Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2024; 12(1): 98-105

Received: 06-11-2023
Accepted: 10-12-2023

Mónica Ardiles
Programa de Magíster en Ciencias mención Entomología, Universidad Metropolitana de Ciencias de la Educación Santiago, Chile

Christian R González
Instituto de Entomología, Universidad Metropolitana de Ciencias de la Educación Santiago, Chile

Corresponding Author:
Mónica Ardiles
Programa de Magíster en Ciencias mención Entomología, Universidad Metropolitana de Ciencias de la Educación Santiago, Chile

Assessment of the susceptibility and alterations caused by lufenuron in larvae, pupae, and adults of *Hermetia illucens* (Linnaeus) (black soldier fly) (Diptera: Stratiomyidae)

Mónica Ardiles and Christian R González

DOI: https://doi.org/10.22271/j.ento.2024.v12.i1b.9283

Abstract
Indoor breeding of edible insects has emerged as a sustainable alternative to fulfill the growing demand for proteins and lipids in animal feed. The quality and safety of the diet used to feed these insects are highly relevant, as they directly influence the increase in biomass, growth of the insect, and safety of the larvae and by-products obtained from it. The presence of insecticides, such as lufenuron, which belongs to the class of benzoylphenyl ureas, inhibits chitin synthesis and interferes with the molting process in arthropods. It could potentially be found in the diets used for feeding. This study introduced lufenuron into the diets of 7-day-old *Hermetia illucens* larvae at varying concentrations (0.069, 0.149, 0.282, 0.878, and 1.066 mg/kg. The survival of the larvae and pupae was evaluated, and the alterations generated were observed using light microscopy. The main alterations observed were morphological alterations on mouth abrasion on larvae, an inability to complete molting to pupae, and pupal abdomen wounds. In adults, wing deformation and genital injuries occur in both males and females. The findings of this study suggest that lufenuron exposure leads to mortality in the larvae and pupae of *H. illucens*. Additionally, insecticides cause significant morphological alterations in the larvae, pupae, and adult stages of insects.

Keywords: *Hermetia illucens*, chitin synthesis inhibitor, lufenuron, morphology, growth regulator

Introduction
Edible insects are considered environmentally friendly because of their more efficient resource utilization compared with conventional livestock. This can potentially reduce the environmental impacts of food production (Godfray *et al.*, 2010; Van Huis *et al.*, 2013) [1, 2]. In addition, edible insects can feed on a wide range of wastes that can be transformed into ingredients with high energy, protein, fat, and vitamin values (Rumpold and Schlüter, 2013) [3] and can be used as ingredients in human and animal food (Fasolin *et al.*, 2019) [4]. These insects can also be used in biofuel production (Zheng *et al.*, 2012) [5] and their excreta can be used as soil improvers (Klammsteiner *et al.*, 2020) [6].
Hermetia illucens, commonly known as the black soldier fly, is a saprophagous fly species in the family Stratiomyidae. The larvae of this species are well known for their ability to feed on and break down a variety of organic waste materials. They are often used in waste management and environmental applications because of their remarkable capacity to convert various types of organic wastes into valuable products. Black soldier fly larvae are highly efficient at consuming and processing organic matter, including agro-industrial waste, animal excreta (manure), food scraps, and fish farming waste. These larvae can convert the waste materials they consume into nutrient-rich biomass, which can be further processed into products such as animal feed, biofuels, and fertilizers (Bessa *et al.*, 2021; Hoek-van den Hil *et al.*, 2022; Meijer *et al.*, 2021; Purschke *et al.*, 2017) [7, 8, 9, 10]. Because of their unique ecological role and waste conversion capabilities, black soldier fly larvae have gained attention as a potential solution for reducing organic waste and its associated environmental impacts. Their ability to rapidly break down waste and transform it into valuable resources has led to research and initiatives aimed at harnessing their potential in various waste management and sustainable development projects (EFSA Scientific Committee, 2015) [11].
Excreta, which include waste from animals or plant husbandry, often contain traces of

antibiotics, pesticides, and other chemicals. These substances may be present in excreta owing to the use of veterinary treatments or agronomic practices in the production chain. Antibiotics are commonly used in animal husbandry to treat and prevent diseases, whereas pesticides are used in agriculture to control pests and increase crop yields. The presence of antibiotics and pesticides in excreta raises important concerns about their potential environmental impacts, including the possibility of soil, water, and ecosystem contamination. When animals are treated with antibiotics, some antibiotics can pass through their digestive systems and be excreted in waste. Similarly, pesticides used in agricultural fields can make their way into animal excreta if animals graze or consume crops treated with these chemicals. (Berendsen *et al*., 2015; Jansen *et al*., 2019) [12, 13].

Currently, the most commonly used insecticides for pest control and human health protection are insect growth regulators (IGR) (Rösner, Wellmeyer and Merzendorfer 2020) [14]. These insecticides are more selective, less persistent, environmentally friendly, and has relatively low toxicity to humans and non-target organisms (Sun *et al*., 2015; Zhu *et al*., 2016) [15, 16], and can interfere with the growth, development, and reproduction of insects. The three most important groups are juvenile hormone analogs, ecdysone antagonists, and chitin synthesis inhibitors. Lufenuron is a chitin synthesis inhibitor belonging to the benzoyl-phenyl urea (BPU) group. When included in the diet of *Drosophila melanogaster* larvae, lufenuron causes mortality and abnormal cuticle development, and surviving individuals die in the next instar, presumably because of inadequate cuticle synthesis, in addition to structural changes in the peritrophic matrix (Kelkenberg *et al*., 2015; Merzendorfer 2013; Wilson and Cryan 1997) [17, 18, 19].

