Toxicity of essential oils of *Piper marginatum* Jacq. against *Tetranychus urticae* Koch and *Neoseiulus californicus* (McGregor)

Nicolle Ribeiro[1], Claudio Camara[1*], and Clecio Ramos[2]

ABSTRACT

Tetranychus urticae Koch (Acari: Tetranychidae) is an economically important pest of agricultural and ornamental crops worldwide. It has been shown that many of natural plant-based pesticides have fewer side effects compared with synthetic chemicals. The essential oils of plants have been broadly studied for pest-control, including toxic and repellent effects, antifeedant, ovicidal, and other properties. Essential oils from stems, flowers, and leaves of *Piper marginatum* Jacq. were evaluated in the laboratory regarding their acaricidal potential against the two-spotted spider mite (*T. urticae*) and the results were compared with eugenol as a positive control. The oils were also evaluated with regard to the susceptibility of the natural enemy of *T. urticae* (*Neoseiulus californicus* McGregor). Based on LC_{50} estimates, oils from stems and flowers exhibited the same toxicity and differed significantly from the leaf oil with LC_{50} 0.37, 0.56, and 3.77 µL L^{-1}, respectively. None of the oils tested exhibited toxicity greater than or equal to that of the positive control. The oil mortality rate was significantly lower for *N. californicus* (50% to 70%) than for *T. urticae* (> 95%). The *P. marginatum* oils also deterred oviposition. Among the chemical constituents tested, sesquiterpenes were more toxic with an LC_{50} of 2.89 µL L^{-1} than phenylpropanoids *Z*-asarone and *E*-asarone with LC_{50} 6.64 and 8.51 µL L^{-1}, respectively. The acaricidal properties, oviposition deterrence and selectivity make these oils strong candidates for use as the active ingredient in a plant-based acaricidal agent.

Key words: Acaricidal activity, chemical constituents, Piperaceae, predator mite, two-spotted spider mite.

[1]Universidade Federal Rural de Pernambuco, Departamento de Agronomia, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, 52171-900 Recife, Pernambuco, Brasil.

[*]Corresponding author (claudio_agc@hotmail.com).

[2]Universidade Federal Rural de Pernambuco, Departamento de Ciências Moleculares, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, 52171-900 Recife, Pernambuco, Brasil.

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INTRODUCTION

Among the different species of mites of the family Tetranychidae, the two-spotted spider mite (*Tetranychus urticae* Koch; Acari: Tetranychidae) is considered the most important. This cosmopolitan pest feeds on several plant species, including crops such as tomato, cucumber, melon, strawberry, corn, soybean, apple, grape, and citrus fruit (Gerson and Weintraub, 2012). The first records of the two-spotted spider mite in agricultural systems in the state of Pernambuco, Brazil, date back to the 1980s. Since then, this pest has caused considerable harm to tomato, melon, strawberry, and grape crops, exerting a negative impact on productivity and economic returns for farmers.

The main form of control for *T. urticae* is the use of synthetic pesticides, together with biological control involving the generalist mite *Neoseiulus californicus* McGregor, which is a natural predator of *T. urticae* found in different regions of Brazil (Fraulo and Liburd, 2007). However, the continual use of conventional acaricides has caused serious damage due to environmental contamination as well as the elimination of *N. californicus* and various pollinating insects (Motazedian et al., 2012). To reduce these negative effects, alternative methods for the control of *T. urticae* are being tested, including the use of essential oils (Motazedian et al., 2012). Essential oils are promising agents for the control of agricultural pests, as these substances act on the nervous system of the target insect, causing physiological and behavioral responses (Mossi et al., 2013).

