

Article

Effects of Insect Growth Regulators on Mortality, Survival, and Feeding of *Euprosterina elaeasa* (Lepidoptera: Limacodidae) Larvae

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Abstract: The potential of insecticides that affect the growth and insect development to control *Euprosterina elaeasa* was evaluated. Fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide were assessed against *E. elaeasa* larvae for toxicity, survivorship, and feeding inhibition in the laboratory, and mortality in semifield conditions. Concentration–mortality bioassays demonstrated that insect growth regulators (IGRs) have a lethal effect on this insect, with pyriproxyfen ($LC_{50} = 0.141 \text{ g L}^{-1}$) being the most effective, followed by fenoxycarb ($LC_{50} = 0.199 \text{ g L}^{-1}$), methoxyfenozide ($LC_{50} = 0.233 \text{ g L}^{-1}$), and tebufenozide ($LC_{50} = 0.259 \text{ g L}^{-1}$). The survival rate was 99.8% in the control group, compared to 44.6%, 42.9%, 42.2%, and 39.5% in insects treated with pyriproxyfen, fenoxycarb, methoxyfenozide, and tebufenozide, respectively. IGRs caused feeding inhibition in *E. elaeasa* larvae 3 h after exposure. Furthermore, mortality in semifield conditions was similar to the results found in the laboratory. Our findings suggest that fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide are effective against *E. elaeasa* and, therefore, we confirm the potential of these IGRs for the control of this pest.

Keywords: ecdysone receptor agonists; feeding inhibition; juvenile hormone mimics; lethal concentration; limacodid pest; toxicity



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1. Introduction

Euprosterina elaeasa Dyar (Lepidoptera: Limacodidae) is a significantly harmful oil palm (*Elaeis guineensis* Jacq.) pest in South American countries [1]. This herbivore damages oil palm leaf and consumes an area of 400 mm²/larva, injuries are recognizable by the irregular cutting in foliar area and causing about 80% of defoliation [2]. Additionally, feeding activities of *E. elaeasa* larvae were implicated in assisting infection of *Pestalotiopsis* fungal species [3]. In commercial oil palm plantations, defoliation by insects causes up to 50% loss in harvest, by rasping the superficial foliar area or by consuming the parenchymal tissue, drastically reducing plant size, biomass, and palm oil production [4]. In this case, detrimental defoliation caused by *E. elaeasa* occurs on the top level of the canopy, and the palm trees require at least 3 years to recover the foliar area [2].

In South America, the utilization of pesticides is a widespread method to minimize the high pest populations and quick infestation of oil palm crops [5,6]. In this scenario, organophosphate insecticides such as acephate, metamidophos, and monocrotophos are applied through trunk injection in palm trees to control *E. elaeasa* [7]. However, minimal quantities of these insecticides were found in palm oil in a preliminary study [8]. Organophosphate insecticides have hazardous environmental effects such as agricultural and food contamination [9], ozone layer deterioration [10], residual long-term exposure [11], the development of target-site resistance and cross-resistance in pests [12], and limited safety to natural enemies, including predators and parasitoids [13]. More sustainable options for acephate, metamidophos, and monocrotophos are needed to avoid the organophosphate

insecticides implemented for decades to control this insect [14]. Seeking environmentally friendly molecules and with low toxicity to non-target organisms to control *E. elaeasa* is essential considering the negative effects of the use of neurotoxic substances applied directly to oil palm [15,16].

The use of biorational agents is an important tool for the alternative management of oil palm pests [17] and their application was proven to reduce populations [18]. The current suite of biorational insecticides includes chitin synthesis inhibitors, ecdysone receptor agonists, and juvenile hormone mimics, distinguished by physiological activity interfering with the growth and development of insects [19–21]. The effects of these insecticides have been investigated to control oil palm pests such as *Eupalamides cyparissias* Fabricius (Lepidoptera: Castniidae) in Brazil [22], *Leptopharsa gibbicarina* Froeschner (Hemiptera: Tingidae) in Colombia [17], and *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) in Malaysia [23]. Insect growth regulators (IGRs) act in insect development and mediate the balance of ecdysone [21] or juvenile hormone [20]. Especially, IGRs play a role in insect physiological processes, altering specific pathways of hormonal control that are related to molting [24], metamorphosis [25], and normal development [26].

