spinosad was studied using the leaf dip method. Treatment with doses of spinosad sublethal concentrations (LC_{10}, LC_{20}, LC_{30}, LC_{40} of 0.026, 0.045, 0.065, 0.090 ppm, respectively) was applied. A significant difference in the effects was observed between the sublethal concentrations (LC_{10}, LC_{20}, LC_{30}, and LC_{40}) and the control in the content of carbohydrate and glycogen, and between the control vs. LC_{30} and LC_{40} in the content of protein. A significant decrease in glutathione S-transferase activity with the increase of spinosad concentration, no significant differences in the activities of α- and β-esterases, and a significant increase in the enzyme activity of phenoloxidase were observed. Effects of LC_{10} and LC_{30} spinosad concentrations on some biological parameters showed that percentage of larval pupation and female fecundity significantly decreased in the concentration of LC_{30}.

Keywords: lesser mulberry pyralid; energy reserves; detoxifying enzyme; fecundity

Lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae), is a destructive pest of mulberry occurring in India, China, Korea, Japan, Malaysia, Pakistan, Uzbekistan, and Burma (Madyarov *et al.* 2006). This pest causes severe damages to mulberry trees also in northern Iran. Mulberry leaves are used for mass rearing of the silkworms (*Bombyx mori* L.) and so this pest can interfere with sericulture industry in Iran (Jaafari Khaljiri *et al.* 2006).

Spinosad is a bio-insecticide (Copping & Menn 2000) that is derived from fermentation of a bacterium *Saccharopolyspora spinosa* Mertz and Yao. This insecticide is a mixture of spinosyns A ($C_{41}H_{65}NO_{10}$) and D ($C_{42}H_{67}NO_{10}$) (Sparks *et al.* 1997) and is highly toxic through contact and ingestion on a number of pests in the orders of Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, and Hymenoptera (Sparks *et al.* 1995). It affects nicotinic acetylcholine receptors (nAChRs) and gamma amino butyric acid (GABA) receptors sites in the insect nervous system (Salgado 1997).

Traditionally, measurement of the acute toxicity of insecticides to insect has relied on the determination of the acute lethal concentration LC_{50} or lethal dose LD_{50}. However, in addition to the direct effect of median lethal dose or concentration induced by pesticides, their sublethal effects on pest must be considered for determination of their impact on next generation. Sublethal effects are expressed as physiological or behavioural impacts on individuals that survive exposure to a pesticide (Desneux *et al.* 2007). Physiological effects may be manifested as reductions in fecundity (Zalizniak & Nugegoda 2006) and in fertility (Liu & Trumble 2005). Behavioural changes may affect feeding and oviposition (Fujiwara *et al.* 2002). Sublethal doses of insecticides may be potentially toxic to different instars and stages of insects through diverse effects such as interfering with the function of glutathione S-transferases (GST), carboxylesterase, and other metabolic enzymes, or changing the behavioural patterns associated with feeding, migration, reproduction, and/or the exchange of chemical information (Haynes 1988; Lee 2000).

Rumpf *et al.* (1997) showed that there is a correlation between the degree of acetylcholinesterase (AChE) and GST inhibition and corresponding mortality caused by insecticide in lacewings (Neuroptera: Hemerobiidae and Chrysopidae). Accordingly, GSTs have attracted attention in insects because of their involvement in the defense towards insecticides (Clark *et al.* 1986; Grant & Matsumura 1989; Reidy *et al.* 1990; Fournier *et al.* 1992). Induction of GST by pyrethroids has also been reported for the honey bee (Yu *et al.* 1984), *Spodoptera frugiperda* (Punzo 1993), and German cockroach (Hemingway *et al.* 1993). It has been demonstrated that in the house fly, phenobarbital (Ottea & Plapp 1981) and several insecticides induce the activity of GST (Hayaoka & Dauterman 1982). DDT was found to be the most active in inducing transferase activity and it was also found that flies with induced GST were more tolerant to several OP insecticides (Motoyama & Dauterman 1980).

General esterases are a large and diverse group of hydrolases that hydrolyse numerous substrates including esters and certain non-ester compounds. 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).

Phenoloxidase (PO) (EC.1.14.18.1) is an oxidative enzyme in insects playing an important role in the development and immunity of insects (Ashida & Brey 1995). Some studies have demonstrated that PO can be inhibited or activated by some pesticides. Nasr *et al.* (2011) showed that oxymatrine, chlorfluazuran, and chlorpyrifos significantly decreased *Bombyx mori*'s PO activities compared with control. In *Lymantria dispar,* spinosad significantly changed PO activities in an activation-inhibition fashion for both 3rd and 5th instar larvae (Yan *et al.* 2012b). Also, the effects of atabron on *Ostrinia furnacalis* larvae showed that the activity of cuticular PO was increased by 276% (Wu & Shang 1992).

The objective of this research was to investigate the sublethal effects of spinosad as a biological control agent on some biochemical parameters such as GST activities, non-specific esterase (α- and β-esterase), PO, and energy reserves in 5th larval instars of *G. pyloalis*. The effects of LC_{10} (0.026 ppm) and LC_{30} (0.065 ppm) on fecundity and survival rate of 5th larval instars were studied, too.

MATERIAL AND METHODS

Insect cultures. The 5th instar larvae of G. *pyloalis* were collected from mulberry trees (Shine Ichinoise cultivar) in the city of Rasht (Iran) and taken to the laboratory. They were reared in the laboratory conditions at 25 ± 1°C, 70 ± 10% relative humidity (RH), and 16:8 h light (L) : darkness (D) photoperiod in plastic boxes (18 × 15 × 7 cm). As female and male insect emerged, they were sexed in plastic containers (18 × 15 × 7 cm) and fed with 10% honey solution (10 ml honey in 100 ml water). The fresh mulberry leaves (leave petioles were placed into vials containing water and the mouth of the vial was covered with cotton) were used for oviposition. The leaves containing eggs were transferred in a plastic box (18 × 15 × 7 cm). After egg hatching, the larvae were transferred on fresh leaves with a camel brush. The leaves were changed every day.

