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ORIGINAL ARTICLE

Insecticidal activity of lemongrass essential oil and its major compounds on velvet caterpillar

Camila Bonatto Vicenço[1], Wendel Silvestre[2*], Gabriel Fernandes Pauletti[1,2]

Abstract - The present work aimed to evaluate the insecticidal activity of *Cymbopogon citratus* essential oil and its major compounds (citral and myrcene) on *Anticarsia gemmatalis*. The essential oil, citral, myrcene, and a mixture of citral and myrcene were tested at the concentrations of 0.1, 0.3, 0.5, 0.7, and 0.9 % v/v, plus two negative controls (distilled water and Tween-80® 0.5 % v/v) and a positive control (novaluron 0.075 % w/v). Insect mortality was evaluated in 24, 48, 72, and 96 h. According to the results, *C. citratus* essential oil and the citral-myrcene mixture at 0.9 % v/v were effective in the control of *A. gemmatalis*, with 96 % and 88 % mortality, respectively, in the first 24 h. At this concentration, citral caused 100 % mortality after 72 h, whereas myrcene had no effect on the caterpillars even after 96 h of exposure. Thus, *C. citratus* essential oil can be a potential option for the alternative control of *A. gemmatalis*.

Keywords: Citral. *Cymbopogon citratus*. *Anticarsia gemmatalis*. Myrcene. Synergism. Terpenes.

Atividade inseticida do óleo essencial de capim limão e seus compostos majoritários sobre a lagarta-da-soja

Resumo - O presente trabalho teve como objetivo avaliar a atividade inseticida do óleo essencial de *Cymbopogon citratus* e de seus compostos majoritários (citral e mirceno) sobre *Anticarsia gemmatalis*. O óleo essencial, citral, mirceno e uma mistura de citral e mirceno foram testados nas concentrações de 0,1, 0,3, 0,5, 0,7 e 0,9 % v/v, mais dois controles negativos (água destilada e Tween-80® 0,5 % v/v) e um controle positivo (novaluron 0,075 % m/v). A mortalidade dos insetos foi avaliada em 24, 48, 72 e 96 h. De acordo com os resultados, o óleo essencial de *C. citratus* e a mistura de citral-mirceno a 0,9 % v/v foram eficazes no controle de *A. gemmatalis*, com mortalidade de 96 % e 88 %, respectivamente, nas primeiras 24 h. Nesta concentração, o citral causou 100 % de mortalidade após 72 h, enquanto o mirceno não teve efeito nas lagartas mesmo após 96 h de exposição. Assim, o óleo essencial de *C. citratus* pode ser uma opção potencial para o controle alternativo de *A. gemmatalis*.

Palavras-chave: Citral. *Cymbopogon citratus*. *Anticarsia gemmatalis*. Mirceno. Sinergismo. Terpenos.

[1] Laboratory of Pest Management, University of Caxias do Sul, Brazil.
[2] Laboratory of Studies of the Soil, Plant, and Atmosphere System and Plant Metabolism, University of Caxias do Sul,
[*] Corresponding author: wpsilvestre@ucs.br.

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Introduction

Anticarsia gemmatalis Hübner, 1818 (Lepidoptera: Noctuidae), commonly known as 'velvet caterpillar', is one of the main defoliator pests that attack soybean (*Glycine max*), capable of causing extensive economic damage to the crop. The reduction in plant productivity is caused by the reduction of the photosynthetically active leaf area due to leaf consumption by the caterpillars. Under high infestation loads, the plants can be completely defoliated, causing their death (PRAÇA; MORAES; MONNERAT, 2006; VICENÇO *et al.*, 2021).

This pest insect in generally controlled using synthetic chemical pesticides, especially from the classes of benzoylureas, pyrethroids, organophosphates, and diamides (BRASIL, 2021). However, the indiscriminate and recurrent use of these substances ends up fostering the selection of resistant individuals, reducing pesticide efficiency, besides the deleterious effects to non-target organisms, as animals and humans and the contamination of soil and groundwaters (DENT; BINKS, 2020).

Nowadays, there is an increasing effort to reduce the use of synthetic pesticides, whether through changes in crop management, use of biological and alternative control, or the development of new plant varieties that are resistant to pests (ZHU *et al.*, 2016; SUDO *et al.*, 2017). Among the possible approaches, there is the use of biomolecules as pesticides or natural repellents. Regarding these biomolecules, terpenes stand out as antimicrobial and antioxidant agents, being secondary plant metabolites produced as protection and defense against pests and herbivores (WAR *et al.*, 2012; GAJGER; DAR, 2021). Essential oils, complex terpene mixtures produced and stored by plants, constitute a possible control option since terpenes in general have a low toxicity to vertebrates and have minimum environmental persistence, volatilizing quickly, besides the susceptibility to oxidation by solar UV radiation and atmospheric oxygen (SHAH *et al.*, 2019; STOLERU; BREBU, 2021).

Several essential oils are composed of terpenes that have some kind of biological activity. These compounds may interfere in metabolism, both isolated and mixed (synergistic effect), hampering physiological and behavioral insect processes (VIEGAS JÚNIOR, 2003; MENEZES, 2005), such as growth inhibition, feeding, or oviposition (PAVELA; BENELLI, 2016).

Synergistic effect corresponds to a potentiation of the activity of two or more substance relative to their activities when isolated. Synergism occurs when the effect of the substances together is greater than the summation of the isolated effects (TOSI; NIEH, 2019). Taking into account that essential oils are a mixture of several terpenes, it must be considered that most of the biological activity of an essential oil may result of synergistic or additive effects among the terpenes that compose the mixture (SCALERANDI *et al.*, 2018).

