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Scientific Article

Bioactivity of extracts of *Ocimum campechianum* in the development of *Ascia monuste orseis*

Bioatividade de extratos de *Ocimum campechianum* sobre o desenvolvimento de *Ascia monuste orseis*

Ana C. V. dos Santos[1], Lucas M. Lopes[1], Jamila F. Mendonça[1], Adalberto H. de Sousa[1]*

[1]Center for Biological and Nature Sciences, Universidade Federal do Acre, Rio Branco, AC, Brazil.

ABSTRACT - The caterpillar *Ascia monuste orseis* (Godart) (Lepidoptera: Pieridae) stands out as one of the main defoliating pests of brassicas. To mitigate the damage and reduce the impact on human health and the environment that results from the persistent and indiscriminate use of chemical insecticides, it is crucial to consider the use of botanical insecticides as a fundamental alternative for pest control. The aim was therefore to investigate the stimulus-response of the hydroalcoholic crude extract of *Ocimum campechianum* and of its dichloromethane (DCM) fraction on *A. monuste orseis*. Leaf consumption (cm^2) of caterpillars exposed to the extract, caterpillar mortality in 24 h, total caterpillar mortality, pupal mortality, pupal period duration, and morphological malformation in adults were evaluated. Feeding the caterpillars for eight days with cabbage impregnated with the crude extract and its DCM fraction resulted in decreased caterpillar feeding, increased larval mortality (90%), lengthening of the pupal period (two days), and an increase in adult deformation (50%). Therefore, the crude extract of *O. campechianum* and its DCM fraction have potential for use in the alternative control of *A. monuste orseis*.

Keywords: Bioinsecticide. Alternative control. Chicken basil. Cabbage leafworm. Feeding preference.

RESUMO - A lagarta *Ascia monuste orseis* (Godart) (Lepidoptera: Pieridae), destaca-se como uma das principais pragas desfolhadoras das brássicas. Para mitigar os danos e reduzir os impactos na saúde humana e no meio ambiente, decorrentes do uso persistente e indiscriminado de inseticidas químicos, é crucial considerar a utilização de inseticidas botânicos como uma alternativa fundamental para seu controle. Deste modo, o objetivo foi investigar o estímulo-resposta do extrato bruto hidroalcoólico de *Ocimum campechianum* e de sua fração diclorometano (DCM) sobre *A. monuste orseis*. Avaliou-se o consumo foliar (cm^2) das lagartas expostas aos extratos, mortalidade de lagartas em 24 h, mortalidade total de lagartas, mortalidade de pupas, duração do período de pupa e malformação morfológica em adultos. A alimentação das lagartas de oito dias, com a couve impregnada com o extrato bruto e sua fração DCM, resultou em diminuição da alimentação das lagartas, aumento da mortalidade larval (90%), alongamento do período pupal (2 dias) e aumento da deformação em adultos (50%). Portanto, o extrato bruto e sua fração DCM de *O. campechianum* apresentam potencial para uso no controle alternativo de *A. monuste orseis*.

Palavras-chave: Bioinseticida. Controle alternativo. Alfavaca-de-galinha. Curuquerê-da-couve. Preferência alimentar.

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***Corresponding author:**
<adalberto.sousa@ufac.br>

INTRODUCTION

Ocimum campechianum Mill. is a species of the Lamiaceae family, *Ocimum* genus. This species is an important source of essential oils (EOs), found in its leaves, inflorescences, and seeds, which contain antioxidant and aromatic phenolic compounds of interest to the food, pharmaceutical, and perfume industries (PANDEY; SINGH; TRIPATHI, 2014; SILVA et al., 2011).

Extracts of the plant are used in traditional medicine to treat diseases of the respiratory tract, rheumatism, paralysis, epilepsy, and mental illness. In addition, they contain compounds that natural insecticides, nematicides, and fungicides (SILVA; SILVA; MATOS, 2004; PEREIRA; MOREIRA; LIMA, 2009).

