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Effect of extracts of *Citrus sinensis* (Rutaceae) and *Trichilia havanensis* (Meliaceae) in mortality and repellence of *Tetranychus urticae* (Acari: Tetranychidae)

Efecto de extractos de *Citrus sinensis* (Rutaceae) y *Trichilia havanensis* (Meliaceae) en mortalidad y repelencia de *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

This work aimed to evaluate the effect of the plant extract from *Citrus sinensis* peel and *Trichilia havanensis* seeds, on mortality and repellency in different biological states of *Tetranychus urticae* under laboratory conditions. Solutions of the extracts at 500, 1000, and 1500 mg•L^{-1} were applied to a known number of eggs, larvae, and female adults of *T. urticae* in foliar discs of bean (*Phaseolus vulgaris* L.). The data analysis of mortality percentage and the repellence (Kruskal-Wallis test and comparison of medians, α = 0.05) showed that the *T. havanensis* extract presented the highest percentage of mortality in eggs with 45.0 % at 1500 mg•L^{-1}, and the *C. sinensis* extract presented the highest mortality in larvae with 40.0 % at 1000 mgL^{-1} and adults with a 50.0 % at 1500 mg•L^{-1}. The highest percentage of repellency was the *C. sinensis* extract at 1500 mg•L^{-1}, with 30.0 % in larvae and 60.0 % in adults. The results indicate that the extracts have the potential to develop effective products for managing *T. urticae*.

Keywords: Egg mortality, larvae repellency, chemical characterization, vegetal extract.

RESUMEN

Este trabajo tuvo como objetivo evaluar el efecto de los extractos de pericarpio de *Citrus sinensis* y semillas de *Trichilia havanensis* en la mortalidad y repelencia de diferentes estados biológicos de *Tetranychus urticae* en condiciones de laboratorio. Soluciones preparadas con los extractos en concentraciones de 500, 1000 y 1500 mg•L^{-1}fueron aplicadas a un número conocido de huevos, larvas y hembras adultas de *T. urticae* en discos foliares de frijol *(Phaseus vulgaris* L.). El análisis de los datos del porcentaje mortalidad y repelencia (prueba de Kruskal Wallis (α = 0.05)) mostraron que el extracto de *T. havanensis* ocasionó la mortalidad de huevos más alta con el 45 % a 1500 mg•L^{-1}, el extracto de *C. sinensis* presentó el mayor porcentaje de mortalidad en larvas y adultos con el 40.0 % a 1000 mg•L^{-1} y 50.0 % a 1500 mg•L^{-1}, respectivamente. El extracto de *C. sinensis* mostró el mayor porcentaje de repelencia con el 30.0 % en larvas y 60.0 % en adultos a 1500 mg•L^{-1}. Los resultados indican que los extractos tienen el potencial para desarrollar productos efectivos en el manejo de *T. urticae*.

Palabras clave: mortalidad en huevos, repelencia en larvas, caracterización química, extracto vegetal.

INTRODUCTION

Tetranychus urticae Koch (Acari: Teranychydae), commonly known as "red spider mite", is a phytophagous mite considered an important pest for agriculture due to its short life cycle, high reproductive activity, great capacity to adapt to the environment and the host plant, and the parthenogenesis common in this species (Páramo *et al*., 1986; Santamaria *et al*., 2020). In Mexico, it is distributed throughout the country in crops of beans, blackberries, and strawberries (CABI, 2018). Wu *et al*. (2019) evaluated the resistance of *T. urticae* to acaricides like abamectin, fenpyroximato, and spirodiclofen, and showed a complex adaptation to these compounds by genes for enhanced metabolic detoxification in 100 % of the populations tested. The compounds of the acaricides used to manage the red mite have essential issues for human health and the environment (Hernández-Antonio and Hansen, 2011; Blanco-Muñoz *et al*., 2016). Currently, it has been pointed out the use of different plants for pest control since they do not have adverse effects on health nor the environment; it has also been reported their effectiveness in different phytophagous by the secondary metabolites that constitute it like terpenes, aldehydes, and alcohols (Flores-Villegas *et al*., 2019).

Citrus sinensis Obseck (Rutaceae) is important in Mexico due to its use in several industrial sectors. Their waste usually

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ends as soil and water contaminants, so their reuse to obtain their secondary metabolites for agricultural use can minimize the environmental impact generated. In addition, its compounds such as limonene, α-pinene, myrcene, and linalool, are attributed to bactericidal, fungicidal, and insecticidal properties (De Santana *et al*., 2021).