Cymbopogon citratus (DC.) Stapf, commonly known as 'lemongrass', is an aromatic plant belonging to Poaceae family. Both *C. citratus* leaves and its essential oil are used in traditional medicine, cooking, aromatherapy, and agriculture (SHAH *et al.*, 2011; OLORUNNISOLA *et al.*, 2014). According to United States Evironmental Protection Agency (EPA) and World Health Organization (WHO), *C. citratus* is classified as a safe species for human consumption (EKPENYONG; AKPAN; NYOH, 2015).

Relative to the potential uses of *C. citratus* essential oil as an alternative pesticide, literature studies cited the antifungal (SESSOU *et al.*, 2012; ITANKAR; TAUQEER; DALAL, 2019), insecticidal and repellent

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properties of this essential oil against insects from different orders, such as *Agrotis ipsilon* (Lepidoptera: Noctuidae), *Ulomoides dermestoides* Fairmaire (Coleoptera: Tenebrionidae), and *Musca domestica* (Diptera: Muscidae) (PINTO *et al.*, 2015; PLATA-RUEDA *et al.*, 2020; MOUSTAFA *et al.*, 2021).

The objective of the present work was to evaluate the insecticidal activity of different concentrations of *C. citratus* leaf essential oil and the identified major compounds (citral and myrcene), isolated and combined, on *Anticarsia gemmatalis*.

Material and Methods

Breeding of *A. gemmatalis* individuals

A. gemmatalis caterpillars were bred under controlled conditions, being fed with an artificial diet formulated in accordance with Greene, Leppla, and Dickerson (1976). The insects were kept under controlled environmental conditions (25 ± 2 °C, 75 ± 5 % relative humidity, and photoperiod of 14 h of light and 10 h of dark). Third-instar caterpillars were used in the bioassays; instar identification was carried out following the procedures of Vicenço *et al.* (2021).

Obtainment of essential oil and major compounds

Cymbopogon citratus plants were grown at the Experimental Site and Farm School of UCS (geographical coordinates: 29°08'28.9"S, 50°59'29.3"W, and an altitude of 806 m above sea level), being harvested in December 2020. After harvest, the samples underwent visual inspection, being excluded inorganic contaminants and decaying plant material. The selected material was dried in a kiln with forced air circulation for 96 h at room temperature (20 – 25 °C).

The dried plant material underwent essential oil extraction by steam distillation for 2 h, following the procedures of ANVISA (2010). The volume of obtained essential oil was measured using a 25 mL glass graduated cylinder, the yield was calculated using the method described by Pauletti *et al.* (2020). The obtained essential oil was stored in an amber glass flask and kept under refrigeration (4 ±2 °C) in a cold chamber until the bioassays; a small oil sample (0.5 mL) was sent to chromatographic analysis.

The synthetic major compounds myrcene (CAS 123-35-3, 98.1 % purity) and citral (CAS 5392-40-5, 96.4 % purity) were purchased from Sigma-Aldrich (EUA) and stored under refrigeration (4 ± 2 °C) until the bioassays.

Chromatographic analysis

The samples were analyzed by GC/MS (qualitative analysis) and GC-FID (quantitative analysis), based on the procedures described by Silvestre *et al.* (2019). The GC-FID analyses were carried out using a Hewlett Packard 6890 gas chromatograph, equipped with an HP-Chemstation software and an HP-Innowax fused silica column (30 m x 320 µm i d., 0.50 µm film thickness). Temperature programming was 40 °C for 8 min, 40 to 180 °C at 3 °C·min^{-1}, 180 to 230 °C at 20 °C·min^{-1}, 230 °C for 10 min; injector temperature of 250 °C; split ratio 1:50; flame ionization detector at 250 °C; hydrogen as carrier gas at 34 kPa; injected sample volume of

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1.0 µL, diluted in hexane (1:10).

The GC/MS analyses were carried out using an HP-6980 gas chromatograph coupled to a HP-MSD5973 selective mass detector, equipped with HP Chemstation and Wiley 275 spectra library. It was used an HP-Innowax fused silica column (30 m x 320 µm i. d., 0.50 µm film thickness). Temperature program was the same of GC-FID analysis; interface at 280 °C; split ratio 1:100; helium as carrier gas at 56 kPa and flow rate of 1.0 $mL \cdot min^{-1}$; ionization energy of 70 eV; injected volume of 1.0 µL, diluted in hexane (1:10).

The essential oil components were identified by comparison of the obtained mass spectra with the Wiley library (GC/EM) and by comparison of the linear retention indexes (LRI) with literature data (NIST). The LRI values were calculated with the Van den Dool and Kratz equation using a standard solution of alkanes (C8-C30). Quantification was carried out using 1-octanol as internal standard, injecting 25 µL of a solution 30.22 $g \cdot L^{-1}$ 1-octanol in hexane (755 µg of 1-octanol injected in each analysis). The determination of the contents of each compound was carried out using calibration curves for each chemical class and the peak area of each component, as proposed by Rebelo *et al.* (2022).

