

INSECTICIDAL ACTIVITY OF CITRONELLA GRASS ESSENTIAL OIL ON *Frankliniella schultzei* AND *Myzus persicae*

Atividade inseticida do óleo essencial de capim-citronela sobre *Frankliniella schultzei* e *Myzus persicae*

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ABSTRACT

The thrips, *Frankliniella schultzei*, and green peach aphid, *Myzus persicae*, cause direct damage to plants of economic importance and transmit phytoviruses, causing large economic losses. Chemical constituents of essential oils present a wide range of biological activities. The aim of this work was to evaluate insecticidal activity of essential oil from citronella grass, *Cymbopogon winterianus*, on *F. schultzei* and *M. persicae*. This essential oil was obtained by steam distillation and components were identified by GC/FID and GC/MS. A Potter spray tower was used to spray insects with the essential oil. The major constituents are geraniol (28.62%), citronellal (23.62%) and citronellol (17.10%). Essential oil of *C. winterianus* at 1% (w v⁻¹) causes mortality in *F. schultzei* and *M. persicae* at 34.3% and 96.9%, respectively. The LC₅₀ value for *M. persicae* was 0.36% and LC₉₀ 0.66%. Thus, citronella grass essential oil at 1% (w v⁻¹) is more toxic to *M. persicae* than *F. schultzei*. This essential oil shows promise for developing pesticides to manage *M. persicae*.

Index terms: *Cymbopogon winterianus*, thrips, green peach aphid, volatile components, natural insecticide.

RESUMO

O trips, *Frankliniella schultzei*, e o pulgão-verde, *Myzus persicae*, além de causarem danos diretos a plantas de importância econômica, também são importantes transmissores de fitovirose, acarretando grandes perdas econômicas. Os constituintes químicos dos óleos essenciais têm sido cada vez mais estudados, pois apresentam uma ampla gama de atividades biológicas. Neste trabalho, objetivou-se avaliar a atividade inseticida do óleo essencial de capim-citronela, *Cymbopogon winterianus*, sobre *F. schultzei* e *M. persicae*. O óleo essencial foi obtido por hidrodestilação e a identificação dos seus componentes foi realizada por CG/DIC e CG/EM. Uma torre de Potter foi utilizada para pulverizar os insetos com óleo essencial. Os componentes majoritários encontrados são geraniol (28,62%), citronelal (23,62%) e citronelol (17,10%). O óleo essencial de *C. winterianus* a 1% (m v⁻¹) causa mortalidade de *F. schultzei* e *M. persicae* em 34,3% e 96,9%, respectivamente. O valor estimado de CL₅₀ para *M. persicae* foi 0,36% e CL₉₀ de 0,66%. Assim, o óleo essencial de capim-citronela a 1% (m v⁻¹) é mais tóxico para *M. persicae* do que para *F. schultzei*. Este óleo essencial mostra-se promissor para o desenvolvimento de inseticidas para o manejo de *M. persicae*.

Termos para indexação: *Cymbopogon winterianus*, trips, pulgão-verde, componentes voláteis, inseticida natural.

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INTRODUCTION

The thrips, *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae), presents piercing/sucking mouthparts, causing necrosis and deformation in the plant tissues it feeds on. Due to injuries caused by feeding and transmitting phytoviruses, this insect causes great economic losses to crops such as tomatoes, cotton, soybeans and grapes (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 tomatoes and is a more efficient GRSV vector than *F. occidentalis* (Pergande). *F. schultzei* was the second most frequently identified species (35.6%) in tomato flowers (BORBÓN et al., 2006). Between 1996 and 2006, tospoviruses caused annual average losses of \$9 million to tomato and pepper crops in the state of Georgia (USA) (RILEY et al., 2011).

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Another pest of economic importance is the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). This aphid sucks sap from leaves and young shoots, causing intense leaf curling in regions with high populations. However, the main injury this pest causes is transmitting viruses to plants (GALLO et al., 2002). *M. persicae* transmits *Potato virus Y* (PVY), *Tomato yellow top virus* (ToYTV) and *Tomato bottom yellow leaf virus* (TBLYV) to tomato crops. In Brazil, PVY causes between 20 and 70% of tomato crop losses and ToYTV can reduce the production of flowers and fruits by about 85% (KUROZAWA; PAVAN, 2005). On eggplant leaves at a temperature of 25° C, this insect completes its development in just 5.9 days (CHAGAS FILHO et al., 2005).

Because *F. schultzei* and *M. persicae* have short life cycles (PINENT; CARVALHO, 1998; CHAGAS FILHO et al., 2005), these insects have great potential to develop chemical resistance to insecticides. As a result of the emergence of resistant populations (FURIATTI; LAZZARI, 2003; LUOGEN et al., 2005; DOĐRAMACI; TINGEY, 2008), demand has increased for agricultural pest control methods that offer safety to humans, selectivity to natural enemies, biodegradability, economic viability and low environmental impact (VIEGAS, 2003).

