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INSECTICIDAL POTENTIAL OF CITRUS AND MANGO ESSENTIAL OILS AND SELECTED CONSTITUENTS ON SILVERLEAF WHITEFLY[1]

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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* (LC_{50} = 0.70 µL L^{-1} air) and *C. limon* (LC_{50} = 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.

POTENCIAL INSETICIDA DOS ÓLEOS ESSENCIAIS DE CITRUS E MANGA E CONSTITUINTES SELECIONADOS SOBRE MOSCA BRANCA

RESUMO - *Bemisia tabaci* é uma praga cosmopolita e responsável por causar prejuízos aos agricultores no Polo agrícola de Petrolina-PE. Os efeitos letal e subletal dos vapores dos óleos essenciais obtidos por hidrodestilação de quatro espécies de *Citrus* e do látex M*angifera indica* var. rosa e espada, foram investigados sobre *B. tabaci*. Análise química por cromatografia gasosa acoplado à espectrometria de massa dos óleos permitiu a identificação de 71 constituintes, sendo limonene o principal dos óleos de *Citrus* e terpinolene para os de *M. indica*. A susceptibilidade de *B. tabaci* foi maior para os óleos de *Citrus aurantiifolia* (CL_{50} = 0,70 µL L^{-1} air) e *C. limon* (CL_{50} = 1,77 µL L^{-1} air), que apresentaram o mesmo nível de toxicidade entre si. Além da toxicidade, os óleos de *Citrus* e *M. indica* também atuaram na redução da fecundidade de *B. tabaci*. A ação letal e subletal dos constituintes, Linalol, *α*-terpineol, *α*-pineno, *β*-pineno, terpinoleno e limoneno também é discutida. A toxicidade associada com a redução da fecundidade dos óleos aqui investigados é uma grande vantagem para o manejo de *B. tabaci*. No entanto, para o uso prático destes óleos como novo inseticida, mais pesquisas são necessárias sobre questões de segurança para a saúde humana e formulação para melhorar a potencialidade inseticida, estabilidade e custo benéfico.

Palavras-chave: Atividade inseticida. Fumigação. Fecundidade. Limoneno. Terpinoleno.

