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Table: 1, Figs : 5

Potential of allelochemicals from basil (*Ocimum micranthum* Willd) to control whitefly (*Aleurodicus cocois* (Curtis, 1846)) in cashew nut crop (*Anacardium occidentale* L.)

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ABSTRACT

We evaluated the potential of essential oil (EO) of basil *Ocimum micranthum* Willd as an insecticide against whitefly *Aleurodicus cocois* (Curtis, 1846) (Hemiptera, Aleyrodidae) and determined its chemical composition using GCMS. The E.O. toxicity was analyzed by spraying it on insects present on dwarf cashew seedlings. EO at 1% concentration exhibited the highest toxicity against *A. cocois* and faster mortality (6.82 h with a confidence interval of 6.34-7.30 h). Eugenol, elemicin, and beta-caryophyllene were the major allelochemicals found in the EOs of *O. micranthum*. We found that the EOs of *O. micranthum* are very promising in controlling the whitefly *A. cocois*.

Keywords: *Aleurodicus cocois*, allelopathy, *Anacardium occidentale*, basil, cashew nut, essential oils, lethal time, mortality, *Ocimum micranthum*, whitefly

INTRODUCTION

Allelopathy has shown its potential for development of new pesticides via allelochemicals such as essential oils (EOs) from plants (14,20,46,47). Many plant species produce noxious allelochemicals, which are toxic to other plant species or herbivores. Furthermore, these allelochemicals can be a potential alternative to conventional synthetic insecticides (44). Most of these allelochemicals are very potent insecticides and are often very specific in low doses. In addition, pesticides from plants are safe alternative to control pests because they have low human toxicity, have high degree of biodegradbility (reduces the risk of adverse ecological effects) and do not induce insecticide resistance (53,56). However, there are few limitations in the production of these allelochemicals as insecticides for pest management on a large scale, due to the lack of studies that evaluate the potential geographical regions favourable for producing the raw materials.

Ocimum micranthum Willd is an annual (family Lamiaceae) and is commonly known as basil (32,33,38,50). *Ocimum* species are extensively used in food and perfume industries (10,54). Furthermore, the allelochemicals from *Ocimum* species are considered as pharmacologically beneficial due to their antioxidant capacity (43) and are toxic to small insects, which may be exploited in pest management. Therefore, we investigated the potential of EOs of *O. micranthum* as source of insecticide against whitefly *Aleurodicus*

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cocois (Curtis, 1846) (family Aleyrodidae). Family Aleyrodidae (Order: Hemiptera) includes small insects, including whiteflies. Worldwide, whiteflies are major pests in agricultural crops (9,18,39) and cause serious problems in several plants species, including ornamental and field crops (9,25).

The *A. occidentale* L. tree produces the cashew apple fruit, which bears a single-seeded nut in its bottom, covered with a hard gray shell. It is major crop of tropical agriculture in several countries [Brazil, India, Indonesia, Vietnam, Nigeria, and Africa (3,5,7,19,57)]. Cashew nut is good source of unsaturated fatty acids, fibre, sterols, vitamins, and amino acids, which are beneficial for human health (49).

The whitefly *A. cocois* (Curtis 1846) (Hemiptera: Aleyrodidae) is pest of cashew nut, mango, avocado, banana, vines, and ornamental plants (27,40). Currently it is the most important pest of cashew *Anacardium occidentale* (Family: Anacardiaceae) in southeast and northeast of Brazil (6,13,34) (Figure 1). Most of the devastating plant diseases are attributed to viruses transmitted by vectors. Some whiteflies can be a vector of several important families of plant viruses (39). In the absence of vectors, these diseases would be of little importance. Therefore, the control measure is dependent on vectors (12).

Considering the insecticidal potential of EO of *O. micranthum* and the lack of insecticides to control the *A. cocois* infestation in cashew crop, this study aimed to evaluate the biotoxicity of EO of *O. micranthum* against whitefly *A. cocois*, to determine its chemical composition, and to find the potential regions for producing insecticides from *O. micranthum* EO.

