

ORIGINAL ARTICLE

The effect of *Hortia oreadica* Groppo (Rutaceae) extracts on the growth, feeding and digestive enzymes of *Anticarsia gemmatalis* Hübner [1818] (Erebidae)

Liliane Evangelista Visotto^{1*}, Vinícius Guimarães Nasser², Vanessa Gisele Pasqualotto Severino³, Márcio Santos Soares⁴, Vinícius Ribeiro Faria², Pedro Ivo Vieira Good God²

¹Institute of Biological and Health Sciences, Universidade Federal de Viçosa Rio Paranaíba, Brazil

²Institute of Agricultural Sciences, Universidade Federal de Viçosa Rio Paranaíba, Brazil

³Chemistry Institute, Universidade Federal de Goiás, Avenida Esperança, Goiania, Brazil

⁴Institute of Exact and Technological Sciences, Universidade Federal de Viçosa Rio Paranaíba, Brazil

Vol. 66, No. 1: 111–120, 2026

DOI: 10.24425/jppr.2026.158068

Received: March 20, 2025

Accepted: July 11, 2025

Online publication: March 23, 2026

*Corresponding address:

Magdalena Karbowska-Dzięgielewska

Responsible Editor:

lvisotto@ufv.br

Abstract

The effects of four extracts of *Hortia oreadica* Groppo (Rutaceae) on the biological parameters, nutritional indices, and activity of digestive enzymes of *Anticarsia gemmatalis* Hübner [1818] (Erebidae) were evaluated. Newly hatched caterpillars were subjected to extracts from *H. oreadica* extracted with hexane (HE), dichloromethane (DE), dichloromethane fractionated with ethyl acetate (DEAE) and dichloromethane fractionated with dichloromethane (DDE) at concentrations 0, 100, 500 and 1000 $\mu\text{g} \cdot \text{ml}^{-1}$. The results showed that treatments significantly increased the cumulative mortality rate of *A. gemmatalis* and that HE 100 $\mu\text{g} \cdot \text{ml}^{-1}$ was the most effective, causing 92% of insects' deaths. The period of larval development was significantly shorter with treatments HE 100 and 500 $\mu\text{g} \cdot \text{ml}^{-1}$ and longer with DEAE (all concentrations). The pupal weight was lower in the treatments HE 1000 $\mu\text{g} \cdot \text{ml}^{-1}$ and in all doses tested of the DEAE treatment. The morphological integrity of pupae and adults was not affected. The lowest values of relative food consumption rate, relative metabolic rate, and relative growth rate were observed in the HE 100 $\mu\text{g} \cdot \text{ml}^{-1}$ treatment. In most treatments, there was a reduction in the activity of total proteases, serine and cysteine proteases, and amylase. The results suggest that the HE extract of *H. oreadica* is the most efficient in controlling *A. gemmatalis*, due to the presence of deterrent metabolites that affect the insect's digestion and nutrition and consequently promote adverse effects on its development and mortality.

Keywords: botanical extracts, pest control, secondary metabolites, soybean caterpillar

Introduction

Many plants are inexorable sources of bioactive substances that can be incorporated into integrated pest management (Abdullah and El-Rokh 2023). The most promising botanical species for use in pest control belong to the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiateae and Canellaceae (Ngegba *et al.* 2022). The Rutaceae family is made up of many genera, of which the genus *Hortia* stands out. It is comprised of 12 species distributed from Panama and northern

South America (especially the Amazon) to the center east of Brazil (Groppo *et al.* 2008).

Secondary metabolites are reported for *Hortia* such as derivatives of dihydrocinnamic acid, alkaloids (furoquinolines, 2-quinolones and β -indolopyridoquinazolines), coumarins (simple, furanocoumarins and pyranocoumarins), terpenoids (sesquiterpenes, triterpenes and limonoids), amides and flavonoids (flavones and flavanone) (Severino *et al.* 2009; Severino

et al. 2014). Limonoids or tetra-nor-triterpenes represent the maximum level in the sequence of terpenoid production in plants that are not normally attacked by insects. At the lower level, monoterpenes with a relatively simple structure, such as limonene, myrcene, and 1,2-epoxy-pulegone, exert protective functions on the plants that produce them. Most studies on the mechanism of action of higher terpenoids in insects refer to activities such as growth inhibitors or retardants, damage to maturation, reduction of reproductive capacity, and appetite suppressants (Ukoroije and Otayor 2020). In the present study, the species *Hortia oreadica* Groppo (Fig. 1), a bushy shrub with a well-developed, yellowish underground system, was selected.



Fig. 1. Species *Hortia oreadica* Groppo (Rutacea) in Pirineus State Park, Goiás, Brazil (Source: Medeiros J.D. Guia de campo: Vegetação do Cerrado 500 espécies. MMA, 2011. <https://www.flickr.com/search/?text=hortia+oreadica>)

Despite the abundance of secondary metabolites present in this species, there are no reports of their action against insect pests.

The advantage of using natural products as an adjuvant in insect control is due to their biodegradation, low toxicity to animals (especially humans and domestic animals), and their efficiency against several species of insect pests. Botanical extracts have a complex chemical constitution that can affect the insect in different ways (Ngegba *et al.* 2022). Several studies report that crude plant extracts, either isolated or as active combinations, affect feeding, development, reproduction oviposition, and survival (Couto *et al.* 2019). They can cause anatomical deformations at different stages of the insect's life cycle (Abdelgaleil and El-Sabrou 2018), in addition to detrimental histopathological defects in cellular tissues such as intestinal epithelial cells (Cui *et al.* 2019).

Extracts of *H. oreadica*, extracted with organic solvents such as hexane, dichloromethane and ethyl acetate, were evaluated on the soybean caterpillar, *Anticarsia gemmatalis* Hübner [1818] (Lepidoptera: Erebididae). These solvents allow the selective extraction of compounds with different polarities, increasing the diversity of metabolites obtained, which is essential in the initial screening of compounds with biological activity. Thus, non-polar metabolites such as terpenoids, fatty acids, and hydrocarbons accumulate in the hexane fraction. Low polarity metabolites, e.g., terpenoids and less polar alkaloids, and some lipophilic flavonoids remain in dichloromethane. Metabolites of intermediate polarity, e.g., flavonoids, coumarins, and moderately polar alkaloids preferentially accumulate in ethyl acetate (Ukoroije and Otayor 2020).

