INSECTICidal POTENTIAL OF CITRUS AND MANGo ESSENTIAL OILS AND SELECTED CONSTITUENTS ON SILVERLEAF WHITEFLY

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ABSTRACT - Bemisia tabaci is a cosmopolitan pest responsible for causing harm to crops in the agricultural hub of Petrolina in the state of Pernambuco, Brazil. We investigated the lethal and sublethal effects of vapors from essential oils obtained through hydrodistillation of the peels of four species of Citrus and the latex from Mangifera indica (var. “rosa” and “espada”) on B. tabaci. The chemical analysis by Gas chromatography coupled to mass spectrometry of the oils led to the identification of 71 constituents, with limonene as the major component of the Citrus oils and terpinolene as the major component of the M. indica oils. B. tabaci was more susceptible to Citrus aurantiifolia (LC₅₀ = 0.70 µL L⁻¹ air) and C. limon (LC₅₀ = 1.77 µL L⁻¹ air) oils, which had the same level of toxicity. Citrus and M. indica oils also led to a reduction in the fecundity of the pest. The lethal and sublethal action of the constituents linalool, α-terpineol, α-pinene, β-pinene, terpinolene and limonene is also discussed. The toxicity of the oils investigated herein associated with the reduction in fecundity is a considerable advantage in the management of B. tabaci. However, for practical use of these oils as a novel insecticide to proceed, further research is required to address safety issues for human health and determine the formulation to improve the insecticidal potency, stability and cost-benefit ratio.

Keywords: Insecticidal activity. Fumigation. Fecundity. Limonene. Terpinolene.
INTRODUCTION

The agricultural hub in the state of Pernambuco in northeast Brazil has exhibited accelerated growth since the installation of irrigated systems in the municipality of Petrolina in the 1970s. To increase the production of vegetable crops for the supply of large cities, protected farming technology, such as non-climatized greenhouses, was introduced in the 1990s (REIS, 2005). Nonetheless, crop losses continue to be frequent in protected crops and, depending on the time of the year, are quite high due to the attacks of pests, such as the silverleaf whitefly, *Bemisia tabaci* biotype B (Genn.) (Homoptera: Aleyrodidae). This occurs because *B. tabaci* has high reproductive potential as well as rapid adaptability to new hosts and environments due to its considerable genotypic plasticity (MALUMPHY, 2004). The main form of controlling this pest is through the use of conventional insecticides. However, the continuous use of such products has contaminated the ecosystem (DAMALAS; ELEFTHEROHORINOS, 2011) and led to populations of the pest to develop resistance to the active ingredients of conventional products, making its control increasingly more difficult (BARRO et al., 2011).

Due to the recognition of the biological properties of essential oils against several arthropods (ISMAN; GRIENEISEN, 2014), the interest in this plant derivative has increased significantly since the 1990s. However, few reports are found in the literature reporting the properties of essential oils from *Citrus* and *Mangifera* species on *B. tabaci*. Northeast Brazil is a producing region of tropical fruits belonging to the families Anacardiaceae and Rutaceae, such as mango, lemon and tangerine, which are known for the production of essential oils. These fruits are consumed fresh or through industrially processed items in the form of juices, jams, sweets and ice cream, which generates an enormous volume of agroindustrial waste. Thus, a viable alternative to conventional pesticides may be found in novel molecules or efficient, environmentally safer products made from the waste found in novel molecules or efficient, viable alternative to conventional pesticides may be found in novel molecules or efficient, environmentally safer products made from the waste of these fruits with the aim of formulating a pesticide for the control of *B. tabaci* in protected crops of Petrolina.

Our research group reported the susceptibility of *Tetranychus urticae* (Koch) (Acari: Tetranychidae), which is an important agricultural pest, to fumigation with the vapors of essential oils from the peels of *Citrus sinensis* L. Osbeck (Rutaceae) and *Citrus aurantium* (L.) Burm.f. (Rutaceae) (ARAÚJO JÚNIOR et al., 2010). More recently, we reported that essential oils from the peels of *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae) and *Citrus reticulata* Blanco (Rutaceae) were active against *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) through the cuticle, digestive and respiratory systems (FOUAD; CAMARA, 2017). Here, our interest is to determine the chemical composition of four species of *Citrus* and two varieties of *Mangifera indica* L. (Anacardiaceae) grown in northeast Brazil and evaluate toxicity by fumigation and the effect on *B. tabaci* fecundity. The lethal and sublethal effects of six constituents of the oils are also discussed.

MATERIAL AND METHODS

The experiments were performed under laboratory conditions from January to December 2013.

Collection of plant material

*Citrus* spp. and *Mangifera indica* L. fruits were collected in the municipality of Santana do Mundaú, Alagoas State (9°10′16.5″S 36°12′15.1″W), and in a fragment of Atlantic forest of Pernambuco (08°12′40.1″ S 34° 95′22.3″ W), respectively. The plants were identified by a botanist from the Federal Rural University of Pernambuco - UFRPE. Vouchers of both samples were mounted and deposited in the Vasconcelos Sobrinho Herbarium of UFRPE under the following numbers: 48734 = *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae); 48736 = *Citrus limon* (L.) Burm.f. (Rutaceae); 48738 = *Citrus reticulata* Blanco (Rutaceae); 48740 = hybrid, *Citrus reticulata* Blanco x *Citrus sinensis* Osbeck (Rutaceae), 363 = *Mangifera indica* var. “rosa” and 364 = *Mangifera indica* var. “espada”.