MATERIALS AND METHODS

Plants of *O. micranthum* were collected from the Medicinal Plants Garden, Empresa Brasileira de Pesquisa Agropecuária Unidade Agroindústria Tropical, Fortaleza, Ceará, Brazil (3°44′S; 38°33′W; 19.5 m above sea level). In this study, we used dwarf cashew seedlings Clone CCP 76 obtained from the experimental field of Unidade Agroindústria Tropical, Pacajus, Ceará, Brazil (4°11’26,62’’S; 38°29’50,78’’W).

I. Extraction and analysis of EO

The EO of *O. micranthum* was extracted by hydrodistillation method using a Clevenger apparatus. Each sample consisted of 1 kg of dried leaves distilled for 240 min (23).

Gas chromatographic (GC) analysis was performed using a GCMS coupled to the detector type Ion Trap operating in the EI mode at 70 eV with a scan mass range of 40-600 m/z, model 450-GC/MS-240 (Varian/Agilent Technologies). Separations were accomplished using a VF5-MS 30 m × 0.25 µm × 0.25 mm DI column under the following conditions: carrier gas: He (1 mL.min-1); injector and interface temperatures of 250 °C and 280 °C, respectively, in a split mode (1:100). The oven temperature program was from 70 °C to 180 °C at 4 °C min-1, and raised to 250 °C at 10 °C min-1. Individual components were identified by comparing their Kovats (1) and linear Retention indexes (56), by co-injection with a C7-C30 n-alkanes series, mass spectra of the literature (1) and computerized MS-database using NIST libraries.

II. Insects

The adult *A. cocois* were randomly collected from leaves of cashew crops of Fortaleza for the infestation. To rear *A. cocois*, we used iron cages covered with organza bags (2.56 × 2.05 × 1.56 m). To obtain the eggs and nymphs of *A. cocois*, cashew seedlings were placed inside the cages that contained adults of *A. cocois*. After 24 h, the seedlings were removed from the cages and kept in greenhouse [29.29 ± 0.66 °C with a relative humidity of 58.74 ± 11.17%] for the eggs to hatch. The leaves with the eggs of *A. cocois* were wrapped in organza bags to prevent access of natural enemies (predators and parasitoids). The number of nymphs was recorded using a stereoscopic microscope (10×).

III. Toxicity bioassays

The EO solution of *O. micranthum* was prepared by mixing 10.0 mg/mL EO with 0.125% v/v of Tween 20. The dilutions used were: 1.25, 2.5, 5.0, and 10.0 mg of EO distilled in 1 mL water (mg/mL). The insecticide imidacloprid (PROVADO 200 SC, BAYER SA, São Paulo-SP) (0.5 mL/L) and distilled water were used as positive and negative controls, respectively. In all treatments, distilled water and Tween 20 were used as emulsifiers.

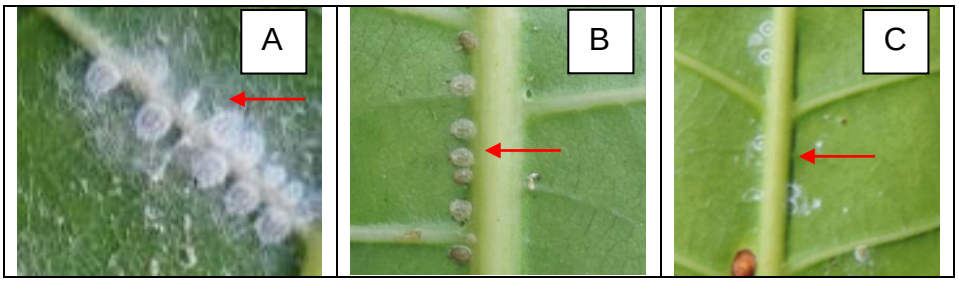

Figure 1. *Aleurodicus cocois* nymphs (A) before spraying with 10.0 mg/mL essential oil (EO) of *Ocimum micranthum*, (B) after spraying with 10.0 mg/mL EO of *O. micranthum*, and (C) after removing by touching slightly with a fine brush or by wind effects.