Anticarsia gemmatalis, also known as velvetbean caterpillar, is considered one of the main pests of soybean crops, *Glycine max* (L.) Merr. (Fig. 2). Even at low population densities, this insect causes extensive damage to crops, ranging from defoliation to complete destruction of the plant (Praça *et al.* 2006). It is widely



Fig. 2. *Anticarsia gemmatalis*, one of the main soybean defoliator caterpillars (A). Leaf damage caused by the soybean caterpillar, due to consumption of the leaf limb and veins (B). (Source: <https://maissoja.com.br/voce-sabe-identificar-os-danos-pelas-pragas-em-soja/>)

recognized that soybean cultivation plays a significant role in the consumption of insecticides in Brazil and around the world. Pest control in this crop is predominantly carried out through the application of synthetic insecticides and, in most cases, with little diversification in management strategies (Perobelli 2025). Therefore, it is essential to seek information about the potential of substances from other sources for use in pest control in soybeans.

Here, the response of velvetbean caterpillar to increasing dietary concentrations of different extracts of *H. oreadica*, in terms of development, consumption and efficiency in the use of food and activity of digestive enzymes was reported. Due to the richness of alkaloids, limonoids, and derivatives of dihydrocinnamic acid, negative consequences of the extracts in the parameters of *A. gemmatalis* were expected.

Materials and Methods

Preparation of *Hortia oreadica* extracts

According to Severino *et al.* (2009), the plant parts were dried, crushed and percolated with the solvents hexane (HE) and dichloromethane (DE). Subsequently, the dichloromethane extract was fractionated with ethyl acetate (DEAE) and dichloromethane (DDE), generating two sub-extracts. The extraction in each solvent was carried out for 3 days, with a solvent change every 24 h. The extracts were concentrated in an evaporator and kept at 4°C, to prevent their degradation.

Rearing of *Anticarsia gemmatalis* and treatments

The artificial diet used to raise insects consisted of cooked red kidney beans, brewer's yeast, wheat germ, soy protein, casein, agar and water. These ingredients were mixed with the aid of a blender, then ascorbic acid, sorbic acid, nipagin (methylparaben), 40% formaldehyde and a vitamin solution (composed of niacinamide, calcium pantothenate, thiamine, riboflavin, pyridoxine, folic acid, biotin, inositol, and water) were added, until a homogeneous paste was formed, and stored at 10°C. The *H. oreadica* extracts were incorporated into the diet at concentrations of 100, 500 and 1000 $\mu\text{g} \cdot \text{ml}^{-1}$, when it was at a temperature of approximately 50°C. The control treatment was carried out only with an artificial diet, without the addition of extract. The treatments, codes and their concentrations are summarized in Table 1.

Table 1. Treatments, codes and concentrations of *Hortia oreadica* extracts evaluated in the study

Treatments	Codes	Concentrations [$\mu\text{g} \cdot \text{ml}^{-1}$]
Control	-	0
Hexane Extract	HE	100, 500, 1000
Dichloromethane Extract	DE	100, 500, 1000
Dichloromethane fractionated with Ethyl Acetate Extract	DEAE	100, 500, 1000
Dichloromethane fractionated with Dichloromethane Extract	DDE	100, 500, 1000

Bioactivity of *Hortia oreadica* on the biological parameters of *Anticarsia gemmatalis*

To assess the bioactivity of the extracts of *H. oreadica*, the artificial diet of each treatment was offered ad libitum to 1st instar larvae (newly hatched caterpillars) from the prepupal stage. A single newly hatched larva was placed in one plastic container (size 150 ml), and tests were conducted at $25 \pm 2^\circ\text{C}$, an relative humidity (RH) of $55 \pm 5\%$ and a photoperiod of 12 h. Larval cycle duration, larval mortality, pupal weight, pupal mortality, pupal and adult deformation were evaluated for *A. gemmatalis* exposed to increasing doses of each extract (100, 500 and 1000 $\mu\text{g} \cdot \text{ml}^{-1}$). The deformity of pupae and adults was analyzed by visual observation. The pupa was considered to be deformed if it showed any of the following characteristics: atypical immobility or lethargy, alterations to the cuticle such as blackening, necrosis or wrinkling. For adult moths, the deformities observed included wrinkled or shrunken wings and the inability to emerge completely from the pupa. The experimental design was in randomized blocks, with 5 treatments being tested (control, HE, DE, DEAE and DDE) with 5 replications; each replicate consisted of 10 individual caterpillars ($n = 50$).

Bioactivity of *Hortia oreadica* on the food consumption and utilization of *Anticarsia gemmatalis*

Larvae of the 2nd instar were individualized, and 1 g of artificial diet was offered daily to each treatment until the development of the 6th instar. The gravimetric technique (60°C, 24 h) was used to determine the dry weight of feces, larvae of the 6th instar, and the diets offered and consumed. The indices relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), approximate

digestibility (AD), and relative metabolic rate (RMR) were calculated, according to Parra *et al.* (2012). The experimental design was in randomized blocks, with one control and four treatments being tested (HE, DE, DEAE and DDE) with four replications; each replicate consisted of five individual caterpillars ($n = 20$). The following calculations were performed:

$$\text{relative consumption rate (RCR, g} \cdot \text{g}^{-1} \cdot \text{day}^{-1}) = \text{I/Bm} \times \text{T},$$

$$\text{metabolic rate (RMR, g} \cdot \text{g}^{-1} \cdot \text{day}^{-1}) = \text{M/Bm} \times \text{T},$$

$$\text{relative growth rate (RGR, g} \cdot \text{g}^{-1} \cdot \text{day}^{-1}) = \text{B/Bm} \times \text{T},$$

$$\text{efficiency of conversion of ingested food (ECI, \%)} = (\text{B/I}) \times 100,$$

$$\text{efficiency of conversion of digested food (ECD, \%)} = (\text{B/I-F}) \times 100,$$

$$\text{approximate digestibility (AD, \%)} = (\text{I-F/I}) \times 100,$$

where T = feeding period in days, I = substrate intake (g) during T, B = larval weight gain (g) during T, F = undigested food + excreted product (g), M = (I-F) - B = food metabolized during T (g), I-F = food assimilated during T (g), and Bm = mean larval weight during T (g).

Bioactivity of *Hortia oreadica* on digestive enzymes of *Anticarsia gemmatalis*

To assess the effect of the extracts of *H. oreadica* on digestive enzymes, the artificial diet of each treatment was offered ad libitum from the 1st instar larvae through to the 5th instar stage. Ten larvae from the 5th instar were water-rinsed and ice-chilled for dissection in 10^{-3} M HCl at 4°C. The intestines were macerated and then centrifuged at $12\,000 \times g$ for 5 min and kept under refrigeration until the end of the assays. Protein concentration was measured following Bradford (1976), using solutions of 0–0.2 mg · ml⁻¹ of bovine serum albumin (BSA) as standard.