Species of the genus *Piper* (Piperaceae) are widely distributed throughout South America, including fragments of the Atlantic Forest in the state of Pernambuco (northeastern Brazil), and have potential for the control of agricultural pests. These plants are known for the production of essential oils and the accumulation of secondary metabolites, such as phenylpropanoids, lignans, amides, and alkaloids, which are used in the defense against herbivory (Scott et al., 2008). Among the species of *Piper* that occur in Pernambuco, *Piper marginatum* Jacq. is an aromatic shrub that grows abundantly around the borders of the Atlantic forest and is known as ‘‘malvaisco” in northeastern Brazil (states of Pernambuco, Paraíba, and Rio Grande do Norte) (Chahal et al., 2011). A preliminary chemical investigation of oils from stems, flowers, and leaves of *P. marginatum* conducted by our research group revealed the presence of phenylpropanoids and sesquiterpenes as the predominant chemical classes. These oils demonstrate potent insecticidal activity on 4th instar larvae of

RESEARCH

the mosquito *Aedes aegypti* and also serve as a deterrent to oviposition (Autran et al., 2009). The insecticidal activity of *P. marginatum* oil has been reported for other arthropods, such as *Solenopsis saevissima* (Souto et al., 2012), *Tenebrio molitor* (Fazolin et al., 2007), *Sitophilus zeamais* (Estrela et al., 2006; Coitinho et al., 2011), *Tyrophagus putrescentiae* and *Suidasia pontifica* (Assis et al., 2011). However, no previous study has evaluated the acaricidal action of *P. marginatum* oil on *T. urticae* and its natural enemy, *N. californicus*.

As part of our investigation into the acaricidal properties of essential oils from aromatic plants that occur in the Atlantic Forest in the state of Pernambuco, Brazil, the aim of the present study was to conduct a laboratory test of the acaricidal properties of essential oil vapors from different parts of *P. marginatum* (stems, flowers, and leaves) on *T. urticae* and *N. californicus*, comparing with eugenol, used as positive control. The relative toxicity of the sesquiterpenes β-caryophyllene, and patchoulol and the phenylpropanoids *Z*-asarone and *E*-asarone is also discussed.

MATERIALS AND METHODS

Collection of plant material

Stems, flowers, and leaves of *P. marginatum* were collected from a fragment of the Atlantic forest in the city of Recife (8°7'30" S, 34°52'30" W), Pernambuco, Brazil. The plant was identified by Dr. Margareth F. de Sales of the Biology Department of the Rural Federal University of Pernambuco (UFRPE) through comparisons with previously identified samples and a voucher specimen was deposited at the Vasconcelos Sobrinho Herbarium of the Federal Rural University of Pernambuco (accession number: 48210).

Isolation of essential oils

Essential oils from stems, flowers, and leaves of *P. marginatum* (100 g) were isolated using a modified Clevenger-type apparatus and hydrodistillation for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers and kept under refrigeration at 5 °C until the acaricidal assays.

The compounds for investigation of acaricidal potential were selected based on the chemical identification of *P. marginatum* oils determined previously by our research group (Autran et al., 2009) (Table 1) and their commercial availability. Compounds with a high degree of purity (97% to 99%) were acquired commercially from Sigma (St. Louis, Missouri, USA), together with eugenol, which was used as the positive control.

Table 1. Percentage of compounds selected for investigation from essential oils of *Piper marginatum*.

Compounds	Stem	Inflorescence	Leaf
β-Caryophyllene	25.7	23.4	16.0
Z-Asarone	8.5	4.5	30.4
E-Asarone	32.6	22.1	6.4
Patchoulol	6.8	13.1	7.5

Constituents listed in order of elution on a non-polar DB-5 column.

Insect rearing

Specimens of *T. urticae* used for the bioassays were reared on jack bean (*Canavalia ensiformis* [L.] DC.) without any exposure to acaricidal agents at the UFRPE Agronomy Department. All bioassays were performed at 25 ± 1 °C, 65 ± 5% RH and a 12:12 h photoperiod.

The population of *N. californicus* used in the bioassays was collected from the municipality of Bonito (State of Pernambuco) and maintained at the UFRPE Agricultural Acarology Laboratory with no exposure to acaricidal agents. The method described by Monteiro (2002) with modifications was used to rear this predatory mite. The specimens were reared in plastic arena maintained in BOD with a mean temperature of 27 °C and a 12:12 h photoperiod. Each arena was made of a polyethylene foam disc soaked in distilled water. A filter paper disc was placed on the polyethylene foam and a jack bean (*C. ensiformis*) leaf was placed on the filter paper with the margin surround by moistened cotton to prevent the escape of the mites. Cotton fibers were placed on the jack bean leaf to stimulate oviposition. *Tetranychus urticae* and pollen from the castor oil plant (*Ricinus communis* L.; Euphorbiaceae) were offered every 2 d.

