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CROP SCIENCE

Toxicity of Essential Oil of *Mentha piperita* (Lamiaceae) and its Monoterpenoid Menthol Against *Tetranychus urticae* Kogan 1836 (Acari: Tetranychidae)

LAUANA P. DE SOUZA, VITOR ZUIM, PRISCILA STINGUEL, PATRÍCIA F. PINHEIRO & HUGO B. ZAGO

Abstract: Essential oils from plants have remarkable biological properties, for example as insecticides and acaricides. Here we provide chemical analysis and evaluate the toxicity of the essential oil of *Mentha piperita* (Lamiaceae) and its main constituent menthol against *Tetranychus urticae* Kogan 1836 (Acari: Tetranychidae), a polyphagous pest present in agricultural landscapes. The essential oil was obtained from *M. piperita* leaves via hydrodistillation. Subsequently, concentration-response bioassays in adult females (fumigation and contact) were conducted to evaluate the lethal effect on the mite with three exposure intervals. We also evaluated the reproductive performance of females after exposure. Both substances were lethal in the fumigation bioassay, in addition, the essential oil was about 6-fold more toxic than menthol after 24 and 48 h of exposure. The fecundity of *T. urticae* females decreased inversely proportional to the increase of the used concentrations. Essential oil contact tests showed sublethal effects, with low mortality and reproductive stimulation of *T. urticae* females. Therefore, menthol and *M. piperita* essential oil can be considered potential acaricides for *T. urticae* by fumigant exposure due to the deleterious effect in adults and reduction in the number of individuals in subsequent generations, that represents a promising management tool.

Key words: botanical insecticides, major compound, peppermint, two-spotted spider mite.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Kogan 1836 (Acari: Tetranychidae), is the most important mite pests in agriculture, infects a variety of plants, including flowers, fruit trees, and vegetables with significant damage to agricultural production (Grbic et al. 2011, Piraneo et al. 2015, Van Leeuwen et al. 2015). The main way of controlling this pest has been through conventional acaricides and insecticides. This common management combined with species characteristics, such as high reproductive rates and short life history, has contributed to the rapid development of resistance to a wide range of acaricides and insecticides chemical classes, which makes *T. urticae* the first species in the resistance rank in terms of the total number of compounds to which their populations have become resistant (Stavrinides & Hadjistylli 2009, Tang et al. 2014, Ferreira et al. 2015, Wu et al. 2019).

The integration of others control strategy, for example phytoinsecticides use, can be an alternative for replacing routinely used chemical molecules. In this context, some botanical products are emerging as eco-friendly alternatives, and some are in large-scale use in plant protection (Isman 2006). Among the botanicals, plant essential oils (EOs) have some advantages that make them suitable for mites management that include, (1) multiple mode of action and sites of action which enable to develop resistance less rapidly than conventional acaricides and insecticides (Tong et al. 2013); (2) low residue persistence due to its high volatility (Isman 2006); (3) these compounds are considered relatively non-toxic to mammals and non-target organisms (e.g., natural enemies) (Turchen et al. 2016). Particularly, research on active plant compost are significant in providing information on the potential in bioprospecting new active ingredients for the management of arthropod pests.

Among essential oils from different plants, EO of *Mentha piperita* L. has stood out due to its antibacterial, antifungal, and antioxidant properties (Iscan et al. 2002, Arslan & Dervis 2010, Hussain et al. 2010, Soltani & Aliabadi 2012, Beyki et al. 2014, Sun et al. 2014). *Mentha piperita* popularly known as peppermint belongs to Lamiacea family, is widely grown for use of fresh leaves in cooking, applied to flavor the pharmaceuticals, oral preparations (e.g., dental creams), and confectionery industry (Singh & Pandey 2018).