The use of BPU in Chile is focused on the agroindustry through the use of the insecticide Sorba 050 EC™, Syngenta S.A (Syngenta, 2021) [20], for pest control in agriculture, and its use has been authorized in the country since 2006. In the salmon industry, this insecticide is used to control *Caligus rogercresseyi* (Copepoda: Siphonostomatoida: Caligidae) through lufenuron and hexaflumuron. Both ingredients, along with hydrogen peroxide, are used as the main treatments for sea lice control. Lufenuron is applied to smolt salmon food before it is transported to the sea, while hexaflumuron is applied to the sea by bathing it in sea cages (Bravo and Treasurer, 2023) [21]. Inhibitors of chitin synthesis, such as lufenuron and hexaflumuron, disrupt chitin biosynthesis in arthropods. The effectiveness of these compounds is attributed to chitin formation due to abnormal endocuticular deposits, which can affect tracheae development (Tanani, Hasaballah and Hussein 2022) [22], causing abortive molting, abnormal phenotypes (Merzendorfer 2013) [18], increased adult lethality, and a lack or decrease in egg hatching in arthropods (Zhu *et al*., 2016) [16]. Some of these inhibitors affect the formation and function of the peritrophic matrix in the digestive system (Merzendorfer 2013) [18].

Therefore, the first objective of this study was to evaluate the mortality rate of *H. illucens* larvae, pupae, and adults when exposed to different concentrations of lufenuron; the second objective was to visually the morphological damage caused by lufenuron exposure on *H. illucens.*

Materials and Methods

Biological Materials

A new strain of the cross was obtained from a colony belonging to Natpro SpA (Los Lagos, Chile) and from native individuals collected in Chile. The new strain was bred for three generations and the flies were fed a diet commonly used for Gainesville house flies.

Choosing the lufenuron concentration

The lufenuron concentrations used in the treatments were chosen based on the regulatory guidelines for veterinary drugs in food intended for human consumption in Chile. Specifically, these guidelines are outlined in Resolution N°1,560 published by the Ministry of Health of the Republic of Chile (Ministry of Health of the Republic of Chile, 2019) [23]. This Resolution sets the maximum residue limits (MRL) for veterinary drugs in food products intended for human consumption. As for lufenuron, it has been established that the MRL allowed is 1.35 mg/kg for muscle tissue with the skin of farmed fish. According to this MRL, a matrix of concentrations that were below the allowed MRL, which differed among them, was established to obtain a response curve of the growth process in *H. illucens* larvae and its incidence in the morphology of the larvae, pupae, and adults.

Insecticide concentrations were used to create formulations for the feed administered during larval rearing. These concentrations were compared to a control group that received feed without lufenuron.

Feed Spiked

For the bioassay, a formulation based on lufenuron (Sigma-Aldrich, St. Louis, MO, USA; 99.5%) was used. Concentrations of lufenuron at 0.069, 0.149, 0.282, 0.878, and 1.066 mg/kg were diluted in distilled water at 20 °C to maintain solubility (Lewis *et al*., 2016) [24] and then spiked with feed formulation based on the Gainesville house fly diet (Hogsette, 1992) [25], which consisted of wheat bran (50%), corn flour (20%), and alfalfa flour (30%). A moisture content of 70% was used in the diet. Once the ingredients were dosed, they were mixed with an Einhell brand manual mixer (1,600 Watt), and the temperature was maintained at 20 °C.

Bioassay

All treatments were performed in triplicate. Per replicate, 700 seven-day-old larvae (post-hatching) were reared on plastic trays (21.5 cm x 15.5 cm x 8 cm). In each tray, 80 g of feed was formulated with the formulation corresponding to the treatment and the larvae were inoculated on top of the feed. A layer of anti-mosquito netting was placed over the tray, covering the larva and feed, and elastic bands were used to secure the anti-mosquito netting in place to prevent any larvae from escaping during the rearing period. The trays were distributed over carts and placed in an incubator room belonging to the company Natpro SpA. (set at 28 °C and 60% RH). The larvae were then introduced into the prepared feed in each trial. Feeding was administered daily ad libitum, without overfeeding or underfeeding. When 50% of the larvae were observed, feeding was suspended at the prepupal stage (sixth instar). The larvae were then manually separated from the substrate by sifting. The substrate consisted of a mixture of leftover feed and larval excreta. For each replicate, 30 individuals of larva L5, pupa, and adult were chosen to observe morphological alterations caused by lufenuron under a Leica EZ 4 W stereo microscope with a 5.0 × Megapixeles camera.

To evaluate larvae L5, we used the information described by Bruno *et al*. (2020) [26] for pupae, Barros *et al*., (2019) [27], and

Fachin and Hauser (2022) [28] for adults.