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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 Anarcadiaceae 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 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 (08º12’40.1” S 34º 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 reticulate* 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. reticulate* 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 ºC) 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 °C at a rate 3 °C min^{-1}. Injector and detector temperatures were 260 °C. 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 C_8-C_{40} 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, LC_{50} (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	TC	LT	LS	ME	MR
			% ± SE	% ± SE	% ± SE	% ± SE	% ± SE	% ± SE
Yield (%) ± SE			2.05±0.07	1.31±0.21	0.81± 0.06	0.48±0.03	5.60 ± 0.13	9.12 ± 0.16
α-Thujene[RI, MS]	924	925	0.59±0.02	0.10±0.00	0.68±0.01	-	-	-
α-Pinene[RI, MS, CO]	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[RI, MS]	945	941	-	-	-	3.84±0.12	-	-
Camphene[RI, MS]	946	954	-	-	-	0.29±0.01	-	0.30 ± 0.02
Sabinene[RI, MS]	969	970	1.08±0.02	2.52±0.08	-	-	-	1.90 ± 0.09
β-Pinene[RI, MS, CO]	974	982	1.67±0.09	-	9.89±0.34	18.14±0.68	2.21 ± 0.03	28.42 ± 0.80
Myrcene[RI, MS, CO]	988	992	4.61±0.08	6.50±0.13	0.58±0.05	2.50±0.02	-	-
δ-2-Carene[RI, MS]	1001	997	-	-	-	-	0.61 ± 0.00	-
p-Mentha-1 (7),8-diene[RI, MS]	1003	1002	4.12±0.11	0.68±0.01	-	-	-	-
δ-3-carene[RI, MS]	1008	1005	-	-	-	-	6.43 ± 0.11	2.09 ± 0.01
α-Terpinene[RI, MS,CO]	1014	1012	-	-	-	-	3.52 ± 0.09	1.00 ± 0.08
Limonene[RI, MS, CO]	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[RI, MS, CO]	1025	1025	-	-	-	-	0.90 ± 0.00	0.70 ± 0.00
(*E*)-*β*-ocimene[RI, MS]	1044	1039	-	-	-	-	-	0.60 ± 0.07
γ-Terpinene[RI, MS, CO]	1050	1050	-	-	-	-	0.43 ± 0.01	0.20 ± 0.01
p-Mentha - 3,8-diene[RI, MS]	1068	1066	-	0.01±0.00	-	0.29±0.01	-	-
p-Mentha -2,4(8)-diene[RI, MS]	1085	1070	9.80±0.21	1.46±0.05	5.53±0.09	1.15±0.02	-	-
Terpinolene[RI, MS, CO]	1086	1093	1.37±0.03	0.10±0.00	1.26±0.03	-	70.14±0.61	39.24±0.29
Linalool[RI, MS, CO]	1095	1103	4.41±0.12	3.56±0.07	3.00±0.07	0.10±0.00	-	-
exo-Fenchol[RI, MS]	1118	1118	-	-	0.19±0.00	1.06±0.02	-	-
cis-Limonene oxide[RI, MS]	1132	1136	-	-	0.49±0.02	0.29±0.00	-	-
trans-Limonene oxide[RI, MS]	1137	1140	-	-	0.68±0.08	-	-	-
(*E*)-Myroxide[RI, MS]	1140	1146	-	-	0.49±0.01	0.58±0.00	-	-
β-Pinene oxide[RI, MS]	1154	1151	-	-	-	1.34±0.11	-	-
iso-Menthone[RI, MS]	1158	1154	0.29±0.00	0.10±0.00	-	-	-	-
Terpinen-4-ol[RI, MS]	1174	1177	0.78±0.01	0.27±0.01	2.62±0.09	2.21±0.08	-	-
p-Cymen-8-ol[RI, MS]	1179	1180	-	-	-	-	-	-
α-Terpineol[RI, MS]	1186	1191	1.08±0.08	0.28±0.00	5.04±0.14	2.78±0.08	-	-
n-Decanal[RI, MS]	1201	1206	1.76±0.10	0.49±0.00	0.29±0.00	-	-	-
cis-Carveol[RI, MS]	1226	1225	-	-	0.19±0.00	-	-	-
Nerol[RI, MS]	1227	1234	0.20±0.00	-	0.68±0.02	0.38±0.01	-	-
Neral[RI, MS]	1235	1246	-	-	2.43±0.08	0.28±0.00	-	-
Geranial[RI, MS]	1264	1261	-	-	0.78±0.00	2.55±0.00	-	-
Isopulegyl acetate[RI, MS]	1274	1271					0.54 ± 0.01	0.10 ± 0.01
Neryl formate[RI, MS]	1280	1276	-	-	2.72±0.08	2.59±0.09	-	-
δ-Elemene[RI, MS]	1335	1336	0.10±0.00	-	0.19±0.00	-	-	-
Citronellyl acetate[RI, MS]	1350	1351	0.09±0.00	0.02±0.00	-	-	-	-
Nerila acetate[RI, MS]	1359	1361	-	-	3.30±0.14	0.38±0.01	-	-
Geranyl acetate[RI, MS]	1379	1380	-	-	1.16±0.09	0.38±0.00	-	-
Daucene[RI, MS]	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 C_8 –C_{40} 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* ; MR: *Mangifera indica* var. "rosa" (MR) and ME: *M. indica* var. "espada" (ME).

Table 1. Continuation.

