Bulletin of the National Research Centre

RESEARCH Open Access

Effect of sublethal doses of some insecticides and their role on detoxication enzymes and protein-content of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

Seham Mansour Ismail

Abstract

Background: The indiscriminating and intensive use of insecticides to control the cotton leafworm (CLW), *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), usually induces high levels of resistance. The insecticides are the principal method for controlling this pest because of its critical role in reducing insects when the economic threshold (ETL); therefore, the effectiveness of these insecticides should be maintained. So, the target of the present work was directed to focus on studying the change in the activities of some important enzymes as a result of sublethal treatment concentrations (viz. LC_{25} values) of tested new insecticides (profenofos, cyfluthrin, emamectin benzoate, lufenuron, and spinetoram). The expected results could offer better understanding and more specific information about the resistance development in field populations of CLW because resistance is a significant challenge to pest control workers and these results may contribute to making the right decision at the right time.

Materials and methods: Bioassays were performed on fourth instar larvae of *S. littoralis* field populations compared with the laboratory strain to assess the activity of the emamectin benzoate, lufenuron, and spinetoram by LC_{25} and study the biochemical activities of some detoxification enzymes, like acetylcholinesterase (AChE), glutathione S-transferases (GST), alkaline phosphatase (ALP), and acid phosphatase (ACP) in fourth larval instar which was treated with LC_{25}; protein content is also determined.

(Continued on next page)

Correspondence: Seham.Ismail@arc.sci.eg
Insect Population Toxicology Department, Central Agricultural Pesticides Laboratory, Agriculture Research Center, Dokki, Giza, Egypt

Springer Open

© The Author(s). 2020 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

(Continued from previous page)

Results: The emamectin benzoate was the most toxic compound followed by lufenuron, cyfluthrin, spinetoram, and profenofos with the corresponding LC_{50} values of 0.05, 49.18, 70.99, 130.26, and 156.78 ppm respectively. The results showed that all the tested insecticides at LC_{25} value gave a slight inhibition of the acetylcholinesterase (AChE) activity, except profenofos was the most potent one. The activity of glutathione S-transferase (GST) of larvae treated with spinetoram has significantly increased, whereas the enzyme activity was significantly inhibited following cyfluthrin and non-significantly inhibited following profenofos, lufenuron, and emamectin benzoate application. There were no significant differences between treated and untreated larvae in ACP activity. In contrast, the alkaline phosphatase (ALP) activity of larvae treated with tested insecticides significantly increased, while the activity was inhibited following cyfluthrin application. On the other hand, spinetoram, emamectin benzoate, and lufenuron exhibited significant increment in the protein content, whereas there was no significant effect following either cyfluthrin or profenofos treatments.

Conclusions: In summary, the present results suggest that not only the lethal effects but also the sublethal effects of newly tested insecticides could have a negative influence on the dynamics of CLW; thus, these sublethal effects can be integrated into pest control to reduce the overuse and misuse of insecticides. This effect appears reduction in the activity of detoxification enzymes, resulting in response to the tested insecticide by the lowest concentration of (sublethal doses). Also, the inhibition of detoxification enzymes, which represents defensive reactions in insects, is playing important role in reducing resistance in *S. littoralis*, which is one of the most dangerous pests of all agricultural crops in the world. Therefore, these results were valuable for the practical use of these insecticides in IPM programs.

Keywords: Acetylcholinesterase, Acid phosphatase, Alkaline phosphatase, Glutathione S-transferases, Protein content, *Spodoptera littoralis*

