ARTHUR VIEIRA RIBEIRO

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

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APROVADA: 20 de fevereiro de 2017.

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CONTENTS

ABSTRACT

RIBEIRO, Arthur Vieira, M.Sc., Universidade Federal de Viçosa, February, 2017. ***Corymbia* and *Eucalyptus* essential oils with insecticide activity to *Ascia monuste* and its selectivity to two non-target organisms**. Adviser: Marcelo Coutinho Picanço.

Due to an increase in environmental and health issues because of the excessive use of synthetic pesticides, many studies are being developed in order to select plant essential oils (EOs) for pest control. These compounds are pointed as safe control agents, since they have low toxicity to non-target organisms and are non-persistent in the environment. Thus, this study was carried out with the aim to select potential EOs from plants of the Myrtaceae family for the control of *Ascia monuste* and to evaluate their selectivity to two non-target organisms (*Solenopsis saevissima* and *Tetragonisca angustula*). Twelve EOs, extracted by hydrodistillation from *Corymbia* and *Eucalyptus* plants, were tested in this study. The terpenes 1,8-cineole, α-pinene, citronellal, ρ-cymene, α-eudesmol and α-phellandrene were the most common compounds (identified by GC-MS/FID). All toxicity bioassays were performed by topical application. *C. citriodora* EO had the highest insecticidal activity against *A. monuste* (LD_{50} = 20.61 μg. mg^{-1}), and also presented a fast action (LT_{50} < 10 minutes). Citronellal was the main compound of *C. citriodora* EO (86.8% of the oil constitution) and exhibited toxicity similar to this EO (LD_{50} = 22.44 μg. mg^{-1}). Hence, the toxicity of the *C. citriodora* EO is mostly explained by the citronellal activity. This EO was selective in favor of the predatory ant *S. saevissima* but caused high mortality of the pollinator bee *T. angustula*. Therefore, *C. citriodora* EO is a promising model to the development of insecticides against *A. monuste*. However, its application must rely on the principles of ecological selectivity, aiming to mitigate its impact over pollinators.

RESUMO

RIBEIRO, Arthur Vieira, M.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Óleos essenciais de *Corymbia* e *Eucalyptus* com atividade inseticida à *Ascia monuste* e sua seletividade a dois organismos não-alvo**. Orientador: Marcelo Coutinho Picanço.

Com o aumento dos problemas ambientais e de saúde pelo uso excessivo de pesticidas sintéticos, pesquisas vêm sendo realizadas com o intuito de selecionar óleos essenciais (OEs) de plantas para o controle de pragas. Estes compostos são apontados como uma forma segura de controle, pois apresentam baixa toxicidade a organismos não-alvo e menor efeito residual. Assim, este estudo foi realizado com o objetivo de selecionar potenciais OEs de plantas da família Myrtaceae para o controle de *Ascia monuste* e avaliar a seletividade a dois organismos não-alvo (*Solenopsis saevissima* e *Tetragonisca angustula*). Doze OEs, extraídos por hidrodestilação de plantas dos gêneros *Corymbia* e *Eucalyptus*, foram testados neste estudo. Os componentes mais comuns foram 1,8-cineol, α-pineno, citronelal, ρ-cimeno, α-eudesmol e α-felandreno (identificados por CG-EM/DIC). Todos os bioensaios de toxicidade foram realizados através de aplicação tópica. O OE de *C. citriodora* apresentou a maior atividade inseticida contra *A. monuste* (DL_{50} = 20.61 µg. mg^{-1}), além de uma rápida ação (TL_{50} < 10 minutos). O citronelal foi o constituinte majoritário do OE de *C. citriodora* (86.8% da constituição do óleo) e mostrou toxicidade similar a este OE (DL_{50} = 22.44 µg. mg^{-1}). Logo, a toxicidade do OE de *C. citriodora* é explicada, sobretudo, pela atividade do citronelal. Este OE foi seletivo em favor da formiga predadora *S. saevissima*, mas causou alta mortalidade à abelha polinizadora *T. angustula*. Portanto, o OE de *C. citriodora* é um modelo promissor para o desenvolvimento de inseticidas contra *A. monuste*. Entretanto, sua aplicação deve ser realizada de acordo com os princípios da seletividade ecológica, mitigando seu impacto sobre polinizadores.

INTRODUCTION

Plant EOs have been used since antiquity, mainly for medical purposes (Raut and Karuppayil, 2014; Schmidt, 2009) and because of their many properties such as insecticidal, fungicidal, bactericidal, virucidal, acaricidal (Bakkali et al., 2008; Koul et al., 2008; Regnault-Roger et al., 2012), nematicidal (Renco et al., 2014) and herbicidal (Amri et al., 2013) activities. Those substances are volatile secondary metabolites from aromatic plants that may act in their direct defenses against herbivores and pathogens, or in indirect defenses as communicative substances to attract other organisms (Franz and Novak, 2009; Zuzarte and Salgueiro, 2015). EOs are complex mixtures of organic substances produced by secretory cells, ducts or cavities, glandular hairs or trichomes, and stored in a wide range of plant tissues (Başer and Demirci, 2007; Franz and Novak, 2009; Sangwan et al., 2001). Their constitution varies largely among plant species, and are generally classified as terpenes, terpenoids and phenolic compounds (Pavela, 2015; Pavela and Benelli, 2016).

Due to an increase in environmental and health issues because of indiscriminate use of synthetic pesticides, the adoption of EOs became more attractive, especially by the consumers' growing demand for safer food and products (Ebadollahi, 2011). Several studies have reported their larvicidal, adulticidal and antifeedant activity, deterrent effects, repellent action and insect cycle alteration (Tripathi et al., 2009). Especially on horticulture crops, EOs are desirable in pest management programs since they are non-persistent in the environment, are usually selective to non-target insects and have low toxicity to humans (Dey and Gupta, 2016).

The Brassicaceae family is one of the major horticulture crops worldwide. In America, the butterfly *Ascia monuste* (Godart) (Lepidoptera: Pieridae) is an important pest of crops from this family, causing losses up to 100%, often related to population outbreaks (Alam, 1992). Therefore, considering the suitability of EOs use in horticulture crops, they have high potential to be incorporated in *A. monuste* Integrated Pest Management (IPM).

