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Selectivity of entomopathogenic fungi Metarhizium anisopliae isolate IBCB 425 (Hypocreales: Clavicipitaceae) on the predatory lacewings Ceraeochrysa cincta (Schneider, 1851) and Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae)

Ana Paula Ferreira
Universidade Estadual de Maringá
Bruno Vinícius Daquila
bv.ds@hotmail.com
Universidade Estadual de Maringá
Elton Luiz Scudeler
Universidade Estadual Paulista (Unesp)
Natieli Jenifer Mateus-Corniani
Universidade Estadual de Maringá
Caroline Fetzner
Universidade Estadual de Maringá
Edmar Antônio Correia
Santa Terezinha Sugar Mill
Adriana Aparecida Sinópolis Gigliolli
Universidade Estadual de Maringá
Julio Cesar Polonio
Universidade Estadual de Maringá
Helio Conte
Universidade Estadual de Maringá

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Abstract

Metarhizium anisopliae is widely used in biological control programs against insect pests, although information on its compatibility with natural enemies remains limited. This study evaluated the selectivity of *M. anisopliae* isolate IBCB 425 (Hypocreales: Clavicipitaceae) toward the predatory lacewings *Ceraeochrysa cincta* (Schneider, 1851) and *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). Larvae of both species were fed *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) eggs treated with 4×10^7, 8×10^7, or 4×10^8 conidia/mL until the prepupal stage. Larval mortality, pupal, adult, and egg inviability, oviposition, and midgut morphology were evaluated using statistical, histological, and ultrastructural analyses. Under the experimental conditions, exposure to *M. anisopliae* did not cause detectable adverse effects. Larval mortality ranged from 4 to 8% in *C. cincta* and from 6 to 8% in *C. externa*. Pupal inviability ranged from 2 to 6% in both species, whereas adult inviability ranged from 6 to 8% in *C. cincta* and from 4 to 6% in *C. externa*. Egg inviability remained low, ranging from 0.92 to 2.53% in *C. cincta* and from 0.50 to 0.65% in *C. externa*, with no significant differences among treatments. Oviposition also did not differ from the control, and no histological or ultrastructural alterations were observed in the larval midgut. These findings indicate that *M. anisopliae* isolate IBCB 425 is selective toward both lacewing species, supporting its compatibility with these predators in integrated pest management programs.

1. INTRODUCTION

Microorganisms play a fundamental role in the development of more sustainable agricultural systems by promoting the growth of economically important crops while reducing the environmental impacts associated with conventional management practices (Wang et al. 2021; Mendéz-González et al. 2022; Lana et al. 2023; Tiwari and Park 2024; Li et al. 2025). Among their numerous applications, fungal bioinsecticides have gained widespread commercial use due to their ability to enhance nutrient availability and uptake by plants, as well as to provide effective control of insect pests (Daquila et al. 2021; Brancini et al. 2022; Iida et al. 2023; Panwar and Szczepaniec 2024; Sheng and Leger 2024; Gupta et al. 2025; Ferreira et al. 2026). Many of these products are based on *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), an entomopathogenic fungus widely used in the biological control of insect pests, whose selectivity toward non-target organisms depends on the fungal isolate and the biological characteristics of the host (Zimmermann 1993; Mascarin et al. 2019; Friuli et al. 2023; Shaukat et al. 2023; Zottele et al. 2023).

The infection process of *M. anisopliae* begins with conidial adhesion to the insect cuticle, followed by germination and appressorium formation. This specialized infection structure enables the fungus to secrete hydrolytic enzymes and secondary metabolites that facilitate host penetration and blastospore production within the hemocoel, ultimately resulting in insect immobilization and death (Li et al. 2022; Liu et al. 2023; Vidhate et al. 2023). Following host death, the fungus emerges through the cuticle and produces new conidia, whose dissemination is promoted by both biotic and abiotic factors. This

environmental persistence reduces the need for repeated bioinsecticide applications, thereby enhancing long-term pest management efficacy (Yasin et al. 2017; Shahbaz et al. 2024; Vivekanandhan et al. 2024).

The biocontroller potential of *M. anisopliae* has been demonstrated against *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), commonly known as the sugarcane borer, a major pest responsible for direct and indirect losses in sugarcane (*Saccharum* spp.) and other grasses (Leão et al. 2015; Oliveira et al. 2018; Silva et al. 2022; Rocha et al. 2024; Reis et al. 2025; Ferreira et al. 2026). However, agroecosystems infested by *D. saccharalis* also harbor a wide diversity of natural enemies, including parasitoids and predators, which play essential roles in regulating pest populations and maintaining ecological balance (Roubinet et al. 2017; Whitehouse et al. 2018; Keasar et al. 2023; Othim et al. 2024; Prévilee et al. 2024; Barascou et al. 2025; Kansman et al. 2025; Robert et al. 2025; Ye et al. 2025).

Among these beneficial organisms, green lacewings (Chrysopidae) are recognized as important polyphagous predators naturally occurring in a wide range of economically important crops (Jumbo et al. 2019; Botti et al. 2022; Guan et al. 2022; Scudeler et al. 2022; Li et al. 2023; Wang et al. 2023). Among the most relevant species are *Ceraeochrysa cincta* (Schneider, 1851) and *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) (Santos et al. 2020; Dami et al. 2023). During the larval stage, both species prey on eggs and early instars of several insect pests, making them highly effective biological control agents (Pappas et al. 2011).

During larval development, *C. cincta* and *C. externa* do not excrete metabolic waste, which accumulates in the posterior region of the midgut until adult emergence (Scudeler et al. 2020). This physiological characteristic results from the incomplete development of the hindgut during the larval stage, rendering it nonfunctional until adulthood (Scudeler et al. 2013, 2016; Daquila et al. 2023). Because these predators exhibit generalist feding habits, they frequently consume prey contaminated with toxins, microbial metabolites, or entomopathogenic microorganisms, leading to the accumulation of these agents within the digestive system (Daquila et al. 2023). Consequently, such accumulation may induce damage to the midgut or result in deleterious effects expressed during subsequent developmental stages, as previously reported (Scudeler et al. 2014, 2016, 2020; Souza et al. 2020; Caballero-Gallardo et al. 2023).

Although entomopathogenic fungi are generally regarded as highly selective biological control agents against insect pests, studies investigating their effects on non-target organisms remain scarce (Ngegba et al. 2022; Ahirwar and Singh 2023; Jiang and Wang 2023; Oliveira et al. 2024; Peñaflor et al. 2024). Therefore, assessing the selectivity of fungal bioinsecticides toward natural enemies is essential to ensure their compatibility with other biological control strategies and to avoid compromising the ecological services provided by these beneficial organisms in agroecosystems (Pec et al. 2024; Qasim et al. 2024; Kafle and Joshi 2025; Sui et al. 2025).

In this context, the present study aimed to evaluate the effects of *M. anisopliae* isolate IBCB 425 on the biology and midgut of the predatory lacewings *C. cincta* and *C. externa* through observational analyses,

histochemical, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Therefore, elucidating the effects of *M. anisopliae* on natural enemies is critical for assessing the environmental safety of fungal bioinsecticides and supporting their responsible integration into sustainable pest management programs.