Background

During the last few decades, many species of insects have acquired resistance to insecticides. This considered resistance is proved to be one of the major obstacles in the successful control of insects and the biggest challenge faced by the pesticide research today due to the critical role of insecticide in reducing insect when the economic threshold (ETL) is exceeded, and insecticides are currently the principal method for controlling the Egyptian cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), in Egypt and will likely continue to be used until more biologically based management systems could be developed (Subramanian and Shankarganesh 2016). Therefore, it was necessary to study the effect of some new insecticides that appeared in the Egyptian market on *S. littoralis*, in terms of their toxicity and their effect on enzymes that plays a defensive role in changing the response to insecticide in *S. littoralis* becomes extremely tolerant of the action of insecticides because of the intensive treatments of these insecticides. Therefore, new insecticides with modes of action that differed from conventional insecticide are highly desirable in integrated pest management (IPM) programs among these insecticides are insect growth regulators (IGRs), avermectin, and spinosyn insecticide group (Subramanian and Shankarganesh, 2016; Wei et al., 2018). Different mechanisms of resistance to organophosphate and pyrethroid insecticides have been identified in *S. littoralis*, including enhanced metabolism, nerve insensitivity, reduced penetration, and target site insensitivity; it is caused by defensive enzymes in the insect (Rong et al., 2017). Among the defensive enzymes are glutathione S-transferases (GST) and phosphatases (APs). GST is an enzyme widespread in both prokaryotic and eukaryotic cells and catalyzes the glutathione conjugation reaction with reduced glutathione (GSH) (Armstrong, 1997; Listowsky et al., 1998).

The present work aims to evaluate the sub-lethal effects of the five compounds (profenofos, cyfluthrin. emamectin benzoate, lufenuron, and spinetoram) on the fourth instar larvae of *S. littoralis* field populations compared with the laboratory strain. Also, these compounds may manipulate the physical and biochemical processes of the insect by influencing relative enzymes. Therefore, the activities of acetylcholinesterase (AChE), glutathione S-transferases (GST), alkaline phosphatase (ALP), acid phosphatase (ACP), and protein content were determined in surviving larvae after treatment.

Materials and methods

Toxicity studies

Test insects

Laboratory strain Laboratory strain (L-strain) of *S. littoralis* was obtained from central laboratory of pesticides, Agricultural Research Center (ARC) Cairo, Egypt, and reared on castor oil leaves under laboratory conditions (27 ± 2 °C and 65 ± 5% RH) for several years, according to Eldefrawi et al. (1964).

Field strain Field strain (F-strain) of *S. littoralis* used in this study was obtained from the cotton field at Vaccus

District, El-Sharkia Governorate (Egypt) during the season 2019. Samples were brought to the laboratory as egg masses. The hatched larvae were reared on castor oil leaves under the same conditions.

Test insecticides

Profenofos (Selecron® 72% EC); lufenuron (Match® 5% EC), and emamectin benzoate (Admire® 20% SC) were obtained from Syngenta Co; cyfluthrin (Baythroid® 5% EC) was obtained from Sumitomo Chemical Co, and spinetoram (Radiant® 12% SC) was obtained from Dow Agro Sciences Co.

Bioassays

Bioassays were performed on the 4th instars larvae both L4- and F4-strains to assess the activity of the five-mentioned insecticides. A series of aqueous concentrations for each insecticide were prepared using the commercial formulations. The leaf dipping technique was used to determine the median lethal concentration (LC_{50}) values; fresh castor oil leaves were cut into discs (2 cm^2). Each disc was dipped for 30 s in one of the prepared concentrations. The treated leaves had discs dried under laboratory conditions before being offered to the larvae. Ten larvae (40–50 mg/larvae), replicated 3× were used for each concentration. Larvae were fed on leaves immersed in only water as a control. Newly moulted 4th larval instars for L- and F-strains were fed on the treated leaves in a glass jar covered with muslin for 24 h with the treated discs insecticides. Another untreated one replaced the treated leaves. Mortality percentages were recorded after 24 h of treatment for profenofos and cyfluthrin and after 72 h for lufenuron, emamectin benzoate, and spinetoram. The corrected mortality was calculated by using Abbott's (1925) formula. Data were subjected to Probit analysis, as described by Finney (1971). Resistance ratio (RR) as field/laboratory (F/L) was calculated by dividing the LC_{50} value of the F-strain over that of the L-strain.

Biochemical studies

Preparation of samples

The survived larvae after 24 h of treatment were fed on discs treated with the corresponding LC_{25} values concentrations of the tested insecticides. The LC_{25} values were 0.332, 4.732, 7.75, 12.09, and 15.35 ppm for emamectin benzoate, lufenuron, cyfluthrin, profenofos, and spinetoram respectively. The collected midguts of the L4 were rinsed and homogenized in distilled water (DW) using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 min. Homogenates were centrifuged at 6000 rpm for 10 min at 5 °C; the supernatant was used as an enzyme for AChE, GST, ALP, ACP and the determination of protein content.