Species of the *Ocimum* genus are being increasingly recognized for their ecological importance and potential in integrated pest management strategies, particularly in the control of defoliating caterpillars (SANTOS et al., 2024). This genus includes a variety of species, each with a unique composition of essential oils and extracts that have insecticidal properties. Singh et al. (2014) and Mota et al. (2017) found that the main chemical compounds in the essential oil of *O. campechianum* were the phenylpropanoids eugenol (41.05%) and elemicin (16.09%) and the sesquiterpene β-caryophyllene (14.10%). Sousa (2004) identified the presence of eugenol, monoterpenes, sesquiterpenes, triterpenoids, and polyphenols (phenylpropanoid glycosides and flavonoids) in methanolic and benzene extracts of fresh *O. campechianum* leaves.

The use of plants from the *Ocimum* genus in pest management is based on an understanding of their bioactive compounds, which have been shown to affect various physiological and behavioral processes in insects (JARAMILLO; DUARTE; DELGADO, 2014; CAAMAL-HERRERA et al., 2018). These include reduced feeding, egg laying, and locomotion, as well as direct toxic effects that

lead to increased mortality rates among pest populations (CABALLERO-GALLARDO et al., 2014; MOTA et al., 2017; SANTOS et al., 2024). The potential of *Ocimum* species to contribute to the sustainable control of defoliating caterpillars not only meets the immediate need for effective pest management solutions, but also supports broader objectives of environmental conservation and protection of human health (ZHAO et al., 2016).

The aim of this study was to evaluate the bioactivity of the crude extract of *O. campechianum* leaves and its dichloromethane fraction (DCM) in the development of *A. monuste orseis*.

MATERIAL AND METHODS

Crude extract

Leaves of *O. campechianum* were collected in the municipality of Boca do Acre, Amazonas, in the geographical coordinates of 8°44'11"S; 67°23'24"O. An exsiccate was prepared and sent to the UFACPZ Herbarium at UFAC, under number 20643. The leaves were separated from the other plant structures and dried at room temperature (25 °C ± 2 °C) for seven days until constant weight was reached. They were then ground into powder in a knife mill (SL - 30, Piracicaba, São Paulo, Brazil).

The fragmented material (320.00 g) was placed in a 2.000 mL Erlenmeyer flask with 1.50 L of ethanol/water solution (7:3). Extraction was performed using an ultrasound machine and the process was repeated four times, each extraction lasting 120 minutes. The extract obtained was filtered and the solvent recovered in a rotary evaporator at 60 rpm. The extract was placed under ventilation until constant weight was achieved (FERNANDES et al., 2009). The crude extract was subjected to fractionation processes, first with the chromatographic system (filter column), and another aliquot was subjected to the liquid-liquid extraction process (partitioning).

Obtaining the Dichloromethane Extract (DCM)

Preparation of the filter column (silica gel)

An amount of 300.00 g of silica gel 60, particle size of between 0.04 and 0.063 mm (230 to 400 mesh), was placed in a 500.00-mL glass separating funnel containing absorbent cotton at the bottom. Packing was performed using 200.00 mL of hexane, after which the dead volume was calculated. Then, using a glass rod with a rubber tip, the outer edges of the separating funnel were touched to eliminate air bubbles and the solvent (hexane) was kept approximately 3 cm above the stationary phase (FERNANDES et al., 2009).

Using the filter column, an aliquot of the crude extract (10.00 g) was added homogeneously to the top of the stationary phase. Absorbent cotton was placed over the extract macerated in silica to protect it and the fractionation process began. Ethyl acetate (AC) solvent (900.00 mL) was used for the elution, which was recovered in a rotaevaporator to obtain the extracts. Subsequently, chromatography was performed with ethanol/water (7:3) (900.00 mL).

Fractionation of the AC sub-extract

To perform silica gel column chromatography, a glass column (80.00 cm) was packed with silica gel 60 and hexane. The AC sub-extract in silica gel was placed on at the top of the column. Elution was performed with hexane/ dichloromethane solution (1:1). Twelve fractions were obtained by chromatography and preliminarily evaluated for their biological activity on three-day-old *A. monuste orseis* caterpillars. Only fractions 1, 2, and 3 showed larvicidal activity. These were then fractionated in a new column.

Sub-fractionation of fractions 1 to 3 from the AC sub-extract

The collected fractions 1 to 3 were grouped and subjected to a second chromatographic step in a silica gel column and eluted with hexane/dichloromethane (2:1). Fractionation resulted in 10 fractions, which were preliminarily evaluated on three-day-old *A. monuste orseis* caterpillars. Larvicidal activity was found only in fractions 2 and 4.