In agroecosystems, *A. monuste* populations are regulated by natural control agents like the generalist predatory ant *Solenopsis saevissima* (Ramos et al., 2012). Hence, an *A. monuste* IPM might consider the EOs selectivity to *S.*

saevissima. Furthermore, during the flowering growth stage, some Brassicaceae plants, such as Canola (*Brassica napus*, *Brassica rapa* or *Brassica juncea*), are important nectar and pollen sources for pollinator insects (Westcott and Nelson, 2001; Woodcock et al., 2013). This fact enhances the necessity to assess EOs selectivity to these insects, including the bee *Tetragonisca angustula*, an important generalist pollinator in tropical regions (Iwama and Melhem, 1979; Morgado et al., 2011).

Currently, many studies report the effects of EOs from plants of the Myrtaceae family (which includes both *Corymbia* and *Eucalyptus* genera) to several organisms, such as insect pests (Batish et al., 2008; Ebadollahi, 2013). However, no study has yet tested the effect of Myrtaceae plants EOs in *A. monuste*, nor their selectivity to *S. saevissima* and *T. angustula*.

Thus, this study was carried out with the goal to select potential EOs from plants of the Myrtaceae family and evaluate their toxicity against *A. monuste* and two non-target organisms (*S. saevissima* and *T. angustula*).

MATERIAL AND METHODS

Plant material

EOs were extracted from leaves of three species of *Corymbia* (*C. citriodora*, *C. henryi* and *C. maculata*) eight of *Eucalyptus* (*E. andrewsii*, *E. resinifera*, *E. sphaerocarpa*, *E. phaeotricha*, *E. cinerea*, *E. pyrocarpa*, *E. punctata* and *E. siderophloia*) and a hybrid (*E. alba* x *E. tereticornis*). The plants were around 30 years old and growled at the campus of the Federal University of Viçosa (UFV), Viçosa, Minas Gerais State, Brasil (20°48'45" S, 42°56'15" W, 600 m above sea level and tropical weather). The leaves used for EO extractions were collected at the end of the dry season, in November 2013.

Extraction and analysis of EOs

The EOs were extracted by hydrodistillation for a period of three hours, in triplicate, using a modified Clevenger apparatus. Each replicate was 100 g of leaves (cut in small pieces) mixed in 1 L of distilled water. The plant material was stored in a freezer at -15 ± 4 °C until complete the extraction procedure. The EOs were stored in glass amber vials and maintained at -5 ºC until the analysis of chemical composition.

Analysis of EOs components was performed by gas chromatography (Shimadzu GC-17A) coupled to flame ionization detection (quantification) and mass spectrometry (identification). The quantification of the EOs components was accomplished using a RTX-5 fused silica column (30 m × 0.25 mm, film thickness of 0.25 μm) at a constant nitrogen flow rate of 1.8 mL. min^{-1}. The oven's initial temperature was programmed to be 40 °C (isothermal for 4 min), followed by an increase of 3 °C. min^{-1} up to 240 °C, remaining isothermal at this temperature for 15 min. The sample injection volume was 1.0 μL (10 mg. mL^{-1} in dichloromethane) with a split ratio of 1:10 and column pressure of 115 kPa. The concentration of each component was calculated as the percentage of its corresponding peak area in relation to the total area of all peaks observed in the chromatogram.

The identification of EOs components was performed at the same chromatographic conditions used for the quantification process, except for the carrier gas and column pressure: helium and 100 kPa, respectively. The EO components' retention indexes (RI) (Adams, 2007) were compared to a standard alkane series (C9-C26) and their mass spectrum compared with those on record in the Wiley library database (Wiley 7.0 and NIST 11) or in the literature.

General experimental condition

Toxicity bioassays were conducted with second-instar larvae of *A. monuste* and adults of the predatory ant *S. saevissima* and the pollinator bee *T. angustula* at the Integrated Pest Management Laboratory of the UFV. The larvae of *A. monuste* were obtained from a rearing maintained in a greenhouse located in the UFV campus following the methodology described in Bastos et al. (1997) and Neves et al. (1996). The adults of the ants and the pollinator bee were collected from natural nests located at the UFV campus.

The experimental design of the bioassays was completely randomized. Each replication was a Petri dish (9 cm in diameter x 2 cm in height) containing 10 insects. Each individual insect was exposed to 0.5 μL of solution (each EO diluted with acetone as solvent), topically applied, via a Hamilton microsyringe. The EOs and solvent quantities, for each treatment, varied accordingly to the dose tested and the average weight of each insect. The solvent acetone (99.5%, Vetec) was used as negative control.

The average weight of each insect was determined by measuring, on an analytical balance, the mass of 30 insects (Gehaka, AG200). The Petri dishes were placed in a biochemical oxygen demand (BOD) incubator at 25 ± 5 °C, relative humidity of 75 ± 5% and a photoperiod of 12 h.

Screening toxicity bioassay

A screening bioassay was performed to select the most lethal EOs against *A. monuste*. Each treatment consisted of an EO in the dose of 30 µg of EO. mg^{-1} of insect, with four replicates. This dose was adopted because, on bioassays to select substances with insecticidal activity, the doses from 10 to 50 µg of EO. mg^{-1} of insect are used (Alvarenga et al., 2012; Moreira et al., 2007). The insecticidal activity of a commercial neem oil formulation (azadirachtin A/B 12 g. L^{-1}, E.I.D. Parry Limited) was evaluated as a positive control. Insect mortality was recorded 72 h after exposure to treatments. This moment was used for evaluations because we previously verified that the mortality stabilized after this period.

Insects were considered dead when they did not move after touched by a fine brush. EOs that caused mortality higher than 80% were selected for subsequent bioassays, since it is the criteria used in Brazil to consider an insecticide efficient to its registration (Bacci et al., 2007).