Bioassay. One-day-old 5th instar larvae were used in bioassay. Leaf dip (3.5 cm in diameter) method was used to determine the susceptibility of larvae to spinosad (Entrust naturalyte®, Dow AgroSciences, Indianapolis, USA) (80% spinosad-mixture of spinosyn A and D and 20% other ingredients). Five concentrations (0.05, 0.1, 0.2, 0.3, and 0.4 mg/l) of spinosad were prepared with distilled water, and control containing distilled water was also used. Triton X-100 was added as an emulsifier at 0.05% to all concentrations. Mulberry leaf disks were dipped in diluted solutions for 45 s and dried at room temperature for 1 hour. Ten 5th instar larvae were placed in a plastic container (14 × 5 × 12 cm) containing two leaf disks treated with the same concentration of spinosad. Each concentration was applied in six replications. The plastic containers were maintained at 25 ± 1°C, 60 ± 10% RH, and 16 h L : 8 h D photoperiod. Mortality was recorded after 48 hours. Concentration–mortality data were analysed by probit analysis using POLO-PC (LeOra Software 1997).

Carbohydrate determination. Carbohydrate was extracted according to the Van Handel (1965) method. One larva was homogenized in 62.5 µl of sodium sulphate solution (2% Na_2SO_4) and mixed with 468.75 µl of chloroform/methanol (1 : 2 v/v). The homogenate was centrifuged at 8000 *g* for 10 minutes. Then 150 µl of the supernatant was transferred into a micro tube and 100 µl of distilled water and 500 µl of anthrone reagent (0.05% in sulphuric acid) were added to each tube and heated in a 90°C water bath for 10 minutes. The blank consisted of 100 µl distilled water, 500 µl anthrone reagent, and 150 µl Na_2SO_4 (2%) and chloroform/methanol. The absorbance was recorded at 630 nm using microplate reader (Stat Fax 3200®; Awareness Technology Inc., Florida, USA). Carbohydrate content was measured with maltose as the standard.

Lipid determination. Lipid content was determined according to the method by Van Handel (1965). Two larvae were homogenised in 100 μl of Na_2SO_4 (2%) and then were mixed with 750 μl of chloroform/methanol (1 : 2 v/v). The homogenate was centrifuged at 8000 *g* for 10 min. 125 μl of the supernatant was transferred into a micro tube and heated in an oven at 40°C until solvent complete evaporation. Then, 125 μl of sulphuric acid (98%) (Merck, Darmstadt, Germany). was added to each tube and heated at a 90°C water bath for 10 minutes. After that, 30 μl of the sample was transferred to a microplate and incubated with 270 μl vanillin reagent (0.006 g vanillin, 4 ml phosphoric acid, 1 ml distilled water). After 30 min incubation with reagent, the reddish colour was developed and absorbance was recorded at 545 nm. The lipid level was calculated by reference to standard curve prepared using cholesterol.

Protein determination. Each larva was homogenised in 100 μl of phosphate buffer (pH 7.0, 20mM). Then the homogenate was centrifuged at 12 000 *g* for 12 min, 10 μl of supernatant was mixed with 500 μl Bradford reagent (10 mg Coomassie blue G250, 5 ml ethanol, 10 ml phosphoric acid). After 30 min the absorbance was recorded at 630 nm and protein content was determined using BSA (serum albumin) as standard.

Glycogen determination. Glycogen content was determined according to the method by Van Handel (1965). Each larva was homogenised in 62.5 μl of Na_2SO_4 (2%) and 468.75 μl of chloroform/methanol (1 : 2 v/v). The homogenate was centrifuged at 8000 *g* for 10 min and the supernatant was discarded. The pellet was washed three times in 80% methanol, then the pellet was dissolved in 125 μl of distilled water, and heated at 70°C for 5 minutes. 100 μl of the sample was added to 500 μl of anthrone reagent. After mixing, the mixtures were heated at 90°C for 10 min and then cooled. The absorbance was recorded at 630 nm, and the glycogen level was calculated using glycogen as standard.

Glutathione S-transferases. The specific activity of GST was determined according to the method of Habig *et al.* (1974). CDNB (1-chloro-2, 4-dinitrobenzene) and DCNB (1,2-dichloro-4-nitrobenzene) were used as substrates. The whole body larva was homogenised in 100 μl phosphate buffer (pH 7.0, 20mM), and then centrifuged at 12 000 *g* at 4°C for 12 minutes. 10 μl of supernatant was transferred into a microplate and mixed with 110 μl of phosphate buffer (pH 7.0, 20mM), 80 μl of substrates CDNB or DCNB (100mM), and 100 μl of GSH (10mM). A blank containing all reactants except for enzyme was used. Increase in absorbance was recorded at 340 nm continuously.

Esterase. The activities of α- and β-esterases were measured according to methods of Van Asperen (1962) using α-naphthyl acetate (α-NA) and β-naphthyl acetate (β-NA) (10mM) as substrates. The whole larval body was homogenised in 100 μl phosphate buffer solution (pH 7.0, 20mM) containing 0.01% Triton X-100, and samples were centrifuged at 10 000 *g* at 4°C for 15 minutes. 12.5 μl of supernatant was transferred to a microplate and mixed with 112.5 μl phosphate buffer (pH 7.0, 20mM), 50 μl substrate, and 50 μl Fast Blue RR salt (1mM). Absorbances at 450 and 540 nm were measured using a microplate reader (Stat Fax® 3200; Awareness Technology Inc., Florida, USA) every 30 s for α-NA and β-NA, respectively. Standard curves using different concentrations of naphthol mixed with fast blue RR salt were included to enable quantification of the amount of naphthol produced during the esterase assay.