Enzyme assay

Acetylcholinesterase (AChE) (EC 3.1.1.7) activity was determined according to the method of Ellman et al. (1961) with slight modification using acetylthiocholine iodide as substrate, while glutathione S-transferases (GST) (EC 2.5.1.18) activity was measured by the simplified procedure of Vessey and Boyer (1984) using 4-chloro-1,3-dinitrobenzene (CDNB) as substrate, both alkaline phosphatase (ALP) (EC 3.1.3.1) and acid phosphatase (ACP) (EC 3.1.3.2) were determined according to Bessey et al. (1946) using sod- ρ-nitrophenyl phosphate as a substrate. Protein content was measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Statistical analysis

All the quantitative estimation of toxicity and biochemical parameters were based on three replicated, and the values were expressed as mean ± SE. The data were statistically analyzed separately for each experiment and were subjected to ANOVA using SPSS 12.0 software (Statistical Package for Social Sciences, USA). Mean values were compared using Duncan's multiple range test (1955).

Table 1 Toxicity of the tested compounds against 4th larval instar of *S. littoralis*

Insecticides	Strains	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits		Slope (mean ± SE)	RR*
				Lower	Upper		
Profenofos	L	0.323	1.28	0.124	0.551	1.13 ± 0.19	122.48
	F	12.09	156.78	32.08	496.45	0.61 ± 0.22	
Cyfluthrin	L	0.282	1.04	0.656	1.455	1.19 ± 0.19	68.26
	F	7.75	70.99	19.17	298.70	0.70 ± 0.22	
Lufenuron	L	2.62	5.22	4.208	8.07	1.62 ± 0.39	9.42
	F	4.732	49.18	2.512	16.54	0.66 ± 0.20	
Emamectin benzoate	L	0.04	0.117	0.092	0.146	0.96 ± 0.20	5.32
	F	0.332	0.623	0.346	0.957	2.47 ± 1.23	
Spinetoram	L	11.50	36.50	16.96	283.96	1.34 ± 0.33	3.57
	F	15.35	130.26	29.46	297.61	0.73 ± 0.24	

RR* Resistance ratio

Table 2 In vivo effect of the tested compounds on the activity of AChE and GST of *S. littoralis* larvae

Treatment	AChE[1]		GST[2]	
	Activity (μmole)	% of control	Activity (nmole)	% of control
Control	1.832[d]	–	35.64[b]	–
Profenofos	0.389[a]	21.28[a]	29.69[b]	83.31[b]
Cyfluthrin	1.065[b]	58.13[b]	19.77[a]	55.47[a]
Lufenuron	1.591[c]	86.84[c]	82.15[c]	230.50[c]
Emamectin benzoate	1.635[c]	89.25[c]	92.39[d]	259.23[d]
Spinetoram	1.651[d]	90.12[c]	98.41[d]	276.14[e]

[1]Activity is expressed as μmole acetylthiocholine hydrolyzed/min. mg protein
[2]Activity is expressed as nmole CDNB conjugated formed/min. mg protein
Means in the same column followed by the same letters are not significantly at $P = 0.05$

Results

Toxicity study

Results of bioassay of the tested insecticides showed that the toxicity of these compounds could be arranged ascendingly according to LC_{50} values as follows: emamectin benzoate (0.623 ppm), lufenuron (49.18 ppm), cyfluthrin (70.99 ppm), spinetoram (130.26 ppm), and profenofos (156.78 ppm), for F-strain, while the L-strain values, 0.117, 5.22, 1.04, 36.50 and 1.28 ppm for the same tested insecticides respectively (Table 1). Also, data showed that the F-strain exerts high resistance levels towards profenofos and cyfluthrin resistance ratios were 122.48 and 68.26, respectively. The 4th instar larvae of the F-strain showed no resistance to emamectin benzoate and spinetoram, and resistance ratios 9.42 towards lufenuron.