C. citriodora* EO constituents against *A. monuste

A toxicity bioassay was performed in order to verify the possible synergism effect between the *C. citriodora* EO constituents against *A. monuste*. The treatments in this bioassay were the LD_{90} of *C. citriodora* and its constituents, isolated, in the corresponding dose that they occur in the oil, with six replicates. Mortality was evaluated 72 h after exposure to treatments. Insects were considered dead when they did not move after touched by a fine brush.

Citronellal, citronellol, isopulegol, α-pinene, β-pinene and *trans*-caryophyllene were purchased from Sigma-Aldrich and used as received.

Lethal dose for *A. monuste*

The treatments used in these tests consisted of *C. citriodora* EO and its major constituent (e.g., citronellal) and azadirachtin, with six replicates. The tested doses varied from 1 to 50 µg. mg^{-1} of insect.

Mortality was evaluated 72 h after exposure to treatments. Insects were considered dead when they did not move after touched by a fine brush.

Lethal time for *A. monuste*

The treatment used in this bioassay was the LD_{90} of *C. citriodora* EO, with 10 replicates. Mortality was evaluated continually from the moment of treatment exposure up to 10 min, then every minute up to 30 min, followed by 30 min of interval up to 120 h after exposure. Insects were considered dead when they did not move after touched by a fine brush.

Selectivity to non-target organisms

In this test, adults of *S. saevissima* (ant natural predator) and *T. angustula* (pollinator bee) were exposed to LD_{90} of *C. citriodora* EO, with six replicates. A mixture of water (50%) and honey (50%) and pure water were added to the petri dishes as a food source.

Mortality was evaluated 72 h after exposure to treatments. Insects were considered dead when they did not move after touched by a fine brush.

Statistical analysis

Mortality data observed in the screening bioassay were submitted to analysis of variance and treatment means were grouped by the Scott-Knott test at $P < 0.05$ (Scott and Knott, 1974) using R-Studio software version 0.99. 896. A correlation analysis between the EOs mortalities and all of its constituents was conducted using Sigma Plot software version 12.5.

For synergism bioassay, to verify if there was a significant difference between treatments mortality, an ANOVA followed by Tukey's test at $P < 0.05$ was performed using SAS software (PROC GLM, SAS).

The results from lethal dose bioassays were submitted to a Probit analysis using SAS software (PROC PROBIT, SAS). Observed mortality in the treatments was corrected to that occurred in the control. Curves with probabilities greater than 0.05 by the χ^2 test were accepted. The relative toxicity index (obtained dividing the LD_{50} values by the LD_{50} of azadirachtin) was used

to compare LD_{50} values of *C. citriodora* EO and its major constituent citronellal to the LD_{50} value of azadirachtin.

Survival analysis was performed using the Kaplan-Meier estimators to obtain the survival curves in the lethal time bioassay. Survival curves were compared by log-rank test. This procedure was carried out on Sigma Plot software version 12.5.

The mortality observed in the selectivity bioassays was analyzed with the unpaired Student's t-test using SAS software (PROC TTEST, SAS).

RESULTS

Chemical composition of the EOs

The chemical composition of EOs has a great variability among the plant species. Fifty-five compounds were identified and quantified, representing 89.3% to 97.5% of the EOs composition. The most common compounds found were 1,8-cineole (3.5-70.8%),α -pinene (0.5-68.1%), citronellal (86.8%), ρ-cymene (1.1-19.7%), α-eudesmol (0.7-30.9%) and α-phellandrene (6.4-15.1%).

Screening toxicity bioassay

There was a significant difference in larvae mortality between treatments in the screening bioassay ($F_{13;43}$ = 33.69; P < 0.001). Only the *C. citriodora* EO exhibited mortality higher than 80% and it was grouped with the positive control azadirachtin, according to the Skott-Knott test (P < 0.05) (Figure 1). Thus, only azadirachtin and *C. citriodora* EO along with its constituents were used in the subsequent bioassays.

There was a significant and positive correlation between the mortality caused to larvae of *A. monuste* by the EOs and the constituents isopulegol (r = 0.753, P = 0.00473), citronellal (r = 0.753, P = 0.00473), citronellol (r = 0.753, P = 0.00473) and *trans*-caryophyllene (r = 0.618, P = 0.0322).

C. citriodora EO constituents against *A. monuste*

There was a significant difference in larvae mortality between treatments ($F_{7;47}$ = 94.59; P < 0.001) in the synergism bioassay. Citronellal mortality was statistically similar to that occurred upon exposition to *C. citriodora* EO. The other treatments did not differ from the control group (Fig. 2).

Lethal dose for *A. monuste*

Both *C. citriodora* EO and citronellal showed similar curve slopes (Fig. 3). The positive control azadirachtin has lower curve slope compared to the curve slopes of *C. citriodora* EO and citronellal (Table 2). Azadirachtin has the lowest LD_{50} (5.44 µg. mg^{-1}). *Corymbia citriodora* EO is 3.79 times less toxic (LD_{50} = 20.61 µg. mg^{-1}) than the commercial product azadirachtin, followed by its main constituent citronellal (4.12 times less toxic) (Table 2).

Lethal time for *A. monuste*

Corymbia citriodora EO at the dose of 39.98 µg. mg^{-1} reduced significantly the survival of *A. monuste* (Log-Rank χ^2 =141.4, df = 1, $P < 0.001$) (Fig. 4). The time required to kill 50% of *A. monuste* larvae was 0.2 h.

Selectivity to non-target organisms

The dose of 39.98 µg. mg^{-1} of *C. citriodora* EO caused no significant mortality to *S. saevissima* compared to the control ($P = 0.484$). On other hand, the same dose caused mortality of 100% to *T. angustula*, which is significantly higher than the control and the mortality to *S. saevissima* ($P < 0.001$) (Fig. 5).

DISCUSSION

The chemical composition of EOs of plants is highly variable. Several studies have pointed to this chemical variability, associating the EOs composition with their toxicity to several organisms (Benelli et al., 2017; Cheng et al., 2009; Ebadollahi, 2016). This occurs because more than one compound can cause insect mortality, acting on more than one site of action (Bakkali et al., 2008; Isman, 2000). Chemical composition of EOs is dependent on biotic and abiotic factors, such as taxa, season, growing conditions, phenological stage, genetic factors, and the EO extraction's method (Masotti et al., 2003; Sampaio et al., 2016; Yang et al., 2005). In this work, a great variability was observed in the chemical composition of the EOs from *Corymbia* and *Eucalyptus* species, with the predominance of monoterpenes and sesquiterpenes compounds.

