Industrial Crops & Products 128 (2019) 399–404

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Effects of paracress (*Acmella oleracea*) extracts on the aphids *Myzus persicae* and *Lipaphis erysimi* and two natural enemies

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ARTICLE INFO

Keywords:
Botanical insecticide
Green peach aphid
Mustard aphid
Diaeretiella rapae
Orius insidiosus

ABSTRACT

This study was conducted to evaluate the toxicity of paracress (*Acmella oleracea*) extracts to the aphids *Myzus persicae* (Sulz.) and *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae), and the extracts selectivity to the aphid parasitoid *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae) and to the predator Orius *insidiosus* (Say) (Hemiptera: Anthocoridae). We evaluated water and ethanol extracts from *A. oleracea*. The ethanol extract caused 90% mortality of both aphid species within 70 h and reduced their fecundity, whereas the water extract was inactive. In addition, this extract caused no mortality of the natural enemies. In an attempt to identify bioactive compounds in the ethanol extract, three alkamides, spilanthol, undeca-2*E*-en-8,10-diynoic acid isobutylamide (compound **2**) and (2*E*)-*N*-(2-methylbutyl)-2-undecene-8,10-diynamide (compound **3**), were isolated and tested on *L. erysimi*. From the isolated alkamides, spilanthol and compound **2** showed high insecticidal activity against *L. erysimi*, indicating that they contributed to the observed extract activity. The results of this study demonstrate the potential of the ethanol extract of *A. oleracea* as a botanical insecticide in the management of aphids in brassicas.

1. Introduction

Aphids (Hemiptera: Aphididae) are one of the main groups of agricultural pests. These insects are important pests of brassica crops, such as kale (*Brassica oleracea* var. *acephala*), cabbage (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), cress (*Lepidium ruderale* L.) and mustard (*Brassica juncea* L.) (Ordás and Cartea, 2008). The main aphids that attack brassica crops include the species *Myzus persicae* (Sulz.) and *Lipaphis erysimi* (Kalt.). These insects suck sap from plants, introduce toxins and transmit viruses (van Emden and Harrington, 2007), causing curling and chlorosis of leaves and shoots (Sylvester, 1987). The green peach aphid, *M. persicae*, is cosmopolitan and extremely polyphagous, feeding on more than 500 hosts from 40 plant families (Sanchez et al., 2011; van Emden and Harrington, 2007). The mustard aphid, *L. erysimi*, attacks leaves, terminal stems and flowers of many brassica species (Lamb et al., 1993).

In commercial fields, brassica pests are mainly controlled by synthetic pesticide applications (Saucke et al., 2000). However, the use of plant-derived compounds as natural pesticides has received greater attention due to their reduced effects on non-target organisms, and low environmental impacts and health risk to growers and consumers when compared to synthetic ones (Gurr et al., 2012; Isman, 2006). In addition, the market expansion of organic products and the emergence of pest insecticide resistance has also increased the search for new botanical products to control insect pests in agriculture (Isman, 2004; Regnault-Roger et al., 2012). In this context, several studies have demonstrated the insecticidal activity of plant extracts against agricultural pests, including aphids (Akhtar et al., 2012; Görür et al., 2008; Xiaorong and Taiping, 2008; Yi et al., 2012).

Acmella oleracea Murr. (Asteraceae), commonly referred to as paracress, is a tropical herbaceous plant largely used as food flavoring and in cosmetics as anti-aging ingredients (Alcantara et al., 2014). Its extract is also known by its therapeutic, bactericidal and antifungal properties (Alcantara et al., 2014; Yamane et al., 2016). The activity of *A. oleracea* has been studied extensively, but only a limited number of studies have assessed its insecticidal activity.

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https://doi.org/10.1016/j.indcrop.2018.11.040
Received 4 July 2018; Received in revised form 15 November 2018; Accepted 17 November 2018
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In agroecosystems, aphid populations are naturally regulated by organisms such as the generalist predator *Orius insidiosus* Say (Hemiptera: Anthocoridae) (Harwood et al., 2007; Rutledge and O'Neil, 2005) and the aphid parasitoid *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae) (Blande et al., 2008; Ferreira et al., 2018). Thus, these organisms should be considered in aphid control programs. This fact enhances, therefore, the necessity to investigate the selectivity to those non-target organisms of potential insecticides to be used on aphid control.