Biochemical studies

Effect on AChE The in vivo effect of tested insecticides on the activity of AChE in larvae of *S. littoralis* is presented in Table 2. It was found that all the tested insecticides slightly inhibited the activities of AChE except that the profenofos was the only one most inhibitor.

Effect on GST Results listed in Table 2 show that the cyfluthrin inhibited the activity of GST in *S. littoralis* by 55.47, while spinetoram significantly induced the enzyme activity by 276.14% of control.

Phosphatases ALP activity in larvae treated with profenofos, lufenuron, emamectin benzoate, or spinetoram had significantly increased. However, its activity was significantly dropped following the treatment with cyfluthrin (Table 3). Moreover, Table 3 showed that there were no significant differences in the activity of ACP between treated and untreated larvae.

Effect on total protein Results in Table 4 showed that there was a significant enhancement in the protein content of the larvae treated with spinetoram. However, cyfluthrin and profenofos treatments were similar to those of the control.

Discussion

Insecticide resistance in Lepidopteran is a major concern throughout the world because several species of it have been reported to have developed resistance to several classes of insecticides Wei et al. (2018). The present study revealed high levels of resistance to profenofos and cyfluthrin in the tested field population; at the same time, emamectin benzoate, spinetoram, and lufenuron showed low levels of resistance. The efficiency of different tested compounds in the control of the L4 and F4 of the *S. littoralis* varied tremendously according to the different modes of action for the tested compounds. These results agreed with those obtained by Ismail (2008), who found that the *S. littoralis* larvae collected from a cotton field that was heavily sprayed with conventional insecticides showed strong resistance to OPs and pyrethroids. In contrast, the LC_{50} values of the F-strains were 120×

Table 3 In vivo effect of the tested compounds on the activity of ACP and ALP of *S. littoralis* larvae

Treatment	ALP		ACP	
	Activity (U/L)	% of control	Activity (U/L)	% of control
Control	30.26[b]	–	12.79[c]	–
Profenofos	37.48[b]	123.86[b]	12.56[c]	98.20[b]
Cyfluthrin	20.88[a]	69.00[a]	11.40[b]	89.13[a]
Lufenuron	105.21[c]	347.69[c]	10.42[a]	81.47[a]
Emamectin benzoate	122.12[d]	402.57[d]	10.31[a]	80.61[a]
Spinetoram	138.11[e]	456.41[e]	10.24[a]	80.06[a]

Activity of ALP or ACP is expressed as unit/liter supernatant
Means in the same column followed by the same letters are not significantly at $P = 0.05$

Table 4 In vivo effect of the tested compounds on the activity of protein content of *S. littoralis* larvae

Treatment	Protein content[1]	
	Activity (mg/g)	% of control
Control	16.22[a]	–
Profenofos	15.22[a]	93.83[a]
Cyfluthrin	17.88[a]	110.23[b]
Lufenuron	25.24[b]	155.61[c]
Emamectin benzoate	25.99[bc]	160.23[d]
Spinetoram	26.35[c]	162.45[d]

[1]Protein content is expressed as mg/g tissue
Means in the same column followed by the same letters are not significantly at $P = 0.05$

and 102× more resistant than the L-strain to the OP chlorpyrifos and the pyrethroid cypermethrin, respectively. Also, our results are similar to El-Sheikh (2015) reported that the F-strain showed low resistance ratios to emamectin benzoate, lufenuron, and spinosad. Meanwhile, fenvalerate- and cypermethrin-resistant larvae of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) showed higher activities of esterases, APs, and methyl paraoxon hydrolase, compared with susceptible larvae (Oppenoorth, 1985).