Phenoloxidase. PO activity was measured with the method of Robb (1984). The whole larval body was homogenised in 100 μl of phosphate buffer solution (pH 7.0, 20mM) and samples were centrifuged at 12 000 *g* at 4°C for 10 minutes. 10 μl of supernatant was transferred to a microplate and mixed with 90 μl phosphate buffer (pH 7.0, 20mM) and 100 μl L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate (100mM). Absorbance was recorded at 490 nm every 30 s, using microplate reader (Stat Fax 3200®; Awareness Technology Inc.).

Effect of sublethal concentration on fecundity and survival. Mulberry leaves were dipped in spinosad solution at LC_{10} = 0.026 ppm and LC_{30} = 0.065 ppm concentrations and 5th larval instars were exposed to treated leaf disks for 48 hours. Then the survived larvae were fed with fresh mulberry leaves and allowed to pupate. Pupae were placed individually into small containers (14 × 5 × 12 cm). A pair of female and male moths with the same age, which emerged from treated larvae, was placed into a container with moistened cotton soaked in a 10% honey solution for adult nutrition and a mulberry leave for oviposition. Five pairs of moths were used for each treatment. The eggs laid on leaves were counted daily and the leaves were replaced every day until the death of the females.

Data analysis. Data obtained from the experiments were analysed using the Analysis of variance (ANOVA; SAS 2003). Means were compared by Tukey's range test, accepting significant differences at $P < 0.05$.

RESULTS

Determination of sublethal concentrations

The susceptibility of the 5th instar larvae of *G. pyloalis* to spinosad was analysed by leaf dip bioassay and mortality at 0.05, 0.1, 0.2, 0.3, and 0.4 ppm concentrations recorded after 48 h and sublethal concentrations of LC_{10}, LC_{20}, LC_{30}, and LC_{40} were estimated (Table 1).

Table 1. Determination of sublethal concentrations of spinosad on 5th instar larvae of *Glyphodes pyloalis*

Sublethal concentration (ppm)	Concentration	Confidence limit at 95%
LC_{10}	0.026	(0.012–0.041)
LC_{20}	0.045	(0.025–0.063)
LC_{30}	0.065	(0.042–0.086)
LC_{40}	0.09	(0.064–0.114)
LC_{50}	0.124	(0.095–0.153)

Effect on energy reserves

Carbohydrate. Treatment of the 5th instar larvae of *G. pyloalis* with LC_{10}, LC_{20}, LC_{30}, and LC_{40} of spinosad showed that increasing the sublethal concentration decreased the carbohydrate content. The highest and lowest carbohydrate content was related with control (0.5 mg/larvae) and LC_{40} (0.04 mg/larvae), respectively (Figure 1). ANOVA showed a significant difference between sublethal concentrations with the control ($F = 99.17$, df = 4, 19, $P < 0.0001$).

Lipid. Treatment with LC_{10} dose of spinosad resulted in the increased lipid content in larvae. But treatment with LC_{20}, LC_{30}, and LC_{40} doses reduced the lipid content. The highest reduction in the lipid content was achieved when treated with LC_{40} (0.23 mg/larvae). ANOVA ($F = 10.87$, df = 4, 14, $P < 0.001$) showed significant differences between the control, LC_{20}, LC_{30}, and LC_{40} (Figure 2).

Protein. The protein content was reduced in larvae treated with LC_{30} and LC_{40} compared to control. The protein content of control was 0.1 mg/larvae and after treatment with spinosad, it was 0.09, 0.09, 0.07, and 0.06 mg/larvae in LC_{10}, LC_{20}, LC_{30}, and LC_{40}, respectively. ANOVA ($F = 21.79$, df = 4, 14, $P < 0.0001$) showed significant differences between treatments. However, control, LC_{10}, and LC_{20} were at the same level and were significantly different with LC_{30} and LC_{40}.

Glycogen. Treatment of the 5th instar *G. pyloalis* larvae with LC_{10}, LC_{20}, LC_{30}, and LC_{40} of spinosad showed that increase in the sublethal concentration of spinosad decreased the glycogen content. The glycogen content of control was 0.06 mg/larvae, the amounts of glycogen content in LC_{10}, LC_{20}, LC_{30}, and LC_{40} were 0.04, 0.03, 0.02, and 0.01 mg/larvae, respectively. ANOVA ($F = 19.49$, df = 4, 19, $P < 0.0001$) showed a significant difference between the control and the sublethal concentrations.