Among the 12 plant species evaluated in this study, only *C. citriodora* has shown to be promising in the control of *A. monuste*, causing similar

mortality to the positive control azadirachtin. In fact, many studies have reported the potential of *C. citriodora* to control insect pests such as *Spodoptera frugiperda* (Souza et al., 2010), *Sitophilus zeamais* (Ootani et al., 2011) and *Nasutitermes corniger* (Lima et al., 2013), associating this activity with the main constituents of the EO, the citronellal and citronellol.

The components isopulegol, citronellal, citronellol and trans-caryophyllene seem the most responsible for *A. monuste* mortality. These constituents were identified only in *C. citriodora* EO, thus, the insecticidal activity of this oil is probably associated with the presence of such compounds. However, only the citronellal caused significant mortality to *A. monuste* (Fig. 2). This constituent represents, by itself, more than 85% of the chemical composition of the *C. citriodora* EO (Table 1). According to Bakkali et al. (2008), the biological properties of EOs are usually related to the activity of its major components. Here, the dose-mortality curve of citronellal was similar to that of the EO (Fig. 3), with similar slopes, indicating the occurrence of this effect. On the other hand, the low mortality caused by the other constituents might be due to the low concentration of these compounds in the EO. Moreover, many other studies indicated the potential of citronellol (Kaufman et al., 2010), isopulegol (Lee et al., 2003) and *trans*-caryophyllene (Yang and Lee, 2012) in the control of pest organisms. In this context, studies should be carried out to explore the insecticidal activity of these other substances.

Lower curve slope indicates that the substance presents, proportionally, higher variation in the dose for the same increase in mortality, which guarantees minor errors associated with its application (Blazka, 2007). However, azadirachtin is a formulated commercial product. These formulations involve the addition of substances such as solvents and adjuvants that alter the physicochemical characteristics of the insecticide (e.g., increased cuticle penetration) (Rathburn, 1985) and, consequently, increase control effectiveness. Thus, the study of insecticidal formulations containing *C. citriodora* EO is recommended.

The toxicity of EOs to insects has been attributed to terpenes, terpenoids and related phenolic compounds (Hummelbrunner and Isman, 2001; Isman and Machial, 2006). In addition, the neurotoxic pathway has being indicated as the mode of action of these compounds, and the probable sites of action are the octopaminergic and cholinergic systems and GABA receptors (Abdelgaleil et al.,

2016; Isman, 2004; Tong and Coats, 2012). The rapid insecticidal activity (LT_{50} = 0.2 h) observed for *A. monuste* (Fig. 4) indicates the neurotoxic activity of the *C. citriodora* EO. This quick effect is desirable for pest control, especially in the occurrence of population outbreaks (Berryman, 1982).

The effect of the *C. citiodora* EO on non-target organisms showed selectivity to the predatory ant *S. saevissima*, but not to the pollinator bee *T. angustula* (Fig. 5). Therefore, it is recommended that the control *A. monuste* with this EO must follow the principles of ecological selectivity (Hull and Beers, 1985), in which the application of insecticides should be carried out at dusk to avoid the period of higher pollinator activity (i.e., warmer periods of the day) (de Bruijn and Sommeijer, 1997). Additionally, EOs have low persistence in the environment due to their rapid degradation, which also contributes to their selectivity (Moreno et al., 2012). Furthermore, the development of formulations that provide more stability of *C. citriodora* EO against environmental variability is also desirable in order to ensure the control efficiency (Pinto et al., 2016; Răileanu et al., 2013).

In addition to the toxic activity, repellent and feeding deterrent effects of *C. citriodora* EO were also observed for some insects (Maia and Moore, 2011; Olivero-Verbel et al., 2010; Souza et al., 2010). This fact increases the possibility that these effects may occur to the pest, as well as to its natural enemies and pollinators. Hence, it is necessary the development of studies to assess these possible effects.

The EO of *C. citriodora* and its major constituent citronellal presented potential as natural insecticides against *A. monuste*. The use of the EO for pest control is promising since it is already a commercialized product, mainly for the cosmetic and pharmaceutical industry. Another prospect is the utilization of the citronellal isolated. It has similar toxicity to the EO and the advantage of a regular concentration, which may vary in the EO.

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Table 1. Chemical composition (%) of EOs from 12 aromatic plant species.