Bioassays

The tests were carried out based on the procedures described by Vicenço *et al.* (2021). The essential oil and major compounds were added to the artificial diet, at different concentrations, plus the negative and positive controls. Five concentrations were tested: 0.1 %, 0.3 %, 0.5 %, 0.7 %, and 0.9 % v/v, based on the study of Hahn *et al.* (2018). The concentrations of the major compounds (citral and myrcene) were the same as the essential oil, being adjusted according to the content in the essential oil and the purity of the synthetic compound, following equation 1.

$$C = D \times \frac{T}{P} \qquad (1)$$

Where 'C' is the adjusted concentration (% v/v), 'D' is the essential oil concentration (% v/v), 'T' is the compound content in the essential oil (%), and 'P' is the purity of the synthetic compound (%).

All treatments (essential oil and major compounds) were emulsified with Tween-80® (0.5 % v/v). Novaluron (0.075 % w/v), active ingredient indicated for the control of *A. gemmatalis* in the juvenile phase, was used as a positive control. Distilled water and Tween-80® 0.5 % v/v were the negative controls.

The insects were kept under controlled environmental conditions (25 ± 2 °C, 75 ± 5 % relative humidity, and photoperiod of 14 h of light and 10 h of dark) in the breeding room, in 100-mL plastic cups with cap, with one caterpillar per cup. The provided artificial diet was not replaced during the experiment. Insect mortality was evaluated daily for four days, corresponding to 24 h, 48 h, 72 h, and 96 h.

Experimental design and statistical analysis

The bioassays followed a bifactorial randomized design. The concentration and kind of substance (essential oil or major compounds) were the two factors evaluated. Fifty individuals were used in each treatment,

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grouped in five replicates of ten individuals each. Data normality was assessed using Kolmogorov-Smirnov test. The parametric data underwent analysis of variance (ANOVA), followed by Fisher's Least Significant Difference (LSD) post hoc test at 5 % error probability ($p = 0.05$), using the Statistical Package for the Social Sciences 17.0 (SPSS) software.

Results and Discussion

Chemical profile of the essential oil

The essential oil yield of the dried *C. citratus* leaves was 0.93 % v/w (volume by weight). The chromatographic analyzes identified sixteen compounds; the detailed chemical profile of the essential oil is presented in Table 1.

Table 1. Results of the chromatographic analysis (CG-DIC and CG/MS) of *Cymbopogon citratus* essential oil, obtained by steam distillation for 2 h.

Compound	Calc. IRL	Lit. IRL	Content (wt.%)
myrcene	1169	1167	40.33
γ-terpinene	1241	1240	0.86
o-cymene	1257	1254	0.50
sulcatone	1344	1347	1.46
fenchone	1409	1410	0.17
α-thujone	1422	1422	0.22
menthone	1473	1474	0.18
camphor	1528	1531	0.18
linalool	1555	1551	0.88
bornyl acetate	1593	1590	0.44
β-caryophyllene	1611	1610	0.47
neral[1] (citral B)	1696	1694	20.54
geranial[1] (citral A)	1728	1725	31.42
geranyl acetate	1766	1765	0.20
nerol	1815	1817	0.22
geraniol	1856	1856	1.34
Monoterpenes			41.69
Oxygenated monoterpenes			55.15
Sesquiterpenes			0.47
Others			2.10
Total identified			99.42
Not identified			0.58

Calc. IRL: calculated linear retention index; Lit. IRL: linear retention index according to the literature (NIST). [1] – isomers of citral, were jointly considered as 'citral' throughout this work.

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According to Table 1, the identified major compounds in the essential oil were the *cis* (neral) and *trans* (geranial) isomers of citral, with 20.5 wt. % and 31.4 wt. %, respectively, corresponding to 51.9 wt. % citral, followed by myrcene, with 40.3 wt. %. Relative to the chemical classes, the essential oil was mainly composed of hydrocarbon and oxygenated monoterpenes, with small amounts of sesquiterpenes.

The GC-FID chromatogram of *C. citratus* essential oil with peak identification is presented in Figure 1.