Each experimental sample consisted of one leaf of cashew seedling in tubes with 10 nymphs of second instar of *A. cocois* (Figure 1). Using the manual sprayer these leaves were sprayed with (5 mL solution) as per the treatments. The data of mortality were corrected by natural mortality in control (2) formula and subjected to Kruskal-Wallis One Way Analysis of Variance and median compared by Tukey test. Thereafter, we evaluated the lethal concentration and lethal time (LT) for the EO against nymphs of *A. cocois.*

IV. Lethal concentration

The experimental design was completely randomized with 7 replications. The number of dead and living individuals was registered up to 24 h after application of treatments. Insects were considered dead, if they changed their color from pale yellow to brown, do not have white wax on the body and show desiccation with stalled growth (Figure 1). Mortality data of *A. cocois* mortality in *O. micranthum* concentrations were corrected by Abbott's formula (2) and and submitted to probit analysis ($p > 0.05$).

V. Lethal time

The experimental design was completely randomized with seven replication. In this experiment, we used 10.0 mg/mL EO of *O. micranthum*, insecticide imidacloprid (0.5 mL/L) with distilled water as negative control. The insect mortalities were evaluated from 0 to 48 h after the start of bioassay. Insects with the characteristics described above were considered dead.

VI. Potential geographical locations for supply of raw materials of *O. micranthum*.

Potential locales for supply of raw materials were based on the Global Biodiversity Information Facility. A total of 844 registers for *O. micranthum* localized in Central and South America were obtained (21).

VII. Statistical analysis

The data did not fit the homogeneity of variance and normality of the errors. Thus, the data of lethal concentration were subjected to Kruskal-Wallis one-way analysis of variance on ranks of variance and the averages were compared by the Tukey honest significant difference (HSD) test ($p < 0.05$).

The experimental data, the insect survival curves were estimated using the Kaplan-Meier product limit method (46). From the survival curves, we estimated the lethal time required to kill 50% insects (LT50) for 1% v/v EO of *O. micranthum* and imidacloprid against *A. cocois*. The LT_{50} was considered different when their average was outside the confidence interval of 95% probability of another substance.

RESULTS AND DISCUSSION

Chemical composition of *O. micranthum*

The chemical composition of the sixteen EO of *O. micranthum* were confirmed (Table 1). The major allelochemicals in the EO of *O. micranthum* were eugenol (41.05%), elemicin (16.09%), and beta-caryophyllene (14.10%) representing 71.24% (Table 1). The allelochemicals (beta-elemene, methyl eugenol, alfa-caryophyllene, beta-selinene, beta-bisabolene bicyclogermacrene, spathulenol, caryophyllene oxide) contents was low (1 - 7%). While eucalyptol, linalool, alloaromadendrene, germacrene D and globulol contents was < 1%.

Toxicity bioassays

EO at 10.0 mg/mL concentration exhibited the highest toxicity against *A. cocois*, similar to imidacloprid, while 5.0 and 2.5 mg/mL concentration exhibited intermediary toxicity (between >30 and <50%), and 1.25 mg/mL was not toxic ($H = 29.47$; $df = 4$; and $p < 0.001$) (Figure 2,3).

Lethal concentration

The data of mortality of nymphs of *A. coccois* was adjusted to probit analysis ($\chi^2 = 2.61$; $df = 2$; $p = 0.27$). The letal concentration CL_{50} and CL_{90} were of 3.63 (confidence interval 3.24-4.06) and 3.63 (confidence interval 7.84-11.04).

