

Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species

Shaiene C Moreno,^{a*} Geraldo A Carvalho,^a Marcelo C Picanço,^b Elisangela GF Morais^c and Rogério M Pereira^b

Abstract

BACKGROUND: Tropical plants are recognised sources of bioactive compounds that can be used for pest control. The objective of this study was to evaluate the biological activity of compounds present in *Acmella oleracea* (Asteraceae) against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which is the main pest of tomato crops in Latin America. The selectivity of these compounds to the predator *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) and to the pollinator *Tetragonisca angustula* (Latr.) (Hymenoptera: Apidae: Meliponinae) was also of interest.

RESULTS: A bioassay screening with hexane and ethanol extracts from 23 plants was performed. The hexane extract of *A. oleracea* was the most active of the extracts and was selected for further study. The following three alkalimides were isolated from a hexane extract of the aerial parts of *A. oleracea*: spilanthol, (*E*)-*N*-isobutylundeca-2-en-8,10-diyndamide and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyndamide. All of the isolated compounds showed insecticidal activity, with spilanthol being the most active ($LD_{50} = 0.13 \mu\text{g mg}^{-1}$) against *T. absoluta*. The alkalimides were selective to both beneficial species studied.

CONCLUSION: The crude hexane extract of *A. oleracea* showed high insecticidal activity and can be used to control *T. absoluta* in organic or conventional crops. Quantification of LD_{50} values of isolated compounds against *T. absoluta* showed that alkalimides could serve as potent insecticides for *T. absoluta* control programmes. Spilanthol was the main alkalimide active isolated. This alkalimide is the most promising as it has the highest insecticidal activity and is selective to non-target organisms.

© 2011 Society of Chemical Industry

Keywords: botanical pesticide; insect control; secondary metabolites; bioactive alkalimides; tomato leafminer

1 INTRODUCTION

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a neotropical oligophagous insect that attacks solanaceous crops.¹ Since the 1960s, it has become one of the key pests of tomato crops in most South American countries.² Recently, *T. absoluta* has also become a serious threat to tomato production in the Mediterranean region.³ Following detection in the Spanish tomato-growing area at the end of 2006, *T. absoluta* spread quickly to other European and northern African countries.^{4,5} The larvae attack tomato plants during all growth stages, producing large galleries in their leaves, burrowing stalks, apical buds and green and ripe fruits.

Chemical control has been the main method of control used against *T. absoluta*. Horticultural growers have attempted to decrease the damage caused by *T. absoluta* by applying insecticides more than 2 times a week during a single cultivation period.² *Tuta absoluta* control is a challenge owing to the nature of the damage it causes and its rapid ability to develop resistance towards conventional insecticides.^{1,6} Thus, there is an urgent

need to develop safe alternatives to conventional insecticides for the protection of tomato plants against *T. absoluta*.

The use of ecofriendly and easily biodegradable plant products with natural insecticidal activity has increased in recent years. To control pests without disturbing the environment, natural products have been screened for potential sources of insecticides. Plant materials with insecticidal properties have been used to kill insects throughout the world for generations. These plants are considered to be an alternative to conventional pesticides

* Correspondence to: Shaiene C Moreno, Departamento de Entomologia, Universidade Federal de Lavras, 27200-000, Lavras, MG, Brazil.
E-mail: shaiene.moreno@ifrj.edu.br

a Departamento de Entomologia, Universidade Federal de Lavras, Lavras, MG, Brazil

b Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG, Brazil

c Embrapa Roraima, Distrito Industrial, Boa Vista, RR, Brazil

because of their low toxicity to warm-blooded mammals as well as their high volatility. Botanical insecticides may be safer for the environment than synthetic insecticides, and they are usually easily processed and used by farmers and small industries.⁷

Tropical plants are recognised sources of bioactive compounds, but less than 1% have been chemically investigated.⁸ They can be used for pest control as plant extracts, horticultural oils or as a source of molecules for pesticide synthesis such as pyrethroids and neonicotinoids.

Acmella oleracea (L.) RK Jansen [synonyms: *Spilanthes oleracea* L. and *Spilanthes acmella* auct. non (L.) Murr.] is an annual plant of the family Asteraceae (Compositae) originating in the tropics of Brazil. The distribution covers tropical and subtropical areas around the world, and it is known in English as toothache plant or paracress, and in Portuguese as jambú.⁹ The inflorescence is composed of yellow flowers, and the leaves have a pungent flavour accompanied with tingling and numbness. The plant has been used in cooking and in popular medicine, mainly for stammering, toothache, stomatitis and throat complaints.

The plant contains alkaloids including spilanthol, which is the principal pungent compound. This chemical compound is known for having several chemical and pharmaceutical applications. It has shown anti-inflammatory, antibacterial, antifungal, diuretic, sialagogic and larvicidal properties.⁹ The activity of *A. oleracea* has been studied extensively. However, only a few studies have assessed the insecticidal activity of compounds from this plant. Furthermore, the majority of these studies have focused on human health pests.^{10–13} These studies show that compounds of *A. oleracea* have high insecticidal activity against insect vectors of diseases. Therefore, the potential use of this plant species for management of agricultural pests requires further investigation.

New compounds should provide selectivity to non-target species, especially predators and pollinators, in addition to efficiency against insect pests. Attack by natural enemies is the most frequent source of mortality for phytophagous arthropods in agroecosystems, and the conservation of these organisms is an essential component in integrated pest management (IPM).¹⁴ Furthermore, pollination is central for successful reproduction in most plants. Thus, pollinators should be preserved because they support the maintenance of biodiversity in the ecosystems they inhabit.¹⁵ Although most tomato plants need no assistance with pollinating, the presence of insects that visit flowers is important to promote the vibrancy of the flowers and allow self-pollination. In agroecosystems there are many species that are part of these groups, including the predator *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) and the pollinator *Tetragonisca angustula* (Latr.) (Hymenoptera: Apidae: Meliponinae). The predation by *S. saevissima* has played an important role in reducing pest insects in agricultural systems. Way and Khoo¹⁶ cited species of the genus *Solenopsis* as important agents of biological control in the tropics and subtropics. *Tetragonisca angustula* is one of the most common stingless bees in the Neotropical region. Stingless bees are generalist foragers and are efficient native pollinators of the American flora.¹⁵

Considering the potential of tropical plant species for pest control and the importance of *T. absoluta*, the aims of this study were to screen plants with insecticide activity to *T. absoluta*. The goal was to isolate, identify and assess the bioactivity of insecticide compounds present in the bioactive plant against this key insect pest of tomato crops. Furthermore, the selectivity of these compounds to the beneficial insects *S. saevissima* and *T. angustula* was of interest.

