Original Article

Cymbopogon citratus (Poaceae) ESSENTIAL OIL ON *Frankliniella schultzei* (Thysanoptera: Thripidae) AND *Myzus persicae* (Hemiptera: Aphididae)

ÓLEO ESSENCIAL DE Cymbopogon citratus *(Poaceae) SOBRE* Frankliniella schultzei *(Thysanoptera: Thripidae) E* Myzus persicae *(Hemiptera: Aphididae)*

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ABSTRACT: Phytovirus vectors *Frankliniella schultzei* (Trybom, 1920) (Thysanoptera: Thripidae) and *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) feed on crops of great economic importance brings large economic losses worldwide to cultivated species such as tomato and cotton. *F. schultzei* transmits *Tospovirus*, *Groundnut ring spot virus* (GRSV) and *Tomato spotted wilt virus* (TSWV) to tomato plants and *M. persicae* transmits *Potato virus* Y (PVY), *Tomato yellow top virus* (ToYTV) and *Tomato bottom yellow leaf virus* (TBYLV) to tomato crops. Chemical constituents of essential oils have been increasingly studied because they present a wide range of biological activities. The aim of this work was to characterize *Cymbopogon citratus* essential oil and evaluate its potential insecticide activity against *F. schultzei* and *M. persicae*. The essential oil was obtained from fresh leaves by hydrodistillation using a Clevenger apparatus. Its yield (1.04%) was determined relative to the dry mass of the plant. Qualitative analysis was performed by gas chromatography coupled to mass spectrometry and chemical constituent content was determined by gas chromatography with a flame ionization detector. Nine compounds were identified, with geranial (49.98%) and neral (37.78%) being the major components. The insects were sprayed with *C. citratus* essential oil at different concentrations using a Potter tower. The LC_{50} values for *M. persicae* and *F. schultzei* were 0.28% and 1.49%, respectively. Essential oil from *C. citratus* is a promising natural alternative for developing pesticides to manage *M. persicae*.

KEYWORDS: Green peach aphid. Lemongrass. Natural insecticide. Thrips. Volatile oil.

INTRODUCTION

The thrips, *Frankliniella schultzei* (Trybom, 1920) (Thysanoptera: Thripidae), present a scraper-sucking buccal apparatus. Because of its feeding habits, this insect causes silvering, necrosis and deformation of the plant tissues that it uses. Due to plant injuries caused by feeding and transmitting phytoviruses, this insect brings large economic losses worldwide to cultivated species such as tomato, cotton, soy and grape (MONTEIRO; MOUND; ZUCCHI, 2001; GALLO et al., 2002; MOREIRA; ARAGÃO, 2009; FERNANDES et al., 2011; MOREIRA et al., 2012). *F. schultzei* transmits *Tospovirus*, *Groundnut ring spot virus* (GRSV) and *Tomato spotted wilt virus* (TSWV) to tomato plants (BORBÓN; GRACIA; PÍCCOLO, 2006). Between 1996 and 2006, tospoviruses caused annual average losses of $9 million USD to tomato and pepper crops in the state of Georgia (USA) (RILEY et al., 2011).

The green peach aphid, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), forms large colonies on the abaxial side of leaves of cultures of cotton, potato, cruciferous vegetables, tobacco, papaya, cucurbits, tomato, eggplant and pepper. These insects continually suck sap from new leaves and branches, causing intense leaf folding and curling when its population is elevated. The principal injury caused by this pest is the transmission of viruses to plants (GALLO et al., 2002). This insect completes its development in only 5.9 days on eggplant leaves at a temperature of 25 ºC (CHAGAS FILHO et al., 2005) and easily develops resistance to synthetic insecticides. *M. persicae* transmits *Potato virus* Y (PVY), *Tomato yellow top virus* (ToYTV) and *Tomato bottom yellow leaf virus* (TBYLV) to tomato crops. In Brazil, PVY causes crop losses between 20 and 70% and ToYTV can reduce the production of flowers and fruits by about 85% (KUROZAWA; PAVAN, 2005).