Table1. Chemical composition of essential oil of *Ocimum micranthum.*

Compound	KI*	Composition (%)
Eucalyptol	1004	0.58
Linalool	1074	0.31
Eugenol	1352	41.05
Beta-elemene	1387	4.63
Methyl eugenol	1396	1.31
Beta-caryophyllene	1420	14.10
Alfa-caryophyllene	1458	2.71
Alloaromadendrene	1463	0.70
Germacrene D	1483	0.55
Beta-selinene	1491	3.20
Bicyclogermacrene	1497	6.49
Beta-bisabolene	1507	1.43
Elemicin	1549	16.09
Spathulenol	1581	3.07
Caryophyllene oxide	1587	2.43
Globulol	1590	0.56

*Kovats index calculated using the Van den Dool and Kratz (56).

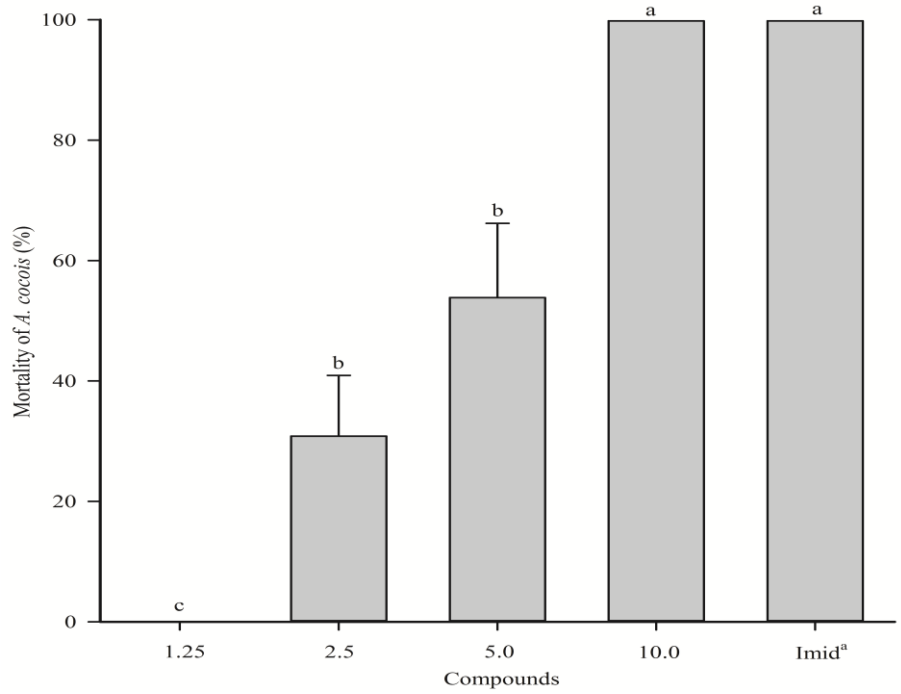

Figure 2. Mortality (mean ± SE) of *Aleurodicus cocois* exposed to doses (mg/mL) of *Ocimum micranthum* essential oil, imidacloprid insecticide, and negative control. The histograms with the same letters are not different according to the Tukey test ($p < 0.05$). Imid[a]: Imidaclopid.