Chemicals

Constituents were used as standards in the identification of volatile compounds in the oils investigated and eugenol was used as the positive control. Linalool, α-terpineol, α-pinene, β-pinene, terpinolene and limonene were selected for the bioassays due to the fact that these compounds were identified oils, are commercially available and have biological properties that have been reported in the literature (RATHORE; NOLLET, 2017).

Isolation of essential oils

Essential oils from fresh fruit peels of *C. aurantiifolia* (100 g), *C. limon* (100 g), *C. reticulata* Blanco (100 g), *C. reticulata* Blanco x *Citrus sinensis* (100 g), and latex of fruits of *M. indica* var. “rosa” (100 g) and *M. indica* var. “espada” (100 g), were obtained by hydrodistillation using a modified Clevenger apparatus for 4 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept at a low temperature (-5°C) until the repellent assays and analysis. Total oil yields were expressed as percentages (g/100 g of fresh plant material).
All experiments were carried out in triplicate.

Gas chromatography-FID analysis

Gas Chromatography (GC) identification was carried out using a Hewlett-Packard 5890 Series II GC apparatus equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) (J and W Scientific). The oven temperature was programmed from 60 to 240 ºC at a rate 3 ºC min⁻¹. Injector and detector temperatures were 260 ºC. Hydrogen was used as the carrier gas at a flow rate of 1 mL min⁻¹ in split mode (1:30). The injection volume was 0.5 µL of diluted solution (1/100) of oil in n-hexane. The percentage of each compound was obtained from GC-FID peak areas in the order of the DB-5 column elution and expressed as the relative percentage of the area of the chromatograms. Analysis was conducted in triplicate.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the essential oils was carried out using a Varian 220-MS IT GC system with a mass selective detector, mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da. fitted with the same column and temperature program as that for the GC-FID experiments, with the following parameters: carrier gas = helium; flow rate = 1 mL min⁻¹; split mode (1:30); injected volume = 1 µL of diluted solution (1/100) of oil in n-hexane.

Identification of components

Identification of the components was based on GC-MS retention indices with reference to a homologous series of C₁₀-C₄₀ n-alkanes calculated using the Van den Dool and Kratz equation (DOOL; KRATZ, 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST version 14 and WILEY version 11) and co-injection with authentic standards as well as other published mass spectra (ADAMS, 2007). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

Acquisition and rearing of Bemisia tabaci biotype b

Specimens of Bemisia Tabaci Biotype B were obtained from the Agronomic Institute of Campinas (IAC) in São Paulo/Brazil, and since then maintained in the Laboratory of Chemical Investigation of Natural Insecticides of the Federal Rural University of Pernambuco, Brazil. B. tabaci organisms were reared at a temperature of 25 ± 1 ºC, relative humidity of 65 ± 5% and a 12-h photoperiod and without any exposure to insecticides. The breeding method was adapted from Ribeiro et al. (2010).

Fumigant toxicity

The fumigant method used to assess the toxicity of the essential oils (Citrus spp. and Mangifera indica) vapors against B. tabaci was as that employed by Ribeiro et al. (2010). Hermetically sealed glass containers with capacity of 1.0 L were used as test chambers. Leaflets from the common bean cultivar “Carioca”, collected 25 to 40 days after the seeds had been sown, were used as supports. To keep leaflet turgor, these leaflets were placed in vials (5 cm height) with cotton plugs moistened with distilled water, then transferred to the fumigation chamber. With the aid of a vacuum adapter, about 15 pairs of whitefly were placed in each fumigation chamber. A filter paper strip (5 x 2 cm) that worked by releasing the oil that was being evaluated was attached to the center of the inner side of the fumigation chamber lid. Different oil and eugenol concentrations were added with the aid of an automatic pipette. The concentrations of essential oils ranged from 1.0 to 7.0 µL L⁻¹ air (C. reticulata), 3.0 to 9.0 µL L⁻¹ air (C. sinensis x C. reticulata), 0.125 to 5.0 µL L⁻¹ air (C. aurantiifolia), 0.8 to 4.5 µL L⁻¹ air (C. limon), 2.0 to 12.0 µL L⁻¹ air (M. indica var. “rosa”) and 0.5 to 6.0 µL L⁻¹ air (M. indica var. “espada”). The concentrations of compounds for oils ranged from 0.5 to 3.0 µL L⁻¹ air (linalool), 0.2 to 3.0 µL L⁻¹ air (α-terpineol), 4.0 to 16.0 µL L⁻¹ air (α-pinene), 4.0 to 14.0 µL L⁻¹ air (β-pinene), 2.0 to 6.0 µL L⁻¹ air (terpinolene), 2.0 to 8.0 µL L⁻¹ air (limonene) and 0.04 to 1.0 µL L⁻¹ air (eugenol). Nothing was applied in the control treatment. Immediately after the application of the oil/compound, the fumigation chamber was closed and covered with PVC plastic wrap. The number of mortality in treatments and controls was recorded after 24 h. To avoid direct contact of the insects with the filter paper, the fumigation chamber opening was surrounded by a piece of voile.

