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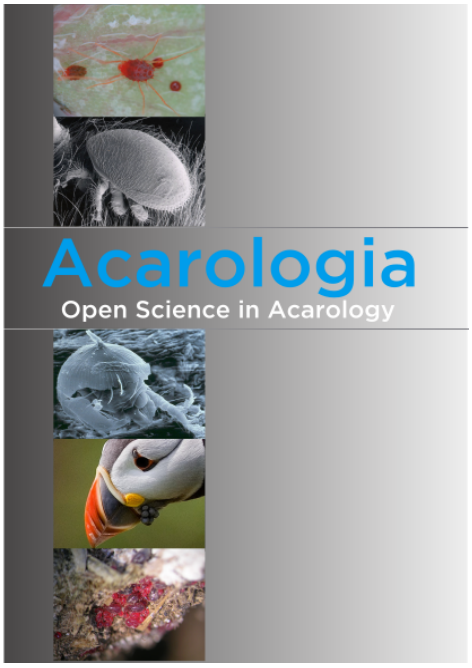

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Effect of the essential oil from the latex of the fruit *Mangifera indica* L. on *Tetranychus urticae* Koch (Acari, Tetranychidae)

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Original research

ABSTRACT

Tetranychus urticae Koch is a cosmopolitan pest that causes damage to crops in protected farming activities in the semiarid region of the state of Pernambuco, Brazil. We investigated the lethal and sublethal effects of the essential oil from the latex of the mango fruit [*Mangifera indica*, Espada and Rosa (MESPA and MROSA) varieties] and selected monoterpenes on *T. urticae*. The yield of the MROSA oil was higher (9.22 ± 0.15%). The GC/MS analysis of the oils enabled the identification of 26 constituents. Terpinolene (70.14 ± 0.61%) was the major compound identified in the MESPA oil; *ß*-pinene (38.22 ± 0.80%) was the major constituent of the MROSA oil, followed by terpinolene (29.44 ± 0.29%). The mite was more susceptible to the oils and constituents through fumigation, with no difference between the two varieties. By residual contact, the MROSA oil was 2.7-fold more toxic than the MESPA oil. Terpinolene was the most toxic constituent by fumigation, whereas *ß*-pinene and a-pinene were the most active by residual contact. The selected compounds from *M. indica* also affected the behavior of the mite, exerting an influence on fecundity, feeding preference and egg-laying preference. The positive control (Azamax®) was more efficient at reducing the fecundity of the mite than the oils, but the MROSA oil was more toxic by fumigation and residual contact. The effects of fumigation and residual contact combined with the change in behavior may be a considerable advantage in the integrated management of *T. urticae*. For the practical use of these oils as novel acaricides, however, further investigations are needed to evaluate the effects on non-target organisms and the cost-benefit ratio for the formulation of a product to be used on protected crops in the semiarid region of the state of Pernambuco, Brazil.

Keywords botanical acaricide; fecundity; fumigation; residual contact; terpinolene

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Introduction

Irrigated systems in the semiarid region of northeastern Brazil are major producers of tropical fruits, such as the mango (*Mangifera indica* L.), which originated in Asia. In 2017, Brazil produced more than a million tons of mangos and 75% of the production came from the northeastern region of the country (IBGE, 2017). This fruit is consumed *in natura* or in the form of industrially processed products, such as juices, jams, sweets and sorbets.

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The fruit has a viscous liquid with a milky appearance denominated latex that exudes upon harvesting. Depending on how the harvesting is performed, this latex can "burn" the peel, which depreciates the commercial value of the fruit (Robinson et al., 1993). A recent investigation addressing the biological properties of the essential oil from the latex of mango has revealed antimicrobial (Fontenelle *et al.*, 2017), antifungal (Perumal *et al.*, 2017), leishmanicidal and cytotoxic (Ramos *et al.*, 2014) properties. However, no studies have investigated the acaricidal potential or the effects on the behavior of *Tetranychus urticae* Koch (Acari: Tetranychidae).

The twospotted spider mite, *T. urticae,* is a major agricultural pest that has caused considerable harm to vegetable crops in protected farming systems, which were installed in the semiarid region of the state of Pernambuco, Brazil, in the 1990s (Reis, 2005). The main form of control of this pest is through the use of synthetic acaricides; the continual use of these products in irrigated and protected farming activities in the region has led to populations of *T. urticae* resistant to one of the most widely used acaricides: Ortus® (a.i. fenpyroximate) (Iwassaki *et al.*, 2015).