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	RI_c	RI
α-Thujene	-	1.4	-	0.5	-	-	-	-	-	0.3	1.5	2	924	924
α-Pinene	68.1	10.9	9.8	27.2	0.6	9.7	40.6	7.7	14	1.9	2.2	0.5	930	932
Camphene	-	-	-	-	-	0.5	-	-	-	-	-	-	943	946
β-Pinene	0.6	0.5	-	0.5	0.9	-	0.7	-	0.4	0.8	0.6	-	972	974
β-Myrcene	-	-	-	0.9	-	-	-	-	-	-	1.5	1.3	990	988
α-Phellandrene	-	6.4	-	6.8	-	-	-	-	-	-	15.1	9.3	1002	1002
α-Terpinene	-	1.4	-	0.5	-	-	-	-	-	0.4	1.2	1.9	1014	1014
p-Cimene	-	13.5	-	3	-	12.9	-	-	2.5	1.1	16.7	19.7	1022	1020
β- Phellandrene	-	5.3	-	-	-	-	-	-	-	-	-	14.7	1027	1025
trans-*β*-Ocimene	-	-	-	-	-	-	-	-	-	-	1.8	-	1043	1044
γ-Terpinene	-	-	-	1	-	-	-	-	-	1.1	1.1	-	1057	1054
Terpinolene	-	-	-	-	-	-	-	-	-	-	0.6	-	1086	1086
1,8-Cineole	3.5	-	70.8	55.6	-	59.3	48.2	70.7	11.9	41.8	37.9	-	1029	1026
Linalol	-	1.5	-	-	-	-	-	-	-	-	1	1.2	1100	1095
endo-Fenchol	-	-	-	-	-	0.5	-	-	-	-	-	-	1111	1114
Diydro-sabina ketone	-	5.9	-	-	-	-	-	-	-	-	3.1	10	1122	1117
trans-Pinocarveol	-	-	-	-	-	3.4	-	-	-	-	-	-	1136	1135
trans-*p*-Menth-2-en-1-ol	-	-	-	-	-	-	-	-	-	-	2.1	-	1138	1136
cis-*β*-Terpineol	-	4	-	-	-	-	-	-	-	-	-	6.8	1140	1140
Isopulegol	-	-	-	-	4.7	-	-	-	-	-	-	-	1143	1145
Citronellal	-	-	-	-	86.8	-	-	-	-	-	-	-	1154	1148
Pinocarvone	-	-	-	-	-	1.7	-	-	-	-	-	-	1160	1160
Borneol	-	-	-	-	-	1.1	-	-	-	-	-	-	1164	1165
Terpinen-4-ol	-	3.1	-	0.7	-	0.4	-	-	0.9	1.2	3.3	4.4	1175	1174
α-Terpineol	-	1.1	5.5	1	-	2.5	-	5.2	1.1	1.2	2.2	1.5	1190	1186
cis-Piperitol	-	-	-	-	-	-	-	-	-	-	-	2	1194	1195
γ-Terpineol	-	1.2	-	-	-	-	-	-	-	-	-	-	1194	1199
trans-Piperitol	-	-	-	-	-	-	-	-	-	-	1.2	3.2	1207	1207
Citronellol	-	-	-	-	3.3	-	-	-	-	-	-	-	1229	1223
α-Terpinyl acetate	-	-	8.5	-	-	1.8	-	-	-	-	3	-	1349	1346
trans-Methyl cinnamate	-	-	-	-	-	-	-	-	-	-	-	7.2	1384	1376
α-Gurjunene	0.6	-	-	-	-	-	-	-	-	-	-	-	1408	1409

Table 1. Continued.

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	RI_c	RI
trans-Caryophyllene	-	-	-	-	1.2	0.5	-	-	-	-	-	-	1417	1417
Aromadendrene	3	0.9	-	-	-	-	-	3.5	-	-	-	-	1437	1439
Alloaromadendrene	0.7	0.6	-	-	-	-	-	-	-	-	-	-	1459	1459
Viridiflorene	1.2	-	-	-	-	-	-	-	-	-	-	-	1494	1496
α-selinene	-	1.6	-	-	-	-	-	-	-	-	-	-	1496	1498
Germacrene B	0.4	-	-	-	-	-	-	-	-	-	-	-	1563	1559
Elemol	-	-	-	-	-	-	-	-	0.6	13.1	-	-	1550	1548
Spathulenol	-	2.5	-	-	-	-	-	-	0.6	1.2	1	1.1	1578	1577
Caryophyllene oxide	-	-	-	-	-	-	2.5	4.8	-	-	-	-	1583	1582
Thujopsan-2-*α*-ol	-	1.4	-	-	-	-	-	-	-	-	-	-	1586	1586
Viridiflorol	0.7	0.9	-	-	-	-	-	-	-	-	-	-	1592	1592
Guaiol	8.8	-	-	-	-	-	-	-	-	-	-	-	1597	1600
10-*epi*-*γ*-Eudesmol	0.9	0.1	-	-	-	-	1.3	-	-	0.8	-	-	1622	1622
γ-Eudesmol	-	0.5	-	-	-	-	-	-	15.1	8.9	-	2.8	1632	1630
α-Acorenol	-	6.6	-	-	-	-	-	-	-	-			1629	1632
Hinesol	-	1.3	-	-	-	-	-	-	-	1.1	-	-	1640	1640
β-Eudesmol	-	-	-	-	-	0.6	-	-	-	-	-	2.5	1650	1649
α-Eudesmol	0.7	8.6	-	-	-	-	-	-	30.9	10.5	-	2.6	1653	1652
Valerianol	-	-	-	-	-	-	-	-	-	8.4	-	-	1658	1656
Selin-11-en-4-*α*-ol	-	8.1	-	-	-	-	-	-	-	-	-	-	1656	1658
7-*epi*-*α*-Eudesmol	0.4	-	-	-	-	-	-	-	15.6	-	-	-	1660	1662
Bulnesol	2.9	-	-	-	-	-	-	-	-	1.6	-	-	1670	1670
Total identified (%)	92.5	89.3	94.6	97.7	97.5	94.9	93.3	91.9	94.2	96.6	97.1	94.7	-	-

Plant species: I = *Corymbia maculata*; II = *Eucalyptus siderophloia*; III = *Eucalyptus cinerea*; IV = *Eucalyptus punctata*; V = *Corymbia citriodora*; VI = *Eucalyptus resinifera*; VII = *Eucalyptus phaeotricha*; VIII = *Eucalyptus alba* x *Eucalyptus tereticornis*; IX = *Eucalyptus pyrocarpa*; X = *Corymbia henryi*; XI = *Eucalyptus sphaerocarpa*; XII = *Eucalyptus andrewsii*. RI_c = Calculated Retention Index; RI = Retention Index from the literature (Adams, 2007).

Table 2. Toxicity (LD_{50} and LD_{90}) of essential oil of *C. citriodora*, its major constituent citronellal and azadirachtin to *A. monuste* after 72h of exposure to topic application.

Treatment	n	LD_{50} (µg. mg^{-1})[a] (FL_{95})	LD_{90} (µg. mg^{-1})[a] (FL_{95})	χ^2	df	*P*-value	RTI_{50}[b]
C. citriodora	360	20.61 (17.83-22.87)	39.98 (35.38-48.20)	7.46	4	0.113	3.79
Citronellal	360	22.44 (20.28-24.44)	43.86 (38.84-52.27)	2.17	4	0.703	4.12
Azadirachtin	480	5.44 (4.63-6.31)	25.59 (19.70-36.92)	3.02	6	0.805	-

[a] LD: lethal dose (µg of compound. mg^{-1} of insect); FL: 95% fiducial limits.