Statistical Analysis

Statistical analysis was conducted using the RStudio software (R Core Team, 2022) [29]. One-way analysis of variance (ANOVA) was performed to assess whether there were statistically significant differences between the treatment and control groups in terms of the observed effects, such as morphological alterations or survival rates. After performing ANOVA, post-hoc Tukey's HSD tests were used to compare treatments with the control.

The analysis of the data obtained was performed by applying the Levene Test, which assesses whether the variances of the data are statistically equal across different groups or treatments. Analysis of variance (ANOVA) with 95% confidence was performed to detect statistically significant differences. When significant differences were detected, Tukey's post-hoc test was applied with 95% confidence.

Results

The effects of lufenuron on the survival of the larvae and pupae are shown in Figure 1 and Figure 2. Larval survival in the fifth instar was affected by the presence of lufenuron regardless of the concentration to which the individuals were exposed. In pupae, treatment with 1.066 (mg/kg lufenuron) generated a survival rate of 43.6%.

The presence of alterations in the larvae treated with 0.878 and 1.066 mg/kg lufenuron further supports the idea that this specific concentration range of the insecticide had a noticeable impact on the development and well-being of the larvae. The general morphology of *H. illucens* larvae is a cylindrical and elongated body consisting of 12 well-defined segments (including the cephalic capsule) surrounded by setae and sensilla. The head is hemicephalic, prognata. Dorsally, it presents a double tridentate hook-shaped sclerite in the distal area of the head. Conical, robust labrum with a small bulge in the distal area where it houses some sensilla. It has two pronounced gene lobes that protect the mandibular-maxillary apparatus. The ventral oral cavity occupies the first third of the head. Globular thorax, meso- and metathorax covered with spine-like sensilla (dorsal and ventral views). Thoracic segment, with setae and rounded ornaments on the cuticle (Barros *et al*., 2019) [27]. The abdomen is composed of eight segments that are similar in shape to the thorax. It is covered by a spine-like sensilla and is located ventrally on the anterior margin, except for the eighth segment (Barros *et al*., 2019) [27]. The eighth segment, which is larger than the rest, has a chimney spiracle that is much larger than functional spiracles. It is surrounded by a row of setae (Barros *et al*., 2019) [27]. The anal opening is surrounded by short, thick, spine-shaped setae (Barros *et al*., 2019) [27].

The larvae exhibited necrosis, which refers to the death of cells or tissues, in various part of the mouthparts. This includes the maxillary and mandibular parts, membranous ventral lobe, prementum, epipharynx (part of the mouthparts), and hypopharynx (also part of the mouthparts) (Figure 3). Malformation was observed in the thoracic and abdominal regions. Specifically, there was a malformation in the metathorax (the posterior segment of the thorax) and the first abdominal segment (Figures 4, 5) and a notable feature was discontinuity of the metathoracic segment, which merged with the first abdominal segment. This abnormality was unique to larvae treated with a higher concentration of lufenuron (1.066 mg/kg). These effects are consistent with the disruption of chitin synthesis, which can lead to severe developmental abnormalities and structural deficiencies in arthropods.

The pupae that were fed 0.149, 0.282, and 1.066 (mg/kg) lufenuron during their larval stage experienced mortality associated with incomplete ecdysis. (Figure 6). The general morphology of the pupae is coarctate, with 12 well-defined segments. It is brown to dark brown in color. The surface of his body is rigid and sclerotic. Completely sclerotic head capsule, lateral antennae, and mouthparts covered by sclerotic plaques. Body surrounded by dense brown to golden plumosity. The abdomen is composed of eight segments that are similar in shape to that of the thorax. With absence of spine-like sensilla. The eighth segment is larger than the rest; it has a chimney spiracle that is much larger than the functional spiracles and is surrounded by a row of setae. Anal opening without a spine-like sensilla. In the 0.149, 0.282, 0.878, and 1.066 mg/kg lufenuron groups, a larger wound and increased fluid secretion were observed in the eighth abdominal segment in the ventral section (Figure 7). These observed effects on the eighth abdominal segment suggest that lufenuron exposure may disrupt the normal development and integrity of this segment. The presence of a larger wound and increased fluid secretion could be indicative of tissue damage or physiological stress resulting from the effect of lufenuron on chitin synthesis and overall development.

In adults, a specific type of wing deformation called "wing curling" was observed more frequently in all treatments, except for the control group and a treatment involving a specific concentration of lufenuron (0.069 mg/kg) (Figure 8). The general morphology of adult *H. illucens* is a large species, which can measure between 13 and 20 mm, a black body, and a black head with a white stripe in the front area. Median occipital sclerite with two white spots. Naked eyes, uniform and small ommatidia, developed ocellar triangle, dichoptic in both sexes (Oliveira, Doelle and Smith 2016; Üstüner, Hasbenli and Rozkošný 2017) [30, 31]. The maxillary palps are located in the front area of the head, lateral to the rostrum (Pezzi *et al*., 2021) [32]. Antennae blackish, pedicel cylindrical, bent at the base. The surface is rough and covered with microtrichia on both sides. The female had a short sensilla at the base of the pedicel, whereas the male had a long sensilla. The pedicel has a truncated conical shape that is wider distally. The flagellum is composed of eight flagellomeres with a spatulate apical shape (Pezzi *et al*., 2017) [33]. Thorax black, not hunchbacked, calyptra not elongated. Prominent shiny black scutellum with humeral callus. Post-wing callus and posterior margin of black scutellum (Roy *et al*., 2018) [34]. The wings can be black to brown in color. Four median veins arise from the discal cell and disappear as they approach the wing margin. Halteres whitish and greenish in distal area. Black legs with white tarsi, upper half of tibia white without spines. Black abdomen with oblong translucent tergites 1 and 2 (Oliveira, Doelle and Smith 2016) [30]. The genitalia of the female had two long segmented cerci, an elongated subgenital plate, and an acute angle distally. Subtriangular genital furcation with an unusually wide median opening. Leaf-shaped posterolateral projections (Üstüner, Hasbenli and Rozkošný 2003) [35]. The genitalia of males present a relatively short epandrium with a proximally emarginate shape, genital capsule (Synsternite) with two posterolateral lobes, one on each side, and pair of reduced gonostyles (Roy *et al*., 2018) [34]. Gonostyle with a finger-like shape with projections in the distal and proximal margins and a median lobe subtriangular proctiger. Well-developed

