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Susceptibility of *Tetranychus urticae* Koch (Acari: Tetranychidae) to essential oil mixtures

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Abstract
The two-spotted spider mite *Tetranychus urticae* is an important pestin many cultures of economic relevance. The search for methods to control this plague like the use of essential oils can reduce the application of synthetic acaricides. Therefore the aim of this work was to evaluate the lethal, sublethal and synergetic effects of essential oils *Cymbopogon citratus* (lemongrass), *Mentha piperita* (pepper mint) *Syzygium aromaticum* (clove) and *Citrus limonum* (sicilian lemon) on adult females of *Tetranychus urticae* Koch (Acari: Tetranychidae). The results show that essential oils and oil mixtures present toxicity on mites. Essential lemongrass oil mixture with Clove oil showed synergetic effectwithLC_{50} and LC_{90} of 0.91% (v v^{-1}) and 2.85% (v v^{-1}), respectively. The binary mixtures of pepper mint essential oils with lemongrass, and pepper mint with Clove presented about 60 to 64% of toxicity effect. Mixtures of essential oils have had significant results for future use in the production of a toxic agent.

Keywords: Atripedacarid, aromatic plants, monoterpene, sesquiterpene

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), is a poly phytophagous species which can eat 1100 plant species, and is also an important plague in greenhouse and field cultivation [1]. Most useful methods to control two-spotted spider mite are synthetic acaricides and predator mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) [2-4].

The use of synthetic acaricides can take serious risks to not target organisms, as mammals, aquatic animals, birds and human beings [5, 6]. As an alternative to synthetic acaricides, essential plants oils constitute excellent candidates to infestation control, involving many ways to present a smaller toxicity in mammals and lower persistence in the environment [7].

The essential oil extracted from aromatic plants has been shown as a hopeful alternative to control many stored grains infestation, such as: *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae); *Rhyzopertha dominica* (Fabricius) 1972 (Coleoptera: Bostrycidae); *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) and *Lasioderma serricorne* ©Fabricius 1792 (Coleoptera: Anobiidae) (Tripathi *et al.* 2009). Several studies describe essential oils toxicity on various pest insects, published till now [8-11].

The aim of this work was to evaluate the lethal, sub lethal and synergetic effect in essential oils made from *Cymbopogon citratus* (D.C.) Stapf (Poaceae) (lemongrass), *Menthapiperita* L. (Lamiaceae) (Pepper mint), *Syzygium aromaticum* (L.) Merr. & LM Perry (Myrtaceae) (Clove) and *Citrus limonum* Risso (Rutaceae) (Sicilian lemon) about *T urticae*.

2. Materials and methods

2.1 Biological material

The *T urticae*mite was raised in jack bean plants, *Canavalia ensiformis* (L.) DC (Fabaceae), in Climatized rooms at 25 ± 1 ºC, 65 ± 3% RH and photo phase of 12h.

2.2 Essential oils acquisition

The essential oils are of commercial origin and were acquired from the company Phytoterápica Ltda, located at Nova Cantareira street, 2627 - Tucuruvi– São Paulo, Zip code: 02341-000, São Paulo - Brazil.

2.3 Essential oils solutions preparation and mixtures
The mixture of essential oils, *Cymbopogon citratus* (lemongrass), *Menthapiperita* (pepper mint), *Syzygium aromaticum* (Clove) and *Citrus limonum* (Sicilian Lemon) were made proportionally as shown in Table 1

Table 1: Essential oils mixture proportions

Mixtures	Mixture Proportion	EssentialOils
1	1:1:1:1	[a] lemongrass + [b] pepper mint + [c] clove + [d] sicilian lemon
2	1:1	lemongrass + peppermint
3	1:1	lemongrass + clove
4	1:1	lemongrass + sicilianlemon
5	1:1	peppermint + clove
6	1:1	peppermint + sicilianlemon
7	1:1	clove + sicilianlemon

Scientific name of the plant: [a] *Cymbopogon citratus*, [b] *Menthapiperita*, [c] *Syzygium aromaticum*, [d] *Citrus limonum*

The preparation of essential oils and the respective mixtures (Table 1) was held to 2.0% (v v^{-1}), Tween® 80 (0.05% v v^{-1}), ethanol 0.25% (v v^{-1}) and diluted in distilled water to 97.70 % (v v^{-1}). Tween® 80 (0.05% v v^{-1}), ethanol 0.25% (v v-^{1}) and distilled water 99.70 % (v v^{-1}) were used to the control.

