Enhanced insecticide-resistance spectrum in green lacewing predator, *Chrysoperla zastrowi sillemi* (strain PTS-8) and its potential role in the management of sucking pests of cotton

M. Ashwini[1], M. Mohan[2], G. Sivakumar[2] and T. Venkatesan[2,*]

[1]Department of Entomology, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bellary Road, Bengaluru 560 065, India
[2]ICAR-National Bureau of Agricultural Insect Resources, Hebbal, Bengaluru 560 024, India

The green lacewing or aphid lion, *Chrysoperla zastrowi sillemi* (Esben-Petersen) is an important predator of sucking pests, and eggs and neonate larvae of lepidopteran pests under many crop ecosystems of India. In the present study, enhanced insecticide resistance spectrum in an insecticide-resistant population of *C. zastrowi sillemi* (strain PTS-8) was evaluated against four commonly used insecticides on cotton. The insecticide resistant *C. zastrowi sillemi* PTS-8 showed 16.4-, 14.8-, 12.7- and 7.2-fold resistance against chlorpyriphos 20% EC, cypermethrin 10% EC, acetamipirid 20% SP and chlorantraniliprole 18.5% SC respectively, compared to the susceptible strain. Biochemical assays revealed an elevated level of three major detoxifying enzymes, viz. carboxylesterase (1.48-fold), glutathione *S*-transferase (1.27-fold) and cytochrome p450 monooxygenase (1.36-fold) in PTS-8 strain compared to the susceptible strain. The field survival and biocontrol potential of PTS-8 strain were significantly better on cotton plants treated with recommended dose of insecticides. The study indicated the potential role of insecticide-resistant natural enemies under biointensive IPM programmes to avoid compatibility conflict with insecticides.

Keywords: *Chrysoperla zastrowi sillemi*, cotton, detoxifying enzymes, insecticide resistance, sucking pests.

CHRYSOPERLA ZASTROWI SILLEMI (Esben-Petersen; Neuroptera: Chrysopidae), commonly called green lacewing or aphid lion, is a potential predator of insect pests in various crop ecosystems of India[1–5]. Under real field conditions, the predators and parasitoids succumb to a cocktail of insecticides either through direct sprays or by consuming the poisoned prey. In many cases, the sprays are lethal to the predator, which in turn causes pest resurgence and secondary outbreak of minor pests. Nevertheless, natural enemies can develop resistance when they are constantly exposed to insecticides[6]. When the insecticides have become an unavoidable source of pest management, integrating the insecticide-resistant natural enemies with the insecticides will enable us to manage the pests in a sustainable manner with delay in resistance development against insecticides in insect pests.

Among the natural enemies, the populations of green lacewing have revealed a significant level of resistance development against different insecticide[7–9]. Studies were carried out to determine the level of insecticide resistance in different populations of *C. zastrowi sillemi* from India. A pesticide resistant strain of *C. zastrowi sillemi* (PTS-8) was commercialized which was found highly resistant to endosulfan, acephate and fenvalerate[5]. Synergism studies revealed the enhanced detoxifying enzymes (general esterases, glutathione *S*-transferases and monooxygenases) in the resistant strain of *C. zastrowi sillemi*[5].

The scenario of pesticide usage keeps changing with time and it is important to test the enhanced insecticide resistance spectrum of *C. zastrowi sillemi* against new molecules. The augmentative releases of *C. zastrowi sillemi* are usually practised in cotton fields against sucking pests, and eggs and neonate larvae of lepidopteran pests[10–12]. Metabolic enzyme mediated resistance may lead to cross-resistance of different insecticides which have a different mode of action[13,14]. Hence, in the present study we evaluated the enhanced insecticide resistance spectrum in strain PTS-8 of *C. zastrowi sillemi* against commonly recommended insecticides to determine its compatible use with insecticides under Integrated Pest Management (IPM) programmes.

Materials and methods

Rearing of C. zastrowi sillemi

Culture of the larval and adult stages of the predator *C. zastrowi sillemi* was maintained as described by Venkatesan *et al.*[5]. The susceptible and resistant populations of the predator were continuously maintained in the laboratory. The insecticide-resistant larval population was

*For correspondence. (e-mail: tvenkat12@gmail.com)

exposed to field-recommended dosage of acephate (0.67 g/l of water) and imidacloprid (0.4 ml/l of water) once in three generations.