[b] RTI: Relative toxicity index.

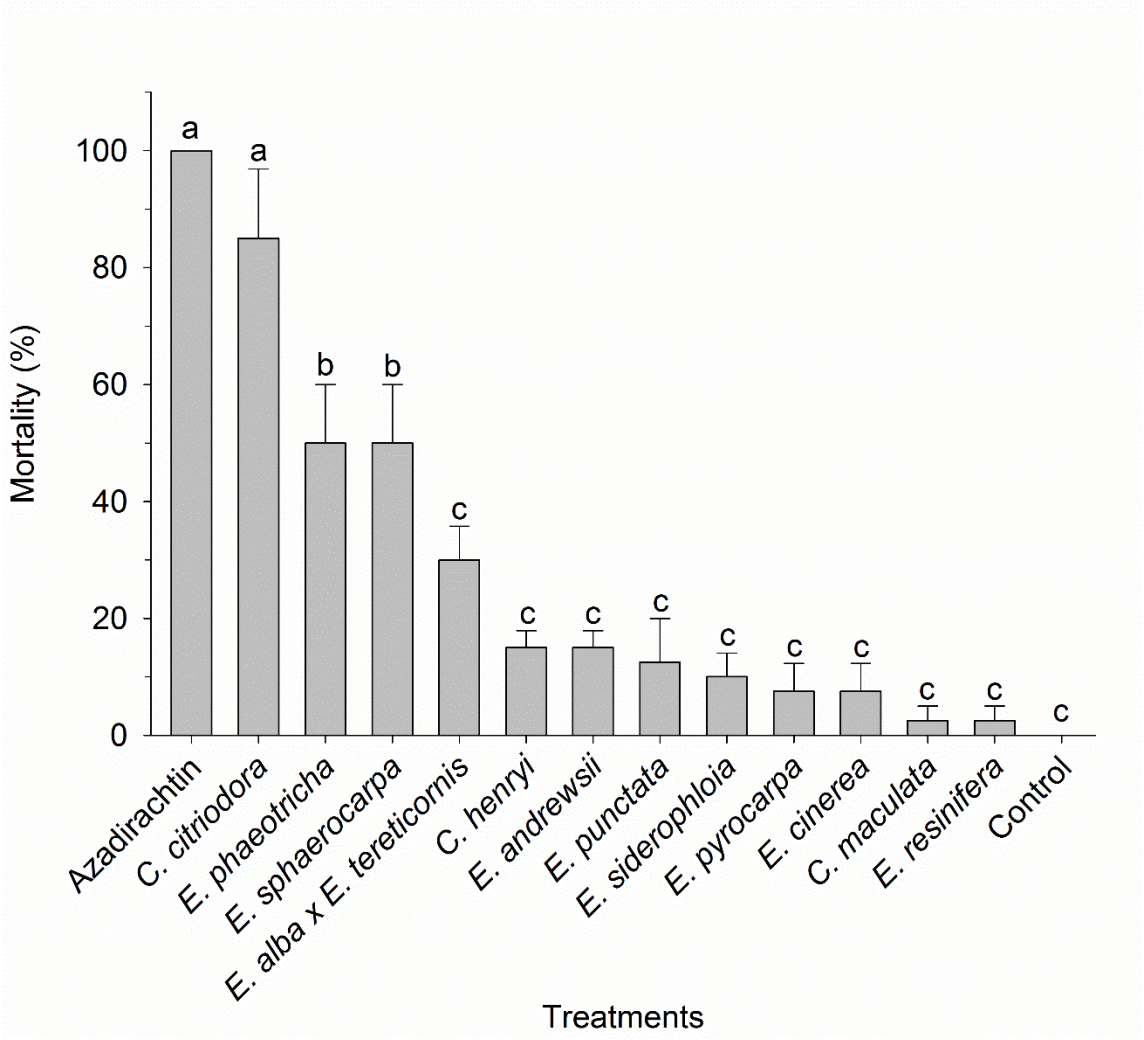

Fig. 1. Mortality (%) of larvae of *Ascia monuste* 72 h after topical exposure to 12 essential oils and azadirachtin at the dose of 30 µg. mg^{-1}. Means followed by the same letter do not differ by the Skott-Knott test ($P < 0.05$).