Dichloromethane Extract (DCM)

The dichloromethane (DCM) extract was obtained using a partition system (solvent/solvent), in which an aliquot (15.00 g) of the crude extract was solubilized in 500.00 mL of ethanol/water solution (7:3). The liquid-liquid extraction was partitioned three consecutive times with DCM (100.00 mL), totaling 300.00 mL. Subsequently, the partitioned liquid-liquid extraction was transferred to a separatory funnel and gently shaken after resting. Then, the immiscible organic phase (OP) was separated from the aqueous phase (AP). The solvents were recovered in a rotaevaporator and then the organic phase extract (OP) was placed under ventilation until constant weight (ANDRADE et al., 2005).

Bioassays

The bioassays were conducted under constant conditions of temperature (25±2 ºC), relative humidity (70±5%), and photoperiod (12 hours), in the Integrated Pest Management (MIP) laboratory at UFAC, Rio Branco Campus, using as the test organism *A. monuste orseis* caterpillars aged eight days after hatching.

Eggs of *A. monuste orseis* were collected every two days from the cabbage patch in the UFAC vegetable garden, which was grown without the use of chemical control for pests and diseases. The collected eggs were taken to the laboratory and placed in plastic containers containing absorbent cotton moistened with distilled water to prevent the eggs from dehydrating. The containers were kept under the conditions in which the bioassays were conducted. After hatching, the caterpillars were fed fresh cabbage leaves free of chemical extracts, and were used in the bioassays when they were eight days old.

The crude extract of *O. campechianum* and its DCM

fraction were evaluated on eight-day-old *A. monuste orseis* caterpillars. The caterpillars were kept in one-liter plastic jars, in groups of 30, receiving cabbage free of chemical treatment as food, until the seventh day after hatching, when they were used in the bioassays. Seven-day-old caterpillars are in the first larval instars and therefore need food to complete their cycle (BITTENCOURT-RODRIGUES; ZUCOLOTO, 2009).

Five caterpillars were placed in 250.00 mL plastic jars containing three cabbage disks with an area of 18.40 cm^2 each that were previously immersed for five seconds in solutions of the crude extract and the DCM fraction. The caterpillars remained close to the disks for 24 hours. After this period, the live and dead caterpillars were counted. The consumed area of the remaining disks was calculated and these were discarded. The dead caterpillars were discarded and the survivors were individualized and fed untreated cabbage until the end of the caterpillar stage.

The effects of the crude extract and the DCM fraction were evaluated at concentrations of 12.50, 25.00, and 50.00 mg mL^{-1}. No application was made in the control treatment. Six replicates with five caterpillars were used for each concentration and control, totaling 30 experimental units. The experimental design used was completely randomized (CR) with six replications, in a 2 x 4 factorial scheme (extracts x concentrations). The variables evaluated were the following: leaf consumption (cm^2), caterpillar mortality in 24 h, total caterpillar mortality, pupal mortality, pupal period duration, and morphological malformation in adults.

The assumptions of ANOVA were analyzed and, where necessary, the data were transformed and then subjected to regression analysis. When the interaction was not significant, the means of the extracts were compared using the t-test at 5%.

RESULTS AND DISCUSSION

There was a significant interaction ($P \leq 0.05$) between the extracts and the concentrations used regarding leaf consumption (cm^2), total caterpillar mortality, pupal duration, and morphological malformation in adults, but there was no significant interaction ($P \geq 0.05$) regarding caterpillar mortality in 24 hours and pupal mortality (Table 1).

Table 1. F calculated for consumption (cm^2), caterpillar mortality in 24 h and total mortality (%), pupal mortality (%), pupal period (days), and adult malformation (%) as a result of *A. monuste orseis* caterpillar feeding on cabbage treated with the crude extract of *O. campechianum* and its DCM fraction.

Treatments	Leaf consumption (cm^2)	Caterpillar mortality 24 h (%)	Total caterpillar mortality (%)	Pupal mortality (%)	Pupal period (days)	Malformation in adults (%)
Extract (E)	102.98*	8.55*	18.52*	0.88^{ns}	6.93*	1.50^{ns}
Concentration (C)	39.17*	11.39*	34.91*	3.79*	30.81*	2.01^{ns}
E x C	15.53*	2.25^{ns}	6.00*	$2.^{24ns}$	16.60*	4.87*
CV (%)	21.52	5.00	6.35	5.08	2.77	9.29

(*) significance at 5% and(^{ns}) not significant, CV = coefficient of variation.