Fumigant toxicity and fecundity test

The fumigant method used to assess toxicity of *P. marginatum* oils vapors against *T. urticae* were the same as that employed by Araújo et al. (2012). The concentrations of oils and compounds ranged from 0.005 to 8 μL L^{-1} air for oils, 0.2 to 14 μL L^{-1} air for the constituents and 6.4×10^{-5} to 1.2 μL L^{-1} air for eugenol (positive control). The fumigant assay method used to measure the toxicity of the oils to *N. californicus* was that described by Araújo et al. (2012) with modifications. Each leaf disc was infested with 10 specimens of *N. californicus*, totaling 30 mites per Petri dish. Fifteen specimens of *T. urticae* and castor oil plant pollen were offered as food on each leaf disc. The concentrations of *P. marginatum* essential oils tested were those that led to a 95% mortality rate in the tests with *T. urticae*: 0.8, 1.0, and 8.0 μL L^{-1} air for oil from stems, flowers, and leaves, respectively.

The *T. urticae* oviposition deterrent effect of the vapors from *P. marginatum* oils was determined using a method adapted from Pontes et al. (2007a). 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, totaling five females per Petri dish. The essential oils and chemical compounds 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 of essential oils and compounds that significantly reduced oviposition in the fumigation tests were used in the fecundity tests: 0.005, 0.2, and 1.0 μL L^{-1} air for the oils from *P. marginatum* stems, flowers, and leaves, respectively; 6.4 ×10^{-5} μL L^{-1} air for eugenol; and 0.2, 2.5, 5.0, and 8.0 μL L^{-1} air for the compounds β-caryophyllene, patchoulol, *Z*-asarone, and *E*-asarone, respectively. No substances were used in the negative control. 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 replicates. The number of eggs in treatments and controls was recorded after 24 h.

Statistical analysis

The mortality data for the oils from stems, flowers, and leaves of *P. marginatum* and eugenol were analyzed with the Probit model using the POLO-PC software program (LeOra Software, 1987) for the determination of the lethal concentration necessary for 50% mortality (LC_{50}) of the mite population, with the calculation of 95% confidence levels. Toxicity ratios were determined based on the method described by Robertson and Preisler (Robertson and Preisler, 1992). In the fecundity bioassays, the *t*-test was used to compare data on the number of eggs per female in treatments and controls. The *t*-test was also used to compare *N. californicus* and *T. urticae* mortality data.

RESULTS

The vapors from the *P. marginatum* oils and selected chemical constituents were toxic to *T. urticae*. The estimated LC_{50} values demonstrate the same degree of toxicity for stem and flower oils, which were approximately 10-fold more toxic in comparison to the leaf oil. However, none of the oils was more potent than the positive control, which was 92.5-fold more toxic than the stem oil (Table 2).

Among the compounds tested, *T. urticae* was most susceptible to sesquiterpene β-caryophyllene, found in the leaf oil in the concentration of 16.0% (Tables 1 and 2). The phenylpropanoid *Z*-asarone, major compound of leaf oil (30.4%), showed less toxicity than the oil and similar toxicity with its isomer *E*-asarone (6.4%). None of the isolated compounds exhibited greater or equal acaricidal activity in comparison to the positive control.