2. MATERIALS AND METHODS

2.1. Insects

All insects used in this study were obtained from the mass-rearing colony maintained at the Laboratory of Biological Control and Insect Bioprospecting, Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Maringá, Paraná, Brazil (23°25'30" S, 51°56'20" W). Neonate larvae of *C. cincta* and *C. externa* (predators), aged 0–12 h, and eggs of *D. saccharalis* (prey), aged 0–24 h, were individually placed in 145 mL plastic containers and maintained in a climate-controlled room at 25 ± 2°C, 70 ± 10% relative humidity, and a 12:12 h (L:D) photoperiod (Araújo 1987; Scudeler 2013), until the bioassays were performed.

2.2. Commercial bioinsecticide

The commercial bioinsecticide Hizium 100 WP, manufactured and formulated by Oligos® Biotecnologia Fabricação de Defensivos Agrícolas Ltda. (São José do Rio Preto, São Paulo, Brazil), was used in all bioassays. The product is registered with the Brazilian Ministry of Agriculture and Livestock (MAPA) under registration n^{o}. 7722 and contains *M. anisopliae* isolate IBCB 425 at a concentration of 1×10^{10} viable conidia/g.

2.3. Determination of conidial concentration and viability

The concentration and viability of *M. anisopliae* conidia in the commercial bioinsecticide were determined following the methodology described by Alves (1986). Initially, 1 g of Hizium 100 WP was suspended in 9 mL of sterile distilled water and thoroughly homogenized. Conidial concentration was estimated by transferring a 10 µL aliquot of the suspension to a Neubauer hemocytometer and counting the conidia under an Olympus CX43 light microscope (Olympus, Tokyo, Japan). The number of conidia was converted to conidia/mL using the standard hemocytometer calculation.

Conidial viability was assessed by determining colony-forming units (CFUs). Serial dilutions of the original suspension were prepared to obtain concentrations of 10^8, 10^7, 10^6, and 10^5 conidia/mL. Subsequently, 100 µL of each dilution was spread onto potato dextrose agar (PDA; Kasvi Laboratory Products, Pinhais, Paraná, Brazil) in Petri dishes. The plates were incubated at 28°C for 48 h in a germination chamber (SS Scientific, S.S. Santana - Tecnologia Científica). Three technical replicates were prepared for each dilution. Viability was estimated by counting colonies on plates containing no more than 300 colonies, and the results were expressed as viable conidia/mL.

2.4. Bioassays

Three concentrations of the commercial bioinsecticide were used: 0.5, 1, and 5 g L^{-1}, corresponding to 4 × 10^7, 8 × 10^7, and 4 × 10^8 conidia/mL, respectively. These concentrations were selected based on previous studies demonstrating the bioactivity of *M. anisopliae* on *D. saccharalis* eggs (Ferreira et al. 2026), a major pest causing substantial losses in sugarcane crops, where predatory lacewings occur naturally (Scudeler et al. 2020).

After solutions preparation, the bioassays were performed following the protocol described by Daquila et al. (2023). Glass slides containing batches of *D. saccharalis* eggs (*n* = 20) were immersed in the respective solutions for 30 s. For the control treatment, slides with eggs were immersed in sterile distilled water. Subsequently, slides containing control or treated eggs were individually placed into 145 mL plastic containers (Fig. 1a-I). One neonate larva of *C. cincta* or *C. externa* was then introduced into each container according to the corresponding treatment (Fig. 1a-II). Each treatment consisted of five replicates, each comprising 10 plastic containers (*n* = 50 larvae per treatment) (Fig. 1b).

Fresh slides of control or treated *D. saccharalis* eggs were supplied every 72 h throughout the experiment. Larvae of *C. cincta* and *C. externa* were allowed to fed *ad libitum* until the prepupal stage. All bioassays were conducted in a climate-controlled room maintained at 25 ± 2°C, 70 ± 10% relative humidity, and a 12:12 h (L:D) photoperiod.

2.5. Larval mortality and pupal and adult inviability

Following the bioassays, larvae of *C. cincta* and *C. externa* were observed every 24 h until cocoon formation. Larvae that failed to produce a cocoon within 360 h were recorded as dead. After cocoon formation, a small cotton ball moistened with distilled water was placed inside each plastic container to maintain adequate humidity. Pupae that failed to produce adults within 360 h were classified as inviable. Before 8–12 h after emergence, adults were transferred to 1.8 L^{-1} plastic containers and provided with an artificial diet consisting of honey and brewer's yeast (1:1), along with distilled water supplied in a cotton-plugged vial (Scudeler et al. 2013). Adults that died within 24 h after emergence were classified as inviable. Morphological evaluations were performed under a Zeiss stereomicroscope (Carl Zeiss, Oberkochen, Germany) at 16× magnification.

2.6. Histochemical analysis

Third instar larvae of *C. cincta* (*n* = 10) and *C. externa* (*n* = 10) (control and treated) were collected and immobilized at − 10°C for 3 min. The midguts were then dissected in insect physiological saline (NaCl, KCl, $Na_2HPO_4 \cdot 7H_2O$, KH_2PO_4; 80:2:21.7:2, w/w) under a Zeiss stereomicroscope (Carl Zeiss, Oberkochen, Germany). The samples were fixed in alcoholic Bouin's alcoholic solution (picric acid, formaldehyde, and acetic acid; 15:5:1, v/v) for 24 h at room temperature, dehydrated through a graded ethanol series (70%, 80%, 90%, and 100%, v/v), cleared in xylene, and embedded in histological paraffin (Dinâmica®, Indaiatuba, São Paulo, Brazil). The samples were sectioned at 6 µm using a Leica RM2255 rotary microtome (Leica Biosystems) and stained with Hematoxylin and Eosin (H/E) (Junqueira and Junqueira

1983). Sections were examined using an Axioskop 40 microscope (Carl Zeiss, Göttingen, Germany), and images were captured with an AxioCam MRc digital camera (Carl Zeiss, Oberkochen, Germany).

2.7. Transmission electron microscopy (TEM)

Midguts (*n* = 5) from third instar larvae of *C. cincta* and *C. externa* control and treated were dissected as previously described. After the samples were fixed in Karnovsky's solution [2.5% glutaraldehyde (v/v), 4% paraformaldehyde (v/v), and phosphate buffer (pH 7.3)] for 24 h at room temperature. After primary fixation, the samples were post-fixed in 1% osmium tetroxide (w/v) prepared in the same buffer for 2 h, rinsed with distilled water, and contrasted with 0.5% aqueous uranyl acetate (w/v) for 2 h. The samples were then dehydrated through a graded acetone series (50%, 70%, 90%, and 100%, v/v) and embedded in Araldite® resin (Huntsman Advanced Materials, Salt Lake City, UT, USA). Ultrathin sections were obtained using a Leica EM UC7 ultramicrotome (Leica Biosystems), contrasted with 1% lead citrate (w/v) and 1% uranyl acetate (w/v), and examined using a Tecnai™ Spirit transmission electron microscope (FEI Company, Eindhoven, The Netherlands) at the Center for Microscopy, Research Support Centers Complex, State University of Maringá, Paraná, Brazil.