Considering the biological properties of essential oils and the effects on different arthropods (Koul *et al.*, 2008), the present study describes the chemical composition of the essential oil from the latex of two varieties of mango as well as its toxicity (through fumigation and residual contact) to *T. urticae* and the effects on the behavior of the mite in terms of feeding and egg-laying preference. Lethal and sublethal effects of the major constituents identified in the oils (α-pinene, β-pinene, limonene and terpinolene) are also discussed. All results were compared to the plant-based insecticide Azamax® and the synthetic insecticide Ortus® used as positive controls.

Materials and methods

Collection of plant material

Magifera indica (L.) fruits were collected from a fragment of Atlantic Forest in the state of Pernambuco (08°12'40.1" S, 34°95'22.3" W). The plants were identified by botanist Dr. Maria R.C.S. de Melo (University Federal Rural of Pernambuco - UFRPE). Vouchers of both samples were mounted and deposited in the Vasconcelos Sobrinho Herbarium of the UFRPE under numbers: 363 = *Mangifera indica* var. Rosa and 364 = *Mangifera indica* var. Espada.

Isolation of essential oils

Essential oils from the latex of *M. indica* fruit [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 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.

Chemicals

Constituents used as standards in the identification of volatile compounds in the oils investigated were purchased from Sigma-Aldrich (Brazil). α-pinene, β-pinene, terpinolene and limonene were selected for the bioassays due to the fact that these compounds were identified in the oils and are commercially available, with known biological properties (Rathore and Nollet, 2017). Ortus® 50 g i.a./L SC Arysta Lifescience (fenpyroximate) and Azamax® 12 g i.a./L EC E.I.D. Parry (azadirachtin) were used as controls.

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 × 0.25 mm × 0.25 µm film thickness) (J & W Scientific). The oven temperature was programmed from 60 to 240 °C at a rate 3 °C min^{-1}. Injector and detector temperature was 260 °C. Hydrogen was used as 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. All analyses were 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 (van Den Dool and 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 *Tetranychus urticae*

Specimens of *T. urticae* were originally collected from grapevines (*Vitis vinifera* L.) in the municipality of Petrolina-PE (09°12′43″ S; 40°29′12″ W) and since maintained in the laboratory at the Agronomy Department of the *Universidade Federal Rural de Pernambuco*, Brazil. The mites were reared at a temperature of 25 ± 1 °C, relative humidity of 65 ± 5% and a 12 h photoperiod, with no exposure to acaricides. The breeding method was the same as that employed by Ribeiro *et al.* (2016).

Fumigant assay

The fumigant method used to assess the toxicity of the *Mangifera* oil vapors to *T. urticae* was as the same as that employed by Araújo *et al.* (2012). Hermetically sealed glass recipients with a capacity of 1.0 L were used as test chambers. Females of *T. urticae* on *Canavalia ensiformes* leaf disks 2.5 cm in diameter were exposed to the *Mangifera* oil, constituents and positive control. A fine brush was used to transfer the mites onto the leaf disks. The leaf disks were placed on filter paper disks saturated with water in Petri dishes (9 cm) to maintain the turgor of the disks and avoid the escape of mites. The experiment was performed in triplicate. One replicate consisted of 30 specimens placed on three leaf disks (10 mites per disk) in a Petri dish. The oil, constituents and positive control (eugenol) were applied with a pipette to a piece of filter paper (5 × 2 cm) attached to the underside of the lid of the recipient. The concentrations of *Mangifera* oils ranged from 3.0 to 15.0 µL L^{-1} air (*Mangifera indica* var. Espada) and 3.0 to 18.0 µL L^{-1} air (*Mangifera indica* var. Rosa). The concentrations of compounds ranged from 1.0 to 30.0 µL L^{-1} air (α-pinene), 1.0 to 14.0 µL L^{-1} air (β-pinene), 0.2 to 4.0 µL L^{-1} air (terpinolene), 1.0 to 16.0 µL L^{-1} air (limonene), 1.0 to 500 µL L^{-1} air (Azamax®) and 1.0 to 150 µL L^{-1} air (Ortus®). Immediately after the application of the oil/compound, the fumigation chamber was closed and covered with PVC plastic wrap. Mortality was determined under a dissecting microscope 24 hours after treatment. All the mites were considered dead if legs did

not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone. All treatments were replicated three times.