The consumption of cabbage disks by the caterpillars decreased as the concentration of the extracts increased ($P \leq 0.05$); however, in the DCM fraction the decrease was greater in with the crude hydroalcoholic extract. The cabbage disks treated with the DCM fraction were consumed 2.27, 6.44 and 32.67 cm^2 less than those treated with the crude extract at concentrations of 12.5, 25.0, and 50.0 mg mL^{-1}, respectively (Figure 1).

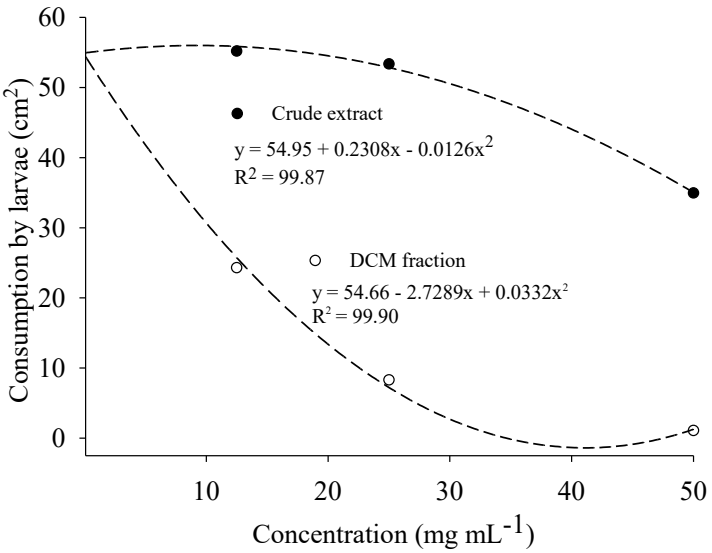

Figure 1. Consumption of cabbage (cm^2) treated with crude extract of *O. campechianum* and its and DCM fraction by *A. monuste orseis* caterpillars in 24 h of exposure, in the laboratory (25 ± 2 ºC, RH = 70±5%, photoperiod 12 h).

Singh et al. (2014) also observed that the growth and development of *Helicoverpa armigera* larvae were significantly slowed down when they fed on *Ocimum kilimandscharicum* leaves, which demonstrates the presence of phagodeterrent metabolites in different species of the *Ocimum* genus. *Ocimum* species have an abundancy of diverse secondary metabolites, including terpenes, phenylpropanoids, phenolics, some of which may be involved in defensive functions that inhibit insect feeding (SARATE et al., 2012).

Caterpillar mortality in 24 hours increased with increasing concentrations of the crude hydroalcoholic extract and the DCM fraction. However, the DCM fraction caused greater toxicity (P≤0.05), resulting in approximately 37% mortality at the highest concentration (50.00 mg mL^{-1}), compared to 13% for the crude extract. Thus, the DCM fraction was 2.85 times more toxic to *A. monuste orseis* caterpillars than the crude extract (Figure 2). It should be noted that there was no significant fit for the probit model.

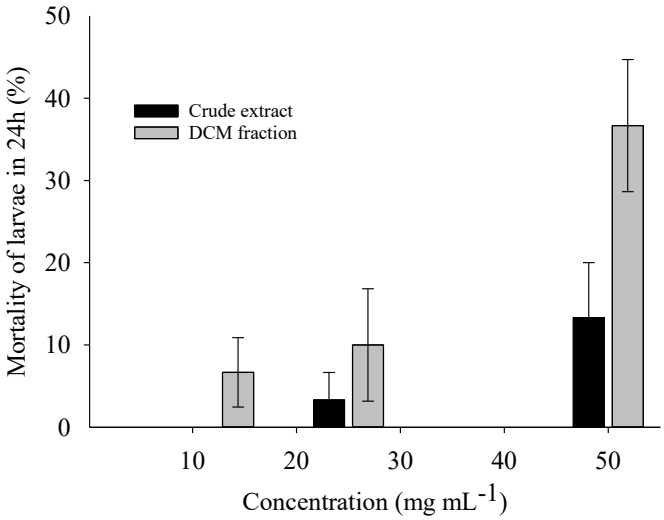

Figure 2. Mortality of *A. monuste orseis* caterpillars (%) after 24 hours of exposure to cabbage disks treated with the crude extract and DCM fraction of *O. campechianum*, in the laboratory (25±2 ºC, RH = 70±5%, photoperiod 12 h).