2.8. Scanning electron microscopy (SEM)

Control and treated cocoons (*n* = 5) were collected and dehydrated in a drying oven (FANEM 315SE, São Paulo, São Paulo, Brazil) at 35°C for 24 h. The samples were then sputter-coated with gold using a Shimadzu IC-50 ion coater (Shimadzu, Kyoto, Japan) and examined with a Shimadzu SS-550 SUPERSCAN scanning electron microscope (Shimadzu, Kyoto, Japan) at the Center for Microscopy, Research Support Centers Complex, State University of Maringá, Paraná, Brazil.

2.9. Effects on the reproduction of *C. cincta* and *C. externa*

To evaluate the effects of *M. anisopliae* on the reproduction of *C. cincta* and *C. externa*, adults from each treatment (control and treated groups) were transferred to 1.8 L^{-1} plastic containers provided with an artificial diet consisting of honey and brewer's yeast (1:1, w/v) and a cotton-plugged vial containing distilled water (Scudeler et al. 2013). Subsequently, 15 pairs were established for each treatment and maintained individually in separate containers. Sheets of bond paper were attached to the underside of the container lids to serve as oviposition substrates. The oviposition substrates and artificial diet were replaced every 72 h over a 30 days period.

Eggs were counted after each collection and transferred to 1.8 L^{-1} plastic containers lined with paper towels moistened with distilled water until larval hatching. All containers were maintained in a climate-controlled room at 25 ± 2°C, 70 ± 10% relative humidity, and a 12:12 h (L) photoperiod. Egg inviability was determined after 7 days of incubation, and eggs that failed to hatch within this period were considered inviable.

2.10. Statistical analyses

Data normality and homogeneity of variances were assessed using the Kolmogorov-Smirnov and Bartlett tests, respectively. As the data did not meet the assumptions of normality and homogeneity of variances, the treatments were analyzed using the Kruskal-Wallis test followed by Dunn's post-hoc test. The significance level was set at $\alpha = 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) (IBM Corporation 2017). Graphs were edited using Inkscape™ version 1.1 (Inkscape Project 2021).

3. RESULTS

3.1. Conidial concentration and viability

Conidial quantification using a Neubauer hemocytometer revealed an initial concentration of 8×10^9 conidia/mL of *M. anisopliae* isolate IBCB 425. Conidial viability was evaluated by the ability to produce colonies after 48 h of incubation. At the 10^5 dilution, the commercial formulation produced more than 300 colonies per plate, corresponding to a minimum viable conidial concentration of 3×10^8 viable conidia/mL. These results confirmed that the product exhibited sufficient conidial viability for use in the bioassays.

3.2. Larval mortality

At the end of 360 h, larval mortality in *C. cincta* was 4%, 8%, 6%, and 4% for the control and the 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively. These values did not differ significantly among treatments (Kruskal–Wallis test: $\chi^2 = 0.746$, $p = 0.862$) (Fig. 2a). Similarly, larval mortality in *C. externa* reached 6%, 6%, 8%, and 8% for the control and the 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively, with no significant differences among treatments (Kruskal–Wallis test: $\chi^2 = 0.905$, $p = 0.824$) (Fig. 2b).

3.3. Analysis of the midgut of *C. cincta* and *C. externa*

Light microscopy revealed no morphological or histological differences in the midgut of control and *M. anisopliae*-treated larvae of *C. cincta* or *C. externa*. In both species, the midgut was cylindrical and presented a well-defined lumen containing eosinophilic luminal contents and insoluble food residues enclosed by the peritrophic matrix (Figs. 3a, d, g and j). The epithelium was pseudostratified and composed of columnar and regenerative cells with conspicuous basophilic nuclei, supported by muscle fibers (Figs. 3b, e, h and k).

Transmission electron microscopy likewise revealed no ultrastructural alterations in the midgut epithelium of treated larvae compared with their respective controls. In both species, the midgut exhibited a well-defined lumen lined by an epithelium composed of columnar and regenerative cells with prominent nuclei (Figs. 3c, f, i and l). The cytoplasm of the columnar cells contained lipid droplets, glycogen deposits, spherites, and mitochondria (Figs. 3c, f, i and l). A well-developed basal labyrinth was

observed in the basal region of the columnar cells, whereas numerous microvilli were present at the apical surface. The epithelium was supported externally by muscle fibers (Figs. 3c, f, i and l).

3.4. Pupal and adult inviability of *C. cincta* and *C. externa*

The pupal inviability of *C. cincta* after 360 h was 2%, 6%, 4%, and 4% for the control and the 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively. No significant differences in pupal inviability were detected among treatments (Kruskal-Wallis test: $\chi^2 = 1.583$, $p = 0.663$) (Fig. 2a). Similarly, pupal inviability in *C. externa* was 2%, 6%, 6%, and 6% for the control and the 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively, with no significant differences among treatments (Kruskal-Wallis test: $\chi^2 = 3.659$, $p = 0.301$) (Fig. 2b).

Within 24 h after emergence, adult inviability in *C. cincta* was 6%, 8%, 8%, and 8% for the control and 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively. The Kruskal-Wallis test revealed no significant differences in adult inviability among treatments ($\chi^2 = 0.183$, $p = 0.980$) (Fig. 2c). Adult inviability in *C. externa* was 4%, 4%, 4%, and 6% for the control and the 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively. No significant differences among treatments were detected (Kruskal-Wallis test: $\chi^2 = 0.576$, $p = 0.902$) (Fig. 2d).

3.5. Changes in cocoons and adults of *C. cincta* and *C. externa*

Scanning electron microscopy revealed no apparent differences in cocoon morphology between the control and *M. anisopliae*-treated groups of *C. cincta* (Figs. 5a and b) or *C. externa* (Figs. 5c and d). In both species, the cocoons were spherical and composed of densely woven silk threads (Figs. 5e-h). Likewise, no morphological differences were observed in adults emerging from the control and treated groups (Figs. 5i-l). Adults of both species exhibited a fully developed head, thorax, and abdomen. The head bore one pair of antennae and one pair of compound eyes, whereas the thorax possessed three pairs of legs and two pairs of wings. The abdomen was elongated and longer than the combined length of the head and thorax (Figs. 5i-l).

3.6. Effects on oviposition and egg inviability of *C. cincta* and *C. externa*

Exposure of larvae to *M. anisopliae* did not affect the reproductive performance of the resulting adults of *C. cincta* or *C. externa*. The mean oviposition of *C. cincta* was 192.86, 145.73, 139.2, and 151.26 eggs for the control and 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively. No significant differences were detected among treatments in the oviposition number of this insect (Kruskal-Wallis test: $\chi^2 = 6.475$, $p = 0.091$) (Fig. 6a). For C. externa, the mean oviposition was 536.33, 449.86, 503.06, and 526.6 eggs for the control and 4×10^7, 8×10^7, and 4×10^8 conidia/mL treatments, respectively. No

significant differences were detected among treatments in the oviposition number of *C. externa* (Kruskal-Wallis test: $\chi^2 = 4.395$, $p = 0.222$) (Figs. 6b).

