

BIOSAFETY OF POLYMER-BASED AZADIRACTIN-SILICA NANO-FORMULATION TO TRICHOGRAMMA PRETIOSUM AND CHRYSOPERLA ZASTROWI SILLEMI

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ABSTRACT

IPM strategies require sustainable alternatives to conventional chemical pesticides to minimise non-target risks while delivering similar efficacy. While botanical insecticides present a promising alternative, their field utility is limited by photodegradation and low persistence. To address this, an Azadirachtin-Silica-Chitosan nano-formulation was developed using mesoporous silica nanoparticles (MSNs) and characterised to assess particle size and stability. Before field adoption, we should evaluate the ecotoxicological impact of this nano-formulated biopesticide on beneficial arthropods. This study evaluated the impacts of the Azadirachtin-Silica-Chitosan nano-formulation on two key biocontrol agents: *Trichogramma pretiosum* and *Chrysoperla zastrowi sillemi*. Based on previous laboratory experiments conducted for thrips, this study utilized single and double concentrations of LC₅₀ and LC₉₀ (100, 200, 1250 and 2500 ppm). Bioassays assessed exposure effects on mortality and development compared with commercial Neemazal T/S (3 mL/L) and the insecticide cyantraniliprole 10.26 OD (1.8 mL/L). Results showed that at lower concentrations of 100 and 200 ppm, the nano-formulation was essentially harmless to the development of both biocontrol agents; however, at higher concentrations of 1250 and 2500 ppm, low to moderate toxicity was observed, highlighting the need for concentration management to protect non-target organisms. The higher doses have limited practical applicability, proving the safety of the nano-formulation towards non-target organisms at a field-applicable rate.

KEYWORDS: Azadirachtin, Silica nanoparticles, Nano-formulation, *Trichogramma pretiosum*, *Chrysoperla zastrowi sillemi*, Non-target toxicity.

1. INTRODUCTION

Modern agriculture faces the dual challenge of maximising crop productivity while minimising the ecological footprint of pest management. For decades, chemical pesticides have been the predominant method for controlling pests and pathogens, and the only control option for certain invasive pest species [1]; they are also widely accepted as part of IPM strategies that complement biological control [2]. Although reports of simultaneous effects yielding long-term control exist [3], their impact on non-target organisms, such as predators and parasitoids, is inevitable [4]. Arthropods are among the most ecologically relevant non-target organisms, occupying central positions as pollinators, predators, parasitoids and decomposers [5]. Consequently, this indiscriminate use of synthetic chemicals disrupts natural biological control, trapping farmers in a cycle of chemical dependency and secondary pest outbreaks.

To reduce these non-target effects, researchers have encouraged a shift toward botanical insecticides [6]. Among these, azadirachtin, a complex tetranorterpene from the neem tree, has emerged as one of the most potent and eco-friendly bioinsecticides, demonstrating its efficacy against major crop pests. However, field deployment is constrained by poor aqueous solubility, susceptibility to thermal and photolytic degradation, and low environmental persistence [7]. These drawbacks require frequent reapplications, limiting economic viability and operational consistency in sustainable crop protection.

To overcome the drawbacks of conventional pesticides and biopesticides, researchers used nanotechnology to engineer smart delivery systems. Developing slow-release formulations presents an effective strategy to improve performance and minimise non-target toxicity [8]. To enhance the durability and effectiveness of phytomolecules, integration of suitable carrier systems is essential. In this regard, mesoporous silica nanoparticles (MSNs) are promising carriers because of their thermal stability, high surface area, and compatibility with biological tissues [9].

Despite the advantages of these nano-formulated biopesticides, claims about the safety of nanopesticides for these non-target organisms should be scrutinised before advocacy [10]. Emerging ecotoxicological reports

indicate that these biopesticides are toxic to non-target organisms at levels below standard limits, calling into question their credibility relative to chemical pesticides [11,12]. Even nanoformulated biopesticides may threaten natural enemies in agricultural ecosystems. Although these nanoformulated botanical pesticides may fall on the lower end of the toxicity spectrum, studies should still evaluate their effects in depth on non-target organisms. This study assessed the impact of an azadirachtin-based nano-formulation on two key non-target insects: *Trichogramma pretiosum*, an endoparasitoid, and *Chrysoperla zastrowi sillemi* (Esben-Peterson), a predator in the agricultural ecosystem.