Effect on GST activity

In untreated 5th instar larvae, the activity of GST was 62.03 (CDNB as substrate) and 5.14 (DCNB as substrate) µmol/min/mg protein. Following the

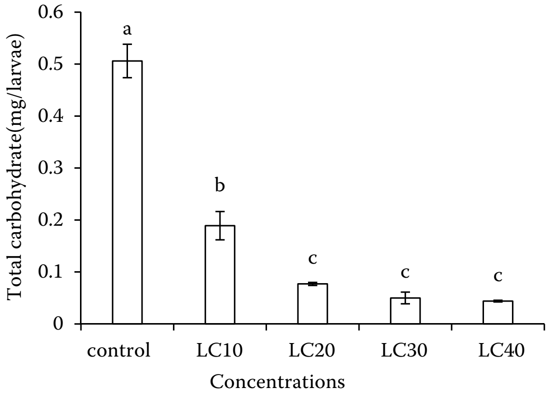

Figure 1. Effect of sublethal concentrations of spinosad on mean total carbohydrate (± SE) in 5th larval instar of *Glyphodespyloalis*

Different letters indicate that the carbohydrate contents are significantly different from each other by Tukey's test ($P < 0.05$)

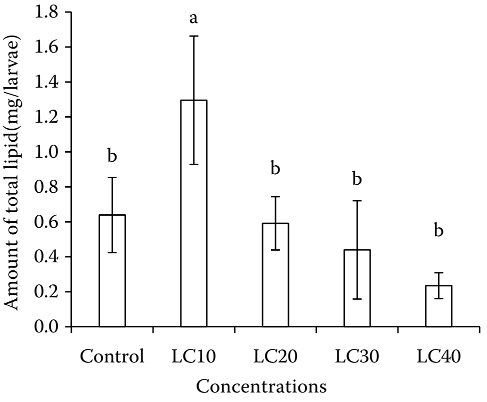

Figure 2. Effect of sublethal concentrations of spinosad on total lipid (mg/larvae) in 5th larval instar of *Glyphodes pyloalis*

Different letters indicate that the lipid contents are significantly different from each other by Tukey's test ($P < 0.05$)

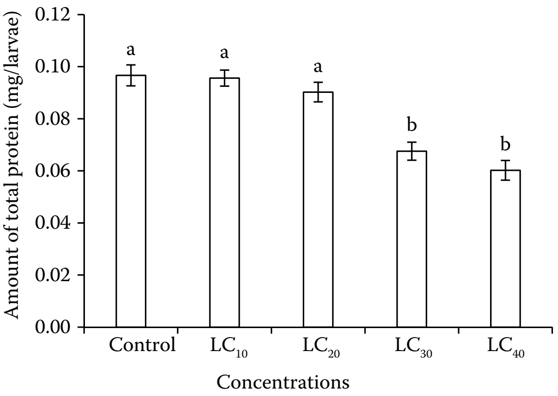

Figure 3. Effect of sublethal concentrations of spinosad on total protein (mg/larvae) in 5th larval instar of *Glyphodes pyloalis*

*Different letters indicate that the protein contents are significantly different from each other by Tukey's test ($P < 0.05$)

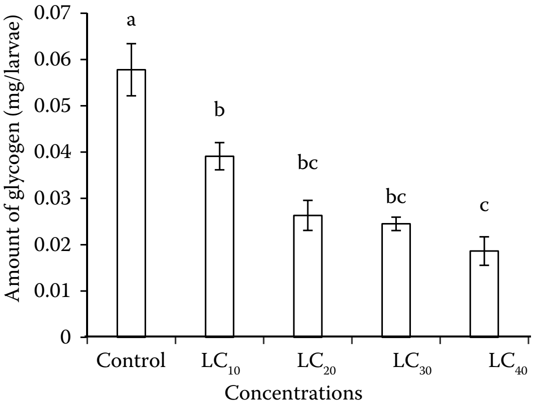

Figure 4. Sublethal effect of spinosad on total glycogen (mg/larvae) in 5th larval instar of *Glyphodes pyloalis*

*Different letters indicate that the glycogen contents are significantly different from each other by Tukey's test ($P < 0.05$)

treatment of 5th instar larvae with sublethal doses of spinosad, the GST activity in larvae treated with LC_{40} was significantly decreased to 15.90 and 8.16 µmol/min/mg protein, when CDNB and DCNB were used as substrates, respectively. On the other hand, CDNB and DCNB showed variable effects on the activity of the enzyme. ANOVA ($F = 21.41$, df = 4, 14, $P < 0.0001$) showed that GST activity was affected by sublethal concentrations of spinosad when CDNB was used as substrate but there was no significant difference between the treatments in the case of DCNB ($F = 1.32$, df = 4, 14, $P < 0.3290$) (Figure 5).

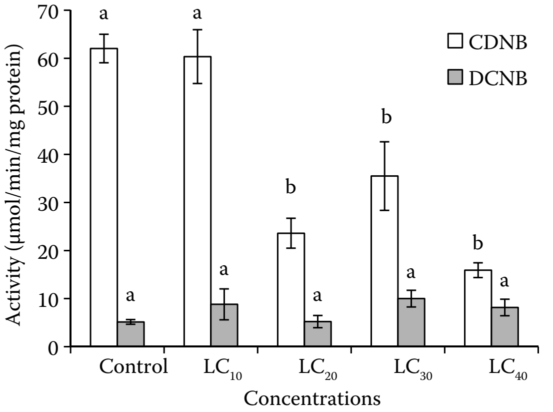

Figure 5. Effect of spinosad on specific activities of glutathione S-transferase (GST) in *Glyphodes pyloalis* using CDNB (1-chloro-2,4-dinitrobenzene) and DCNB (1,2-dichloro-4-nitrobenzene) as substrate

*Different letters indicate that the specific activities of the enzymes are significantly different from each other by Tukey's test ($P < 0.05$)

Effect on esterase activity

ANOVA for α-esterase ($F = 1.86$, df = 4, 14, $P > 0.1947$) and β-esterase ($F = 0.92$, df = 4, 14, $P > 0.4884$) showed that esterase activities in treated 5th instar larvae did not show any significant differences between the control and sublethal concentrations (Figure 6).