Egg inviability ranged from 0.92% to 2.53% in *C. cincta* and from 0.50% to 0.65% in *C. externa* across the control and *M. anisopliae* treatments. Considering the data no significant differences were detected among treatments developed with *C. cincta* ($\chi^2 = 1.270$, $p = 0.736$) or *C. externa* ($\chi^2 = 0.749$, $p = 0.862$) (Figs. 6c and d).

4. DISCUSSION

In this study, predation on *D. saccharalis* eggs treated with *M. anisopliae* isolate IBCB 425 suspensions at different concentrations did not negatively affect the predators *C. cincta* and *C. externa*. Similar results were reported for *C. externa* larvae fed *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs contaminated with *M. anisopliae* isolate ESALQ E9 at concentrations of 10^7, 10^8, and 10^9 conidia/mL, with no adverse effects on the viability of the predator's larvae, pupae, or adults (Dias et al. 2019, 2020). Likewise, when *C. externa* larvae were fed *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae) nymphs contaminated with *M. anisopliae* IBCB 425 at concentrations of 10^5 and 10^8 conidia/mL, no negative effects on the biology of this natural enemy were observed (Souza et al. 2015). Similar findings were reported when *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) eggs contaminated with *M. anisopliae* at concentrations of 10^5, 10^7, and 10^8 conidia/mL were offered to *C. externa* larvae (López and Osorio 2019). Likewise, *C. cincta* larvae feed *Sitotroga cerealella* (Lepidoptera: Gelechiidae) eggs contaminated with *M. anisopliae* E9 at a concentration of 2.1×10^7 conidia/mL showed no changes in the viability of larvae, pupae, or adults (Cardoso et al. 2007). However, none of these studies evaluated the midgut or the reproductive performance of the predators used.

In addition to lacewings, predators from other orders exposed to different species of entomopathogenic fungi also showed no adverse effects on their biology. For example, *Harmonia axyridis* (Palas 1773) (Coleoptera: Coccinellidae), when applied in combination with the entomopathogen *Metarhizium releyi* MrSIGZL-1 for the control of *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) and *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae), exhibited no negative effects on its biological performance (Sui et al. 2025). Similar results were reported when *Chilocorus bipustulatus* (Coleoptera: Coccinellidae) and *Exochomus nigripennis* (Coleoptera: Coccinellidae) were used in combination with *Alternaria muriposa* (Ascomycota: Pleosporaceae) for the control of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) (El Aalaoui et al. 2025).

The insect gut is considered a major entry route for pathogens and therefore possesses several defense mechanisms. The midgut, for example, is lined by a thin chitinous membrane (the peritrophic matrix), which protects against mechanical damage and invading microorganisms, such as entomopathogenic fungi. However, if this barrier is breached, more complex immune mechanisms are activated, including innate and acquired immunity, with innate immunity responding through both humoral and cellular

pathways (Feldhaar and Gross 2008). In this study, the absence of midgut damage in *C. cincta* and *C. externa* may be explained by three hypotheses: (i) the low concentration of conidia present in contaminated *D. saccharalis* eggs; (ii) inhibition of fungal development by enzymes produced either by the gut microbiota or by the host itself; or (iii) the hosts' defense mechanisms.

Considering the low concentration of conidia in the prey (*D. saccharalis* eggs), previous studies identified the colonization of the yolk and embryos of *D. saccharalis* by hyphae and blastospores of *Beauveria bassiana* (Hypocreales: Sordariomycetes) and *M. anisopliae*, resulting in the interruption of embryonic development; however, little is known about the number of conidia present in the yolk, as no studies have quantified the concentration of these microorganisms in this tissue (Ferreira et al. 2026). These findings highlight the need for further studies on the adaptive potential and the abundance of microorganisms and their propagules in the eggs and embryos of different insect pests. Such studies would make it possible to determine the concentration of conidia in the yolk region and estimate the amount ingested by *C. cincta* and *C. externa* larvae, which remain unknown. This information would help demonstrate that low conidial concentrations, such as those used in the present study, do not cause damage to the midgut of non-target organisms.

Although our histochemical and ultrastructural analyses did not reveal the presence of symbiotic microorganisms in the midgut of *C. cincta* and *C. externa*, Chen et al. (2006) and Dantas et al. (2021) reported the presence of symbiotic bacteria, fungi, and yeasts in *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) and *C. externa*, respectively. Symbionts are generally classified into two categories: obligate and facultative. Obligate symbionts are associated with host nutrition and are located within the cytoplasm of specialized cells known as mycetocytes, which are found in the mycetoma, their primary site of residence. Facultative symbionts are primarily associated with host defense and reproduction and may occur in various tissues and in the hemolymph. However, in some cases, facultative symbionts may become obligate symbionts (Karkuzhali et al. 2025). In addition, enzymes synthesized either by the symbionts or by their hosts (lacewings) may contribute to the degradation of microbial agents by breaking down proteins present in pathogenic microorganisms, thereby preventing them from disrupting host homeostasis (Feldhaar and Gross 2008; Gurung et al. 2019; Zhang et al. 2024).

Larvae of *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae) fed on prey contaminated with pyriproxyfen (Scudeler et al. 2024), *Azadirachta indica* (Meliaceae) (Scudeler et al. 2013), and *Cymbopogon nardu*s (L.) (Caballero-Gallardo et al. 2023) exhibited alterations in the uniformity of the different layers composing the insect cocoon, changes were likely associated with damage to the Malpighian tubules (Scudeler et al. 2024). In the present study, cocoons spun by *C. cincta* and *C. externa* larvae fed on prey contaminated with *M. anisopliae* IBCB 425 showed no structural alterations when examined by ultrastructural analysis. Similar results were reported for cocoons spun by *C. claveri* larvae fed *D. saccharalis* eggs contaminated with *Bacillus thuringiensis* isolate *aizawai* GC-91 (Bacillales: Bacillaceae) (Daquila et al. 2023). This preservation of cocoon structure is likely associated with the

selectivity of these biological control agents toward predatory lacewings, in contrast to the effects reported for synthetic compounds and botanical extracts.

Adult female *C. cincta* and *C. externa* originating from larvae fed with *M. anisopliae* contaminated preys showed no differences in the number of oviposition events compared with the control group, likewise, no differences were observed in egg viability. Similar results were reported for *C. claveri* females whose larvae had been fed eggs contaminated with *B. thuringiensis aizawai* GC-91 (Daquila et al. 2023). The absence of reproductive effects may be related to the delayed maturation of the reproductive system in these insects. Newly emerged females require additional time for ovarian maturation, as is typical of other insects of the order Neuroptera (Stelzl and Devetak 1999). During the adult stage, they must also supplement the nutritional reserves accumulated during the larval stage by consuming a diet rich in sugars, amino acids, and lipids (Scudeler et al. 2020), in contrast to females of other insect orders, such as Lepidoptera (Perveen 2017). Therefore, the maintenance of reproductive performance following larval exposure to *M. anisopliae* suggests that this entomopathogenic fungus is compatible with these predatory lacewings and is unlikely to compromise their ecological role under the conditions evaluated in the present study.