Residual contact assay

Leaf discs were painted using the method employed by Neves and da Camara (2016). Tests were conducted on Petri dishes (10 cm diameter) and solutions were prepared by diluting the essential oil in methanol. Discs (2.5 cm diameter) were cut from leaves of greenhouse-grown jack bean. Twenty µL of each concentration was painted on the underside of the disc with a micropipette. Each disc was individually placed on the bottom of a Petri dish atop a disc of filter paper (diameter: 10 cm) wetted with distilled water. Ten adult female mites were placed into each Petri dish and the dishes were covered. All experiments were also performed with open Petri dishes to observe the possible action of the volatile compounds of the oils and compounds tested. Mortality was determined under a dissecting microscope 24 hours after treatment. Control mites were held on leaf discs painted with the carrier solvent alone. All treatments were replicated three times. In the residual assays, concentrations of the *Mangifera* oils ranged from 5 to 250 µL mL^{-1} (*Mangifera indica* var. Espada) and 2.5 to 90.0 µL mL^{-1} (*Mangifera indica* var. Rosa). The concentrations of compounds ranged from 0.76 to 130 µL mL^{-1} (α-pinene), 0.87 to 141 µL mL^{-1} (β-pinene), 16 to 271 µL mL^{-1} (limonene), 44 to 689 µL mL^{-1} (terpinolene), 5 to 126 µL mL^{-1} (Azamax®) and 2 to 112 µL mL^{-1} (Ortus®). Mortality was determined under a dissecting microscope 24 hours after treatment. All the mites were considered dead if legs did not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone. All treatments were replicated three times.

Fecundity Bioassay

The effect of *Mangifera* oil vapors on *T. urticae* fecundity was determined using a fumigation bioassay method (see Ribeiro *et al.*, 2016). Five jack bean leaves (1.5 cm) were placed equidistant in a Petri dish (10 cm) containing filter paper saturated with water. Each leaf disc was infested with an adult *T. urticae* female. The *Mangifera* oils, selected constituents and positive control were applied to strips of filter paper (10 × 2 cm) attached to the inner surface of the lid of the fumigation chamber with the aid of an automatic pipette. The lowest concentrations were 3.0 µL L^{-1} air for *Mangifera indica* oil var. Espada, 3.0 µL L^{-1} air for *Mangifera indica* oil var. Rosa, 1.0 µL L^{-1} air for α-pinene, 1.0 µL L^{-1} air for β-pinene, 0.2 µL L^{-1} air for terpinolene, 1.0 µL L^{-1} air for limonene, 1 µL L^{-1} air for Azamax® and 1 µL L^{-1} air for Ortus®. Immediately after the application of the oil/compound, the fumigation chamber was closed and covered with PVC® plastic wrap. An entirely randomized design was employed, with five replicates, totaling 10 repetitions. The number of eggs in the treatments and controls were recorded after 24 h.

Two-choice assay

The two-choice method was slightly modified from Araújo *et al.* (2012). To determine the preference for feeding and egg laying by *T. urticae* females, bioassays were carried out in arenas made up of Petri dishes (10 cm) containing polyethylene foam moistened with distilled water and a filter paper disk. Two disks of *C. ensiformis* leaf (2.5 cm) were placed in the arena on filter paper. The leaf disks were united by a glass slide (18 mm). Twenty µl of solution containing 1% essential oil, selected constituents or positive control diluted with methanol were placed on one leaf disk. The other disk was treated with methanol alone (negative control). Thirty *T. urticae* females were released in the center of each Petri dish on the glass slide between the two leaf disks. After 48 h, analyses were performed through counts of the mites and eggs on the treated and non-treated leaf disks. All treatments were repeated ten times.

Statistical analysis

To estimate the curve slopes of the LC_{50} (lethal concentration) of each *Mangifera* oil and selected constituents, mortality data were submitted to PROBIT analysis (Finney, 1971) using the Statistical Analysis System (version 9.0) (SAS Institute, 2002). The fecundity bioassay data were submitted to analysis of variance using PROC ANOVA with the means compared by Tukey's test ($P < 0.05$) estimated using SAS (SAS Institute, 2002). The data on feeding and egg-laying preferences were submitted to t-test using PROC TTEST SAS (SAS Institute, 2002).

Results

Yields and chemical profile of essential oils

Yields and chemical constituents of the oils from the latex of the fruit of two varieties of *Mangifera indica* ["Espada" (MESPA) and "Rosa" (MROSA)] are listed in Table 1. Excellent yields were obtained from the latex of both varieties. The yield from MROSA (9.22%) was 1.67-fold higher than the yield from MESPA. The GC-MS analysis of the oils revealed a total of 26 chemical constituents, corresponding to 95.40% and 94.79% of the chemical composition of the MESPA and MROSA oils, respectively. Monoterpenes constituted the dominant class in both oils, accounting for 88.29% for the MESPA oil and 88.18% of the MROSA oil. Eighteen of the 26 compounds were found in both oils. Terpinolene (70.14%) was the main constituent of the MESPA oil, whereas *β*-pinene (38.22%), terpinolene (29.44%) and *α*-pinene (11.50%) were the main constituents in the MROSA oil.