2 MATERIALS AND METHODS

2.1 Insects

The bioassays were performed with second-instar larvae of *T. absoluta* and adults of *S. saevissima* and *T. angustula*. Larvae of *T. absoluta* were obtained from a laboratory rearing located at the campus of the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. Adults of *S. saevissima* and *T. angustula* were collected from nests located around the campus of the UFV.

2.2 Plant screening

2.2.1 Plant extract preparation

Table 1 describes the plants that were used for extraction and toxicity bioassays. The plants were chosen on the basis of available literature, popular or indigenous knowledge and chemotaxonomy. The plant material was identified in the botanical park of the Federal University of Acre.

Samples of 1.0 kg from the canopy of each plant species were collected in Rio Branco, AC, Brazil (plants of the Amazon Biome), and in Viçosa, MG, Brazil (plants of the Cerrado and of general occurrence). Each sample was lyophilised, and the dried material was crushed and placed in a 2 L Erlenmeyer flask, with enough hexane to submerge the plant material. After 48 h, the solvent was removed under filtration. Ethanol extraction was performed by grinding the samples with the solvent (100% ethanol) and waiting for 48 h. The hexane and ethanol extracts were concentrated under low pressure and reduced temperature (<50 °C). The yield for each extract is shown in Table 1. The plant extracts were stored at low temperature for subsequent bioassays.

2.2.2 Screening bioassay

A set of screening bioassays was performed to identify the bioactive plant extracts to *T. absoluta*. The stored extracts were diluted with acetone to a dose of 10 µg mg⁻¹ body mass. The average weight was obtained by measuring the mass of ten groups containing ten insects each on an analytical balance. The experimental design was completely randomised with six replications. Each experimental unit consisted of a glass petri dish (9.5 cm × 2.0 cm) containing ten insects.

The bioassays were conducted by topical application. For each insect, a 10 µL Hamilton microsyringe was used to add 0.5 µL of a solution of the test extract. In a control experiment under the same conditions, 0.5 µL of hexane was applied on each insect.

After the application, the insects were kept in individual petri dishes containing tomato leaflets (cv. Santa Clara) as food. The petri dishes were placed in an incubator at 25 ± 0.5 °C and 75 ± 5% relative humidity with a photoperiod of 12 h. The mortality counts were made after 6, 12 and 24 h of treatment. Mortality included dead individuals as well as those without movements. Mortality data were subjected to analysis of variance, and the averages were compared by the Scott–Knott grouping analysis test ($P < 0.05$).

2.3 Bioactivity of compounds from *A. oleracea*

2.3.1 Extract preparation of *A. oleracea*

The hexane extract of *A. oleracea*, which showed the highest insecticidal activity in the screening bioassay, was selected for isolation and structure elucidation of its bioactive compounds. A total of 2.0 kg of dried and powdered aerial parts of *Acmella oleracea* was used for this purpose. The solvent (hexane) was changed every 2 days for 45 days. The extraction continued until the solvent was colourless. The filtered extract obtained was concentrated in a rotary vacuum evaporator under low pressure and reduced temperature (<50 °C).

Table 1. Identification of plants used in screening bioassays (scientific name and family) and yield of hexane and ethanol extracts obtained from 1.0 kg of the plant aerial parts

No.	Scientific name	Family	Yield (g)	
			Hexane extract	Ethanol extract
<i>Plants of the Amazon Biome</i>				
1	<i>Acmella oleracea</i> L.	Asteraceae	10.74	15.04
2	<i>Banara guianensis</i> Aubl.	Flacourtiaceae	22.74	26.54
3	<i>Banara nitida</i> Spruce ex Benth.	Flacourtiaceae	4.2	10.49
4	<i>Clavija weberbaueri</i> Mez.	Theophrastaceae	5.42	35.88
5	<i>Copaifera duckei</i> Dwyer	Caesalpinioideae	7.42	18.44
6	<i>Eugenia egensis</i> DC.	Myrtaceae	7.42	18.44
7	<i>Mayna parvifolia</i> Sleumer	Flacourtiaceae	16.95	9.23
8	<i>Piper aduncum</i> L.	Piperaceae	7.6	11.58
9	<i>Piper augustum</i> Rudge	Piperaceae	7.86	21.33
10	<i>Ryania speciosa</i> Vahl.	Flacourtiaceae	8.96	77.34
11	<i>Siparuna amazônica</i> Mart. ex A. DC.	Monimiaceae	10.43	36.3
<i>Plant of the Cerrado Biome</i>				
12	<i>Curatela americana</i> L.	Dilleniaceae	13.26	19.87
<i>Plants of general occurrence</i>				
13	<i>Ageratum conyzoides</i> L.	Asteraceae	12.00	25.24
14	<i>Allamanda cathartica</i> L.	Apocynaceae	5.31	4.51
15	<i>Argemone mexicana</i> L.	Papaveraceae	5.98	6.48
16	<i>Artemisia vulgaris</i> L.	Asteraceae	4.47	6.81
17	<i>Bauhinia variegata</i> L.	Caesalpinioideae	11.48	32.02
18	<i>Bougainvillea glabra</i> Choisy	Nyctaginaceae	7.56	9.63
19	<i>Calendula officinalis</i> L.	Asteraceae	4.30	5.81
20	<i>Chenopodium ambrosioides</i> L.	Chenopodiaceae	4.75	6.38
21	<i>Coriandrum sativum</i> L.	Apiaceae	5.59	7.52
22	<i>Spathodea campanulata</i> P. Beauv.	Bignoniaceae	12.70	28.76
23	<i>Tropaeolum majus</i> L.	Tropaeolaceae	6.32	7.21