Trichilia havanensis Jacq (Meliaceae), commonly known as ciruelillo in Puebla, Mexico, is native to the northeastern highlands of the state; it is used as a pest control method for grains in storage and religious ceremonies in various indigenous communities of the country (Arenas and Rodríguez-Hahn, 1990; López-Olguín *et al*., 1997; Villavicencio-Nieto *et al*., 2010). Finally, the extract consists mainly of limonoids such as havanensin and trichilenone, placing the plant as a potential pesticide (Chan *et al*., 1973). For these reasons, this research aimed to obtain, characterize, and evaluate the mortality of eggs, larvae, and adults and their repellency by *C. sinensis* and *T. havanensis* extracts against *T. urticae* under laboratory conditions.

MATERIAL AND METHODS

Plant material

Orange (*C. sinensis*) peel was used, separated from the rest of the pulp, washed, and dried at 18 ± 5 °C. Finally, its size was reduced to 2 cm^2.

Fruits of *T. havanensis* were collected in 2019 in Cuetzalan, Puebla, Mexico; their fruits were dried at ambient temperature (18 ± 5 °C) for 20 d, the seed was separated from the pericarp, it was ground with a Nixtamatic NG-02 grain mill, and the product was sieved through a number 30 sieve (Mont-Inox).

Obtaining plant extracts

The orange peel extract was obtained by distillation with a Clevenger-type apparatus; the vegetal extract and the hydrolate (subproduct from the distillation) were stored at -18 °C and 20 °C, respectively.

The extract of *T. havanensis* was obtained by ethanolic maceration (preliminary tests showed a low yield of obtaining the extract using the distillation method); 100 g of ground and a sieved seed were placed in an Erlenmeyer flask with a 1 L capacity, and 200 mL of ethanol were added at 25 °C with stirring (60 rpm), performing concentrations every 24 h for two d. Subsequently, the ethanol was separated from the sedimented plant material, and a purification process was given in the Hei-VAP Core rotary evaporator at a temperature of 80 °C for the water bath and 5 °C for the refrigerant medium at a speed of 80 rpm for 90 min. The purified plant extract was stored at - 18 °C. The obtained hydrolate from the preliminary tests was stored at 8 °C and used in subsequent bioassays.

Characterization of compounds from extracts of *C. sinensis* and *T. havanensis*

Determination of antioxidant activity

The antioxidant activity of each extract was determined with the DPPH technique; the absorbance was measured of a standard sample prepared with the compound 2,2-diphenyl-1-picrylhydrazyl as blank and a solution of the DPPH radical with each plant extract. The inhibition percentage was calculated using formula 1 (Jaramillo *et al*., 2012):

$$\% \text{ Inhibition} = \frac{\text{Absorbance of standard sample} - \text{Absorbance of extract}}{\text{Absorbance of standard sample}} \quad (1)$$

Identification of plant extracts

The volatile components were analyzed and determined using an Agilent GC–MS system (6850N/5975, Santa Clara, CA) consisting of a GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS capillary column (0.25 µm film thickness, 30 m length and 0.25 mm diameter), and a mass selective detector operating in the electron impact ionization mode (70 eV). GC–MS analysis was performed as follows: 1 mL/min carrier gas (helium) flow rate, 10:1 flux ratio, and injector temperature of 200 °C. The oven temperature was held at 40 °C for 3 min, then programmed to increase from 40 °C to 150 °C at a rate of 45 °C/min and 200 °C for 2 min. The scanning mass range was m/z 43-350. The volatile components of the essential oil were identified by comparing their mass spectral fragmentation patterns with those of analogous compounds in the NIST database and MS data in the literature.

Search and obtaining of *T. urticae*

The mites for the bioassays were obtained from a passion fruit crop (*Passiflora edulis* Sims Passifloraceae) that did not have any treatment against pests. Therefore, the infected leaves were transported to the agroecological pest management laboratory of the Agroecology Center of the Science Institute of Benemérita Universidad Autónoma de Puebla, where the organisms that had the morphological characteristics of *T. urticae* were separated (Ferragut and Santoja, 1989). The separated mites were placed in healthy and clean bean plants (*Phaseolus vulgaris* L. Fabaceae) inside a transparent acrylic container covered with tricot cloth, kept in conditions of 26 ± 1 °C, and relative humidity of 60 ± 5 %, where they were reproduced for laboratory bioassays.