Effect on phenoloxidase activity

In the present study, the PO activity showed a greater activity level in larvae treated with LC_{30} and

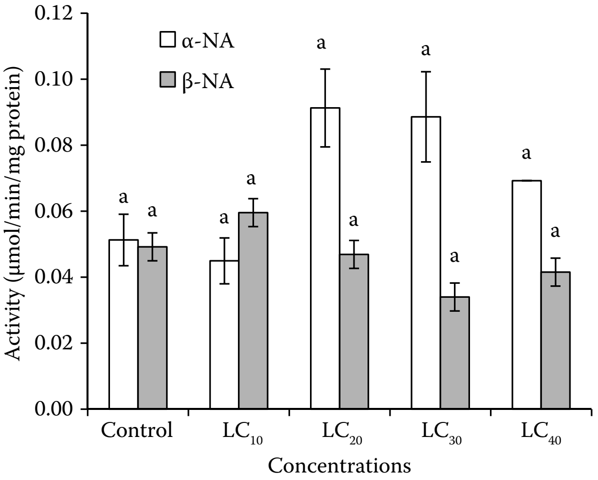

Figure 6. Sublethal effect of spinosad on activities of α- and β-esterase in *Glyphodes pyloalis*

*Different letters indicate that the specific activities of the enzymes are significantly different from each other by Tukey's test ($P < 0.05$)

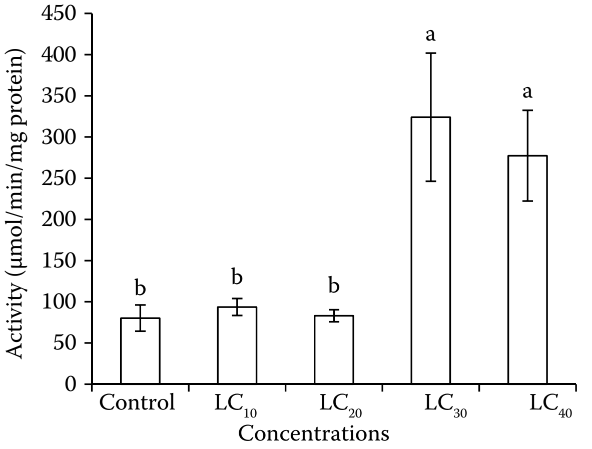

Figure 7. The effect of spinosad on activity of phenoloxidase (PO) in *Glyphodes pyloalis*

*Different letters indicate that the specific activity of PO is significantly different by Tukey's test ($P < 0.05$)

LC_{40} compared with LC_{10}, LC_{20}, and control. ANOVA ($F = 7.46$, df = 4, 14, $P < 0.005$) of PO activity showed significant differences between treatments (Figure 7).

Sublethal effects of spinosad on fecundity and percentage of pupation of *G. pyloalis*

The total numbers of eggs laid by each female were evaluated after treatment of larvae with LC_{10} and LC_{30}. The female fecundity of the survivors was declined, when the larvae were treated with LC_{30}. The eggs laid per female were reduced by 41% in insect treated with LC_{30}. Our result showed a significant difference between treatments ($F = 11.43$, df = 2, 14, $P < 0.002$). Fecundity of parent females in LC_{30} was significantly lower than of those in LC_{10} and control (Figure 8).

Percentage of 5th instar larvae pupated was affected by LC_{30} concentration ($F = 9.54$, df = 2, 38, $P < 0.0005$). Pupation at LC_{30} was significantly lower than at LC_{10} and control (Figure 9).

DISCUSSION

The changes in energy reserves such as carbohydrates, lipids, proteins, and glycogen indicate the susceptibility of the insect to insecticide and its function alterations. Carbohydrates are an important source of energy for insects. Carbohydrates may be converted to lipids, and may contribute to the production of amino acids. Many carbohydrates such as sugars are powerful feeding stimulants (Dadd 1985; Nation 2001; Genc 2002). The result of the present study showed that when *Glyphodes pyloalis* larvae were treated with sublethal concentrations of spinosad, their carbohydrate content decreased significantly. The reduction of carbohydrate may be due to the effect of anti-feedent and increased metabolism under toxicant stress. The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Remia *et al*. 2008).

Proteins are important for individual-level fitness-associated traits such as body size, growth rate, and fecundity, and at higher levels of organisation they

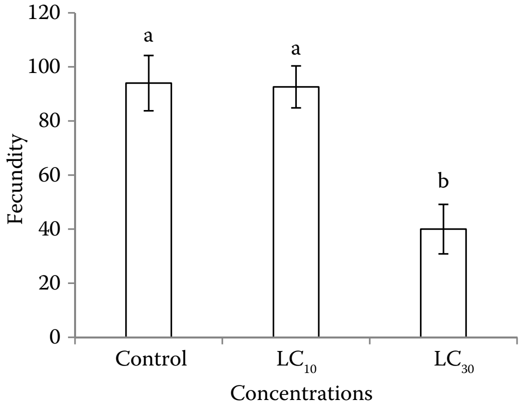

Figure 8. Fecundity of *Glyphodes pyloalis* when 5th instar larva was treated with LC_{10} and LC_{30} of spinosad

*Different letters indicate that fecundity is significantly different by Tukey's test ($P < 0.05$)

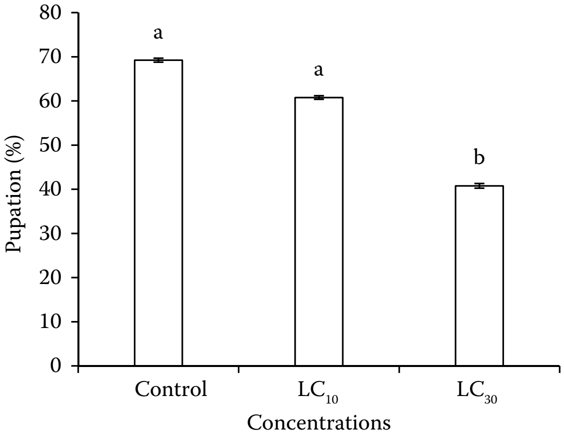

Figure 9. Percentage of 5th instar larvae pupated (± SE) at LC_{10} and LC_{30} concentrations

*Different letters indicate that the percentages of 5th instar larvae pupated are significantly different from each other by Tukey's test ($P < 0.05$)

have been linked to population dynamics, life histories, and even biological diversification (Fagan *et al.* 2002). Our results showed a significant reduction of protein in LC_{30} and LC_{40} than in LC_{10}, LC_{20}, and control. Etebari (2006) showed that many insecticides decrease feeding efficiency and protein amount. Nath *et al.* (1997) suggested that this could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the TCA cycle by retaining free amino acid content in hemolymph.