Regarding use in pest management, *M. piperita* EO has an insecticide effect against stored grain pest (Halit et al. 2012, Mishra et al. 2014, Rajkumar et al. 2019), spotted wing drosophila *Drosophila suzukii* (Diptera: Drosophilidae) (Park et al. 2016), housefly *Musca domestica* L. (Diptera: Muscidae) (Kumar et al. 2012). However, little is known about the effect of the *M. piperita* EO against the two-spotted spider mite. Therefore, in the present study, we provide the chemical analysis of the essential oil of *M. piperita* and evaluated the lethal effect and reproductive performance these EO and its majority compound menthol against *T. urticae* adult females.

MATERIALS AND METHODS

Insect populations

The population of *Tetranychus urticae* was established from collections by late 2012 in strawberry plantations in Guaçuí City (20° 46’ 36.48” S and 41° 40’ 37.92” O, State of Espírito Santo, Brazil). The colonies were kept in cowpea (*Canavalia ensiformes* L., Fabaceae) plants, which served as the substrate for feeding and oviposition. Every 5 days a new plant was offered to the tetraniquids. They have been reared in controlled environmental conditions at 25 ± 1 °C temperature, 70 ± 10% relative humidity, and L12: D12 photo phase, the same conditions in which the bioassays were carried out.

Plant material and extraction of volatile components

Plants of *Mentha piperita* (fresh leaves) were collected from Alegre – ES, in the morning, between March and November 2012. The preparation of the essential oil according to Pinheiro et al. (2013) by hydrodistillation, with an extractor type Clevenger. Briefly, 100 g fresh (hand-crushed) leaves were placed in a round bottom flask (3 L) with 1.5 L distilled water and after distillation, for 3 h the hydrolat was collected. The hydrolat was partitioned with a separatory funnel using pentane. Three extractions were carried out with 30 mL of solvent each, the organic phase was dried with anhydrous sodium sulfate and was filtered, and the solvent removed by a rotary evaporator. The essential oil was stored in an amber bottle and stored at 4 °C. The oil yield was calculated.

Chemical composition of the volatile material

The oil was analyzed by gas chromatography and mass spectrometry (GC-MS), in Shimadzu QP-PLUS-2010 equipment equipped with the selective mass detector, with followed sets:

capillary column fused silica Rtx® -1 (30 m x 0.25 mm of internal diameter); carrier gas helium (1.2 mL min^{-1}); temperature programmed at 60 – 240 °C (3 °C min^{-1}); injector and detector temperatures were set at 220 and 300 °C, respectively. Described in detail in Souza et al. (2015). The individual components were identified by comparison of mass spectra with those available on the Willey 330.000 Spectrotheque database and by the Kovats Index (KI) calculated for each component (Adams 2007). The retention time (RT) is exhibited in Table I.

After knowing the chemical composition of the oil, we explored the pure molecule of the compound that had the largest area to compare its toxicity with that of the essential oil. Monoterpenoid Menthol was obtained from company Sigma Aldrich (Menthol, M2772, Relative density: 0.89 g mL^{-1} at 25 °C; Appearance: solid form).

Activity of Mentha piperita and Menthol against *Tetranichus urticae*

Adult *T. urticae* females (less than 24 h old) were used in the fumigation and contact bioassays under different exposure times in a completely randomized design. To test fumigant action of the compounds, the following adapted procedures from Aslan et al. (2004) were used: glass containers (1.4 L) were fumigation chambers, inside of which were placed three glass containers (10 mL) containing distilled water

Table I. Chemical profile of *Mentha piperita* L. essential oil, collected in Alegre - ES.