2.3.2 Isolation and structural elucidation of bioactive compounds

Fractionation of the hexane extract (28 g) was performed by column chromatography (silica gel 60, 63–200 µm particle size, 70–230 mesh) using hexane with increasing amounts of ethyl acetate and finally with methanol as the eluting solvents. Thin-layer chromatography (TLC) (silica gel 60 F254, 0.25 mm) was used to identify fractions containing similar compounds. The TLC spots were detected under UV (254 and 365 nm) as well as by heating the plates to 100 °C after spraying with phosphomolybdic acid/ethyl alcohol. Eight fractions (A to H) were collected and subjected to bioassay with *T. absoluta* using the same methods as described in Section 2.2.2. The most toxic fractions were purified by preparative TLC of Merck silica gel 60 F254 (20 × 20 cm plates, 0.75 mm adsorbent). The different zones separated through preparative TLC were scraped from the silica plate and were collected in separate test tubes. Ethyl acetate (100 mL) was added to each test tube. Thereafter, the ethyl acetate containing dissolved compounds was filtered twice through filter paper (125 mm diameter) to remove any suspended silica powder completely. The bioactive fraction F was purified using hexane–ethyl acetate (6 : 1) as the mobile phase to yield the following three major bands: I (725 mg), II (56 mg) and III (21 mg). Band I was further purified using hexane–ethyl acetate (1 : 2) to give compounds **1** and **2** (320 and 210 mg respectively). The bioactive fraction G was purified with hexane–ethyl acetate (3 : 1) as eluent to yield compound **3** (27 mg). The IR spectra of isolated compounds were recorded on KBr in a Paragon 1000 FTIR infrared spectrometer (Perkin Elmer, Wellesley, MA) from 600

to 4000 cm⁻¹. GC-MS was conducted with a Shimadzu QP5050A gas chromatograph–mass spectrometer. To identify the isolated compounds, ¹H NMR and ¹³C NMR were recorded in a Bruker WM 400 spectrometer (Bruker Optics Inc., Billerica, MA) using CDCl₃ as a solvent and TMS as an internal standard.

2.3.3 Dose–mortality bioassays

The isolated compounds and the hexane extract of *A. oleracea* were subject to toxicity bioassays against *T. absoluta*, *S. saevissima* and *T. angustula*. The insecticidal activity of neem (*Azadirachta indica* A. Juss) seed kernel hexane extract and of permethrin (92.2% purity; Syngenta), a synthetic derivative of the natural pyrethrins recommended for *T. absoluta* control, were also evaluated and used as positive controls. The experimental design was completely randomised with six replications. Each experimental unit consisted of a glass petri dish (9.5 cm × 2.0 cm) containing ten insects. The average weight of each insect species was obtained by measuring the mass of ten groups containing ten insects each on an analytical balance.

Initially, four doses of each compound were tested to identify the range of concentrations that would provide mortalities greater than zero and less than 100%. Once the range of concentrations was defined, other doses were tested for each compound. The number of doses used to obtain the dose–mortality curves varied from five to eight doses.

Bioassays were conducted by topical application. For each insect, a 10 µL Hamilton microsyringe was used to apply 0.5 µL

Table 2. Contact toxicity of plant extracts at a concentration of 10 µg of extract per mg of insect against *Tuta absoluta* 6, 12 and 24 h after topical application

Plants	Mean percentage mortality ^a					
	6 h after topical exposure		12 h after topical exposure		24 h after topical exposure	
	Ethanol extract	Hexane extract	Ethanol extract	Hexane extract	Ethanol extract	Hexane extract
<i>Acmella oleracea</i>	88.3 (±1.5) Ab	100.0 (±0.0) Aa	88.3 (±1.5) Ab	100.0 (±0.0) Aa	88.3 (±1.5) Ab	100.0 (±0.0) Aa
<i>Ageratum conyzoides</i>	26.7 (±1.9) Bb	45.0 (±2.0) Ba	35.0 (±3.1) Bb	48.3 (±2.8) Ca	35.0 (±3.1) Bb	51.7 (±3.1) Ca
<i>Allamanda cathartica</i>	21.7 (±2.8) Ba	18.3 (±2.1) Da	21.7 (±2.8) Ba	23.3 (±1.9) Ea	25.0 (±3.4) Ba	23.3 (±3.3) Ca
<i>Argemone mexicana</i>	25.0 (±3.1) Ba	20.0 (±2.4) Da	25.0 (±3.1) Bb	33.3 (±2.8) Ea	28.3 (±3.1) Bb	41.7 (±3.1) Ca
<i>Artemisia vulgaris</i>	5.0 (±2.0) Da	6.7 (±1.9) Ea	13.3 (±1.9) Ba	8.3 (±1.7) Gb	15.0 (±2.2) Ca	8.3 (±1.7) Eb
<i>Banara guianensis</i>	26.7 (±1.9) Ba	28.3 (±2.8) Ca	36.7 (±3.3) Ba	33.3 (±3.6) Ea	36.7 (±3.3) Ba	41.7 (±4.0) Ca
<i>Banara nitida</i>	16.7 (±3.7) Ca	13.3 (±2.9) Ea	23.3 (±3.3) Ba	18.3 (±1.7) Fa	23.3 (±3.3) Ba	18.3 (±1.7) Da
<i>Bauhinia variegata</i>	6.7 (±3.1) Da	8.3 (±2.8) Ea	11.7 (±1.7) Ba	15.0 (±2.2) Fa	11.7 (±1.7) Ca	15.0 (±2.2) Da
<i>Bougainvillea glabra</i>	25.0 (±3.1) Bb	42.5 (±2.5) Ba	33.3 (±2.1) Bb	42.5 (±2.5) Da	33.3 (±2.1) Bb	51.7 (±3.1) Ca
<i>Calendula officinalis</i>	13.3 (±1.9) Ca	6.7 (±1.9) Eb	13.3 (±1.9) Ba	15.0 (±2.2) Fa	13.3 (±3.3) Ca	15.0 (±2.2) Da
<i>Chenopodium ambrosioides</i>	15.0 (±2.0) Ca	16.7 (±3.3) Da	16.7 (±3.3) Ba	16.7 (±3.3) Fa	16.7 (±3.3) Ca	21.7 (±3.1) Ca
<i>Clavija weberbaueri</i>	25.0 (±2.2) Ba	28.3 (±2.8) Ca	25.0 (±2.2) Bb	36.7 (±3.3) Ea	25.0 (±2.2) Bb	36.7 (±3.3) Ca
<i>Copaifera duckei</i>	36.7 (±3.3) Bb	50.0 (±4.1) Ba	36.7 (±3.3) Bb	63.3 (±3.3) Ba	36.7 (±3.3) Bb	63.3 (±3.3) Ba
<i>Coriandrum sativum</i>	18.3 (±1.7) Cb	41.7 (±2.8) Ba	21.7 (±1.7) Bb	48.3 (±2.8) Ca	21.7 (±1.7) Bb	66.7 (±3.3) Ba
<i>Curatela americana</i>	31.7 (±2.8) Ba	30.0 (±3.3) Ca	41.7 (±3.1) Ba	30.0 (±3.3) Eb	41.7 (±3.1) Bb	48.3 (±1.7) Ca
<i>Eugenia egensis</i>	30.0 (±2.6) Ba	16.7 (±1.9) Db	30.0 (±2.6) Ba	28.3 (±2.8) Ea	30.0 (±2.6) Ba	36.7 (±2.1) Ca
<i>Mayna parvifolia</i>	26.7 (±3.3) Ba	20.0 (±2.4) Da	26.7 (±3.3) Ba	30.0 (±3.6) Ea	26.7 (±3.3) Ba	30.0 (±3.6) Ca
<i>Piper aduncum</i>	33.3 (±2.1) Ba	35.0 (±2.2) Ca	33.3 (±2.1) Ba	35.0 (±2.2) Ea	33.3 (±2.1) Ba	35.0 (±2.2) Ca
<i>Piper augustum</i>	35.0 (±2.2) Ba	18.3 (±1.5) Db	35.0 (±2.2) Ba	25.0 (±2.2) Eb	35.0 (±2.2) Ba	25.0 (±2.2) Cb
<i>Ryania speciosa</i>	15.6 (±3.9) Cb	33.3 (±3.8) Ca	18.3 (±1.7) Bb	33.3 (±3.8) Ea	18.3 (±1.5) Cb	33.3 (±3.8) Ca
<i>Siparuna amazónica</i>	16.7 (±1.9) Ca	20.0 (±2.4) Da	25.0 (±3.1) Ba	28.3 (±3.1) Ea	26.7 (±3.0) Ba	28.3 (±3.1) Ca
<i>Spathodea campanulata</i>	3.3 (±1.9) Db	13.3 (±1.9) Ea	6.7 (±3.3) Cb	26.7 (±1.9) Ea	6.7 (±3.3) Db	26.7 (±1.9) Ca
<i>Tropaolum majus</i>	31.7 (±3.1) Ba	33.3 (±2.1) Ca	31.7 (±3.1) Ba	33.3 (±2.1) Ea	31.7 (±3.1) Ba	33.3 (±2.1) Ca
Control ^b	0.0 (±0.0) Ea	0.0 (±0.0) Fa	0.0 (±0.0) Da	0.0 (±0.0) Fa	0.0 (±0.0) Ea	0.0 (±0.0) Fa