There was a significant interaction (P≤0.05) regarding total caterpillar mortality, and it followed the mortality trend for caterpillars exposed for 24 hours. Mortality increased with increasing concentrations of both the crude extract and the DCM fraction. However, the percentage of larval mortality was higher with the DCM fraction, with 50 mg mL^{-1} causing around 90% mortality. On the other hand, the same concentration of the crude hydroalcoholic extract resulted in a 40% mortality (Figure 3). There was also no significant fit for the probit model.

Singh et al. (2014) observed that caterpillars feeding on *O. kilimandscharicum* leaves showed impaired growth and increased mortality. The authors identified phenylpropanoids and sesquiterpenes as the main classes of compounds in the plant.

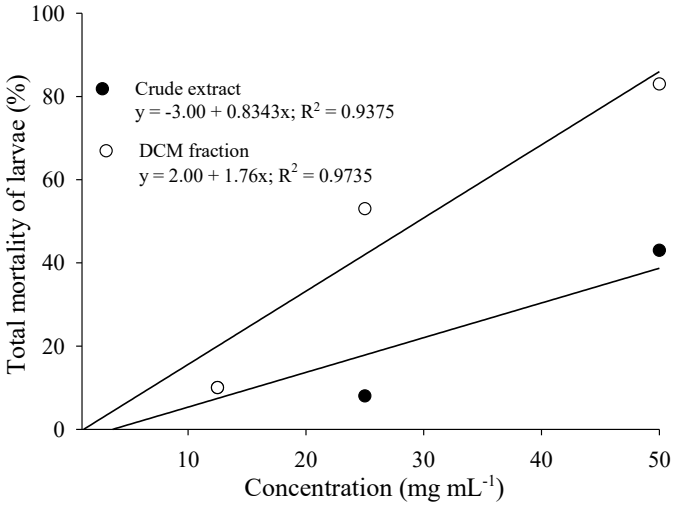

Figure 3. Total larval mortality (%) as a function of consumption of cabbage disks treated with the crude extract and DCM fraction of *O. campechianum* by *A. monuste orseis* caterpillars.

High total mortality was obtained using the DCM fraction, even at the highest concentration (50.00 mg mL^{-1}) at which consumption was extremely low, around 1.00 cm^2 of cabbage. The explanation for this result may be the greater amount of insecticidal metabolites at this concentration; thus, even though the caterpillars consumed little cabbage, it was enough to cause their mortality.

It should be noted that although the caterpillars fed more on the disks treated with the crude hydroalcoholic extract (Figure 1), the associated mortality was 50% lower (Figure 2). The DCM extract is a low-polarity fraction of the crude extract, which means that while in the crude extract all the metabolites produced by the plant, including the primary metabolites, are present in insufficient amounts, the DCM fraction contains only the low-polarity secondary metabolites. Thus, the concentration of the compounds that act as insecticides in the DCM fraction is higher than that in the crude extract, which explains the lower larval mortality in the latter.

Lucena et al. (2017) found that the hexane and ethyl acetate extracts of *Piper aduncum* induced higher mortality rates in *Anticarsia gemmatalis* and *Spodoptera frugiperda* caterpillars than the ethanolic extract. The authors attributed the results to the higher concentration of phenylpropanoids in those extracts. These results show that extracts produced with less polar solvents have greater larvicidal activity than more polar extracts, which is in line with the findings of the present study.

With regard to the pupal mortality variable, there were no significant differences regarding the interaction and the extracts ($P \geq 0.05$), but there was a significant difference regarding the concentrations ($P \leq 0.05$). The highest percentage of pupal mortality (27%) was obtained at a concentration of 25.00 mg mL^{-1} (Figure 4). However, the data did not fit the proposed regression models.