2.4 Chemical composition determination of essential oils
The essential oils samples were analysed by gas chromatography with an ionization flame detector (GC-FID) (Shimadzu device GC-2010 Plus) and by gas chromatography attached to mass spectrometry (GC-MS) (Shimadzu device GCMS-2010). The essential oils samples were exposed under two chromatography conditions in those two analyses: fused silica capillary column (30 mm × 0.25 mm) and N_2 (analysed by GC-FID) or He (analysed by GC-MS) as carrier gas. GC-MS analysis were done with an equipment operated by electronic impact with energy impact of 70eV; scanning speed 1.000; scan interval of 0.50 fragments / second and fragments detected from 29 to 400 (m/z).

The component identification was made by a comparison between its mass spectrum with the available data in the spectrum database Willey7, NIST05, NIST05s, with a co-injection of pure essential oil components pattern and by the retention indices with linear temperature programming (Linear Temperature Programmed Retention Indexes, LTPRI). A homogeneous mixture of linear alkanes (C7 to C40) was used to calculate the LTPRI and the amount calculated to each compound was likened to the literature amounts [12].

2.5 High toxicity test by contact
Essential oils toxicity and its mixtures were tested in adult females of *T. urticae* from the adapted method of Paes *et al.* [13].

Jack bean leaf discs with 4.5 cm of diameter were put in a 6.5-cmdiameter acrylic Gerbox® with a 5-cmdeep layer and in agar-agar water solution at 3% (m/v). A calibrated airbrush was use data pressure of 15 lb.pol-², at a distance of 60 centimeters to spread essential oils and their mixtures.

Each foliar disc was infested by 10 adult females (24 hours old) obtained by lab creation. Ten repetitions were evaluated for each essential oil and its related mixtures (Table 1).

The bio test was led in climatized chambers (temperature of 25±1 ºC, 70±10% RH and photo phase of 12 hours). After 24 hours of exposure to the treatments, the number of dead females was counted. The mites were touched by a thin brush to confirm the mortality. Still mites or those with a displacement shorter than their own body size were considered dead.

2.6 Lethal concentration estimate
In a preliminary test, the mixtures of essential oils were used in a concentration of 2% (v v^{-1}) to check the toxicity about the female adults of *T. urticae*. The essential oils and the mixtures that obtained a minimum mortality of 80% were submitted to a lethal concentration estimation, with the following concentration (v v^{-1}): 0,0 (control); 0.4; 0.5; 0.6; 0.8; 1.0; 1.25; 1.6% (v v^{-1}). The lethal concentration LC_{50} and LC_{90} were estimated by Probit progression [14].

2.7 Sublethal effect test
After 24 hours of essential oils application in lethal effect by contact bio test, the female two-spotted spider mite survivors were taken off the leaf discs with the help of thin brushes. Posteriorly, 50 eggs were accounted with the help of Binocular Tecnival ®magnifying glasses. The eggs were kept in climatized chambers (temperature of 25±1 ºC, 70±10% RH and photo phase of 12 hours) for five days to check their hatch.

2.8 Data analysis
The completely randomized design (CRD) was used for the test of the lethal effect (DIC), with comparative averages by Scott-Knott test ($p \leq 0.05$) and the correct mortality rates using Abbott formula (1925) in the [15]. However an adjusted mortality analysis about the treatments was not applied to the sublethal effect.