Compound	KI (Tab.)	KI (Cal.)	RT (min)	Area peak (%)
α-Trujene	924	924	8.46	0.15
α-Pinene	932	930	8.68	0.50
Sabinene	969	966	10.21	0.07
β-Pinene	974	968	10.31	1.01
Myrcene	988	985	11.15	0.32
1,4-Cineole	1012	1017	12.65	5.41
Limonene	1024	1020	12.75	2.08
(Z)-β-Ocimene	1032	1031	13.24	0.06
γ-Terpinene	1054	1050	14.11	0.15
Terpinolene	1086	1078	15.51	0.04
Linalool	1095	1088	16.02	0.16
2-Methylbutyl-2-Methylbutyrate	1100	1092	16.27	0.04
2-Methylbutyl-Isovalerate	1103	1095	16.45	0.02
Menthone	1148	1134	18.21	31.07
2-Isopropyl-5-Methylcycloexanone	-	1140	18.52	4.84
Menthol	1167	1165	**19.71**	**42.35**
d-p-Menth-4(8)-en-3-one	-	1210	21.99	1.73
Menthol acetate	1273	1278	25.11	5.97
Menthyl acetate	1294	1289	25.64	0.20
α-Copaene	1374	1367	29.11	0.07
β-Elemene	1389	1380	29.73	0.08
(Z)-Caryophyllene	1408	1404	30.84	1.20
(Z)-β-Farnesene	1440	1447	32.57	0.07
Total				97.59

KI (Tab.) = Kovats Index tabulated; KI (Cal.) = Kovats Index calculated; RT = retention time; Constituents in bold is for highlighting the major compounds.

which served as a support for a leaf disc (2.5 cm in diameter) of *C. ensiformes*, fix by petiole on the glasses with the aid of hydroponic cotton. On the upper edge of the fumigation chamber was attached a tape of filter paper (2 cm x 5 cm), where the essential oil was deposited with the aid of an automatic pipettor (concentrations: 0, 5, 10, 15, 20, and 25 µL L^{-1} of air to *M. piperita*; and 0, 33.7, 68.5, 137.1, 277.5, and 561.8 µL L^{-1} of air to menthol). Nothing was applied at concentration 0 µL L^{-1} (control). After the application of the oil, the upper part of the fumigation chamber was sealed with three layers of PVC-type plastic and the chambers were stored in a controlled environment (described above). Each treatment, represented by different concentrations, was composed of three replicates (fumigation chambers) with 30 adult females of *T. urticae* each (n = 90 females/treatment).

In bioassay with exposure by contact, only *Mentha piperita* was used because the menthol pure forms a waxy layer on the leaf surface preventing the mite from feeding, and thus, mortality could not be attributed to treatment. Arenas were made from leaf discs (4 cm in diameter) of cowpea and placed with bottom face up inside Petri dishes (8 cm in diameter) on wetted filter paper discs. The edges of the leaves were covered with moistened hydrophilic cotton strips to prevent the mite escape. Solutions were prepared with distilled water and Tween (0.05%) at concentrations of 0.03, 0.06, 0.09, 0.12 and 0.15 µL of *M. piperita* oil per square centimeter of leaf (Miresmailli et al. 2006). An aliquot of 20 µl of each solution was applied with an automatic pipettor in each arena and spread over the surface of the leaf disc with a glass rod. For the control, distilled water + tween (0.05%) was used. After drying the treated leaves at room temperature for 20 min, 10 adult *T. urticae* females were transferred to the cowpea disks and the open plates were placed in a controlled environment (described above). Each treatment, represented by different concentrations, was composed of five replicates (leaf arenas) with 10 adult females of *T. urticae* each (n = 50 females/treatment).

To both fumigation and contact bioassays, mortality (mites unable to walk longer than body length after light touch with fine bristle brush) and fecundity (number of eggs present in leaf disc) were evaluated after 24, 48 and 72 h of exposure to treatments.

Statistical analysis

Normality and homoscedasticity of the data were checked using Shapiro-Wilk and Bartlett tests, respectively. Concentration–mortality curves were estimated using probit analysis with the PROBIT procedure in the SAS statistical software package (SAS Institute, 2001), after adjusting the female percentage mortality using Abbott's formula (Abbott 1925). The toxicity (essential oil and menthol) of compounds were compared based on the estimated LC_{50} (i.e., the lethal concentration capable of killing 50% of tested two-spotted spider mite). Mean fecundity values were submitted to regression analysis.