sternum 10, shorter than epandrium, round-shaped cerci, elongated at tip Aedeagal complex very thin and dilated in basal part (Üstüner, Hasbenli and Rozkošný 2003) [35]. The presence of wing curling deformations in adults, particularly in response to specific treatments, highlights the potential physiological and developmental effects of pesticide exposure. This type of deformity can have functional consequences for affected arthropods and may provide insight into the specific ways in which lufenuron or other treatments affect the development of these organisms.

In the genitalia of the female, a wound with liquid release was observed in the pleura of the ninth abdominal tergite (Figure 9). This damage was observed only in the 0.282, 0.878, and 1.066 mg/kg lufenuron treatments. In the male sample obtained from the 1,066 mg/kg lufenuron treatment group, a specimen with a malformation in one of its flagella was observed. In the form of an elongated appendage attached at the same point as the rest of the flagellum (Figure 10). In this treatment, damage was also observed in the male genitalia cerci, absence of both cerci, and asymmetry in one of them (Figure 11). The observation of a wound with liquid release in the genitalia of females, particularly in the pleura of the ninth abdominal tergite, suggests that exposure to treatments (presumably involving lufenuron) may have resulted in physical damage or stress to these reproductive structures.

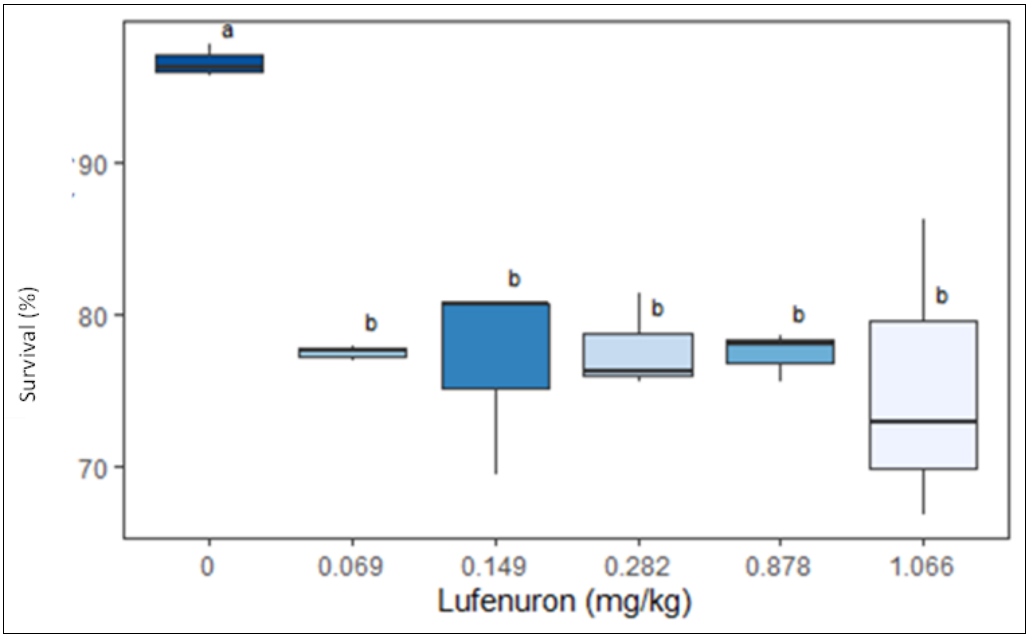

Fig 1: Box plot of percentage of surviving larvae of *Hermetia illucens* reared on diets contaminated with different concentrations of lufenuron