Compounds	RIL	RIC	TM	TC	LT	LS	ME	MR
			% ± SE	% ± SE	% ± SE	% ± SE	% ± SE	% ± SE
Yield (%) ± SE			2.05±0.07	1.31±0.21	0.81± 0.06	0.48±0.03	5.60 ± 0.13	9.12 ± 0.16
β-Cubebene[RI, MS]	1387	1388	-	0.08±0.01	0.01±0.00	-	-	-
β-Elemene[RI, MS]	1389	1389	0.05±0.00	-	0.01±0.00	-	-	-
Ethyl geranate[RI, MS]	1394	1396	-	-	-	0.77±0.01	-	-
β-Longipinene[RI, MS]	1400	1400	-	-	-	-	0.82 ± 0.02	2.90 ± 0.10
Cycloseychellene[RI, MS]	1406	1407	-	-	-	-	0.41 ± 0.01	0.20 ± 0.00
α-cis-Bergamotene	1411	1414	-	-	0.28±0.00	-	-	-
β-Caryophyllene[RI, MS, CO]	1417	1421	-	-	1.16±0.10	0.31±0.00	-	-
α-trans-Bergamotene[RI, MS]	1432	1434	-	-	2.91±0.10	0.30±0.00	-	-
γ-Elemene[RI, MS]	1434	1438					0.28 ± 0.00	1.50 ± 0.08
α-Guaiene[RI, MS]	1437	1436	-	-	-	1.15±0.00	-	-
Citronellyl propanoate[RI, MS]	1444	1442	-	-	-	-	-	0.13 ± 0.03
α-Clovene[RI, MS]	1452	1449	-	-	-	-	0.61 ± 0.08	0.30 ± 0.01
(*E*)-*β*-Farnesene[RI, MS]	1454	1453	0.10±0.00	-	0.48±0.03	2.88±0.15	-	-
β-Santalene[RI, MS]	1457	1457	-	-	0.20±0.00	-	-	-
Cumacrene[RI, MS]	1470	1471	-	0.03±0.00	0.01±0.00	-	-	-
γ-Gurjunene[RI, MS]	1475	1472	-	-	-	-	3.68 ± 0.13	1.34 ± 0.02
Geranyl propanoate[RI, MS]	1476	1474	-	-	0.41±0.01	0.38±0.00		
γ-Muurolene[RI, MS]	1478	1477	0.20±0.00	0.10±0.00	-	-		
γ-Muurolene[RI, MS]	1478	1477					0.42 ± 0.00	-
γ-Himachalene[RI, MS]	1481	1478					0.60 ± 0.04	0.21 ± 0.01
Valencene[RI, MS]	1496	1497	-	-	0.38±0.00	0.29±0.00	-	-
(*E*,*E*)- *α*-Farnesene[RI, MS]	1505	1502	0.10±0.00	0.20±0.00	-	-	-	-
β-Bisabolene[RI, MS]	1505	1510	-	-	4.17±0.17	-	-	-
β-Sesquiphellandrene[RI, MS]	1521	1525	-	-	-	4.03±0.17	-	-
δ-Cadinene[RI, MS]	1522	1529	0.10±0.00	0.10±0.00	-	-	-	-
Germacrene B[RI, MS]	1559	1554	0.20±0.00	-	-	-	-	-
Caryophyllene oxide[RI, MS, CO]	1582	1583	-	-	0.68±0.08	-	-	-
Humulene epoxide II[RI, MS]	1608	1606	-	-	0.20±0.00	1.34±0.10	-	-
Selin-11-en-4-*α*-ol[RI, MS]	1658	1653	-	-	0.18±0.01	-	-	-
epi-β-Bisabolol[RI, MS]	1670	1666	-	-	0.27±0.00	-	-	-
epi-α-Bisabolol[RI, MS]	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_8 –C_{40} 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* ; 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	LC_{50} µL L^{-1} air (CI 95%)	χ^2	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						
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-terpineol	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; c^2: 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-terpineol 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-terpineol) 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, MALACRINÒ 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 LC_{50} of 0.91 mL/cm^3 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 product reduce the fecundity of the whitefly when compared to the negative control ($F = 560.53$; $DF = 14$; $P < 0.0001$).

Table 3. Fecundity (eggs female^{-1} day^{-1}) of *Bemisia tabaci* exposed to essential oils of *Citrus* and *Mangifera indica,* and applications of selected constituents in laboratory after 24 h.

Essential oil or selected constituents	N	eggs female^{-1} day^{-1} (means±SE)	E (%)
Negative Control	74	9.66±0.15 h*	-
C. reticulata	73	1.67±0.12 c	82.71
C. sinensis x *C. reticulata*	73	0.49±0.05 a	94.93
C. aurantiifolia	73	1.40±0.07 c	85.51
C. limon	73	1.32±0.09 c	86.34
M. indica var. "rosa"	74	8.71±0.15 g	9.83
M. indica var. "espada"	73	8.42±0.14 g	12.84
eugenol	75	7.20±0.20 f	25.47
Linalool	73	3.41±0.27 d	64.70
α-terpineol	74	3.48±0.13 d	63.98
α-pinene	72	2.88±0.14 d	70.19
β-pinene	73	6.31±0.13 e	34.68
terpinolene	74	0.51±0.06 ab	94.72
Limonene	74	1.17±0.08 bc	87.89

*Means followed by the same letter are not significantly different (Tukey test, $p<0.05$); F = 560.53; DF = degree of freedom = 14; P < 0.0001; N = number of female mites; E = reduction in female mites' fecundity.

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 α-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 of *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 from essential oils on the fecundity of *B. tabaci*. However, previous reports describe the effect by fumigation on the fecundity of other agricultural pests that occur in protected farming environments. MELO et al. (2018) showed that sublethal concentrations of the oil from *Aristolochia trilobata* L. (Aristolochiaceae) and the constituents limonene, ρ-cymene, linalool and sulcatyl acetate reduced the fecundity of *Tetranychus urticae*. In another investigation, BORZOUI et al. (2016) report the reduction in the fecundity of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) when exposed to sublethal concentrations of the vapors of oils from *Artemisia khorassanica* Podl. (Asteraceae) and *Vitex pseudo-negundo* Hausskn. (Lamiaceae).

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. 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.

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REFERENCE

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 aurantiifolia* 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 C*itrus 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.

MALACRINÒ, 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 Anacardaciae crude extracts on the dengue hemorrhagic vector, *Aedes aegypti*. **Tropical Biomedicine**, 31: 297-304, 2014.