Total proteolytic activity was determined using azocasein as substrate at a final concentration of 2% (w/v), following a 30 min incubation of the reaction when the reaction was interrupted by adding trichloroacetic acid (10%, w/v). Samples were centrifuged at $8000 \times g$ for 5 min and 1 M NaOH was added to the supernatant, determining the general proteolytic activity at 440 nm. The serine protease activity was determined using *N*- α -benzoyl-L-Arg-*p*-nitroanilide (L-BAPNA) as substrate at a final concentration of 0.5 mM in 0.1 M Tris-HCl buffer (pH 8.2) containing 20 mM CaCl₂ and 1% dimethyl formamide (DMF, w/v). Cysteine protease activity was determined as described for serine protease, but with adding 1.0 mM benzamidine, a specific inhibitor of serine protease (Erlanger *et al.* 1961).

Amylase activity was determined by incubating the samples with starch, according to the manufacturer's

instructions, using the enzymatic kit K003 (Amylase) from the company Bioclin® (QUIBASA- Química Básica Ltda, Belo Horizonte, Minas Gerais, Brazil). Activities were expressed as specific activity of amylase units per mg of protein (AU · mg⁻¹ protein). Amylase units (AU) refer to the amount of amylase that hydrolyzes 10 mg of starch in 30 min at 37°C (Caraway 1959).

Lipase activity was determined according to the manufacturer's instructions, using the enzymatic kit K025, (Lipase) from the company Bioclin® (QUIBASA - Química Básica Ltda, Belo Horizonte, Minas Gerais, Brazil). This method is based on the activity of lipases on a glycerol ester, releasing a chromogenic compound quantified at 410 nm. Lipase activities were expressed as specific activity in international units per mg of protein (IU · mg⁻¹ protein). IU refers to the amount of lipase that releases 1 mmol of fatty acids min⁻¹ (Cherry and Crandall 1932).

Statistical analysis

Statistical analysis of these data was performed using R version 3.2.2 (R Core Team 2015). Analysis of variance was used on all data (ANOVA) and means were separated by the Scott-Knott test.

Results

Effect of *Hortia oreadica* extracts on insect survival and development

The effect of extracts of *H. oreadica* on the cumulative mortality is shown in Figure 3. All treatments significantly increased the cumulative mortality rate of *A. gemmatalis* ($p \leq 0.0001$, *df* residual = 52, *F* value = 5.5979, $n = 50$), except DEAE (1000 $\mu\text{g} \cdot \text{ml}^{-1}$). Among the treatments, HE 100 $\mu\text{g} \cdot \text{ml}^{-1}$ was the most effective (92% of dead insects), followed by HE 500 $\mu\text{g} \cdot \text{ml}^{-1}$ (84%) and HE 1000 $\mu\text{g} \cdot \text{ml}^{-1}$ (82%).

The other biological parameters evaluated are presented in Table 2. The period of larval development was significantly shorter with treatments HE (100 and 500 $\mu\text{g} \cdot \text{ml}^{-1}$) and higher with DEAE (all concentrations) than the control (Table 2). In the larval phase there was greater mortality of the insects in all treatments with extracts ($p \leq 0.0001$, *df* residual = 52, *F* value = 7.4981, $n = 50$), while in the pupal stage, mortality was reduced in most treatments, due to high larval mortality ($p \leq 0.0001$, *df* residual = 52, *F* value = 3.7352, $n = 50$). The pupal weight was lower with treatments HE 1000 $\mu\text{g} \cdot \text{ml}^{-1}$ and in all doses tested of the DEAE treatment, while the other treatments did not differ from the control (Table 2). The morphological integrity of pupae and adults was not affected by the treatments (Table 2).

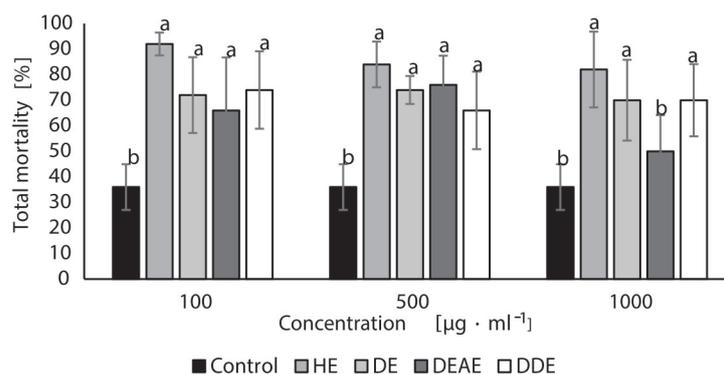


Fig. 3. Percentage cumulative mortality of *A. gemmatilis* after treatment with extracts of *Hortia oreadica*. Means followed by different letters above bars indicate significant differences between treatments (ANOVA at $p \leq 0.01$, using Scott Knott test, $n = 50$). HE – hexane extract; DE – dichloromethane extract; DEAE – dichloromethane fractionated with ethyl acetate extract; DDE – dichloromethane fractionated with dichloromethane extract

Table 2. Effect of extracts of *Hortia oreadica* on the biological parameters of *Anticarsia gemmatilis*