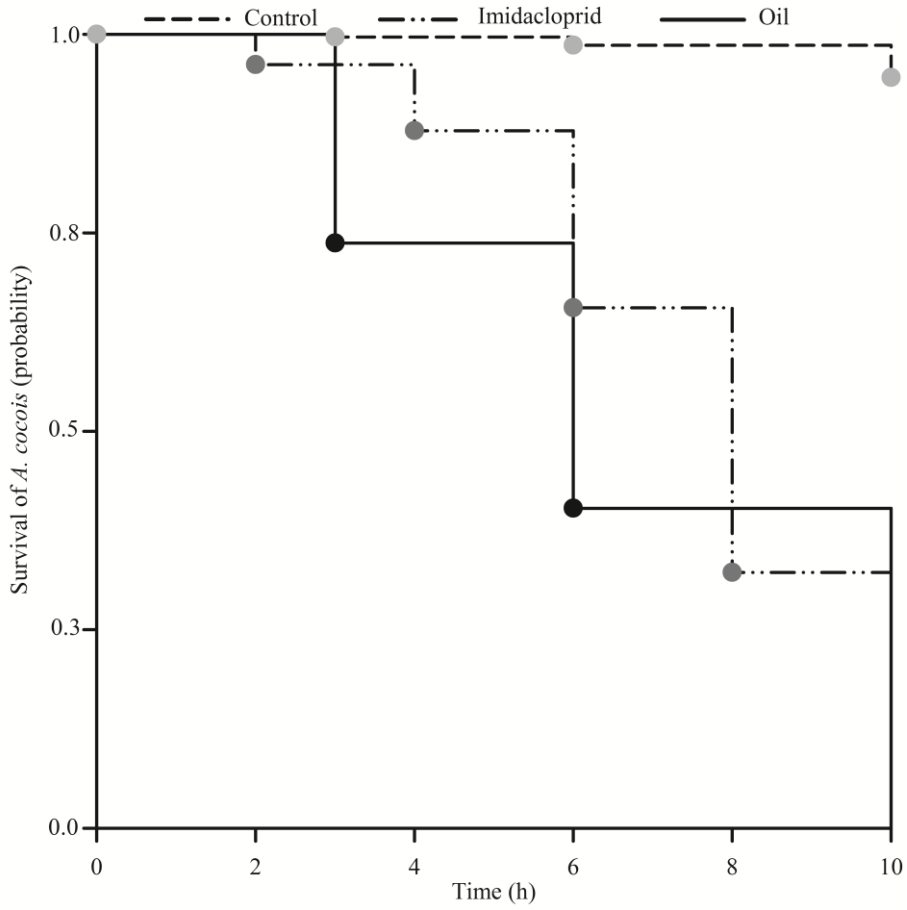

Figure 3. Survival curve of *Aleurodicus cocois* exposed to 10.0 mg/mL essential oil of *Ocimum micranthum*, imidacloprid insecticide, and negative control.

The EO at 10.0 mg/mL concentration showed faster mortality (LT50 = 6.82 h with a confidence interval of 6.34-7.30 h) than imidacloprid (LT50 = 7.63 h with a confidence interval of 7.31-7.95 h) (Figure 4).

Lethal time

There were significant differences between the survival time of *A. cocois* when treated with EO and imidacloprid (Figure 4). The survival time of *A. cocois* exposed to the 10.0 mg/mL EO was significantly reduced over time (Log-rank test: $\chi^2 = 265.22$; dfn = 2, and $p < 0.001$).