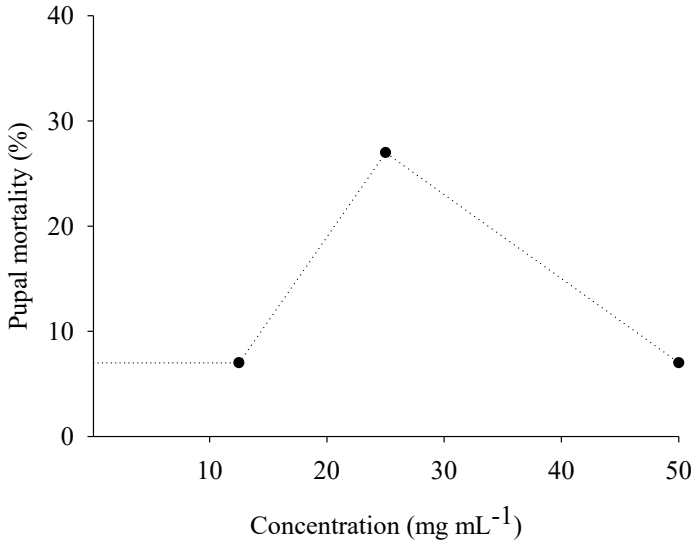

Figure 4. Average percentage of pupal mortality as a function of consumption of cabbage disks treated with crude extract and DCM fraction of *O. campechianum* during the larval period of *A. monuste orseis*, in the laboratory (25±2 ºC, RH = 70±5%, photoperiod 12 h).

There was a significant interaction between the extracts and the concentrations for the duration of the pupal period ($P \leq 0.05$). It was observed that regardless of the extract used, increasing concentrations lengthened the pupation period. There was an increase of around one day between the highest and lowest concentrations for both extracts (Figure 5). However, the DCM extract began to lengthen the period at 12.50 mg mL^{-1}, while the crude extract began to lengthen at twice this concentration.

Lucena et al. (2017) observed that the addition of ethanolic and ethyl acetate extracts of *P. aduncum* to the diet of *S. frugiperda* did not cause the pupal stage to lengthen even at the highest concentration used (15.00 mg mL^{-1}); however, the hexane extract lengthened the period by about one day with the lowest concentration (1.00 mg mL^{-1}). This is in line with the results of the present study, in which pupal stage lengthening was greater in the less polar extract (DCM). Food substrates that provide a longer insect cycle lead to lengthening of the biological development of individuals, thereby providing longer development phases and lower survival (SANTOS et al., 2024). These results are promising for management strategies to control *A. monuste orseis*.

The increase in the duration of the larval and pupal stages by different extracts can be explained by the presence of growth inhibitors or toxic substances such as phenylpropanoids and sesquiterpenes, which are toxic to insects and can affect their life cycle (MAPELI et al., 2015; SANTOS et al., 2024). Crucial processes of development and metamorphosis occur during the pupal stage, in which the larva transforms into an adult insect. Changes in this period interfere with the correct formation of the insect's tissues, organs, and external structures, such as the wings, exoskeleton, and sensory systems (CARVALHO-NETO et al., 2017; NOGUEIRA et al., 2018). In the laboratory, extending the development period of lepidopterans increased the duration of exposure of these pests to their natural predators, thus reducing the number of their generations (SINGH et al., 2014).

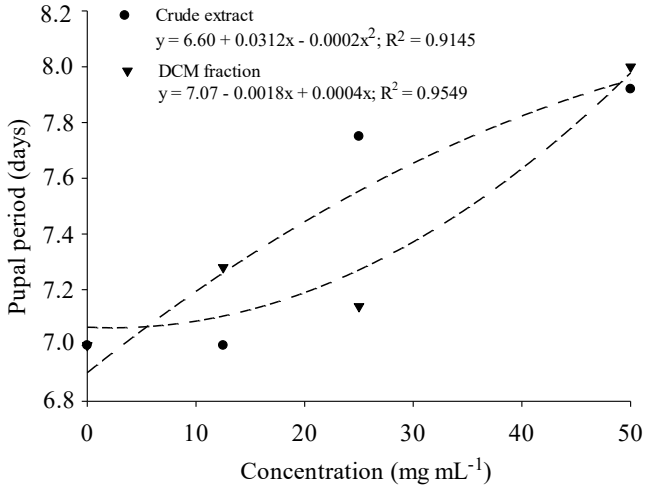

Figure 5. Average pupal period of *A. monuste orseis* as a function of consumption of cabbage disks treated with crude extract and DCM fraction of *O. campechianum* (25±2 ºC, RH = 70±5%, photoperiod 12 h).

