Original Research Article

Sublethal Effects of Chitosan-O-Arginine, a chitosan based insecticidal compound against Green lacewing *Chrysoperla zastrowi sillemi* (Esben-Petersen)

ABSTRACT

Aims: To study the sublethal effects of chitosan-o-arginine against the green lacewing *Chrysoperla zastrowi sillemi*, when treated and untreated *P. xylostella* was given as feed.

Study design: Factorial Completely Randomized Design

Place and Duration of study: Post Graduation Research Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore and July, 2023.

Methodology: Laboratory experiments explored the effects of sublethal doses of Cs-O-Arg to the third instar grubs of *C. zastrowi sillemi* when fed with treated and untreated larvae of diamondback moth (DBM) which according into different types of exposures.

Results: The results showed that no mortality was observed when the untreated third instar *C. zastrowi sillemi* grub was fed with treated *P. xylostella* larvae. But, when the same untreated grub was fed with treated *P. xylostella* larvae at LC_{25}, LC_{35} and LC_{45} concentrations, there was 6.67 per cent mortality of *C zastrowi sillemi* grub at all the concentrations, after 48 and 72 hours of exposure. but, when the treated *C. zastrowi sillemi* grubs (LC_{35} and LC_{45}) were fed with untreated *P. xylostella*, there was only 3.33 and 10.00 per cent mortality of *C. zastrowi sillemi* grubs, respectively. Whereas, Cs-O-Arg caused 33.33 per cent mortality when LC_{45} treated grubs were provided with LC_{45} treated DBM larvae as feed, whereas the treatment with LC_{25} concentrations of Cs-O-Arg resulted in the highest pupation percentage (76.67%) and adult emergence rate (73.33%) in *C. zastrowi sillemi* grubs when fed with DBM larvae treated with the same concentration.

Conclusion: Chitosan derivative insecticide causes a mild adverse impact on the survival of the economically significant insect predator *C. zastrowi sillemi*, which hold valuable implications for guiding decisions regarding the compatible utilization of insecticides alongside *C. zastrowi sillemi* or other natural predators within integrated pest management strategies.

Key words: *Chrysoperla zastrowi sillemi, Plutella xylostella, Chitosan-O-Arginine, Median Lethal Concentration, Sublethal effects*

1. INTRODUCTION

The green lacewing, *Chrysoperla zastrowi sillemi* (Neuroptera: Chrysopidae), often known as the aphid lion or golden eye, is a versatile and universal predator well-suited to laboratory mass production. This species can effectively predate, feed on a wide range of insects, and have excellent prey-searching ability and an insatiable appetite [1]. *C. zastrowi sillemi*'s outstanding skills have positioned it as a viable biocontrol agent in a number of pest management activities. This species preys on a variety of soft-bodied insect pests such as aphids, scales, thrips, mealybugs, whiteflies, and mites, as well as the eggs and young larvae of numerous lepidopteran pests. Its remarkable resistance to many insecticide classes increases its potential as a biocontrol agent [2,3].

The Diamondback Moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a prominent insect pest that causes enormous economic damage to Brassicaceae crops both internationally and in India [4]. This pest has forced farmers to use a wide range of insecticides to protect their Brassicaceae crops from DBM infestations and the resulting economic losses. Unfortunately, DBM has developed resistance to practically all pesticides used against it due to the broad and intensive use of these chemicals. The widespread use of insecticides has also produced severe environmental problems, such as air pollution and residue-related challenges in crop products [5]. Hence, alternative options are being continuously explored to bring adequate checks on the DBM population perpetuation and, at the same time, minimize insecticide usage.

Previously, the new bioactive chemical substance Chitosan-O-Arginine (Cs-O-Arg), generated from chitosan by L-arginine acylation, showed promising insecticidal efficacy against DBM [6]. The effect of Cs-O-Arg on non-target species must be investigated before it can be used on a broad basis on farmer holdings. *C. zastrowi sillemi* is present in all cropping systems; hence, as a model insect, the effect of Cs-O-Arg was studied on them. The current study cataloged the sublethal effects of Cs-O-Arg on the green lacewing, *C. zastrowi sillemi*.