Fumigant and residual contact bioassay

The results of the fumigant and residual contact bioassays of the oils from the latex of *M. indica* var. "Espada" (MESPA), *M. indica* var. "Rosa" (MROSA) and selected constituents (*α*-pinene, *β*-pinene, terpinolene and limonene) are displayed in Table 2. The oils and constituents were more toxic by fumigation than by residual contact. The MROSA and MESPA oils did not differ significantly in terms of toxicity by fumigation. By residual contact, however, the MROSA oil was 2.7-fold more toxic than the MESPA oil.

Terpinolene was the most toxic constituent by fumigation, followed by *β*-pinene. In contrast, the mite was more tolerant to α-pinene and limonene, with no significant difference in the toxicity due to these two hydrocarbon monoterpenes. The greatest susceptibility of the mite by residual contact was found for *α*-pinene and *β*-pinene, which had the same toxic effect, followed by limonene. Terpinolene had the least effect in the residual contact assays. The positive controls of a synthetic (Ortus®) and plant-based (Azamax®) products, which are used for the control of *T. urticae* on different crops, were not toxic by fumigation. No mortality was found when the mites were exposed to Ortus® up to a concentration of 150 $\mu L\ L^{-1}$ of air; low fumigant activity (mortality rate: 2.50%) was found when the mites were exposed to the same concentration of Azamax®. By residual contact, Ortus® was more effective than the oils and selected constituents, whereas Azamax® was 3.3-fold less toxic than the MROSA oil and had the same level of toxicity as the MESPA oil. Regarding the constituents investigated, *β*-pinene and limonene had the same toxicity as Azamax®, whereas α-pinene was 1.6-fold more toxic than Azamax®.

Fecundity of mite after fumigation

Table 3 displays the number of eggs laid per female when exposed to the MESPA and MROSA oils, selected constituents and positive controls (Azamax® and Ortus®) at the lowest concentration in which the number of eggs differed significantly from the negative control ($F = 186.68$; $DF = 8$; $P < 0.0001$). No differences in the number of eggs laid were found

when *T. urticae* females were exposed to the MESPA and MROSA oils. Among the selected constituents, *β*-pinene and limonene had the same effect on the mite fecundity and were the most efficient, followed by α-pinene and terpinolene. The oils had a greater effect on fecundity than Ortus® (synthetic acaricide). All selected constituents, with of the exception terpinolene, promoted a greater reduction in the number of eggs compared to Ortus®. Azamax® (plant-based acaracide) was the most efficient, promoting a greater reduction in the number of eggs compared to the oils. Among the constituents tested, only limonene promoted a greater reduction in the number of eggs laid per female than Azamax®.

Table 1 Chemical composition of essential oils from latex of fruits of *Mangifera indica* var. Rosa (MROSA), *M. indica* var. Espada (MESPA).