With regard to the occurrence of defects in adults, there was a significant interaction between extracts and concentrations (P≤0.05). In the case of caterpillars fed on cabbage disks treated with the DCM fraction, it was found that the occurrence of adults with some defect (teratogenicity) ranged from 0.00% to 50.00%, while the range obtained with the crude extract was 7.50% to 51.70%. However, the DCM fraction caused the highest occurrence of defects at 50.00 mg mL^{-1}, while with the crude extract this occurred at 25.00 mg mL^{-1} (Figure 6).

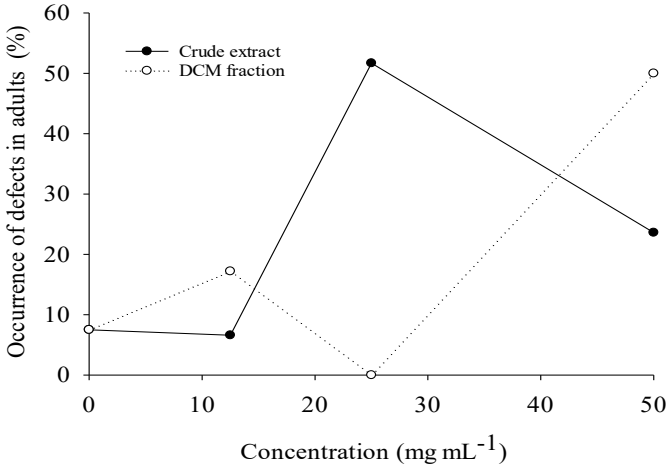

Figure 6. Occurrence of defects in adults of *A. monuste orseis* as a function of consumption of cabbage disks treated with crude extract and DCM fraction of *O. campechianum*, in the laboratory (25±2 ºC, RH = 70%, photoperiod 12 h).

Lucena et al. (2017) observed morphological and physiological changes in *S. frugiperda* using extracts of *P. aduncum* of different polarities. The authors attributed these effects to the activity of phenylpropanoids and sesquiterpenes, which they believe cause toxic interference with the biochemical and physiological functions of insects. Sousa (2004) identified the presence of the phenylpropanoid eugenol in the methanolic and benzene extracts of *O. campechianum* leaves, as well as monoterpenes, sesquiterpenes, and triterpenoids. These compounds may be responsible for the teratogenicity observed in adult insects of *A. monuste orseis* (Figure 7).

The use of the crude extract and DCM fraction of *O. campechianum* to control *A. monuste orseis* is a promising approach to sustainable agriculture and integrated pest management. These extracts, rich in bioactive compounds such as terpenes and phenols, have been shown to be effective in inhibiting the growth and larval development of various agricultural pests (JARAMILLO; DUARTE; DELGADO, 2014; CAAMAL-HERRERA et al., 2018). The importance of these extracts lies in their role as an ecological alternative to synthetic pesticides, to reduce the environmental impact and the risks to human health and pollinators.

Figure 7. Malformation in adults of *A. monuste orseis*.

With a view to large-scale application, the use of *Ocimum* extracts could be integrated in agricultural production systems as an additional tool in the arsenal against pests, thus helping to reduce dependence on synthetic insecticides and promoting the sustainability of agricultural ecosystems. Large-scale use, however, requires further studies into the efficacy, formulation, stability, and method of application of the extracts to ensure economic viability and acceptance by farmers.

CONCLUSION

The crude extract and the DCM fraction of *O. campechianum* reduce feeding in *A. monuste orseis* caterpillars, with the greatest reduction obtained with the DCM fraction.

Both the crude hydroalcoholic extract and the DCM fraction of *O. campechianum* were shown to be toxic, causing mortality of caterpillars and pupae of *A. monuste orseis*.

Feeding *A. monuste orseis* caterpillars with cabbage treated with the crude extract (25.00 mg mL^{-1}) and the DCM fraction (50.00 mg mL^{-1}) resulted in morphological malformations in the adults.

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