Since many toxicants show secondary actions at high concentrations, the sublethal dose (LC_{25}) was used in this study to measure the biochemical changes in the L4 larvae of *S. littoralis* following treatment with five compounds mentioned previously. Current results show that the profenofos recorded a significant decrease in the activity of AChE; this inhibition may be occurred as a result of the blocking of the action potential of the nervous system caused by the toxic effect of larvae treated with profenofos because organophosphorus insecticides are neurotoxic by acting as inhibitors of neuronal cholinesterase activity. Generally, this indicated that the number of active sites on the AChE of the larvae was increased in the F-strain; this explains the low susceptibility of F-strain for profenofos, our results agreement with Richardson et al. (2001); El-Sheikh (2012); Abd El-Aziz and Shaurub (2014). On the contrary, GST activity was increased in treated larvae; this enzyme plays a role in detoxification mechanism in insects; therefore, may be an overproduction led to the toxic effect of treated larvae. This results parallel with Motoyama (1978), Oppenoorth et al. (1979); Kristensen (2005), they reported that GST plays a physiological role in detoxication and elimination of toxic compounds, and therefore, the increment of GST activity may protect against toxic compounds; also, Wang et al. (2010) reported that GST was higher in treated larvae than their control. On the other hand, the present study showed significantly increased ALP activity, and decreased ACP activity; the reaction on ALP and ACP activities might be due to tissue damage, where the enhanced activity could be related to the influence of glucocorticoids or could be released from ruptured cells due to the effect of pesticide (Abou-Donia et al., 1986); also, Trowsdale et al. (1990) reported that the increment of ACP activity seems to result from enhanced enzyme turnover under pesticide stress. In contrast, the reaction of its activity may be related to leakage of the enzyme into the extracellular compartment.

The total protein is a major component necessary for an organism to develop, grow, and perform its vital activities; thus, the effect on protein leads to a cell disorder. From this study, a significant increase in the total protein content was detected in treated larvae with tested compounds; thus, this effect leads to a cell disorder. Our result agreement with Muthusamy and Ramkumar (2011) reported that changing the behavioral patterns associated with enzymes and/or the exchange of chemical information can be due to alteration in total protein reserves of the larvae tissues which could be partly due to the stress of the tested compounds of an imbalance between the rate of protein synthesis and the rate of biodegradation.

Conclusion

The Egyptian cotton leafworm, *Spodoptera littoralis*, a destructive insect pest for a wide range of host plants. Because of the extensive and continuous use of traditional insecticides, this led to the development of resistance in this insect, so there is a constant demand to use new control agents. Therefore, from this study, it could be recommended to use emamectin benzoate, lufenuron, and spinetoram because these compounds have modes of action that differed from conventional insecticides like profenofos and cyfluthrin. These results are valuable for the practical use of these compounds in Integrated Pest Management (IPM) programs to reduce the phenomenon of resistance in this insect pest.

Author's contributions
The author contributed to the production and writing of the manuscript. The author(s) read and approved the final manuscript.

Funding
There was no funding for this work.

Availability of data and materials
All data generated during this study are included in this published article.

Ethics approval and consent to participate
The manuscript does not contain any studies involving human participants, human data or human tissue.

Consent for publication
Not applicable.

Competing interests
The author declares that there are no competing interests.

Received: 23 October 2019 Accepted: 27 February 2020
Published online: 05 March 2020