N	Compounds	RIL[1]	RIC[2]	MI[3]	MESPA (% ± SE)	MROSA (% ± SE)
	Yield (%) ± SE				5.50 ± 0.12	9.22 ± 0.15
1	*α*-Pinene	932	933	RI, MS, CI	1.03 ± 0.09	11.50 ± 0.21
2	Camphene	946	954	RI, MS	-	0.30 ± 0.02
3	Sabinene	969	970	RI, MS	-	1.90 ± 0.09
4	*β*-Pinene	974	982	RI, MS, CI	2.21 ± 0.03	38.22 ± 0.80
5	*δ*-2-Carene	1001	997	RI, MS	0.61 ± 0.00	-
6	*δ*-3-carene	1008	1005	RI, MS	6.48 ± 0.11	2.11 ± 0.01
7	*α*-Terpinene	1014	1012	RI, MS, CI	3.52 ± 0.09	1.00 ± 0.08
8	Limonene	1024	1021	RI, MS, CI	1.42 ± 0.10	1.31 ± 0.03
9	Sylvestrene	1025	1025	RI, MS, CI	0.90 ± 0.00	0.70 ± 0.00
10	(*E*)-*β*-ocimene	1044	1039	RI, MS	-	0.60 ± 0.07
11	*γ*-Terpinene	1050	1050	RI, MS, CI	0.43 ± 0.01	0.20 ± 0.01
12	Terpinolene	1086	1093	RI, MS, CI	70.14 ± 0.61	29.44 ± 0.29
13	*p*-Cymen-8-ol	1179	1180	RI, MS	0.51 ± 0.01	0.30 ± 0.00
14	*trans*-Chrysanthenyl	1235	1234	RI, MS	0.39 ± 0.02	-
15	*cis*-Chrysanthenyl	1261	1258	RI, MS	0.10 ± 0.00	0.33 ± 0.03
16	Isopulegyl acetate	1274	1271	RI, MS	0.54 ± 0.01	0.10 ± 0.01
17	(*E*)-patchenol	1328	1329	RI, MS	-	0.10 ± 0.00
18	*β*-Patchoulene	1379	1382	RI, MS	0.30 ± 0.01	0.10 ± 0.01
19	*β*-Longipinene	1400	1400	RI, MS	0.82 ± 0.02	2.90 ± 0.10
20	Cycloseychellene	1406	1407	RI, MS	0.41 ± 0.01	0.20 ± 0.00
21	*γ*-Elemene	1434	1438	RI, MS	0.28 ± 0.00	1.50 ± 0.08
22	Citronellyl propanoate	1444	1442	RI, MS	-	0.13 ± 0.03
23	*α*-Clovene	1452	1449	RI, MS	0.61 ± 0.08	0.30 ± 0.01
24	*γ*-Gurjunene	1475	1472	RI, MS	3.68 ± 0.13	1.34 ± 0.02
25	γ-Muurolene	1478	1477	RI, MS	0.42 ± 0.00	-
26	*γ*-Himachalene	1481	1478	RI, MS	0.60 ± 0.04	0.21 ± 0.01
	Total				95.40 ± 0.51	94.79 ± 0.85
	Monoterpenes				88.29 ± 0.46	88.18 ± 0.41
	Sesquiterpenes				7.11 ± 0.18	6.61 ± 0.10
	Fatty acid derivatives				-	-

[1] Retention indices from literature;[2] 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;[3] Method of identification: RI = Retention Index; MS = Mass Spectroscopy; CI = Co-Injection with authentic compounds.

reference for feeding and egg laying by *T. urticae* females

Figures 1 and 2 show the results of the two-choice test when *T. urticae* females were exposed to the oils and selected constituents. The MESPA oil, MROSA oil, α-pinene and *β*-pinene had no effect on feeding or egg-laying preferences. In contrast, limonene and terpinolene exerted significant effects on these preferences. No significant differences in preferences were found in the comparison between the plant-based acaricide (Azamax®) and the negative control.

Discussion

Yields and chemical profile of essential oils

The yields obtained for the essential oils from the latex of MESPA and MROSA are in agreement with previous reports by Ramos *et al.* (2014) for these two varieties. The chemical composition of the essential oils from different parts of the *M. indica* plant is well documented (Andrade *et al.*, 2000; Alwala *et al.*, 2010; Ansari *et al.*, 2004; Franco *et al.*, 2004; Pino *et al.*, 2005; Pino and Mesa, 2006). However, studies reporting the chemical composition of the oil from the latex of mango fruit are rare. For example, Loveys *et al.* (1992) investigated the properties of latex from the fruit of two mango varieties (Kensington and Irwin) and found that the "burning" caused by the latex to the peel is due to its major constituent (terpinolene) and other volatile components. In a more recent study, Ramos *et al.* (2014) found that terpinolene

Table 2 Toxicity towards *Tetranychus urticae* females by fumigation (μL L^{-1} of air) and residual contact (μL mL^{-1}) of essential oils from latex of fruits of *Mangifera indica* var. Rosa (MROSA), *M. indica* var. Espada (MESPA), selected constituents and positive controls (Azamax® and Ortus®).

Essential oil	Bioassay	N[1]	DF[2]	Slope±SE[3]	LC_{50}[4] (IC[5] 95%)	$\chi^{2,6}$
MROSA	Fumigant	626	4	4.68±0.62	9.13(6.11-10.78)	9.36
	Residual	630	5	1.99±0.14	14.10(12.08-16.25)	6.41
MESPA	Fumigant	633	4	4.71±0.56	7.72(5.41-9.18)	9.08
	Residual	630	5	1.79±0.12	37.51(31.93-43.57)	7.87
Positive Control						
Azamax®	Fumigant	630	5	-	>150	-
	Residual	780	5	1.55±0.11	47.07(40.30-55.98)	8.14
Ortus®	Fumigant	-	-	-	-	-
	Residual	630	5	1.31±0.15	6.37(3.95-9.22)	10.34
Compounds						
α–pinene	Fumigant	724	6	3.81±0.28	13.64(10.46-15.71)	10.4
	Residual	804	7	1.57 ± 0.12	29.37(21.47-38.10)	12.11
β–pinene	Fumigant	629	5	2.95±0.25	5.92(4.94-7.61)	8.61
	Residual	807	7	1.53 ± 0.12	34.00(27.88-48.02)	13.01
Terpinolene	Fumigant	623	5	2.17±0.18	2.07(1.61-2.62)	5.29
	Residual	804	7	3.39 ± 0.29	263.06(221.31-	5.21
Limonene	Fumigant	629	5	9.53±1.05	11.52(10.05-12.53)	8.62
	Residual	804	7	2.74 ± 0.19	60.43(50.12-70.18)	4.12