Insecticides and chemicals

Based on the recommendations of the Central Insecticide Board Registration Committee, Government of India (www.cibrc.gov.in), chlorpyriphos, cypermethrin, acetamiprid and chlorantraniliprole were selected for studying the enhanced insecticide resistance spectrum in strain PTS-8 of *C. zastrowi sillemi* and the associated metabolic enzyme assays. Commercial formulations, viz. chlorpyriphos 20% EC, cypermethrin 10% EC, acetamiprid 20% SP and chlorantraniliprole 18.5% SC were used in the study. The other chemicals and reagents used in the enzyme studies were purchased from Sigma-Aldrich Chemical Co, USA.

Dose–response bioassays

Dose–response bioassays with the insecticides were conducted by taking the field-recommended dosage of each insecticide as the base concentration, and the other concentrations above and below it. The concentrations were further increased in the insecticides, where 50% mortality was not obtained (Table 1). In each concentration, 100–120 (early second instar) grubs were released on a petri plate and the required concentration of insecticides was sprayed using hand sprayer (0.5 lit. capacity) for 3–5 s. After spraying, the larvae were placed on a tissue paper in order to remove excess insecticides. Such treated larvae were released in a glass vial (3 cm × 1.5 cm) and provided with UV-treated (to paralyse the embryo and prevent hatching) *Corcyra cephalonica* eggs. The larvae treated with distilled water were used as control. The larval mortality was recorded 24 and 48 h after treatment. The moribund larvae were also considered as dead[5,15].

Preparation of whole-body larval homogenate

Twenty four-day-old grubs each for resistant and susceptible populations were homogenized thoroughly in 200 µl ice-cold 50 mM phosphate buffer, pH 6.8. The samples were centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant was used as the enzyme source. The total protein content of the enzyme sample was determined by Bradford Coomassie Brilliant Blue (CBB) G-250 dye binding method using bovine serum albumin (BSA) as the standard[16].

Table 1. Concentrations of insecticides used for bioassay studies

Insecticide	Concentration tested (ppm)
Chlorpyriphos 20% EC	62.5, 125, 250, 500, 1000, 2000, 4000
Cypermethrin 10% EC	12.5, 25, 50, 100, 200, 400, 800, 1,600, 3,200, 6,400, 12,800, 25,600
Acetamiprid 20% SP	5, 10, 20, 40, 80, 160, 320, 640, 1,280, 2,560, 5,120, 10,240
Chlorantraniliprole 18.5% SC	6.87, 13.75, 27.5, 55, 110, 220, 440, 880, 1,760, 3,520, 7,040, 14,080, 28,160, 56,320

Metabolic enzyme assays

The carboxylesterase activity was determined using α-naphthyl acetate as a substrate[17]. The α-naphthol formed after the reaction was measured at 595 nm and quantified using α-naphthol standard curve. The qualitative changes in carboxylesterase isozyme profile were determined in resistant and susceptible populations by performing native polyacrylamide gel electrophoresis (PAGE)[18]. To visualize the bands, the gel was stained with freshly prepared staining solution containing 0.05% α-naphthyl acetate and 0.1% fast blue B salt in 50 mM phosphate buffer, pH 6.8.

The activity of glutathione *S*-transferase was determined spectrophotometrically using 1-chloro-2,4-dinitrobenzene as substrate[19] and the molar extinction coefficient value of 9.6 mM^{-1} cm^{-1}. The activity of cytochrome p450 monooxygenase was determined by *p*-nitroanisole demetylase assay[20]. Molar extinction coefficient ε value of *p*-nitrophenol (18.2 mM^{-1} cm^{-1}) was used to calculate enzyme activity and expressed as µmol/min/mg of protein.