^a Means followed by the same lower-case letter in a row (for comparison between ethanol and hexane extracts) or by the same upper-case letter in a column are not significantly different by the Scott–Knott grouping analysis test at $P > 0.05$.

^b Only acetone was used in the control.

of a solution of the test compound dissolved in acetone. In a control experiment, carried out 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 the appropriate food. *T. absoluta* were fed tomato leaflets (cv. Santa Clara), while *S. saevissima* and *T. angustula* both received a mixture of honey (50%) and pure water (50%). The mixture of honey and water was supplied in plastic containers that were 1.5 cm in diameter and 1.0 cm in height.

The petri dishes were placed in an incubator at 25 ± 0.5 °C and $75 \pm 5\%$ relative humidity with a photoperiod of 12 h. The mortality counts were made after 24 h. Mortality included both dead individuals and those that were no longer moving. Dose–mortality data were subjected to probit analysis using SAS software (PROC PROBIT; SAS) to estimate dose–mortality curves.¹⁷ Curves that had probabilities greater than 0.05 by the χ^2 -test were accepted.¹⁸

2.3.4 Risk assessment to non-target insects

To determine the magnitude of selectivity of the compounds to the beneficial insects, the selectivity ratio was calculated using the formula $S_L R_{50} = LD_{50}$ of the insecticide for the beneficial insect per LD_{50} of the insecticide for *T. absoluta*. Values of 1 and <1 indicate that the chemical is non-selective to the beneficial insect. Values of >1 indicate that the chemical is selective and/or harmless to the

beneficial insect.¹⁹ Using the dose–mortality curves, mortalities caused to beneficial insects by the doses of the compounds that caused 80% mortality in *T. absoluta* were also estimated.

3 RESULTS

3.1 Bioactivity of plant extracts (plant screening)

The hexane extract from aerial parts of *A. oleracea* exhibited the highest activity of all extracts, causing 100.0% ($N = 60$) mortality in *T. absoluta* at a concentration of 10 µg of extract per mg of insect after 6 h of exposure. The mortality caused by the solvents was zero (0.0%) in all of the bioassays (Table 2).

The ethanol extract of *A. oleracea* also showed high activity (88.3% mortality) against *T. absoluta*, and was the second most active extract. The hexane and ethanol extracts of the remainder of the plants tested showed low insecticidal activity towards *T. absoluta* (Table 2).

On the basis of these results, the hexane extract of *A. oleracea* was selected for isolation and structure elucidation of its bioactive compounds.

3.2 Isolation and structural elucidation of compounds from *A. oleracea*

To obtain bioactive compounds, the hexane extract (28 g) was fractionated by a bioactivity-guided fractionation approach, and

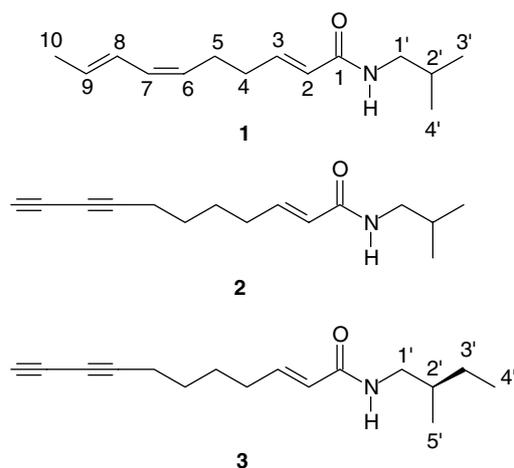


Figure 1. Structure of the three alkamides isolated from *Acmella oleracea*: (2*E*,6*Z*,8*E*)-*N*-isobutyldeca-2,6,8-trienamide (**1**), (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide (**2**) and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide (**3**).

eight fractions (A to H) were obtained. The eight fractional groups were evaluated for their insecticide activity against *T. absoluta* larvae. Fractions F and G eluted with hexane–ethyl acetate (1 : 1) were biologically active, causing 100% mortality 6 h after administration of a dose of 10 $\mu\text{g mg}^{-1}$ body mass. The remainder of the fractions (A, B, C, D, E and H) caused mortalities of less than 40%.