Fecundity bioassay

The effects of vapors of essential oils (Citrus spp. and M. indica) on the fecundity of B. tabaci eggs were determined using the fumigation bioassay method employed by Ribeiro, Camara and Ramos (2016). Hermetically sealed glass containers with capacity of 1.0 L were used as test chambers. Leaflets from the common bean cultivar “Carioca”, collected 25 to 40 days after the seeds had been sown, were used as supports. To keep leaflet turgor, they were placed in vials (5 cm height) with cotton plugs moistened with distilled water, then transferred to the fumigation chamber. With the aid of a vacuum adapter, about 15 pairs of whitefly were placed in
each fumigation chamber. A filter paper strip (5 x 2 cm) that worked by releasing the oil that was being evaluated was attached to the center of the inner side of the fumigation chamber lid. Different oil and eugenol concentrations were added with the aid of an automatic pipette. The lowest concentrations were 1.0 µL L⁻¹ air (C. reticulata), 3.0 µL L⁻¹ air (C. sinensis x C. reticulata), 0.125 µL L⁻¹ air (C. aurantiifolia), 0.8 µL L⁻¹ air (C. limon), 2.0 µL L⁻¹ air (M. indica var. “rosa”) and 0.5 µL L⁻¹ air (M. indica var. “espada”). The concentrations of compounds for the oils were equal to 0.5 µL L⁻¹ air (linalool), 0.2 µL L⁻¹ air (α-terpineol), 4.0 µL L⁻¹ air (α-pinene), 4.0 µL L⁻¹ air (β-pinene), 2.0 µL L⁻¹ air (terpinolene), 2.0 µL L⁻¹ air (limonene) and 0.04 µL L⁻¹ air (eugenol). Nothing was applied in the control treatment. Immediately after the application of the oil/compound, the fumigation chamber was closed and covered with PVC® plastic wrap. A completely randomized design was employed, with five replicates, totaling 10 repetitions. The number of eggs in the treatments and controls were recorded after 24 h. To avoid direct contact of the insects with the filter paper, the fumigation chamber opening was surrounded by a piece of voile.

Statistical analysis

To estimate the curve slopes, LC₅₀ (lethal concentration) of each essential oil (Citrus spp. and M. indica) and selected constituents, mortality data were submitted to PROBIT analysis (FINNEY, 1971) using SAS software (version 9.0) (SAS INSTITUTE, 2002). The concentrations used were calculated based on the logarithmic series proposed by Robertson et al. (2017). The fecundity bioassay data were submitted to analysis of variance using PROC ANOVA with the means compared by the Tukey test (P < 0.05) estimated using Statistical Analysis System software (SAS INSTITUTE, 2002).

RESULTS AND DISCUSSION

Yields and chemical profile of essential oils

The yields of the essential oils from the peels of Citrus species and latex of M. indica varieties obtained through hydrodistillation are presented in Table 1. The highest yields were found for the oils from the latex of the mangoes. The yield of the “rosa” variety (9.12 ± 0.16%) was 1.63-fold greater than that of the “espada” variety (5.60 ± 0.13%). These yields are in agreement with previous reports found in the literature (RAMOS et al., 2014).

Regarding the yields of the essential oils from the peels of fruits of the Citrus species, the highest values were found for tangerine (C. reticulata x C. sinensis) (2.05 ± 0.03%) and mandarin orange (C. reticulata) (1.31 ± 0.21%), followed by lemon (C. limon) (0.81 ± 0.06%) and lime (C. aurantiifolia) (0.48 ± 0.03%). These yields are in agreement with the values reported in the literature, which vary from 0.005 to 2.04% for tangerine and orange (OTHMAN et al., 2016) and from 0.23 to 2.2% for lemon and lime (GHOORCHIBEIGI et al., 2017; TCHAMENI et al., 2018).

The GC/MS analysis of the oils of the four species of Citrus enabled the identification of 56 compounds accounting for 96.80 ± 1.13, 96.53 ± 1.80, 95.43 ± 0.98 and 92.05 ± 1.10% of the chemical composition of oils from the peels of C. reticulata x C. sinensis, C. reticulata, C. aurantiifolia and C. limon, respectively. The oils were characterized by an abundance of monoterpenes, with limonene as the major component. This result is in agreement with reports on the oil from the peel of C. aurantiifolia from Vietnam (DANG et al., 2016) and Cameroon (TCHAMENI et al., 2018), C. limon in Iran (GHOORCHIBEIGI et al., 2017) as well as C. reticulata in Vietnam (DANG et al., 2016) and Egypt (HAMDAN; MOHAMED; EL-SHAZLY, 2016).

Twenty-one compounds were found in M. indica, accounting for 94.52 ± 0.61 and 93.96 ± 0.75% of the chemical composition of the oils from the latex of the “espada” and “rosa” varieties, respectively. Terpinolene was the major constituent in both oils. This is in agreement with findings described by Loveys et al. (1992), who investigated oils from the latex of two other varieties of mango (Kensington and Irwin). Moreover, the occurrence of terpinolene has been reported in the chemical composition of oils from different organs of the plant, particularly the fruit (PINO et al., 2005).
Table 1. Yields and chemical profiles of essential oils from peels of *Citrus* species and latex of *Mangifera indica* varieties.