2. MATERIALS AND METHODS

2.1. Materials: Azadirachtin technical grade powder (40% purity) was obtained from Ecobiocides and Botanicals Pvt. Ltd., Tamil Nadu, India. All other chemicals and reagents used in the experiments were of analytical grade and procured from standard commercial suppliers. The colonies of *T. pretiosum* and *C. zastrowi sillemi* were procured from the Biocontrol Laboratory, Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, and used for the experiments.

2.2. Preparation and characterisation of nano-formulation: Azadirachtin - Silica - Chitosan nano-formulation was prepared by high-energy ultrasonication. The oil phase containing azadirachtin and surfactants was added dropwise to the aqueous phase containing silica nanoparticles and chitosan, with stirring for 3 hours, yielding a macroemulsion, followed by ultrasonication with a probe sonicator (LABMAN PRO900 – 25kHz, 650 W, 6 mm probe) in pulse mode for 15 minutes to obtain a nano-formulation. The nano-formulation was characterised via particle size, dispersity index and zeta potential using Dynamic Light Scattering (DLS) in a Malvern Zetasizer (Malvern Panalytical Ltd., UK).

2.3. Effect on adult emergence of *T. pretiosum*: The safety of the developed nano-formulation towards the parasitoid, *Trichogramma pretiosum*, was tested following the procedure given by Jalali and Singh [13], with modifications. The Azadirachtin - Silica - Chitosan nano-formulation was sprayed on parasitised egg cards using an atomiser, at single and double LC₅₀ (100 and 200 ppm) and LC₉₀ (1250 and 2500 ppm) concentrations obtained from a previous experiment (leaf-dip bioassay) conducted for thrips. Each treatment was replicated three times. After spraying, the egg cards were shade-dried for 10 minutes and placed in test tubes. These were compared with Neemazal T/S (3ml/L), cyantraniliprole 10.26 OD (1.8ml/L), and an untreated control. The number of parasitoids that emerged was recorded, and per cent adult emergence was calculated using the formula:

$$\text{Per cent emergence} = \frac{\text{Number of wasps emerged}}{\text{Total number of eggs}} \times 100$$

2.4. Effect on egg emergence, grub survival, pupation and adult emergence of *C. zastrowi sillemi*: The toxic effect of the developed nano-formulation on the eggs of *C. zastrowi sillemi* was assessed using the method described by Krishnamoorthy [14]. 30 stalked eggs/treatment of *C. zastrowi sillemi* were used for each replication, and each treatment was replicated three times. The nano-formulation was sprayed at 1X and 2X LC₅₀ (100 and 200 ppm) and LC₉₀ (1250 and 2500 ppm) concentrations using a hand atomiser. These were compared with Neemazal T/S (3ml/L), cyantraniliprole 10.26 OD (3.8ml/L), and a control sprayed with distilled water. The number of grubs hatched were observed and per cent hatchability was calculated.

$$\text{Per cent hatchability} = \frac{\text{Number of grubs hatched}}{\text{Total number of eggs}} \times 100$$

Third-instar grubs were sprayed with the formulation at the above-mentioned concentrations and provided with *Corcyra* eggs. We observed grub mortality at 6, 12, and 24 hours after treatment (HAT), and calculated per cent mortality. We also recorded observations on pupation and adult emergence.

$$\text{Per cent mortality} = \frac{\text{Number of dead grubs}}{\text{Total number of grubs}} \times 100$$

2.5. Statistical analysis: R studio (4.2.3) was used for analysis. The mortality data was subjected to arcsine transformation and Abbott's correction was carried out [15]. The significance of differences between treatment means was assessed using Tukey's Honest Significant Difference (HSD) test at p=0.05.

3. Results

3.1. Preparation and characterisation of nano-formulation: Azadirachtin – Silica – Chitosan nano-formulation was prepared and characterised to assess particle size and stability. The nano-formulation had a particle size of 156.5 nm, a polydispersity index (PDI) of 0.169, and a zeta potential of +75.8 mV (**Figure 1 (a) and (b)**).

3.2. Effect on adult emergence of *T. pretiosum*: At the lowest tested concentration (Single LC₅₀, 100 ppm) of Azadirachtin–Silica–Chitosan nano-formulation, adult emergence was 75.42%, reducing to 71.47% at double LC₅₀ and 55.77% at single LC₉₀ (1250 ppm) and at 2X concentration (2500 ppm), resulted in a minimum emergence rate of 40.06%. However, this was higher than emergence from cyantraniliprole 10.26 OD-treated eggs, which produced only 32.93% emergence. Neemazal T/S resulted in the minimum reduction over the control, with 79.53% emergence (**Figure 2**).