3. Results
3.1 Essential oils chemical composition
The individual chemical components with 1% superior relative area were identified by comparison between relative retention of the peaks with the library mass spectrum of essential oils, and by comparison between LTTPR index and literature data (Table 2). The major components with a bigger relative area were eugenol (85.09%), menthol (69.90%), geranial (66.47%) and limonene (76.62%) Fig 1. 19 components were identified, and the essential oil which presented a number of more components was *Menthapiperita* (pepper mint).

Among the components of four essential oils, 15 were monoterpenes, two were sesquiterpenes and two were phenolics. Among the monoterpenes, limonene (76.62%) showed a bigger relative area and was identified only in the sicilian lemon essential oil. The Clove essential oil showed a phenolic component (eugenol) with a relative area of 85.09% (Table 2).

Table 2: Essential oils composition identification by LTPRI Index and Mass Spectrometry (GC / MS) [a]

EssentialOils	Components	Retentionindex [b]	RelativeArea (%)[d]	RetentionRate[c]
Syzygiumaromaticum (clove)	Linalool	17.155	2.62	1102
	Eugenol	29.045	85.09	1360
	trans-Caryophyllene	31.540	2.91	1416
	Eugenylacetate	36.460	2.15	1531
Menthapiperita (peppermint)	Eucalyptol	13.680	2.84	1030
	Isomenthone	19.590	14.73	1158
	Isopulegol	20.045	6.07	1163
	Menthol	20.500	69.90	1167
	Menthylacetate	26.215	4.04	1295
	trans-Caryophyllene	31.515	1.09	1416
Cymonpogoncitratus (lemongrass)	Camphene	9.730	3.42	947
	Geraniol	24.525	6.80	1252
	Geranial	25.180	66.47	1273
	Geranylacetate	30.280	4.77	1386
Citruslimonum (sicilianlemon)	α-Pinene	9.165	2.81	933
	β-Pinene	10.985	9.77	975
	β-Myrcene	11.930	1.52	993
	Limonene	13.575	76.62	1028

[a] The compounds were identified by LTPRI Index (GC/FID) and Mass Spectrometry (GC/MS), [b] Tabulated Retention index [16],
[c] Retention index calculated from data obtained by sampling of saturated n-alkanes (C7-C40), [d] Compounds with relative areas >1% were identified