Survival studies on insecticide resistant C. zastrowi sillmi (strain-PTS-8)

An experiment was conducted to test the survival of insecticide-resistant *C. zastrowi sillemi* (PTS-8) under field net-house conditions (insect proof, 100 mesh netting with size of 12 × 12 × 15 ft) during 2017–18 on the cotton variety DCH-32. Cotton plants (50 days old) were raised on pots under natural conditions and natural infestation of sucking pests. It was found that the plants were naturally infested with the sucking pests, viz. *Aphis gossypii*, *Bemisia tabaci*, *Thrips tabaci* and *Phenacoccus solenopsis*, and predators, viz. *Cheilomenes sexmaculata*, *Cryptolaemus montrouzieri* and ants. Adequate care was taken to remove such natural enemies. The sucking pests-infested potted cotton plants were transferred to wooden cages individually and placed in the net house. Before release of the predator, the plants were sprayed with different concentrations of insecticides. Four-day-old insecticide-resistant and susceptible *C. zastrowi sillemi* grubs @ 20 per plant were released on the insecticide sprayed plants after 3 h of spray. In addition to the sucking pests, adequate quantity of UV-exposed eggs of *C. cephalonica* were also provided in a card (4 × 2 cm) that was stapled on the plants as a feed for the *C. zastrowi sillemi* grubs.

Table 2. Relative toxicity of four insecticides to the second instar grubs of resistant (PTS-8) and susceptible populations of *Chrysoperla zastrowi sillemi*

Insecticide	Population	LC_{50} (ppm)	95% Fiducial limit (ppm)		Slope ± SE	χ^2 value (d*f*)	*P**	RR#
			Lower	Upper				
Chlorpyriphos 20% EC	PTS-8	1,172.0	867.87	1,679.24	1.53 ± 0.203	1.568(5)	0.905	16.37
	Susceptible	71.56	32.70	113.43	2.00 ± 0.229	3.418(5)	0.636	–
Cypermethrin 10% EC	PTS-8	1,633.92	1,231.02	2,192.13	4.3 ± 0.134	4.832(10)	0.902	14.75
	Susceptible	110.73	78.85	151.46	2.41 ± 0.125	3.492(10)	0.967	–
Acetamiprid 20% SP	PTS-8	1,790.99	1,318.88	2,534.00	4.31 ± 0.146	5.267(10)	0.920	12.74
	Susceptible	140.40	103.39	190.20	2.72 ± 0.113	4.543(10)	0.920	–
Chlorantranili prole 18.5% SC	PTS-8	20,936.12	14,263.21	34,469.68	4.6 ± 0.145	2.325(12)	0.999	7.15
	Susceptible	2,927.10	2,248.82	3,835.19	4.94 ± 0.153	5.906(12)	0.921	–

**P* ≥ 0.05 indicates a good fit between observed and expected regression lines in a probit analysis.
S.E. Standard error; d*f*, Degrees of freedom. #Resistance ratio = LC_{50} of resistant PTS-8 population/LC_{50} of susceptible population.

Three concentrations of each insecticide were tested against both the populations in three replications along with water-sprayed control. The LC_{90} dose of each insecticide was taken as a base concentration along with half and double the LC_{90} doses for both resistant and susceptible populations along with water-sprayed control. Observations on the survival of the grubs were made 1, 3, 5 and 7 days after spraying on the whole plant.

Statistical analysis of data

The larval mortality data were subjected to Abbott's correction[21]. The corrected data were analysed by probit analysis[22] to determine the LC_{50} values using SPSS software version 23, plotting the probit mortality against log dose in ppm. The resistance ratio against each insecticide was determined by dividing the LC_{50} values of the resistant population with the susceptible population. The level of resistance for each insecticide was determined based on the classification of resistance given by Kim *et al.*[23]. Each enzyme assay was performed with three replicates and significant difference of mean enzyme activity in the resistant and susceptible populations was determined based on Student's *t*-test. Data on survival of larvae in different insecticidal-treated plants were subjected to analysis of variance. Means were compared using Duncan's multiple range test.

Results

Enhanced insecticide resistance spectrum studies

The insecticides were screened for their enhanced resistance in laboratory-reared resistant population of *C. zastrowi sillemi* (strain PTS-8) and susceptible population. Among the four insecticides tested, the highest resistance ratio was recorded for chlorpyriphos (16.4-fold), followed by cypermethrin (14.7-fold), acetamiprid (12.7-fold) and the lowest for chlorantraniliprole (7.2-fold) in the resistant population compared to the susceptible population (Table 2). Based on the classification of resistance by Kim *et al.*[23], the population of *C. zastrowi sillemi* was moderately resistant to chlorpyriphos, cypermethrin, acetamiprid and showed low resistance to chlorantraniliprole.