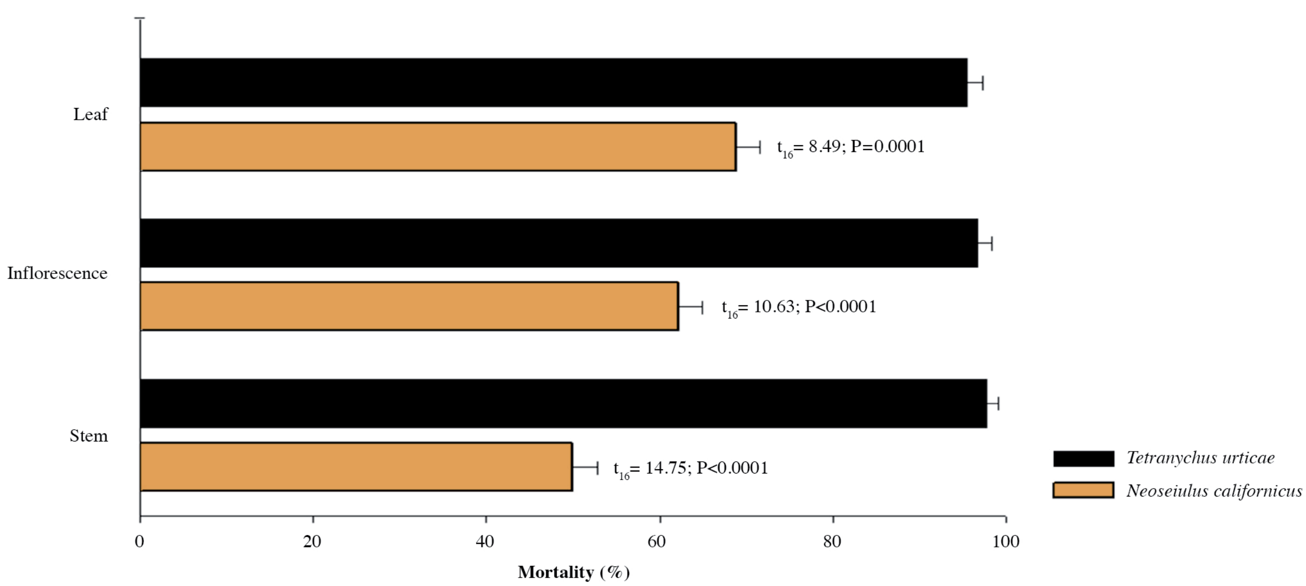
Figure 1 displays the susceptibility of the predatory mite *N. californicus* to the *P. marginatum* oils at concentrations

Table 2. Toxicity by fumigation (LC_{50}, μL L^{-1} air) of the *Piper marginatum* essential oils and major constituents against *Tetranychus urticae*.

Oil/Compound	N	DF	Slope ± SD	LC_{50} (CI at 95%)	χ^2	TR_{50}
EU	640	3	0.85 ± 0.076	0.004 (0.002-0.005)	1.52	-
Stem	540	3	5.64 ± 0.58	0.37 (0.27-0.46)	7.61	105.84* (16.9-659.3)
Inflorescence	540	3	6.46 ± 0.74	0.56 (0.42-0.65)	6.28	159.2* (25.4-995.4)
Leaf	540	3	3.85 ± 0.49	3.77 (2.12-4.12)	6.94	1070.6* (145.7-7866.1)
β-Caryophyllene	540	3	2.44 ± 0.28	2.89 (1.51-4.30)	6.81	820.78* (57.56-11702.94)
Patchoulol	630	4	2.31 ± 0.20	4.00 (2.79-5.24)	8.96	1134.9* (164.2-7840.9)
Z-Asarone	540	3	6.93 ± 0.75	6.64 (5.05-7.70)	6.84	1883.9* 258.2-13744.5)
E-Asarone	630	4	5.39 ± 0.54	8.51 (7.14-9.61)	8.80	2413.3* (333.3-17471.9)

EU: Eugenol used as positive control, N: number of mites, DF: degrees of freedom, SD: standard deviation, CI: confidence interval; χ^2: Chi-squared, TR: toxicity ratio calculated by Robertson & Preisler method.
*Significant when confidence interval does not include 1.

Figure 1. Mean mortality rate of *Tetranychus urticae* and *Neoseiulus californicus* submitted to *Piper marginatum* oils at concentrations that caused greater than 95% of specimens *T. urticae* in the fumigation bioassay (stem = 0.8, flower = 1.0, and leaf = 8.0 μL L^{-1} air).



that led to ≥ 95% *T. urticae* mortality (stems = 0.8, flowers = 1.0, and leaves = 8.0 $\mu L\ L^{-1}$ air). Mean *T. urticae* mortality was significantly higher than mean *N. californicus* mortality, suggesting that the *P. marginatum* oils were selective to *T. urticae*.