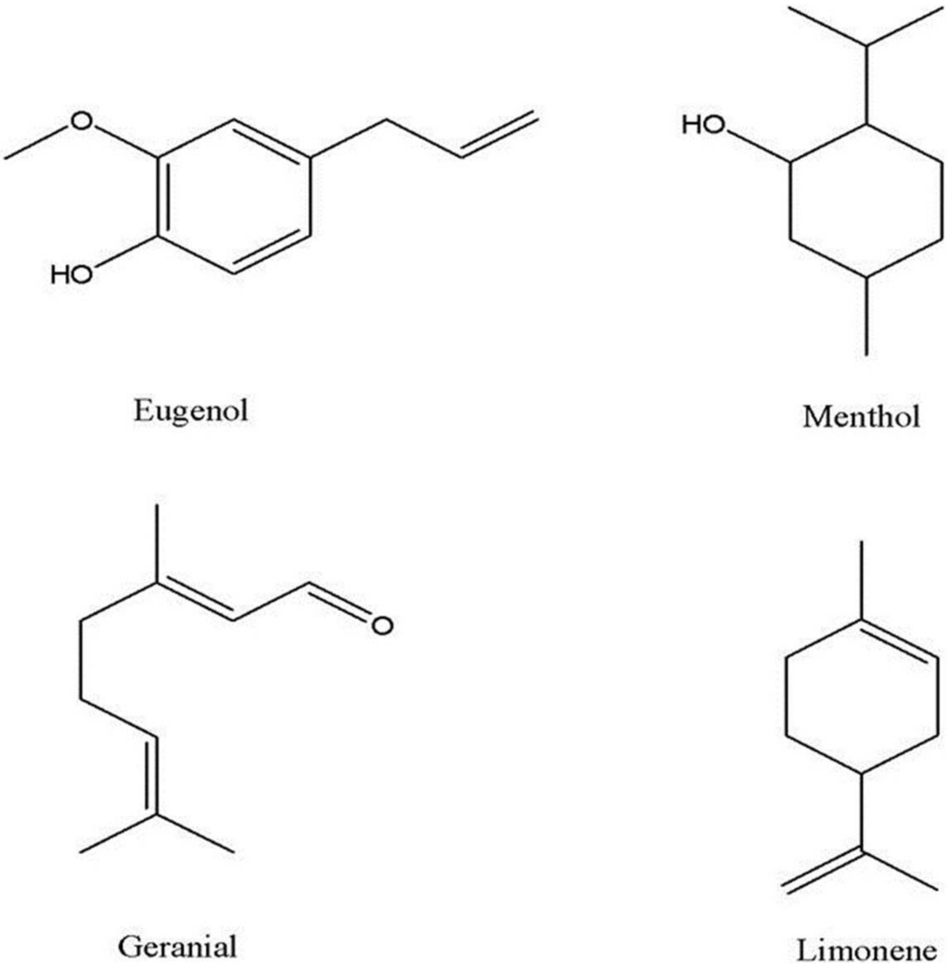

Fig 1: Chemical structure of the main components

3.2 Essential oil lethal effect

The results showed that the 2% vv^{-1} mixture concentration presents a toxicity on adult female and on the eggs of *T. urticae* (Fig. 2 and Fig 3). In advance, the mixtures of lemongrass (+) pepper mint and pepper mint (+) clove showed a toxic effect between 60 and 64%. However, the mixtures of lemongrass (+) pepper mint (+) clove (+) sicilian lemon, lemongrass (+) sicilian lemon, pepper mint (+) sicilian lemon and clove (+) Sicilian lemon provided toxicity under 60%.

The mixture of lemongrass (+) clove oils showed a synergetic effect, with LC_{50} and LC_{90} of 0.91% ($v\ v^{-1}$) and 2.85% ($v\ v^{-1}$), respectively (Table 3).

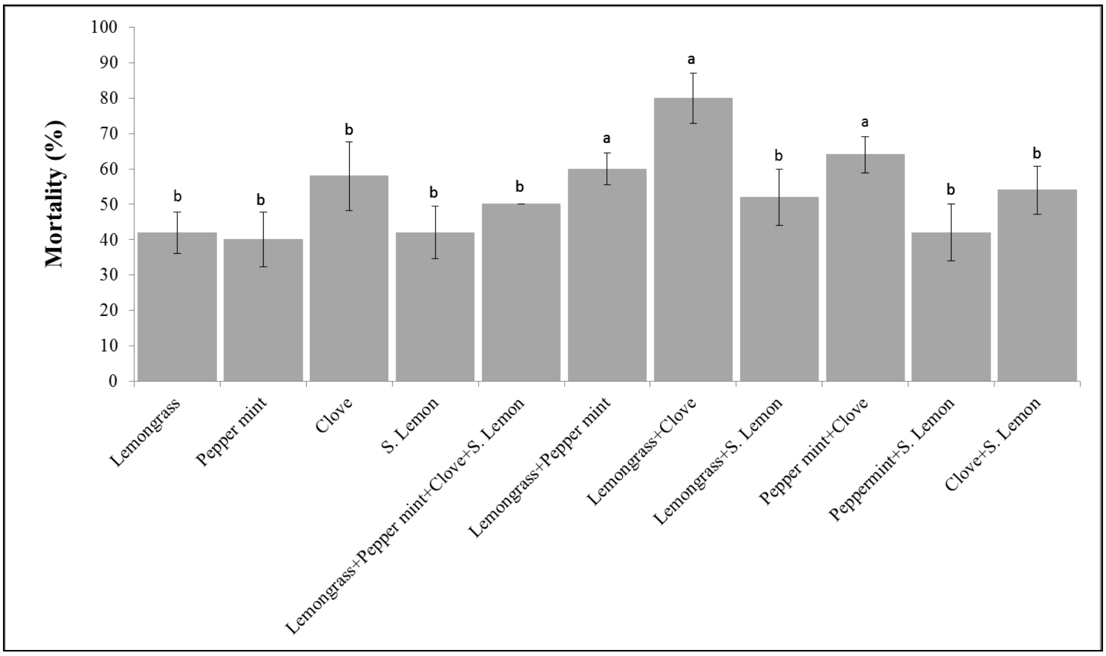

Fig 2: Essential oils toxicity and their mixtures checked in *T. urticae* adult females, Treatments followed by the same letters do not differ statistically from each other by the Tukey test at 5% probability