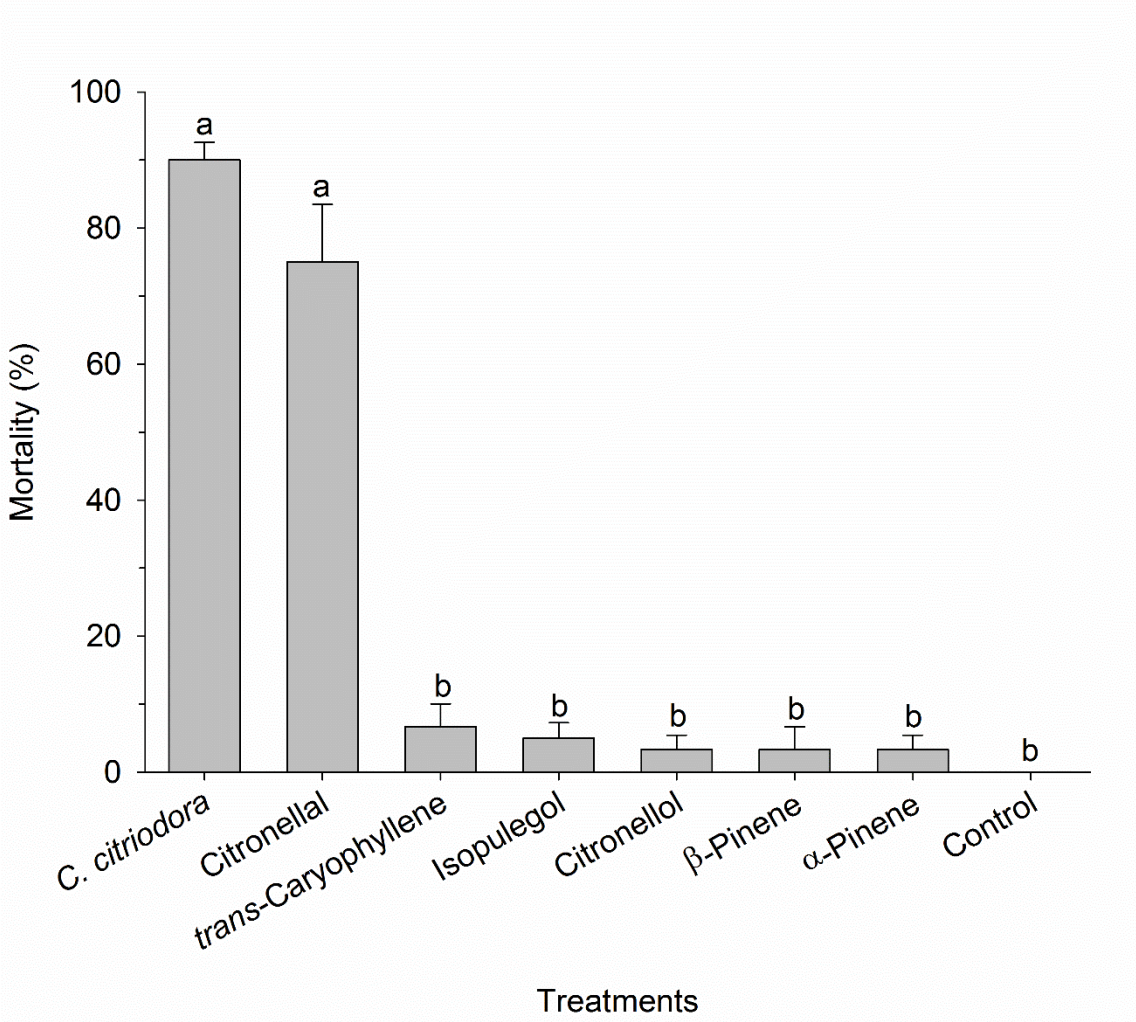

Fig 2. Mortality (%) of larvae of *Ascia monuste* to *Corymbia citriodora* essential oil and its constituents in the corresponded dose that they occur in the oil, 72 h after exposure. Means followed by the same letter do not differ by the Tukey's test ($P < 0.05$).

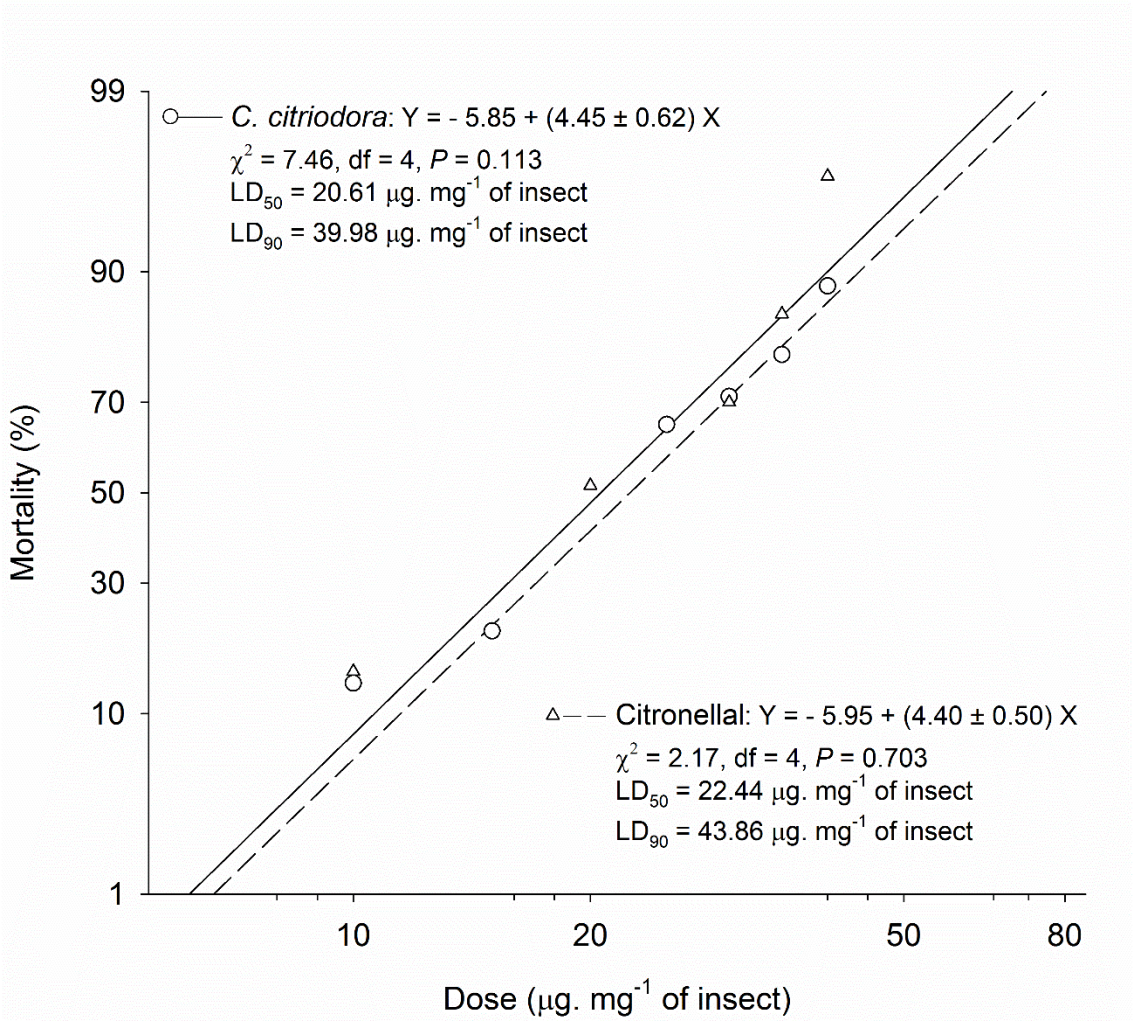

Fig. 3. Dose-mortality regression lines of *Corymbia citriodora* essential oil and its major constituent citronellal to *Ascia monuste*.

