Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Enhancing toxicity of pyrethroids by oxidase and esterase inhibitors in *Spodoptera littoralis* (Boisd.) larvae

Seham Mansour Ismail*

Insect Population Toxicology Department, Central Agricultural Pesticides Laboratory, Agriculture Research Center, Giza, Egypt

DOI: 10.24425/jppr.2024.149162

Received: May 22, 2023
Accepted: October 18, 2023
Online publication: April 04, 2024

*Corresponding address:
Seham.Ismail@arc.Sci.eg

Responsible Editor:
Paweł Sienkiewicz

Abstract

Although pyrethroids are increasingly being used to control a number of agricultural insect pests, especially the cotton leafworm, *Spodoptera littoralis* (Boisd.), pyrethroid resistance is a major obstacle limiting effective control. With the aim of maintaining the effectiveness of pyrethroids in managing pests, a study was undertaken to evaluated the effectiveness of oxidase and esterase inhibitors for synergizing pyrethroids in *S. littoralis* larvae. Compared with the insecticide-susceptible strain (L-SS) of *S. littoralis*, the resistance ratio (RR) in the field population (F-RS) was 271.43–fold to cypermethrin. The use of profenofos as an esterase inhibitor significantly increased larval susceptibility to cypermethrin in the F-RS strain, with a synergy ratio (SR) of up to 192.57–fold. Significant inhibition of esterase by profenofos in the F-RS strain was found *in vivo*. Also, piperonyl butoxide (PB) as an oxidase inhibitor had slight effect of cypermethrin toxicity, so its addition is not a solution for pyrethroid resistance. Thus, modifying the toxicity of cypermethrin by mixing it with organophosphorus compounds (OPs) increased its toxicity and decreased the population of *S. littoralis*, which is a successful strategy for managing pyrethroid resistance.

Keywords: pyrethroid resistance, resistance management, synergism

Introduction

Cotton leafworm, *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae), known as cotton leafworm is a polyphagous and widely distributed pest of many economic crops (e.g., cotton, clover, corn and vegetables). *S. littoralis* larvae is considered the most destructive stage, with more than 80 species attacking about 40 families of several important agricultural crops, causing significant economic losses, including reducing the quantity and quality of the crop by affecting plant growth and increasing the risk of entry of pathogens (CABI 2019; Ismail 2022). Cotton leafworm management has primarily relied on synthetic insecticides, however, it has developed resistance to most classes of conventional insecticides, including organophosphates, carbamates, and synthetic pyrethroids, as a result of extensive field application, this represents a major threat to successful control of this pest (Ismail 2022).

Pyrethroids have been the most widely used insecticides for nearly 30 years to protect crops from pest infestation, and they are known to affect sodium conductance. Extensive use of insecticides has led to the development of resistance in >440 insect species worldwide, including more than 50 that show resistance to pyrethroids that have resulted in a loss of efficacy (Carvalho *et al.* 2013; Ismail and Morshedy 2017). In addition, given the difficulty associated with developing new insecticide molecules that are effective in managing resistance at the field level, there is a need to maintain the effectiveness of currently used pesticides, including pyrethroids.

Spodoptera littoralis populations may have multiple mechanisms that confer resistance to pyrethroid, including increased metabolic detoxification (Ishaaya *et al.* 1983; Gunning *et al.* 1999; Ismail 2020), delayed cuticular penetration (DeVries and Georghiou 1981),