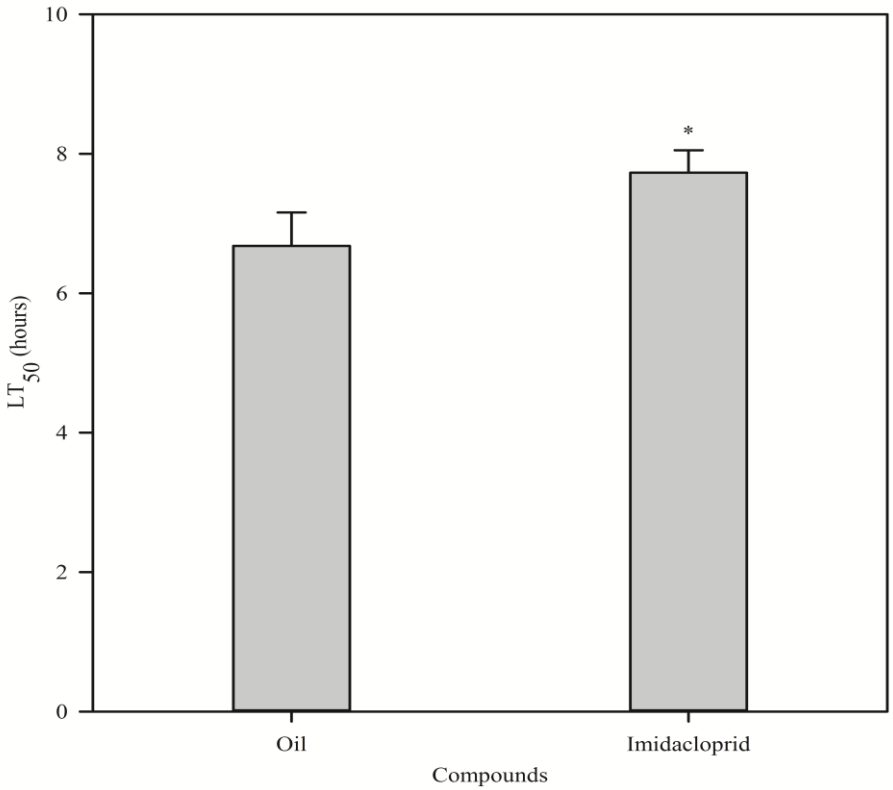

Figure 4. Time to kill 50% of *Aleurodicus cocois* exposed to 10.0 mg/mL essential oil of *Ocimum micranthum* and imidacloprid insecticide.

Regions suitable for the cultivation of *O. micranthum*

Figure 5 shows the 844 registers of *O. micranthum* distributed in 16 countries in tropical zone in Central and South America.

In this study, for the first time, we have found the effectiveness of EO of *O. micranthum* against whitefly *A. cocois*. It can be a good alternative to also control other species of whiteflies. The chemical composition of the EO of *O. micranthum* shows the predominance of eugenol, elemicin and beta-caryophyllene. These compounds are prevalent in plants of Lamiaceae family and some plants of this family also have insecticidal, microbial, and antioxidant activities (4,51).

The mortality of *A. cocois* increased proportionally with the increasing concentration of EO of *O. micranthum.* The mortality of *A. cocois* may be related to the high contents of compounds (eugenol, elemicin, and beta-caryophyllene). Eugenol and beta-caryophyllene are terpenoids and elemicin is a phenolic compound. These classes of compounds have insecticidal, microbial, antioxidant and anti-hemorrhagic activities (8,10,41,48). However, it is necessary to evaluate the toxicity of each compound alone and in combination. The volatile monoterpenes in EOs of plants are responsible for their defence against arthropods, herbivores and pathogenic fungi (30).