Mortality bioassay in laboratory

The experimental unit consisted of a 1.5 cm diameter bean leaf disc with a certain number of *T. urticae* eggs, larvae, or adults placed inside a 9 cm diameter Petri dish with the bottom covered with two layers of wet filter paper to saturation. The turgor of the leaf disc was maintained, placing the Petri dish inside a cylindrical polypropylene box 5 cm high by 12 cm in diameter. A clip connected a piece of absorbent material to the Petri dish. Water flow between the Petri dish's filter paper and the outer box's water reservoir was maintained (Rodríguez-Cabrera *et al*., 2022). Each treatment had 10 repetitions, and the experimental design was completely randomized.

Preparation of solutions derived from extracts

Solutions were prepared at concentrations of 500, 1000, and 1500 $mg \cdot L^{-1}$ of each plant extract, and the hydrolate was used undiluted. Twenty-five mL of each treatment was prepared,

and 0.025 mL of Surfatol + 6® were added to each one and passed through the Shaker (JT-14®) for 10 min to obtain a homogeneous solution. Two controls were included: distilled water and distilled water mixed with surfatol, to determine any mortality derived from surfatol.

Evaluation of mortality in eggs

Two females and three males of *T. urticae* were placed in each experimental unit, and oviposition was allowed for 12 hours (to obtain a minimum of 10 eggs per disc). After that time, adults were removed, and the laid eggs were counted and treated with plant extracts at the established concentrations. For its application, a manual sprinkler with a capacity of 25 mL was used to spray the leaf discs homogeneously. Once the discs were treated, they were allowed to dry in the open air for 15 min and placed in the breeding room at 26 ± 1 °C, relative humidity of 60 ± 5 %, and a photoperiod of 12:12 light and dark. The hatching of the eggs was checked 6 d after the application of the treatments. The number of dead and hatched eggs was recorded by direct observation with a Carl Zeiss stereo microscope (Rodríguez-Cabrera *et al*., 2022).

Evaluation of mortality in neonate larvae

For this, 120 females and males were collected and placed on bean leaves previously placed in 90 mm diameter Petri dishes with 2 - 3 moist filter paper discs. Females were allowed to lay eggs for 24 h after removing them from the leaves. After 5 d, the eggs hatched, obtaining larvae of homogeneous age (< 12 h). The plant extract was applied to each Petri dish with the help of a manual sprinkler at 10 cm at each concentration established in the treatments. They were dried at ambient temperature, and with the use of a brush of camel hair, the larvae were distributed in foliar discs with 10 larvae per disc. The experimental units were randomly placed in the breeding chamber under controlled conditions of 26 ± 1 °C, 60 ± 5 % relative humidity, and a 12:12 h photoperiod of light and darkness. Dead individuals were counted at the time the first protonymphs were observed (4 d). Those larvae that did not respond with movement when touched three times with the brush were considered dead. Individuals that escaped from leaf discs were not considered for data analysis.

Evaluation of mortality in adult females

The respective treatment was applied to the leaf disc of each experimental unit containing 10 adult females (Figure 1) with the help of a manual sprinkler and allowed to dry under ambient conditions for 15 min. Then, the experimental units were randomly placed in the breeding chamber, where they remained under the already indicated temperature, relative humidity, and photoperiod conditions. Finally, dead mites (those that did not respond to the touch of the brush) were counted at 24 h.

Laboratory repellency bioassays

The experimental unit consisted of a leaf disk sectioned into two equal parts. One section was submerged for 10 seconds in a solution of 25 mL of distilled water and 0.025 mL of surfatol, while the other section was immersed in the solution with the respective treatment (plant extracts at different concentrations) for 10 sec, both teams were allowed to dry in ambient conditions. Finally, both sections were merged again to form a leaf disk.

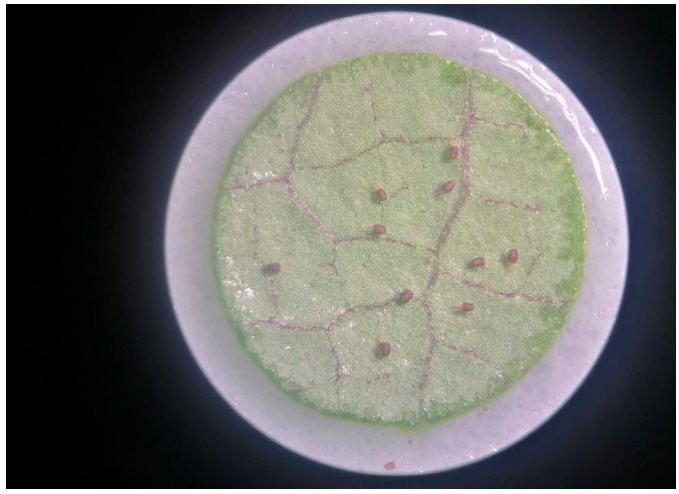

Figure 1. *T. urticae* females on leaf disc.
Figura 1. Hembras de *T. ruticae* en disco foliar.