| Compounds                   | RIL | RIC | TM % ± SE | TC % ± SE | LT % ± SE | LS % ± SE | ME % ± SE | MR % ± SE |
|-----------------------------|-----|-----|-----------|-----------|-----------|-----------|-----------|-----------|
| α-Thujene<sup>RL, MS</sup>  | 924 | 925 | 0.59±0.02 | 0.10±0.00 | 0.68±0.01 | -         | -         | -         |
| α-Pinene<sup>RL, MS, CO</sup> | 932 | 933 | 3.14±0.08 | 2.04±0.10 | 3.78±0.11 | 0.77±0.02 | 1.03±0.09 | 11.50±0.21|
| α-Fenchene<sup>RL, MS</sup> | 945 | 941 | -         | -         | 3.84±0.12 | -         | -         | -         |
| Camphene<sup>RL, MS</sup>   | 946 | 954 | -         | -         | 0.29±0.01 | -         | -         | 0.30±0.02 |
| Sabinene<sup>RL, MS</sup>   | 969 | 970 | 1.08±0.02 | 2.52±0.08 | -         | -         | -         | 1.90±0.09 |
| β-Pinene<sup>RL, MS, CO</sup> | 974 | 982 | 1.67±0.09 | -         | 9.89±0.34 | 18.14±0.68| 2.21±0.03 | 28.42±0.80|
| Myrcene<sup>RL, MS, CO</sup> | 988 | 992 | 4.61±0.08 | 6.50±0.13 | 0.58±0.05 | 2.50±0.02 | -         | -         |
| δ-2-Carene<sup>RL, MS</sup> | 1001| 997 | -         | -         | 0.61±0.00 | -         | -         | -         |
| p-Mentha-1(7),8-diene<sup>RL, MS</sup> | 1003| 1002| 4.12±0.11 | 0.68±0.01 | -         | -         | -         | -         |
| δ-3-carene<sup>RL, MS</sup> | 1008| 1005| -         | -         | 6.43±0.11 | 2.09±0.01 | -         | -         |
| α-Terpinene<sup>RL, MS, CO</sup> | 1014| 1012| -         | -         | 3.52±0.09 | 1.00±0.08 | -         | -         |
| Limonene<sup>RL, MS, CO</sup> | 1024| 1021| 60.96±1.01| 77.79±1.73| 37.73±0.86| 40.70±1.13| 1.42±0.10| 1.31±0.03 |
| Sylvestrene<sup>RL, MS, CO</sup> | 1025| 1025| -         | -         | 0.90±0.00 | 0.70±0.00 | -         | -         |
| (E)-β-ocimene<sup>RL, MS</sup> | 1044| 1039| -         | -         | 0.60±0.07 | -         | -         | -         |
| γ-Terpinene<sup>RL, MS, CO</sup> | 1050| 1050| -         | -         | 0.43±0.01 | 0.20±0.01 | -         | -         |
| p-Mentha-3,8-diene<sup>RL, MS</sup> | 1068| 1066| -         | 0.01±0.00 | 0.29±0.01 | -         | -         | -         |
| p-Mentha-2,4(8)-diene<sup>RL, MS</sup> | 1085| 1070| 9.80±0.21 | 1.46±0.05 | 5.53±0.09 | 1.15±0.02 | -         | -         |
| Terpinolene<sup>RL, MS, CO</sup> | 1086| 1093| 1.37±0.03 | 0.10±0.00 | 1.26±0.03 | 0.34±0.01 | 70.14±0.61| 39.24±0.29|
| Linalool<sup>RL, MS, CO</sup> | 1095| 1103| 4.41±0.12 | 3.56±0.07 | 3.00±0.07 | 0.10±0.00 | -         | -         |
| eexo-Fenchol<sup>RL, MS</sup> | 1118| 1118| -         | -         | 0.19±0.00 | 1.06±0.02 | -         | -         |
| cis-Limone oxide<sup>RL, MS</sup> | 1132| 1136| -         | -         | 0.49±0.02 | 0.29±0.00 | -         | -         |
| trans-Limone oxide<sup>RL, MS</sup> | 1137| 1140| -         | -         | 0.68±0.08 | -         | -         | -         |
| (E)-Myroxide<sup>RL, MS</sup> | 1140| 1146| -         | -         | 0.49±0.01 | 0.58±0.00 | -         | -         |
| β-Pinene oxide<sup>RL, MS</sup> | 1154| 1151| -         | -         | 1.34±0.11 | -         | -         | -         |
| iso-Menthone<sup>RL, MS</sup> | 1158| 1154| 0.29±0.00 | 0.10±0.00 | -         | -         | -         | -         |
| Terpinen-4-ol<sup>RL, MS</sup> | 1174| 1177| 0.78±0.01 | 0.27±0.01 | 2.62±0.09 | 2.21±0.08 | -         | -         |
| p-Cymen-8-ol<sup>RL, MS</sup> | 1179| 1180| -         | -         | -         | -         | -         | -         |
| α-Terpineol<sup>RL, MS</sup> | 1186| 1191| 1.08±0.08 | 0.28±0.00 | 5.04±0.14 | 2.78±0.08 | -         | -         |
| n-Decan<sup>RL, MS</sup> | 1201| 1206| 1.76±0.10 | 0.49±0.00 | 0.29±0.00 | -         | -         | -         |
| cis-Cardio<sup>RL, MS</sup> | 1226| 1225| -         | 0.19±0.00 | -         | -         | -         | -         |
| Nerol<sup>RL, MS</sup> | 1227| 1234| 0.20±0.00 | -         | 0.68±0.02 | 0.38±0.01 | -         | -         |
| Geranial<sup>RL, MS</sup> | 1235| 1246| -         | 2.43±0.08 | 0.28±0.00 | -         | -         | -         |
| Isopulegyl acetate<sup>RL, MS</sup> | 1274| 1271| -         | 0.54±0.01 | 0.10 ± 0.01| -         | -         | -         |
| Neryl formate<sup>RL, MS</sup> | 1280| 1276| -         | 2.72±0.08 | 2.59±0.09 | -         | -         | -         |
| δ-Elemene<sup>RL, MS</sup> | 1335| 1336| 0.10±0.00 | -         | 0.19±0.00 | -         | -         | -         |
| Citronellyl acetate<sup>RL, MS</sup> | 1350| 1351| 0.09±0.00 | 0.02±0.00 | -         | -         | -         | -         |
| Nerila acetate<sup>RL, MS</sup> | 1359| 1361| -         | 3.30±0.14 | 0.38±0.01 | -         | -         | -         |
| Geranyl acetate<sup>RL, MS</sup> | 1379| 1380| -         | 1.16±0.09 | 0.38±0.00 | -         | -         | -         |
| Daucene<sup>RL, MS</sup> | 1380| 1425| -         | 0.10±0.00 | -         | -         | -         | -         |