Treatments	Dose [$\mu\text{g} \cdot \text{ml}^{-1}$]	Larval developmental period [days]	Pupal weight [g]	Deformation [%]		Mortality [%]	
				pupae	adult	larvae	pupae
Control	0	12.40 \pm 2.66 c	0.24 \pm 0.03 a	8.00 \pm 4.89 a	2.00 \pm 2.00 a	14.00 \pm 5.09 d	22.00 \pm 3.74 a
HE	100	9.32 \pm 2.65 d	0.22 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	90.00 \pm 2.00 a	0.00 \pm 0.00 b
HE	500	10.28 \pm 4.28 d	0.22 \pm 0.03 a	4.00 \pm 2.44 a	2.00 \pm 2.00 a	82.00 \pm 3.74 a	2.00 \pm 2.00 b
HE	1000	13.48 \pm 3.44 c	0.18 \pm 0.04 b	10.00 \pm 0.00 a	2.00 \pm 2.00 a	76.00 \pm 7.48 a	6.00 \pm 2.44 b
DE	100	11.94 \pm 2.80 c	0.22 \pm 0.02 a	10.00 \pm 0.00 a	6.00 \pm 2.44 a	68.00 \pm 7.34 b	4.00 \pm 2.44 b
DE	500	12.24 \pm 2.63 c	0.22 \pm 0.04 a	12.00 \pm 3.74 a	2.00 \pm 2.00 a	66.00 \pm 5.09 b	8.00 \pm 3.74 b
DE	1000	12.06 \pm 2.65 c	0.22 \pm 0.04 a	18.00 \pm 8.00 a	2.00 \pm 2.00 a	48.00 \pm 5.83 c	22.00 \pm 9.16 a
DEAE	100	15.62 \pm 3.75 b	0.20 \pm 0.07 b	4.00 \pm 2.44 a	0.00 \pm 0.00 a	60.00 \pm 7.07 b	6.00 \pm 4.00 b
DEAE	500	17.68 \pm 5.27 a	0.19 \pm 0.03 b	16.00 \pm 6.78 a	4.00 \pm 2.44 a	48.00 \pm 12.40 c	28.00 \pm 8.00 a
DEAE	1000	16.80 \pm 5.05 a	0.20 \pm 0.14 b	7.00 \pm 3.66 a	2.00 \pm 2.00 a	38.00 \pm 7.34 c	12.00 \pm 4.89 b
DDE	100	12.04 \pm 2.99 c	0.24 \pm 0.03 a	4.00 \pm 2.44 a	0.00 \pm 0.00 a	54.00 \pm 8.71 c	20.00 \pm 4.47 a
DDE	500	12.20 \pm 2.82 c	0.24 \pm 0.03 a	6.00 \pm 4.00 a	2.00 \pm 2.00 a	54.00 \pm 10.29 c	12.00 \pm 4.89 b
DDE	1000	13.10 \pm 2.26 c	0.25 \pm 0.04 a	2.00 \pm 2.00 a	4.00 \pm 2.44 a	66.00 \pm 6.78 b	4.00 \pm 2.44 b
<i>p</i> value		< 0.0001	< 0.05	0.06	0.06	< 0.0001	< 0.0001
<i>df</i> residual		63	25	52	52	52	52
<i>F</i> value		24.117	2.060	1.848	0.840	7.498	3.735

Means \pm SD. Means followed by different letter in the column are significantly different (ANOVA at $p \leq 0.05$, using Scott Knott test, $n = 50$). HE – hexane extract; DE – dichloromethane extract; DEAE – dichloromethane fractionated with ethyl acetate extract; DDE – dichloromethane fractionated with dichloromethane extract

Effect of *Hortia oreadica* extracts on insect nutritional parameters

The results of the nutritional parameters of the different treatments are presented in Table 3. In general, RCR, RMR, and AD of treatments with extracts were significantly lower than the control. The indices of ECI and ECD were significantly higher in treatments with extracts. The RGR varied between treatments, but it is possible to state that the hexane (HE) and dichloromethane extracts partitioned with ethyl acetate

(DEAE) were those that most negatively affected the insect's RGR (Table 3). As expected, in some treatments such as HE (100 and 1000 $\mu\text{g} \cdot \text{ml}^{-1}$) and DEAE (500 and 1000 $\mu\text{g} \cdot \text{ml}^{-1}$), insects consumed less food and showed reduced growth. However, lower food consumption was also observed with treatments HE (500 $\mu\text{g} \cdot \text{ml}^{-1}$), DE (100 and 500 $\mu\text{g} \cdot \text{ml}^{-1}$), DEAE (100 $\mu\text{g} \cdot \text{ml}^{-1}$) DDE (all concentrations), but at a rate of growth equal to or greater than the control. In these cases, there was greater efficiency in converting food into energy for the insect's development (Table 3).

Table 3. Nutritional indices of *Anticarsia gemmatalis* submitted to treatments with *Hortia oreadica* extracts

Treatments	RCR	RMR	RGR	ECl	ECD	AD
	[mg · mg ⁻¹ · day ⁻¹]	[mg · mg ⁻¹ · day ⁻¹]	[mg · mg ⁻¹ · day ⁻¹]	[%]	[%]	[%]
Control	0.53 ± 0.03 a	0.30 ± 0.05 a	0.09 ± 0.01 b	18.26 ± 2.94 c	24.71 ± 5.48 d	74.87 ± 7.75 a
HE 100	0.19 ± 0.01 d	0.02 ± 0.01 d	0.06 ± 0.00 c	36.04 ± 2.13 a	72.77 ± 12.03 a	50.26 ± 5.43 d
HE 500	0.28 ± 0.01 c	0.07 ± 0.01 c	0.09 ± 0.00 b	31.17 ± 2.23 b	56.16 ± 5.10 b	55.63 ± 2.37 c
HE 1000	0.49 ± 0.16 a	0.26 ± 0.13 a	0.08 ± 0.02 c	16.99 ± 5.19 c	26.08 ± 10.11 d	67.25 ± 7.28 b
DE 100	0.30 ± 0.07 c	0.02 ± 0.01 c	0.11 ± 0.02 a	37.96 ± 3.14 a	87.03 ± 11.44 a	44.13 ± 5.37 d
DE 500	0.25 ± 0.00 c	0.03 ± 0.02 c	0.09 ± 0.00 b	37.64 ± 2.68 a	80.98 ± 14.39 a	47.44 ± 6.37 d
DE 1000	0.49 ± 0.06 a	0.30 ± 0.04 a	0.06 ± 0.02 c	13.73 ± 2.80 c	18.70 ± 4.46 d	74.09 ± 4.24 a
DEAE 100	0.35 ± 0.03 c	0.10 ± 0.03 c	0.09 ± 0.01 b	25.81 ± 4.35 b	48.03 ± 12.72 c	54.89 ± 6.02 c
DEAE 500	0.27 ± 0.01 c	0.07 ± 0.02 c	0.07 ± 0.00 c	26.26 ± 4.10 b	49.95 ± 12.61 c	53.99 ± 8.23 c
DEAE 1000	0.32 ± 0.02 c	0.14 ± 0.02 c	0.06 ± 0.01 c	20.73 ± 4.23 c	32.20 ± 7.87 d	65.44 ± 6.19 b
DDE 100	0.49 ± 0.09 a	0.19 ± 0.05 b	0.11 ± 0.02 a	23.79 ± 2.14 b	37.98 ± 6.18 c	63.62 ± 7.57 b
DDE 500	0.41 ± 0.03 b	0.13 ± 0.07 c	0.10 ± 0.02 b	24.85 ± 7.21 b	48.25 ± 24.08 c	56.23 ± 11.18 c
DDE 1000	0.41 ± 0.03 b	0.10 ± 0.06 c	0.11 ± 0.01 a	28.20 ± 4.17 b	57.79 ± 22.75 b	52.33 ± 10.68 c
<i>p</i> value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<i>df</i> residual	12	12	12	12	12	12
<i>F</i> value	24.329	25.644	86.885	31.297	22.310	14.905