In this respect, the use of essential oils for controlling insects has obtained satisfactory results (SOARES et al., 2008; LIMA et al., 2009; LIMA et al., 2011). Essential oil from *Cymbopogon winterianus* Jowitt, popularly known as citronella grass, has an acaricide (OLIVO et al., 2008) and repellent effect (LABINAS; CROCOMO, 2002). Acaricidal properties of this oil are attributed to the presence of volatile substances such as citronellal, geraniol and citronellol (MARTINS, 2006). Therefore, the aim of this study was to evaluate the effect of *C. winterianus* essential oil on controlling *F. schultzei* and *M. persicae*.

MATERIAL AND METHODS

Plant material acquisition

Fresh leaves of *C. winterianus* were collected during morning (March 2012) 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 (20° 44' 49" S, 41° 27' 58" W) and an altitude of 250 m. *C. winterianus* was identified by DSc. Tatiana Tavares Carrijo (Department of Biology/CCA/UFES) and exsiccation (n. 21537) 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. winterianus* 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.

Essential oil chemical characterization

Volatile components of *C. winterianus* 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 220° C and 300° C, respectively. The initial column temperature was 60° C, programmed for an increase of 3° C per min until reaching a maximum temperature of 240° C (CASTRO et al., 2004). Each essential oil component was identified by comparing their Kováts retention index relative to a standard alkane series (C₉-C₂₆) and comparing its mass spectrum with reference data from the equipment database (NIST/EPA/NIH08 Mass Spectral Library) and the literature (ADAMS, 2007). Kováts retention indexes of each compound were calculated using equation 01 (LUBECK; SUTTON, 1983):

$$KI = 100Z - 100 \frac{\log t'_R X - \log t'_R Z}{\log t'_R (Z+1) - \log t'_R Z} \quad (01)$$

Where: Z is the carbon number of the alkane eluting just before the analyte, t'_R X is the retention time of the analyte, t'_R Z is the retention time of the alkane eluting just before the analyte, t'_R (Z+1) is the retention time of the alkane eluting just after the analyte.

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 250° C, 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

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 ± 1° C, relative humidity of 70 ± 10% and photoperiod of 12 h in acrylic cages with a square base measuring 121.0 cm² 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

M. persicae was obtained from kale plants, *Brassica oleracea* var. *acephala* DC., in the municipality of Alegre, Espírito Santo State, Brazil. They were reared in an incubator at 25 ± 1° C, 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.

Insecticide activity test

Test solution was prepared by dissolving 1.5 g of citronella grass essential oil in a mixture of acetone (3 mL) and Tween 80® (15 µL). The volume was completed to 150 mL with distilled water for a mixture with essential oil at 1% (w v⁻¹). A 150 mL solution with this same composition, but lacking essential oil, was used as a control.

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 transferred 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 ± 1° C, 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 10 replicates containing 10 insects each was used. The corrected mortality was calculated in relation to the control with the Abbott (1925) formula. Data were subjected to analysis of variance and means were compared by the Fisher's test at 5% probability.

Estimate of lethal concentration (LC)

The lethal concentration was estimated only for *M. persicae*. Nine concentrations of the citronella grass

essential oil were used (0, 0.020, 0.050, 0.100, 0.163, 0.257, 0.391, 0.595, 0.906 and 1.240% (w v⁻¹)), spaced on a logarithmic scale. Lower and upper limits were determined by preliminary tests. The control consisted of 2% (v v⁻¹) acetone and 0.01% (w v⁻¹) Tween 80 diluted in distilled water. This stage of the bioassay was performed according to the procedures adopted in the insecticidal activity test. Mortality was assessed until the fifth day. A completely randomized design with five replicates each containing 10 insects 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 the essential oil

The yield of the essential oil was 2.02% (w w⁻¹). Essential oil obtained from fresh leaves of *C. winterianus* showed yield of 0.5% (GONÇALVES et al. 2010). Chemical composition for *C. winterianus* essential oil is described (Table 1). The main components found in the citronella grass essential oil in the present study were geraniol (28.62%), citronellal (23.62%) and citronellol (17.10%); these compounds are classified as monoterpenes. The presence of citronellal and citronellol was also detected by Labinas and Crocomo (2002) and by Quintas-Júnior et al. (2008), with the latter also reporting the presence of geraniol (40.06%) as the main component of citronella grass essential oil, followed by citronellal (27.44%) and citronellol (10.45%). The predominance of those same components in the essential oil of *C. winterianus* was also determined by Leite et al. (2011), who found citronellal (36.19%), geraniol (32.82%) and citronellol (11.37%).

In addition to the identification of citronellol and geraniol, elemol, a sesquiterpene, was also identified in *C. winterianus* by Akhila (1986) as one of the major components in this essential oil. This component was also identified in this study, corresponding to 5.27% of the oil (Table 1).

The quantity and chemical composition of essential oils produced by plants can vary within a species as a function of environmental conditions, interactions between plant/plant, plant/microorganisms, plants/insects and factors such as age, development and abiotic factors including light, temperature, rainfall, nutrition, season and collection time (MORAIS, 2009).

