

Comparative toxicity of biopesticides, synthetic insecticides and fungicides to mycophagous predator *Illeis cincta* F. (Coleoptera: Coccinellidae)

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Abstract

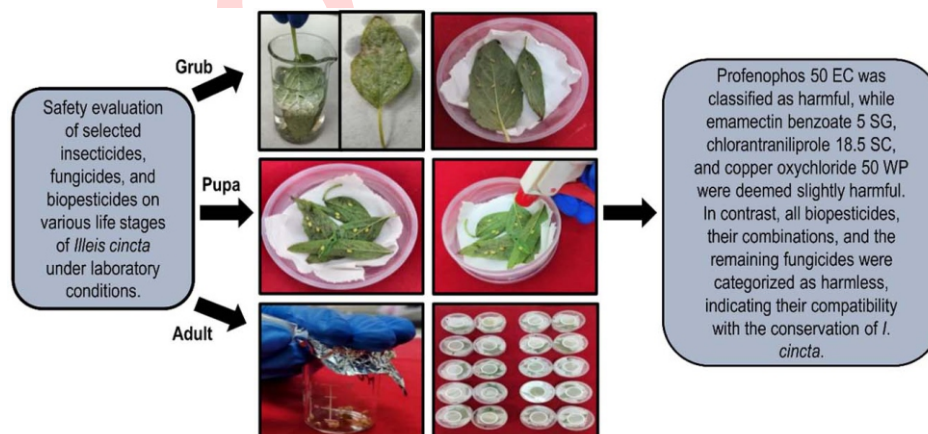
Aim: The present investigation was undertaken to evaluate the comparative toxicity of selected insecticides, fungicides, and biopesticides on different life stages of *Illeis cincta* (Coleoptera: Coccinellidae) a mycophagous predator.

Methodology: Bioassays were conducted under laboratory conditions (27±2°C, 60-70% RH) using a Completely Randomized Design (CRD) with thirteen treatments, each replicated thrice. Leaf-dip method was employed for 2nd instar grubs, topical application for pupae and the dry film residue method for adults. Mortality was recorded over five days and categorized according to IOBC/WPRS toxicity classification.

Results: Profenophos 50 EC was classified as harmful (>99% mortality), causing complete mortality in adults and high mortality in 2nd instar grubs and pupae. Emamectin benzoate 5 SG, chlorantraniliprole 18.5 SC, and copper oxychloride 50 WP were categorized as slightly harmful (30-79% mortality). In contrast, difenoconazole 25 EC, cymoxanil 8 + mancozeb 64 WP, *Bacillus thuringiensis* var. *kurstaki* (Bt-127 SC), *Beauveria bassiana* SC, *Metarhizium rileyi* (1×10⁸ CFU ml⁻¹), *Bacillus thuringiensis* var. *kurstaki* + *Beauveria bassiana* SC, *Bacillus thuringiensis* var. *kurstaki* + *Metarhizium rileyi* SC, and azadirachtin 0.03 EC were rated harmless (<30% mortality), with no significant adverse effects at any life stage of *I. cincta*.

Interpretation: These findings highlight the ecological safety of biopesticides and selective fungicides as viable alternatives for pest management. Their use can contribute to the conservation of beneficial predators like *I. cincta* and reduce dependence on broad-spectrum chemical pesticides in agroecosystems.

Key words: Bioassay, Biopesticides, Fungicides, *Illeis cincta*, Insecticides, Toxicity



Introduction

Powdery mildew fungi, classified under the family *Erysiphaceae* and order *Erysiphales*, comprise over 900 species across more than 80 genera (Braun, 2011). These fungi are obligate biotrophs, dependent on living host cells for nutrition and reproduction without inducing direct cell death (Vogel et al., 2004). Powdery mildew is among the most prevalent plant diseases globally, affecting a wide range of crops, including cereals, vegetables, fruits, oilseeds, legumes, and ornamentals (Liu and Braun, 2022). Currently, powdery mildew management largely depends on resistant crop varieties and chemical fungicides. However, the effectiveness of resistance breeding is being challenged by the emergence of new pathogen races (Kim et al., 2020). Additionally, the prolonged and intensive use of fungicides has led to resistance development (Vielba-Fernández et al., 2020) and environmental concerns due to residual accumulation (Qin et al., 2021). As a sustainable alternative, biological control has emerged as a promising approach for suppressing powdery mildew through the use of antagonistic organisms (Sarhan et al., 2020). These strategies reduce chemical inputs and support agroecological stability (Tsegaye et al., 2018).