SE: Standard Error; RIL: Retention indices from the literature; RIC: Retention indices calculated from retention times in relation to those of a series of C₆–C₄₀ n-alkanes on a DB-5 capillary column; Method of identification: RI: Retention Index; MS: Mass Spectroscopy; CO: Co-Injection with authentic compounds; LT: *Citrus aurantiifolia*; LS: *C. limon*; TM: *C. sinensis* x *C. reticulata*; TC: *C. reticulata*; ME: Mangifera indica var. “rosa” (MR) and ME: *M. indica* var. “espada” (ME).
Table 1. Continuation.

| Compounds                              | RIL | RIC | TM % ± SE | TC % ± SE | LT % ± SE | LS % ± SE | ME % ± SE | MR % ± SE |
|----------------------------------------|-----|-----|-----------|-----------|-----------|-----------|-----------|-----------|
| β-Cubebene<sub>RL, MS</sub>           | 1387| 1388| -         | 0.08±0.01 | 0.01±0.00 | -         | -         | -         |
| β-Elemene<sub>RL, MS</sub>            | 1389| 1389| 0.05±0.00 | -         | 0.01±0.00 | -         | -         | -         |
| Ethyl geranate<sub>RL, MS</sub>       | 1394| 1396| -         | -         | -         | 0.77±0.01 | -         | -         |
| β-Longipinene<sub>RL, MS</sub>        | 1400| 1400| -         | -         | -         | -         | 0.82±0.02 | 2.90±0.10 |
| Cycloseychellene<sub>RL, MS</sub>     | 1406| 1407| -         | -         | -         | -         | 0.41±0.01 | 0.20±0.00 |
| α-cis-Bergamotene<sub>RI, MS</sub>    | 1411| 1414| -         | -         | 0.28±0.00 | -         | -         | -         |
| β-Caryophyllene<sub>RL, MS, CO</sub>  | 1417| 1421| -         | -         | 1.16±0.10 | 0.31±0.00 | -         | -         |
| α-trans-Bergamotene<sub>RL, MS</sub>  | 1432| 1434| -         | -         | 2.91±0.10 | 0.30±0.00 | -         | -         |
| γ-Elemene<sub>RL, MS</sub>            | 1434| 1438| -         | -         | -         | 0.28±0.00 | 1.50±0.08 | -         |
| α-Guaiene<sub>RL, MS</sub>            | 1437| 1436| -         | -         | -         | 1.15±0.00 | -         | -         |
| Citronellyl propanoate<sub>RL, MS</sub>| 1444| 1442| -         | -         | -         | -         | 0.13±0.03 | -         |
| α-Clovene<sub>RL, MS</sub>            | 1452| 1449| -         | -         | -         | -         | 0.61±0.08 | 0.30±0.01 |
| (E)-β-Farnesene<sub>RL, MS</sub>      | 1454| 1453| 0.10±0.00 | -         | 0.48±0.03 | 2.88±0.15 | -         | -         |
| β-Santalene<sub>RL, MS</sub>          | 1457| 1457| -         | -         | 0.20±0.00 | -         | -         | -         |
| Cumacrene<sub>RL, MS</sub>            | 1470| 1471| -         | 0.03±0.00 | 0.01±0.00 | -         | -         | -         |
| γ-Gurjunene<sub>RL, MS</sub>          | 1475| 1472| -         | -         | -         | 3.68±0.13 | 1.34±0.02 | -         |
| Geranyl propanoate<sub>RL, MS</sub>    | 1476| 1474| -         | -         | 0.41±0.01 | 0.38±0.00 | -         | -         |
| γ-Muurolene<sub>RL, MS</sub>          | 1478| 1477| 0.20±0.00 | 0.10±0.00 | -         | -         | -         | -         |
| γ-Muurolene<sub>RL, MS</sub>          | 1478| 1477| -         | -         | -         | 0.42±0.00 | -         | -         |
| γ-Himachalene<sub>RL, MS</sub>        | 1481| 1478| -         | -         | -         | 0.60±0.04 | 0.21±0.01 | -         |
| Valencene<sub>RL, MS</sub>            | 1496| 1497| -         | -         | 0.38±0.00 | 0.29±0.00 | -         | -         |
| (E,E)-α-Farnesene<sub>RL, MS</sub>    | 1505| 1502| 0.10±0.00 | 0.20±0.00 | -         | -         | -         | -         |
| β-Bisabolene<sub>RL, MS</sub>         | 1505| 1510| -         | -         | 4.17±0.17 | -         | -         | -         |
| β-Sesquiphellandrene<sub>RL, MS</sub> | 1521| 1525| -         | -         | -         | 4.03±0.17 | -         | -         |
| δ-Cadinene<sub>RL, MS</sub>           | 1522| 1529| 0.10±0.00 | 0.10±0.00 | -         | -         | -         | -         |
| Germacrene<sub>B, RL, MS</sub>        | 1559| 1554| 0.20±0.00 | -         | -         | -         | -         | -         |
| Caryophyllene oxide<sub>RL, MS, CO</sub>| 1582| 1583| -         | -         | 0.68±0.08 | -         | -         | -         |
| Humulene epoxide II<sub>RL, MS</sub>  | 1608| 1606| -         | -         | 0.20±0.00 | 1.34±0.10 | -         | -         |
| Selin-11-en-4-α-ol<sub>RL, MS</sub>   | 1658| 1653| -         | -         | 0.18±0.01 | -         | -         | -         |
| epi-β-Bisabolol<sub>RL, MS</sub>      | 1670| 1666| -         | -         | 0.27±0.00 | -         | -         | -         |
| epi-α-Bisabolol<sub>RL, MS</sub>      | 1683| 1682| -         | -         | 0.38±0.00 | -         | -         | -         |