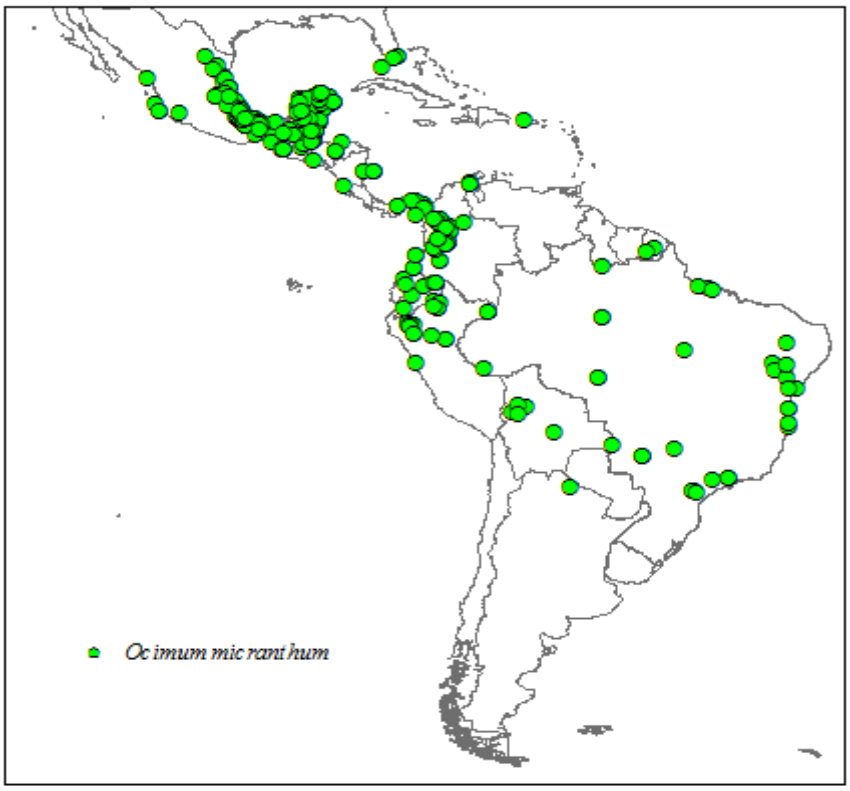

Figure 5. The distribution of *Ocimum micranthum* in Central and South America.

The highest mortality of *A. cocois* occurred at 10.0 mg/mL *O. micranthum* EO treatment that was similar to imidacloprid insecticide. In addition, the EO of *O. micranthum* at 10.0 mg/mL concentration showed faster toxicity than imidacloprid. EOs are lipophilic compounds that can easily penetrate the body of an insect (22,53). Furthermore, the rapid action of EOs against some pests is indicative of a neurotoxic mode of action (15,16,28,45).

The high toxicity of EO to nymphs of *A. cocois* (CL_{50} = 3.63 mg/mL) can be due to their complex composition. The presence of compounds of different chemical groups in the EO of *O. micranthum* can represent barrier to metabolism of *A. cocois*, once this compound can have different action sites, e.g. eugenol, major compound of EO the *O. micranthum*, acts through the octopaminergic system by activating the receptors for octopamine (17), linalool inhibits the enzyme acetylcholinesterase (37). The electrophysiological experiments have confirmed that EO of *O. basilicum* broke off neuronal electrical activity in insects by decreasing the amplitude of action potentials and reducing both the post hyperpolarization phase and firing frequency of action potentials (24,47). In addition, the phytochemicals of EO influenced the non-neurotoxic modes of action [antifeedant action, inhibition of molting, growth reduction, loss of fecundity and

respiratory inhibition of insects (8,35).] Corroborating our results, previous works verified high toxicity of EO of *Ocimum* spp. to larvae of *Aedes eagypti* (11) and to adults of coffee berry borer *Hypothenemus hampei* (36), fumigant and repellent effects on adults of *Rhyzopertha dominica*, *Oryzaephilus surinamensis* and *Callosobruchus chinensis* (39).

The rapid action and effectiveness against *A. cocois* caused by 10.0 mg/mL EO of *O. micranthum* may be related to the interaction of EO with the lipid bilayer of the cuticle leaving the cuticle less selective, causing cellular damage and causing loss of water, ions, and other cellular constituents (29,31). These characteristics were observed in *A. cocois* after treatments with the 10.0 mg/mL EO of *O. micranthum* and also they did not have white wax on the body. Insecticides can kill insects through suffocation (by blocking the spiracles) or disruption of cuticular waxes and membranes in the integument leading to desiccation (26).

The occurrence of *O. micranthum* in 16 countries localized in Central and South America indicatesd that these regions are suitable sources for the production of EO of *O. micranthum* as an insecticide. The results of our study may be helpful in these regions to take advantage of new opportunities in *O. micranthum* crops through the production of insecticides. We highlighted that further studies are necessary to evaluate the impact on non-target organisms and the economic viability of production of EO of *O. micranthum*.

The EO of *O. micranthum* is effective to control nymphs of whitefly *A. cocois* and has shown very promising insecticidal activity, thus creating new effective product to control the whiteflies and indirectly control of diseases transmitted by them.

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