Among biocontrol agents, members of the family Coccinellidae (Coleoptera), commonly known as ladybird beetles, are well-known predators of soft-bodied insect pests such as aphids, mealybugs, whiteflies, mites, and lepidopteran eggs (Sarwar, 2016). In addition to their predatory role, certain coccinellids exhibit mycophagy - feeding on powdery mildew fungi - and thus serve as an effective biocontrol agents (Sutherland and Parrella, 2009). Notably, species within the tribe Psyllorborini are obligate mycophages, feeding exclusively on conidia and hyphae of powdery mildew throughout all mobile life stages (Ahmad et al., 2003). This behaviour is believed to have evolved from coccidophagous ancestors (Giorgi et al., 2009; Lundgren, 2009). Among these, *Illeis cincta* Fabricius (Coleoptera: Coccinellidae) has been identified as a prominent natural enemy of powdery mildew in several cropping systems. It has been reported on linseed (Prasad and Rai, 1988), niger (Dharpur et al., 1990), sunflower (Jagadish et al., 2006), mulberry (Krishnakumar and Maheswari, 2004), red gram and castor (Bhuvaneshwari et al., 2006), and tree species such as *Dalbergia sissoo* and *Xanthium strumarium* (Thite et al., 2013).

Recent reports have also documented its presence on cluster bean, pigeon pea (Dola and Korat, 2016), okra (Roy and Raghavender, 2024) and eggplant (Ekanayake et al., 2025). Due to its broad host range, wide distribution, and strong feeding efficiency, *I. cincta* is considered an important biocontrol agent against powdery mildew (Krishnakumar and Maheswari, 2004; Patankar et al., 2009; Dharshini and Jagadish, 2018). However, the widespread use of synthetic pesticides in agriculture poses a potential threat to non-target beneficial organisms, including *I. cincta*. Given its ecological role and frequent occurrence on powdery mildew-infected crops, it is essential to assess the impact of pest management inputs on its survival and

functionality. Therefore, the present study aimed to evaluate the effects of commonly used insecticides, fungicides, and biopesticides on the survival of different life stages of *I. cincta* under laboratory conditions.

Materials and Methods

A laboratory bioassay was conducted during rabi 2025 in the Crop Protection Section of ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, under controlled environmental conditions ($27 \pm 2^\circ\text{C}$ and 60-70% RH). Sesame plants were raised in pots, and powdery mildew spores were dusted onto the leaves to initiate infection. Field-collected adults of *Illeis cincta* were subsequently released onto the infected plants for culture maintenance. For taxonomic confirmation, representative specimens were sent to a coccinellid taxonomic expert at the ICAR-National Research Centre for Banana (ICAR-NRCB), Tiruchirapalli. Various developmental stages of *I. cincta* were examined and documented using a stereozoom microscope (ZEISS Stereo Discovery V20).

The experiment was laid out in a Completely Randomized Design (CRD) with thirteen treatments, each replicated thrice. The doses of insecticides, fungicides, and biopesticides were selected based on their respective recommended field dosages. The treatment details are as follows: T₁ - Profenophos 50 EC (2 ml l⁻¹), T₂ - Emamectin benzoate 5 SG (0.4 g l⁻¹), T₃ - Chlorantraniliprole 18.5 SC (0.3 ml l⁻¹), T₄ - Cymoxanil 8 + Mancozeb 64 WP (2.5 g l⁻¹), T₅ - Difenconazole 25 EC (1 ml l⁻¹), T₆ - Copper oxychloride 50 WP (3 g l⁻¹), T₇ - *Bacillus thuringiensis* var. *kurstaki* (Bt-127 SC, 3 ml l⁻¹), T₈ - *Beauveria bassiana* SC (1 ml l⁻¹), T₉ - *Metarhizium rileyi* (1×10^8 CFU ml⁻¹), T₁₀ - *Bacillus thuringiensis* var. *kurstaki* + *Beauveria bassiana* SC (3 ml l⁻¹), T₁₁ - *Bacillus thuringiensis* var. *kurstaki* + *Metarhizium rileyi* SC (3 ml l⁻¹), T₁₂ - Azadirachtin 0.03 EC (300 ppm, 5 ml l⁻¹), and T₁₃ - untreated control. The combined formulations of *Bacillus thuringiensis* var. *kurstaki* + *Beauveria bassiana* SC and *Bacillus thuringiensis* var. *kurstaki* + *Metarhizium rileyi* SC were ready-to-use storable formulations developed and patented (Patent No. 315134) by Vimala Devi et al. (2020).