RESULTS

Essential oil composition

The average yield of the essential oil was 0.75% (w w^{-1}) of the fresh mass. Chromatographic analysis of the essential oil of *Mentha piperita* demonstrated standard chemical compounds expected for the species. Oil had 97.59% of its constituents identified. Among these compounds, the most abundant were menthol (42.35%) and menthone (31.07%), and the other compounds were detected with a percentage between 0 and 6% (Table I).

Activity of *Mentha piperita* and Menthol against *Tetranychus urticae*

The exposition of essential oil of *M. piperita* and Menthol on *Tetranychus urticae* adult, showed promising results for the control of this pest mite, as it caused mortality and reduced fertility in females.

For the fumigant toxicity bioassay, both substances caused toxicity (Table II). Larger doses were necessary to cause mortality of 50% of the population within 24 h of exposure, and these values have been reduced according to evaluations in longer times. With 24 and 48 h of exposure the essential oil of *M. piperita* was about 6 times more toxic than the monoterpene menthol, however, menthol was more toxic with 72 h of exposure (Table II). It was also observed that fertility was affected by essential oil and monoterpene menthol, as the number of eggs/females was inversely proportional to the concentrations used at different periods of fumigation (Fig. 1).

For the contact toxicity bioassay, only the essential oil of *M. piperita* has been tested. The acaricidal activity was milder, reaching 46% of female mortality, with 72 h of exposure and in the higher oil concentration (i.e., 0.15 µL cm^{-2}) (Fig. 2a). Curiously, contact exposure did not reduce the fecundity of females. In contrast, initial concentrations (i.e., 0.03 and 0.06 µL cm^{-2}) caused an increase in the number of eggs per female and at higher concentrations, these values did not differ from the control (Fig. 2b).

DISCUSSION

In the present study, we showed that *M. piperita* EO and its major constituent menthol present rapid toxic action on *T. urticae* females, in addition to a reduction in female fecundity. Thus, showing promise in the management of this important mite pest. The chemical profile obtained in our study (23 compounds) was higher than reported by Rajkumar et al. (2019) (16 compounds), Saeidi & Mirfakhraie (2017) (11 compounds), Bassolé et al. (2010) (17 compounds), similar to that found by Pang et al. (2020) (21 compounds) and was lower than reported by Sun et al. (2014) (51 compounds). It is consonant among the authors that menthol and menthone are present in the chemical profile as major compounds. Many additional compounds identified in our study and by Sun et al. (2014) were detected with low percentages and this may be associated with extraction methods, Clevenger apparatus, pharmacopeia distillation apparatus, and sensitivity of capillary column of the CG-MS. In addition, the interspecific variation in the chemical constitution of the EO may be

Table II. **Fumigant toxicity of *Mentha piperita* L. essential oil and its major compound against *Tetranychus urticae* after 24, 48, and 72 h of exposure.**

Sample	Exposure time (h)	Mites in bioassay (N)	LC_{50} (LC_{90})	95% confidence limit LC_{50} (LC_{90})		Slope ± SE	X^2	*df*
				LCL	UCL			
Mentha piperita [a]	24	450	11.04 (16.90)	9.32 (14.77)	12.39 (22.05)	6.92 ± 0.76	4.39	3
	48	450	9.49 (14.38)	8.78 (13.39)	10.75 (15.81)	7.10 ± 0.75	2.32	3
	72	360	7.55 (10.74)	7.00 (10.05)	8.02 (11.76)	8.38 ± 0.99	0.36	3
Menthol [a]	24	404	69.66 (1,744.94)	42.69 (861.79)	98.88 (2,628.09)	0.92 ± 0.15	2.69	3
	48	391	57.30 (116.85)	40.45 (101.12)	64.05 (142.69)	4.16 ± 0.43	0.39	3
	72	413	2.25 (46.07)	0.22 (10.11)	10.11 (75.28)	1.05 ± 0.32	1.89	3

[a] Units LC_{50} and LC_{90} = µL L^{-1} air; LCL = lower confidence limit; UCL = upper concentration limits, X^2 = chi-square test, df = degree of freedom.

related to the features of the plant (i.e., the origin of the material, part and age of the plant, harvest time) and abiotic factors (i.e., clime) (Rohloff et al. 2005, Figueiredo et al. 2008, Hussain et al. 2010).