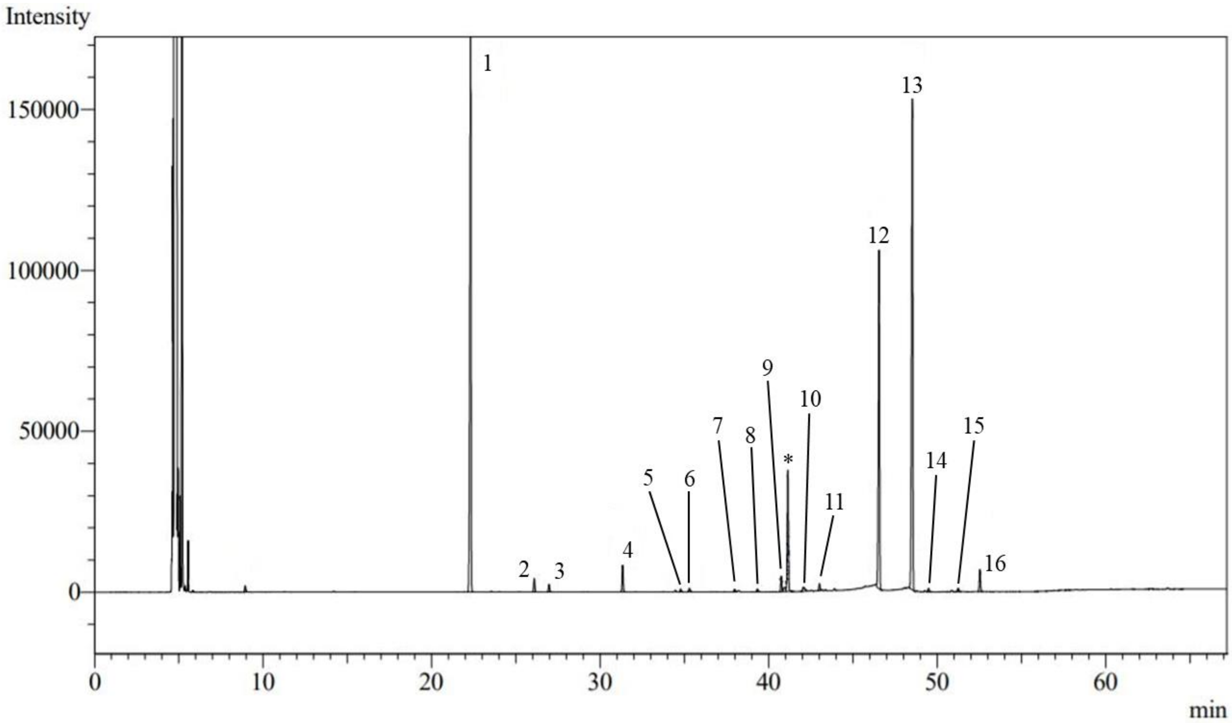

Figure 1. CG-FID chromatogram of *Cymbopogon citratus* essential oil obtained by steam distillation for 2 h. 1 – myrcene; 2 – γ-terpinene; 3 – o-cymene; 4 – sulcatone; 5 – fenchone; 6 – thujone; 7 – menthone; 8 – camphor; 9 – linalool; 10 – bornyl acetate; 11 – β-caryophyllene; 12 – neral (*cis* isomer of citral); 13 – geranial (*trans* isomer of citral); 14 – geranyl acetate; 15 – nerol; 16 – geraniol; * – internal standard (1-octanol).

Kimutai *et al.* (2017) reported a chemical profile similar to the one observed in this work, with citral as the major compound (30.2 wt. %), followed by myrcene (14.2 wt. %) for *C. citratus* essential oil from Kenia, whereas Fogné *et al.* (2017) reported a citral content of 83.9 wt. %, with 35.8 wt. % of neral and 48.1 wt. % of geranial. The citral chemotype is regarded as characteristic of *C. citratus* essential oil, with variations in the secondary major compounds (AVOSEH *et al.*, 2015).

Bioassays

The mortality percentages of *A. gemmatalis* individuals exposed to the essential oil and major compounds by ingestion during the experiment are compiled in Table 2.

It is important to observe that, in the first 24 h of exposure at the concentration of 0.9 % v/v, the essential oil of *C. citratus* caused 96 % caterpillar mortality, followed by the mixture of citral and myrcene with 88 %, whereas citral alone caused 68 % mortality and myrcene alone had no effect (zero mortality). It could be seen

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that, at the concentration of 0.7 % v/v, the mixture of citral and myrcene had a higher mortality (88 %), followed by the essential oil (68 %), and citral (60 %). Since essential oils are complex mixtures of several terpenes, the minor compounds may have had an effect on the digestive sorption of the major compounds (citral and myrcene), or even had delayed the start of the toxic effects, a phenomenon that has not occurred at a higher essential oil concentration (Shahriari *et al.*, 2020; Asadi *et al.*, 2021).

Table 2. Mortality of *Anticarsia gemmatalis* caterpillars under exposure by ingestion to increasing concentrations of *Cymbopogon citratus* essential oil, citral, and myrcene added to artificial diet.

Treatment	Concentration	Mortality (%)			
		24 h	48 h	72 h	96 h
C. citratus essential oil	0.1 % v/v	0 ± 0 Ae	0 ± 0 Ae	2 ± 1 Be	2 ± 0 Be
	0.3 % v/v	28 ± 4 Ad	28 ± 4 Ad	32 ± 4 Ad	32 ± 4 Ad
	0.5 % v/v	32 ± 4 Bc	60 ± 3 Bc	62 ± 2 Cc	62 ± 4 Cc
	0.7 % v/v	68 ± 4 Bb	86 ± 5 Bb	86 ± 5 Bb	86 ± 5 Bb
	0.9 % v/v	96 ± 5 Aa	100 ± 0 Aa	100 ± 0 Aa	100 ± 0 Aa
citral	0.1 % v/v	0 ± 0 Ae	0 ± 0 Ae	0 ± 0 Be	0 ± 0 Be
	0.3 % v/v	10 ± 1 Bd	26 ± 4 Ad	28 ± 4 Bd	28 ± 0 Bd
	0.5 % v/v	50 ± 3 Ac	78 ± 4 Ac	80 ± 5 Bc	80 ± 0 Bc
	0.7 % v/v	60 ± 2 Cb	82 ± 5 Cb	86 ± 4 Bb	86 ± 1 Bb
	0.9 % v/v	68 ± 5 Ca	96 ± 4 Ba	100 ± 0 Aa	100 ± 0 Aa
myrcene	0.1 % v/v	2 ± 0 Ae	2 ± 0 Ae	2 ± 0 Be	2 ± 0 Be
	0.3 % v/v	2 ± 0 Ce	2 ± 0 Ce	2 ± 0 De	2 ± 0 De
	0.5 % v/v	0 ± 0 Ce	0 ± 0 De	0 ± 0 De	0 ± 0 De
	0.7 % v/v	0 ± 0 De	2 ± 0 De	2 ± 0 Ce	2 ± 0 Ce
	0.9 % v/v	0 ± 0 De	0 ± 0 Ce	0 ± 0 Be	0 ± 0 Be
citral + myrcene	0.1 % v/v	0 ± 0 Ae	2 ± 0 Ae	14 ± 3 Ae	14 ± 0 Ae
	0.3 % v/v	8 ± 4 Bc	12 ± 4 Bd	18 ± 2 Cd	18 ± 0 Cd
	0.5 % v/v	48 ± 4 Ab	76 ± 4 Ac	86 ± 4 Ac	86 ± 1 Ac
	0.7 % v/v	88 ± 5 Aa	94 ± 5 Ab	96 ± 4 Ab	96 ± 0 Ab
	0.9 % v/v	88 ± 5 Ba	100 ± 0 Aa	100 ± 0 Aa	100 ± 0 Aa
Distilled water		0 ± 0 e	0 ± 0 e	0 ± 0 e	0 ± 0 e
Tween-80® 0.5 % v/v		0 ± 0 e	0 ± 0 e	0 ± 0 e	0 ± 0 e
Novaluron 0.075 % w/v		0 ± 0 e	0 ± 0 e	100 ± 0 a	100 ± 0 a
p-value for treatments		< 0.001	< 0.001	< 0.001	< 0.001
p-value for concentrations		< 0.001	< 0.001	< 0.001	< 0.001
p-value for interaction effect		< 0.001	< 0.001	< 0.001	< 0.001
Coefficient of variation (%)		13.2	11.8	7.7	7.7