3.3. Effect on egg emergence of *C. zastrowi sillemi*: The nano-formulation produced a higher hatchability rate of 89.99% at 100 ppm, closely matching the Neemazal (93.33%) and control. Even at 200 ppm and 1250 ppm, egg hatchability dropped only moderately to 83.33% and 60.00%, respectively. However, at the maximum

exposure level (2500 ppm), egg hatchability dropped to 46.67%, showing slightly harmful effects. Eggs exposed to cyantraniliprole 10.26 OD showed the lowest hatching rate (36.66%) (**Table 1**) (**Figure 3**).

3.4. Effect on grub survival of *C. zastrowi sillemi*: Direct exposure to the Azadirachtin - Silica - Chitosan nano-formulation showed higher toxicity to grubs than to eggs; however, it remained within an acceptable range at lower concentrations. At 100 ppm, grub survival was 96.55%, on par with Neemazal T/S (98.55%), and declined concentration-dependently to 89.65% and 72.41% at 200 and 1250 ppm, and to 44.83% at 2500 ppm, becoming slightly harmful at 2500 ppm. Grubs' survival was recorded to be 37.93% when treated with cyantraniliprole 10.26 OD (**Table 2**) (**Figure 4**).

3.5. Effect on pupation and adult emergence of *C. zastrowi sillemi*: At 100 ppm of Azadirachtin–Silica–Chitosan nano-formulation, pupation was 89.36%, comparable to Neemazal T/S (89.56%); however, as the concentration of the nano-formulation increased, pupation dropped from 88.57% at 200 ppm and 80.77% at 1250 ppm to 76.92% at the double LC₉₀ (2500 ppm). While cyantraniliprole 10.26 OD suppressed pupation to 54.61%. For pupae developed under the Azadirachtin–Silica–Chitosan treatment, adult emergence ranged from 92.31% at 100 ppm to 69.45% at 2500 ppm, higher than cyantraniliprole 10.26 OD (66.67%). (**Table 2**) Adult malformations were observed at higher concentrations of the nanoformulation and cyantraniliprole 10.26 OD (**Figure 5 & 6**).

4. DISCUSSION

This study evaluated the effect of the Azadirachtin - Silica - Chitosan nano-formulation against two important natural enemies, *T. pretiosum* and *C. zastrowi sillemi*, and compared its impact with, Neemazal T/S, and cyantraniliprole 10.26 OD. The results showed a concentration-dependent effect on adult emergence of *T. chilonis*, as well as on egg hatchability, grub survival, pupation, and adult emergence of *C. zastrowi sillemi*.

Lower concentrations of the nano-formulation (100 and 200 ppm) showed low toxicity, comparable with Neemazal T/S and the control, while higher concentrations (1250 and 2500 ppm) showed low to moderate toxicity; however, they were less toxic than cyantraniliprole as per IOBC standards [16].

For *T. pretiosum*, at the pests' LC₅₀, the nano-formulation was statistically insignificant compared to the commercial Neemazal T/S, and retained more than 90% of the control emergence, agreeing with the classification of azadirachtin as harmless to trichogrammatids, with >80% emergence of *T. cacoeciae* [17], and 82.33% emergence of *T. exiguum* not significantly below control [18]. The reduction at double LC₉₀, however, exceeds any previous reports for conventional azadirachtin at comparable multiples and indicates that the delivery of the molecule in the silica-chitosan matrix prolongs or intensifies exposure developing inside the host eggs.

In *C. zastrowi sillemi*, a similar concentration-dependent toxicity pattern was observed. The resilience of the embryonic stage under the nanomatrix is consistent with the harmless classification of silica nanoparticles to lacewing eggs up to 10000 ppm [19], and with the lack of sustained hatchability effects over generations of chitosan-alginate exposure [20]. The higher toxicity to grubs reflects greater contact exposure for mobile larvae and aligns with azadirachtin's route-dependent hazard, which was mild by ingestion but harmful by residual contact in chrysopid larvae [21].

Pupation was sensitive to the treatments, while adult emergence from formed pupae was relatively high. This intact ecdysis but blocked larval-pupal transition is consistent with azadirachtin's inhibition of prothoracicotrophic hormones and disruption of larval-pupal moult [21]. The greater toxicity of the ternary nano-formulation than each component indicates a synergistic interaction, likely due to prolonged azadirachtin release from the silica-chitosan matrix.