The bioactive fraction F was purified by preparative TLC (hexane–ethyl acetate, 6 : 1) to yield the following three major bands: I (725 mg, RF 0.65), II (56 mg, RF 0.45) and III (21 mg, RF 0.25). Band I was biologically active and was further purified by preparative TLC (hexane–ethyl acetate, 1 : 2) to give compounds **1** and **2** (320 and 210 mg respectively). The bioactive fraction G was purified by preparative TLC (hexane–ethyl acetate, 3 : 1) to yield compound **3** (27 mg).

Compound **1**, (2*E*,6*Z*,8*E*)-*N*-isobutyldeca-2,6,8-trienamide or spilanthol (Fig. 1) was isolated as a colourless oil. The IR spectrum showed the presence of a secondary amide group (3340, 1636 and 1550 cm^{-1}), a double bond conjugated to an amide carbonyl group (1678 cm^{-1}) and a conjugated diene group with *Z*, *E* or *E*, *Z* configuration (987 and 953 cm^{-1}). The MS spectrum had a molecular ion peak at m/z 221, which indicates the molecular formula $\text{C}_{14}\text{H}_{23}\text{NO}$. GC-EIMS 70 eV, m/z (rel. int.): 221 [$\text{M}]^+$ (20), 206 (3), 141 (100), 126 (23), 98 (23), 81 (87), 69 (10), 53 (10). The ^{13}C NMR (CDCl_3) and the ^1H NMR (CDCl_3) spectra showed spilanthic acid. On the amine moiety, the typical signals at δ 3.15 (2H, t, H-1'), 1.78 (1H, m, H-2'), and 0.93 (6H, d, H-3', 4') in ^1H NMR and δ 46.9 (C-1'), 28.6 (C-2') and 20.1 (C-3',4') in ^{13}C NMR indicated the presence of an isobutylamino group. All of the spectral data were in agreement with those of spilanthol (**1**) in the literature.²⁰

Compound **2** was isolated as a colourless crystal. In the ^1H NMR spectrum, characteristic signals at δ 3.18 (dd, H-1'), 1.79 (m, H-2') and 0.91 (d, H-3' and H-4') indicated the isobutylamide moiety. This compound was identified as (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide (Fig. 1) by comparing its ^1H NMR spectral data with published values.²¹ The ^{13}C NMR spectrum (Table 4) was consistent with published data.¹⁰

Compound **3**, (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide (Fig. 1), was isolated as a colourless oil. The IR spectrum presented absorption bands attributable to a triple bond

(2225 cm^{-1}) in addition to a secondary amide group (3299, 1627 and 1554 cm^{-1}) and a double bond conjugated with an amide carbonyl group (1669 cm^{-1}). The ^1H NMR spectrum revealed signals at δ 5.77 (d, $J = 15$ Hz) and 6.80 (dt, $J = 15$ Hz and 7 Hz) which have been attributed to olefinic protons H-2 and H-3 respectively. On the amine moiety, a pair of 1H ddq signals at δ 1.17 and 1.41 are attributed to methylene protons of C-3', and a pair of ddd signals at δ 3.14 and 3.27 are attributed to methylene protons of C-1' owing to the presence of asymmetric carbon at C-2'. The ^{13}C NMR spectrum gave rise to 16 carbon signals. Five carbon signals at δ 45.2, 35.1, 27.1, 11.3 and 17.2 confirmed a 2-methylbutylamine moiety. The ^{13}C NMR and ^1H NMR signals correspond well to the literature.²⁰

3.3 Bioactivity of isolated compounds

The dose–mortality results from insecticide application in larvae of *T. absoluta* showed low χ^2 and high *P*-values (<7.7 and >0.103 respectively), indicating the suitability of the probit model for fitting the dose–response curves and consequently obtaining estimates of the mortality parameters LD_{50} and LD_{80} (Table 3).

Compound **1** (spilanthol) exhibited the highest toxicity to *T. absoluta*, with the lowest LD_{50} . Furthermore, spilanthol (**1**) was approximately 5 times more toxic than permethrin and approximately 321 times more potent than *A. indica* extract (Table 3).

The compounds (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide (**2**) and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide (**3**) showed insecticidal activity similar to that of the commercial insecticide permethrin. In comparison with the extract of *A. indica*, compounds **2** and **3** were respectively about 62 and 52 times more toxic to *T. absoluta* (Table 3).

The *A. oleracea* extract was less toxic than the isolated compounds, but it showed good insecticidal activity. It was 23 times more toxic than the neem extract (Table 3).

3.4 Selectivity of isolated compounds

The dose needed to kill 50% of the test population (LD_{50}) was determined for the beneficial insects (Table 4) and used to calculate selectivity ratios of the insecticides for the two beneficial insects (Table 5). The *A. oleracea* extract and compounds **1**, **2** and **3** were selective to the predator *S. saevissima* and the pollinator *T. angustula* relative to *T. absoluta*, with a selectivity ratio ($S_{\text{LR}_{50}}$) greater than 1.0 (Table 5). For the *A. oleracea* extract, the doses that caused 50% mortality of *T. absoluta* larvae were respectively 36 and 39% lower than the doses that caused the same mortality to *S. saevissima* and *T. angustula*. The estimated mortalities of *S. saevissima* and *T. angustula* by the LD_{80} of this extract to *T. absoluta* were 56 and 55% respectively (Table 5).

For compounds **1**, **2** and **3**, the doses that caused 50% mortality of *T. absoluta* larvae were respectively 38, 39 and 64% lower than the doses that caused the same mortality to *S. saevissima*. Furthermore, the doses were 169, 37 and 35% lower than the doses that caused the same mortality to *T. angustula*. The estimated mortality of *S. saevissima* and *T. angustula* by the LD_{80} of these compounds to *T. absoluta* ranged from 55 to 68%. However, the LD_{50} of permethrin for *T. absoluta* was respectively 15.4 and 2366.7 times higher than the LD_{50} for *S. saevissima* and *T. angustula*. These results indicate that permethrin is harmful to the beneficial insects. The estimated mortality of *S. saevissima* and *T. angustula* by the LD_{80} of this insecticide to *T. absoluta* was 100% (Table 5).