Means followed by the same letter, lowercase within the treatment, and uppercase within the concentration, do not differ statistically by Fisher's LSD test at 5 % probability error (p = 0.05).

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Thus, at higher concentrations (0.7 % and 0.9 % v/v), both the essential oil and the mixture of citral and myrcene were effective in controlling *A. gemmatalis* within 24 h. A similar behavior was observed by Silvestre *et al.* (2021) when evaluating the insecticidal activity of *Callistemon speciosus* essential oil (major compound 1,8-cineole) on this pest insect. Plata-Rueda *et al.* (2020, 2021) reported a quick action of citral (neral and geranial) and their derivatives (neryl/geranyl acetate) on *Ulomoides dermestoides* and *A. gemmatalis*. It is also important to observe that the essential oil caused a higher mortality at 0.9 % v/v, which is desirable considering the possibility of using this material without the need of further purification, unlike the use of citral an myrcene, which would require prior purification or being chemically synthesized and purified before use.

After 48 h of exposure, there was an increase in the mortality of *A. gemmatalis* individuals exposed to the essential oil, citral, and the mixture of citral and myrcene at the concentration of 0.5 % v/v, suggesting a slower poisoning probably due to the smaller amounts of terpenes in the artificial diet comparatively to the concentrations of 0.7 % and 0.9 % v/v, which caused toxic effects in the first 24 h. Myrcene caused no significant caterpillar mortality within 48 h, regardless of the concentration, indicating that this monoterpene probably had no toxic effect on this species (BEDINI *et al*., 2015).

After 72 h, there were no significant changes in insect mortality for the concentrations of 0.7 % and 0.9 % v/v; there was a small increase in mortality for the concentrations of 0.3 % and 0.5 % v/v. It is also noteworthy to comment that novaluron (positive control) acted between 48 h and 72 h, killing all individuals exposed to it.

As commented by Silvestre *et al.* (2021), the toxic mechanism of novaluron occurs through inhibition of chitin synthesis. This active compound must be absorbed and transported to the target tissues for its toxic effect to occur, a slower process than the one observed for terpenes, which is believed to act by a neurotoxic mechanism, which is faster (ENAN, 2001; REGNAULT-ROGER, 2013). In this sense, the essential oil had a faster action, causing complete (100 %) mortality in the first 48 h, a desirable effect, especially for use in situations in which a quick control is necessary.

Vicenço *et al.* (2020), studying the insecticidal effect of the essential oil of two species of the *Schinus* genus on *A. gemmatalis*, observed a similar behavior for *S. molle* essential oil, which had a high monoterpene content (approx. 85 wt. %), attributing the lower efficiency of this oil due to the faster volatilization of the monoterpenes compared to sesquiterpenes.

There was no mortality increase between 72 h and 96 h, so that the concentrations below 0.5 % v/v of all treatments caused mortality percentages below 33 %. As observed, higher concentrations (above 0.5 % v/v) of both the essential oil and the mixture of citral and myrcene were effective in the control of the caterpillars, with mortality above 85 % in the first 24 h.

This suggests that citral and myrcene can act in a synergistic way when applied in the same proportion as the essential oil since citral, when applied alone, caused a lower mortality than both the essential oil and the mixture of citral and myrcene, and myrcene was completely ineffective when applied alone. It is also important to consider a possible contribution of the minor compounds in the potentiation of essential oil toxicity, even if these substances were present in quite smaller amounts compared to the major compounds (GARCÍA-DÍEZ *et*

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al., 2017; SCALERANDI *et al.*, 2018). Andrade-Ochoa *et al.* (2018), assessing the relationships of biological activity of several terpenes alone and in binary mixtures, reported that binary mixtures of myrcene with *trans*-anethole, limonene, carvacrol, and thymol have had a synergistic insecticidal effect against pupae and larvae of *Culex quinquefasciatus* (Diptera, Culicidae), Khorram *et al.* (2011) observed that binary mixtures of myrcene with α-pinene, β-pinene, camphene, and γ-terpinene had an additive effect since there was no increase of the insecticidal activity relative to the activity of the individual compounds.