Means ± SD. Means followed by different letter in the column are significantly different (ANOVA at $p \leq 0.01$, using Scott Knott test, $n = 20$). HE – hexane extract; DE – dichloromethane extract; DEAE – dichloromethane fractionated with ethyl acetate extract; DDE – dichloromethane fractionated with dichloromethane extract; RCR – relative consumption rate; RMR – relative metabolic rate; RGR: relative growth rate; ECl – efficiency of conversion of ingested food; ECD – efficiency of conversion of digested food and AD – approximate digestibility

Effect of *Hortia oreadica* extracts on insect digestive enzymes activity

The effect of *H. oreadica* extracts on the specific activities of *A. gemmatalis* digestive enzymes is shown

in Table 4. The specific activity of total protease was significantly lower in the treatments HE, DE (all concentrations), and DEAE (100 and 500 $\mu\text{g} \cdot \text{ml}^{-1}$), but increased in DEAE (1000 $\mu\text{g} \cdot \text{ml}^{-1}$), and in the DDE

Table 4. Effect of *Hortia oreadica* extracts on the specific activities of digestive enzymes of 5th instar *Anticarsia gemmatalis* caterpillar

Treatments [$\mu\text{g} \cdot \text{ml}^{-1}$]	Specific activities				
	total protease [A440 · mg ⁻¹ protein]	serine protease [nM · s ⁻¹ · mg ⁻¹ protein]	cysteine protease [nM · s ⁻¹ · mg ⁻¹ protein]	amylase [AU · mg ⁻¹ protein]	lipase [IU · mg ⁻¹ protein]
Control	0.05 ± 0.00 b	93.43 ± 4.40 a	46.82 ± 1.95 b	105.55 ± 9.42 a	23.11 ± 3.74 c
HE 100	0.02 ± 0.01 c	33.73 ± 4.51 d	31.81 ± 6.02 c	46.34 ± 6.43 d	22.21 ± 2.75 c
HE 500	0.02 ± 0.00 c	62.73 ± 6.65 b	25.68 ± 6.26 d	61.43 ± 5.31 c	21.51 ± 3.16 c
HE 1000	0.01 ± 0.00 d	16.56 ± 1.36 f	12.73 ± 2.80 f	19.19 ± 0.79 f	23.34 ± 3.71 c
DE 100	0.03 ± 0.00 c	87.09 ± 4.15 a	36.75 ± 11.44 c	84.09 ± 3.92 b	19.87 ± 4.38 c
DE 500	0.01 ± 0.00 d	46.81 ± 7.84 c	18.75 ± 4.62 e	28.43 ± 4.57 e	18.34 ± 2.87 c
DE 1000	0.01 ± 0.00 d	22.65 ± 3.23 e	11.93 ± 2.99 f	55.36 ± 5.94 c	18.81 ± 3.64 c
DEAE 100	0.02 ± 0.00 c	20.87 ± 4.15 e	29.25 ± 1.28 d	18.22 ± 1.45 f	30.22 ± 2.46 b
DEAE 500	0.03 ± 0.01 c	31.65 ± 0.89 d	42.36 ± 5.01 b	13.10 ± 2.97 g	38.12 ± 3.26 a
DEAE 1000	0.08 ± 0.00 a	31.38 ± 6.69 d	25.23 ± 3.76 d	7.10 ± 0.45 h	34.66 ± 0.23 a
DDE 100	0.04 ± 0.00 b	41.93 ± 2.48 d	53.90 ± 2.70 a	24.50 ± 3.16 e	20.60 ± 2.57 c
DDE 500	0.06 ± 0.00 b	46.09 ± 7.76 d	38.01 ± 2.16 c	21.59 ± 2.14 f	37.30 ± 1.44 a
DDE 1000	0.05 ± 0.01 b	13.11 ± 3.50 f	17.10 ± 7.77 e	4.86 ± 1.65 h	18.63 ± 2.68 c
<i>p</i> value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
<i>df</i> residual	64	70	70	68	65
<i>F</i> value	23.21	73.90	35.03	198.48	22.60

Means ± SD. Means followed by different letter in the column are significantly different (ANOVA at $p \leq 0.05$, using Scott Knott test, $n = 30$). HE – hexane extract; DE – dichloromethane extract; DEAE – dichloromethane fractionated with ethyl acetate extract; DDE – dichloromethane fractionated with dichloromethane extract

treatment (all concentrations) there was no difference. There was a significant reduction in serine-specific activity, except for the DE ($100 \mu\text{g} \cdot \text{ml}^{-1}$) treatment. Similar results were obtained for the activity of cysteine protease, with the exception of the DEAE ($500 \mu\text{g} \cdot \text{ml}^{-1}$) and DDE ($100 \mu\text{g} \cdot \text{ml}^{-1}$) treatments. Specific amylase activity was significantly lower in all extract treatments. However, lipase activity increased in DEAE (all concentrations) and DDE ($500 \mu\text{g} \cdot \text{ml}^{-1}$); the other treatments did not differ from the control (Table 4).

Discussion

The use of plant extracts and their metabolites in pest control has been extensively studied (Sackett *et al.* 2007; Abdullah *et al.* 2023; Nhung and Quoc 2024). The occurrence of secondary metabolites in the specie *H. oreadica*, including alkaloids, limonoids, coumarins and derivatives of dihydrocinnamic acid, highlights its potential as a source of bioactive compounds. In the present study, the effects of crude (HE and DE) and fractionated (DEAE and DDE) extracts of *H. oreadica*, on the biological parameters, nutritional indices and digestive enzyme activities of *A. gemmatalis* were investigated. The results demonstrated that the secondary metabolites present in the extracts exert adverse effects on insects, including reduced food intake, decreased food digestibility, and inhibition of key enzymes involved in the digestive process. These physiological effects consequently impair larval development and lead to increased mortality rate.

All *H. oreadica* extracts caused significant mortality, especially during the larval stage of *A. gemmatalis*. The HE treatment recorded a cumulative mortality rate of over 80%, probably because hexane extracts nonpolar compounds, such as terpenes (Boncan *et al.* 2020) or lipids (Kaczmarek *et al.* 2022), recognized for their insecticidal activity. Treatments with HE and DEAE extracts also induced significant changes in larval development duration and pupal weight, corroborating the findings reported by Lucena *et al.* (2017), who observed high mortality rates (93.3% and 90%) in *A. gemmatalis* larvae following exposure to hexane and ethyl acetate extracts of *Piper aduncum* L. (Piperaceae), respectively. The authors attribute this finding to the high concentration of phenylpropanoids in the extracts, with apiol being the major compound (Lucena *et al.* 2017).