or decreased sensitivity of the voltage-dependent sodium channels of the nervous system (Pauron *et al.* 1989). Given the array of mechanisms by which pyrethroid insecticides may fail to control *S. littoralis* infestations, improving the efficacy of pyrethroids is required for their control. Ishaaya *et al.* (1983) found that enzyme inhibitors, piperonyl butoxide (PB)-oxidase inhibitors and provinofos-esterase inhibitor were effective in controlling field-collected *S. littoralis* that were resistant to pyrethroid. Young *et al.* (2006), Romero *et al.* (2009), Gonzalez-Morales and Romero (2019) showed that pre-application of PB reduced deltamethrin, cypermethrin and lambda-cyhalothrin resistance in field strains of *Helicoverp armigera* (Hübner), *Bemisia tabaci* (Gennadius) and *Cimex lectularius* (L.). Martin *et al.* (2003), Ahmed *et al.* (2009) showed that pre-treatment with OP insecticides significantly increased pyrethroid toxicity in field-collected strains of *H. armigera* and *S. litura* (Fab.). In addition, pyrethroids are often applied to insect populations that are also exposed to OP compounds, creating synergy between them and enhancing pyrethroid efficacy. However, studies on the effects of PB and OPs with pyrethroids as enzyme inhibitors in *S. littoralis* have been very limited. Hence the objectives of this study were to determine the resistance level to cypermethrin, the synergistic effects of two enzyme inhibitors on insecticide-susceptible (L-SS) and field population (F-RS), and the effect of enzyme inhibitors on esterase activity of *S. littoralis* larvae.

Materials and Methods

Collection and mass rearing of *Spodoptera littoralis*

Two *S. littoralis* strains were used for this study: 1. Laboratory susceptible strain (L-SS) was obtained from the Central Agricultural Pesticides Laboratory, Egypt, used as a reference insecticide-susceptible strain. This strain has been maintained in the laboratory for over 15 years, with no history of insecticides exposure; 2. In 2022, egg masses were collected from cotton fields in El Senbellawein City (30°47′47.868″N, 31°27′39.4704″E), El-Dakahlia Governorate, Egypt, used as a field population (F-RS).

This population (F-RS) has a history of exposure to organophosphate (chlorpyrifos or profenofos), a pyrethroid (deltamethrin, cypermethrin or alpha-cyahlothrin) and one of the several newer insecticides (spinosad, fipronil or chlorfenapyr) treatments prior to its collection. Populations were reared in the laboratory for one generation to obtain sufficient numbers of insects for bioassays. All life stages of both strains were maintained in a climate-controlled room (25 ± 2°C and 65 ±5% relative humidity with a 16 : 8 h light: dark photoperiod). Larvae were fed with castor bean leaves (*Ricinus communis* L.).

Used chemicals

The cypermethrin and profenofos (≈98 and 99.2% technical grade, respectively) in this study were supplied by Chemical Service, West Chester, USA.

Piperonyl butoxide (PB, 90% technical grade) was supplied by Aldrich Chemical Company, Inc.). The solvent acetone (analytical grade) and the surfactant Triton X-100 were purchased from Fisher Scientific, Loughborough, UK. Substrates and reagents used in enzyme assays were procured from Sigma-Aldrich (St. Louis, USA).

Larvae treatment

The effect of cypermethrin on the *S. littoralis* (30 ± 2.5 mg; 3rd instar larvae) was evaluated using topical application. After the preparation of a stock solution in acetone, six concentrations (0.5, 1.0, 2.0, 5.0, 10, and 20 $\mu g \cdot l^{-1}$) were prepared. Larvae of each population (L-SS and F-RS) were treated with 1 µl of the insecticide solution in acetone, administered topically to the dorsal region of the abdomen using a hand microapplicator (Burkard Manufacturing, Rickmansworth, UK). In total, five replicates were performed for each dose (n = 50). Control groups received acetone alone. Treated larvae were immediately placed in the chamber under conditions identical to those used for rearing. Larvae were fed untreated castor bean leaves and mortality was determined 48 h later. Larvae, which did not respond to fine brush touching, were judged to be dead.