Received: 14/03/13
Accepted: 14/09/13

To control *F. schultzei* and *M. persicae*, chemical and biological controls are recommended in addition to selective application and environmental management (GALLO et al., 2002; GRAVENA; BENVENGA, 2003). Thus, there is increasing demand for alternative methods of agricultural pest control that offer improved human safety, selectivity to natural enemies, biodegradability, economic viability and reduced environmental impacts (VIEGAS, 2003).

In this context, satisfactory results have been obtained from the use of diverse essential oils for insect management, such as the fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) (LIMA et al., 2009), the dengue vector mosquito, *Aedes aegypti* (L., 1762) (Diptera: Culicidae) (ACIOLE et al., 2011), the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae) (CAMARILLO et al., 2009), the maize weevil, *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae) (OOTANI et al., 2011) and the cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) (BREDA; OLIVEIRA; ANDRADE, 2010). Therefore, products based on essential oils may be created for pest management (CHIASSON; BOSTANIAN; VINCENT, 2004).

The essential oil of lemongrass, *Cymbopogon citratus* (D.C.) Stapf (Poaceae), presents insecticidal activity against the caterpillar (SOARES et al., 2011), mosquito (FURTADO et al., 2005) and fly (KHANIKOR; BORA, 2011) and repels beetles (HORI, 2003). These characteristics are attributed to the presence of the constituents present in the essential oil extracted from the leaves, mainly citral, an isometric mixture of neral and geranial (KHANIKOR; BORA, 2011).

In light of the importance of essential oils as an alternative form of pest control, the objective of this work was to identify the constituents of the essential oil of the leaves of *C. citratus* and evaluate their potential insecticidal activity against *F. schultzei* and *M. persicae.*

MATERIAL AND METHODS

Plant material acquisition.

Fresh leaves of *C. citratus* were collected during morning in an experimental greenhouse of the Center of Agricultural Sciences of the Federal University of Espírito Santo (CCA/UFES), in the municipality of Alegre, Espírito Santo State, Brazil (20º 44' 49'' S, 41º 27' 58'' W) and an altitude of 250 m, in june 2012. *C. citratus* was identified by Amélia Carlos Tuler and exsiccation (n. 21629) has been deposited at the herbarium of the Federal University of Espírito Santo-(VIES)-Alegre Campus.

Extraction of the essential oil.

Essential oil of *C. citratus* was obtained by steam distillation at the Center for Scientific and Technological Development in Phytosanitary Management of Pests and Diseases (NUDEMAFI) at CCA/UFES according to Wasiky and Akisue (1969).

A sample of fresh plant material (300 g) was transferred to a distillation flask containing distilled water (2 L). The flask was attached to a Clevenger apparatus, which was attached to the condenser. Steam distillation continued for 2 h after initial boiling. The organic phase was collected by liquid-liquid extraction of the hydrolate (100 mL) with pentane (30 mL). An excess amount of anhydrous sodium sulfate was added to remove water from the sample, which was then filtered. The filtrate was transferred to the rotary evaporator apparatus (MARCONI, MA-120 series) to acquire the essential oil (CASTRO et al., 2004). The yield was calculated using the amount of essential oil (g) obtained relative to the fresh weight (g) of the leaves.

Chemical characterization of essential oil.

Volatile components of *C. citratus* were identified by gas chromatography coupled with mass spectrometry (GC-MS) with a mass selective detector, model QP-PLUS-2010 (SHIMADZU). The chromatographic column used was a fused silica capillary column with stationary phase Rtx-5MS, 30 m long and 0.25 mm in internal diameter, using helium as the carrier gas. Temperature of the injector and detector were 220ºC and 300ºC, respectively. The initial column temperature was 60ºC, programmed for an increase of 3ºC per min until reaching a maximum temperature of 240ºC (CASTRO et al., 2004). Each essential oil component was identified by comparing their Kóvats retention index relative to a standard alkane series (C_9-C_{26}) and comparing its mass spectrum with reference data from the equipment database (NIST/EPA/NIH 08 Mass Espectral Library) and the literature (ADAMS, 2007).