Table 3: Lethal concentration of mixture *Cymbopogon citratus* (lemongrass) + *Syzygium aromaticum* (clove) on *T. urticae* adult females

Treatment	N[a]	GL[b]	Slop±SE[c]	χ^{2d}	Lethal concentration ((v v[1]) IC 95%[e])	
					LC_{50}	LC_{90}
leLemongrass+Clove	880	6	2.58 ± 0,10	6.05	0.91(0.84±1.00)	2.85(2.34±3.72)

[a] InsectNumber
[b]Degreesoffreedom
[c] Slope of the line
[d] Chi square
[e] Reliable Span

3.3 Essential oils sublethal effect

To the Sublethal effect, the essential oil of Clove and the mixtures Lemongrass (+) pepper mint made 100% of the eggs unfeasible. However, the mixtures Lemongrass (+) pepper mint (+) Clove (+) Sicilian Lemon and Clove (+) Sicilian Lemon made the eggs unfeasible because of Clove essential oils. Nevertheless, when Clove (+) and Pepper mint were mixed it showed an antagonism. The other treatments made between 40 and 80% of the eggs unfeasible Fig. 3.

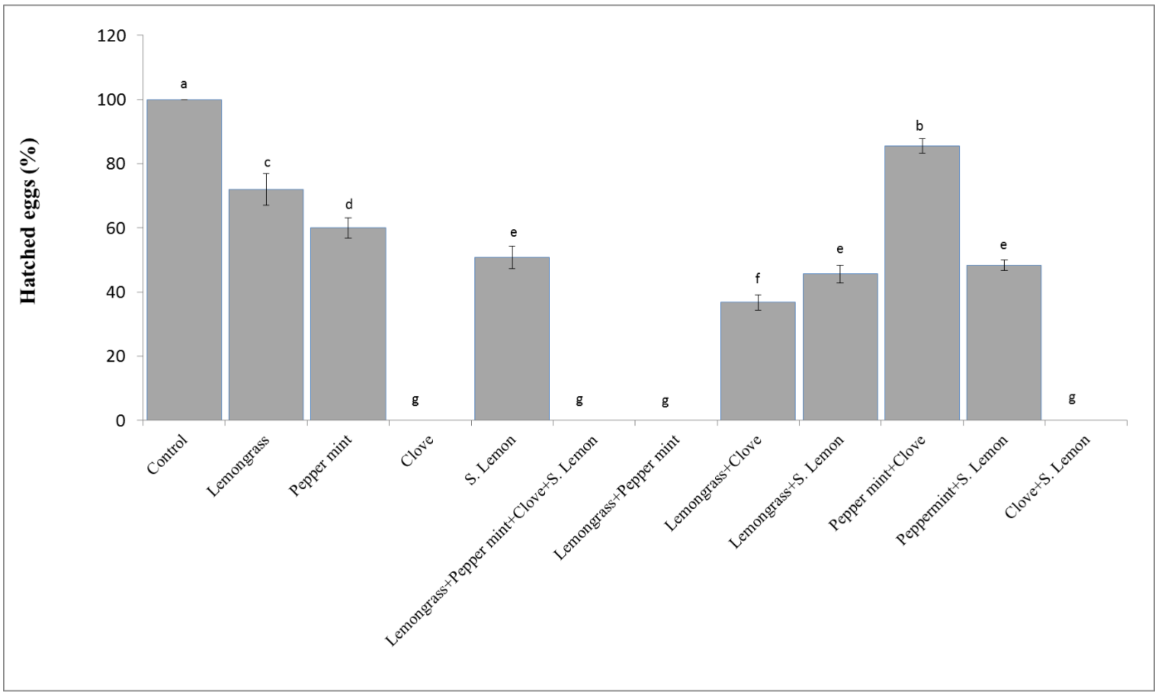

Fig 3: Sublethal effect of essential oils and their mixtures on *Tetrychus urticae* (Acari: Tetranychidae) eggs, Treatments followed by the same letters do not differ statistically from each other by the Tukey test at 5% probability