Toxicity bioassays have revealed that *M. piperita* EO exhibited promising results for control of the mite pest, as it caused deleterious effects *T. urticae* females ($LC_{50\,(24h)}$ = 11.04 µL L^{-1}). Acaricidal activity of EOs from plants of the genus *Mentha* has already been explored in previous research against *T. urticae* and other greenhouse pests (Stepanycheva et al. 2019), and the estimated LC_{50} values fluctuated according to the plant species. For example, the concentrations that caused 50% mortality from *T. urticae* were 1.3 and 20.08 µL L^{-1}, for *M. spicata* (Pavela et al. 2016) and *M. longifolia* (Motazedian et al. 2012), respectively.

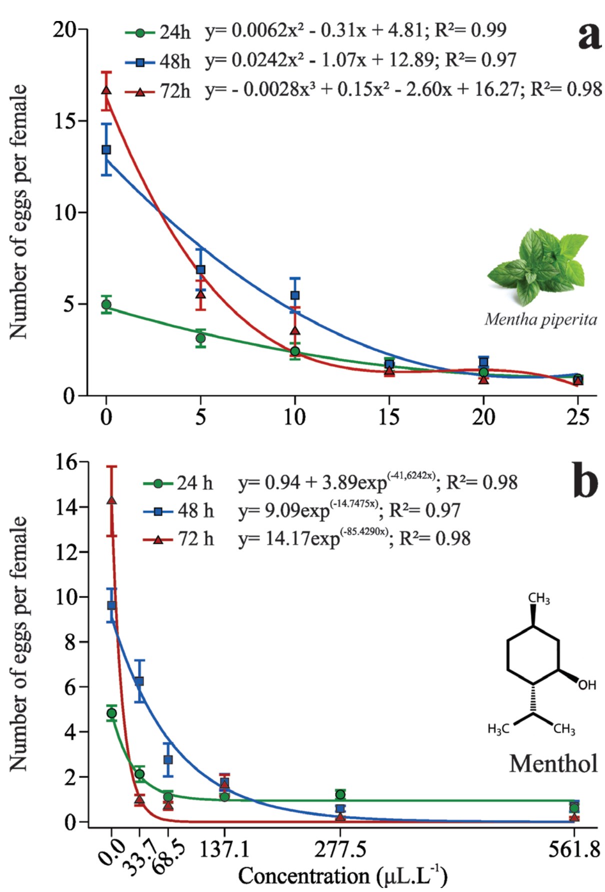

Figure 1. Female fecundity (mean ± SE) of *Tetranychus urticae* after fumigant exposure (24, 48 or 72 h) by *Mentha piperita* essential oil (a) and Menthol (b).

Pavela et al. (2016) provide the chemical profile of the *M. spicata* EO, with carvone (68.5%) and limonene (19.7%) being the major compounds and menthone (1.7%) and menthol (1.1%) only present. This huge difference in the majority compounds found in plant species gives clues to unequal LCs.

In this study, the toxicity of the essential oil was superior to the toxicity of its major compound menthol. These results allow us to assume that menthol contributes to the toxic effect of EO, however, the best results in trials using the EO suggest the presence of intrinsic effects of synergism and additive between other OE compounds. In fact, it has been already observed this behavior for three stored product insects (*Tribolium castaneum, Lasioderma serricorne*, and *Liposcelis bostrychophila*) and shows that interactions among constituents interfere in the final bioactivity of the EO and its complexity is possibly accentuated by the high number of compounds (Pang et al. 2020). Although menthol was less toxic in the first 48 h, it is noticed that this compound has a greater persistence compared to essential oil, which is progressively volatilized. The substances chemical property hypothetically explains the increased lethality of menthol after 72 h exposure. About four decades ago, Larson & Berry (1984) has been observed a significant reduction in the oviposition and survival of *T. urticae* when applying vapors with 5% solutions of menthol. Acaricidal and repellent activity of menthol to two-spotted spider mite has also been reported by Tak & Isman (2017).