IGRs are chemical substances classified as juvenile hormone mimics and ecdysone disruptor agonists (Insecticide Resistance Action Committee; groups 7 and 18) [27]. Particularly, effective molecules such as fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide are used substantially to manage lepidopteran defoliating pests [20,21,24–26]. Different classes of neurotoxic insecticides are used to control *E. elaeasa*; however, the availability and use of biorational agents as ecdysone receptor agonists and juvenile hormone analogs is an option for insect pest management programs. We assumed that IGRs such as fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide would have suppressive effects on *E. elaeasa* larvae, which could be due to their capability to affect the survival, feeding, and development of this pest.

This research assessed the effects of fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide on *E. elaeasa*, evaluating their toxicity, survivorship, and feeding inhibition in the laboratory, and mortality in semifield conditions.

2. Materials and Methods

2.1. Insects

Adults of *E. elaeasa* ($n = 3975$; $\sigma = 2134$, $\text{♀} = 1841$) were collected from 5-year-old oil palm crops ($07^{\circ}20' \text{ N}$, $73^{\circ}54' \text{ W}$) and brought to the Crop Protection Laboratory of the Monterrey Oil Palm AS Plantation (Puerto Wilches, Colombia) for rearing. Adults were fed a liquid diet composed of 25% honey, 25% sucrose, and 50% deionized distilled water (ddH₂O) soaked in a cotton ball. For mating and reproduction, adults (one male and one female) of *E. elaeasa* were isolated in polystyrene boxes ($35 \times 35 \times 35 \text{ cm}$) containing liquid diet and oil palm leaflets. For egg development, eggs laid on the leaflets were placed in Petri dishes (90 mm diameter) and maintained at $28 \pm 2 \text{ }^{\circ}\text{C}$, $79\% \pm 35\% \text{ RH}$, and 12:12 h light/dark cycle until larva emergence. Emerged larvae were placed in polystyrene boxes (750 mL) and fed on oil palm leaflets. Healthy, 24 h old *E. elaeasa* third-instar larvae were used in the laboratory and semifield bioassays.

2.2. Concentration–Mortality Bioassay

Four IGRs were adjusted in 100 mL of ddH₂O to obtain five dilutions (0.075, 0.15, 0.3, 0.6, and 1.2 g L^{-1}): fenoxycarb ($250 \text{ a.i. g L}^{-1}$ Insegar WG, Syngenta Crop Protection AG, Panamá, Panamá), methoxyfenozide ($240 \text{ a.i. g L}^{-1}$ Intrepid SC, Dow Agrosiences LLC, Soledad, Colombia), pyriproxyfen ($100 \text{ a.i. g L}^{-1}$ Epingle SC, Summit Agro S.A.S., Bogotá, Colombia), and tebufenozide ($240 \text{ a.i. g L}^{-1}$ Confirm SC, Summit Agro S.A.S., Bogotá, Colombia). Serial dilutions of each IGR in addition to the control (only ddH₂O) were prepared to evaluate toxicity and calculate the concentration–mortality relationship and lethal concentrations (LC_{25} , LC_{50} , LC_{75} , and LC_{95}). Subsequently, each insecticide dilution ($1 \mu\text{L}$) was applied to the thorax of 50 *E. elaeasa* larvae using a micropipette

(Eppendorf[®], Hamburg, Germany). After IGRs exposure, larvae were individualized in polystyrene boxes (13 × 17 cm), maintained in a climatized room, and fed with oil palm leaves. Three replicates with 40 larvae each were utilized for each of the five dilutions evaluated (600 larvae per IGR) and the number of dead larvae was counted after IGR exposure for 6 d.

2.3. Time–Mortality Bioassay

Euprosterina elaeasa larvae were topically exposed to the estimated lethal concentration (LC₅₀ and LC₉₅) of each IGR prepared in ddH₂O. A control was performed using ddH₂O. Similar exposure procedures were realized as described in the concentration–mortality bioassay. Three replicates of 40 larvae were utilized for each IGR lethal concentration and the number of live larva was registered every 8 h for 6 d.