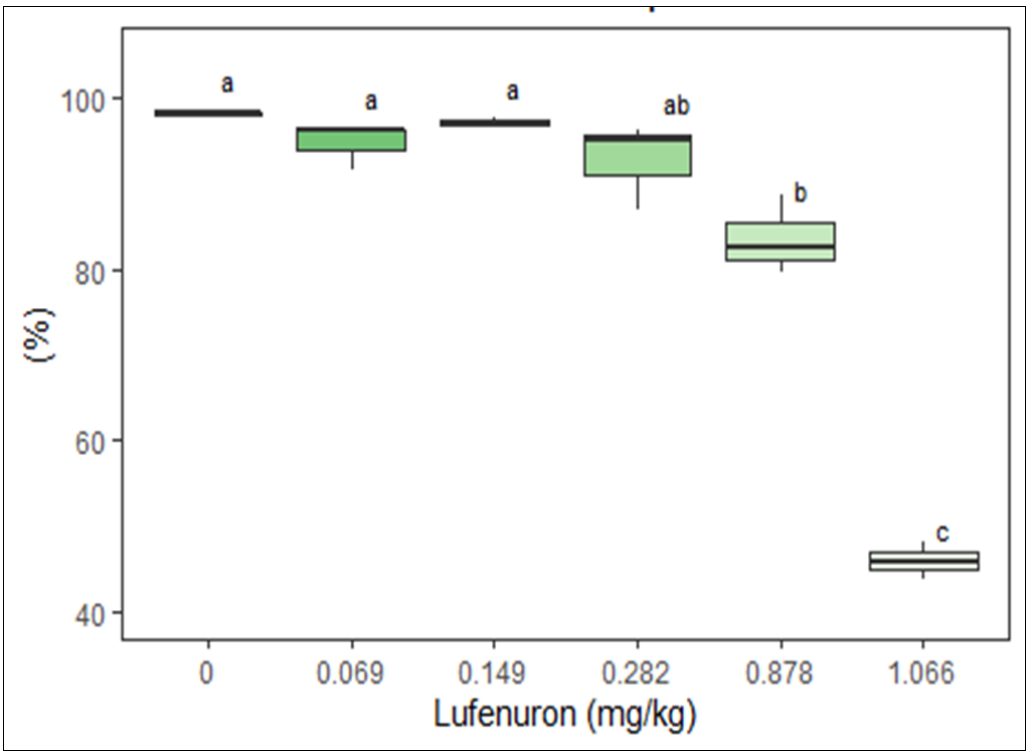

Fig 2: Box plot of survival pupae percentage of *Hermetia illucens* reared on diets contaminated with different concentrations of lufenuron

Fig 3: Morphological aspect mouth part of the fifth-instar larvae of *Hermetia illucens* from the group exposed to lufenuron at a concentration of 1,066 mg/kg

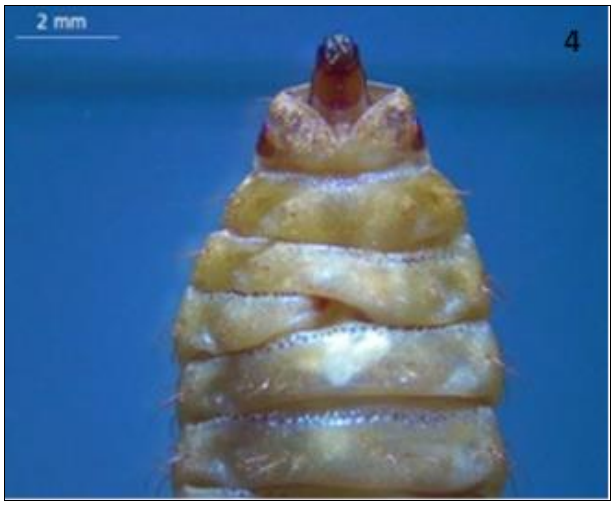

Fig 4: Ventral

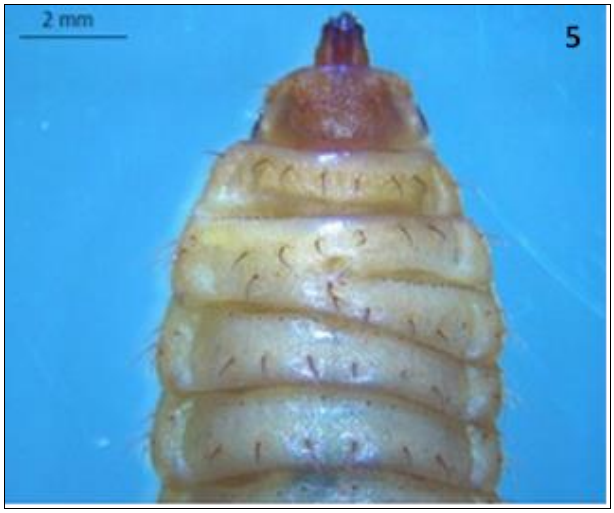

Fig 5: Dorsal view of morphological damage of fifth-instar larvae of *Hermetia illucens*. From the group exposed to lufenuron at a concentration of 1,066 mg/kg

Fig 6: Ventral view of incomplete ecdysis of Hermetia illucens pupae from the group exposed to lufenuron at concentrations of 0.149, 0.282, and 1.066 mg/kg

Fig 7: A larger wound with increased secretion of fluid on the eighth abdominal segment in the ventral section of pupae of *Hermetia illucens* from the group exposed to lufenuron at concentrations of 0.149, 0.282, 0.878, and 1.066 mg/kg

Fig 8: Wings deformations in adult of *Hermetia illucens* were in observed in all treatments except the control and treatment with 0.149 mg/kg lufenuron

Fig 9: Wound with release of liquid was observed in the pleura of the ninth abdominal tergite of *Hermetia illucens* in the groups exposed to lufenuron at concentrations of 0.282, 0.878 and 1.066 mg/kg