Detoxification of insecticides

All the detoxifying enzymes showed significantly higher levels of activity compared to the susceptible population based on Student's *t*-test (Table 3). The resistant population showed 1.48-, 1.27- and 1.36-fold increase in carboxylesterase, glutathione *S*-transferase and cytochrome p450 monooxygenase activity respectively, compared to the susceptible population. The esterase banding pattern of the resistant and susceptible populations revealed the presence of an extra band (E_1) in the resistant population compared to the susceptible population (Figure 1).

Survival of predator under insecticide stressed conditions

All the insecticides tested showed significant difference in survival of resistant population compared to the laboratory-reared population at different concentrations (Table 4). The number of larvae that survived at 3860, 7720 and 15,440 ppm of chlorpyriphos at 7 DAS (days after spraying) was 17.67, 16.00 and 8.00 in resistant population and 6.67, 5.33 and 1.33 in susceptible population respectively. For cypermethrin, the concentrations used were 61,101, 122,203 and 244,406 ppm, where the larval survival at 7 DAS was 17.33, 16.67 and 8.33 in resistant population and 6.00, 4.67 and 1.67 in susceptible population respectively. At 7360, 14,720 and 29,440 ppm of acetamiprid, the number of larvae that

Table 3. Metabolic enzyme activities of *C. zastrowi sillemi* populations

C. zastrowi sillemi population	Carboxylesterase (μ mol min^{-1} mg^{-1})	Glutathione *S*-transferase (μ mol min^{-1} mg^{-1})	Cytochrome p450 monooxygenase (μ mol min^{-1} mg^{-1})
Resistant (PTS-8)	1.228 ± 0.012 (1.48)	0.084 ± 0.001 (1.27)	0.0168 ± 0.0001 (1.36)
Susceptible laboratory reared	0.831 ± 0.003	0.066 ± 0.001	0.0123 ± 0.0001

The given values of enzyme activity of resistant population differ significantly from susceptible population ($P < 0.05$) based on Student's *t*-test. Values in brackets depict the fold increase activity compared to susceptible population.

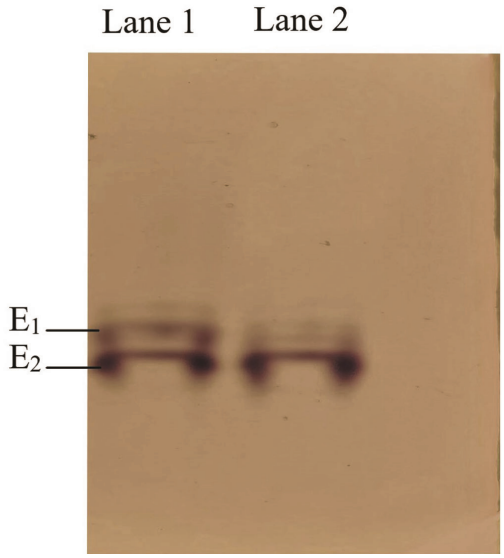

Figure 1. Esterase banding pattern in resistant population. PTS-8 (lane 1) and susceptible population (lane 2) of *Chrysoperla zastrowi sillemi* (E1, Esterase 1; E2, Esterase 2).

survived at 7 DAS was 17.33, 16.00 and 7.67 in resistant population compared to 6.667, 4.667 and 1.667 in susceptible population respectively. The number of larvae that survived at 123,829, 247,659 and 495,318 ppm of chlorantraniliprole was 17.33, 16.33 and 7.67 in resistant population and 6.00, 4.33 and 2.33 in susceptible population respectively.

The maximum survival of the grubs from the resistant population was recorded at half the concentration of the recommended dosage of all the four insecticides. The larval survival in the recommended dosage of cypermethrin and chlorantraniliprole treatment was on par with half the concentration. At double the concentration of the recommended dosage, the survival was less than 50%. However, survival of the insecticide-resistant grubs was significantly better than the susceptible ones at all the concentrations of the four insecticides.