2.4. Feeding Inhibition

Larvae of *E. elaeasa* were individualized in Petri dishes with a piece of oil palm leaf treated with LC₅₀ or LC₉₅ of each IGR and ddH₂O as the control. Leaves were obtained from 6-year-old oil palms and cut into pieces (10 mm²), sterilized with 2.5% sodium hypochlorite, washed three times with ddH₂O, and air-dried. Subsequently, the leaf pieces were immersed for 20 s in a solution of the LC₅₀ and LC₉₀ of each IGR or control, air-dried for 10 min, and the larvae were in contact with the leaf piece for 3 h. The area consumed by each insect was photographed with a D40 Nikon digital camera (Nikon Corporation, Tokyo, Japan) equipped with a 150 mm macrofocus and SB-700 Speedlight flash. The images were analyzed using the digital analysis software QUANT v. 1.0 (Federal University of Viçosa, Viçosa, Brazil). The leaf area consumed by *E. elaeasa* was measured in mm² with pixels based on the RGB histogram (red, 743 nm; green, 525 nm; blue, 417 nm). Twenty repetitions per lethal concentration of each IGR and control were performed.

2.5. Mortality in Semifield Conditions

The experiment was performed in 6-year-old oil palm crops (ASD, cv Dura × Pisifera) in Monterrey Oil Palm AS Plantation, with an average temperature of 28.17 °C, 73–87% relative humidity, 1545–2127 h annual sunlight, and 2176 mm annual rainfall. Palm trees ($n = 100$, 20 per treatment) were selected and 40 *E. elaeasa* larvae were placed on a leaf (no. 17, in accordance with the phyllotaxy position) of each palm tree [28] and enclosed in organza fabric (0.75 × 1.5 m) for 24 h to guarantee natural relocation. IGRs prepared to the LC₉₅ level in ddH₂O in addition to the control (only ddH₂O) were used as treatments with five replications. After 1 d of larvae isolated in organza fabric, 250 mL of each treatment (IGRs or control) was sprayed on both sides of the leaf with a Royal Condor[®] pump spray (Soacha, Colombia). Organza fabric pieces were removed before treatment application and again placed until the final experiment duration. Oil palm leaves were carefully cut and the number of dead *E. elaeasa* larvae was quantified. Larval mortality caused by IGRs or control was registered for 6 d.

2.6. Statistical Analysis

The concentration–mortality data were submitted to Probit analysis. Kaplan–Meier survival analysis and the log-rank test were used to compare time–mortality data between IGRs and curves plotted with Prism 8.0 software [29]. Data on the anti-feeding effect and mortality in semifield conditions were arcsine-transformed and submitted to one-way analysis of variance, while Tukey’s HSD ($p < 0.05$) test was also used to compare means. Concentration–mortality, antifeeding effect, and mortality in semifield assay data were analyzed with SAS 9.0 software [30].

3. Results

3.1. Concentration–Mortality Bioassay

The mortality data were suitable for a Probit model fit ($p > 0.05$), showing IGRs toxicity to *E. elaeasa* and allowing us to estimate lethal concentrations (Table 1). For the estimated LC_{50} , testing showed that pyriproxyfen with $LC_{50} = 0.141$ (0.117–0.171) $g L^{-1}$ and fenoxycarb with $LC_{50} = 0.199$ (0.162–0.241) $g L^{-1}$ were the most effective IGRs for *E. elaeasa*, followed by methoxyfenozide with $LC_{50} = 0.233$ (0.188–0.285) $g L^{-1}$, and tebufenozide with $LC_{50} = 0.259$ (0.217–0.308) $g L^{-1}$. Mortality was 0.1% in the control group.

Table 1. Toxicity of insect growth regulators (IGRs) by contact exposure on *Euprosterna elaeasa* larvae (df = 5). Lethal concentration (LC) values were estimated based on concentration–mortality bioassay using Probit analysis. EC, estimated concentration; CI, confidence interval.

