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Disturbance of phosphatase and transaminase activities in grubs of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) by certain insecticidal compounds

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Abstract

Background: Serious damage of red palm weevil to date palm encourages its control by various measures. The present work was carried out to investigate the effects of some insecticides of different categories (Pyriproxyfen, Neemazal, and Spinetoram) on the activities of phosphatases (acid phosphatase; ACP, alkaline phosphatase; ALP) and transaminases (glutamic oxaloacetic transaminase; GOT, glutamine pyruvic transaminase; GPT) in both haemolymph and fat body of the red palm weevil *Rhynchophorus ferrugineus* 5th instar larvae.

Results: After 24-h treatment by sublethal concentrations LC50 and LC75: 1067.5, 2317.5 ppm (Pyriproxyfen), 14,600, 27,100 ppm (Neemazal), and 18.37, 88.60 ppm (Spinetoram). Depending on the present results, the enzyme activities in both haemolymph and fat body tissues had been altered. Spinetoram inhibited the activities of both phosphatase and transaminase enzymes in the two tissues with an exception for the transaminases in haemolymph tissue at LC_{50}. On the other hand, Pyriproxyfen stimulated GOT activity in the two tissues but inhibited GPT activity with no exception. While, phosphatases were inhibited with few exception. Neemazal LC_{75} stimulated phosphatases in the two tissues while transaminases were fluctuated.

Conclusion: Spinetoram exhibited the most potent compound for inhibiting the enzyme activities.

Keywords: ACP, ALP, GOT, GPT, Neemazal, Pyriproxyfen, Spinetoram

Background

The red palm weevil, *Rhynchophorus ferrugineus* (RPW), is a serious pest of date palm (*Phoenix dactylifera*). The insect is native to Southern Asia and Melanesia. It was introduced to the Arabian Gulf countries during the mid-1980s (Bozbuga & Hazir, 2008). In some details, it reached Eastern Saudi Arabia in 1985 and then extended to other countries. Also, it was discovered in Egypt in 2000 (Ferry & Gomez, 2002). Serious damages have been caused by this weevil to some palm genera. Thus, control measures should be achieved (EPPO (European, Mediterranean Plant Protection Organization), 2008).

Excessive uses of chemically conventional insecticides usually cause many problems to all living creatures, including human, so there is an indispensable need to discover a safe alternative control compounds with new mode of action. Insect growth regulators (IGRs), plant extracts, and microbial products are considered one of these alternatives. Spinetoram is a microbial product that is derived from soil-dwelling bacteria (*Saccharopolyspora spinosa*). It exerts its toxic action by contact or ingestion (Sparks et al., 1998). It has a targeting nicotinic acetylcholine receptor as well as -aminobutyric acid-gated chloride channels causing insect paralysis (Salgado, Watson, & Sheets, 1997; Sarfraz, Dosdall, & Keddie, 2005; Watson, 2001). Pyriproxyfen (juvenile hormone analogue, JHA) is one of IGRs that exert their insecticidal

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effects by disturbing the normal activity of the endocrine system (Oberlander, Silhacek, Shaaya, & Ishaaya, 1997; Smet, Rans, & Loof, 1990). Plant extracts are considered as attractive alternatives to synthetic chemical insecticides for minimizing the threats to the environment and human health (Koul & Walia, 2009). Neem seed and leaf extracts, such as azadirachtin and its formulation, Neemazl (20% azadirachtin content), are vigorous as antifeedants, repellents, growth disruptors, molting inhibitors, and oogenesis suppressors (Dorn, Rademacher, & Sehn, 1986; Garcia & Rembold, 1984; Ghoneim, Mohamed, & Bream, 2000; Koul, 1984; Rembold & Sieber, 1981; Richer, Bohm, & Kleeberg, 1997). The disruptive effects of Pyriproxyfen, Neemazal, and Spinetoram had been previously evaluated against survival of RPW (Hamadah & Tanani, 2013) and its hemogram (Hamadah & Tanani, 2017).