Effect of inhibitors on cypermethrin toxicity

The toxicity of the cypermethrin in mixtures was evaluated in the presence of synergist, PB-oxidase inhibitor and profenofos-esterase inhibitor. A similar study was conducted with topical application of different mixtures in order to determine the synergistic effects on cypermethrin toxicity. In the combination assays, the LD_{50} dose of cypermethrin 1.05 and 285 µg per larva were used for the L-SS and F-RS populations, respectively. Nonlethal doses of PB (0.1 µg per larva), and profenofos (0.0032 µg per larva) were established in preliminary bioassays. Combinations of cypermethrin with profenofos or PB were examined in two formats: 1. Cypermethrin/PB, the mixture of insecticide and synergist was combined at 1 : 1 (V : V) ratio; 2. Cypermethrin/profenofos, combination prepared at ratio of 1 : 1 (V : V). In parallel assays the amount of profenofos or PB in these assays caused no more than 5% mortality. Treated larvae of each strain (L-SS and

F-RS) were placed in groups of ten larvae, with five replications in each mixture treatment. Post-treatment procedure was as described above, and mortality was determined after 2 days.

Crude extract preparation

Third instar larvae 24 h after treatment with each mixture treatment tested were used for enzyme extraction (*in vivo*). Samples were prepared from 10 larvae of each strain (whole body), pooled and homogenized in 0.5 ml of ice-cold buffer (0.1 M phosphate buffer, pH 7.0 and 1% Triton X-100). The homogenate was centrifuged at 4°C, 10,000 rpm for 15 min, and the supernatant was used as an enzyme source in all cases.

Esterase assay and protein determination

Total esterase activity was determined spectrophotometrically by hydrolysis of p-nitrophenylacetate (pNPA) substrate (van Asperen 1962). The incubation mixture contained pNPA (30 mM, 200 µl), phosphate buffer (0.1 M, pH 7.0), and tissue homogenate (20 µl). The reaction rate was determined for 3 min at 25°C by monitoring the optical density of the incubation mixture at 450 nm. The amount that catalyzed the hydrolysis of pNPA per minute at room temperature was used for calculating specific activity, which was expressed as nmol · mg · min^{-1} (van Asperen 1962). The average of three measurements was used to calculate the value of each activity.

Protein concentration was measured at 595 nm following the Bradford (1976) method, using bovine serum albumin as standard.

Data analysis

The probit analysis (POLO-PC Program, LeOra Software LLC, Petaluma, CA, USA) was used to calculate LD_{50} values for the insecticide cypermethrin in both population (LeOra 2003). RR = LD_{50} of insecticide in field population (F-RS)/LD_{50} of insecticide in susceptible population (L-SS). SR = LD_{50} without synergist/ LD_{50} with synergist (PB or profenofos). Enzyme data were analyzed using the analysis of variance (ANOVA), followed by Tukey's test at $p < 0.05$ significant level using SAS program (SAS Institute 2004).

Results

Cypermethrin resistance level in field-collected larvae of *Spodoptera littoralis*

The toxicity of cypermethrin to field population (F-RS) and laboratory susceptible population (L-SS) shown in Table 1. After 48 h of topical treatment, the LD_{50} was 1.05 and 285 µg per larva, respectively for L-SS and F-RS populations. The F-RS population was 271.43-fold resistant to cypermethrin in comparison to L-SS population.

Toxicity of cypermethrin alone and in combination against susceptible and field populations of *Spodoptera littoralis* larvae

In synergy tests, a decrease in resistance was observed in the field population (F-RS) (Table 2). The inhibitor piperonyl butoxide (PB) or profenofos increased larval

Table 1. Resistance to cypermethrin in the field-collected population (F-RS) of *Spodoptera littoralis*

Population	Slope (± SE)	LD_{50} 95% confidence limit [µg · $larva^{-1}$]*	χ^2 [df]	*p*-value	RR
L-SS	2.34 (0.22)	1.05 (0.86–1.13)	0.22 (5)	0.96	
F-RS	1.09 (0.08)	285 (167–486)	0.12 (5)	0.40	271.43

*data represent averages of five replicates (10 larvae per replicate); RR – resistance ratio (LD_{50} F-RS/ LD_{50} L-SS), L-SS – susceptible population

Table 2. Toxicity of cypermethrin alone and in combination with profenofos and piperonyl butoxide (PB) to a susceptible (L-SS) and resistant (F-RS) populations of *Spodoptera littoralis*