Given this context, this study was carried out *i*) to evaluate the effects of *A. oleracea* extracts and its constituents on the aphids *M. persicae* and *L. erysimi*, and *ii*) to assess the selectivity of the extracts on the predator *O. insidiosus* and the parasitoid *D. rapae*.

2. Materials and methods

2.1. Plant extracts

Samples of 20 g from *A. oleracea* canopy were collected on the campus of the Federal University of Viçosa (UFV), Minas Gerais, Brazil (20°48′45″ S, 42°56′15″ W, 600 m above sea level and tropical weather), where this plant is permanently cultivated. *Acmella oleracea* samples were used in two different extracts (water or ethanol extracts). For extraction, each sample was dried, crushed and placed in a 1-L Erlenmeyer flask with 200 mL of solvent (ethanol or deionized water). After 48 h, the extracts were filtered using a Buchner Funnel (90 mm diameter) and Grade 1 filter paper. Subsequently, 100 mL of each filtered extract (ethanol or water extract) was diluted in 900 mL of deionized water and used immediately in the bioassays. This concentration (10%) was used because it is a standard in screening bioassays of botanical insecticide activity against aphids (Erdoğan and Yildirim, 2016; Rando et al., 2011; Santana et al., 2012).

2.2. Insects

The aphids *L. erysimi* and *M. persicae* used in the bioassays were obtained from a culture maintained in a laboratory located at the UFV Campus (Fidelis et al., 2018). The parasitoid *D. rapae* was also reared in the laboratory. To establish the culture, mummies of *M. persicae* parasitized by *D. rapae* were collected in brassica crops, put into Petri dishes (90 × 20 mm) and immediately brought to the laboratory. The insects were then placed inside wooden cages closed with organza fabric. The cages contained kale leaves infested with *M. persicae* from the laboratory culture. The leaf petioles were immersed in 100 mL containers filled with water. Every three days, new kale leaves infested with aphids were added to the cages. The predator *O. insidiosus* was collected from vegetable crops near the UFV Campus using aspirators and immediately brought to the laboratory. The insects were acclimated in laboratory-controlled conditions for two hours prior to the bioassays.

2.3. Experimental procedure

Four sets of bioassays were conducted. The first was a bioassay to determine the effects of *A. oleracea* extracts on the survival and biology of M. persicae and *L. erysimi*. The second bioassay was conducted to establish time-mortality curves of the most active plant extract for M. persicae and *L. erysimi*. The third bioassay investigated the effects of the most active plant extract on the natural enemies *D. rapae* and O. *insidiosus*. In the fourth set of bioassays, three N-isobutylamides isolated from *A. oleracea* were tested on *L. erysimi* to determine their roles in the observed insecticidal activity.

Two preliminary bioassays were conducted to ensure that the experiments could be carried out without interference. In the first preliminary bioassay, the phytotoxicity of the extracts to the kale leaves was evaluated. The observations were taken daily for a period of 15 days after application of treatments and deleterious effects were not observed on the leaves. The second bioassay was performed to confirm if the solvent ethanol would not affect aphid survival. In this trial, we sprayed kale leaves containing ten aphids each (in six replicates) with 10% ethanol and water and evaluated the survival of both species (*M. persicae* and *L. erysimi*) in seven periods (ten minutes and one, four, six, 12, 48 and 72 h). We found that there was no difference in the survival of the two species when sprayed with water and 10% ethanol, i.e., ethanol did not affect the survival of the aphids.

2.3.1. Effect of Acmella oleracea extracts on the survival and biology of the aphids

The experiments regarding the effects of *A. oleracea* extracts (0.01 g mL^{-1}) on M. persicae and *L. erysimi* were conducted on neonate nymphs on kale leaves with its petioles immersed in 100-mL containers filled with water. For this purpose, apterous female adults were maintained on kale leaves for a period of 24 h. Then, the insects were removed with the aid of a fine soft hair brush and ten newborn nymphs were kept on each leaf.

The experimental design was completely randomized with six replications. Each experimental unit consisted of a kale leaf containing ten nymphs. The kale leaves and aphids were sprayed until run-off with the *A. oleracea* extracts with a backpack sprayer. In the control, leaves and insects were sprayed with deionized water in a procedure identical to that of the extract application. The leaves were then stored in a climate controlled chamber at 25 ± 1 °C, 65 ± 5% relative humidity (RH), and 12 h photophase.