Lipids in living organisms consist of free and bound fatty acids, short and long chain alcohols, steroids and their esters, phospholipids, and other groups of compounds. Insects are able to convert carbohydrates into lipids, and many insects can synthesise lipids and accumulate them in fat body tissue. Fatty acids, phospholipids, and sterols are components of cell walls in addition to having other specific functions (Nation 2001). Our results showed a significant increase of lipid at LC_{10} and decrease at LC_{20}, LC_{30}, and LC_{40}. Bennett & Shotwell (1972) suggested that the infected larvae might produce enzyme that utilises lipids for energy requirement. Our results also showed a significant decrease of glycogen content in 5th instar *G. pyloalis* larvae treated with LC_{10}, LC_{20}, LC_{30}, and LC_{40} of spinosad concentrations. Changes in the amount of glycogen could be due to the upset of the homeostatic mechanism in insects by insecticides (Nath 2003; Oguri & Steele 2007).

Our results also agree with Nehad *et al.* (2008) that reported significant reduction of total carbohydrates (–65.06% as compared to untreated group) of homogenate of 4th larval instars of *Spodoptera littoralis* after treatment with LC_{50} of Radiant® (a new generation of spinosyn) after 24 hours. El-Sheikh (2012) reported that treating 4th instar larvae of *Spodoptera littoralis* with LC_{50} spinosad significantly reduced total protein contents by about 31.5% after 24 hours.

GSTs are a family of enzymes that catalyse the nucleophilic attack of the sulphur atom of glutathione on the electrophilic centre of many chemical compounds (Mannervik & Danielson 1988). The GSTs, in addition to their enzymatic activities, can bind with high affinity a variety of hydrophobic compounds (Daniel 1993). Our results showed that activity of GST was reduced in sublethal concentrations of LC_{20}, LC_{30}, and LC_{40} compared with control.

Results obtained here are similar to those shown by Yan *et al.* (2012a) who studied bioactivity and LC_{50} of spinosad against 3rd and 5th instar larvae of *Lymantria dispar* L. and its effect on activities of the detoxifying enzymes. Carboxylesterase, GST, multi-function oxidase (MFO), and alkaline phosphatase (ALP) were increased first significantly and then decreased in different time intervals ($P < 0.05$).

Liu *et al.* (2012) studied the insecticidal activity and toxicity mechanism of spinosad on *Malacosoma neustria testacea* larvae by leaf membrane method and its effects on the activities of detoxifying and protective enzymes were measured 3, 6, 12, and 24 h after treatment. Their results showed that GST activity in 4th and 5th instar larvae was first inhibited, then induced, and finally inhibited. Therefore, spinosad could effectively disrupt and interfere not only with the detoxifying and protective enzymes but also normal physiological metabolism and showed extremely high toxicity against this pest.

In the present study, we evaluated the sublethal effect of spinosad on PO activity levels (as representative of the innate immune response). The PO showed a greater activity level in larvae treated with LC_{30} and LC_{40} compared with $LC_{10,}$ LC_{20}, and control. Our results agree with Wu and Shang (1992). They reported that PO activity in 5th instar larvae of *O. furnacalis* treated with atabron was significantly increased. Also, spinosad affected the PO activities in *Lymantria dispar* larvae (Yan *et al.* 2012b). Valadez-Lira *et al* (2011) showed that exposure to *Bacillus thuringiensis* resulted in significantly increased PO activity in 2nd instar of *Plodia interpunctella*.

Our result showed significant decrease in fecundity of parent females and significant decrease in percentage of pupation. Galvan *et al.* (2005) reported that exposure to spinosad in the multi-coloured Asian lady beetle decreased the survival of first instars, extended the time for first instars to become adult, and reduced female fertility. Wang *et al.* (2008) reported that fecundities of parent females of *Helicoverpa armigera* (Lepidoptera: Noctuidae), which emerged from second instars treated with 0.04 and 0.16 mg/kg of spinosad, were significantly lower than those of control. The reduction in fecundity has been related to physiological and morphological changes in both males and females. Khosravi *et al* (2011) also reported that fecundity of *G. pyloalis* treated with LC_{50} and LC_{20} (on newly ecdysed 4th instar larvae) of *Artemisia annua* was reduced. Results obtained here are similar to those shown by Yin (2008) who studied sublethal effects of spinosad on *Plutella xylostella*

(Lepidoptera: Yponomeutidae). The fecundity of parent females treated with LC_{25} and LC_{50} doses for 48 h was significantly lower than of those given the control treatment, by 32.6 and 49.2%, respectively.

Sublethal doses of spinosad affected some biological and biochemical parameters of *G. pyloalis*. Sublethal concentrations of spinosad significantly inhibited larval growth, pupation, and fecundity, also affected the amount of carbohydrates, lipids, protein, and glycogen, and changed activities of detoxification enzymes and PO activity. The results of evaluating the sublethal effect of spinosad suggested that substantial physiological events in the life of *G. pyloalis* larvae are involved in responding to the action of the insecticide. Spinosad may provide more benefits to an integrated pest management program for *G. pyloalis*.