Quantification of chemical constituents in the essential oil was performed using a SHIMADZU GC-2010 Plus gas chromatograph, equipped with a flame ionization detector (GC-FID). The carrier gas used was nitrogen with the Rtx-5MS capillary column, measuring 30 m long and 0.25 mm in internal diameter. Temperatures of the injector and

detector were fixed at 240 and 250ºC, respectively. The temperature program of the furnace was the same as that used in the GC-MS analyses. A 10 mg sample was diluted in dichloromethane (1 mL), followed by a 1 µL injection (CASTRO et al., 2004).

Acquisition and rearing of insects.

F. schultzei was collected from jack-bean flowers, *Canavalia ensiformis* L., located at CCA/UFES in the municipality of Alegre. Insects were maintained in an incubator at 25 ± 1ºC, relative humidity of 70 ± 10% and photoperiod of 12 h in acrylic cages with a square base measuring 121.0 cm^2 and 3.2 cm in height. Moistened filter paper was placed in the bottom of the cage to maintain humidity. Every two days one cotyledon leaf measuring roughly 18 cm in height of the natural host, jack bean, was provided to feed the nymphs. These leaves were collected and approximately 3 cm of the petiole was inserted in a cotton swab followed by anesthetic vials of 0.7 x 5.0 cm (diameter and height) containing distilled water to maintain turgidity. Plants were grown in 72-cell styrofoam trays containing a mixture of soil and cattle manure in a 6:1 proportion.

The cages were sealed with plastic wrap containing small holes. After copulation (48 h after the emergence of adults), the insects were sexed and 60 females were placed in each cage, since oviposition is reduced when females are in the presence of males. Cotyledon leaves containing castor bean pollen, *Ricinus communis* L., at the base for improved reproduction performance were offered everyday for feeding and oviposition of the females. Eggs were collected daily and transferred to new cages (RONDELLI et al., 2012).

M. persicae was obtained from kale plants, *Brassica oleracea* L. var. *acephala*, in the municipality of Alegre, Espírito Santo State, Brazil. They were reared in an incubator at 25 ± 1ºC, relative humidity of 70 ± 10% and photoperiod of 12 h on 8.0 cm diameter leaf discs of organic kale, in Petri dishes measuring 9.0 x 1.3 cm (diameter and height) and lined with filter paper. Every two days the plates were cleaned 70% alcohol and the filter paper and leaf discs were exchanged, while the insects were transferred with the aid of a bristle brush.

Estimate of lethal concentration (LC).

To estimate the lethal concentration, we used seven concentrations of essential oil of lemongrass spaced on a logarithmic scale, in accordance with each pest. The upper and lower limits were determined by preliminary tests. To solubilize the essential oil, 2% (v/v) acetone and 0.01% (m/v) Tween 80 were diluted in distilled water. The control consisted of 2% (v/v) acetone and 0.01% (m/v) Tween 80 diluted in distilled water.

F. schultzei nymphs between 48 and 72 h of age in the first or second instar were used for bioassay purpose (PINENT; CARVALHO, 1998). Ten *F. schultzei* nymphs were transfered to the abaxial face of jack-bean cotyledonary leaves (repetition) with the aid of a fine-bristle brush and funnel with a cut tip, since they are very agile. The test solutions containing essential oil were sprayed on both sides of the leaf. A cotton swab was placed on the petiole of the jack-bean leaves and placed in an anesthetic vial containing distilled water to maintain leaf turgidity. The dishes were sealed with plastic wrap containing six pin holes to allow gas and moisture exchange.

The bioassay was carried out with *M. persicae* nymphs between 24 and 48 h of age in the first or second instar (CHAGAS FILHO et al., 2005). Ten *M. persicae* insects were placed on the abaxial face of organic kale leaf discs measuring 4.5 cm in diameter (repetition). Essential oil was sprayed on both sides of the leaf and the Petri dish was closed with its own lid.

The leaves containing the insects were placed in Petri dishes measuring 9.0 x 1.3 cm coated with filter paper and sprayed using a Potter tower with a pressure of 15 lb in^{-2} and 6 mL of the solution for each leaf side (12 mL/repetition), spreading an average volume of 1.62 mg cm^{-2} (RONDELLI et al., 2011). Plates were maintained in a climatic chamber at 25 ± 1ºC, relative humidity of 70 ± 10% and photoperiod of 12 h, at the NUDEMAFI. Leaves were exchanged on the second day and as needed. Mortality was assessed until the fifth day.