Bioassay techniques: To evaluate treatment effects on the 2nd instar grub stage, the leaf dip method described by Manimegalai (2008) was used. Powdery mildew-infected sesame leaves were dipped in the respective treatment solutions, air-dried, and placed in plastic containers (9 × 4 cm). Ten 2nd instar grubs were released per container and fed on the treated leaves. For adults, dry film residue method described by Wu et al. (2018) was followed. Glass vials of 40 ml capacity were uniformly coated with 0.5 ml of treatment solution and rotated manually to distribute the solution evenly. After drying, ten adults were introduced into each vial and covered with muslin cloth. Following one hour of exposure, the adults were transferred into plastic containers (9 × 4 cm) and fed with fresh mildew-infected leaves daily. Mortality data was recorded daily for five consecutive days and calculated by the formula given below.

Mortality (%) = (Number of dead grubs/adults ÷ Total number of grubs/adults) x 100.

The impact of treatments on pupae was assessed using the topical application method described by Fogel *et al.* (2016). Ten one-day-old pupae were placed on sesame leaves inside plastic containers (9 × 4 cm) and sprayed using an atomizer to ensure uniform coverage. After air drying for one hour, the containers were maintained under controlled conditions. Observations were recorded daily, and pupal mortality was determined based on failure of adult emergence. Based on the observed mortality, treatments were classified according to the IOBC/WPRS (International Organisation for Biological Control, West Palaearctic Regional Section) Guidelines (Skouras *et al.*, 2023) into the following classes: harmless (<30% mortality), slightly harmful (30-79% mortality), moderately harmful (80-99% mortality) and harmful (>99% mortality).

Statistical analysis: Percentage mortality data were corrected by substituting 0% and 100% values with (1/4n) and (100 – 1/4n), respectively, prior to arcsine transformation. The transformed data were subjected to ANOVA, and the treatment means were compared using the Least Significant Difference (LSD) test at 5% level of significance.

Results and Discussion

The developmental stages and key morphological features of *Illeis cincta* observed under laboratory conditions are illustrated in Fig. 1. The effect of various treatments on the 2nd

instar grub stage of *I. cincta* was evaluated at 24, 48, 72, 96 and 120 hrs after treatment (HAT), and significant differences in mortality were observed across treatments (Table 1). Among the tested compounds, profenophos 50 EC (2 ml l⁻¹) was categorized as moderately harmful (80-99% mortality), causing 93.3% mortality by 120 HAT. This aligns with the study of Nidheesh *et al.* (2020), who reported substantial toxicity to *Cryptolaemus montrouzieri*, confirming its unsuitability in conservation biological control programs, which can be attributed to its organophosphate mode of action involving acetylcholinesterase inhibition, leading to rapid neuromuscular disruption and mortality. Emamectin benzoate 5 SG (0.4 g l⁻¹) caused 70.0% mortality and chlorantraniliprole 18.5 SC (0.3 ml l⁻¹) caused 43.3%, both falling under the slightly harmful category (30-79% mortality). These results are in agreement with the reports of Kares *et al.* (2017) and Solangi *et al.* (2007), who reported moderate toxicity of emamectin benzoate to various coccinellids, and with Cong *et al.* (2023), who observed comparable effects of chlorantraniliprole in *Coccinella septempunctata*, which may be attributed to the neurotoxic action of emamectin benzoate on GABA- and glutamate-gated chloride channels and disruption of calcium homeostasis by chlorantraniliprole through ryanodine receptor activation, resulting in impaired muscle function and reduced survival.

In contrast, all the tested biopesticides-*Bacillus thuringiensis* var. *kurstaki* (Bt-127 SC) (3 ml l⁻¹), *Beauveria bassiana* SC (1 ml l⁻¹), *Metarhizium rileyi* (1×10⁸ CFU ml⁻¹) and azadirachtin 0.03 EC (300 ppm, 5 ml l⁻¹) - caused no mortality and were classified as harmless (<30% mortality). These findings are