Monoterpenes present in the oil, such as limonene, citronellal and geraniol activate plant chemical defenses against insect pests (IBRAHIM et al., 2001; HUMMELBRUNNER; ISMAN, 2001; GALLARDO et al.,

2012). Furthermore, geraniol has anti-septic activity, inhibiting the growth of fungi and bacteria (CHEN; VIJOEN, 2010).

Table 1 – Chemical components of the essential oil of citronella grass, *Cymbopogon winterianus*, presented as calculated and tabulated Kovats indices and percentage of the compound.

Compound	KI cal ^a	KI tab ^b	% Area
Limonene	1036	1031	0.95
Linalool	1104	1098	0.67
Isopulegol<neo>	1153	1145	1.53
Citronellal	1161	1153	23.62
Citronellol	1236	1228	17.10
Neral	1249	1240	0.49
Geraniol	1263	1255	28.62
Geranial	1278	1270	0.63
Terpinen-4-ol acetate	1341	1340	5.72
citronella acetate	1360	1354	1.09
NI ^c	1362	–	3.33
Eugenol	1365	1356	0.15
Geranyl Acetate	1383	1389	1.69
Beta-Elemene	1397	1391	0.53
Germacrene-D	1485	1480	0.83
Delta-Cadinene	1531	1524	1.25
Elemol	1557	1549	5.27
NI ^c	1583	–	1.45
Beta-Eudesmol	1641	1649	0.52
Torreal	1651	1645	1.44
alpha -Cadinol	1663	1653	2.96

^aCalculated Kovats Index. ^bTabulated Kovats Index (ADAMS, 2007). ^cUnidentified.

Insecticide activity test

The citronella grass essential oil at a concentration of 1% (w v⁻¹) was toxic to both pests evaluated, however a higher corrected mortality (96.9 ± 1.57%) (mean ± standard error) was observed for *M. persicae* than *F. schultzei* (34.3 ± 3.77%) (F_{1,18} = 235.96; P < 0.0001). In the control with *F. schultzei*, the observed mortality was 5.7 ± 3.67% and in the control with *M. persicae*, the observed mortality rate was 8.0 ± 2.92%.

The insecticidal activity of *C. winterianus* essential oil (500 µg L⁻¹) was evaluated against *Aedes aegypti* (L.) (Diptera: Culicidae) larvae and presented 100% mortality

after 48 h (MENDONÇA et al., 2005). Furthermore, the acaricide action of the principal components of the essential oil of *C. winterianus* was evaluated separately against engorged adult females and larvae from the tick *Boophilus microplus* (Canestrini, 1887), which showed that citronellal and geraniol have significantly stronger action than citronellol, indicating a shared action between them (MARTINS, 2006).

In addition to mortality, essential oils can cause deformations in different stages of insect development, repellence and deterrence. When essential oils interact with the integument of insects, it may affect digestive and neurological enzymes (ISMAN, 2006).

Estimate of the lethal concentration (LC)

The LC₅₀ of the citronella grass essential oil on *M. persicae* was 0.36% and the confidence interval at 95% probability was 0.30 to 0.41. The LC₉₀ was 0.66% and the confidence interval was 0.57 to 0.81. The slope of the concentration-response curve of the citronella grass essential oil on *M. persicae* was high (4.79). High values of the slope of the concentration-response curve indicate that a small variation in concentration of the essential oil promotes large variations in mortality. The number of insects used in the test was 360, with five degrees of freedom and a chi-squared value of 4.21.

Citronella grass essential oil applied to *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) at 0.5% sprayed on the surface of a Petri dish caused nearly 100% larval mortality (LABINAS; CROCOMO, 2002). When cardboard disks were immersed for five minutes in *C. winterianus* essential oil at a concentration of 0.2% (v v⁻¹) and offered to the termite species *Nasutitermes corniger* Motschulsky (Isoptera: Termitidae), 100% insect mortality was observed after 24 h (SOARES et al., 2008).

Values of the LC₅₀ and LC₉₀ calculated for citronella grass essential oil on larvae of *A. aegypti* were 98 and 172 µg L⁻¹, respectively (MENDONÇA et al., 2005). In another study at a concentration of 50 µg L⁻¹, this oil caused 60% mortality in *A. aegypti* larvae after 24 h (AMER; MEHLHORN, 2006). Essential oils also have a repellent effect on various pests (LABINAS; CROCOMO, 2002; ISMAN, 2006), which is an important feature of virus vectors such as those studied in this work.

Verifying insecticidal activity in essential oils and determining their active components may serve as a basis for creating new commercial insecticides, seeking to develop and expand alternative methods to increase production in agroecosystems and reduce synthetic insecticide use.

CONCLUSIONS

Results obtained show that the essential oil of citronella grass at 1% (w v⁻¹) caused significant mortality to *M. persicae* (96.9 ± 1.57%), and was less toxic to *F. schultzei* (34.3 ± 3.77%). The LC₅₀ value for *M. persicae* was 0.36% and LC₉₀ 0.66%. Therefore, this essential oil is a promising option for developing pesticides to manage *M. persicae*.

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