References

Abbott WS (1925) A method for computing the effectiveness of an insecticide. J. Econ. Entomol *18*:265–267
Abd El-Aziz NM, Shaurub HE (2014) Sub-lethal effects of spinetoram on the activities of some detoxifying enzymes in the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). African Entomol *22*:136–143
Abou-Donia MB, Abdo KM, Timmonsand PR, Proctor JE (1986) Brain acetylcholinesterase, acid phosphatase and 2,3-cyclic nucleotide 3-phosphohydrolase and plasma butrylcholinesterase activities in hens treated with a single dermal neurotoxic dose of S,S,S-tri-n-butyl phosphorotrithioate. Toxicol. Appl. Pharmacol *82*:461–473
Armstrong RN (1997) Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem. Res. Toxicol *10*:2–18
Bessey DA, Lowery OH, Brock MI (1946) A method for the rapid determination of Alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem *164*: 321–339
Duncan DB (1955) Multiple rang and multiple F test. Biometerics. *11*:1–42
Eldefrawi ME, Toppozada A, Mansour N, Zeid M (1964) Toxicological studies on the Egyption cotton leafworm, *Prodenia litura*. I. Susceptibility to different larval instars of *Prodenia* to insecticides. J. Econ. Entomol *57*:591–593
Ellman GI, Courtncy DK, Andres V, Featherstone MR (1961) A new and rapid Egyption colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol *7*:88–95
El-Sheikh EA (2015) Comparative toxicity and sublethal effects of emamectin benzoate, lufenuron and spinosad on *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae). Crop Protect *67*:228–234
El-Sheikh TAA (2012) Comparative toxicity and biochemistry of organophosphates and pyrethroid compounds on both laboratory and field strain of the cotton leafworm *Spodoptera littoralis* (Boisd.). Biol. Sci *4*:141–151
Finney DJ (1971) Probit analysis, 3rd edn. Cambridge Univ. Press, Cambridge, England., p 318
Ismail SM (2008) Biochemical studies of Na^+,K^+-ATPase and acetylcholinesterase sensitivity to phenothrin and thiodicarb among different Egyptian field populations of *Spodoptera littoralis*. Alex. Sic. Exchange J *29*:26–35
Kristensen M (2005) Glutathione S-transferase and insecticide resistance in laboratory strains and field populations of *Musca domestica*. J. Econ. Entomol *98*:1341–1348
Listowsky YI, Abramovitz M, Homma H, Niitsu Y (1998) Intracellular binding and transport of hormones and xenobiotics by glutathione S-transferase. Drug Metab. Rev *19*:305–318
Lowry OH, Rosebrough JN, Farry LAL, Randall JR (1951) Protein measurements with folin phenol reagent. J. Bio. Chem. *193*:265–271
Motoyama N, Kulkarni AP, Hodgson E, Dauterman WC (1978) Endogenous inhibitors of glutathione S-transferase in house fly. Pestic. Biochem. Physiol *9*: 225
Muthusamy SK, Ramkumar R (2011) Pesticide detoxifying mechanism in field population of *Spodoptera litura* (Lepidoptera: Noctuidae) from South India. Biol. Sci *3*:51–57
Oppenoorth FG (1985) In: KerKut GA, Gilbert LI (eds) Biochemistry and genetics of insecticide resistance, In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol 12. Pergamon Press, UK, pp 731–773
Oppenoorth FG, K. van DorPas TJ, Houx NWH (1979) Glutathione S-Transferase and hydrolytic activity in a tetrachlovinphos resistance. Pestic. Biochem. Physiol *11*:167–188
Richardson JR, Chambers HW, Chambers JE (2001) Analysis of the additivity of in vitro inhibition of cholinesterase by mixtures of chlorpyrifos-oxon and azinophos-methyl-oxon. Toxicol. Appl. Pharmacol *172*:128–139
Rong Y, Dan-Dan Z, Shuai Z, Li-Qi Z, Xin W, Cong-Fen G, Shun-Fan W (2017) Monitoring and mechanisms of insecticide resistance in *Chilo suppressalis* (Lepidoptera: Crambidae), with special reference to diamides. Pest Management Sci *73*:1169–1178
Subramanian, S. and Shankarganesh, K. 2016. Insect hormones (as pesticides). Ecofriendly Pest Management for Food Security, 613-650
Trowsdale J, Marttin D, Bicknell D, Campbell I (1990) Alkaline phosphatases. Biochem. Soc. Trans *18*:178–180
Vessey DA, Boyer TD (1984) Differential activation and inhibition of different forms of rat liver glutathione-s-transferase by the herbicides 2,4-dichlorophenoxy acetate (2,4-D) and 2,4,5-trichlorophenoxy acetate (2,4,5-T). Toxicol. App. Pharmacol *73*:492–499
Wang YK, Yong Z, Yan WH, Ming XX, Xian LT (2010) Influences of three diets on susceptibility of selected insecticides and activities of detoxification esterases of *Helicoverpa assulta* (Lepidoptera: Noctuidae). Pest Biochem. Physiol *96*:51–55
Wei J, Zhang L, Yang S, Xie B, An S, Liang G (2018) Assessment of the lethal and sublethal effects by spinetoram on cotton bollworm. PloS One *13*:1–11

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

► Convenient online submission
► Rigorous peer review
► Open access: articles freely available online
► High visibility within the field
► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com