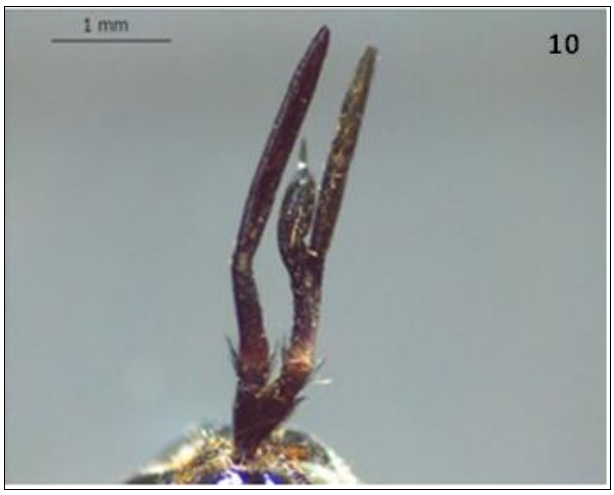

Fig 10: Malformation of the male antenna in a flagellomere. In the form of an elongated appendage and attached to the same point as the rest of the flagellomeres *Hermetia illucens* from the group exposed to lufenuron at a concentration of 1.066 mg/kg

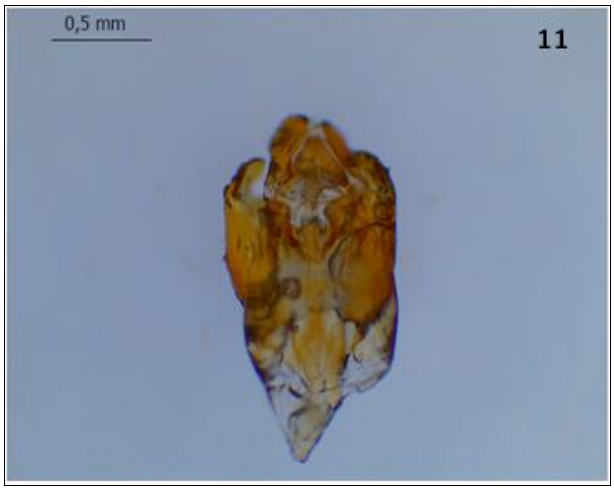

Fig 11: Male genitalia with a cerci asymmetry malformation on adults of *Hermetia illucens* from the group exposed to lufenuron at a concentration of 1.066 mg/kg

Discussions

The inhibition of chitin synthesis leads to deficient cuticle formation, resulting in weak, malformed, or irregular exoskeletons. This compromises the ability of arthropods to function properly and exposes them to various environmental stresses. (Hammock and Quistad, 1981; Mommaerts, Sterk and Smagghe 2006) [36, 37]. The present study determined the potential impact of the insecticide lufenuron, which belongs to the group of chitin synthesis inhibitors, benzoyl phenyl ureas, which was tested on 5 concentrations (0.069, 0.149, 0.282, 0.878 and 1.066 mg/kg lufenuron) plus the control (lufenuron-free) on the feeding of 7-day-old *H. illucens* larvae.

The results presented here show that the mortality of fifth stage (L5) larvae exposed to increasing concentrations of lufenuron was significantly higher than that of the control larvae. However, mortality did not increase with the concentration of lufenuron (Figure 1). At the pupal stage, lufenuron concentrations greater than or equal to 0.878 mg/kg (treatments 5 and 6) resulted in a significant increase in the percentage of non-emerged pupae (Figure 2). These results are consistent with those observed in *Drosophila melanogaster* larvae (Diptera: Drosophilidae). Newly emerged larvae (0-4 hours) fed high lufenuron doses (0.3-0.5 ppm showed a negative effect on the survival of larvae, pupae, and even adult flight, with a subsequent decrease in the number of eggs collected (Wilson and Cryan 1997) [19]. Fonseca *et al*., (2015) [38] applied a sublethal dose of 4.04 mg lufenuron/L to the diet of 10-day-old *Diatraea flavipennella* (Lepidoptera: Crambidae) larvae. It was observed that this concentration, the lowest in this test, caused movement difficulties in the individuals and various morphological deformations in the larvae, such as incomplete molting, exuvial retention, and air bubbles or liquid in the cephalic area. These findings are similar to the deformations observed in the *H. illucens* larvae test (Figure 5). In *Ephestia figulilella* (Lepidoptera: Pyralidae), the mode of application of lufenuron was different from that described by Fonseca *et al*., (2015) [38]. The insecticide Match™% 50 EC from Syngenta Crop Protection was sprayed at concentrations of 500, 750, 1,000, 1,250, and 1,500 mg/l of lufenuron during the last larval stage of the species. In addition to detecting the mortality of individuals, malformations were found. The treated larvae presented a dark coloration in the distal part of the abdomen, and in addition, they were not able to feed and died. Other findings include malformations in pupae and dark coloration deformation in adults (Khajepour, Izadi and Asari 2012) [39].

Since lufenuron is used in salmon farming, the Scottish Executive (2002) [40] reported that benzoylphenyl urea agents have poor absorption across the gastrointestinal tract of salmon. Samuelsen *et al*., (2015) [41] found that approximately 90% of benzoylphenyl urea compounds are teflubenzuron. administered on medicated feed and eaten by the fish, is excreted in its active form and spread to the environment. This represents a major ecological concern for non-target species, such as wild crustaceans that live near salmon farms.