Population	Treatment	Slope (± SE)	LD_{50} 95% confidence limit [µg · $larva^{-1}$]	χ^2 [df]	SR
L-SS	cypermethrin	2.34 (0.22)	1.05 (0.86–1.13)	0.22 (5)	
	cypermethrin + PB	0.9162 (0.024)	0.0568 (0.0025–0.0129)	0.24 (5)	18.49
	cypermethrin + profenofos	0.9166 (0.063)	0.0061(0.004–0.0014)	0.24 (5)	172.13
F-RS	cypermethrin	1.09 (0.08)	285 (167–486)	0.12 (5)	
	cypermethrin + PB	1.37 (0.16)	6.89 (4.22–12.18)	0.29 (5)	41.36
	cypermethrin + profenofos	0.77 (0.41)	1.48 (0.52–3.03)	0.02 (5)	192.57

SR – synergistic ratio (LD_{50} cypermethrin alone/ LD_{50} cypermethrin + PB or profenofos)

Table 3. The inhibition of piperonyl butoxide (PB) and profenofos on activity of esterase in both susceptible (L-SS) and resistant (F-RS) populations of *Spodoptera littoralis*, *in vivo*

Population	Treatment	Enzyme activity [nmol · min · mg^{-1}]	
		Esterase ± SE	
		pNPA	Inhibition ratio [%]
L-SS	cypermethrin	47.9 ± 7.41a	
	cypermethrin + PB	38.9 ± 3.13b	18.8
	cypermethrin + profenofos	4.22 ± 0.80c	91.2
F-RS	cypermethrin	92.0 ± 15.32a	
	cypermethrin + PB	54.1 ± 4.95b	41.2
	cypermethrin + profenofos	8.66 ± 0.84c	90.6

Values are mean (±SE) of three replicates. Means followed by the same letters in same column are not significantly different (Tukey's test, $p < 0.05$)

sensitivity to cypermethrin in F-RS population with low resistance causing a reduction in LD_{50} compared to susceptible population (L-SS). The toxicity of the mixture of cypermethrin + profenovos against *S. littoralis* was the most toxic among the other mixtures tested, showing 1.48 µg per larva and 192.57-fold as LD_{50} and synergistic ratio (SR) respectively. PB with cypermethrin resulted in a slight synergy of 41.36-fold. Overall, the toxicity of the mixture of cypermethrin + profenofos proved to be the highest in the current study compared to the combination of cypermethrin + PB against *S. littoralis* larvae.

Effects of enzyme inhibitors on the activity of esterase in susceptible and field populations, *in vivo*

Compared with the susceptible population (L-SS) of *S. littoralis*, the field population (F-RS) displayed low esterase activity (Table 3). Significant inhibition of esterase activity by profenofos 90.6 and 91.2%, respectively in both F-RS and L-SS populations of *S. littoralis* were found, *in vivo*. In contrast, PB showed a slight decrease in esterase activity.

Discussion

Pyrethroids, a class of traditional insecticides, are still widely used in the control of lepidopteran pests in many crops. Resistance to pyrethroids has evolved after many years of continuous and extensive use. Moderate to high resistance to pyrethroids has been reported in field populations of *H. armigera* (Hübner), *Chloridea virescens* (F.), *Plutella xylostella* (L.), *S. frugiperda* (J.E. Smith), *S. litura* (F.) and *S. littoralis* (Boisd.) (Gunning *et al.* 1999; Terán-Vargas *et al.* 2005; Khaliq *et al.* 2007; Carvalho *et al.* 2013; Gandhi *et al.* 2016; Ismail 2022). Effective management of *S. littoralis*, a devastating pest worldwide, relies heavily on chemical insecticides, such as pyrethroids. To enhance the efficacy of pyrethroids, this study was conducted to determine the resistance level to cypermethrin, the synergistic effects of two enzyme inhibitors on field population (F-RS), and the effect of enzyme inhibitors on the esterase activity of *S. littoralis* larvae.