Acid phosphatase (ACP) and alkaline phosphatase (ALP) are hydrolytic enzymes, which hydrolyze phosphomonoesters under acid or alkaline conditions, respectively. ACP, as a lysosomal marker enzyme (Csikós & Sass, 1997), is abundant in the decomposed tissues and organs subjected to cytolysis (Sahota, 1975). ALP, as a marker enzyme for brush border membrane (Wolfersberger, 1984), is accountable for cytolysis of tissues during the insect development (Dadd, 1970). Glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) are also known as aspartic transeferase (AST) and alanine transaminase (ALT) respectively. The transaminases are key enzymes in the formation of non-essential amino acids, gluconeogenesis, metabolism of the nitrogen compound, and associated with protein metabolism (Mordue & Goldworthy, 1973). The aim of the current study was to evaluate the effects of Pyriproxyfen, Neemazal, and Spinetoram on the activities of ALP, ACP, GOT, and GPT in the RPW larvae.

Materials and methods

Insect culturing

The red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) culture was established originally by collecting a sample of pupae from a date palm field. The weevil was reread on sugarcane stems under constant laboratory conditions (29 °C ± 2 and 60–70% RH). Also, the light intensity of about 30 fc is provided with fluorescent tubes (Rahalkar, Harwalkar, & Rananavare, 1972; Ranavare, Shantharan, Harwalkar, & Rahalhar, 1975).

Insecticidal compounds

All chemicals, Spinetoram 5% (radiant), Pyriproxyfen 10% (admiral), and Neemazal (20% azadirachtin), were offered by the Central Laboratory of Insecticides, Agricultural Research Centre, Giza, Egypt. In a previous study against the same weevil, two sublethal concentration levels were determined for each compound, LC_{50} and LC_{75}: 1067.5 and 2317.5 ppm (pyriproxyfen), 14,600 and 27,100 ppm (Neemazal), as well as 18.37 and 88.60 ppm (Spinetoram) (Hamadah & Tanani, 2013). In the present study, the two concentrations were prepared and tested against the enzymatic activities in larvae of the same weevil.

Determination of the enzymatic activities

Enzyme sample preparations

Fat body For the determination of the GOT, GPT, ACP, and ALP activities, fat body was collected from 5th instar larvae after 24-h treatment. The fat body was weighed (0.1 g) and then homogenized in a saline solution (the fat body of one insect/1 ml saline solution 0.7%) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was used directly or frozen at − 20 °C (maximum 7 days) until use. Three replicates were similarly prepared.

Haemolymph For the determination of the GPT, GOT, ACP, and ALP activities, haemolymph was collected from 5th instar larvae after 24-h treatment. Haemolymph (0.1 ml) was drawn into Eppendorf Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. For whole blood assays, the diluted haemolymph was frozen for 20 s to rupture the hemocytes. The haemolymph samples were then centrifuged at 2000 rpm for 5 min at 4 °C, and only the supernatant fractions were used for assay directly or frozen at − 20 °C (maximum 7 days) until use. Three replicates were similarly prepared.

Enzyme measurement procedures

Acid phosphatase activity ACP activity was determined according to the method of Tietz (1986) using a kit of Bioadwic. The enzyme activity was measured calorimetrically at 405 nm using spectrophotometer.

Alkaline phosphatase activity ALP activity was determined according to the method of Klein, Read, and Babson (1960) using a kit of Quimica clinical aplicada S.A. The enzyme activity was measured calorimetrically at 550 nm using spectrophotometer.

Glutamic oxaloacetic transaminase activity GOT activity was determined according to the method of Harold (1975) using a kit of Bioadwic. The enzyme activity was measured calorimetrically at 546 nm using spectrophotometer.

Glutamic pyruvic transaminase activity GPT activity was determined according to the method of Harold (1975) using a kit of Bioadwic. The enzyme activity was measured calorimetrically at 546 nm using spectrophotometer.