Observations regarding aphid survival, nymphal stage duration and adult fecundity were made by direct counting of insects at 12 h intervals up to 312 h (13 days) using a 30X binocular stereo microscope (6X - 50X Motic K401) and a fine soft hair brush to handle insects. Exuviae were removed from leaves before observations and insects were considered dead when they did not move upon stimulation. Adult fecundity was determined by counting newborn nymphs until the death of all adults. After each observation, those newborn nymphs were carefully removed from leaves so they did not influence posterior evaluations. Fecundity was then calculated from the average number of newborn nymphs per female aphid.

2.3.2. Time-mortality bioassays

The first stadium nymphs of each aphid species were subjected to time-mortality bioassays with the *A. oleracea* ethanol extract. This experiment was conducted because of the high mortality rates caused by this extract. The experimental unit consisted of a kale leaf containing ten nymphs, using the same procedure described in Section 2.3.1. Deionized water was used as control. Six independent replicates were used for each combination of insect species, ethanol extract or the control, and length of exposure. The kale leaves and aphids were sprayed until run-off with the *A. oleracea* ethanol extract with a backpack sprayer. In the control treatment, plants and insects were sprayed with deionized water. The leaves containing insects were placed in a climatic chamber at 25 ± 1 °C, 65 ± 5% RH and 12 h photophase. Mortality assessments were conducted at regular and independent exposure intervals (i.e., with separate replicates at each time interval) that had been pre-established after preliminary tests (ten minutes, and one, four, six, 12, 48 and 72 h after the experiment setup).

2.3.3. Effect of Acmella oleracea extract on natural enemies

These bioassays were performed using the natural enemies *D. rapae* and *O. insidiosus* adults. The experimental design was completely randomized with six replications. Each experimental unit consisted of a kale leaf disk (diameter 90 mm) containing ten natural enemies of a species. The leaf disks were sprayed until run-off with the *A. oleracea* ethanol extract or deionized water as control with a backpack sprayer. We assessed only the ethanol extract of *A. oleracea* because it showed the highest insecticidal activity against the aphids.

Treated leaves were dried at room temperature for two hours and

placed on the bottom of glass Petri dishes (90 × 20 mm). Adults were transferred to the Petri dishes using aspirators. The Petri dishes were then covered with organza fabric, which was tied with a rubber band to prevent insects from escaping. The covered Petri dishes were maintained at 25 ± 1 °C, 65 ± 5% RH and 12 h photophase. Mortality, defined as immobility upon stimulation with a fine soft hair brush, was recorded 48 h after treatment.

2.3.4. Toxicity to Lipaphis erysimi of alkamides isolated from Acmella oleracea

The alkamides used in this bioassay had been previously isolated from the *A. oleracea* extract and identified as spilanthol (Compound **1**), undeca-2*E*-en-8,10-diynoic acid isobutylamide (Compound **2**), and (2*E*)-*N*-(2-methylbutyl)-2-undecene-8,10-diynamide (Compound **3**) (Moreno et al., 2012).

This bioassay was conducted by topical application to evaluate the insecticidal activity of *A. oleracea* isolated alkamides against the aphid *L. erysimi*. Topical application was necessary because only a small amount of the compounds was isolated. The bioassay was performed with only *L. erysimi* due to the small size of the M. persicae nymphs, which did not allow topical application.

The experimental design was completely randomized with six replications. Each experimental unit consisted of a glass Petri dish (90 mm x 20 mm) containing ten newborn nymphs transferred from the laboratory culture with the aid of a fine soft hair brush. The average weight of insects was obtained by measuring, on an analytical balance, the mass of ten groups containing 10 insects each. For each individual insect, 0.5 µL of a solution of the test compound dissolved in acetone at the dose of 1 µg of compound per mg of insect was applied via a 10-µL Hamilton micro syringe. In the control experiment, which was conducted under the same conditions, 0.5 µL of acetone was applied to each insect. After application, the insects were kept in individual Petri dishes containing kale leaf disks (diameter 90 mm) as food. The Petri dishes were placed in a climatic chamber at 25 ± 0.5 °C, 65 ± 5% RH and 12 h photophase. The mortality counts were made after 48 h. Insects were considered dead if they did not move while stimulated by a fine soft hair brush.