IGRs	No. Insects	LC	EC ($g L^{-1}$)	95% CI ($g L^{-1}$)	Slope \pm SE	χ^2 (p -Value)
Fenoxycarb	120	LC_{25}	0.109	0.079–0.137	1.815 \pm 0.23	1.66 (0.64)
	120	LC_{50}	0.199	0.162–0.241		
	120	LC_{75}	0.363	0.297–0.470		
	120	LC_{95}	0.859	0.630–1.378		
Methoxyfenozide	120	LC_{25}	0.120	0.086–0.152	1.486 \pm 0.20	4.42 (0.21)
	120	LC_{50}	0.233	0.188–0.285		
	120	LC_{75}	0.451	0.362–0.603		
	120	LC_{95}	1.167	0.824–1.997		
Pyriproxyfen	120	LC_{25}	0.079	0.061–0.096	2.281 \pm 0.29	6.23 (0.11)
	120	LC_{50}	0.141	0.117–0.171		
	120	LC_{75}	0.251	0.204–0.333		
	120	LC_{95}	0.578	0.419–0.942		
Tebufenozide	120	LC_{25}	0.154	0.121–0.185	1.760 \pm 0.23	5.77 (0.12)
	120	LC_{50}	0.259	0.217–0.308		
	120	LC_{75}	0.434	0.360–0.554		
	120	LC_{95}	0.914	0.690–1.387		

3.2. Time–Mortality Bioassay

The IGRs significantly reduced *E. elaeasa* survival rates after 6 d exposure and were differentially at LC_{50} (log-rank test, $\chi^2 = 16.97$; df = 4; $p < 0.001$) (Figure 1A). *Euprosterna elaeasa* survival declined from 99.8% in the control to 44.6% with tebufenozide, 42.9% with methoxyfenozide, 42.2% with fenoxycarb, and 39.5% with pyriproxyfen. *Euprosterna elaeasa* survival was significantly impaired by IGRs at LC_{95} (log-rank test, $\chi^2 = 18.52$; df = 4; $p < 0.001$; Figure 1B). *Euprosterna elaeasa* survival declined from 99.8% in the control to 35.2% with methoxyfenozide, 31.7% with tebufenozide, 26.3% with fenoxycarb, and 9.61% with pyriproxyfen.

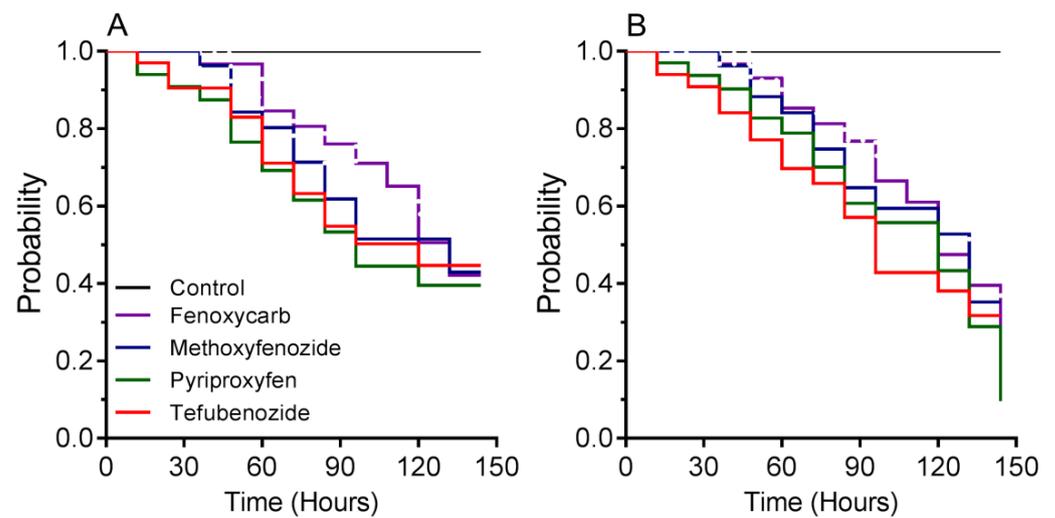


Figure 1. Kaplan–Meier survival curves for contact exposure of *Euprosterina elaeasa* larvae with insect growth regulators (IGRs) at different lethal concentrations: (A) LC₅₀ and (B) LC₉₅.