Statistical analysis of data

Data obtained were analyzed by the Student's *t* distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means ± SD.

Results

In general, the activities of ACP, ALP, GPT, and GOT had been found greater in haemolymph than in fat body of control larvae of *Rh. ferrugineus*. On the other hand, ACP activity was greater than ALP activity in haemolymph and vice versa in fat body of controls. While GOT was greater than GPT, irrespective to the tissue. The enzyme activities of the treated 5th instar larvae exhibited the same trend, with few exceptions.

ACP

Table 1 showed the results of the ACP activity in both tissues of *Rh. ferrugineus* after 24-h treatment of early 5th instar larvae with LC_{50} and LC_{75} of all tested compounds. The enzyme activity insignificantly declined in haemolymph after treatment with Pyriproxyfen and Spinetoram but LC_{50} of Neemazal significantly inhibited the enzyme activity (change % of − 45). The enzyme activity in fat body significantly varied between increase and decrease but the highest activity was recorded at LC_{50} of pyriproxyfen (30.95 ± 2.38 U/L) and lowest activity was determined at LC_{50} of Spinetoram (5.80 ± 0.28 U/L), in comparison with 16.55 ± 0.82 U/L of control larvae. On the other hand, the enzyme activity decreased by Spinetoram in both tissues, regardless the concentration level.

ALP

As shown in Table 2, Neemazal had no significant effect on the enzyme activity in haemolymph. The other compounds remarkably prohibited the enzyme activity and the highest decrease was measured at LC_{75} of Spinetoram (change % − 66.68) followed by LC_{50} of Pyriproxyfen (change % − 59.99). However, ALP in fat body exhibited variable activity by Pyriproxyfen (4.32 ± 0.53, 8.93 ± 0.99 U/L, at LC_{75} and LC_{50}, respectively) and Neemazal (11.37 ± 0.96 and 11.37 ± 0.96 U/L, at LC75 and LC50, respectively), compared to the control larvae (6.11 ± 0.34 U/L). Spinetoram detrimentally prohibited the enzyme activity (change %: − 32.41 and − 38.13, at LC_{75} and LC_{50}, respectively). However, the enzyme decreased by Spinetoram in both tissues at two concentrations, with no exception.

GPT

Results of the affected GPT activity by the tested compounds were illustrated in Table 3. After treatment with Pyriproxyfen, the enzyme activity decreased in both tissues, with no exception. In haemolymph, the enzyme activity was extremely induced at LC_{75} of Neemazal (848.33 ± 10.41 U/L) and at LC_{50} of Spinetoram (658.33 ± 15.28 U/L), compared to 90.00 ± 8.66 U/L in the control larvae. The enzyme activity slightly decreased in fat body, except LC_{75} of both Pyriproxyfen and Spinetoram which considerably inhibited its activity (change %: − 53.9 and − 34.97 U/L, respectively).

GOT

Results of Table 4 clearly show that the enzyme activity was inhibited in both types of tissues by Pyriproxyfen, with no exception. The LC_{50} induced the enzyme activity in haemolymph, irrespective of the tested compounds and the greatly induction was recorded for Spinetoram (181.67 ± 7.64 U/L) and vice versa for LC_{75} that inhibited the enzyme activity. Pyriproxyfen pronouncedly enhanced the enzyme activity (238.33 ± 10.41 U/L), compared to

Table 1 ACP activity (U/L) in haemolymph and fat body of the early 5th instar larvae of *Rh. ferrugineus* after 24-h treatment with LC_{50} and LC_{75} of the tested insecticidal compounds