A completely randomized design with five replicates containing 10 insects in each repetition was used. The lethal concentration was estimated according to the Probit analysis using the Polo-PC program (LEORA SOFTWARE, 1987).

RESULTS AND DISCUSSION

Chemical characterization of essential oil.

The essential oil of the leaves of *C. citratus* obtained by hydrodistillation presented yield of 1.04% (m/m). Oliveira et al. (2011) obtained a similar result (1.39%).

The quantity and chemical composition of the essential oil produced by a plant of the same species may vary as a function of plant/plant,

plant/microorganisms and plant/insect interactions, genetic diversity, age, development stage and abiotic factors such as luminosity, temperature, rainfall, nutrition, season and time of collection (ORTIZ; MARRERO; NAVARRO, 2002; MORAIS, 2009).

Nine compounds were identified in the essential oil of *C. citratus* (Table 1). The majority components found in the essential oil was geranial (49.98%) and neral (37.78%). Franz, Knaak and Fiuza (2011) observed similar quantities of geranial (47.56%) and neral (31.5%). PEREIRA et al. (2008) identified and quantified geranial (31.8%) and neral (40.2%) as majority components of *C. citratus* essential oil.

Table 1. Chemical constituents of the essential oil of lemongrass, *Cymbopogon citratus*, presented as calculated and tabulated Kovats indices and percentage of the compound.

Compound	KI cal[2]	KI tab[3]	% Area
6-methyl-5-hepten-2-one [1]	968	–	0.98
β-Myrcene	985	988	6.59
Cis-Ocimene	1,031	1,032	0.02
Linalool	1,080	1,095	1.35
Trans-Verbenol	1,144	1,140	0.48
Neral	1,215	1,240	37.78
Geraniol	1,239	1,249	2.47
Geranial	1,246	1,264	49.98
2-undecanone	1,274	1,293	0.35

[1] Compound identified only by apparatus library. [2] Calculated Kovats Index. [3] Tabulated Kovats Index (ADAMS, 2007).

In accordance with Leal et al. (2003), *C. citratus* essential oil has citral (a mixture of the isomeres neral and geranial) as the principal compound, around of 65 to 80%. Cimanga et al. (2002) and Costa et al. (2005) observed the presence of citral as constituting the majority of the essential oil of the lemongrass, corroborating the present study. Moreover, compounds such as 6-methyl-5-hepten-2-one, linalool and 2-undecanone were found in the essential oil of *C. citratus* in this work and by Costa et al. (2005).

Estimate of lethal concentration (LC).

Based on CL_{50}, the essential oil of lemongrass was 5.3 times more toxic to *M. persicae* than *F. schultzei* (Table 2). The CL_{90} of this essential oil on *M. persicae* was estimated at 0.85%. The inclination of the concentration-response curve of the essential oil of lemongrass on *M. persicae* was greater than on *F. schultzei* (Table 2). The upper value of inclination of the concentration-response curve indicated that a slight variation in the concentration of the essential oil caused a large variation in mortality. In the control with *F. schultzei*, mortality was 7.0 ± 2.61% (mean±standard-error), and in the control with *M. persicae*, mortality was 6.6 ± 1.71%.

Table 2. Inclination of the concentration-response curve and lethal concentration (LC_{50} and LC_{90}) of the essential oil of lemongrass, *Cymbopogon citratus*, on *Frankliniella schultzei* and *Myzus persicae* nymphs.

Pest	N[1]	Inclination ± SE[2]	LC_{50} (CI of 95%)[3] (%)	LC_{90} (CI of 95%)[3] (%)	DF[4]	$\chi^{2(5)}$
F. schultzei	309	1.72 ± 0.41	1.49 (0.87 – 2.76)	[6]	5	5.30
M. persicae	299	2.68 ± 0.34	0.28 (0.20 – 0.37)	0.85 (0.64 – 1.19)	4	1.96

[1] Number of insects used in the test. [2] Standard-error. [3] Confidence interval of the LC_{50} and LC_{90} at 95% probability. [4] Number of degrees of freedom. [5] Chi-squared test. [6] CL_{90} and IC a 95% were not estimated due to the low mortality.