It was observed in the treatments with HE, DE and DEAE, in which the lowest tested concentration ($100 \mu\text{g} \cdot \text{ml}^{-1}$) led to significantly higher larval mortality than higher concentrations. A similar pattern was observed for pupal mortality in the treatment with

DDE (Table 2). This non-monotonic dose-response suggests the possible occurrence of hormetic effects, a phenomenon characterized by paradoxical biological responses, where low concentrations of toxic substances elicit more pronounced deleterious or inhibitory effects than higher concentrations, which may even promote stimulatory responses. In insects, such responses have been previously reported upon exposure to natural compounds and pesticides, indicating that, at sublethal doses, certain metabolites present in plant extracts may more effectively disrupt critical physiological processes (Cutler *et al.* 2022). Higher concentrations may trigger the activation of physiological defense mechanisms, such as enhanced activity of detoxification enzymes, resulting in an adaptative response that mitigates the toxic effects (Sial *et al.* 2018). Additionally, the volatilization and/or instability of bioactive compounds should also be considered, particularly in complex formulations of natural extracts. Application at higher doses may favor the rapid evaporation or degradation of these compounds, thereby reducing their bioavailability at the site of exposure (Ngegba *et al.* 2022).

Plant secondary metabolites have been linked to antifeedant activity in insects (Abdelgaleil and El-Sabrout 2018; Derbalah *et al.* 2024). Some studies suggest that certain limonoids and alkaloids make foods less attractive or interfere with the digestion capacity of insects (Darrag *et al.* 2022), resulting in decreased food intake and, consequently, reduced survival and reproductive capacity. The present results demonstrated that all tested extracts exhibited antifeedant activity against *A. gemmatalis* (Table 3). This effect may be associated with the presence of limonoids (guyanin, hortiolide D, hortiolide E, and 12β -hydroxyhortiolide) and alkaloids (the furoquinoline alkaloid dictamnine and N-methyl-4-methoxy-quinolin-2-one), which have been previously identified in phytochemical studies of *H. oreadica* (Severino *et al.* 2009; Severino *et al.* 2014). The present findings are consistent with those reported by Solipeta *et al.* (2020), who observed antifeedant activity of limonoids isolated from fruits of *Trichilia connaroides* (Wight & Arn.) Benth. (Meliaceae) against *Spodoptera litura* Fabricius [1775] (Lepidoptera: Noctuidae). The authors reported a high antifeedant effect and insect mortality induced by trichanolide F, a high rate of deformities associated with trichanolide G, and elevated pupal mortality caused by carapanolide U (Solipeta *et al.* 2020). In another study, the negative effects on insect growth, as well as antifeedant and deterrent activities, were attributed to the furoquinoline alkaloids skimmianine and dictamnine against larvae of *Spodoptera litura* and *Trichoplusia ni* Hübner [1803] (Lepidoptera: Noctuidae) (Sackett *et al.* 2007). Notably, dictamnine and furoquinoline alkaloids were also detected in the extracts of *H. oreadica* (Severino *et al.*

2009; Severino *et al.* 2014), further supporting the potential role of these compounds in the observed insecticidal effects. The toxicity of furoquinolines has been associated with their ability to interact with DNA, leading to the formation of adducts via the furan ring, or to interference with the activity of cytochrome P450 oxidases (Sackett *et al.* 2007).

Overall, in this study a reduction in RCR, RMR, AD, accompanied by an increase in ECI and ECD in *A. gemmatalis* larvae treated with *H. oreadica* extracts was observed. The RGR values varied among treatments, with HE, DE, and DEAE exhibiting the lowest RGR. These physiological and nutritional changes are likely attributable to secondary metabolites present in the extracts, as certain compounds isolated from *H. oreadica* have been previously reported to suppress feeding and growth in insects. For example, limonin has demonstrated such effects in *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Liu *et al.* 1990), while dictamnine has been implicated in similar activities against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motschulsky [1855] (Coleoptera: Curculionidae) (Liu *et al.* 2002) and bergapten in *Spodoptera exigua* Hübner [1808] (Lepidoptera: Noctuidae) (Berdegué *et al.* 1997).

The consumption and use of food are crucial for the growth, development, and reproduction of insects. It is known that the behavior, physiology, metabolism (such as enzyme synthesis and lipid accumulation) and even the ecological relationships of insects are associated with a nutritional context, which can be evaluated by determining nutritional indices (Parra *et al.* 2012). Studies show that natural products can negatively affect the nutritional levels of insects and, consequently, their development. One of the hypotheses that explains this fact suggests that certain metabolites present in the extracts influence the insects' feeding control center, leading in most cases to a reduction in these indices proportionally to the concentration of the metabolite in the food (Abdelgaleil and El-Sabroun 2018). For example, essential oils extracted from *Artemisia monosperma* Del. (Asteraceae), *Callistemon viminalis* (Sol. ex Gaertn.) G. Don (Myrtaceae) significantly reduced RGR, ECI, and ECD of *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) larvae, in proportion to the increase in the concentration of the oils (Abdelgaleil and El-Sabroun 2018). However, contrary to expectations, in the present study significantly higher food intake and metabolic rates in the HE and DE treatments at 1000 $\mu\text{g} \cdot \text{ml}^{-1}$ than the lower-dose treatments were observed. This response may be associated with the induction of enzymatic detoxification systems in insects at higher compound concentrations, including cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and esterases (Cutler *et al.* 2022). The activation of these metabolic pathways

demands additional energy, which may result in an increased RMR to support detoxification, as well as a higher RCR as an attempt to compensate for the elevated energy requirement. However, this energy is diverted from growth processes, leading to a reduction in RGR. This pattern is characteristic of the sublethal effects of toxic compounds: the insect increases food intake and energy expenditure, but fails to convert this energy into biomass (Wu *et al.* 2015).