3.3. Feeding Inhibition

The IGRs caused feeding inhibition in *E. elaeasa* larvae, with lower leaf area consumed compared to the control (Figure 2). Food consumption by *E. elaeasa* was significantly different between the IGRs at LC₅₀ ($F = 31.24$; $df = 4,19$; $p < 0.001$), reducing from 21.8 mm² in the control to 17.1 mm² with fenoxycarb, 15.8 mm² with pyriproxyfen, 12.4 mm² with methoxyfenozide, and 6.81 mm² with tebufenozone. For LC₉₅, the area consumed by *E. elaeasa* differed between IGRs ($F = 39.02$; $df = 4,19$; $p < 0.001$), reducing from 22.1 mm² in the control to 15.2 mm² with fenoxycarb, 13.8 mm² with pyriproxyfen, 9.13 mm² with methoxyfenozide, and 3.22 mm² with tebufenozone.

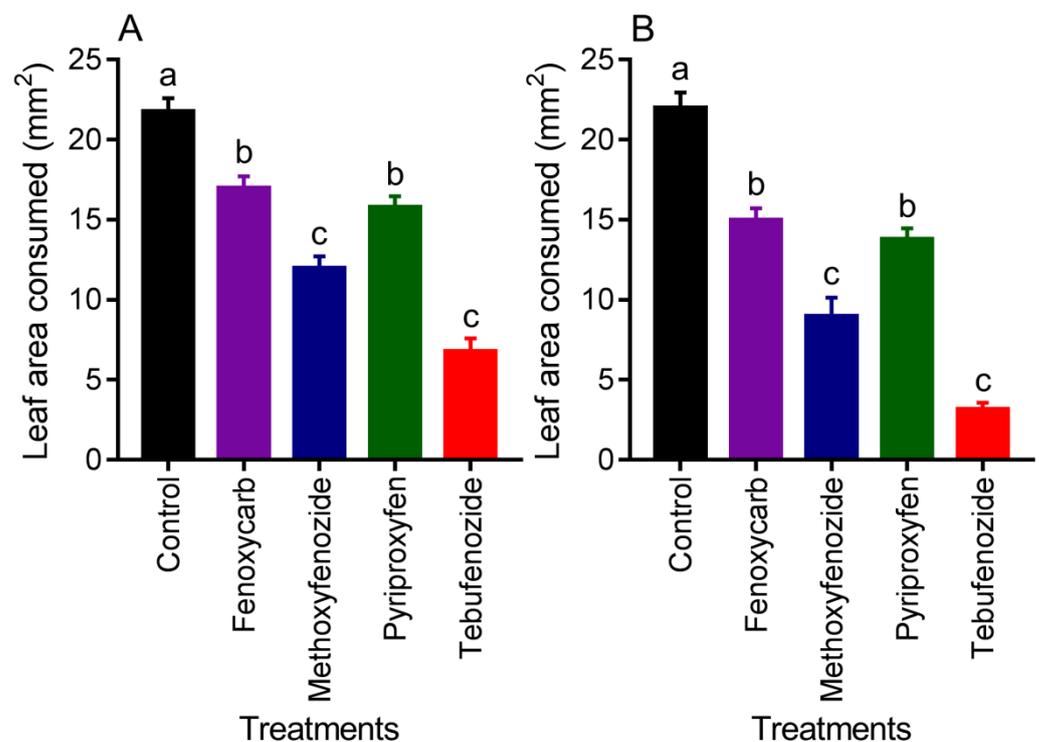


Figure 2. Leaf consumption (mean \pm SEM) by *Euprosterina elaeasa* larvae exposed to insect growth regulators (IGRs): (A) LC₅₀ and (B) LC₉₅. Treatments means with different letters show significant differences by Tukey's HSD test at the $p < 0.05$ level.

3.4. Mortality in Semifield Conditions

The mortality caused by the IGRs tested on *E. elaeasa* larvae in field conditions was variable ($F = 31.36$; $df = 4, 19$; $p < 0.001$; Figure 3). High larval mortality was caused by tebufenozide at $89.6\% \pm 4.9\%$ and methoxyfenozide at $87.4\% \pm 4.7\%$, followed by pyriproxyfen at $79.4\% \pm 3.8\%$ and fenoxycarb at $79.4\% \pm 3.9\%$. Mortality was $3.67\% \pm 0.5\%$ in the control.