Discussion

The insecticide-induced selection pressure causes development of insecticide resistance in the insects. In the present study, field-collected insecticide-resistant grubs of *C. zastrowi sillemi* (strain PTS-8) were further subjected to selection pressure by exposing them to field-recommended dosage of acephate (0.67 g/l of water) and imidacloprid (0.4 ml/l of water) once in three generations under laboratory conditions. Such a resistant predator was exposed to new insecticides, viz. chlorpyriphos, cypermethrin, acetamiprid and chlorantraniliprole. The strain PTS-8, originally collected from Sri Ganganagar, Rajasthan, showed 50.4-, 66.1- and 277.5-fold resistance for endosulfan, fenvalerate and acephate respectively[5], and strain also showed resistance to new insecticides. Similar studies from Pakistan reported 53–160-fold resistance to chlorpyriphos and 1–8-fold resistance to deltamethrin in 15 field populations of *Chrysoperla carnea*[15]. In the adults of *C. carnea*, Sohail *et al.*[24] reported 15-fold resistance to acetamiprid.

Metabolic enzymes are responsible for the detoxification of insecticidal molecules in insects. Esterases, glutathione *S*-transferases and cytochrome p450 monooxygenases are the most common metabolic enzymes involved in insecticide resistance[5,15,25,26]. All the three enzymes showed significantly elevated levels of activity in the resistant population compared to the susceptible population. These metabolic enzymes are known to act together, but only the proportion of enzyme activity varies with insecticides. Synergism studies conducted previously also revealed the role of these three enzymes in insecticide resistance in the same population of *C. zastrowi sillemi*[5]. The role of esterase as the major factor of resistance to synthetic pyrethroids in *C. carnea* was reported in previous studies[25,27]. Association of esterases and monooxygenases with organophosphate and pyrethroid resistance in *C. carnea* field populations in Canada has been reported[7]. The esterase banding pattern also confirmed the difference in esterase activity of resistant and susceptible populations. The difference in esterase activity can be depicted by variation in the number of bands and thickness of the bands[28]. The extra band in the resistant population could be due to induction of additional esterases responsible for hydrolysis of the insecticidal molecule.

Cross-resistance denotes that resistance to one insecticide in insects confers resistance to another insecticide, even when the insect is not exposed to it earlier. Cross-resistance is known to exist between insecticides having similar binding target sites or similar detoxifying pathways[29]. Metabolic enzyme-mediated resistance could confer cross-resistance to a different group of insecticides[14].

Table 4. Survival of insecticide-resistant (PTS-8) and susceptible populations of *C. zastrowi sillemi* on insecticide-treated cotton plants