Conc. (ppm)		Haemolymph		Fat body	
		mean ± SD	Change %	mean ± SD	Change %
Pyriproxyfen	LC_{75} (2317.5)	27.22 ± 2.55 a	− 18.33	13.63 ± 0.34 c	− 17.64
	LC_{50} (1067.5)	22.22 ± 9.62 a	− 33.33	30.95 ± 2.38 d	87.01
Neemazal	LC_{75} (27100)	35.56 ± 2.55 a	6.69	21.96 ± 2.00 b	32.69
	LC_{50} (14600)	18.33 ± 1.67 b	− 45	30.18 ± 2.62 d	82.36
Spinetoram	LC_{75} (88.60)	22.22 ± 2.55 a	− 33.33	11.77 ± 0.25 b	− 28.88
	LC_{50} (18.37)	27.78 ± 9.25 a	− 16.65	5.80 ± 0.28 d	− 64.95
Control		33.33 ± 16.67	–	16.55 ± 0.82	–

Conc. concentration; mean ± SD followed with the letter (a): is not significantly different ($P > 0.05$), (b): significant ($P < 0.05$), (c): very significant ($P < 0.01$), (d): extremely significant ($P < 0.001$)

Table 2 ALP activity (U/L) in haemolymph and fat body of the early 5th instar larvae of *Rh. ferrugineus* after 24-h treatment with LC_{50} and LC_{75} of the tested insecticidal compounds

Conc. (ppm)		Haemolymph		Fat body	
		mean ± SD	Change %	mean ± SD	Change %
Pyriproxyfen	LC_{75} (2317.5)	18.52 ± 14.46 b	− 46.66	4.32 ± 0.53 c	−29.3
	LC_{50} (1067.5)	13.89 ± 6.94 b	− 59.99	8.93 ± 0.99 c	46.15
Neemazal	LC_{75} (27100)	39.35 ± 8.02 a	13.34	11.37 ± 0.96 d	86.09
	LC_{50} (14600)	23.15 ± 4.01 a	− 33.32	1.82 ± 0.32 d	− 70.21
Spinetoram	LC_{75} (88.60)	11.57 ± 8.02 b	− 66.68	4.13 ± 0.69 b	− 32.41
	LC_{50} (18.37)	32.41 ± 8.02 a	− 6.65	3.78 ± 0.33 c	− 38.13
Control		34.72 ± 6.94	–	6.11 ± 0.34	–

Conc., a, b, c, d: see footnote of Table 1

113.33 ± 12.58 U/L of controls. In fat body, the tested compounds enhanced or inhibited the enzyme activity. The considerable induction was determined as 178.57 ± 1.43 U/L at LC_{50} of Pyriproxyfen and as 14.69 ± 2.04 U/L at LC_{50} of Spinetoram, compared to 22.41 ± 2.56 U/L for controls.

Discussion

The use of insecticides may cause multiple sublethal effects on the enzyme activities in insect pests (Sabri, Islam, Hussain, & Saleem, 2017; Singh & Marwaha, 2000). The sublethal effects, in addition to mortality, should be in consideration for evaluating the effects of insecticides on insects (Yin, Wu, Li, Zhang, & Xu, 2008). As reported by (Hassan, H. A: *Biological and biochemical studies on the effect of some botanical extracts on cotton leaf worm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae)*, unpublished), the activities of the tissue-specific enzymes have been used to diagnose the damage of specific tissues and organs resulting from a chemical toxicity. The phosphatases and transaminases usually show the greatest diagnostic potential as a result of their roles in many functions within the insect body. In the current study, the enzyme activities in control *Rh. ferrugineus* larvae were higher in haemolymph than in fat body. In addition, ACP activity was higher in haemolymph while ALP was higher in fat body. Also, GOT activity was higher in both tissues than GPT. Results of the present investigation disagreed with those reported results of higher GPT activity than GOT in normal tissues of some silkworm *species* (Mamatha, Kanji, Hari, Cohly, & Rao, 2008; Pant & Kumar, 1979). In the light of the present study, the major effects of the tested compounds were found to be stimulatory or inhibitory on the enzyme activities in the last instar larvae of *Rh. ferrugineus*, depending on the concentration level.