The number of eggs laid was reduced with the increase in the concentration of the oils and consequent death of the specimens of *T. urticae*. To determine whether this reduction in the number of eggs was due to exposure to the oils or the death of mites, further experiments were performed with the sublethal concentration of each oil and major constituent. Figure 2 displays the mean number of eggs laid per *T. urticae* female when exposed to the different substances. The number of eggs on the discs in the treatments (oils and major constituents) was significantly smaller than the number of eggs in the negative control.

DISCUSSION

In the present study, oils from *P. marginatum* stems, flowers, and leaves and their major components either together or separately caused physiological and behavior responses in *T. urticae*, as demonstrated by fumigant toxicity and oviposition deterrence.

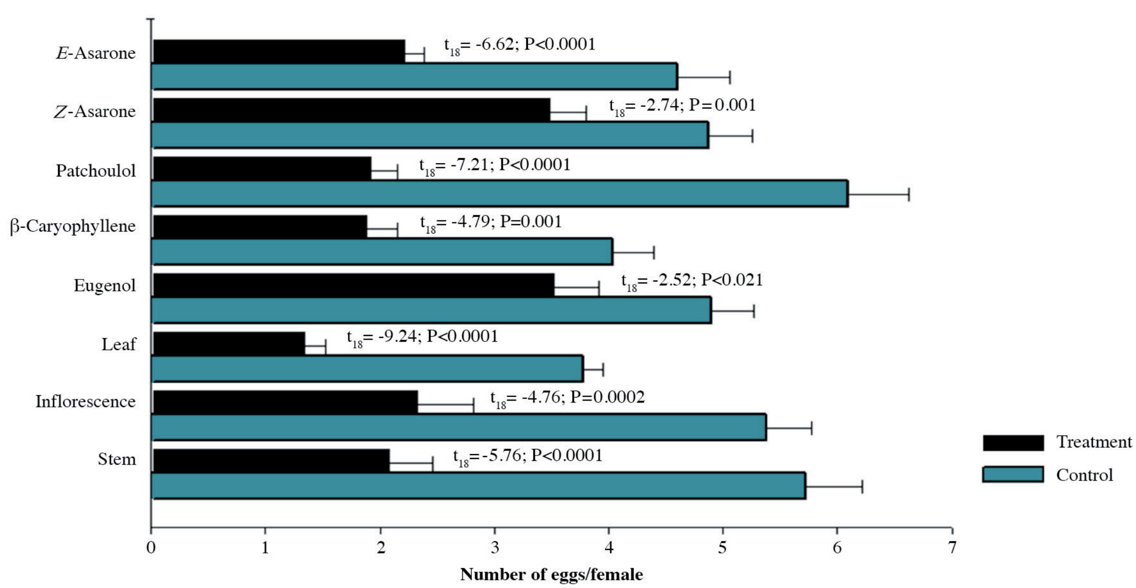
This is the first report of the fumigating action of *P. marginatum* oils on *T. urticae* and its natural enemy *N. californicus*. However, the evaluation of the acaricidal activity of other plant species against *T. urticae* has been investigated previously, such *Protium bahianum*, *Protium heptaphyllum* (Pontes et al., 2007a; 2010), *Xylopia sericea* (Pontes et al., 2007b), *Citrus sinensis*, *Citrus aurantium* (Araújo-Júnior et al., 2010), and *Piper aduncum* (Araújo et al., 2012).

The difference in toxicity among different *P. marginatum* oils is likely due to qualitative and/or quantitative differences in chemical constituents, stem and flower oils were more toxic to *T. urticae* than the leaf oil (Table 1). Among the major components, only β-caryophyllene and patchoulol, which were found in different proportions in stem, flower, and leaf oils, and it demonstrated acaricidal activity. The biological properties of β-caryophyllene have been confirmed in previous reports involving the larvae of *Aedes aegypti* (Diptera: Culicidae) (Dória et al., 2010) and *T. urticae* (Araújo et al., 2012). Moreover, the major constituents are not the only components responsible for the fumigating action observed in the experiments and it is likely that these compounds act in synergy with those found in smaller amounts (Moraes et al., 2012).