[1] number of mites; [2] degree of freedom; [3] Standart Error; [4] lethal concentration; [5] confidence interval; [6] Khi-square.

was the major component of the oil from the latex of two mango varieties grown in Brazil ("Rosa" and "Espada"). These findings are in agreement with the present results regarding the oil from the latex of the MESPA and MROSA varieties.

The major constituent identified in the present investigation (terpinolene) has also been found in several analyses of essential oils from different parts of the *M. indica* plant, especially the fruit (Andrade *et al.*, 2000; Pino *et al.*, 2005). However, δ-3-carene has been reported as the major compound in the essential oil from the fruit of *M. indica* var. Irwin grown in Australia (Loveys *et al.*, 1992). δ-3-Carene was also found in the present investigation, but at a lower proportion (6.48% and 2.11% of the oils from the latex of the fruit of MESPA and MROSA, respectively). This difference in the chemical profile of volatile components may be attributed to two factors: the genetic variability of the plant and pedoclimatic conditions (Figueiredo *et al.*, 2008).

Fumigant and residual contact bioassay

Essential oils are naturally found as secondary metabolites in aromatic plants and are basically composed of terpenes and phenylpropanoids, which play a fundamental role in the plant defense system against microorganisms, herbivores and arthropods as well as in allelopathic interactions (Bakkali *et al.*, 2008).

The greater susceptibility of the mite to the oils investigated in the present study when submitted to fumigation may be explained by the penetration of the vapors through the tracheal system, with subsequent diffusion to the target site in the pest (Lorini *et al.*, 2015). Through residual contact, however, the oil must pass through the layers of the cuticle in order to reach the target site (Enan, 2001). Despite the lack of investigations on the acaricidal effect of the oil from the latex of *Mangifera indica*, several studies have demonstrated that the oils from other plant species are more efficient by fumigation compared to residual contact (Born *et al.*, 2018; Neves and da Camara, 2016; Moraes *et al.*, 2012; Araujo *et al.*, 2012; de Melo *et al.*, 2018).

Table 3 Fecundity (eggs female^{-1} day^{-1}) of *Tetranychus urticae* after exposure to essential oils from latex of fruits of *Mangifera indica* var. Rosa (MROSA). *M. indica* var. Espada (MESPA). selected constituents and positive controls (Azamax® and Ortus®) applications in laboratory after 24 h.

Essential oil	N[1]	eggs female^{-1} day^{-1} (mean ± SE[2])	E[3] (%)
Negative control	50	5.97 ± 0.17 f*	-
MROSA	50	2.35 ± 0.17 c	60.63
MESPA	50	2.05 ± 0.16 c	65.66
Positive Control			
Azamax®	50	1.72 ± 0.11 b	71.18
Ortus®	50	2.74 ± 0.16 d	54.1
Compounds			
α-pinene	50	1.87 ± 0.13 c	68.67
β-pinene	50	1.57 ± 0.15 ab	73.7
Terpinolene	50	4.47 ± 0.13 e	25.12
Limonene	50	1.46 ± 0.14 a	75.54

[1] number of female mites; [2] Standart Error; [3] reduction in female mites fecundity; *Means followed by the same letter are not significantly different (Tukey test. $p<0.05$).