2.3.5. Statistical analyses

A significance level of 0.05 was used for all statistical tests. The data on aphid survival were subjected to a survival analysis using the nonparametric procedure LIFETEST (SAS 9.2, SAS Institute Inc, Cary, USA). This procedure allows the estimation of survival curves obtained through Kaplan–Meier estimators generated from the proportion of aphids that survive from the beginning until the end of an experiment. Survival curves were then compared by log-rank test. Data of nymphal duration, fecundity, and toxicity of compounds from the *A. oleracea* extract were submitted to analysis of variance (ANOVA) followed by Tukey test (PROC GLM, SAS 9.2). Time-mortality data were subjected to probit analysis (PROC PROBIT, SAS 9.2) to obtain times for 50% (LT_{50}) and 95% (LT_{95}) mortality. Mortality data of natural enemies were subjected to Student's *t*-test in order to compare the mortality caused by the ethanol extract with that from the control.

3. Results

3.1. Survival and biology of the aphids

Survival analysis of the aphids exposed to *A. oleracea* extracts indicated significant differences among treatments for both *M. persicae* (log-rank test, $\chi^2 = 104.51$, $df = 2$, $P < 0.0001$) and *L. erysimi* (log-rank test, $\chi^2 = 138.79$, $df = 2$, $P < 0.0001$). While the ethanol extract caused 90% mortality to both species in less than 70 h (three days), the water extract took more than 200 h (eight days) to cause 80% and 60% mortality rates in *L. erysimi* and *M. persicae*, respectively (Fig. 1).

The effects of the water and ethanol extracts on the nymphal stage duration were not significant in comparison with the control for *M. persicae* ($F_{2;97} = 0.94$; $P = 0.40$) and *L. erysimi* ($F_{2;84} = 0.17$; $P = 0.85$). Conversely, the effects of the *A. oleracea* extracts were significant on the fecundity of M. persicae ($F_{2;74} = 7.46$; $P = 0.001$) and *L. erysimi* ($F_{2;56} = 12.43$; $P < 0.001$). The fecundity of M. persicae and *L. erysimi* was reduced when the ethanol extract of paracress was applied according to Tukey's test ($P < 0.05$). On average, compared to the control, the ethanol extract reduced the fecundity by 46 and 65% in M. persicae and *L. erysimi*, respectively. The water extract only reduced the fecundity of *L. erysimi* by 36% and did not affect the fecundity of *M. persicae* (Table 1).

3.2. Time-mortality bioassays

The ethanol extract presented a faster action to *L. erysimi* ($LT_{50} = 3.29$ h, $LT_{95} = 24.85$ h) than to *M. persicae* ($LT_{50} = 8.85$ h, $LT_{95} = 57$ h). Furthermore, the time-mortality curve for *M. persicae* had a lower slope than that for *L. erysimi* (Table 2 and Fig. 1).

3.3. Effect of the ethanol extract on natural enemies

The *A. oleracea* ethanol extract did not affect the mortality of both *D. rapae* ($t = 1.58$, $df = 10$, $P = 0.15$) and *O. insidiosus* ($t = 1.0$, $df = 10$, $P = 0.34$) compared to control (deionized water). Mortality rates were 3.33 ± 2.11% and 1.67 ± 1.67% for *D. rapae* and *O. insidiosus,* respectively.

3.4. Toxicity to Lipaphis erysimi of the isolated alkamides

The bioassay with the three identified compounds showed significant insecticide activity for all compounds against *L. erysimi* ($F_{3;23} = 110.04$, $P < 0.001$). Compound **1** caused the highest mortality (83%), followed by compounds **2** (68%) and **3** (55%) according to Tukey's test ($P < 0.05$) (Table 3).

4. Discussion

Compared to the water extract, the *A. oleracea* ethanol extract caused higher mortality rates in a shorter period of time (Fig. 1). In addition, the ethanol extract reduced the fecundity of *L. erysimi* and *M. persicae*, while the water extract did not (Table 1). Several studies indicate that bioactivity of plant extracts such as lethal and sublethal effects against herbivores can be related to the presence of plant secondary metabolites (Moreira et al., 2007; Olivero-Verbel et al., 2010; Ventrella et al., 2016). Earlier biochemical analysis of *Acmella* spp. revealed that the majority of secondary metabolites in these plants consist of a group of alkamides and their respective isomers (Crouch et al., 2005; Ley et al., 2006; Moreno et al., 2012; Ramsewak et al., 1999). These compounds have polar (hydrophilic) and nonpolar (hydrophobic) moieties and are therefore better dissolved by amphiphilic compounds (i.e., with both hydrophobic and hydrophilic properties) such as ethanol, and less by water (highly polar) (Barbosa et al., 2016; Saeidnia et al., 2015). In other words, ethanol is a better solvent to amphiphilic compounds such as alkamides. Thus, one of the possible reasons for the lower activity of the water extract may be due to a lower content of bioactive compounds (alkamides) in this extract.