C. citratus essential oil was also toxic to the third instar of *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) fed dried eucalyptus leaves immersed in an essential oil solution at a 1% concentration, causing 100% caterpillar mortality (SOARES et al., 2011). For the third instar of the dengue vector mosquito, *A. aegypti*, CL_{50} was 63.89 mg/mL and CL_{90} de 112.21 mg/mL after 24 h, with the essential oil containing 63.6% neral (FURTADO et al., 2005). Geraniol, which represented 2.47% of the oil we evaluated, presented CL_{50} of 124 ppm in a residual contact test with *Exorista sorbillans* Wiedemann (Diptera: Tachinidae) (KHANIKOR; BORA, 2011). Citral repelled *Lasioderma*

serricorne (F., 1792) (Coleoptera: Anobiidae) at a 1 µL concentration, but caused attraction at 0.1 µL (HORI, 2003).

The exposure of the insects to essential oils occurs by fumigation, contact and ingestion (ISMAN, 2006; SOARES et al., 2011). The essential oils act on the nervous system, e.g. by inhibiting the enzyme acetylcholinesterase (RYAN; BYRNE, 1998). Therefore, pollinators and natural enemies may also be intoxicated by products based on essential oils. Moreover, by being volatile, essential oils may not have residual effects in field conditions (greater than one hour), therefore, natural enemies that immigrate to the treated culture hours after treatment will not be intoxicated, as often occurs with conventional insecticides (CHIASSON; BOSTANIAN; VINCENT, 2004; ISMAN, 2006). Therefore, the verification of the insecticidal activity of essential oils and the determination of its active components may serve as a base for forming new commercial insecticides to control such pests.

CONCLUSIONS

The majority components of lemongrass essential oil of evaluated are geranial (49.98%) and neral (37.78%).

The essential oil is more toxic to *M. persicae* than *F. schultzei*, as it causes significant mortality to *M. persicae*. Therefore, the essential oil of lemongrass is a promising natural alternative for developing phytosanitary products to manage *M. persicae*.

ACKNOWLEDGEMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Espírito Santo (FAPES) for financial support.

RESUMO: Os vetores de fitovírus *Frankliniella schultzei* (Trybom, 1920) (Thysanoptera: Thripidae) e *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) se alimentam de culturas de grande importância econômica, trazendo grandes perdas econômicas em todo o mundo para as espécies cultivadas, como tomate e algodão. *F. schultzei* transmite *Tospovirus*, *Groundnut ring spot virus* (GRSV) e *Tomato spotted wilt virus* (TSWV) em tomateiro and *M. persicae* transmite *Potato virus* Y (PVY), *Tomato yellow top virus* (ToYTV) e *Tomato bottom yellow leaf virus* (TBYLV) ao tomateiro. Os constituintes químicos dos óleos essenciais têm sido cada vez mais estudados, pois apresentam uma ampla gama de atividades biológicas. O objetivo deste trabalho foi caracterizar o óleo essencial de *Cymbopogon citratus* e avaliar o seu potencial inseticida sobre *F. schultzei* e *M. persicae*. O óleo essencial foi obtido a partir de folhas frescas por hidrodestilação utilizando um aparelho Clevenger. O seu rendimento (1,04%) foi determinado em relação à massa seca da planta. A análise qualitativa foi realizada por cromatografia gasosa acoplada a espectrometria de massa e o teor dos constituintes químicos foi determinado por cromatografia gasosa com detector de ionização de chama. Nove compostos foram identificados, com geranial (49,98%) e neral (37,78%), sendo os componentes principais. Os insetos foram pulverizados com óleo essencial de *C. citratus* em diferentes concentrações utilizando uma torre de Potter. Os valores de CL_{50} de *M. persicae* e *F. schultzei* foram de 0,28% e 1,49%, respectivamente. Óleo essencial de C. *citratus* é uma alternativa natural promissor para o desenvolvimento de inseticidas para o manejo de *M. persicae*.

PALAVRAS-CHAVE: Capim-limão. Óleo volátil. Inseticida natural. Tripes. Pulgão-verde.

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