References

Ashida M., Brey P. (1995): Role of the integument in insect defense: prophenoloxidase cascade in the cuticular matrix. Proceedings of the National Academy of Sciences USA, **92**: 10698–10702.

Bennett G.A., Shotwell O.L. (1972): Heamolymph lipids of healthy and diseased Japanese beetle larvae. Journal of Insect Physiology, **18**: 53–57.

Clark A.G., Shamaan N.A., Sinclair M.D., Dauterman W.C. (1986): Insecticide metabolism by multiple glutathione S-transferases in two strains of the house fly *Musca domestica* (L.). Pesticide Biochemistry and Physiology, **25**: 169–175

Copping L.G., Menn J.J. (2000): Biopesticide: a review of their action, applications and efficacy. Pest Management Science, **56**: 651–676.

Dadd R.H. (1985): Nutrition: Organisms 4. In: Kerkut G.A., Gilbert L.I. (eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon, Oxford; National Academy Press, Washington: 313–390

Daniel V. (1993): Glutathione S-transferases: gene structure and regulation of expression. CRC Critical Review of Biochemistry and Molecular Biology, **28**: 173–207.

Desneux N., Decourtye A., Delpuech J.M. (2007): The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology, **52**: 81–106.

El-Sheikh T.A.A. (2012): Biological, biochemical and histological effects of spinosad, *Bacillus thuringiensis* var. *kurstaki* and cypermethrin on the cotton leafworm, *Spodoptera littoralis* (Boisd.). Egyptian Academic Journal of Biological Sciences, **4**: 113–124.

Etebari K., Mirhoseini S. Z., Matindoost L. (2005): A study on intraspecific biodiversity of eight groups of silkworm (*Bombyx mori*) by biochemical markers. Insect Science, **12**: 87–94.

Fagan W.F., Siemann E., Mitter C., Denno R.F., Huberty, A.F., Woods H.A., Elser J.J. (2002): Nitrogen in insects: implications for trophic complexity and species diversification. American Naturalist, **160**: 784–802.

Fournier D., Bride J.M., Poirie M., Berge J.B., Plapp F.W. (1992): Insect glutathione S-transferases: Biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides. The Journal of Biological Chemistry, **267**: 1840–1845.

Fujiwara Y., Takahashi T., Yoshioka T., Nakasuji F. (2002): Changes in egg size of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) treated with fenvalerate at sublethal doses and viability of the eggs. Applied Entomology and Zoology, **37**: 103–109.

Galvan T.L., Koch R.L., Hutchison W.D. (2005): Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). Biological Control, **34**: 108–114.

Genc H., Phaon C., Phyciodes P. (2002): Life Cycle, Nutrional Ecology and Reproduction, [Ph.D. Disseretation.] University of Florida, Gainesville.

Grant D.F., Matsumura F. (1989): Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti*. Pesticide Biochemistry and Physiology, **33**: 132–143

Habig W.H., Pabst M.J., Jakoby W.B. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, **249**: 7130–7139

Hayaoka T., Dauterman W.C. (1982): Induction of glutathione S-transferase by phenobarbital and pesticides in various house fly strains and its effect on toxicity. Pesticide Biochemistry and Physiology, **17**: 113–119

Haynes K.F. (1988): Sublethal effects of neurotoxic insecticides on insect behavior. Annual Review of Entomology, **33**: 149–168.

Hemingway S.J.; Dunbar S.J., Monro A.G. (1993): Pyrethroid resistance in German cockroaches (Dictyoptera, Blattelidae): resistance levels and underlying mechanisms. Journal of Economic Entomology, **86**: 1631–1638.

Jaafari Khaljiri Y., Rezaei V., Zargarpour Kazemian P. (2006): Biological study of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae), a new pest of mulberry in Guilan province, Iran. In: 17th Iranian Plant Protection Congress, Karaj, Iran: 257.

Khosravi R., Jalali Sendi J., Ghadamyari M., Yezdani E. (2011): Effect of sweet wormwood *Artemisia annua* crude leaf extracts on some biological and physiological characteristics of the lesser mulberry pyralid, *Glyphodes pyloalis*. Journal of Insect Science, **11**: 1536–2442.

Lee C.Y. (2000): Sublethal effects of insecticides on longevity, fecundity and behaviors of insect pests: a review. Journal of Biosciences **11**: 107–112.

LeOra Software (1987): POLO-PC a User's Quide to Probit or Logit Analysis. LeOra Software, Berkeley.

Liu D., Shanchun Y., Chuanwang C., Yuezhi L. (2012): Effect of spinosad on the detoxifying and protective enzymes of *Malacosoma neustria* testacea. Scientia Silvae Sinicae, **3**: 49–50

Liu D.G., Trumble J.T. (2005): Interactions of plant resistance and insecticides on the development and survival of *Bactericerca cockerelli* [Sulc] (Homoptera: Psyllidae). Crop Protection, **24**: 111–117.

Madyarov Sh.R., Khamraev A.Sh., Otarbaev D.O., Kamita S.G., Hammock B.D. (2006): Comparative effects of wild and recombinant baculoviral insecticides on mulberry pyralid *Glyphodes pyloalis* Wlk. and mulberry silkworm *Bombyx mori*. In: International Congress Biotechnology, Moscow, March 12–16, 2006: 230–231.

Mannervik B., Danielson U.H. (1988): Glutathione transferases – structure and catalytic activity. CRC Critical Review of Biochemistry, **23**: 281–334.