5. CONCLUSION

Overall, the results show that chitosan to be the safest matrix, and the Azadirachtin - Silica - Chitosan nano-formulation requires concentration management to remain IPM compatible. All the treatments, including Neemazal T/S, stayed within harmless-to-slightly harmful categories at every endpoint, whereas cyantraniliprole was consistently the most harmful, supporting the nano-formulation as a less disruptive alternative that conserved *Trichogramma* and *Chrysoperla*. Extrapolation is needed to conduct semi-field validation and sublethal testing of the combined formulation before drawing field-level conclusions and their incorporation into IPM strategies.

CRedit authorship contribution statement

Soundarya Saravanan: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kannan Malaichamy:** Writing – review & editing, Conceptualization, Formal analysis, Supervision, Validation, Resources. **Soundararajan Raga Palanisamy, Harish Sankarasubramanian, Jayakanthan Mannu:** Writing – review & editing, Formal analysis, Validation.

Ethics approval and consent to participate

Not applicable.

Code Availability

Not applicable

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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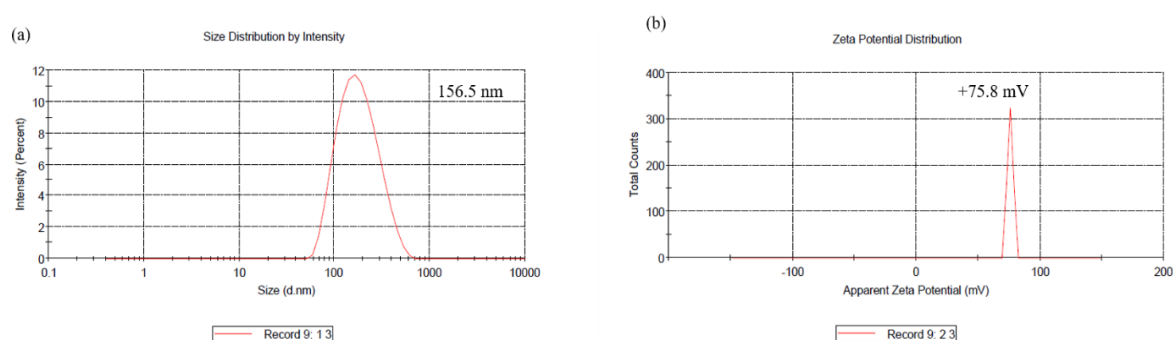


Figure 1. Azadirachtin – Silica – Chitosan nano-formulation: (a) Particle size and (b) zeta potential distribution

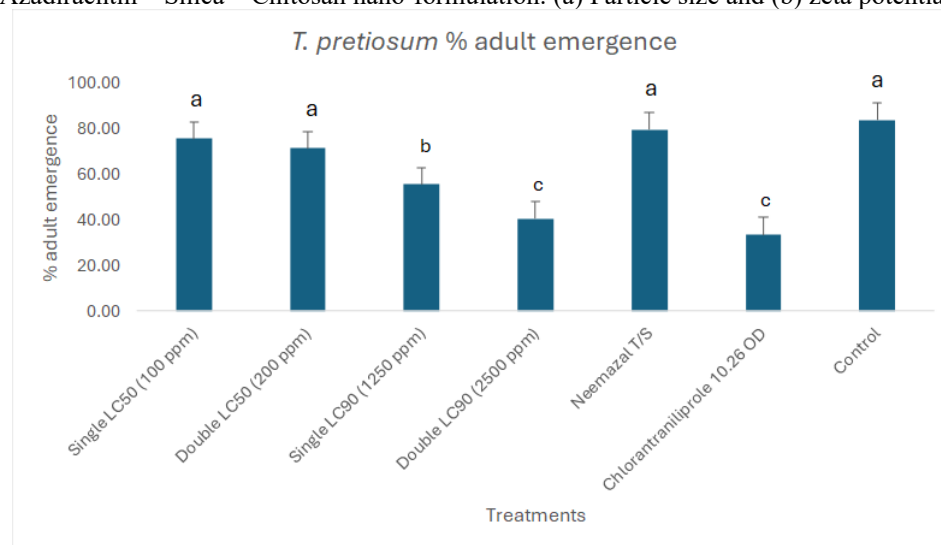


Figure 2. Effect of Azadirachtin – Silica – Chitosan nano-formulation on adult emergence of *T. pretiosum*