4. Discussion

The lethal effect of the essential oils can be added to the mixture preparation, as observed with lemongrass (+) clove, which showed toxicity in *T. urticae* adult female(LC_{50} 0.91 vv^{-1}), similar to synthetic insecticides Clorfenapir and Propargite [17, 18].

Although it is difficult to precisely define the effectiveness of essential oils and their mixtures, the essential oils that contain monoterpene class components in the constitution play a role in lipophilicity by disabling protein synthesis, a major part of the insecticidal activity of substances [19, 20]. Tak *et al.* showed that the major components of the essential oil of *Rosmarinus officinalis* L (Lamiaceae) belonging to the monoterpenes class cause high toxicity by penetrating the *Trichoplusia ni* (Lepidoptera, Noctuidae) larvae [21]. A possible explanation for the high toxicity demonstrated by the mixture of clove and lemongrass would be the presence of components of the monoterpene class geraniol, linalol and geranyl acetate. Another explanation for the increased toxicity of essential oils that have monoterpene class ingredients in their constitution may be due to two main properties: Saturated compounds (contain simple carbon-carbon bonds outside the benzene ring) and presence of hydroxyl groups in the benzene ring [22, 23]. These structural properties allow ingredients of the monoterpene class to penetrate rapidly through the cuticle that inhibits the detoxification in insect metabolism [24]. Monoterpenoids can participate in different metabolic pathways. Linalool and terpinen-4-ol a monoterpenoid inhibit AChE, through positive allosteric modulation of GABA and competition with octopamine for binding to its receptor [25]. Monoterpenoids are able to easily penetrate insects due to their lipophilic property and interfere with the physiological functions of insects [26].

Enan and De-Oliveira *et al.* suggested that the toxicity of essential oil constituents is related to the insect's octopaminergic nervous system. However, there are other suggestions that some monoterpenes may inhibit cytochrome P450-dependent monooxygenases [27, 28]. Therefore, the monoterpene destination locations can be multiple [29].

The essential oils usually consist of complex mixtures of substances [30] that show different effects on insects, high toxicity, natural repellency and sublethal effect [31]. For the sublethal effect, clove essential oil and mixtures of lemongrass (+) pepper mint, lemongrass (+) pepper mint (+) clove (+) sicilian lemon and clove (+) sicilian lemon prevented the hatching of eggs, because the eggs are in contact with the essential oils present in the bean leaves, causing the penetration of the chemical components of the monoterpenes class present in the essential oils. Rezaei *et al.* revealed in their work that essential oil from *C. zeylanicum* the same species of the present work inhibited the hatching ofeggs *T. urticae* [32]. However, monoterpene class components have shown toxicity in eggs, larvae and adults in various insect species of medical, veterinary and agricultural importance [33-36].

However, mixture of essential oils can have an antagonistic effect due to the complexity of their substances [19]. The antagonistic presented in the clove (+) pepper mint mixture may be related to the majority components belonging to different classes, monoterpenes and phenolics. [37] noted in his work that when the essential oils of *Pinusnigra* JF Arnold Italian var. (Pinaceae) were mixed with the essential oil of *Satureja montana* L. subsp. *montana* (Lamiaceae), which had major components of different classes, were antagonistic.

5. Conclusion

Mixtures of essential oils have had significant results for future use in the production of a toxic agent. However, for the formulation of products containing the essential oils in the mixtures of the present work as active ingredient, further studies are needed to evaluate selectivity towards natural enemies and pollinating insects, mammalian toxicity and cost-effective of the product formulated.

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