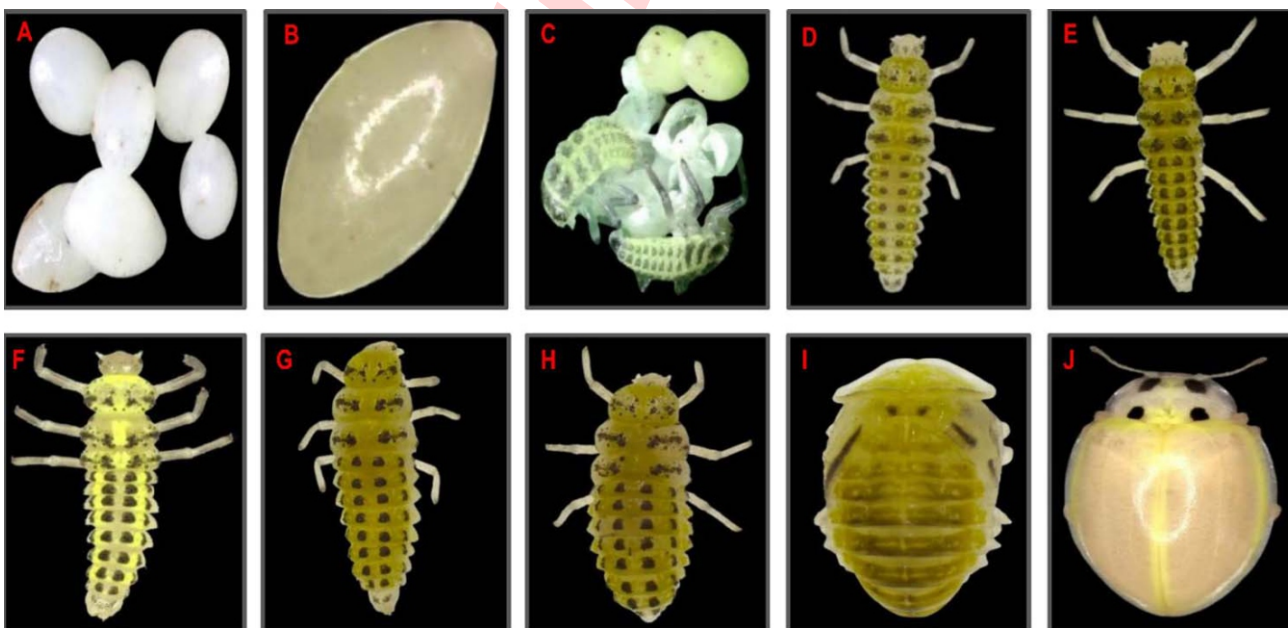


Fig. 1: Developmental stages of the mycophagous predator *Illeis cincta*: (A) Egg mass; (B) Single egg; (C) Newly hatched grub; (D) First instar; (E) Second instar; (F) Third instar; (G) Fourth instar; (H) Prepupa; (I) Pupa; (J) Adult.

Table 1: Effect of different insecticides, fungicides, and biopesticides to 2nd instar grubs of *Illeis cincta* under laboratory conditions

Treatments	Concentration	Grub mortality (%)				
		24HAT	48HAT	72HAT	96HAT	120HAT
T ₁ : Profenophos 50 EC	2 ml l ⁻¹	83.3 (66.1) ^a	83.3 (66.1) ^a	86.7 (69.0) ^a	90.0 (71.5) ^a	93.3 (77.4) ^a
T ₂ : Emamectin benzoate 5 SG	0.4 g l ⁻¹	43.3 (41.1) ^b	50.0 (45.0) ^b	56.7 (49.0) ^b	66.7 (54.8) ^b	70.0 (57.0) ^b
T ₃ : Chlorantraniliprole 18.5 SC	0.3 ml l ⁻¹	20.0 (27.0) ^c	30.0 (33.2) ^c	30.0 (33.2) ^c	33.3 (35.2) ^c	43.3 (41.1) ^c
T ₄ : Cymoxanil 8 + Mancozeb 64 WP	2.5 g l ⁻¹	3.3 (6.8) ^d	6.7 (12.6) ^d	10.0 (18.4) ^d	13.3 (21.1) ^e	16.7 (24.0) ^e
T ₅ : Difenconazole 25 EC	1 ml l ⁻¹	0.0 (0.9) ^d	3.3 (6.8) ^{de}	6.7 (12.6) ^d	10.0 (18.4) ^e	13.3 (21.1) ^e
T ₆ : Copper oxychloride 50 WP	3 g l ⁻¹	3.3 (6.8) ^d	6.7 (12.6) ^d	10.0 (18.4) ^d	20.0 (27.0) ^d	30.0 (33.0) ^d
T ₇ : <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> (Bt-127 SC)	3 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
T ₈ : <i>Beauveria bassiana</i> SC	1 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
T ₉ : <i>Metarhizium rileyi</i>	1×10 ⁸ CFU ml ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
T ₁₀ : <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> + <i>Beauveria bassiana</i> SC	3 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
T ₁₁ : <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> + <i>Metarhizium rileyi</i> SC	3 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
T ₁₂ : Azadirachtin 0.03 EC (300 ppm)	5 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
T ₁₃ : Untreated check	-	0.0 (0.9) ^d	0.0 (0.9) ^e	0.0 (0.9) ^e	0.0 (0.9) ^f	0.0 (0.9) ^f
SEm±	-	2.5	3.1	2.3	1.1	2.5
CD (p=0.05)	-	7.2	8.9	6.7	3.2	7.2