Total: 96.80±1.13 96.53±1.80 95.43±0.98 92.05±1.10 94.52±0.61 93.96±0.75

Monoterpenes: 93.77±1.00 95.46±1.77 83.78±0.80 81.68±1.14 87.41±0.46 87.35±0.41
Sesquiterpenes: 0.78±0.05 0.58±0.01 11.35±0.20 10.37±0.15 7.11±0.18 6.61±0.10
Fatty acid derivatives: 2.25±0.00 0.49±0.00 0.29±0.00 - - -

SE: Standard Error; RIL: Retention indices from the literature; RIC: Retention indices calculated from retention times in relation to those of a series of C<sub>5</sub> – C<sub>40</sub> n-alkanes on a DB-5 capillary column; Method of identification: RI: Retention Index; MS: Mass Spectroscopy; CO: Co-Injection with authentic compounds; LT: Citrus aurantifolia; LS: C. limon; TM: C. sinensis x C. reticulata ; TC: C. reticulata ; MR: Mangifera indica var. “rosa” (MR) and ME: M. indica var. “espada” (ME).
Fumigant bioassay

The insecticidal action found when *B. tabaci* was exposed to the vapors of the oils from the Citrus species and two varieties of *M. indica* varied according to the type of oil (Table 2).

The whitefly was more susceptible to the oils of lime and lemon (*C. aurantiifolia* and *C. limon*), followed by those of *C. reticulata, C. sinensis* x *C. reticulata, M. indica* var. “espada” and “rosa”. With the exception of the oil from *C. aurantiifolia*, which had the same level of toxicity as eugenol, used as the positive control, none of the oils investigated was more active against *B. tabaci* than this phenylpropanoid (Table 2).

Table 2. Fumigant action of essential oils from Citrus species and Mangifera indica varieties, selected compounds and positive control (eugenol) on Bemisia tabaci biotype B

| Essential oils                                      | N  | DF | Slope±SE | LC50 μL L−1 air (CI 95%) | χ²   | p-value |
|-----------------------------------------------------|----|----|----------|--------------------------|------|---------|
| *C. reticulata*                                     | 388| 3  | 4.26±0.85| 3.04 (2.22-3.59)          | 2.19 | 0.41    |
| *C. sinensis* x *C. reticulata*                    | 452| 3  | 7.62±1.16| 5.39 (3.99-6.10)          | 3.80 | 0.33    |
| *C. aurantiifolia*                                  | 305| 3  | 1.75±0.28| 0.70 (0.13-1.43)          | 3.40 | 0.29    |
| *C. limon*                                          | 322| 3  | 3.87±0.69| 1.77 (0.63-2.44)          | 4.18 | 0.18    |
| *M. indica* var. “rosa”                            | 630| 4  | 8.08±1.14| 7.95 (6.08-8.91)          | 9.30 | 0.06    |
| *M. indica* var. “espada”                           | 540| 3  | 6.36±0.81| 3.27 (2.25-3.93)          | 7.40 | 0.09    |

Compounds

| Components   | N  | DF | Slope±SE | LC50 μL L−1 air (CI 95%) | χ²   | p-value |
|--------------|----|----|----------|--------------------------|------|---------|
| Eugenol      | 495| 4  | 1.96±0.32| 0.20 (0.02-0.34)          | 9.37 | 0.06    |
| Linalool     | 540| 3  | 5.06±0.69| 1.60 (0.97-1.99)          | 7.42 | 0.09    |
| α-terpinolene| 540| 3  | 4.93±0.57| 1.43 (1.05-1.73)          | 6.35 | 0.11    |
| α-pinene     | 630| 4  | 9.20±1.13| 11.37 (10.16-12.22)       | 5.15 | 0.17    |
| β-pinene     | 630| 4  | 5.19±0.50| 7.40 (6.17-8.37)          | 6.71 | 0.22    |
| terpinolene  | 540| 3  | 8.36±1.06| 4.21 (3.38-4.73)          | 5.95 | 0.08    |
| Limonene     | 540| 3  | 9.91±1.02| 5.41 (4.81±5.86)          | 3.90 | 0.34    |

N: number of mites; DF: degree of freedom; SE: standard error; LC: lethal concentration values; CI: confidence interval; χ²: chi-square.

Fumigant bioassays were performed to investigate the relative toxicity of some chemical compounds identified in the oils and demonstrated that linalool and α-terpinolene were the most toxic to *B. tabaci*, followed by terpinolene, limonene, β-pinene and α-pinene. These results indicate that the major component of a mixture is not always the most active. Indeed, the minor constituents found in the Citrus (linalool and α-terpinolene) were about 3.38-fold more toxic than the major constituent (limonene).