Based on the $S_{\text{LR}_{50}}$, the neem extract was selective to *S. saevissima*. However, the DL_{80} of neem extract to *T. absoluta*

Table 3. Contact toxicity of *Acmella oleracea* hexane extract and of spilanthol (**1**), (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide (**2**) and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide (**3**) extracted from aerial parts of *A. oleracea* against *Tuta absoluta* 24 h after topical application

Treatments	N ^a	LD ₅₀ (µg mg ⁻¹) (95% FL) ^b	LD ₈₀ (µg mg ⁻¹) (95% FL) ^b	Slope ± SE	χ ²	P-value
<i>Acmella oleracea</i> extract	420	1.83 (1.43–2.04)	2.94 (2.50–3.83)	1.00 ± 0.06	4.50	0.480
Compound 1	600	0.13 (0.09–0.16)	0.56 (0.41–0.84)	0.39 ± 0.01	1.69	0.998
Compound 2	420	0.49 (0.39–0.62)	1.34 (1.06–1.79)	0.53 ± 0.03	2.01	0.987
Compound 3	540	0.81 (0.46–1.18)	1.76 (1.21–3.55)	0.51 ± 0.04	1.06	0.998
<i>Azadirachta indica</i> extract ^c	420	41.73 (37.90–46.99)	98.19 (86.78–114.90)	2.21 ± 0.09	6.27	0.370
Permethrin ^c	360	0.71 (0.38–1.08)	2.57 (1.60–4.72)	0.63 ± 0.04	7.70	0.103

^a Number of insects tested.

^b Lethal dose with 95% fiducial limits (FL).

^c Positive control.

Table 4. Contact toxicity of *Acmella oleracea* hexane extract and of spilanthol (**1**), (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide (**2**) and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide (**3**) extracted from aerial parts of *A. oleracea* against *Solenopsis saevissima* and *Tetragonisca angustula* 24 h after topical application

Treatments	N ^a	LD ₅₀ (µg mg ⁻¹) (95% FL) ^b	LD ₈₀ (µg mg ⁻¹) (95% FL) ^b	Slope ± SE	χ ²	P-value
<i>Solenopsis saevissima</i>						
<i>Acmella oleracea</i> extract	420	2.48 (2.14–2.84)	5.47 (4.71–6.54)	0.68 ± 0.03	3.75	0.967
Compound 1	420	0.18 (0.13–0.23)	1.12 (0.73–2.24)	0.44 ± 0.03	6.02	0.450
Compound 2	360	0.67 (0.54–0.82)	2.22 (1.78–2.88)	0.47 ± 0.03	6.37	0.278
Compound 3	420	1.33 (1.06–1.63)	4.40 (3.53–5.70)	0.47 ± 0.03	6.12	0.354
<i>Azadirachta indica</i> extract ^c	360	72.65 (68.47–76.63)	92.50 (87.39–99.10)	2.76 ± 0.25	1.42	0.999
Permethrin ^c	300	0.046 (0.029–0.073)	1.458 (0.682–4.453)	0.28 ± 0.02	4.69	0.450
<i>Tetragonisca angustula</i>						
<i>Acmella oleracea</i> extract	420	2.55 (2.22–2.86)	4.28 (3.82–4.90)	1.08 ± 0.07	3.62	0.608
Compound 1	420	0.35 (0.29–0.42)	0.81 (0.67–1.01)	0.68 ± 0.02	1.62	0.998
Compound 2	360	0.67 (0.48–0.86)	2.02 (1.55–2.91)	0.55 ± 0.05	7.49	0.112
Compound 3	420	1.10 (0.89–1.33)	2.26 (2.16–3.47)	0.58 ± 0.03	1.81	0.994
<i>Azadirachta indica</i> extract ^c	360	29.52 (25.23–34.15)	71.04 (59.62–88.92)	0.68 ± 0.05	6.93	0.399
Permethrin ^c	300	0.0003 (0.0002–0.0005)	0.002 (0.001–0.004)	0.40 ± 0.02	4.95	0.427

^a Number of insects tested.

^b Lethal dose with 95% fiducial limits (FL).

^c Positive control.

caused a mortality of 84 and 98% to *S. saevissima* and *T. angustula* respectively (Table 5).

4 DISCUSSION

The plant species showing the highest insecticidal activity in the present study was the toothache plant *Acmella oleracea*. Furthermore, the activity was higher in the hexane extract than in the ethanol extract. The insecticide activity of *A. oleracea* extracts has been reported for several insect vectors of diseases such as *Aedes aegypti* Linn, *Anopheles stephensi* Liston, *Anopheles culicifacies* Giles and *Culex quinquefasciatus* Say (Diptera: Culicidae).^{10–13} However, there is no thorough study on the effect of this plant on insect pests of agricultural crops.

The bioactivity of *A. oleracea* is due to alkamides present in the plant. The main active amide in the plant is an isobutylamide, (2*E*, 6*Z*, 8*E*)-*N*-isobutyldeca-2,6,8-trienamide, commonly known as spilanthol.^{22,23} These alkamides have a pungent effect and have been studied for various purposes. The flowers and leaves of *A. oleracea* are used in cooking and in popular medicine, mainly as an analgesic for toothache. The spilanthol is known for having several chemical and pharmaceutical applications in addition to

the analgesic for toothache already mentioned. It is used for the treatment of aphtha and herpes, for stomatitis and infections in the throat, in treatment of tuberculosis, as a sialagogue, as a fungistat and fungicide against *Aspergillus* spp., as an antimutagenic agent and as a cicatrisant.^{24–26}

The results also showed that the alkamides evaluated in this study have the potential to control arthropods of agricultural importance. Three alkamides were identified in the bioactive fractions of the hexane extract of *A. oleracea* [spilanthol, (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide]. This study evaluated the effect of *A. oleracea* on *T. absoluta*, an important pest of tomato in the world. The results showed that all of the compounds isolated had high insecticidal activity that was at least as toxic as permethrin, a pyrethroid recommended for control of *T. absoluta*. Furthermore, the compounds were far more toxic than the neem extract. The high efficiency of these compounds, combined with the ready availability from natural sources and the friendlier environmental footprint, makes this plant an excellent candidate as a future natural insecticide.