Enzyme inhibition

In respect to the enzyme inhibition, LC_{50} of Neemazal reduced both ACP, ALP in haemolymph. In agreement with these results, some authors (Al-Dali, 2007; Younes et al., 2011) reported inhibitory effects of some plant oils on ALP and ACP in treated khapra beetle *Trogoderma ranarium* larvae and grasshopper *Euprepocnemis plorans* nymphs, respectively. Also, Mostafa (1993) recorded a significant reduction in ACP activity after treatment of 4th and 6th instar larvae of cotton leafworm *Spodoptera littoralis* with the neem formulation, Margason-O. Different plant extracts had been recorded as inhibitors for ACP activity in various insects, such as Azadirachtin (Azt.) against the house fly *Musca domestica* (Saeed,

Table 3 GPT activity (U/L) in haemolymph and fat body of the early 5th instar larvae of *Rh. ferrugineus* after 24-h treatment with LC_{50} and LC_{75} of the tested insecticidal compounds

Conc. (ppm)		Haemolymph		Fat body	
		mean ± SD	Change %	mean ± SD	Change %
Pyriproxyfen	LC_{75} (2317.5)	53.33 ± 10.41 c	− 40.74	6.67 ± 1.33 c	− 53.9
	LC_{50} (1067.5)	45.00 ± 8.66 c	− 50	11.90 ± 3.22 a	− 17.76
Neemazal	LC_{75} (27100)	848.33 ± 10.41 d	842.59	13.77 ± 1.20 a	− 4.84
	LC_{50} (14600)	63.33 ± 10.41 b	− 29.63	10.24 ± 1.04 a	− 29.23
Spinetoram	LC_{75} (88.60)	43.33 ± 5.77 c	− 51.86	9.41 ± 0.99 b	− 34.97
	LC_{50} (18.37)	658.33 ± 15.28 d	631.48	10.20 ± 1.63 a	− 29.51
Control		90.00 ± 8.66	–	14.47 ± 2.13	–

Conc., a, b, c, d: see footnote of Table 1

Table 4 GOT activity (U/L) in haemolymph and fat body of the early 5th instar larvae of *Rh. ferrugineus* after 24-h treatment with LC_{50} and LC_{75} of the tested insecticidal compounds

Conc. (ppm)		Haemolymph		Fat body	
		mean ± SD	Change %	mean ± SD	Change %
Pyriproxyfen	LC_{75} (2317.5)	238.33 ± 10.41 d	110.3	24.00 ± 3.33 a	7.1
	LC_{50} (1067.5)	118.33 ± 5.77 a	4.41	178.57 ± 1.43 d	696.83
Neemazal	LC_{75} (27100)	81.67 ± 7.64 b	− 27.94	24.75 ± 3.30 a	10.44
	LC_{50} (14600)	115.00 ± 5.0 a	1.47	16.27 ± 2.17 b	− 27.4
Spinetoram	LC_{75} (88.60)	36.67 ± 7.64 d	− 67.64	15.51 ± 2.23 b	− 30.79
	LC_{50} (18.37)	181.67 ± 7.64 c	60.3	14.69 ± 2.04 b	− 34.45
Control		113.33 ± 12.58	–	22.41 ± 2.56	–

Conc., a, b, c, d: see footnote of Table 1

Naqvi, & Akhtar, 1987) and *S. littoralis* (Ayyangar & Rao, 1990); Margosan-O and Jojoba oil against *M. domestica* (Ghoneim, Abdel-Ghaffar, & Tanani, 2008); *Fagonia bruguieri* extracts against the desert locust *Schistocerca gregaria* (Basiouny, Hamadah, & Tanani, 2010); and Neemazal against the same insect (Hamadah, K. S: *Some developmental, haematological and enzymatic effects of certain plant extracts on the desert locust Schistocerca gregaria (Orthoptera: Acrididae)*, unpublished). In addition to botanicals, Pyriproxyfen was found to inhibit the activities of both phosphatases, in the current study, which agreed with those reported results after treatment of the 5th instar larvae of *Bombex mori* with pyriproxyfen (Etebari, Bizhannia, Sorati, & Matindoost, 2007) as well as after treatment of 4th instar larvae *S. littoralis* with the IGRs Chlorfluazuron and Triflumuron (Abdel-Mageed, El-bokl, Khidr, & Said, 2018). As recorded in the current study, Spinetoram inhibited the activities of both ALP and ACP in haemolymph and fat body of *Rh. ferrugineus* larvae, which parallel to Spinetoram effect on both phosphatases in *S. littoralis* (Assar, Abo El-Mahasen, Dahi, & Amin, 2016; El-Barky, Dahi, & El-Sayed, 2008; El-Sheikh, 2012).