The findings suggest that lufenuron, once taken up by the larvae of *H. illucens*, can have profound effects on the survival of larvae L5 and affect the survival of pupae when exposed to lufenuron at concentrations of 0.878 and 1.066 mg/kg. Lufenuron interferes with chitin synthesis, abnormal endocuticular deposition, and abortive molting. This study showed damage and significant morphological alterations in the larvae, pupae, and adult stages of this insect. These factors can cause profound damage to the *H. illucens* colonies.

Further studies are needed on the effects of BPU insecticides, such as lufenuron, on the metabolic pathways used by insects,

such as *H. illucens*, to deal with this type of substance or other agents. It is recommended to investigate the frass that the larvae generate when they are fed an insecticide, such as lufenuron. It is not known whether the effectiveness of the insecticide is maintained or diminished by ingestion and excretion by larvae.

Conclusion
The study shows that lufenuron causes morphological damage that affects larval rearing, reproduction, and fertility of the female. These factors can cause profound damage to the Indoor breeding of edible insects *H. illucens.*

Acknowledgments
Natpro SpA. Company that supported this study. We thank the assistance of Antonio Rivera (Instituto de Entomología, Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile) and Cristóbal Briceño (Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile) for their opinions and contribution for the development in this study.

Consent to participate
The article did not involve any human participants.

Consent to publish
The submitted work is original.

Author contributions
MA was involved in formal analysis, data collection, and curation, investigation, methodology incl. statistics, software, visualization, writing of original draft, and revision. CRG was involved in conceptualization, data curation, writing of original draft, review, and editing.

Data availability and material
All data (i.e. individual values used in graphics and figures reported in this manuscript) generated or analyzed during this study are included in this article, and will be made available on Zenodo https://zenodo.org/record/8347295

Disclosure of interest
The authors report no conflicts of interest.

Funding
Not applicable.

ORCID
Mónica Ardiles https://orcid.org/0009-0002-6554-5645
Christian R. González http://orcid.org/0000-0003-2582-6071

References
1. Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, *et al*. Food security: The challenge of feeding 9 billion people. Science. 2010;327:812-818.
2. Van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, *et al*. Edible insects: future prospects for food and feed security. Future Prospects for Food and Feed Security Food and Agriculture Organization of the United Nations, 2013, 187.
3. Rumpold BA, Schlüter OK. Nutritional composition and safety aspects of edible insects. Molecular Nutrition and Food Research. 2013;57(5):802-823.
4. Fasolin LH, Pereira RN, Pinheiro AC, Martins JT, Andrade CCP, Ramos OL, *et al*. Emergent food proteins – Towards sustainability, health and innovation. Food Research International. 2019;125:108586.
5. Zheng L, Hou Y, Li W, Yang S, Li Q, Yu Z, *et al*. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. Energy. 2012;47(1):225-229.
6. Klammsteiner T, Turan V, Juárez MFD, Oberegger S, Insam H. Suitability of black soldier fly frass as soil amendment and implication for organic waste hygienization. Agronomy. 2020;10(10):1578.
7. Bessa LW, Pieterse E, Marais J, Dhanani K, Hoffman LC. Food safety of consuming black soldier fly (*Hermetia illucens*) larvae: Microbial, heavy metal and cross-reactive allergen risks. Foods. 2021;10:1934.
8. Hoek-van den Hil EF, van de Schans MGM, Bor G, van der Fels-Klerx HJ. Effects of veterinary drugs on rearing and safety of black soldier fly (*Hermetia illucens*) larvae. Journal Insects Food Feed. 2022;8(10):1097-106.
9. Meijer N, de Rijk T, van Loon JJA, Zoet L, van der Fels-Klerx HJ. Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (*Hermetia illucens*) larvae. PLoS One, 2021, 16(4).
10. Purschke B, Scheibelberger R, Axmann S, Adler A, Jäger H. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain Food Additives & Contaminants: Part A. 2017;34(8):1410-1420.
11. Risk profile related to production and consumption of insects as food and feed. EFSA Journal, 2015, 13(10).
12. Berendsen BJA, Wegh RS, Memelink J, Zuidema T, Stolker LAM. The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta. 2015;132:258-268.
13. Jansen LJM, van de Schans MGM, de Boer D, Bongers IEA, Schmitt H, Hoeksma P, *et al*. A new extraction procedure to abate the burden of non-extractable antibiotic residues in manure. Chemosphere. 2019;224:544-553.
14. Rösner J, Wellmeyer B, Merzendorfer H. *Tribolium castaneum*: A Model for Investigating the Mode of Action of Insecticides and Mechanisms of Resistance. Current pharmaceutical design. 2020;26(29):3554-3568.
15. Sun R, Liu C, Zhang H, Wang Q. Benzoylurea Chitin Synthesis Inhibitors. Journal of agricultural and food chemistry. 2015;63(31):6847-6865.
16. Zhu KY, Merzendorfer H, Zhang W, Zhang J, Muthukrishnan S. Biosynthesis, Turnover, and Functions of Chitin in Insects. Annual Review of Entomology. 2016;61:177-196.
17. Kelkenberg M, Odman-Naresh J, Muthukrishnan S, Merzendorfer H. Chitin is a necessary component to maintain the barrier function of the peritrophic matrix in the insect midgut. Insect biochemistry and molecular biology. 2015;56:21-28.
18. Merzendorfer H. Chitin synthesis inhibitors: Old molecules and new developments. Insect Science. 2013;20(2):121-138.
19. Wilson TG, Cryan JR. Lufenuron, a chitin-synthesis inhibitor, interrupts development of *Drosophila melanogaster*. The Journal of experimental zoology. 1997;278(1):37-44.
20. Syngenta. Sorba® 050 EC Label; c2021.