5. CONCLUSION

Exposure of *C. cincta* and *C. externa* to *D. saccharalis* eggs treated with *M. anisopliae* isolate IBCB 425 (Hizium 100 WP) did not adversely affect the biological performance, midgut ultrastructure, cocoon morphology, or reproductive performance of either predator under the conditions evaluated. These findings indicate that larval ingestion of contaminated prey was not associated with detectable impairment of the development or fitness of these lacewings and support the selectivity of *M. anisopliae* toward these non-target natural enemies. Therefore, the combined use of this entomopathogenic fungus and predatory lacewings appears to be a promising strategy for integrated pest management programs targeting *D. saccharalis*. Nevertheless, additional studies under greenhouse and field conditions are warranted to confirm the compatibility of these biological control agents under more complex ecological scenarios.

Declarations

Conflicts of Interest:

The authors declare that they have no conflicts of interest.

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Author Contribution

Conceptualization: A.P.F., B.V.D. and H.C.Methodology: A.P.F., B.V.D., E.L.S., J.C.P. and H.C.Investigation: A.P.F., B.V.D., N. J. M-C., C.F. and E.A.C.Formal analysis: A.P.F., B.V.D., E.L.S., A. A.S.G., J.C.P. and H.CValidation: A.P.F., B.V.D., E.L.S., J.C.P. and H.C.Resources: A.P.F., B.V.D., E.L.S., J.C.P. and H.C.Writing – original draft: A.P.F., B.V.D., E.L.S., A.A.S.G., J.C.P. and H.C.Writing – review & editing: A.P.F., B.V.D., E.L.S., N. J. M-C., C.F., E.A.C., A.A.S.G., J.C.P. and H.C.Visualization: A.P.F. and B.V.D.Supervision: A.P.F. and B.V.D.Project administration: A.P.F., B.V.D., J.C.P. and H.C.Funding acquisition: B.V.D., J.C.P. and H.C.

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Data Availability

The data presented in this study are original and will be made available by the authors upon request.

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Figures

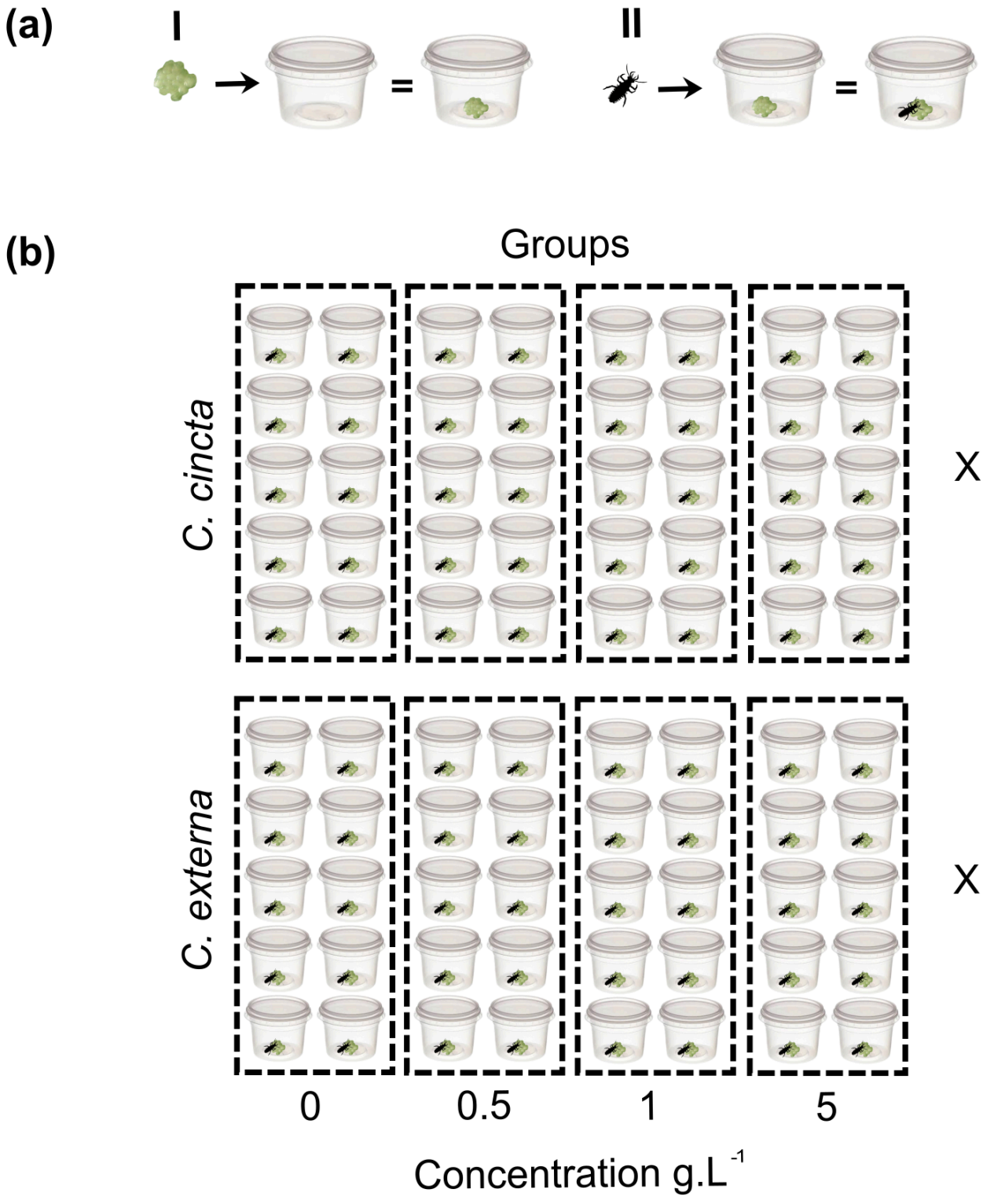


Figure 1

Experimental design of the bioassays. (a-I) Eggs of *Diatraea saccharalis* (control and treated with *Metarhizium anisopliae* isolate IBCB 425) were individually placed in 145 mL plastic containers. (a-II) One neonate larva (1-12 h old) of *Ceraeochrysa cincta* or *Chrysoperla externa* was individually introduced into each container. (b) Treatments (4×10^7, 8×10^7 and 4×10^8 conidia/mL) consisted of five independent replicates of 10 larvae each (n = 50 larvae per treatment). The control treatment consisted in concentration 0 conidia/mL.