With regard to the transaminases, the present study recorded a declined level of GPT activity in haemolymph and fat body of *Rh. ferrugineus* larvae after treatment with Pyriproxyfen. This result agreed with the decreased enzyme activity in *S. littoralis* larvae after treatment with several IGRs (Abdel-Aal, 2003; Abdel-Mageed et al., 2018; Zohry, 2006). Similarly, reduction of GPT activity had been estimated in the pink bollworm *Pectinophora gossypiella*, spiny bollworm *Earias insulana*, and black cutworm *Agrotis ipsilon* by pyriproxyfen (Anan, Mohamed, & Hussein, 1993; Etebari et al., 2007). Moreover, both GOT and GPT activities had been reduced in 4th instar larvae of *Tribolium granarium* after rearing on diet treated with some plant oils (Younes et al., 2011). In the current investigation, the inhibition of both GOT and GPT activities in *Rh. ferrugineus* larvae after treatment with Spinetoram. This result was in accordance with the recorded result in *S. littoralis* larvae after treatment with the same compound (Assar et al., 2016).

Enzyme stimulation

In contrast to the previously discussed inhibition of enzyme activities, enhancement of GOT activity in haemolymph and fat body by Pyriproxyfen had been recorded in the present study on *Rh. ferrugineus* larvae. To some extent, this result was in agreement with some results for the *S. littoralis* after treatment with several IGRs (Abdel-Aal, 2003; Abdel-Mageed et al., 2018; Zohry, 2006); as well as induced GOT activity in *P. gossypiella* (Anan et al., 1993) and *M. domestica* (Assar, Abo El-Mahasen, Khalil, & Mahmoud, 2010) by pyriproxyfen. Also, GPT and GOT levels elevated in *S. littoralis* tissues after treatment with lufenuron or Chlorfluazuron (Abou-Taleb, Zahran, & Gad, 2015) and *Eurygaster integriceps* after treatment with pyriproxyfen (Zibaee, Zibaee, & Sendi, 2011). Both IGRs, Methoprene and Fenoxycarb, induced GOT and GPT activities in *B. mori* 5th instar muscle and silk gland (Mamatha et al., 2008). Also, the major effect of Neemazal was the induction of both GOT and GPT in *S. gregaria* (Hamadah, K. S: *Some developmental, haematological and enzymatic effects of certain plant extracts on the desert locust Schistocerca gregaria (Orthoptera: Acrididae)*, unpublished).

In connection with the phosphatases, Pyriproxyfen (LC_{50}) stimulated both ACP and ALP in the fat body only in the current study. This finding was in agreement with an increased ACP activity by different IGRs, such as pyriproxyfen against *E. insulana* and *P. gossypiella*, *S. littoralis*, and *A. ipsilon* (Abdel-Aal, 2003; Anan et al., 1993; El-Sheikh, 2002). Also, ALP elevated in *S. littoralis* tissues as a response to lufenuron or Chlorfluazuron treatment (Abou-Taleb et al., 2015) and *E. integriceps* by pyriproxyfen (Zibaee et al., 2011). Flufenoxuron induced ALP activity in haemolymph of 4th instar larvae of *S. littoralis* (Abdel-Mageed et al., 2018). In the present work, however, Neemazal (LC_{75}) induced the activities of both phosphatases, irrespective of the tissue type. These results were in line with the inducing effects of some