21. Bravo S, Treasurer J. The management of the sea lice in Chile: A review. Reviews in Aquaculture. 2023;15(4):1749-1764.
22. Tanani MA, Hasaballah AI, Hussein RM. Assessment of the perturbation induced by chitin synthesis inhibitors lufenuron, flufenoxuron and hexaflumuron in the house fly, *Musca domestica vicina* (Diptera: Muscidae). The Journal of Basic and Applied Zoology, 2022, 83(29).
23. Ministry of Health of the Republic of Chile. Sets maximum residue limits of veterinary drugs in food for human consumption. Resolution 1,560 of 2019, September 11; c2019.
24. Lewis KA, TJ, WD and GA. An international database for pesticide risk assessments and management. H and ERAAIJ. 2016;22(4):1050-1064. D 10. 1080/10807039. 2015. 1133242. Lufenuron (Ref: CGA 184699). Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/420.htm
25. Hogsette JA. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. Journal of economic entomology. 1992;85(6):2291-2294.
26. Bruno D, Bonacci T, Reguzzoni M, Casartelli M, Grimaldi A, Tettamanti G, *et al*. An in-depth description of head morphology and mouthparts in larvae of the black soldier fly *Hermetia illucens*. Arthropod structure & development. 2020;58:100969.
27. Barros LM, Gutjahr ALN, Ferreira- Keppler RL, Martins RT. Morphological description of the immature stages of *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae). Microscopy research and technique. 2019;82(3):178-189.
28. Fachin DA, Hauser M. Large Flies Overlooked: the Genus *Hermetia Latreille*, 1804 (Diptera: Stratiomyidae) in the Neotropics, with 11 Synonyms and a New Species to Brazil. Neotropical entomology. 2022;51(5):660-690.
29. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria; c2022. Available from: https://www.R-project.org/.
30. Oliveira FR, Doelle K, Smith RP. External morphology of *Hermetia illucens* Stratiomyidae: Diptera (L.1758) based on electron microscopy. Annual research & review in biology. 2016;9(5):1-10
31. Üstüner T, Hasbenli A, Rozkošný R. The first record of *Hermetia illucens* (Linnaeus, 1758) (Diptera, Stratiomyidae) from the Near East. Studia dipterological. 2003;10(1):181-185.
32. Pezzi M, Scapoli C, Bharti M, Faucheux MJ, Chicca M, Leis M, *et al*. Fine structure of maxillary palps in adults of *Hermetia illucens* (Diptera: Stratiomyidae). Journal of Medical Entomology. 2021;58(2):658-665.
33. Pezzi M, Leis M, Chicca M, Falabella P, Salvia R, Scala A, *et al*. Morphology of the antenna of *Hermetia illucens* (Diptera: Stratiomyidae): An ultrastructural investigation. Journal of Medical Entomology. 2017;54(4):925-933.
34. Roy S, Chakraborty SK, Parui P, Mitra B. Taxonomy of Soldier Flies (Diptera: Stratiomyidae) of Sunderban Biosphere Reserve, India. Proceedings of the Zoological Society. 2018;71(2):121-126.
35. Üstüner T, Hasbenli A, Rozkošný R. The first record of *Hermetia illucens* (Linnaeus, 1758) (Diptera, Stratiomyidae) from the Near East. Studia Dipterologica. 2003;10(1):181-185.
36. Hammock BD, Quistad GB. Progress in Pesticide Biochemistry: Metabolism and mode of action of juvenile hormone, juvenoids and other insect growth regulators. New York: Wiley; c1981.
37. Mommaerts V, Sterk G, Smagghe G. Hazards and uptake of chitin synthesis inhibitors in bumblebees *Bombus terrestris*. Pest Management Science. 2006;62(8):752-758.
38. Fonseca APP, Marques EJ, Torres JB, Silva LM, Siqueira HÁA. Lethal and sublethal effects of lufenuron on sugarcane borer *Diatraea flavipennella* and its parasitoid *Cotesia flavipes*. Ecotoxicology. 2015;24(9):1869-1879.
39. Khajepour S, Izadi H, Asari MJ. Evaluation of two formulated chitin synthesis inhibitors, hexaflumuron and lufenuron against the raisin moth, *Ephestia figulilella*. Journal of Insect Science. 2012;12:102.
40. Executive S. Review and Synthesis of the Environmental Impacts of Aquaculture. Edinburgh; c2002.
41. Samuelsen OB, Lunestad BT, Hannisdal R, Bannister R, Olsen S, Tjensvoll T, *et al*. Distribution and persistence of the anti sea-lice drug teflubenzuron in wild fauna and sediments around a salmon farm, following a standard treatment. Science of the total environment. 2015;508:115-121.