2. MATERIALS AND METHODS

The experiment was carried out in the Post Graduate Research Facility Laboratory of the Department of Agricultural Entomology at the Tamil Nadu Agricultural University (TNAU) in Coimbatore, India. The Biological Control Laboratory, Department of Agricultural Entomology, TNAU, Coimbatore, provided the *C. zastrowi sillemi* for the study. MATSYAFED, Kerala State Co-operative Federation for Fisheries Development Ltd., Neendakara, Kerala, supplied the crude chitosan. Sri Sai Scientific Company in Coimbatore supplied the L-Arginine.

2.1 Synthesis of Chitosan-O-Arginine

The method described by Hefni *et al.* (2020) [7] was used to synthesize chitosan-O-arginine (Cs-O-Arg). Chitosan flakes (3 g, 18 mmol glucosamine units, Mwt 1526.5 g/mol) were initially dispersed in 100 ml of distilled water. L-arginine (6.26 g, 36 mmol, Mwt 174.2 g/mol) was mixed with the chitosan solution in a 1:2 (Chitosan: L-arginine) ratio. Drops of sulfuric acid (5 ml of 2 M H2SO4) were used to catalyze the reaction, while 5 ml of 2 M HCl was added dropwise to increase the solubility of L-arginine. After stirring at 80°C for 4 h, the mixture was cooled to room temperature, and the pH was adjusted to 7 using $NaHCO_3$ solution, resulting in the precipitation of Cs-O-Arg derivatives. The sediment was cleared using acetone, and unreacted acid was removed. The resultant Cs-O-Arg residue was removed with acetone for 24 h in a Soxhlet system. The residue was then dried at 60°C in a hot air oven, sieved, and refrigerated for later use.

2.2 Mass culturing of *P. xylostella*

P. xylostella nucleus culture was acquired from the Postgraduate Research Facility, Department of Agricultural Entomology, TNAU, Coimbatore. The DBM culture was maintained under controlled conditions of 25 ± 2°C temperature, 70-75 per cent relative humidity, and a light-dark cycle of 14:10 h.

DBM pupae were kept in 45x45x45cm egg-laying cages. Adult moths were given a 20% honey solution and 4-5 cm tall mustard seedlings at emergence to aid oviposition. Following hatching, neonatal larvae engaged in leaf-mining on the mustard plants and, as they advanced through instars, were continually fed with new cabbage leaves free of pesticides daily until they reached the pupal stage. The pupae were then transferred to a cage for adult emergence, letting the life cycle continue.

2.3 Evaluation of lethal concentrations of Cs-O-Arg against DBM in laboratory

In the laboratory, a leaf dip bioassay was conducted to determine the lethal concentrations of Cs-O-Arg. The experimental design followed a Completely Randomized Block Design. It comprised seven treatments: five different concentrations of Cs-O-Arg (400, 600, 800, 1000, and 1200 ppm), one chitosan treatment (3000 ppm), and a control. To prepare the Cs-O-Arg concentrations, 8, 12, 16, 20, and 24mg of Cs-O-Arg were diluted in 20 ml of 0.1% glacial acetic acid (Isochem Laboratories, Kochi, India). Uniform leaf discs (6 cm in diameter) were excised from fully grown cauliflower leaves of uninfested potted plants and dipped into various concentrations of Cs-O-Arg and chitosan solutions for 30 seconds, followed by air-drying for 5 to 10 minutes. These treated leaves were then placed in circular plastic containers lined with damp filter papers to maintain leaf turgidity. Subsequently, second-instar DBM larvae were introduced to each treatment using a fine brush, and this process was replicated thrice. Leaves treated with water served as the control. Larval mortality was noted 24, 48, and 72 hours after treatment (HAT). Mortality observations at 72 HAT were used for probit analysis to ascertain lethal concentrations [5].