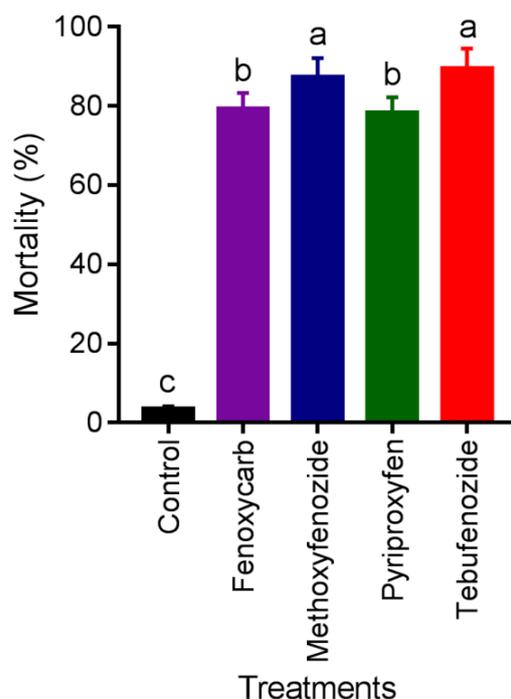


Figure 3. Mortality (mean \pm SEM) of *Euprosterina elaeasa* larvae caused by insect growth regulators (IGRs) applied on oil palm leaves in field conditions. Treatments means with different letters show significant differences by Tukey's HSD test at the $p < 0.05$ level.

4. Discussion

The effectiveness of the four IGRs used in this research was investigated; they caused mortality, lower survival rates, feeding inhibition, and reduced *E. elaeasa* populations. Fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide are toxic to *E. elaeasa* larvae and have a strong effect through topical exposure. IGRs lead to *E. elaeasa* mortality in a concentration-dependent manner, as observed in other pests [21,26,31,32]. However, *E. elaeasa* exposed to fenoxycarb and pyriproxyfen were more susceptible than when exposed to methoxyfenozide and tebufenozide. Therefore, a critical concentration of methoxyfenozide and tebufenozide is required for effective lethality, presumably to overcome insect growth regulation during contact and cuticle penetration. These results indicate that the effects produced by fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide present differences when mediating the physiology of *E. elaeasa* larvae. IGR classes (ecdysone receptor agonists and juvenile hormone analogs) express and exert wide endocrinal imbalances, including insect hormones (20-hydroxyecdysone (20HE), ecdysone (Ec), and juvenile hormone (JH)) involved in the epidermal cells reprogramming to produce specific proteins for the next instar/stage and completion of the molting and metamorphosis processes [24–26]. A set of study results point to disruption of the growth and development of lepidopteran pests such as *Anticarsia gemmatilis* Hübner (Noctuidae) when exposed to tebufenozide [21], *Choristoneura rosaceana* Harris (Tortricidae) when exposed to pyriproxyfen [31], *Lymantria dispar* Linnaeus (Erebidae) when exposed to methoxyfenozide [26], and *Plutella xylostella* Linnaeus (Plutellidae) when exposed to

fenoxycarb [32]. In general, IGRs exhibit toxic effects against *E. elaeasa* larvae, which increase at higher insecticide concentrations.

The significant time variations in *E. elaeasa* survival are produced by the interaction of IGRs attaching to the exoskeleton, penetrating in the hemocoel and being transported by hemolymph, and perturbing the hormonal balance. The time taken for fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide to induce mortality in *E. elaeasa*, from 3 to 6 days, presents slow action on the larva of this pest. In the present research, the comparative effects of the IGRs on *E. elaeasa* were observed at different time periods. These time differences commonly occur due to IGRs' ability to disrupt embryonic development and deform neonates [33], by causing lethal ecdysis deficiencies in instar larval development [34], by inducing morphological epidermal cell alterations [35], and by producing supernumerary molts [36], leading to delayed pupation and adult emergence [37]. Moreover, IGRs were found to inhibit feeding or insect reproduction, and subsequently, interrupt the life cycle [38]. High *E. elaeasa* survival for extended periods suggests that fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide cause adverse effects on larvae via reduction in the insect population. Thus, they may represent a valuable method to avoid the use of neurotoxic substances to control *E. elaeasa*.