The results of the present study demonstrated the inhibitory effect of *H. oreadica* extracts on the activity of total proteases, serine proteases, cysteine proteases and amylase. One of the widely known plant defense mechanisms is the synthesis of enzyme inhibitors. Inhibitors are molecules that associate with enzymes and significantly reduce their catalytic activity. Some inhibitors are associated with digestive proteases and amylases, resulting in a reduction in food consumption, indigestion, food deterrence and, consequently, developmental and reproduction problems and increased mortality of the insect may occur (Sagu *et al.* 2021). The metabolites nepetoidin B, quercetin-3-O-rutinoside, chicoric acid, rosmarinic acid, rosmarinyl glucoside, and salvigen isolated from extracts of *Ocimum basilicum* L. (Lamiaceae), inhibited digestive proteases of *Rhynchophorus ferrugineus* Olivier [1790] (Coleoptera: Dryophthoridae) and showed high insecticidal activity (Darrag *et al.* 2022). Histological changes in the midgut of insects are another possible mechanism of action of natural products that directly affect the activity of digestive enzymes (Taha 2024). The triterpene, toosendanin, extracted from the root bark of *Melia toosendan* Siebold & Zucc. (Meliaceae) damaged the epithelial cells of the midgut of *Mythimna separate* Walker larvae (Lepidoptera: Noctuidae) causing degeneration of microvilli and cellular changes (Li *et al.* 2020). Citral and geranyl acetate, secondary metabolites of lemongrass essential oil isolates, caused midgut histopathological effects in the digestive and globet cells of *A. gemmatalis* (Plata-Rueda *et al.* 2022). *Spodoptera littoralis* treated with garlic (*Allium sativum* L.) and lemon (*Citrus limon* L.) essential oils exhibited ultrastructural variations in the midgut, and these histological changes were responsible for decreasing RCR, RGR, AD, and enzymatic inhibition in insects (Shaurub *et al.* 2020).

Conclusions

The high larval mortality and changes in the development of *A. gemmatalis* are consequences of low food consumption, reduced metabolic rates, and digestibility and inhibition of the activity of digestive enzymes. These effects are probably related to the existence of secondary metabolites in *H. oreadica* extracts that

affect the insect's digestion and use of food. The *n*-hexane extract provided a higher percentage of mortality, reduced larval cycle, and lower food consumption rate, therefore being a promising alternative for controlling soybean caterpillars.

Acknowledgments

The present work was supported by the Research Supporting Foundation of Minas Gerais State (FAPEMIG) [project number APQ 04585-10]. The authors would also like to thank the National Council for Scientific and Technological Development (CNPq) and Coordination of Higher Level Staff Improvement (CAPES) for granting the scholarship and the Federal University of Viçosa – Campus Rio Paranaíba for support and conditions for carrying out this study.

References

- Abdelgaleil S.A., El-Sabrouit A.M. 2018. Anti-nutritional, anti-feedant, growth-disrupting and insecticidal effects of four plant essential oils on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Crop Protection* 7 (2): 135–150. Available on: <http://jcp.modares.ac.ir/article-3-16367-en.html> [Accessed: 19 April 2025]
- Abdullah R.R.H., El-Rokh A.R. 2023. Using the secondary metabolites of some fungi and wild plants as natural pesticides to control cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). *Journal of Plant Protection Research* 63 (3): 318–330. DOI: <https://doi.org/10.24425/jppr.2023.146870>
- Berdegúe M., White K.K., Trumble J.T. 1997. Feeding deterrence of *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae by low concentrations of linear furanocoumarins. *Environmental Entomology* 26 (4): 912–919. DOI: <https://doi.org/10.1093/ee/26.4.912>
- Boncan D.A.T., Tsang S.S.K., Li C., Lee I.H.T., Lam H.M., Chan T.F., Hui J.H.L. 2020. Terpenes and terpenoids in plants: Interactions with environment and insects. *International Journal of Molecular Sciences* 21 (19): 7382. DOI: <https://doi.org/10.3390/ijms21197382>
- Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254. DOI: [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Caraway W.T. 1959. A stable starch substrate for the determination of amylase in serum and other body fluids. *American Journal of Clinical Pathology* 32: 97–99. DOI: [10.1093/ajcp/32.1_ts.97](https://doi.org/10.1093/ajcp/32.1_ts.97)
- Cherry S., Crandall L.A. 1932. The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *American Journal of Physiology* 100: 266–270. DOI: <https://doi.org/10.1152/ajplegacy.1932.100.2.266>
- Couto I.F.S., Silva S.V., Valente F.I., Araújo B.S., Souza S.A., Mauad M., Scalón S.P.Q., Mussury R.M. 2019. Botanical extracts of the Brazilian Savannah affect feeding and oviposition of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). *Journal of Agricultural Science* 11 (5): 322–333. DOI: <https://doi.org/10.5539/jas.v11n5p322>
- Cui C., Yang Y., Zhao T., Zou K., Peng C., Cai H., Wan X., Hou R. 2019. Insecticidal activity and insecticidal mechanism of total saponins from *Camellia oleifera*. *Molecules* 24: 4518. DOI: <https://doi.org/10.3390/molecules24244518>
- Cutler G.C., Amichot M., Benelli G., Guedes R.N.C., Qu Y., Rix R.R., Ullah F., Desneux N. 2022. Hormesis and insects: Effects and interactions in agroecosystems. *Science of the Total Environment* 825: 153899. DOI: <https://doi.org/10.1016/j.scitotenv.2022.153899>
- Darrag H.M., Almuhanha H.T., Hakami E.H. 2022. Secondary metabolites in basil, bio-insecticide, inhibition effect, and in silico molecular docking against proteolytic enzymes of the red palm weevil (*Rhynchophorus ferrugineus*). *Plants* 11 (1087): 2–25. DOI: <https://doi.org/10.3390/plants11081087>
- Derbalah A., Keratum A., Darwesh M., Omar A.F., Salama A., Fatma Hegazy F. 2024. The efficacy of some synthetic monoterpenes and Yucca extract for controlling *Tribolium castaneum* (Herbst) in wheat grain. *Journal of Plant Protection Research* 64 (1): 1–10. DOI: <https://doi.org/10.24425/jppr.2024.149154>
- Erlanger B.F., Kokowsky N., Cohen W. 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics* 95: 271–278. DOI: [https://doi.org/10.1016/0003-9861\(61\)90145-X](https://doi.org/10.1016/0003-9861(61)90145-X)
- Groppo M., Pirani J.R., Salatino M.L.F., Blanco S.R., Kallunki J.A. 2008. Phylogeny of Rutaceae based on two non-coding regions from cpDNA. *American Journal of Botany* 95: 985–1005. DOI: <https://doi.org/10.3732/ajb.2007313>
- Kaczmarek A., Wrońska A.K., Kazek M., Boguś M.I. 2022. Octanoic acid – an insecticidal metabolite of *Conidiobolus coronatus* (Entomophthorales) that affects two majors antifungal protection systems in *Galleria mellonella* (Lepidoptera): Cuticular lipids and hemocytes. *International Journal of Molecular Sciences* 23 (9): 5204. DOI: <https://doi.org/10.3390/ijms23095204>
- Li H., Zhang J., Ma T., Li C., Ma Z., Zhang X. 2020. Acting target of toosendanin locates in the midgut epithelium cells of *Mythimna separate* Walker larvae (Lepidoptera: Noctuidae). *Ecotoxicology and Environmental Safety* 201: 110828. DOI: <https://doi.org/10.1016/j.ecoenv.2020.110828>
- Liu Y.B., Alford A.R., Rajab M.S., Bentley M.D. 1990. Effects and modes of action of citrus limonoids against *Leptinotarsa decemlineata*. *Physiological Entomology* 15 (1): 37–45. DOI: <https://doi.org/10.1111/j.1365-3032.1990.tb00490.x>
- Liu Z.L., Xu Y.J., Wu J., Goh S.H., Ho S.H. 2002. Feeding deterrents from *Dictamnus dasycarpus* Turcz against two stored-product insects. *Journal of Agricultural and Food Chemistry* 50 (6): 1447–1450. DOI: <https://doi.org/10.1021/jf010838l>
- Lucena D.C., Bertholdo-Vargas L.R., Silva W.C., Machado A.F., Lopes T.S., Moura S., Barros N.M. 2017. Biological activity of *Piper aduncum* extracts on *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *Anais da Academia Brasileira de Ciências* 89: 1869–1879. DOI: <http://dx.doi.org/10.1590/0001-3765201720170194>
- Ngegba P.M., Cui G., Khalid M.Z., Zhong G. 2022. Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture* 12 (600): 1–24. DOI: <https://doi.org/10.3390/agriculture12050600>
- Nhung T.T.P., Quoc L.P.T. 2024. Assessment of the insecticidal efficacy of ethanol extract of *Milletia pachyloba* Drake leaves against *Plutella xylostella* Linnaeus moth. *Journal of Plant Protection Research* 64 (2): 165–177. DOI: [10.24425/jppr.2024.150251](https://doi.org/10.24425/jppr.2024.150251)
- Parra J.R.P., Panizzi A.R., Haddad M.L. 2012. Nutritional indices for measuring insect food intake and utilization. p. 13–49. In: “Insect Bioecology and Nutrition for Integrate Pest Management” 2 ed. (A.R. Panizzi, J.R.P. Parra eds.). CRC Press, Brasília, DF, Brazil, 750 pp. DOI: <https://doi.org/10.1201/b11713>
- Perobelli J.E. 2025. Pesticides and public health: discussing risks in Brazilian agroindustrial growth. *Frontiers in Toxicology* 7: 1442801. DOI: [10.3389/ftox.2025.1442801](https://doi.org/10.3389/ftox.2025.1442801)
- Plata-Rueda A., Fiaz M., Brügger B.P., Cañas V., Coelho R.P., Zanon J.C., Martínez L.C., Serrão J.E. 2022. Lemongrass