The synergistic ratio (SRs) for cypermethrin by profenofos in the susceptible and field populations were significantly higher than the SRs by piperonyl butoxide (PB) in the two populations (SRs = 172.13–192.57; 18.49–41.36, respectively). Based on these results the susceptibility of *S. littoralis* larvae to cypermethrin increased when mixed with profenofos. These results are consistent with Martin *et al.* (2003), Ahmed (2007), Ahmed *et al.* (2009) reported synergism synergism of cypermethrin and deltamethrin with profenofos in *S. litura*, *H. armigera* and *Bemisia tabaci* (Genn.). Several studies have also shown a slight increase in pyrethroid toxicity by PB in *H. armigera* and *Cimex lectularius* (L.) (Young *et al.* 2006; Romero *et al.* 2009).

Significant synergism was found in both cypermethrin-resistant (F-RS) and susceptible (L-SS) populations of *S. littoralis* depending on enzyme inhibitors. Among the tested enzyme inhibitors, the profenofos-esterase inhibitor displayed the highest synergistic effects on the toxicity of cypermethrin in both strains (L-SS and F-RS) of *S. littoralis*. The data indicated that esterase inhibition may play a role in the reduction of the resistance to cypermethrin. These results strongly demonstrated that the mixture of pyrethroids and OPs resulted in synergism and enhanced pyrethroid efficacy. Previous studies have shown that profenofos could inhibit esterase activity in *B. tabaci* (Bryne and Devonshire 1991), in *H. armigera* (Gunning *et al.* 1999), and in *S. litura in vivo* (Ahmed et al. 2009) and increase the toxicity to insecticides in these insect species.

Conclusions

This study revealed a high-level of cypermethrin resistance in the field-collected population of *S. littoralis*. Synergistic bioassays and enzyme assays support the involvement of a detoxifying enzyme in cypermethrin resistance. Overall, the findings of this study confirm that OPs can be effective for the control of pyrethroid-resistant cotton leafworms.

References

Ahmad M. 2007. Potentiation/antagonism of pyrethroids with organophosphate insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology* 100 (3): 886–893. DOI: https://doi.org/10.1093/jee/100.3.886

Ahmad M., Saleem M.A., Sayyed A.H. 2009. Efficacy of insecticide mixtures against pyrethroid- and organophosphate-resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae). Pest Management Science 65 (3): 266–274. DOI: https://doi.org/10.1002/ps.1681

Bradford M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248–254. DOI: https://doi.org/10.1006/abio.1976.9999

Bryne F.J., Devonshire A.L. 1991. *In vivo* inhibition of esterase and acetylcholinesterase activities by profenofos treatment in the tobacco whitefly *Bemisia tabaci* (Genn) implications for routine biochemical monitoring of these enzymes. Pesticide Biochemistry and Physiology 40 (3): 198–204. DOI: https://doi.org/10.1016/0048-3575(91)90090-9

CABI. 2019. *Spodoptera littoralis*. Invasive Species Compendium. [Available on: https://doi.org/10.1079/cabicompendium.51070] [Accessed: 22 August 2022].

Carvalho R.A., Omoto C., Field L.M., Williamson M.S., Bass C. 2013. Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLoS One 8 (4): e62268. DOI: https://doi.org/10.1371/journal.pone.0062268

DeVries D.H., Georghiou G.P. 1981. Decreased nerve sensitivity and decreased cuticular penetration as mechanisms of resistance to pyrethroids in a (R)-trans-permethrin selected strain of house fly. Pesticide Biochemistry and Physiology 15 (3): 234–241. DOI: https://doi.org/10.1016/0048-3575(81)90005-5

Gandhi K.B., Patil R.H., Srujana Y. 2016. Field resistance of *Spodoptera litura* (Fab.) to conventional insecticides in India. Crop Protection 88: 103–108. DOI: https://doi.org/10.1016/j.cropro.2016.06.009