The fumigant properties of oils are well known for a wide variety of arthropods (RIBEIRO et al., 2019, MALACRINÔ et al., 2016, PAVELA; BENELLI, 2016), including *B. tabaci* (YANG et al., 2010). However, this is the first report of the fumigant action of oils from *Citrus sinensis* x *C. reticulata*, *C. limon* and *M. indica* (“rosa” and “espada” varieties) on the whitefly. A previous investigation of the toxicity of oils from the key lime (*Citrus aurantiifolia*) and the mandarin orange (*C. reticulata*) grown in South Korea on the Q and B biotypes of *B. tabaci* revealed toxicity by fumigation only for the Q biotype (KIM et al., 2011), with an estimated LC50 of 0.91 mL/cm2 for the *C. aurantiifolia* oil.

By comparing these results to those obtained in the present study, it is possible to see that the essential oil from *C. aurantiifolia* grown in northeast Brazil was more toxic to the whitefly. This difference in toxicity in the experiments conducted in South Korea and the present investigation may be attributed to the different biotypes tested and the possible qualitative and/or quantitative variations in the chemical composition of the oils.

Investigations evaluating the insecticidal action of the oils from the latex of mangos are rare. However, the insecticidal action of other derivatives, such as aqueous extract of the leaves of *M. indica*, has been evaluated against other agricultural pests and insects of interest to human medicine. For instance, Mohammed and Chadde (2007) and Zuharah et al. (2014) verified the efficacy of the aqueous extract from the leaves of *M. indica* against 3rd instar larvae of *Aedes aegypti* L. (Diptera: Culicidae). In another study, Devanand and Rani (2008) evaluated the effectiveness of the aqueous extract from the leaves against two important pests of cotton [*Spodoptera litura* F. (Lepidoptera: Noctuidae)] and castor bean [*Achaea janata* L. (Noctuidae: Lepidoptera)].

Fecundity Bioassay

The number of eggs per *B. tabaci* female when exposed to the *Citrus* and *Mangifera* oils is shown in Table 3. The fecundity tests performed with the oils and selected constituents suggest that, at sublethal concentrations, these products reduce the fecundity of the whitefly when compared to the negative control (F = 560.53; DF = 14; P < 0.0001).
The effect on the fecundity of *B. tabaci* varied according to the type of essential oil. The oil from *C. reticulata x C. sinensis* had the greatest effect, reducing the number of eggs laid per female by 94.93%. The other *Citrus* oils had a somewhat lower effect and did not differ significantly from one another. The oils from the latex of the “rosa” and “espada” varieties of *M. indica* had the least effect on the fecundity of the whitefly, reducing the number of eggs laid per female by 9.83 and 12.84%, respectively.

Regarding the selected constituents, terpinolene was the compound with the most effect on fecundity, reducing the number of eggs laid per *B. tabaci* female by 94.72%, followed by limonene, which achieved an 87.89% reduction in eggs laid. Linalool, α-terpineol and β-pinene achieved similar reductions in fecundity (64.80 to 70.19%), whereas β-pinene had the least effect.

Among the oils tested, those from the species *Citrus* were more effective than the positive control (eugenol). Based on the sublethal effects of the monoterpenes investigated, all selected constituents from the *Citrus* and *M. indica* oils were more effective at reducing the number of eggs laid per *B. tabaci* female than eugenol.

This is the first report of the effect of the vapors of oils from *Artemisia khorrassanica* Podl. (Asteraceae) and *Vitex pseudo-negundo* Hausskn. (Lamiaceae) on *B. tabaci* fecundity, reducing the number of eggs laid per female by 94.72%, followed by limonene, α-terpineol and terpinolene as the major constituent of the *Citrus* oils and terpinolene as the major constituent of the *M. indica* oils. This is the first report of the fumigant properties and effects of these oils on *B. tabaci* fecundity. The findings reveal that the *Citrus* and *M. indica* oils and selected constituents (linalool, α-terpineol, α-pinene, β-pinene, terpinolene and limonene) are potentially useful for the future integrated management of *B. tabaci* in protected environments due to their different mechanisms of action, such as toxicity and a reduction in the fecundity of the target pest. However, further studies are needed to investigate the effects of these essential oils on non-target organisms and the cost-benefit ratio for the formulation of an insecticidal agent containing the essential oils from *Citrus* and *Mangifera* as the active ingredient.

**CONCLUSIONS**

The chemical study of the essential oils from the peels of tangerine, mandarin orange, lemon and lime as well as the latex of two mango varieties demonstrated that the oils were rich in monoterpenes, with limonene as the major constituent of the *Citrus* oils and terpinolene as the major constituent of the *M. indica* oils. This is the first report of the fumigant properties and effects of these oils on *B. tabaci* fecundity.
REFERENCES

ADAMS, R. P. Identification of Essential Oil Components by Gas Chromatography-Mass Spectrometry. 4. ed. Carol Stream: Allured Publishing Corporation, 2007. 804 p.

ARAÚJO JÚNIOR, C. P. et al. Acaricidal activity against Tetranychus urticae and chemical composition of peel essential oils of three Citrus species cultivated in NE Brazil. Natural Product Communications, 5: 471-476, 2010.

BARRO, P. J. et al. Bemisia tabaci: A Statement of Species Status. Annual Review of Entomology, 56: 1-19, 2011.

BORZOUI, E. et al. Lethal and sublethal effects of essential oils from Artemisia khorassanica and Vitex pseudo-negundo against Plodia interpunctella (Lepidoptera: Pyralidae). Environmental entomology, 45: 1220-1226, 2016.