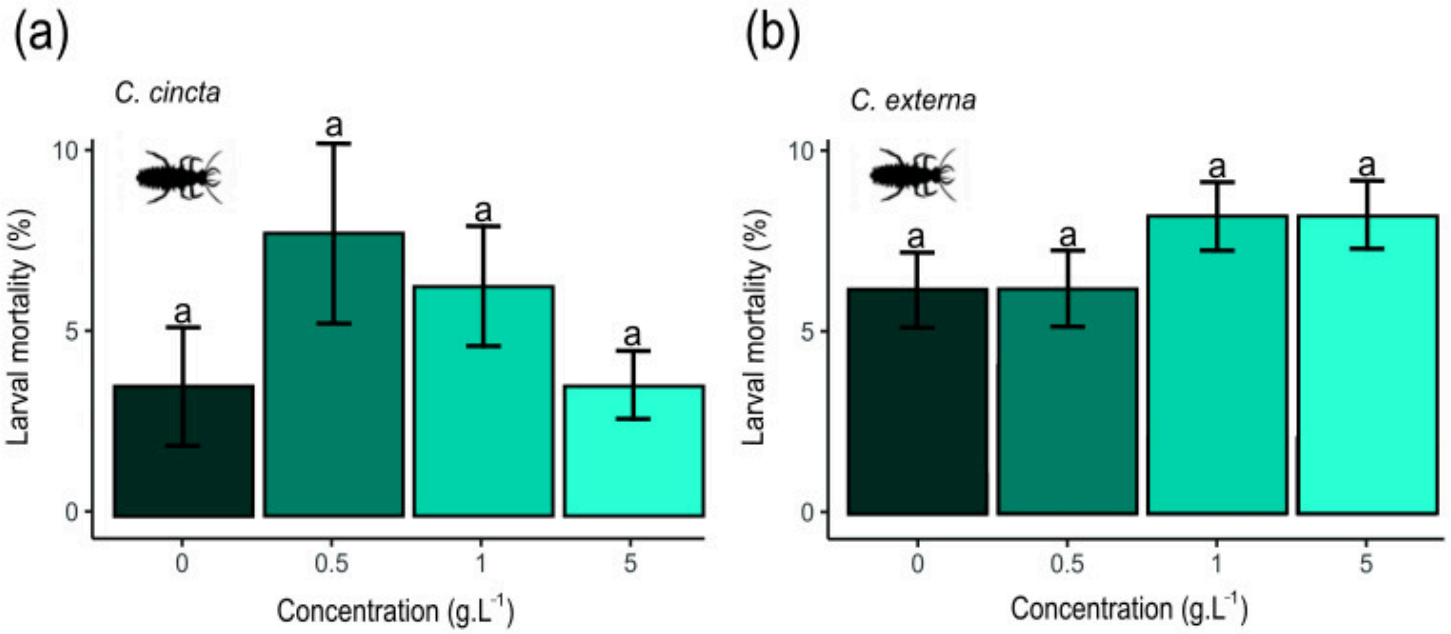


Figure 2

Larval mortality of the predatory lacewings *Ceraeochrysa cincta* (a) and *Chrysoperla externa* (b) fed *Diatraea saccharalis* eggs treated with different concentrations (4×10^7, 8×10^7, and 4×10^8 conidia/mL) of *Metarhizium anisopliae* isolate IBCB 425. Bars sharing the same letter did not differ significantly according to the Kruskal-Wallis test ($p \geq 0.05$). Error bars represent the standard deviation of the mean (± SD). The control treatment contained no fungal conidia (0 conidia/mL).

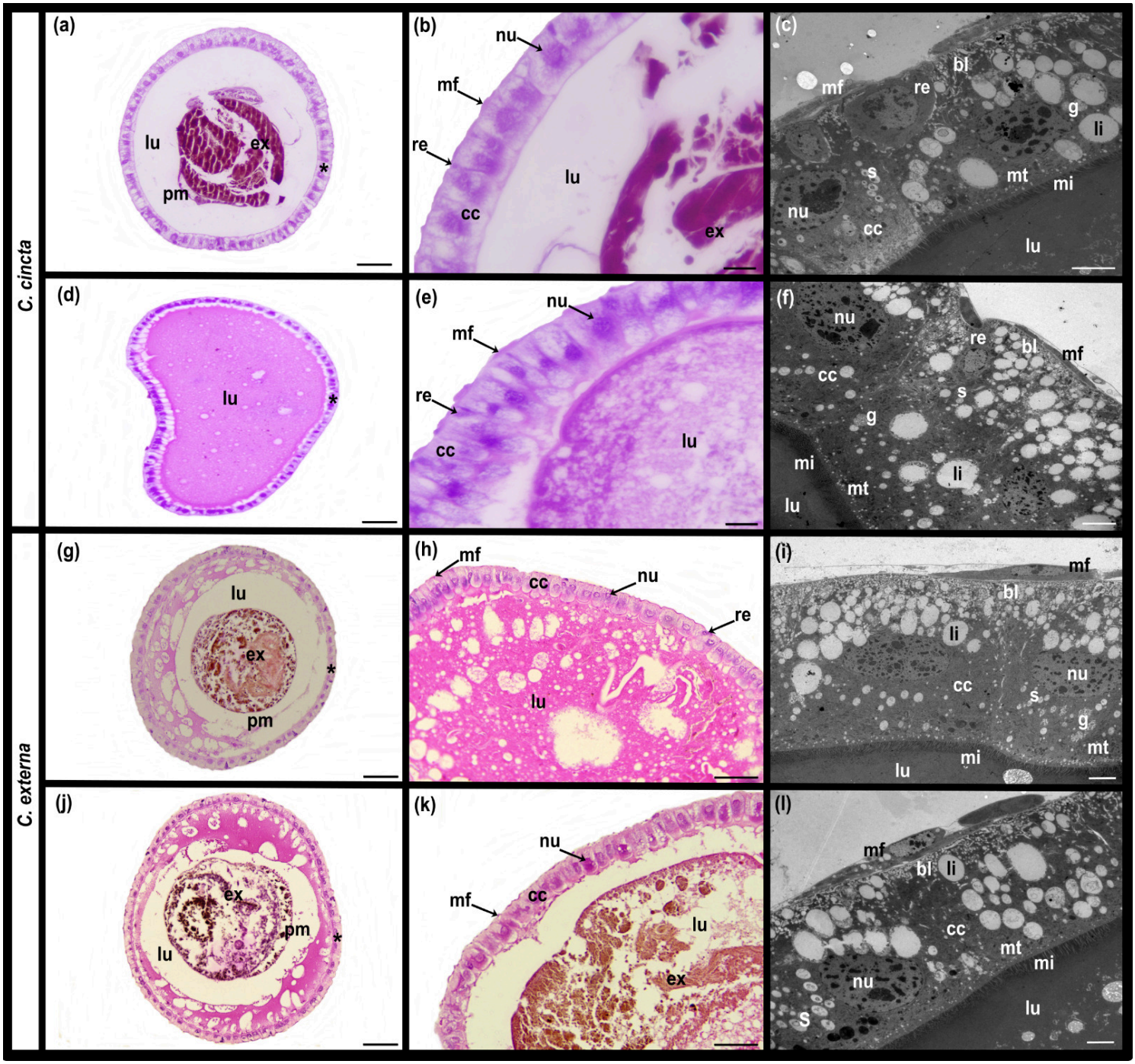


Figure 3

Morphological and histological analysis of midgut larvae of *Ceraeochrysa cincta* and *Chrysoperla externa*. Light microscopy (a, b, d, e, g, h, j and k), stained with Hematoxylin and Eosin (H/E). Transmission electron microscopy (c, f, i and l). Controls (a, b, c, g, h and i). Treatments with 4×10^8 conidia/mL of *Metarhizium anisopliae* isolate IBCB 425 (d, e, f, j, k and l). Circular-shaped midgut formed by a simple epithelium (*) and a distinct lumen (lu) filled with gastric secretions and insoluble solid residues (ex), surrounded by the peritrophic matrix (pm) (a, d, g and j). Epithelium composed of columnar cells (cc) and regenerative cells (re) with prominent nuclei (nu), both supported by muscle fibers (mf) (b, e, h, k). Columnar and regenerative cells with evident nuclei; columnar cells contain lipid droplets (li), glycogen

reserves (g), spherites (s), and mitochondria (mt) within the cytoplasm (c, f, i, l). The apical region of these cells features microvilli (mi) facing the lumen (lu), while the basal region shows a basal labyrinth (bl) (c, f, i, l). The epithelium is supported by bundles of muscle fibers (mf) (c, f, i, l). Scale bars in a, b, d, e, g, h, j and k = 100 µm and, c, f, i and l = 2 µm.