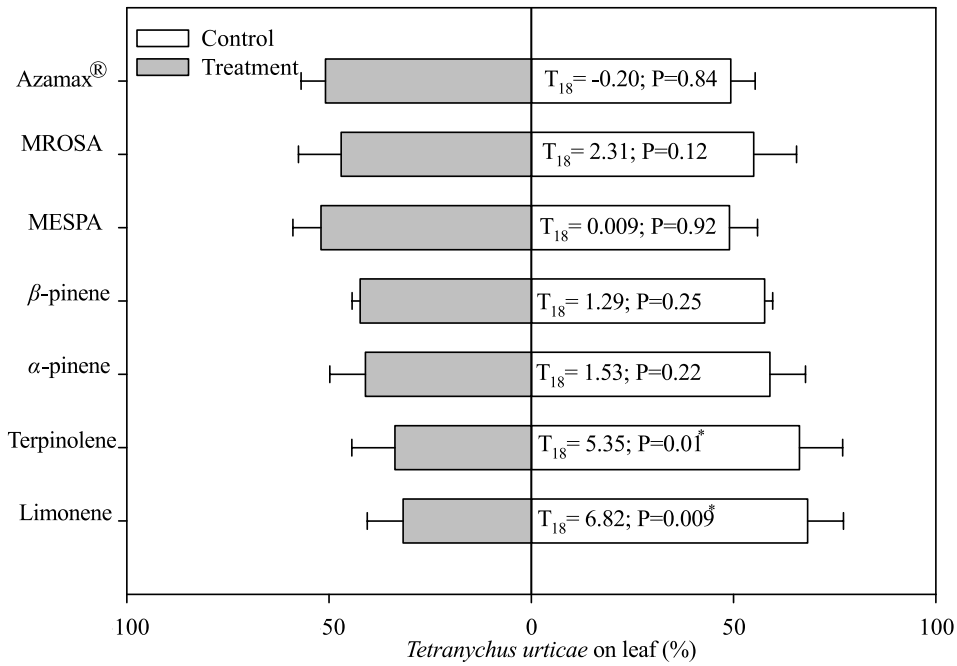

Figure 1 Feeding preference (mean ± SE) of *Tetranychus urticae* exposed to *Mangifera indica* oils and selected constituents for 48 hours. *significantly different ($p<0.05$).

On the other hand, previous studies have investigated the fumigating and residual contact action of selected chemical constituents of the oils (terpinolene, α-pinene, *β*-pinene and limonene) against *T. urticae*. Similar levels of toxicity by fumigation and residual contact as those reported in the present study have been described for terpinolene, *β*-pinene (Born *et al.*, 2018) and limonene (Badawy *et al.*, 2018). However, Tak and Isman (2017) report threefold lower toxicity to *T. urticae* for α-pinene than the level found in the present study for the same monoterpene. This difference in the susceptibility of the pest to the same constituent may be explained by the use of mite populations from different regions (Monteiro *et al.*, 2015; Gouveia *et al.*, 2018).

The synergic potential among the constituents of oils is a well-established phenomenon and explains the toxicity of essential oils to mites and other insects (Miresmailli *et al.*, 2006; Tak and Isman, 2017). This phenomenon was seen in the residual contact tests, as the monoterpenes investigated herein and other constituents of the MROSA oil acted in a synergic manner, promoting 2.08-fold, 2.41-fold, 18.66-fold and 4.28-fold greater toxicity of the oil than that found for α-pinene, β-pinene, terpinolene and limonene, respectively.

An important aspect of the relative toxicity by fumigation of the selected constituents involves the structure-activity relationship of bicyclic isomers, as *β*-pinene was 2.3-fold more toxic than α-pinene. These results are in agreement with findings reported by Lucia *et al.* (2007) and Perumalsamy *et al.* (2009) for these isomers against larvae of the mosquito *Aedes aegypti*, who attribute the greater toxicity of *β*-pinene to the existence of an exocyclic double bond. Among the monocyclic isomers tested, the greatest activity was found for terpinolene, which has two double bonds, one of which is exocyclic.

Regarding the greater susceptibility of the mite to α-pinene and β-pinene by residual contact, the higher levels of these two monoterpenes in the MROSA oil may at least partially explain the greater toxicity of this oil in these assays.