2.4 The sublethal effects of Cs-O-Arg on *C. zastrowi sillemi*

The sublethal impact of Cs-O-Arg on third-instar grubs of *C. zastrowi sillemi* was assessed through chronic exposure until grubs pupate. The grubs were continuously provided with treated second and third-instar DBM larvae as food until the pupation. We used the Cs-O-Arg median lethal concentration for DBM to calculate sublethal concentrations, namely LC_{25}, LC_{35}, and LC_{45} Cs-O-Arg, and prepared the solutions in 0.1% glacial acetic acid solvent. The control group was subjected to distilled water. All solutions received 0.05% Tween 80 (Quality Deals India, Hyderabad, India). The treatments are: 1. Untreated *C. zastrowi sillemi* fed with untreated *P. xylostella,* 2. Untreated *C.*

zastrowi sillemi fed with treated *P. xylostella* (LC_{25}, LC_{35} and LC_{45}); 3. Treated *C. zastrowi sillemi* (LC_{25}, LC_{35} and LC_{45}) fed with untreated *P. xylostella,* and 4. Treated *C. zastrowi sillemi* (LC_{25}, LC_{35} and LC_{45}) fed with treated *P. xylostella* (LC_{25}, LC_{35} and LC_{45}).

A total of six replications were carried out for each treatment. Within each replication, five third instar grubs of *C. zastrowi sillemi*, all within one day of molting, were utilized per treatment. These grubs were positioned on potted cabbage plants and treated via a handheld sprayer, ensuring complete runoff. Subsequently, all treated larvae (late second to early third instar stage) from the various treatment groups were individually placed in sterilized plastic containers. Following this, each *C. zastrowi sillemi* grub in their respective treatments was supplied with second or third-instar DBM larvae, treated with concentrations corresponding to LC_{25}, LC_{35}, LC_{45}, and untreated DBM larvae. The mortality of *C. zastrowi sillemi* was documented at intervals of 24, 48, and 72 hours after treatment. Furthermore, observations were made on larval and pupal periods, the number of pupated grubs, and the emerging adults. Percentages for pupation and adult emergence were calculated based on these observations.

2.5. STATISTICAL ANALYSIS

All the experiments were conducted under Factorial Completely Randomized Design (FCRD). The per cent mortalities in laboratory studies were transformed to arcsine percentage. Data subjected to statistical analysis using SPSS for Windows (version 22) to carry out ANOVA. The mean values of treatments were then separated by Duncan's Multiple Range Test (DMRT). The mortality data were subjected to chi-square test and Probit analysis using SPPS software and estimated the lethal concentration (LC_{25}, LC_{35} and LC_{45}), and their associated confidence intervals [8].

3. RESULTS AND DISCUSSION

3.1 Effect of sublethal doses of Chitosan-O-Arginine on *C. zastrowi sillemi* mortality

Probit analysis revealed that the LC_{25}, LC_{35}, and LC_{45} values for Cs-O-Arg against DBM were 334.80 ppm, 395.78 ppm, and 459.95 ppm, respectively (Table 1). The chi-square analysis confirmed a good fit. The mean mortality percentage in Cs-O-Arg treated *C. zastrowi sillemi*, fed with both treated and untreated DBM larvae, is detailed in Table 2. The findings indicated that nearly all sublethal concentrations of Cs-O-Arg induced larval mortality in *C. zastrowi sillemi*. Table 3 represents mean grub period and mean pupal period of *C. zastrowi sillemi*. While, mean per cent pupation and adult emergence of *C. zastrowi sillemi* is represented in Table 4. The results are documented here under according to the type of exposures.