The results from this study showed that alkamides **1**, **2** and **3** were selective to *S. saevissima* and *T. angustula*. The tolerance of

Table 5. Risk assessment of *Acmella oleracea* hexane extract and of spilanthol (**1**), (*E*)-*N*-isobutylundeca-2-en-8,10-diyamide (**2**) and (*R,E*)-*N*-(2-methylbutyl)undeca-2-en-8,10-diyamide (**3**) extracted from aerial parts of *A. oleracea* on adults of *Solenopsis saevissima* and *Tetragonisca angustula*

Treatment	<i>Solenopsis saevissima</i>			<i>Tetragonisca angustula</i>		
	$S_L R_{50}^a$	Category of insecticide	Mortality (%) ^b	$S_L R_{50}^a$	Category of insecticide	Mortality (%) ^b
<i>Acmella oleracea</i> extract	1.36	Selective	56	1.39	Selective	55
Compound 1	1.38	Selective	68	2.69	Selective	58
Compound 2	1.39	Selective	57	1.37	Selective	62
Compound 3	1.64	Selective	55	1.35	Selective	59
<i>Azadirachta indica</i> extract ^c	1.74	Selective	84	0.70	Non-selective	98
Permethrin ^c	0.00042	Non-selective	100	0.065	Non-selective	100

^a A selectivity ratio at the LD₅₀ ($S_L R_{50}$) of > 1 indicates that the insecticide is selective (more toxic to the pest than to the natural enemy).

^b Mortality estimated by the CL₈₀ (lethal concentration for 80% of populations) of compounds to *Tuta absoluta*.

^c Positive control.

beneficial insects to alkalimides could be related to lower rates of insecticide penetration through the integument, higher rates of insecticide breakdown and relative insensitivity of the target site in natural enemies compared with *T. absoluta*.^{27–31}

The selectivity provided by alkalimides to *S. saevissima* and *T. angustula* suggests that the use of these compounds to control *T. absoluta* presents a low risk to these beneficial insects. Furthermore, the results from this study showed that all compounds had a lower toxicity than permethrin (insecticide already used to control *T. absoluta*) to all non-target species studied. This finding indicates that the alkalimides are less harmful to the beneficial insects. Thus, to preserve the predator and the pollinator investigated in this study, the use of these compounds for pest control can be recommended as a strategy to manage these beneficial insects.

Physiological selectivity is based on the use of insecticides that are more toxic to the target pest than the natural enemies and should always be considered when controlling pests. Furthermore, the principles of ecological selectivity should also be considered.^{32,33} The ecological selectivity is related to the different methods of applying insecticides as a means to minimise exposure of natural enemies to the insecticide.³² It is of utmost importance to use selective insecticides to preserve the beneficial species in the ecosystem, and it is necessary to resort to strategies that will enable the achievement of ecological selectivity even if the use of selective insecticides is not possible. With ecological selectivity, an insecticide can be applied with a methodology designed to make it selective. The low stability of botanical pesticides and consequent rapid degradation in the environment are characteristics that favour ecological selectivity, because they reduce the exposure time of beneficial organisms to toxic compounds.

The mechanism of action of active alkalimides found in *A. oleracea* has not yet been determined. It appears to affect the nervous system, as evident from abnormal movement such as uncoordinated muscular activity. This effect suggests that the compounds disturb nerve conduction somewhere. The analgesic activity of spilanthol in humans has been attributed to an increased GABA release in the temporal cerebral cortex,³⁴ while other bioactive alkylamides are acting on voltage-gated sodium channels.³⁵ The mortality after short exposure to the compounds indicates that alkalimides greatly disturb the ongoing processes of histolysis of larval tissues. Saraf and Dixit¹² observed rapid mortality of pupae of *A. culicifacies*, *C. quinquefasciatus* and *A. aegypti*

when exposed to spilanthol. These results suggest that spilanthol interferes in histolysis and histogenesis processes. Further research is needed to address this question.

Overall, the results of this research indicate that the *A. oleracea* extract is the most promising among the plant extracts studied. The active alkalimides from *A. oleracea* can be a potential alternative for controlling *T. absoluta* and should be studied further for other agricultural pests. All compounds presented high insecticidal activity for the insect pest *T. absoluta* and selectivity for beneficial insects *S. saevissima* and *T. angustula*. Given the vital need for environmentally friendly chemicals that represent new insecticide groups with novel mechanisms of action, low persistence in the field and low toxicity to mammals and non-target species, the feasibility and impacts of using natural chemicals in pest management programmes require further attention. It must be remembered, however, that the biological activity of a chemical is a function of its structure rather than its origin. The biological properties of a chemical depend on its structure and the way in which the chemical is used. Bioactive alkalimides from *A. oleracea* have been found to be harmless to the majority of vertebrates and lethal to invertebrates.^{36,37} Because *A. oleracea* is widely used as both food and folk medicine in its region of origin, it is assumed that its toxicity to humans is extremely low. However, the actual risks of using these natural products should be identified. Therefore, to assess the feasibility and impacts of using *A. oleracea* alkalimides in agriculture, more research on the effects on humans and the environment should be performed.

5 CONCLUSION

The hexane extract of *A. oleracea* showed high insecticidal activity and can be used to control *T. absoluta* in organic or conventional crops. Quantification of LD₅₀ values of isolated alkalimides of *A. oleracea* against *T. absoluta* showed that alkalimides could serve as potent insecticides for *T. absoluta* control programmes. The spilanthol was the main alkalimide active isolated. This alkalimide is the most promising, as it had the highest insecticidal activity and was selective to non-target organisms. Therefore, spilanthol and the other alkalimides isolated are potential pest management tools likely to have their insecticide activity improved through organic synthesis guided by studies of quantitative structure–activity relationships.

ACKNOWLEDGEMENT

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq/Brazil for financial support (doctoral fellowship).