DAMALAS, C. A.; ELEFTHEROHORINOS, I. G. Pesticide exposure, safety issues, and risk assessment indicators. International Journal of Environmental Research and Public Health, 8: 1402-1419, 2011.

DANG, N. H. et al. Chemical composition and α-glucosidase inhibitory activity of vietnamese Citrus peels essential oils. Journal of Chemistry, 2016:1-5, 2016.

DEVANAND, P.; RANI, P. U. Biological potency of certain plant extracts in management of two lepidopteran pests of Ricinus communis L. Journal of Biopesticides, 1: 170-176, 2008.

DOOL, H. V.; KRATZ, P. H. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography A, 11: 463-471, 1963.

FINNEY, D. J. Probit Analysis. 3 ed. Cambridge: University Press, 1971. 304 p.

FOUAD, H. A.; DA CAMARA, C. A. Chemical composition and bioactivity of peel oils from Citrus aurantifolia and Citrus reticulata and enantiomers of their major constituent against Sitophilus zeamais (Coleoptera: Curculionidae). Journal of Stored Products Research, 73: 30-36, 2017.

GHOORCHIBEIGI, M. et al. Chemical composition and radical scavenging activity of Citrus limon peel essential oil. Oriental Journal Of Chemistry, 33: 458-461, 2017.

HAMDAN, D. I.; MOHAMED, M. E.; EL-SHAZLY, A. M. Citrus reticulata Blanco cv. Santra leaf and fruit peel: A common waste products, volatile oils composition and biological activities. Journal of Medicinal Plants Research, 10: 457-467, 2016.

ISMAN, M. B.; GRIENEISEN, M. L. Botanical insecticide research: many publications, limited useful data. Trends in Plant Science, 19: 140-145, 2014.

KIM, S. et al. Contact and fumigant toxicity of plant essential oils and efficacy of spray formulations containing the oils against B- and Q-biotypes of Bemisia tabaci. Pest Management Science, 67: 1093-1099, 2011.

LOVEYS, B. R. et al. Mango sapburn: components of fruit sap and their role in causing skin damage. Functional Plant Biology, 9: 449-457, 1992.

MALACRINÔ, A. et al. Fumigant and repellent activity of limonene enantiomers against Tribolium confusum du Val. Neotropical entomology, 45: 597-603, 2016.

MALUMPHY, C. Diagnostic protocols for regulated pests-Bemisia tabaci. EPPO bulletin, 34: 281-288, 2004.

MELO, J. P. R. et al. Acaricidal properties of the essential oil from Aristolochia trilobata and its major constituents against the two-spotted spider mite (Tetranychus urticae). Canadian Journal of Plant Science, 98: 1342-1348, 2018.

MOHAMMED, A.; CHADEE, D. D. An evaluation of some Trinidadian plant extracts against larvae of Aedes aegypti mosquitoes. Journal of the American Mosquito Control Association, 23: 172-176, 2007.

OTHMAN, S. N. A. M. et al. Essential oils from the Malaysian Citrus (Rutaceae) medicinal plants. Medicines, 3: 1-13, 2016.

PAVELA, R.; BENELLI, G. Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends in plant science, 21: 1000-1007, 2016.

PINO, J. A. et al. Volatile components from mango (Mangifera indica L.) cultivars. Journal of Agricultural and Food Chemistry, 53: 2213-2223, 2005.
RAMOS, E. H. S. et al. Chemical composition, leishmanicidal and cytotoxic activities of the essential oils from Mangifera indica L. var. Rosa and Espada. BioMed Research International, 2014: 1-9, 2014.

RATHORE, H. S.; NOLLET, L. M. Green Pesticides Handbook: essential oils for pest control. 1. ed. Boca Raton: CRC Press, 2017. 570 p.

REIS, N. V. B. Construção de Estufas para Produção de Hortaliças nas Regiões Norte, Nordeste e Centro-Oeste. 1. ed. Brasília, DF: Embrapa Hortaliças, 2005. 16 p.

RIBEIRO, N. C. et al. Acaricidal properties of essential oils from agro-industrial waste products from citric fruit against Tetranychus urticae. Journal of Applied Entomology, 143: 731-743, 2019.

RIBEIRO, N. C. et al. Insecticidal activity against Bemisia tabaci biotype B of peel essential oil of Citrus sinensis var. pear and Citrus aurantium cultivated in northeast Brazil. Natural Product Communications, 5: 1819-1822, 2010.

RIBEIRO, N.; CAMARA, C.; RAMOS, C. Toxicity of essential oils of Piper marginatum Jacq. against Tetranychus urticae Koch and Neoseiulus californicus (McGregor). Chilean Journal of Agricultural Research, 76: 71-76, 2016.

ROBERTSON, J. L. et al. Bioassays with Arthropods. 3. ed. Boca Raton: CRC press, 2017. 212 p.

SAS Institute. The SAS System for Windows Version 9.0. Cary: SAS Institute, 2002.

TCHAMENI, S. N. et al. Using Citrus aurantifolia essential oil for the potential biocontrol of Colocasia esculenta (taro) leaf blight caused by Phytophthora colocasiae. Environmental Science and Pollution Research, 25: 29929-29935, 2018.

YANG, N. et al. Effects of plant essential oils on immature and adults sweetpotato whitefly. Bemisia tabaci biotype B. Crop Protection, 29: 1200-1207, 2010.

ZUHARAH, W. F. et al. Larvicidal efficacy screening of Anacardiacae crude extracts on the dengue hemorrhagic vector, Aedes aegypti. Tropical Biomedicine, 31: 297-304, 2014.