Table 1. Probit analysis with 95% confidence limits for second instar larvae of *P. xylostella* against Cs-O-Arg

Treatment	LC_{25} (ppm)	UL	LL	LC_{35} (ppm)	UL	LL	LC_{45} (ppm)	UL	LL	X^2 value	Y Intercept	R^2
Cs-O-Arg	334.80	448.72	249.81	395.78	502.56	311.7	459.95	559.22	378.31	7.33	Y=4.7303x-7.8015	0.8249

LC_{25}=Lethal Concentration that kills 25 per cent of the exposed larvae; LC_{35}=Lethal Concentration that kills 35 per cent of the exposed larvae; LC_{45}=Lethal Concentration that kills 45 per cent of the exposed larvae; χ^2: Chi Square, df: Degrees of freedom, UL: Upper Limit, LL: Lower Limits

3.1.1. Untreated *C. zastrowi sillemi* fed with untreated *P. xylostella*

Untreated *C. zastrowi sillemi* exhibited no mortality on any day after treatment when it was fed with untreated DBM larvae (Table 2). The mean grub and pupal period were 3.33 and 5.17 days respectively (Table 3). Pupation and adult emergence were also reached cent per cent at this type of exposure (Table 4).

3.1.2. Untreated *C. zastrowi sillemi* fed with treated *P. xylostella* (LC_{25}, LC_{35} and LC_{45})

When untreated *C. zastrowi sillemi* grubs were fed with treated DBM, no mortality was observed at 24 HAT whereas, 6.67 per cent mortality was observed on 48 and 72 HAT when the grubs were fed with treated DBM irrespective of all the concentrations (Table 2). The average grub and pupal period of *C. zastrowi sillemi* were 3.33 and 5.17 days, when LC_{45} treated DBM larvae was provided as feed (Table 3). Pupation and adult emergence per cent were comparatively higher than any other treatments when treated DBM larvae were utilized as diet. LC_{25} treated

DBM larvae when provided as feed for untreated *C. zastrowi sillemi* grubs, 90.00 per cent of the grub population turned to pupae and emerged as adults. While 80.00 per cent of the grubs metamorphized into pupa and 76.67 per cent of them emerged into adults, when LC_{45} treated DBM larvae were provided for the grubs (Table 4).

3.1.3. Treated *C. zastrowi sillemi* (LC_{25}, LC_{35} and LC_{45}) fed with untreated *P. xylostella*

C. zastrowi sillemi grubs, when treated with Cs-O-Arg at LC_{45} concentration, and fed with untreated DBM, a maximum rate of mortality (10.00%) arrived both on 48 and 72 HAT under this type of exposure. Next to it, 3.33 per cent mortality is achieved when LC_{45} and LC_{35} treated grubs were fed with untreated DBM larvae at 24 HAT. While no mortality was occurred in *C. zastrowi sillemi* grubs, when they were treated at LC_{25} concentration and fed with untreated DBM larvae (Table 2). The LC_{45} treated chrysoperla grubs which were fed with untreated DBM larvae completed third instar in 3.83 days and spent 5.83 days in pupal stage before adult emergence (Table 3). While the pupation and adult emergence rate were highest (90.00% and 86.67% respectively), when LC_{25} treated chrysoperla were fed with untreated DBM larvae (Table 4).

3.1.4. Treated *C. zastrowi sillemi* (LC_{25}, LC_{35} and LC_{45}) fed with treated *P. xylostella* (LC_{25}, LC_{35} and LC_{45})

C. zastrowi sillemi treated with LC_{45} and fed with LC_{45} treated DBM larvae, exhibited the highest mortality rate (30.00%) at 24 HAT. However, the LC_{25} treated *C. zastrowi sillemi* grubs displayed the lowest mortality rate (10%) among all treatments when LC_{25} treated DBM was provided for as sustenance. The maximum larval mortality (33.33%), which differs significantly from all other treatments was recorded in LC_{45} treated *C. zastrowi sillemi* grubs fed with LC_{45} treated DBM larvae, both on 24 and 48 HAT (Table 2). There was no significant difference in larval and pupal periods among the treatments in this type of exposure (Table 3). Both pupation and adult emergence reached 76.67 per cent in the treatment where LC_{25} treated *C. zastrowi sillemi* grubs fed with LC_{25} treated DBM larvae while the lowest values (46.67% and 43.33%) were depicted in the predator prey combination where both were treated with LC_{45} concentration (Table 4).