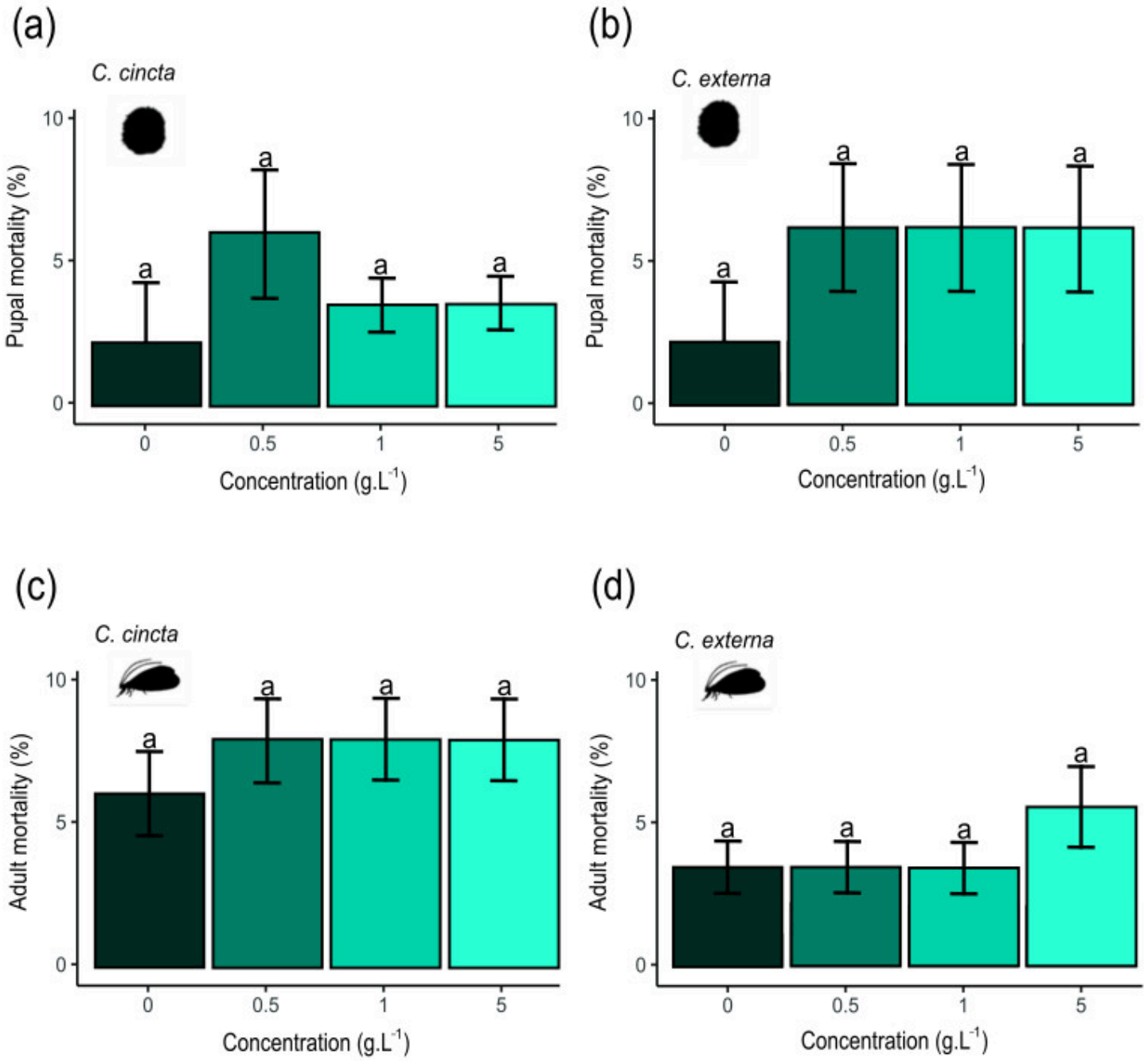


Figure 4

Pupal and adult inviability of the predatory lacewings *Ceraeochrysa cincta* (a, c) and *Chrysoperla externa* (b, d) fed *Diatraea saccharalis* eggs treated with different concentrations of *Metarhizium anisopliae* isolate IBCB 425 (4×10^7, 8×10^7, and 4×10^8 conidia/mL). Bars sharing the same letter did not differ

significantly according to Dunn's post hoc test following the Kruskal-Wallis test ($p \geq 0.05$). Error bars represent the standard deviation of the mean (± SD). The control treatment contained no fungal conidia (0 conidia/mL).