Figures in parentheses are arcsine transformed values, In a column means followed by a common letter are not significantly different at $p = 0.05$ by LSD, HAT - hours after treatment

consistent with the previous reports of Basappa *et al.* (2007) who demonstrated the safety of Bt on coccinellids, while Smith and Krischik (2000) confirmed the safety of azadirachtin and *B. bassiana*. The safety of *B. bassiana* was further supported by Sayed *et al.* (2021) and Kares *et al.* (2017). Although Trizelia *et al.* (2017) noted species-specific sensitivity to *Metarhizium* spp. in *Menochilus sexmaculatus*, *M. rileyi* showed no adverse effects on *I. cincta* in the present study, which corroborates with the findings of Bayissa *et al.* (2016), who reported only 7.5% mortality in *Cheilomenes lunata*. Combination treatments (*Bacillus thuringiensis* var. *kurstaki* + *Beauveria bassiana* SC @ 3 ml l⁻¹ and *Bacillus thuringiensis* var. *kurstaki* + *Metarhizium rileyi* SC @ 3 ml l⁻¹) also caused no mortality, reinforcing their compatibility with predator conservation strategies. Their safety can be attributed to their selective and ingestion- or growth-mediated modes of action, host specificity, and absence of direct neurotoxic effects, which limit acute toxicity to predatory coccinellids such as *I. cincta*.

Among the fungicides, difenoconazole 25 EC (1 ml l⁻¹) and cymoxanil 8 + mancozeb 64 WP (2.5 g l⁻¹) induced less than 17.0% mortality and were deemed harmless (<30% mortality), while copper oxychloride 50 WP (3 g l⁻¹) caused 30.0% mortality, classifying it as slightly harmful (30-79% mortality). These findings align with Zeleny *et al.* (1988), who reported a low impact of selective fungicides on coccinellids. However, greater sensitivity to copper-based fungicides as reported by Lo (2004) and Vostrel *et al.* (2013) supports the slightly elevated mortality observed in the current study, which may be attributed to the non-specific release of copper ions causing oxidative stress and cellular toxicity, unlike other fungicides that primarily target fungal

metabolic pathways without direct neurotoxic effects on insects. The toxicity of treatments was also assessed on pupal and adult stages (Table 2). In the pupal stage, profenophos 50 EC (2 ml l⁻¹) and emamectin benzoate 5 SG (0.4 g l⁻¹) caused 50.0% and 36.7% mortality, respectively - both falling in the slightly harmful category (30-79% mortality). Chlorantraniliprole 18.5 SC (0.3 ml l⁻¹) caused 23.3% mortality and was considered harmless (<30% mortality). All biopesticides and their combinations again resulted in no mortality, confirming their safety, consistent with the previous findings of Smith and Krischik (2000), Bayissa *et al.* (2016), and Sayed *et al.* (2021).

Among the fungicides, pupal mortality ranged from 3.3% (difenconazole 25 EC @ 1 ml l⁻¹) to 13.3% (copper oxychloride 50 WP @ 3 g l⁻¹), all within the harmless category (<30% mortality). These findings are in line with Zeleny *et al.* (1988), although Lo (2004) and Vostrel *et al.* (2013) noted higher toxicity from copper-based fungicides in other coccinellids. In adults, exposure to profenophos 50 EC (2 ml l⁻¹) resulted in complete mortality (100.0%) within 24 hours and was accordingly categorized as harmful (>99% mortality). Emamectin benzoate 5 SG @ 0.4 g l⁻¹ (56.7%) was slightly harmful (30-79% mortality), while chlorantraniliprole 18.5 SC @ 0.3 ml l⁻¹ (26.7%) remained harmless (<30% mortality). These patterns are supported by Solangi *et al.* (2007) and Cong *et al.* (2023), who reported similar trends in other coccinellids. Again, all biopesticides and their combinations caused no mortality, reaffirming their harmless classification (<30% mortality). Fungicides caused mortality ranging from 10.0 to 23.3%, also falling within the harmless range (<30% mortality). Overall, the tested insecticides, fungicides and