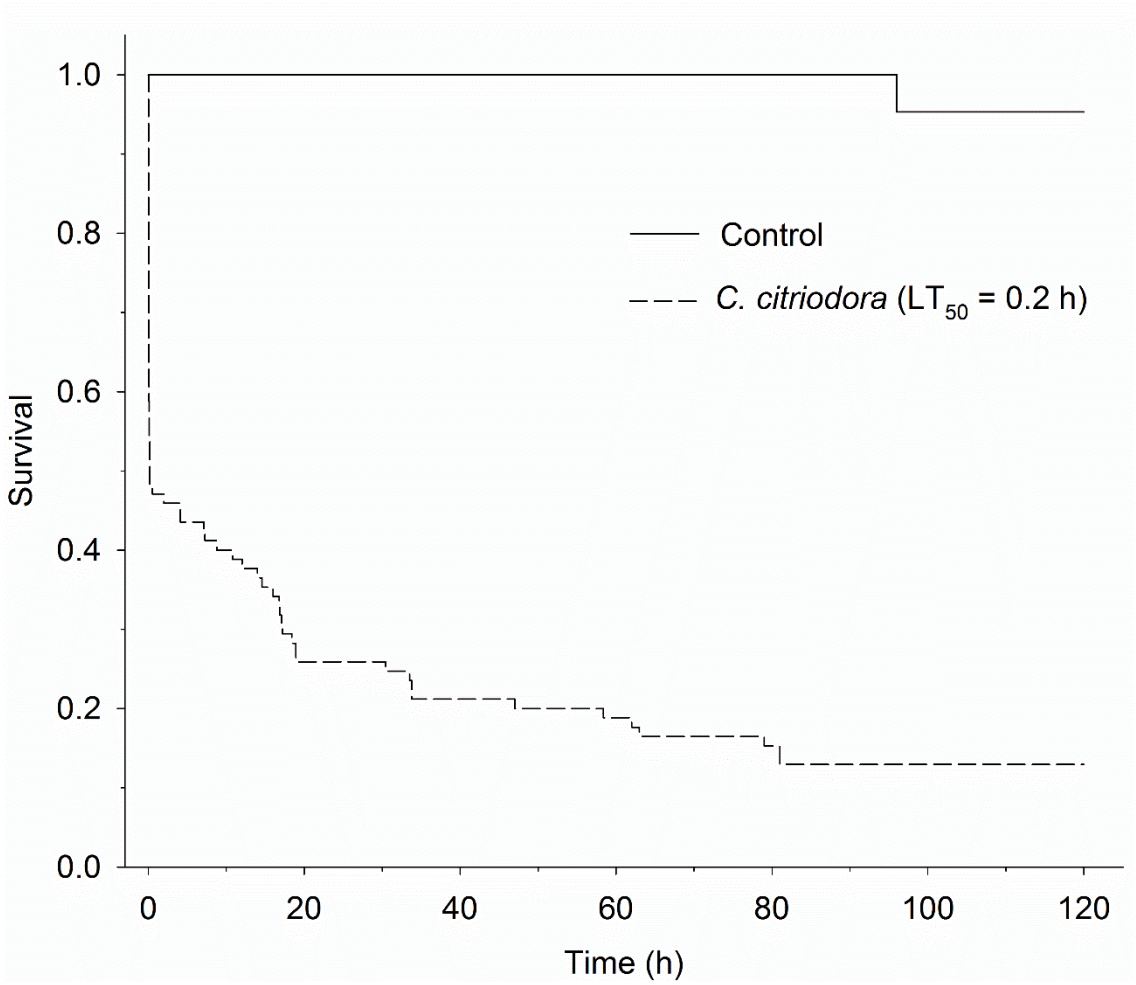

Fig. 4. Survival curves of *Ascia monuste* exposed topically to *Corymbia citriodora* essential oil at the dose of 39.98 µg. mg^{-1}.

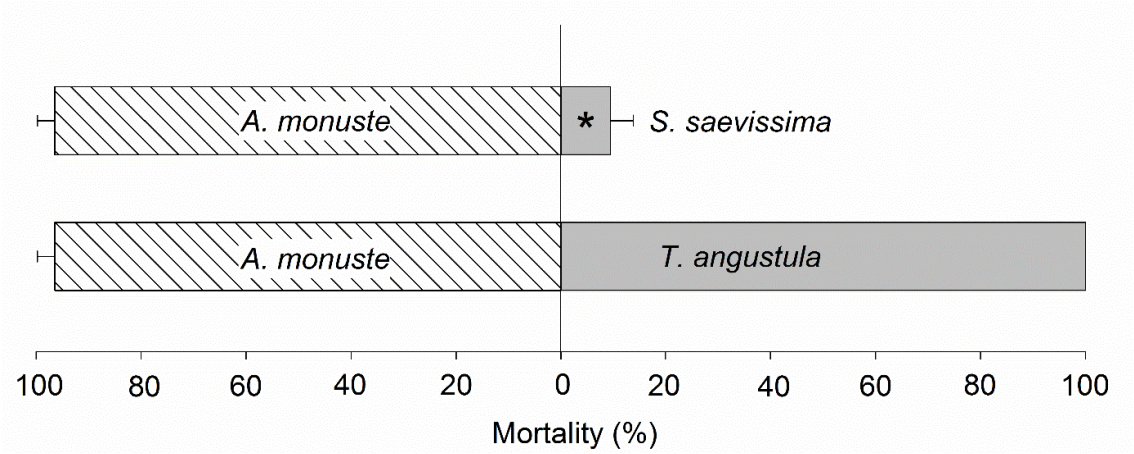

Fig. 5. Mortality (%) caused by DL_{90} (39.98 µg. mg^{-1} of insect) of *Corymbia citriodora* essential oil to the pest *Ascia monuste* and to the non-target organisms *Solenopsis saevissima* and *Tetragonisca angustula*, 72 h after exposure. Histogram followed by * denotes significant difference between species by the Student's t-test ($P < 0.05$).