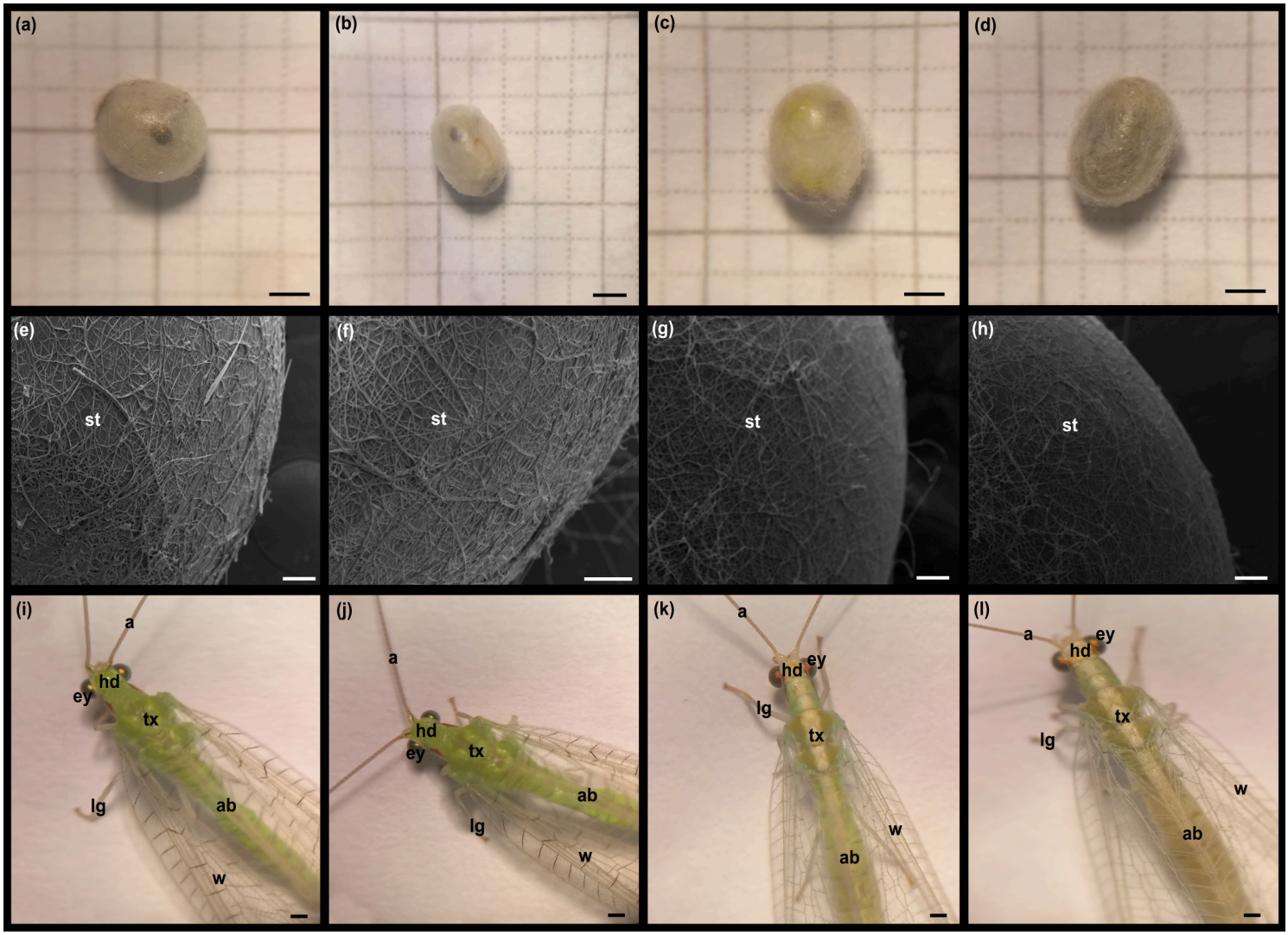


Figure 5

Cocoons and adults of *Ceraeochrysa cincta* and *Chrysoperla externa*. Stereomicroscopy (a-d and i-l). Scanning electron microscopy (SEM) (e-h). *C. cincta* control (a, e and i). *C. cincta* fed *Diatraea saccharalis* eggs treated with a suspension containing 4×10^8 conidia/mL of *Metarhizium anisopliae* isolate IBCB 425 (b, f and j). *C. externa* control (c, g and k). *C. externa* fed *D. saccharalis* eggs treated with a suspension containing 4×10^8 conidia/mL of *M. anisopliae* isolate IBCB 425 (d, h and l). Spherical cocoons composed of silk threads (st) (a-h). Adults in dorsal view showing a well-developed head (hd), thorax (tx), and abdomen (ab), with compound eyes (ey), antennae (an), legs (lg), and wings (w) (i-l). Scale bars in a-d and i-l = 1 mm and, in e-h = 100 µm.

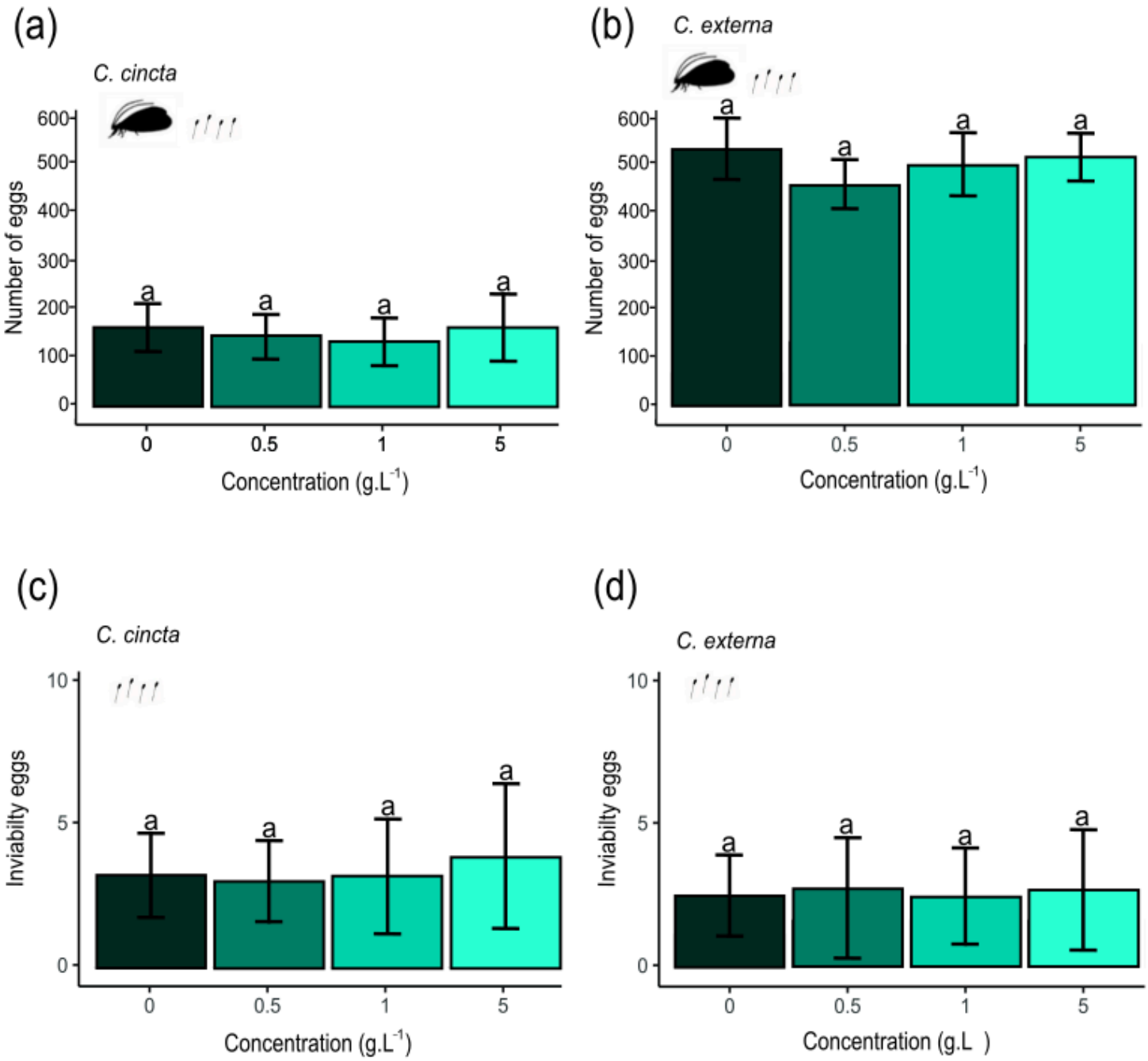


Figure 6

Mean oviposition over 30 days period and egg inviability after 7 days in *Ceraeochrysa cincta* (a and c) and *Chrysoperla externa* (b and d) originating from larvae fed *Diatraea saccharalis* eggs treated with different concentrations of *Metarhizium anisopliae* isolate IBCB 425 (4×10^7, 8×10^7, and 4×10^8 conidia/mL). Same letter did not differ significantly according to Dunn's post hoc test following the Kruskal-Wallis test ($p \geq 0.05$). Error bars represent the standard deviation of the mean (± SD). The control treatment contained no fungal conidia (0 conidia/mL).