Among the major components, the β-caryophyllene was more active against the mite with LC_{50} 2.89 $\mu L\ L^{-1}$ that have been confirmed in previous reports involving the action of β-caryophyllene against mites (Oh et al., 2013). In this study, using the fumigant method the β-caryophyllene exhibited LC_{50} of 3.13 and 3.58 μg cm^{-2} against house dust mites *Dermatophagoides farinae* (Hughes) and *D. pteronyssinus* (Trouessart), respectively.

Eugenol (positive control) is a phenylpropanoid, with *E*-azarone and *Z*-azarone isomers, however eugenol was about 1750 times more toxic than the two isomers. The three phenylpropanoids have similar chemical structures, but eugenol has an allylic chain. The toxicity of phenylpropanoid safrole, a natural insecticide and chemically similar to eugenol, is assigned to the allylic chain (Borchert et al., 1973), and previous report revealed the LD_{50} 0.36 $\mu L\ L^{-1}$ for safrole against *T. urticae* females. These data indicate that the allylic group in the phenylpropanoids can contribute significantly to high toxicity of these compounds against the mite *T. urticae*.

Figure 2. Mean number of eggs per *Tetranychus urticae* female when submitted to vapors from *Piper marginatum* essential oils, major constituents (β-caryophyllene, patchoulol, *Z*-asarone, and *E*-asarone) and positive control (eugenol). The concentrations employed in this test were selected based on the fumigation bioassay in which the number of eggs differed significantly from the negative control.



The use of conventional acaricides is one of the most widely employed methods for the control of *T. urticae* in groves and greenhouses in agricultural systems in the city of Petrolina, Pernambuco, Brazil. However, this pest has developed resistance to such acaricides in recent years. Thus, the use of natural enemies has intensified in greenhouse operations and has become a key measure in the integral control of *T. urticae*. Nonetheless, this practice is not always effective due to the fact that the predator mite is more susceptible to conventional acaricides than *T. urticae* (Choi et al., 2004).

In the present study, *T. urticae* proved to be more susceptible to *P. marginatum* essential oils than its predator *N. californicus*. This finding suggests that the management of *T. urticae* with *N. californicus* is compatible with the use of a plant-based acaricide with *P. marginatum* essential oil as the active ingredient. Similar results have been reported for essential oils from *Carum carvi* (Apiaceae), *Eugenia caryophyllata* (Myrtaceae) and *Ocimum basilicum* (Lamiaceae) on the same predator (Han et al., 2010). However, cited authors found no significant differences in the toxicity of oils from *Cymbopogon nardus* (Poaceae), *Eucalyptus citriodora* (Myrtaceae), *Mentha pulegium* (Lamiaceae), *Mentha ×piperita* (Lamiaceae), *Salvia officinalis* (Lamiaceae), *Mentha spicata* (Lamiaceae) or *Thymus vulgaris* (Lamiaceae) between *T. urticae* and *N. californicus*.

Piper marginatum oils and their major compounds also affected fecundity in *T. urticae* indicating an action on *T. urticae* fecundity at sublethal concentrations. The ability of essential oils to reduce fecundity in *T. urticae* has been reported in previous studies analyzing fumigant toxicity (Pontes et al., 2007a; 2007b; 2010), but with no indication as to whether this reduction in fecundity was due to the properties of the oils or the death of mites. In the present study, the results of the fecundity experiments demonstrated that *T. urticae* females exhibited a behavioral response to the essential oil vapors. While exposure to sublethal concentrations of the *P. marginatum* oils and their major constituents did not impede oviposition, fecundity was drastically reduced.

CONCLUSION

Based on the present findings, essential oils from *Piper marginatum*, especially stem and flower oils, are promising for the control of *Tetranychus urticae*. The acaricidal properties of these oils, along with oviposition deterrence and the less toxic effect on the natural enemy of this mite, *Neoseiulus californicus*, make these oils a strong candidate as the active ingredient in a plant-based acaricide.

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