REFERENCES

- Suinaga FA, Picanço M, Jham GN and Brommonschenkel SH, Causas químicas de resistência de *Lycopersicon peruvianum* (L.) a *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *An Soc Entomol Brasil* **28**:313–321 (1999).
- Souza JC, Reis PR and Salgado LO, Traça-do-tomateiro: histórico, reconhecimento, biologia, prejuízos e controle. Belo Horizonte, Empresa de Pesquisa Agropecuária de Minas Gerais, 15 pp. (1983).
- Seplyarsky V, Weiss M and Haberman A, *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), a new invasive species in Israel. *Phytoparasitica* **38**:445–446 (2010).
- Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA, *et al*, Biological invasion of european tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *J Pest Sci* **83**:197–215 (2010).
- Kılıç T, First record of *Tuta absoluta* in Turkey. *Phytoparasitica* **38**:243–244 (2010).
- Lietti MMM, Botto E and Alzogaray RA, Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop Entomol* **34**:113–119 (2005).
- Belmain SR, Neal GE, Ray DE and Golop P, Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food Chem Toxicol* **39**:287–291 (2001).
- Balick MJ, Elisabetsky E and Laird SA, *Medicinal Resources of the Tropical Forest: Biodiversity and its Importance to Human Health*. Columbia University Press, New York, NY, 440 pp. (1995).
- Sharma G, Gupta V, Sharma S, Shrivastava B and Bairva R, Toothache plant *Spilanthes Acmella* Murr.: a review. *J Natura Conscientia* **1**:135–142 (2010).
- Ramsewak RS, Erickson AJ and Nair MG, Bioactive *N*-isobutylamides from the flower buds of *Spilanthes acmella*. *Phytochemistry* **51**:729–732 (1999).
- Pandey V, Agrawal V, Raghavendra K and Dash AP, Strong larvicidal activity of three species of *Spilanthes* against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* Say). *J Parasitol Res* **102**:171–174 (2007).
- Saraf DK and Dixit VK, *Spilanthes acmella* Murr: study on its extract spilanthol as larvicidal compound. *Asian J Exp Sci* **16**:9–19 (2002).
- Amer A and Mehlhorn H, Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol Res* **99**:466–472 (2006).
- Cornell HV and Hawkins BA, Survival patterns and mortality sources of herbivorous insects: some demographic trends. *Amer Nat* **145**:563–593 (1995).
- Malagodi-Braga KS and Kleinert AMP, Could *Tetragonisca angustula* Latreille (Apinae, Meliponini) be used as strawberry pollinator in greenhouses? *Aust J Agric Res* **55**:771–773 (2004).
- Way MJ and Khoo KC, Role of ants in pest management. *Annu Rev Entomol* **37**:479–503 (1992).
- SAS/STAT User's Guide, Version 9.1. SAS Institute, Cary, NC (2002).
- Young LJ and Young JH, *Statistical Ecology: a Population Perspective*. Kluwer Academic, Boston, MA, 584 pp. (1998).
- Bacci L, Picanço MC, Rosado JF, Silva GA, Crespo ALB, Pereira EJJ, *et al*, Conservation of natural enemies in brassica crops: comparative selectivity of insecticides in the management of *Brevicoryne brassicae* (Hemiptera: Sternorrhyncha: Aphididae). *Appl Entomol Zool* **44**:103–113 (2009).
- Nakatani N and Nagashima M, Pungent alkaloids from *Spilanthes acmella* L. var. *oleracea* Clarke. *Biosci Biotech Biochem* **56**:759–762 (1992).
- Bauer R, Remiger P and Wagner H, Alkaloids from the roots of *Echinacea angustifolia*. *Phytochemistry* **28**:505–508 (1989).
- Ley JP, Blings M, Krammer G, Reinders G, Schmidt CO and Bertram HJ, Isolation and synthesis of acmellonate, a new unsaturated long chain 2-ketol ester from *Spilanthes acmella*. *Nat Prod Res* **20**:798–804 (2006).
- Wu LC, Fan NC, Lin MH, Chu IR, Huang SJ, Hu CY, *et al*, Anti-inflammatory effect of spilanthol from *Spilanthes acmella* on murine macrophage by down-regulating LPS-induced inflammatory mediators. *J Agric Food Chem* **56**:2341–2349 (2008).
- Haw AB and Keng CL, Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant, through proliferation of multiple shoots. *J Appl Hortic* **5**:65–68 (2003).
- Coutinho LN, Aparecido CC and Figueiredo MB, Galls and deformation on jambu (*Spilanthes oleracea*) caused by *Tecaphora spilanthos* (Ustilaginales). *Summa Phytopathol* **32**:283–285 (2006).
- Singh SK, Rai MK, Asthana P, Pandey S, Jaiswal VS and Jaiswal U, Plant regeneration from alginate-encapsulated shoot tips of *Spilanthes acmella* (L.) Murr., a medicinally important and herbal pesticidal plant species. *Acta Physiol Plant* **31**:649–653 (2009).
- Guedes RNC, Resistência de insetos a inseticidas, in *Manejo Integrado de Doenças e Pragas*, ed. by Zambolin L. Editora UFV, Viçosa, MG, Brazil, pp. 101–107 (1999).
- Yu SJ, Selectivity of insecticides to the spined bug (Heteroptera: Pentatomidae) and its lepidopterous prey. *J Econ Entomol* **81**:119–122 (1988).
- Hackman RH, Chemistry of the cuticle, in *The Physiology of Insecta*, ed. by Rockstein M. Academic Press, New York, NY, pp. 216–270 (1974).
- Hollingworth RM, The biochemical and physiological basis of selective toxicity, in *Insecticide Biochemistry and Physiology*, ed. by Wilkinson CF. Plenum Press, New York, NY, pp. 431–506 (1976).
- Leite GLD, Picanço M, Guedes RNC and Gusmão MR, Selectivity of insecticides with and without mineral oil to *Brachygastra lecheguana* (Hymenoptera: Vespidae), a predator of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Ceiba* **39**:191–194 (1998).
- Ripper WE, Greenslade RM and Hartley GS, Selective insecticides and biological control. *J Econ Entomol* **44**:448–459 (1951).
- O'Brien RD, *Toxic Phosphorus Esters*. Academic Press, New York, NY, 434 pp. (1960).
- Rios MY, Aguilar-Guadarrama AB and Gutierrez MD, Analgesic activity of affinin, an alkaloid from *Heliopsis longipes* (Compositae). *J Ethnopharmacol* **110**:364–367 (2007).
- Gertsch J, Immunomodulatory lipids in plants: plant fatty acid amides and the human endocannabinoid system. *Planta Med* **74**:638–650 (2008).
- Pandey V and Agrawal V, Efficient micropropagation protocol in *Spilanthes acmella* L. possessing strong antimalarial activity. *In Vitro Cell Dev Biol Plant* **45**:491–499 (2009).
- Watt PM and Brayer-Brandwijk MC, *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. E&S Livingstone, Edinburgh, UK, 1457 pp. (1962).