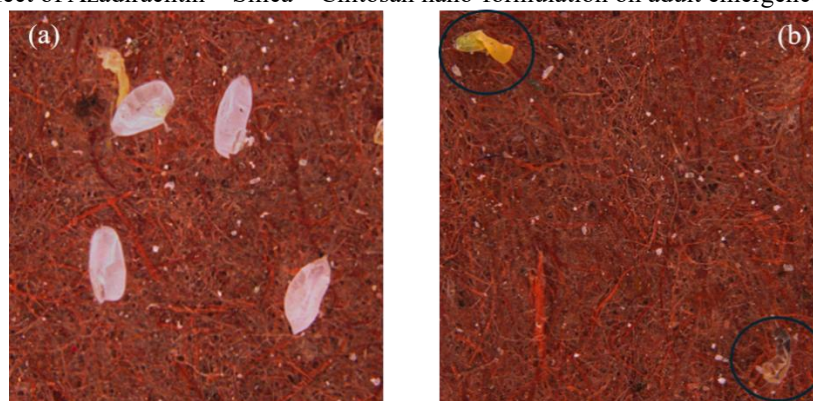


Figure 3. Effect of Azadirachtin – Silica – Chitosan nano-formulation (2500 ppm) on egg hatching of *C. zastrowi sillemi*: (a) hatched eggs (b) unhatched eggs

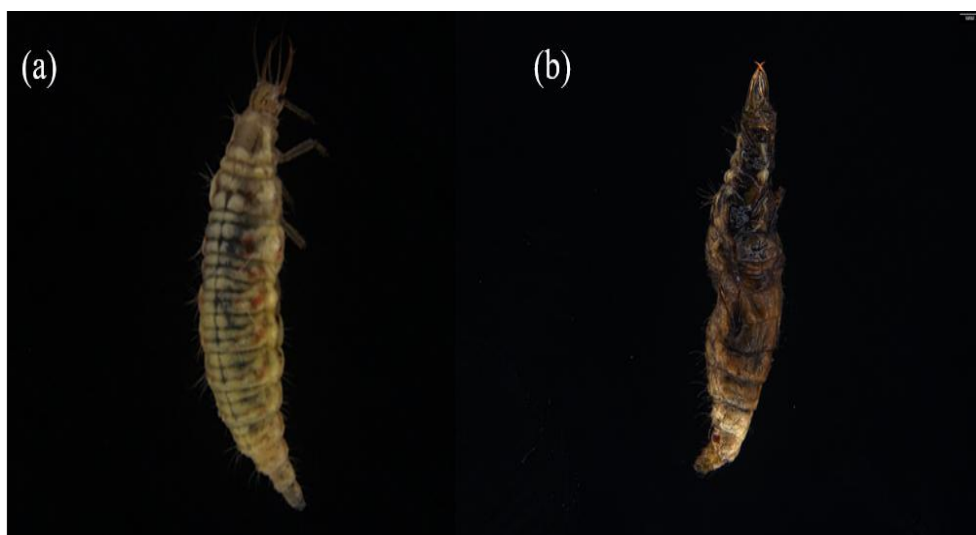


Figure 4. Effect of Azadirachtin – Silica – Chitosan nano-formulation (2500 ppm) on grub survival of *C. zastrowi sillemi*: (a) Normal grub (b) Dead grub



Figure 5. Effect of Azadirachtin – Silica – Chitosan nano-formulation (2500 ppm) on pupal development of *C. zastrowi sillemi*: (a) Normal pupa (b) Pupal case after adult emergence (c) Dead pupa with pupal case (d) Dead pupa without pupal case