Chlorpyriphos					Cypermethrin				
Treatment (dosage (ppm) and (strain)	1 DAS	3 DAS	5 DAS	7 DAS	Treatment (dosage (ppm) and strain)	1 DAS	3 DAS	5 DAS	7 DAS
3860 (PTS-8)	19.33^{a}	19.00^{ab}	18.33^{ab}	17.67^{b}	61101 (PTS-8)	19.000^{a}	18.667^{a}	18.000^{a}	17.333^{ab}
3860 (susceptible)	13.00^{b}	10.67^{c}	8.00^{c}	6.67^{e}	61101 (susceptible)	11.000^{b}	10.667^{b}	8.333^{bc}	6.000^{d}
7720 (PTS-8)	18.33^{a}	18.00^{b}	17.33^{b}	16.00^{c}	122203 (PTS-8)	19.333^{a}	19.000^{a}	18.667^{a}	16.667^{b}
7720 (susceptible)	9.66^{c}	9.00^{d}	7.33^{c}	5.33^{f}	122203 (susceptible)	8.667^{c}	8.333^{c}	7.000^{c}	4.667^{d}
15440 (PTS-8)	12.00^{b}	10.67^{c}	8.67^{c}	8.00^{d}	244406 (PTS-8)	11.000^{b}	10.000^{b}	9.000^{b}	8.333^{c}
15440 (susceptible)	5.00^{d}	4.00^{e}	2.33^{d}	1.33^{g}	244406 (susceptible)	5.333^{d}	4.333^{d}	3.333^{d}	1.667^{e}
Untreated control	19.66^{a}	19.67^{a}	19.33^{a}	19.00^{a}	Untreated control	19.667^{a}	19.667^{a}	19.333^{a}	19.000^{a}
S.Em (±)	0.549	0.488	0.535	0.398	S.Em (±)	0.504	0.418	0.549	0.563
SED	0.777	0.690	0.755	0.563	SED	0.713	0.591	0.777	0.797
CD@5%	1.667	1.480	1.621	1.209	CD@5%	1.529	1.267	1.666	1.709
Acetamiprid					Chlorantraniliprole.				
Treatment (dosage (ppm) and strain)	1 DAS	3 DAS	5 DAS	7 DAS	Treatment (dosage (ppm) and strain)	1 DAS	3 DAS	5 DAS	7 DAS
7360 (PTS-8)	18.667^{a}	17.667^{b}	17.667^{b}	17.333^{b}	123829 (PTS-8)	19.667^{a}	19.333^{a}	18.333^{ab}	17.333^{b}
7360 (susceptible)	9.000^{cd}	8.333^{c}	7.667^{de}	6.667^{e}	123829 (susceptible)	8.333^{c}	8.000^{d}	7.000^{d}	6.000^{d}
14720 (PTS-8)	17.000^{b}	16.667^{b}	16.333^{c}	16.000^{c}	247659 (PTS-8)	18.333^{a}	17.667^{b}	17.000^{b}	16.333^{b}
14720 (susceptible)	8.000^{d}	7.667^{c}	6.667^{e}	4.667^{f}	247659 (susceptible)	7.333^{c}	6.667^{e}	6.000^{d}	4.333^{e}
29440 (PTS-8)	9.667^{c}	8.333^{c}	8.000^{d}	1.667^{g}	495318 (PTS-8)	13.000^{b}	10.667^{c}	8.667^{c}	7.667^{c}
29440 (susceptible)	4.667^{e}	4.000^{d}	2.667^{f}	1.667^{g}	495318 (susceptible)	5.333^{d}	4.667^{f}	3.333^{e}	2.333^{f}
Untreated control	19.667^{a}	19.667^{a}	19.333^{a}	19.000^{a}	Untreated control	19.667^{a}	19.667^{a}	19.333^{a}	19.000^{a}
S.Em (±)	0.333	0.436	0.378	0.282	S.Em (±)	0.577	0.436	0.454	0.418
SED	0.471	0.617	0.535	0.398	SED	0.817	0.617	0.642	0.591
CD@5%	1.011	1.323	1.146	0.855	CD@5%	1.751	1.323	1.378	1.267

DAS, Days after spraying. Means followed by the same alphabet do not differ significantly by DMRT (P = 0.05%).

Overexpression of non-specific enzymes such as monooxygenases results in resistance to a broad spectrum of insecticides[9,30]. The elevated levels of enzyme activity in the resistant population (PTS-8) confirmed the metabolic detoxification of insecticides, and it was evident that the population showed cross-resistance to different insecticides tested.

Field trials under netted conditions revealed significant variability in the survival of resistant population than the susceptible population at different concentrations. The survival of resistant population ranged from 88.4% to 86.7% for half the recommended dose of all the chemicals, 80% to 83% for the recommended dosage and 33.3% to 30% for double the recommended dosage of the insecticides. It was also found that the larvae were able to reach pupal stage at 7 DAS, mostly in the resistant population (PTS-8). Among the pests, *P. solenopsis* and *B. tabaci* were able to survive on the insecticide-sprayed plants which were the feeding source for the chrysopid predators. This reveals the profound ability of strain PTS-8 to resist high doses of insecticides even under field conditions. Studies on insecticide resistance have proved the resistance of *Chrysoperla* towards organophospates, synthetic pyrethroids and neonicotinoides[5,9,15]. It is noteworthy that the strain of predator which would tolerate high doses of insecticides sustains better when integrated under IPM programmes and keeps a check on the pest population regularly.

Conclusion

The present study confirms enhanced insecticide resistance spectrum among organophosphates, synthetic pyrethroids and neonicotinoids in the resistant strain PTS-8 of *C. zastrowi sillemi*. Its field survival at the recommended doses of insecticides indicates the compatibility of biological control and chemicals. Currently, the strain PTS-8 has been commercialized to industrial partners for large-scale multiplication and release. The release of insecticide-resistant predator also has the potential to slow down the rate of evolution of insecticide resistance in pest species and resurgence of sucking pests.

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