Other comparative studies have shown superior menthol toxicity compared to the *M. piperita* EO, in tests with *Drosophila suzukii* (Diptera: Drosophilidae) (Park et al. 2016) and *Tyrophagus putrescentiae* (Acari: Astigmata) (Park et al. 2014, Jeon & Lee 2016). Although less toxic than *M. piperita* EO, menthol had good responses in the control of *T. urticae*, emphasizing its promising use as an active ingredient in acaricides.

The phytoinsecticides are good options for the management of mites-pests hazardous and resistance problems of the commercial insecticides. Many researchers have investigated the target of action of these chemical compounds. A priori, action was indicated on the neurotransmitter and neurohormone octopamine, present in the neuromuscular junctions and body fluid of insects (Singh & Pandey 2018). More recently, activity on acetylcholinesterase and the antioxidant system has been confirmed, which leads to postsynaptic membrane overstimulation in the neurons and the impediment of the primary defense against oxidative stress, respectively (Rajkumar et al. 2019). Confirming the modes of action just mentioned in *T. urticae*, the exploitation of menthol and complete oil as a commercial product represents a viable alternative for the rotation of chemical groups, as they have a different mode of action from the main synthetic acaricides and insecticides currently used (Wu et al. 2019). Most of these synthetic products are growth inhibitors (e.g., etoxazole), mitochondrial electron transport inhibitors (e.g., bifenazate), inhibitors of acetyl-coenzyme A carboxylase (e.g., spirodiclofen), and glutamate-gated chloride channel allosteric modulators (e.g., abamectin) (Piraneo et al. 2015).

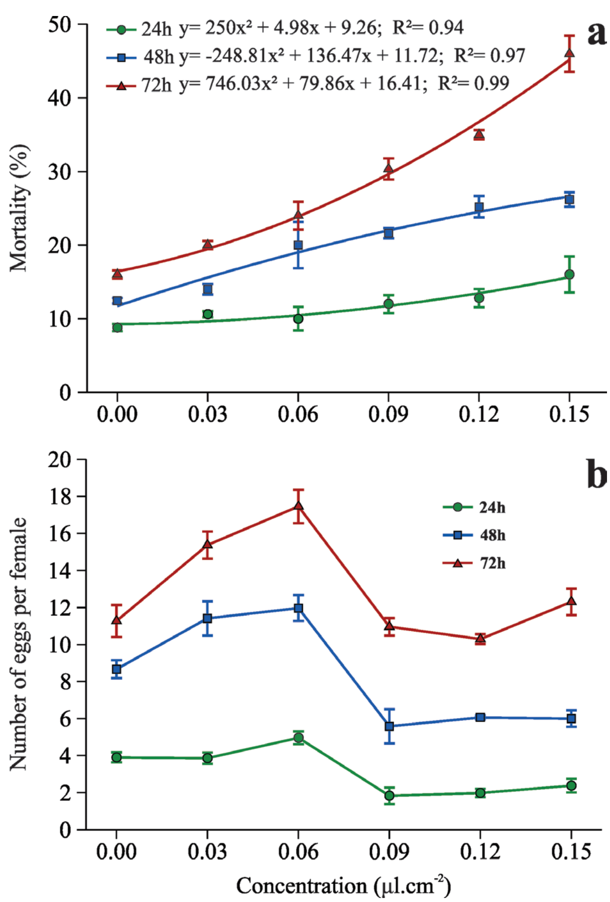

Figure 2. Mortality (mean ± SE) (a) and female fecundity (mean ± SE) (b) of *Tetranychus urticae* after contact exposure (24, 48 or 72 h) by *Mentha piperita* essential oil.