Gonzalez-Morales M.A., Romero A. 2019. Effect of synergists on deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). Journal of Economic Entomology 112 (2): 786–791. DOI: https://doi.org/10.1093/jee/toy376

Gunning R.V., Moores G.D., Devonshire A.L. 1999. Esterase inhibitors synergies the toxicity of pyrethroids in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pesticide Biochemistry and Physiology 63 (1): 50–62. DOI: https://doi.org/10.1006/pest.1998.2388

Ismail S.M. 2020. Effect of sublethal doses of some insecticides and the role on detoxication enzymes and protein-content of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Bulletin of the National Research Centre 44 (35): 2–6. DOI: https://doi.org/10.1186/s42269-020-00294-z

Ismail, S. M. 2022. Laboratory evaluation of chlorpyrifos against resistant and susceptible strains of a moth pest of crop plants, *Spodoptera littoralis* (Lepidoptera: Noctuidae). Proceedings of the Zoological Society 75 (3): 1–9. DOI: https://doi.org/10.1007/s12595-022-00461-2

Ismail S.M., Morshedy M. 2017. Toxicity and kinetic studies of H^+, K^+-ATPase sensitivity to cyfluthrin and fenpropathrin in Egyptian field populations of *Spodoptera littoralis*. Alexandria. Journal of Agricultural Sciences 62 (2): 203–208. DOI: https://doi.org/10.21608/alexja.2017.5777

Khaliq A., Attique M.N.R., Sayyed A.H. 2007. Evidence for resistance to pyrethroids and organophosphates in *Plutella xylostella* (Lepidoptera: Plutellidae) from Pakistan. Bulletin of Entomological Research 97 (2): 191–200. DOI: https://doi.org/10.1017/S0007485307004877

LeOra Software, Polo-PC. 2003. A user' guide to probit and logit analysis. LeOra Software, Bakeley, CA, USA.

Martin T., Ochou O.G., Vaissayre M., Fournier D. 2003. Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from West Africa. *Journal of Economic Entomology* 96 (2): 468–474. DOI: https://doi.org/10.1093/jee/96.2.468

Pauron D., Barhanin J., Amichot M., Pralavorio M., Bergè J.B., Lazdunski N. 1989. Pyrethroid receptor in insect Na^+ channel: altertaion of its properties in pyrethroid-resistance flies. Biochemistry 28 (4): 1673–1677. DOI: https://doi.org/10.1021/bi00430a037

Romero A., Potter M.F., Haynes K.F. 2009. Evaluation of piperonyl butoxide as a deltamethrin synergist for pyrethroid-resistant bed bugs. Journal of Economic Entomology 102 (6): 2310–2315. DOI: http://dx.doi.org/10.1603/029.102.0637

SAS. 2004. Statistical Analysis System (SAS) Institute. SAS/STAT user's guide, Version 9. SAS Institute, Inc., Cary, North Carolina, USA.

Terán-vargas A.P., Rodríguez J.C., Blanco C.A., Martínez-carrillo J.L., Cibrián-tovar J., Sánchez-arroyo H., Rodríguez-delbosque L.A., Stanley D. 2005. Bollgard cotton and resistance of tobacco budworm (Lepidoptera: Noctuidae) to conventional insecticides in southern tamaulipas, Mexico. Journal of Economic Entomology 98 (6): 2203–2209. DOI: httsdoi.org/10.1093/jee/98.6.2203

van Asperen K. 1962. A study of house fly esterase by means of a sensitive colorimetric method. Journal of Insect Physiology 8 (4): 401–416. DOI: https://doi.org/10.1016/0022-1910(62)90074-4

Young S.J., Gunning R.V., Moores G.D. 2006. Effect of pretreatment with piperonyl butoxide on pyrethroid efficacy against insecticide-resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Bemisia tabaci* (Sternorrhyncha: Aleyrodidae). Pest Management Science 62 (2): 114–119. DOI: https://doi.org/10.1002/ps.1127