Table 2. Sublethal toxicity of Cs-O-Arg on *Chrysoperla zastrowii sillemi* (III instar grub) fed with treated and untreated *P. xylostella* (II & III instar larvae)

Cs-O-Arg treatments on Chrysoperla	Cs-O-Arg treatments on *P. xylostella*											
	Mean % mortality of Chrysoperla											
	At 24 HAT				At 48 HAT				At 72 HAT			
	LC_{25}	LC_{35}	LC_{45}	LC_0	LC_{25}	LC_{35}	LC_{45}	LC_0	LC_{25}	LC_{35}	LC_{45}	LC_0
LC_{25}	10.00 ab (18.43)	13.33 b (21.42)	20.00 b (26.57)	0.00 a (0.00)	10.00 ab (18.43)	16.67 ab (24.09)	20.00 b (26.57)	3.33 a (10.52)	13.33 ab (21.42)	16.67 ab (24.09)	23.33 b (28.88)	3.33 a (10.52)
LC_{35}	16.67 b (24.09)	23.33 bc (28.88)	26.67 b (31.09)	3.33 a (10.52)	20.00 ab (26.57)	20.00 c (26.57)	26.67 b (31.09)	3.33 a (10.52)	20.00 b (26.57)	23.33 b (28.88)	30.00 b (33.21)	3.33 a (10.52)
LC_{45}	20.00 b (26.57)	26.67 c (31.09)	30.00 b (33.21)	3.33 a (10.52)	23.33 b (28.88)	26.67 c (31.09)	33.33 b (35.26)	10.00 a (18.43)	23.33 b (28.88)	30.00 b (33.21)	33.33 b (35.26)	10.00 a (18.43)
Untreated	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	6.67 a (14.96)	6.67 a (14.96)	6.67 a (14.96)	0.00 a (0.00)	6.67 a (14.96)	6.67 a (14.96)	6.67 a (14.96)	0.00 a (10.52)

LC_{25}=Lethal Concentration that kills 25 per cent of the exposed larvae; LC_{35}=Lethal Concentration that kills 35 per cent of the exposed larvae; LC_{45}=Lethal Concentration that kills 45 per cent of the exposed larvae; LC_0=Lethal Concentration that kills 0 per cent of the exposed larvae; Means followed by a common letter in a column are not significantly different at p = 0.05 by DMRT; Figures in parentheses are arcsine √P transformed values

Table 3. Sublethal effect of Cs-O-Arg on the growth and developmental period of *C. zastrowii sillemi* (Mean ± SD)

Cs-O-Arg treatments on Chrysoperla	Cs-O-Arg treatments (DBM as prey)							
	Mean III instar grub period (days)				Mean pupal period (days)			
	LC_{25}	LC_{35}	LC_{45}	LC_0	LC_{25}	LC_{35}	LC_{45}	LC_0
LC_{25}	2.17±0.41	2.17±0.41	2.83±0.41	2.67±0.52	4.50±0.55	4.33±0.52	4.67±0.52	4.83±0.41
LC_{35}	2.50±0.55	2.67±0.52	3.33±0.52	2.83±0.75	4.17±0.41	4.50±0.55	4.50±0.55	4.67±0.52
LC_{45}	2.67±0.52	2.67±0.82	3.83±0.41	3.83±0.41	4.67±0.52	5.17±0.75	5.50±0.55	5.83±0.41
LC_0	2.50±0.55	2.67±0.52	3.33±0.52	3.33±0.52	4.33±0.52	4.83±0.41	5.17±0.75	5.17±0.41

LC_{25}=Lethal Concentration that kills 25 per cent of the exposed larvae; LC_{35}=Lethal Concentration that kills 35 per cent of the exposed larvae; LC_{45}=Lethal Concentration that kills 45 per cent of the exposed larvae; LC_0=Lethal Concentration that kills 0 per cent of the exposed larvae

Table 4. Sublethal effect of Cs-O-Arg on the pupation and adult emergence percentage of *C. zastrowii sillemi*