In addition to the lethal effect, fumigation bioassays significantly reduced the number of eggs per female (fecundity) (Fig. 1), which indicates the efficiency of essential oil and menthol in compromising the population growth of *T. urticae*. Inhibition of oviposition in arthropods pests by *Mentha* essential oil also was registered by Kumar et al. (2009) in *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) and Mishra et al. (2014) in *Tribolium castaneum* (Coleoptera: Tenebrionidae). This ovipositional activity by *Mentha* essential oil and menthol proves to be useful, especially against insects that have developed resistance when treated with those responsible for lethal toxicity.

Curiously, the *M. piperita* EO exposure by contact has not kept the reducing effect on female's oviposition observed in fumigant exposure. Sublethal insecticide exposure - concentrations with low lethality as observed by the contact test (Fig. 2a) - can elicit behavioral and/or physiological responses in the target organism (Guedes et al. 2016), like hormesis phenomenon. Hormesis is characterized by a reversion in response between low and high doses of a compound allowing a stimulatory effect under low dose exposure of a compound toxic at higher doses (Guedes & Cutler 2014). This probably occurred in the present study (Fig. 2b). The occurrence of population outbreaks is one of the consequences of hormesis (Cordeiro et al. 2013, Guedes & Cutler 2014). Therefore, through the results obtained by the contact tests, this form of exposure must be avoided in the concentrations tested for the *M. piperita* EO. On the other hand, a product based on *M. piperita* and rosemary oils combined has been marketed in the United States for application via leaf spray for greenhouse pest management under the tradename Ecotec^{TM} (Isman et al. 2011). However, in addition to the intraspecific variation in the chemical profile of plants, the mixture of products can trigger mortality responses different from that obtained by isolated oils.

Phytoinsecticides are beneficial for maintaining the ecosystem as well as human health. It has also been established that some plant essential oils exhibit selectivity to non-target organisms (Regnault-Roger et al. 2012, Turchen et al. 2016), therefore, compatible with biological control. The results indicated that the *M. piperita* EO and the monoterpene menthol alone can be considered as potential acaricides for *T. urticae*, due to the mortality and reduced fecundity of the females caused by the fumigant exposure. So, these compounds can be used as alternatives to conventional insecticides. Contact exposure should not be recommended due to low lethality and reproductive stimulation. However, additional studies are needed to evaluate the safety for non-target organisms, phytotoxicity in host plants, and target site of action in *T. urticae*. In addition, studies to develop advanced encapsulation techniques to improve the effectiveness and stability of botanical insecticides will enable to test the efficiency of this product in greenhouses and open environments allowed the large-scale use of these promising compounds in the management of mites in the field.

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LAUANA P. DE SOUZA[1]
https://orcid.org/0000-0002-5037-2174

VITOR ZUIM[1]
https://orcid.org/0000-0001-9702-8568

PRISCILA STINGUEL[1]
https://orcid.org/0000-0003-1975-3590

PATRÍCIA F. PINHEIRO[2]
https://orcid.org/0000-0003-4019-5773

HUGO B. ZAGO[1]
https://orcid.org/0000-0003-4097-2835

[1]Universidade Federal do Espírito Santo, Centro de Ciências Agrárias e Engenharias, Departamento de Agronomia, Alto Universitário, s/n, Guararema, 29500-000 Alegre, ES, Brazil

[2]Universidade Federal do Espírito Santo, Centro de Ciências Exatas, Naturais e da Saúde, Departamento de Química e Física, Alto Universitário, s/n, Guararema, 29500-000 Alegre, ES, Brazil

Correspondence to: **Vitor Zuim**
E-mail: vitorzuim@gmail.com

Author Contributions

LPS and HBZ conceived and designed the study. HBZ and PFP provided the materials and tools, LPS, VZ and PS performed the experiments and gathered the data, and LPS and VZ analyzed the data. LPS and VZ structured and wrote the manuscript draft, which was read, corrected, and approved by all authors. The manuscript was written through contributions of all authors.