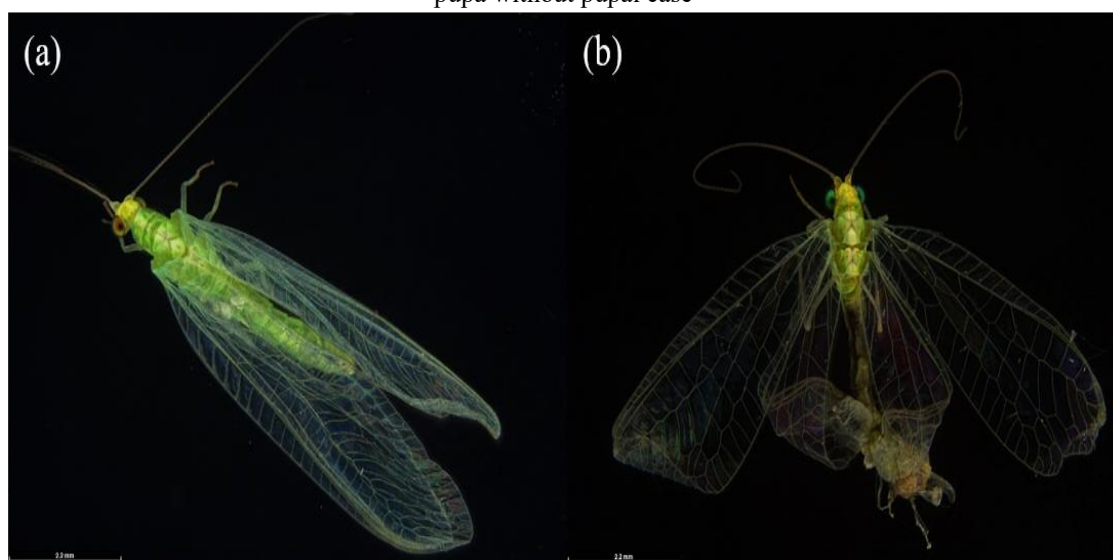


Figure 6. Effect of Azadirachtin – Silica – Chitosan nano-formulation on adult development of *C. zastrowi sillemi*: (a) Normal adult (b) Malformed adult

Table 1. Effect of Azadirachtin – Silica – Chitosan nano-formulation on egg hatchability of *C. zastrowi sillemi*

Treatments	No. of eggs	Total no. of eggs hatched (Nos.)	% Hatchability of eggs	% Reduction over control	Category
Single LC ₅₀ (100 ppm)	30	27 ^{ab} ±1.732	89.997 ^{ab} ±5.774	10.00	Harmless
Double LC ₅₀ (200 ppm)	30	25 ^b ±1.00	83.333 ^b ±3.335	16.67	Harmless
Single LC ₉₀ (1250 ppm)	30	18 ^c ±2.00	60.000 ^c ±6.670	40.00	Slightly harmful
Double LC ₉₀ (2500 ppm)	30	14 ^{cd} ±1.732	46.667 ^{cd} ±5.774	53.33	Slightly harmful
Neemazal T/S (3ml/L)	30	28 ^{ab} ±1.732	73.337 ^{ab} ±5.774	6.67	Harmless
Cyantraniliprole 10.26 OD (1.8 mL/L)	30	11 ^d ±2.00	36.667 ^d ±6.665	60.00	Slightly harmful
Control	30	30 ^a ±0.00	100.00 ^a ±0.00		
Sed		1.309	4.365		
MSD		4.471	14.904		

*Mean of three replications

*<30% mortality-harmless; 30-79 – Slightly harmful; 80-89 – Moderately harmful; >90 – Harmful

Table 2. Effect of Azadirachtin – Silica – Chitosan nano-formulation on grub survival of *C. zastrowi sillemi*

Treatments	Total number of insects	Grub mortality at 24 hours after exposure (%)	Grub survival (%)	Pupation %	Adult emergence (%)	Category
Single LC ₅₀ (100 ppm)	30	3.450 ^c ±3.450	96.550 ^a ±3.450	89.367 ^{ab} ±5.682	92.310 ^{ab} ±7.690	Harmless
Double LC ₅₀ (200 ppm)	30	10.343 ^c ±5.970	89.657 ^a ±5.970	88.570 ^{ab} ±3.184	86.940 ^{ab} ±0.568	Harmless
Single LC ₉₀ (1250 ppm)	30	27.587 ^b ±3.445	72.413 ^b ±3.445	80.770 ^b ±5.682	76.453 ^{ab} ±5.110	Harmless
Double LC ₉₀ (2500 ppm)	30	55.173 ^a ±6.895	44.827 ^c ±6.895	77.017 ^b ±6.805	69.447 ^{ab} ±4.809	Slightly harmful
Neemazal T/S (3ml/L)	30	1.150 ^c ±1.992	98.550 ^a ±1.992	89.567 ^{ab} ±3.811	96.230 ^a ±3.587	Harmless
Cyantraniliprole 10.26 OD (1.8 mL/L)	30	62.070 ^a ±5.976	37.930 ^a ±5.976	54.617 ^c ±5.044	66.032 ^b ±5.740	Slightly harmful
Control	30	0.000 ^c ±0.000	100.00 ^a ±0.000	96.553 ^a ±5.970	96.550 ^a ±3.450	

Sed		3.737	3.737	4.52		
MSD		12.76	12.76	15.434		

*Mean of three replications

*<50% mortality-harmless; 50-79 – Slightly harmful; 80-89 – Moderately harmful; >90 – Harmful