Cs-O-Arg treatments on Chrysoperla	Cs-O-Arg treatments on *P. xylostella*							
	Mean Pupation (%)				Mean Adult emergence (%)			
	LC_{25}	LC_{35}	LC_{45}	LC_0	LC_{25}	LC_{35}	LC_{45}	LC_0
LC_{25}	76.67 ab (61.12)	70.00 b (56.79)	66.67 b (54.74)	90.00 ab (71.57)	76.67 b (61.12)	66.67 b (56.79)	63.33 bc (52.73)	86.67 ab (68.58)
LC_{35}	66.67 a (54.74)	56.67 ab (48.83)	50.00 a (45.00)	86.67 ab (68.58)	66.67 ab (54.74)	53.33 ab (46.91)	50.00 ab (45.00)	83.33 ab (65.91)
LC_{45}	63.33 a (52.73)	50.00 a (45.00)	46.67 a (43.09)	80.00 a (63.43)	60.00 a (50.77)	50.00 a (45.00)	43.33 a (41.17)	76.67 a (61.12)
LC_0	90.00 b (71.57)	86.67 c (68.58)	80.00 b (63.43)	100.00 b (90.00)	90.00 c (71.57)	86.67 c (68.58)	76.67 c (61.12)	96.67 b (79.48)

LC_{25}=Lethal Concentration that kills 25 per cent of the exposed larvae; LC_{35}=Lethal Concentration that kills 35 per cent of the exposed larvae; LC_{45}=Lethal Concentration that kills 45 per cent of the exposed larvae; LC_0=Lethal Concentration that kills 0 per cent of the exposed larvae. Means followed by a common letter in a column are not significantly different at p = 0.05 by DMRT; Figures in parentheses are arcsine √P transformed values

Because synthetic chemicals may penetrate new ecosystems and disturb existing niches, their extensive use causes accidental damage to non-target organisms, directly or indirectly. As a result, it is critical to undertake predator safety studies to determine the selectivity of these compounds. Pesticides' sublethal effects on the physiology and behaviour of arthropods should also be studied in order to have a thorough knowledge of their overall impact.

Sublethal studies with Cs-O-Arg on *C. zastrowi sillemi* were done as part of these considerations. The chrysopid mortality was noticed in a range of 6.67 to 10.0 per cent, when either predator or prey was treated with CS-O-Arg at all the concentrations. But, the most significant grub mortality (33.33%) was seen when both the grubs and DBM larvae were treated consistently at LC_{45} concentration. These results are consistent with the findings of Moscardini *et al.* (2013) [9], who emphasized the importance of sublethal effects evaluation in proving that exposing flower bug *Orius insidiosus* eggs to the marine-origin insecticide, cartap hydrochloride at 0.00125 mg a.i. L^{-1} resulted in 59.09 per cent death. When feeding on aphids treated with various neem formulations, Ahmad *et al.* (2003) [10] reported significant mortality in first-instar larvae of *Coccinella septempunctata*, lowering the predator's foraging ability and longevity.

Similarly, Fogel *et al.* (2016) [11] investigated the selectivity of four insecticides (pyriproxyfen, teflubenzuron, acetamiprid, and cypermethrin) to *Eriopis connexa* Germar (Coccinellidae), a critical horticultural pest management agent. Their results showed that all substances had deadly or sublethal effects on the predator, with neurotoxic insecticides being more toxic than growth regulators and pupal stages being more vulnerable than adults. When exposed to cartap hydrochloride, the predatory assassin insect *Rhyncoris marginatus* died at a rate of 54.63 per cent [12]. Patel (2020). These earlier findings are consistent with the conclusions given in the current study.

4. CONCLUSION

This work is likely the first to show that a chitosan derivative insecticide negatively influences the survival of the commercially important insect predator *C. zastrowi sillemi* upon continuous exposure and feeding contaminated prey. These results have important implications for directing judgments about the safe use of pesticides in conjunction with *C. zastrowi sillemi* or other natural predators in integrated pest management strategies.

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