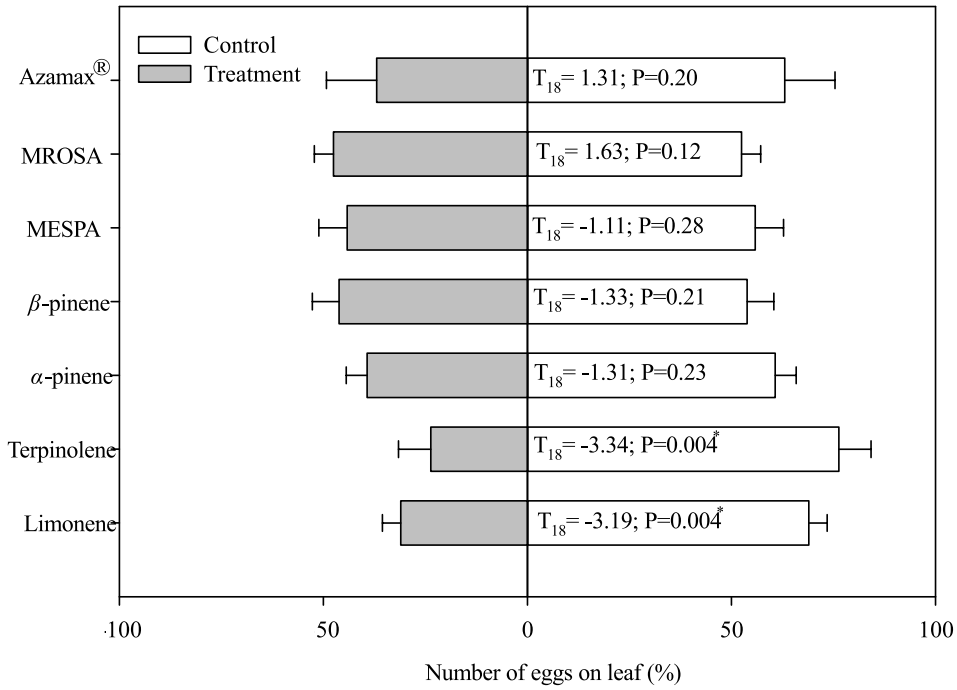

Figure 2 Egg laying preference (mean ± SE) of *Tetranychus urticae* exposed to *Mangifera indica* oils and selected constituents for 48 hours. *significantly different ($p<0.05$).

Fecundity of mite after fumigation

The capacity of sublethal concentrations to reduce the fecundity of *T. urticae* has previously been reported for other essential oils. For instance, *Rosmarinus officinalis* oil led to an 8.90% reduction in fecundity at a concentration of 15.0 µL L^{-1} (Laborda *et al.*, 2013). In another study, Chitgar *et al.* (2013) found a 40% reduction in the number of eggs when *T. urticae* was exposed to *Thymus vulgaris* oil at a concentration of 6.24 µL L^{-1} of air. Therefore, the vapors from the MESPA and MROSA oils had a greater effect on the fecundity of *T. urticae* than the oils from *R. officinalis* and *T. vulgaris*. Although the positive control used in these experiments (Azamax®) had a greater effect than the oils on the fecundity of the mite, the analysis of the selected constituents revealed that limonene and *β*-pinene proved to be promising in the reduction of *T. urticae* fecundity, with comparable results to those achieved with Azamax®.

##Preference for feeding and egg laying by *T. urticae* females Repellency is an important property of an acaricide, as it affects the behavior of the mite. The use of this property in integrated pest management programs has achieved important results, as damage to crops is minimized when the pest is repelled (da Camara *et al.*, 2015). In recent years, there has been a notable increase in investigations on the properties of natural products and their effects on different arthropods (Isman and Grieneisen, 2014). Such studies clarify toxicological issues, but the effects of these natural products on the behavior of pests have been under-investigated.

Although the MESPA and MROSA were not repellent to *T. urticae* at the concentrations tested, previous investigations involving other essential oils report effects on the behavior of this pest. da Camara *et al.* (2015) found that exposure to the oil from *Citrus aurantium* avoided the dispersal of *T. urticae* in a greenhouse for one week. Araújo *et al.* (2010) found that the oil from *Citrus sinensis* var. "Mimo" repelled *T. urticae* at a concentration lower than 2.5%, whereas the oil from *C. sinensis* var. "Pera" had no effect. In another investigation, Araújo *et*

al. (2012) attributed the repellent action of the oil from *Piper aduncum* against *T. urticae* to the constituents (*E*)-nerolidol, *α*-humulene and *β*-caryophyllene.

In the analysis of the selected constituents of the MESPA and MROSA oils, the monocyclic monoterpenes (limonene and terpinolene) were the most repellent. This finding may be attributed to the volatility and conformational flexibility of these compounds regarding the interaction with the active site in the pest. This argument is supported by the lack of repellent action found for the bycyclic compounds (α-pinene and *β*-pinene), which have a more rigid chemical structure.

The chemical profile of the oils from the latex of the fruit of MESPA and MROSA is in agreement with results reported for other varieties of *M. indica* rich in monoterpenes with terpinolene as the major component. This is the first report on the repellent action, effect on fecundity and toxicity, by both fumigation and residual contract, of the oils from the latex of *M. indica* var. "Espada" and var. "Rosa" against *T. urticae*. The findings reveal that the MESPA and MROSA oils and selected constituents (terpinolene and *β*-pinene) are potentially useful for future integrated management of *T. urticae*, with different modes of action (fumigant and residual contact) and effects on the behavior of the pest (reduction in fecundity). However, further investigations are needed to evaluate the effects on non-target organisms and the cost-benefit ratio for the formulation of an acaricidal agent developed from the latex of this fruit for use on organic and protected crops in the semiarid region of the state of Pernambuco, Brazil.

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