botanicals on the ACP enzyme activity in various insects, such as *P. gossypiella* and *E. insulana* (Anan et al., 1993); the corn earworm *Helicoverpa armigra* (Babu, Murugan, & Vanithakumari, 1996); *S. littoralis* (Abdel-Aal, 2003) and *A. ipsilon* (El-Sheikh, 2002; Hassan, H. A: *Biological and biochemical studies on the effect of some botanical extracts on cotton leaf worm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae)*, unpublished). In our study, ALP activity increased to a great extent, in accordance with those reported results of enhanced ALP activity in different insects by various botanicals, such as *Pieris rapae* larvae by methanolic extract of *Silybium marianum* (Hasheminia, Sendim, Jahromi, & Moharramipour, 2013); *S. gregaria* by different extracts of *Nigella sativa* extracts (Ghoneim, Hamadah, & El-Hela, 2016b) and *A. aegypti* larvae (Koodalingam, Deepalakshmi, Ammu, & Rajalakshmi, 2014).

Diverse enzyme activities

As clearly shown in the present study on *Rh. ferrugineus* larvae, the tested insecticidal compounds diversely affected the enzymatic activities depending on the concentration level and on the enzyme itself. As for example, treatment with pyriproxyfen (LC_{50}) stimulated the ALP activity but pyriproxyfen (LC_{75}) inhibited it in fat body. To a great extent, this finding corroborated with those reported results using the same IGR against the flesh fly *Parasarcophaga argyrostoma* (El-Gindi, 2000). On the other hand, Neemazal exhibited similar effect on activities of all enzymes in haemolymph with no exception. To some extent, this result agreed with the results for GOT and ACP activities in fat body of *S. gregaria* using *Nigella sativa* extracts (Ghoneim et al., 2016a, 2016b).

In the light of our results, the inhibition of detoxifying enzymes, including ACP and ALP, indicated that these enzymes play no role in the detoxification against the tested compounds and may induce the susceptibility of *Rh. ferrugineus* larvae against these compounds (Abd-Elaziz & El-Sayed, 2009). On the contrary, increasing activities of these enzymes denoted an increasing capability of this weevil to detoxify the same compounds (Sharifi, Kosari, Zibaee, & Sendi, 2013). However, ineffectiveness of a compound for control an insect pest can be attributed to the increased levels of enzymatic detoxification (Biddinger, Hull, & Mcpheron, 1996). In general, the enzymatic alterations in the current study indicating that the tested insecticidal compounds can be affecting the synthesis or functional levels of these enzymes directly or indirectly by altering the cytomorphology of the cells (Nath, 2000).

Conclusions

The present study, the tested compounds (Pyriproxyfen, Neemazal, and Spinetoram) disturbed the activities of enzymes ACP, ALP, GOT, GPT which play different roles in the insect body. This means that different physiological functions, such as growth, development, reproduction, in the insect body have been disrupted. Disturbance of the enzyme activities can be understood as sublethal effects of the tested compounds but ultimately lead to death. As a conclusion, the most potent compound for inhibiting the enzyme activities was Spinetoram.

Abbreviations

A. ipsilon: *Agrotis ipsilon*; ACP: Acid phosphatase; ALP: Alkaline phosphatase; *B. mori*: *Bombex morai*; *E. insulana*: *Erias insulana*; *E. integriceps*: *Eurygaster integriceps*; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamine pyruvic transaminase; *M. domestica*: *Musca domestica*; *P. gossypiella*: *Pectinophera gossypiella*; *Rh. ferrugineus*: *Rhynchophorus ferrugineus*; RPW: Red palm weevil; *S. gregaria*: *Schistocerca gregaria*; *S. littoralis*: *Spodoptera littoralis*

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All data generated or analyzed during this study are included in this published article.

Authors' contributions

The author read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The author declares that he has no competing interests.

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