As observed by Regnault-Roger (2013), Jankowska *et al.* (2018), and Silvestre *et al.* (2021), terpenes and essential oils act through a neurotoxic effect. This effect has two main mechanisms, the first is by disrupting neuron membranes (and not only nerve cell membranes), causing ionic imbalances and malfunctioning of ion channels and other membrane proteins. The second mechanism is the interaction of the terpenes with enzymes that metabolize neurotransmitters and receptors. This further hinders the functioning of insect nervous system, causing the death of the individuals.

The low mortality observed for myrcene alone is probably the result of a low toxicity of this terpene on *A. gemmatalis*, considering that toxic, repellent, and attractive effects are also influenced by insect species. As observed by Sun *et al.* (2020), myrcene had a repellent effect on *Liposcelis bostrychophila* (Psocodea, Liposcelididae) whereas it had an attractive effect on *Tribolium castaneum* (Coleoptera, Tenebrionidae). Bedini *et al.* (2015) also reported repellent activity of myrcene on *A. gemmatalis* when applied alone, regardless of the concentration used.

Citral is already acknowledged in the literature as having insecticidal effect against several insect species (AVOSEH *et al.*, 2015; OLADEJI *et al.*, 2019). Hsu, Yen, and Wang (2013) reported that the mixture of citral and myrcene had a synergistic repellent effect on *Aedes aegypti* (Diptera, Culicidae). According to Louis-Clément *et al.* (2017), the mixture of citral and myrcene has synergistic antioxidant and antimicrobial effects, acting against protozoa of the *Leishmania* genus and *Anopheles gambiae* (Diptera, Culicidae). A possible synergistic effect between citral and myrcene may be responsible for the high mortality percentages of both *C. citratus* essential oil and the mixture of citral and myrcene relative to them applied isolated.

According to the results, a synergistic insecticidal effect between citral and myrcene may exist, in which the toxicity of the former seems to be potentiated by the latter. After 96 h, all treatments at the concentration of 0.9 % v/v with exception of myrcene killed all of the exposed individuals. In this sense, both *C. citratus* essential oil and the mixture of citral and myrcene can be considered suitable options for use in the alternative control of this species, although more studies are needed. However, considering that *C. citratus* essential oil can be used without further purification, it can be considered a promising option for the alternative control of the pest insect *A. gemmatalis*.

Conflict of Interests

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical Statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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Camila Bonatto Vicenço: https://orcid.org/0000-0003-0713-7707
Wendel Silvestre: https://orcid.org/0000-0002-9376-6405
Gabriel Fernandes Pauletti: https://orcid.org/0000-0001-9850-3682

References

ANDRADE-OCHOA, S. *et al.* Oviposition deterrent and larvicidal activity of seven essential oils and their major components against *Culex quinquefasciatus* Say (Diptera: *Culicidae*): Synergism-antagonism effects. **Insects**, v. 9, n. 1, 25, 2018. https://doi.org/10.3390/insects9010025.

ANVISA. Agência Nacional de Vigilância Sanitária. **Farmacopéia Brasileira**. 5th ed. Brasília: Agência Nacional de Vigilância Sanitária, 2010.

ASADI, M. *et al.* Effects of plant essential oils on the changes of digestive enzymes in the ectoparasitoid, *Habrobracon hebetor* Say, with description of its digestive tube. **Arthropod-Plant Interactions**, v. 15, p. 929–935, 2021.https://doi.org/10.1007/s11829-021-09860-2.

AVOSEH, O. *et al.* Cymbopogon species, ethnopharmacology, phytochemistry and the pharmacological importance. **Molecules**, v. 20, n. 5, p. 7438-7453, 2015. https://doi.org/10.3390/molecules20057438.

BEDINI, S. *et al.* Not just for beer: evaluation of spent hops (*Humulus lupulus* L.) as a source of eco-friendly repellents for insect pests of stored foods. **Journal of Pest Science**, v. 88, p. 583-592, 2015. https://doi.org/10.1007/s10340-015-0647-1.

PESQ. AGROP. GAÚCHA, V.29, N.1, P. 1-15, 2023.
ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received on 11 Mar. 2022. Accepted on 30 Sep 2022.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Sistema de agrotóxicos fitossanitários.** Brasília: Ministério da Agricultura, Pecuária e Abastecimento, 2021. Available from: https://www.gov.br/agricultura/pt-br/acesso-a-informacao/acoes-e-programas/cartas-de-servico/defesa-agropecuaria-agrotoxicos/agrotoxicos-registrados-no-agrofit. Accessed: Jan. 18, 2022.

DENT, D.; BINKS, R. H. **Insect Pest Management**. 3rd ed. Oxfordshire: CAB International, 2020. https://doi.org/10.1079/9781789241051.000.

EKPENYONG, C. E.; AKPAN, E.; NYOH, A. Ethnopharmacology, phytochemistry, and biological activities of *Cymbopogon citratus* (DC.) Stapf extracts. **Chinese Journal of Natural Medicines**, v. 13, n. 5, p. 321-337, 2015. https://doi.org/10.1016/S1875-5364(15)30023-6.

ENAN, E. Insecticidal activity of essential oils: octopaminergic sites of action. **Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology**, v. 130, n. 3, p. 325-337, 2001. https://doi.org/10.1016/S1532-0456(01)00255-1.