The ethanol extract reduced the fecundity of *L. erysimi* and *M. persicae*. Some mechanisms responsible for the reduction in the fecundity of aphids have been reported, including the delay in the onset of reproduction, caused by the increase in the development time of aphids exposed to insecticides (Down et al., 1996). In our study, the ethanol extract did not affect the duration of nymphal development, which refutes this mechanism. Reduction in fecundity of aphids by insecticides may also occur due to reduced development embryos (Lowery and Isman, 1996) and the destruction of bacterial symbionts (including those of the *Buchnera* genus) in the aphid mycetomes (Jayaraj et al.,

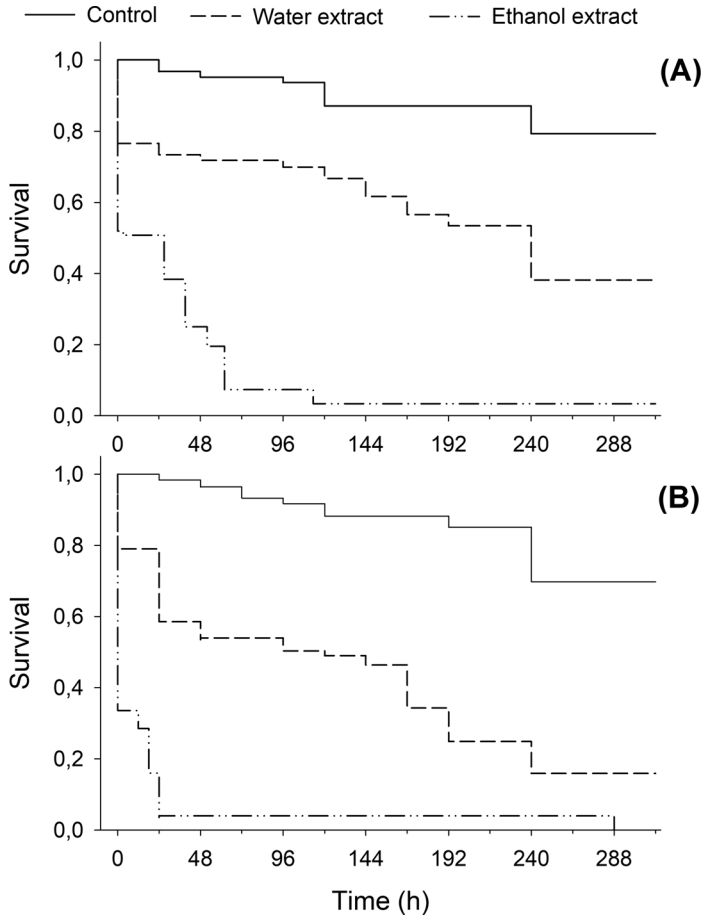


Fig. 1. Survival curves (estimated by the Kaplan-Meier product-limit) of two aphid species, *Myzus persicae* (A) and *Lipaphis erysimi* (B), exposed to *Acmella oleracea* water and ethanol extracts (at 10 g L^{-1} concentration), and to control (deionized water) at 25 °C.

Table 1
Fecundity (nymphs female^{-1}) of *Myzus persicae* and *Lipaphis erysimi* on kale plants when sprayed with paracress (*Acmella oleracea*) water and ethanol extracts (at 10 g L^{-1} concentration), and to control (deionized water) at 25 °C.

Species	Treatment	Fecundity ± SE
M. persicae	Control	23.0 ± 1.8 a
	Water extract	18.6 ± 1.6 a
	Ethanol extract	12.2 ± 1.0 b
L. erysimi	Control	37.0 ± 2.4 a
	Water extract	23.3 ± 1.6 b
	Ethanol extract	13.2 ± 1.1 c

Different letters indicate significant differences among treatments by Tukey's test ($P < 0.05$) for each species.

Table 3
Mortality (%) caused by Spilanthol (compound **1**), undeca-2E-en-8,10-diynoic acid isobutylamide (compound **2**) and (2E)-N-(2-methylbutyl)-2-undecene-8,10-diynamide (compound **3**) to *Lipaphis erysimi* (at 1 µg mg^{-1} concentration) 48 h after topical exposure.

Treatment	N	Mortality ± SE
Compound 1	60	83.33 ± 3.42 a
Compound 2	60	68.33 ± 4.01 b
Compound 3	60	55.00 ± 3.42 c
Control	60	3.33 ± 2.11 d

N = Number of insects tested; Control = acetone.
Different letters indicate significant differences among treatments by Tukey's test ($P < 0.05$).