Table 2: Effect of different insecticides, fungicides, and biopesticides to the pupal and adult stages of *Illeis cincta* under laboratory conditions

Treatments	Concentration	Adult mortality (%)				
		24HAT	48HAT	72HAT	96HAT	120HAT
T ₁ : Profenophos 50 EC	2 ml l ⁻¹	100.0 (89.1) ^a	100.0 (89.1) ^a	100.0 (89.1) ^a	100.0 (89.1) ^a	100.0 (89.1) ^a
T ₂ : Emamectin benzoate 5 SG	0.4 g l ⁻¹	40.0 (39.1) ^b	46.7 (43.1) ^b	50.0 (45.0) ^b	56.7 (48.9) ^b	56.7 (48.9) ^b
T ₃ : Chlorantraniliprole 18.5 SC	0.3 ml l ⁻¹	20.0 (27.0) ^c	23.3 (28.8) ^c	26.7 (31.0) ^c	26.7 (31.0) ^c	26.7 (31.0) ^c
T ₄ : Cymoxanil 8 + Mancozeb 64 WP	2.5 g l ⁻¹	3.3 (6.8) ^d	10.0 (18.4) ^d	13.3 (21.1) ^d	20.0 (27.0) ^{cd}	23.3 (29.0) ^c
T ₅ : Difenoconazole 25 EC	1 ml l ⁻¹	3.3 (6.8) ^d	3.3 (6.8) ^{ef}	3.3 (6.8) ^{ef}	6.7 (12.6) ^e	10.0 (18.4) ^d
T ₆ : Copper oxychloride 50 WP	3 g l ⁻¹	3.3 (6.8) ^d	6.7 (12.6) ^{de}	10.0 (15.3) ^{de}	16.7 (20.2) ^{de}	20.0 (26.1) ^c
T ₇ : <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> (Bt-127 SC)	3 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
T ₈ : <i>Beauveria bassiana</i> SC	1 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
T ₉ : <i>Metarhizium rileyi</i>	1 × 10 ⁸ CFU ml ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
T ₁₀ : <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> + <i>Beauveria bassiana</i> SC	3 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
T ₁₁ : <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> + <i>Metarhizium rileyi</i> SC	3 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
T ₁₂ : Azadirachtin 0.03 EC (300 ppm)	5 ml l ⁻¹	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
T ₁₃ : Untreated check	-	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^d	0.0 (0.9) ^e
SEM±		3.0	2.6	3.0	3.4	1.8
CD (p=0.05)		8.6	7.6	8.6	9.9	5.3

Figures in parentheses are arcsine transformed values, in a column means followed by a common letter are not significantly different at p = 0.05 by LSD, HAT - hours after treatment.

biopesticides exhibited varying levels of toxicity to different life stages of *I. cincta* under laboratory conditions.

Profenophos 50 EC (2 ml l⁻¹) emerged as the most toxic, causing complete adult mortality and high mortality in grubs and pupae, and was thus classified as harmful (>99% mortality). Emamectin benzoate 5 SG (0.4 g l⁻¹), chlorantraniliprole 18.5 SC (0.3 ml l⁻¹), and copper oxychloride 50 WP (3 g l⁻¹) were classified as slightly harmful, as they caused 30-79% mortality in one or more stages. In contrast, all tested biopesticides, including *Bacillus thuringiensis* var. *kurstaki* (Bt-127 SC) (3 ml l⁻¹), *Beauveria bassiana* SC (1 ml l⁻¹), *Metarhizium rileyi* (1×10⁸ CFU ml⁻¹), azadirachtin 0.03 EC (300 ppm, 5 ml l⁻¹) and their combinations, along with fungicides such as difenoconazole 25 EC (1 ml l⁻¹) and cymoxanil 8 + mancozeb 64 WP (2.5 g l⁻¹), consistently caused less than 30% mortality, and were thus classified as harmless.

These findings reinforce the potential of biopesticides and selective fungicides as ecologically safer alternatives. Their use in pest management programs can support the conservation of natural enemies such as *I. cincta*, contributing to more sustainable and environmentally responsible crop protection strategies.

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