Motoyama N., Dauterman W.C. (1980): Glutathione S-transferases: Their role in the metabolism of organophosphorus insecticides. Review Biochemical Toxicology, **2**: 49–69.

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

Nation J.L. (2001): Insect Physiology and Biochemistry. CRC Press, Boca Raton.

Nasr H.M. (2011). Toxicological and biochemical effects of chlorpyrifos, chlorfluazuron and oxymatrine on larvae on *Bombyx mori*. Journal of Agricultural Research of Kafer El-Sheikh University, **37**: 209–222**.**

Nath B.S. (2003). Shifts in glycogen metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticide toxicity. Pesticide Biochemistry and Physiology. **74**: 73–84.

Nath B.S., Suresh A., Mahendra V.B., Kumar R.P. (1997): Changes in metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* L. in response to organophosphorus insecticides toxicity. Ecotoxicology and Environmental Safety, **36**: 169–173.

Nehad M.E., Hassan F.D., Yasser A.E.S. (2008): Toxicological evaluation and biochemical impacts for radient as a new generation of spinosyn on *Spodoptera littoralis* (Boisd.) larvae. Egyptian Academic Journal of Biological Sciences, **1** (2): 85–97

Oguri E., Steele J.E. (2007): A comparative study of the metabolic effects of hyper trehalosemic hormone and 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH) in the American cockroach, *Periplaneta americana*. Pesticide Biochemistry and Physiology, **87**: 196–203.

Ottea J.A., Plapp F.W. (1981): Induction of glutathione S-aryl transferase by phenobarbital in the house fly. Pesticide Biochemistry and Physiology, **15**: 10–13.

Punzo F. (1993): Detoxification enzymes and the effects of temperature on the toxicity of pyrethroids to the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Comparative Biochemistry and Physiology, **105**(C): 155–158

Reidy G.F., Rose H.A., Visetson S., Murray M. (1990): Increased glutathione S-transferase activity and glutathione content in an insecticide-resistant strain of *Tribolium castaneum* (Herbst). Pesticide Biochemistry and Physiology, **36**: 269–276.

Remia K.M., Logaswamy S., Logankumar K., Rajmohan D. (2008): Effect of an Insecticide (Monocrotophos) on some biochemical constituents of the fish *Tilapia mossambica*. Pollution Research, **27**: 523–526

Robb D.A. (1984). Tyrosinase. In: Lontie R. (ed.): Copper Proteins and Copper Enzymes. Vol. 2. CRC Press, Boca Raton: 207–241.

Rumpf S., Hetzel F., Frampton C. (1997): Lacewings (Neuroptera: Hemerobiidae and Chrysopidae) and integrated pest management: enzyme activity as biomarker of sublethal insecticide exposure. Journal of Economic Entomology, **90**: 102–108.

Salgado V.L. (1997): The mode of action of spinosad and other insect control product. Down to Earth, **52**: 14–20.

Sparks T.C., Thompson G.D., Larson L.L., Kirst H.A., Jantz O.K., Worden T.V., Hertlein M.B., Busacca J.D. (1995): Biological characteristics of the spinosyns: new naturally derived insect control agents. In: Proceedings of the Beltwide Cotton Conference, San Antonio, Texas, 4–7 January, 1995. National Cotton Council of America, Memphis: 903–907.

Sparks T.C., Sheets J.J., Skom J.R., Worden T.V. (1997): Penetration and metabolism of spinosyn A in lepidopterous larvae. In: Proceeding 1997 Beltwide Cotton Production Conference Cotton Council, Memphis: 1259–1264.

Valadez-Lira J.A., Alcocer-Gonzalez J.M., Damas G., Nuñez-Mejía G, Oppert B., Rodriguez-Padilla C., Tamez-Guerra P. (2011): Comparative evaluation of phenoloxidase activity in different larval stages of four lepidopteran pests after exposure to *Bacillus thuringiensis.* Journal of Insect Science, **12**: 1536–2442.

van Asperen K. (1962): A study of house fly esterase by means of sensitive colourimetric method. Journal of Insect Physiology, **8**: 401–416.

Van Handel E. (1965): Microseparation of glycogen, sugars, and lipids. Analytical Biochemistry, **11**: 266–271.

Wang D., Gong P., Li M., Qiu X., Wang K. (2008): Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae), Pest Management Science, **65**: 223–227.

Yan J., Zhong H., Junxin Y., Shanchun Y. (2012a): Effect of spinosad to detoxifying enzymes activity in *Lymantria dispar* larva. Scientia Silvae Sinicae, **48**: 82–87.

Yan J., Yan S., Cao C. (2012b): Effects of spinosad on the activity of protective enzymes and chitinase in *Lymantria dispar*. Journal of Beijing Forestry University, **34** (1): 80–85.

Yin X.H., Wu Q.J., Li X.F., Zhang Y.J., Xu B.Y. (2008): Sublethal effects of spinosad on *Plutella xylostella* (Lepidoptera: Yponomeutidae). Crop Protection, **27**: 1385–1391

Yu S.J., Robinson F.A., Nation J.L. (1984): Detoxication capacity in the honey bee *Apis mellifera* L. Pesticide Biochemistry and Physiology, **22**: 360–368.

Wu G., Shang Z. (1992). Effects of atabron on phenoloxidase and chitinase in the cuticle of the *Ostrinia furnacalis* larva. Acta Entomologica Sinica. **35**: 306–311.

Zalizniak L., Nugegoda D. (2006): Effect of sublethal concentrations of chlorpyrifos on three successive generations of *Daphnia carinata*. Ecotoxicology and Environmental Safety, **64**: 207–214.

Received for publication Jule 27, 2013
Accepted after corrections December 30, 2013

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