- essential oil and its components cause effects on survival, locomotion, ingestion, and histological changes of the midgut in *Anticarsia gemmatalis* caterpillars. *Toxin Reviews* 41 (1): 208–217. DOI: <https://doi.org/10.1080/15569543.2020.1861468>
- Praça L.B., Neto S.P.S., Monnerat R.G. 2006. *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae). Biology, sampling and control methods. [*Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae). Biologia, amostragem e métodos de controle]. Brasília: Embrapa Recursos Genéticos e Biotecnologia, 2006. 17 p. DOI: 10.1603/0022-0493-98.1.121 (in Portuguese)
- R Core Team 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- Sackett T.E., Towers G.H.T., Isman M.B. 2007. Effects of furquinoline alkaloids on the growth and feeding of two polyphagous lepidopterans. *Chemoecology* 17: 97–101. DOI: <https://doi.org/10.1007/s00049-007-0367-y>
- Sagu S.T., Landgräber E., Henkel I.M., Huschek G., Homann T., Bußler S., Schlüter O.K., Harshadrai Rawel H. 2021. Effect of cereal α -amylase/trypsin inhibitors on developmental characteristics and abundance of digestive enzymes of mealworm larvae (*Tenebrio molitor* L.). *Insects* 12 (454): 1–16. DOI: <https://doi.org/10.3390/insects12050454>
- Severino V.G.P., Cazal C.M., Forim M.R., Silva M.F.G.F., Rodrigues-Filho E., Fernandes J.B., Vieira, P.C. 2009. Isolation of secondary metabolites from *Hortia oreadica* (Rutaceae) leaves through high-speed counter-current chromatography. *Journal of Chromatography A* 1216 (19): 4275–4281. DOI: <https://doi.org/10.1016/j.chroma.2009.02.009>
- Severino V.G., de Freitas S.D., Braga P.A., Forim M.R., Silva M.F.G.F., Fernandes J.B., Vieira P.C., Venâncio T. 2014. New limonoids from *Hortia oreadica* and unexpected coumarin from *H. superba* using chromatography over cleaning Sephadex with sodium hypochlorite. *Molecules* 19 (8): 12031–12047. DOI: <https://doi.org/10.3390/molecules190812031>
- Shaurub E.H., EL-Sayed A.M., Ali A.M., Mohamed D.S. 2020. Some plant essential oils induce variations in the physiological aspects and midgut ultrastructure of larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *African Entomology* 28 (2): 349–358. DOI: <https://doi.org/10.4001/003.028.0349>
- Sial M.U., Zhao Z., Lan Z., Yanning Z., Mao L., Hongyun J. 2018. Evaluation of insecticides induced hormesis on the demographic parameters of *Myzus persicae* and expression changes of metabolic resistance detoxification genes. *Scientific Reports* 8 (1): 16601. DOI: <https://doi.org/10.1038/S41598-018-35076-1>
- Solipeta D.R., Bandi S., Katragunta K., Mutheneni S.R., Katragadda S.B. 2020. UPLC-MSE guided isolation of new antifeedant limonoids from fruits of *Trichilia connaroides*. *Journal of Agricultural and Food Chemistry* 68 (25): 6826–6834. DOI: <https://doi.org/10.1021/acs.jafc.0c00862>
- Taha H.S.E.D. 2024. Quantification of enzymes and histology changes belongings to the pinl boll worm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) exposed to some insecticide toxicities. *International Journal of Agricultural and Natural Sciences* 17 (1): 30–44. Available on: <https://www.ijans.org/index.php/ijans/article/view/809> [Accessed: 29 April 2024]
- Ukoroiye R.B., Otayor R.A. 2020. Review on the bio-insecticidal properties of some plant secondary metabolites: types, formulations, modes of action, advantages and limitations. *Asian Journal of Research in Zoology* 3 (4): 27–60. DOI: <https://doi.org/10.9734/ajriz/2020/v3i430099>
- Wu K., Zhang J., Zhang Q., Zhu S., Shao Q., Clark K.D., Liu Y., Ling E. 2015. Plant phenolics are detoxified by prophenoloxidase in the insect gut. *Scientific Reports* 5: 16823. DOI: <https://doi.org/10.1038/srep16823>