Euprosterna elaeasa showed a low consumption of oil palm leaves treated with IGRs, suggesting an antifeeding effect. IGRs with antifeeding effects play an important role in herbivorous pests, causing responses such as feeding inhibition, distortions in the midgut histology, and low nutrient absorption [21,39]. In this research, the effect on feeding inhibition by both LC₅₀ and LC₉₀ suggests that the intoxication effect of IGRs is cumulative. Especially, our findings show that methoxyfenozide and tebufenozide cause further cessation in feeding of *E. elaeasa* larvae in comparison with fenoxycarb and pyriproxyfen. Feeding inhibition after exposure to methoxyfenozide and tebufenozide has been also reported in lepidopteran pests, including *Rachiplusia nu* Gueene (Noctuidae) [40] and *Orgyia pseudotsugata* (McDunnough) (Erebidae) [41]. In contrast, moderate feeding inhibition after exposure to fenoxycarb and pyriproxyfen has been reported in *Abraxas suspecta* Warren (Geometridae) [42] and *Spodoptera littoralis* Boisduval (Noctuidae) [43], respectively. The findings obtained here of a reduction in food consumption by *E. elaeasa* larvae, in both lethal concentrations, indicates a possible poisoning per os, perhaps due to alterations in the midgut [21], affecting digestive enzymes secretion [44] and energy metabolism [45] with possible suppression of the detoxification response [43,46], as observed in other insects after oral insecticide exposure [47–51]. In summary, the reduction in food consumption caused by IGRs on treated *E. elaeasa* larvae suggests feeding inhibition impairing the digestive process.

Fenoxycarb, methoxyfenozide, pyriproxyfen, and tebufenozide showed lethality against *E. elaeasa* in oil palm leaves in the field, and the findings are consistent with those found in the laboratory. However, the mortality rate at the larval stage was lower than that obtained under laboratory conditions. Additionally, the findings show that methoxyfenozide and tebufenozide affect a high number of *E. elaeasa* larvae. It is possible that the efficacy of IGRs under field conditions is due to climatic factors [52], translaminar action [53], chemical degradation [54], and the persistence of biorational insecticides in foliage [17]. However, while it is difficult to accurately determine the quantity of insecticide penetrating (by contact or ingestion) each insect, mortality caused by IGRs on *E. elaeasa* was similar to that found with topical application in the concentration–mortality bioassay. The lethality of IGRs and their effectiveness has also been observed with other defoliating pests under field conditions, justifying them as potent bio-rational agents against defoliating pests that can be incorporated in several agricultural systems [24–26,40–43]. The findings demonstrate that these IGRs have a specific mode of action as insecticides affecting a high number of *E. elaeasa* larvae. In particular, methoxyfenozide and tebufenozide are the most effective in the field and the maximum efficiency from IGRs should be used during the larval stage. Testing with these IGRs suggests that applications can dramatically decrease *E. elaeasa* infestation and are an essential element to protect oil palm leaves.

5. Conclusions

The suppressive effects of four IGRs on *E. elaeasa* were investigated. In the laboratory, fenoxycarb and pyriproxyfen were more toxic than methoxyfenozide and tebufenozide; however, tebufenozide and pyriproxyfen drastically affected the survival of this pest through contact exposure at different lethal concentrations. For oral exposure, methoxyfenozide and tebufenozide were more effective in causing feeding inhibition of this insect compared to fenoxycarb and pyriproxyfen. In semifield conditions, methoxyfenozide and tebufenozide caused high mortality after contact/oral exposure, increasing *E. elaeasa* mortality through two routes (dermal and ingestion) of exposure, with the potential to control its field populations. The toxicity caused by these IGRs provides a powerful tool to manage *E. elaeasa* larvae and reduce the insect's damage to oil palm leaves. In the field, *E. elaeasa* was highly susceptible to IGRs, which can be alternatives to replace organophosphate insecticides, directly reducing the defoliation and indirectly reducing the Pestalotiopsis infection caused by feeding activities of this pest in oil palm.

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