FOGNÉ, D. S. *et al.* Susceptibility of MED-Q1 and MED-Q3 biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations to essential and seed oils. **Journal of Economic Entomology**, v. 110, n. 3, p. 1031-1038, 2017. https://doi.org/10.1093/jee/tox100.

GAJGER, I. T.; DAR, S. A. Plant allelochemicals as sources of insecticides. **Insects**, v. 12, n. 3, 189, 2021. https://doi.org/10.3390/insects12030189.

GARCÍA-DÍEZ, J. *et al*. Synergistic activity of essential oils from herbs and spices used on meat products against food borne pathogens. **Natural Product Communications**, v. 12, n. 2, p. 281-286, 2017. https://doi.org/10.1177/1934578X1701200236.

GREENE, G. L.; LEPPLA, N. C.; DICKERSON, W. A. Velvet caterpillar: a rearing procedure and artificial medium. **Journal of Economic Entomology**, v. 69, n. 4, p. 487-488, 1976. https://doi.org/10.1093/jee/69.4.487.

HAHN, R. C. *et al.* Atividade inseticida do óleo essencial de *Cymbopogon citratus* sobre *Anticarsia gemmatalis*. In: ENCONTRO DE JOVENS PESQUISADORES E MOSTRA ACADÊMICA DE INOVAÇÃO E TECNOLOGIA, 26., 2018, Caxias do Sul. **Proceedings…** Caxias do Sul: UCS, 2018. 1 p.

HSU, W. S.; YEN, J. H.; WANG, Y. S. Formulas of components of citronella oil against mosquitoes (*Aedes aegypti*). **Journal of Environmental Science and Health: B**, v. 48, n. 11, p. 1014-1019, 2013.

https://doi.org/10.1080/03601234.2013.816613.

ITANKAR, P. R.; TAUQEER, M.; DALAL, J. S. Toxicological and pharmacological profiling of organically and non-organically cultivated *Cymbopogon citratus*. **Journal of Ayurveda and Integrative Medicine**, v. 10, n. 4, p. 233-240, 2019. https://doi.org/10.1016/j.jaim.2017.04.002.

JANKOWSKA, M. *et al.* Molecular targets for components of essential oils in the insect nervous system - a review. **Molecules**, v. 23, n. 1, 34, 2018. https://doi.org/10.3390/molecules23010034.

KHORRAM, M. S. *et al.* The toxicity of selected monoterpene hydrocarbons as singles compounds and mixtures against different developmental stages of Colorado Potato Beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). **Journal of Entomology**, v. 8, n. 5, p. 404-416, 2011. https://doi.org/10.3923/je.2011.404.416.

KIMUTAI, A. *et al.* Repellent effects of the essential oils of *Cymbopogon citratus* and *Tagetes minuta* on the sandfly, *Phlebotomus duboscqi*. **BMC Research Notes**, v. 10, n. 98, 2017. https://doi.org/10.1186/s13104-017-2396-0.

LOUIS-CLÉMENT, O. E. *et al.* Larvicidal and ovicidal properties against *Anopheles gambiae*, antioxidant and antibacterial activities of the combination of essential oils *Eucalyptus citriodora*, *Cymbopogon giganteus* and *Cymbopogon nardus* from Gabon. **Journal of Multidisciplinary Engineering Science and Technology**, v. 4, n. 8, p. 7887-7894, 2017.

MENEZES, E. L. A. **Inseticidas botânicos: seus princípios ativos, modo de ação e uso agrícola**. Seropédica: Embrapa Agrobiologia, 2005. 53 p.

MOUSTAFA, M. A. M. *et al.* Insecticidal activity of lemongrass essential oil as an eco-friendly agent against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). **Insects**, v. 12, n. 8, 737, 2021. https://doi.org/10.3390/insects12080737.

OLADEJI, O. S. *et al.* Phytochemistry and pharmacological activities of *Cymbopogon citratus*: a review. **Scientific African**, v. 6, e00137, 2019. https://doi.org/10.1016/j.sciaf.2019.e00137.

OLORUNNISOLA, S. K. *et al.* Biological properties of lemongrass: an overview. **International Food Research Journal**, v. 21, n. 2, p. 455-462, 2014.

PAULETTI, G. F. *et al.* Poejo (*Cunila galioides* Benth.) production in five agroecological regions of Rio Grande do Sul. **Brazilian Archives of Biology and Technology**, v. 63, e20190481, 2020. https://doi.org/10.1590/1678-4324-2020190481.

PAVELA, R.; BENELLI, G. Essential oils as ecofriendly biopesticides? Challenges and constraints. **Trends in Plant Science**, v. 21, n. 12, p. 1000–1007, 2016. https://doi.org/10.1016/j.tplants.2016.10.005.

PINTO, Z. T. *et al.* Chemical composition and insecticidal activity of *Cymbopogon citratus* essential oil from Cuba and Brazil against housefly. **Revista Brasileira de Parasitologia Veterinária**, v. 24, n. 1, p. 36-44, 2015. https://doi.org/10.1590/S1984-29612015006.

PLATA-RUEDA, A. *et al.* Insecticidal and repellent activities of *Cymbopogon citratus* (Poaceae) essential oil and its terpenoids (citral and geranyl acetate) against *Ulomoides dermestoides*. **Crop Protection**, v. 137, 105299, 2020. https://doi.org/10.1016/j.cropro.2020.105299.