1967; Lal, 1972). These mechanisms were not studied here, and their occurrence is only hypothesized. Further studies are required to determine the mechanisms underlying the reduced fecundity of *M. persicae* and *L. erysimi* exposed to the *A. oleracea* ethanol extract.

Once the ethanol extract showed greater activity on the aphids, time-mortality curves for the two species were constructed. This extract was effective against both species, although *M. persicae* showed a slight tolerance (> LT_{50}) when compared to *L. erysimi*. The higher tolerance of *M. persicae* may be due to its generalist feeding habits, which exposes this species to a larger number of plant secondary metabolites (Turley and Johnson, 2015). As a result, this aphid exhibits a broad genetic variability, which gives it greater tolerance to insecticidal molecules

Table 2
Lethal times to kill 50 (LT_{50}) and 95% (LT) of two aphid species, *Myzus persicae* and *Lipaphis erysimi*, topically exposed to paracress (*Acmella oleracea*) ethanol extract.

Species	N[a]	Slope ± SE	LT_{50} (h) (95% FL)[b]	LT_{95} (h) (95% FL)[b]	χ^2	*P*
M. persicae	420	0.20 ± 0.04	8.85 (5.30 - 12.40)	57.05 (45.58 - 68.05)	7.61	0.30
L. erysimi	420	0.33 ± 0.03	3.29 (0.41 - 8.32)	24.85 (18.01 - 36.23)	6.53	0.45

[a] Number of insects tested; [b] Lethal time with 95% fiducial limits (FL).

than monophagous and oligophagous species. Populations of this pest have shown resistance to almost all classes of insecticides (Bass et al., 2014; Panini et al., 2017; Voudouris et al., 2016). Such adaptation is mediated through various detoxifying mechanisms (metabolic resistance) and target site insensitivity (Voudouris et al., 2016).

The toxicity of the ethanol extract to natural aphid enemies was also evaluated. The *A. oleracea* ethanol extract, at the same concentration used for aphids, was not toxic to the aphid parasitoid *D. rapae* or the predator *O. insidiosus*. Therefore, this extract can be used in integrated pest management (IPM) systems and in organic agriculture to manage *M. persicae* and *L. erysimi* populations because it is effective against aphids and is relatively nontoxic to natural enemies. Our results provide practical information to improve IPM systems in brassicas through the use of botanical insecticides. Conservation biological control is an important component of integrated pest management and can be achieved with the use of selective insecticides, thus enabling the integration of chemical and biological methods to suppress pest populations in agricultural systems (Bacci et al., 2012; Gurr et al., 2012; Rampelotti-Ferreira et al., 2017).

Previous studies have shown that the bioactivity of *A. oleracea* is due to the presence of alkamides (Carvalho et al., 2010; Cruz et al., 2016; Moreno et al., 2012). In this study, three alkamides were isolated from the *A. oleracea* extract and tested on *L. erysimi*. Our results show that the isolated alkamides had high (spilanthol) to moderate (compound **2** and **3**) insecticidal activity against *L. erysimi*, proving the bioactivity of these compounds and indicating that they contribute to the observed activity of the extract. Some studies have shown that, in addition to the alkamides isolated in this study, *A. oleracea* has other active alkamides (Pandey et al., 2011; Ramsewak et al., 1999; Simas et al., 2013). However, spilanthol is considered to be the main active alkamide of this plant (Pandey et al., 2011; Ramsewak et al., 1999), which is consistent with our results.

In conclusion, given the need for alternatives to control pests with low toxicity to mammals and non-target organisms, the ethanol extract of *A. oleracea* is a potential botanical insecticide in the management of aphids in brassicas. The ease of paracress seedlings production (via seeds or cuttings) (Hind and Biggs, 2003) combined with the simplicity of the ethanol extract preparation may allow farmers to perform the ethanol extraction process themselves at low cost and apply it with backpack or mechanical sprayers. In addition, spilanthol and the compound **2** are promising molecules for a chemical optimization program aiming to develop new formulations for the control of *L. erysimi* and *M. persicae*.

Declarations of interest

None.

Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Coordination of Improvement of Higher Education Personnel (CAPES), National Council of Scientific and Technological Development - Brasil (CNPq), and Research Support Foundation of Minas Gerais State - Brasil (FAPEMIG).

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