PLATA-RUEDA, A. *et al.* Lemongrass essential oil and its components cause effects on survival, locomotion, ingestion, and histological changes of the midgut in *Anticarsia gemmatalis* caterpillars. **Toxin Reviews**, v. 41, n. 1, p. 208-217, 2021. https://doi.org/10.1080/15569543.2020.1861468.

PRAÇA, L. B.; MORAES, S.; MONNERAT, R. G. ***Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae): Biologia, amostragem e métodos de controle**. Brasília: Embrapa Recursos Genéticos e Biotecnologia, 2006. 17 p.

REBELO, R. A. *et al.* Essential oils from leaves of *Vernonanthura montevidensis* (Spreng.) H. Rob.: chemical profile and antimollicute potential. **Natural Product Research**, v. 36, n. 9, p. 2393-2398, 2022. https://doi.org/10.1080/14786419.2020.1831491.

REGNAULT-ROGER, C. Essential oils in insect control. In: Ramawat, K. G.; Mérillom, J. M. (Eds.) **Natural products: phytochemistry, botany, and metabolism of alkaloids, phenolics, and terpenes**. Berlin: Springer-Verlag Berlin Heidelberg, 2013. p. 4087-4107.

SCALERANDI, E. *et al.* Understanding synergistic toxicity of terpenes as insecticides: contribution of metabolic detoxification in *Musca domestica*. **Frontiers in Plant Science**, v. 9, 1579, 2018. https://doi.org/10.3389/fpls.2018.01579.

SESSOU, P. *et al.* Bioefficacy of *Cymbopogon citratus* essential oil against food borne pathogens in culture medium and in traditional cheese wagashi produced in Benin. **International Research Journal of Microbiology**, v. 3, n. 12, p. 406-415, 2012.

ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received on 11 Mar. 2022. Accepted on 30 Sep 2022.

SHAHRIARI, M. *et al.* Bio-efficacy and physiological effects of *Eucalyptus globulus* and *Allium sativum* essential oils against *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). **Toxin Reviews**, v. 39, n.4, p. 422-433, 2020. https://doi.org/10.1080/15569543.2018.1554588.

SHAH, G. *et al.* Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). **Journal of Advanced Pharmaceutical Technology Research**, v. 2, n. 1, p. 3-8, 2011. https://doi.org/10.4103/2231-4040.79796.

SHAH, T. *et al.* Impact of nanomaterials on plant economic yield and next generation. In: Ghorbanpour. M.; Wani, S. H. (Eds.). **Advances in phytonanotecnology: from synthesis to application**. London: Academic Press, 2019. p. 203-214.

SILVESTRE, W. P. *et al.* Fractionation of rosemary (*Rosmarinus officinalis* L.) essential oil using vacuum fractional distillation. **Journal of Food Science and Technology**, v. 56, p. 5422-5434, 2019. https://doi.org/10.1007/s13197-019-04013-z.

SILVESTRE, W. P. *et al.* Insecticidal activity of *Callistemon speciosus* essential oil on *Anticarsia gemmatalis* and *Spodoptera frugiperda*. **International Journal of Tropical Insect Science**, v. 42, p. 1307-1314, 2021. https://doi.org/10.1007/s42690-021-00648-8.

STOLERU, E.; BREBU, M. Stabilization techniques of essential oils by incorporation into biodegradable polymeric materials for food packaging. **Molecules**, v. 26, n. 20, 6307, 2021. https://doi.org/10.3390/molecules26206307.

SUDO, M. *et al.* Optimal management strategy of insecticide resistance under various insect life histories: heterogeneous timing of selection and interpatch dispersal. **Evolutionary Applications**, v. 11, n.2, p. 271-283, 2017. https://doi.org/10.1111/eva.12550.

SUN, J. *et al.* Investigation of pesticidal effects of *Peucedanum terebinthinaceum* essential oil on three stored-product pests. **Records of Natural Products**, v. 14, n. 3, p. 177-189, 2020. https://doi.org/10.25135/rnp.149.19.05.1287.

TOSI, S.; NIEH, J. C. Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto®), on honeybees. **Proceedings of the Royal Society B**, v. 286, n. 1900, 20190433, 2019. https://doi.org/10.1098/rspb.2019.0433.

ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received on 11 Mar. 2022. Accepted on 30 Sep 2022.

VICENÇO, C. B. *et al.* Bioactivity of *Schinus molle* L. and *Schinus terebinthifolia* Raddi. Essential Oils on *Anticarsia gemmatalis* (Hübner 1818). **Brazilian Archives of Biology and Technology**, v. 63, e20200111, 2020. https://doi.org/10.1590/1678-4324-2020200111.

VICENÇO, C. B. *et al.* Insecticidal activity of *Lavandula dentata* L. essential oil on *Anticarsia gemmatalis* (Hübner, 1818). **Brazilian Archives of Biology and Technology**, v. 64, e21210327, 2021. https://doi.org/10.1590/1678-4324-2021210327.

VIEGAS JÚNIOR, C. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. **Química Nova**, v. 26, n. 3, p. 390-400, 2003. https://doi.org/10.1590/S0100-40422003000300017.

WAR, A. R. *et al.* Mechanisms of plant defense against insect herbivores. **Plant Signaling & Behavior**, v. 7, n. 10, p. 1306-1320, 2012. https://doi.org/10.4161/psb.21663.

ZHU, F. *et al.* Insecticide resistance and management strategies in urban ecosystems. **Insects**, v. 7, n. 1, 2, 2016. https://doi.org/10.3390/insects7010002.

ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received on 11 Mar. 2022. Accepted on 30 Sep 2022.