Determination of repellency in neonate larvae

The larvae were obtained as previously described. Then, with the help of a camel hairbrush, 10 neonate larvae were placed in each leaf disc of the experimental units of the 10 replicates of each treatment and kept in the breeding chamber with the conditions already described. After 24 h, the number of individuals in each section of the leaf disc was counted, and the repellency percentage was calculated with the following formula (Aissaoui *et al.*, 2019):

$$\% \text{ Repellency} = \frac{(C-T)}{(C+T)} \text{ x } 100 \quad (2)$$

Where C is the number of individuals in the control section and T is the number of individuals treated with the extract solution at the respective concentration.

Determination of repellency in adult females

The experimental unit was the one previously described, except that 10 adult females were placed in the leaf disk of the sections with the control treatment and the extract. Each treatment had 10 repetitions in each trial, and the experimental design was completely randomized. The individuals in both sections of the leaf were counted at 24 h, and the percentage of repellency was calculated with the expression indicated above.

RESULTS AND DISCUSSION

Characterization of plant extracts

The percentage of the DPPH radical inhibition of the orange peel aqueous plant extract was 8 %. For the *T. havanensis* ethanolic extract, an inhibition percentage of 45 % was

obtained. The results with the extracts of *C. sinensis* have been reported with a low antioxidant activity, possibly due to the limonoid compounds present in the extract (Yu *et al*., 2005). Likewise, the results are like other reports with a low percentage of inhibition (Kamal *et al*., 2013). No information was found on the antioxidant activity of the *T. havanensis* extracts, however, in other species, as is the case of the bark and stem of *Trichilia roka* Chiov. (Meliaceae), the obtained percentage of the DPPH radical inhibition ranged from 30 to 40 % (Nana *et al*., 2013).

The secondary metabolites present in the *C. sinensis* aqueous extract (Table 1) were determined with the data obtained by gas chromatography coupled with mass spectrometry. The main compound is limonene with 75 %, squalene with 16 %, and the rest corresponds mainly to 7-hydroxy-5,6-dimethoxychromene-2-1;2H-1-Benzopyrane-2-1, β-myrcene, n-hexadecanoic acid, which are attributed to bactericidal and insecticidal properties (Abdelgaleil *et al*., 2019; Luna-Guevara *et al*., 2021). The data is consistent with other reports where limonene was the major bioactive compound present in the plant extract of the orange peel. The β-myrcene, the secondary metabolite present in the highest proportion in the plant extract was also reported (Barros-Gomes *et al*., 2021; Conde-Hernández *et al*., 2021).

Table 1. Secondary metabolites present in the *C. sinensis* extract sample.
Tabla 1. Metabolitos secundarios presentes en el extracto de la muestra de *C. sinensis*

Metabolites	Content in the extract (%)
Limonene	75.83
Squalene	16.76
7-hydroxy-5,6-dimethoxychromene-2-1	1.83
beta-mircene	1.29
n-hexadecanoic acid	1.09
Linalool	0.61
Compounds of minor proportion (< 0.6 %)	2.57
Total	99.98

Table 2 shows the results of the chemical characterization of the *T. havanensis* ethanolic extract. As can be seen, the bioactive compounds present in the plant belong to a complex mixture made of esters, mainly acetates. Thymol is the primary compound with 13.97 %, followed by linoleic acid at 11.99 % and (-) Spathulenol at 11.67 %. The rest are compounds at less than 9 %, which are precursors for the limonoids formation of the azadiron, cedrelone, havanensin, trichilin, trichilinin, and vilasinin class, which have been reported in plants belonging to the *Meliaceae* family and constituents of the *T. havanensis* plant extract. The formation of these limonoids is conditioned by contact with light, temperature, as well as by the medium used, as Tang and Luo (2011) reported that the formation of havanens in is carried out under acidic conditions. On the other hand, reports of some other species belonging to the *Trichilia* genus have been found such as *Trichilia emética* Vahl (Meliaceae), whose

Table 2. Secondary metabolites present in *T. havanensis* ethanolic extract.
Tabla 2. Metabolitos secundarios presentes en la muestra del extracto etanolico de *T. havanensis*.

Metabolites	Content in the extract (%)
Tymol	13.97
Linoleic acid	11.99
(-) Spathulenol	11.67
B-cubenene	8.11
n-hexadecanoic acid	3.54
Aromadendrene	3.45
Ylangene	3.38
Gamma-muurolene	2.9
Gemma-cadinene	2.62
Acetic acid	2.33
Aromadendrene oxide (2)	2.24
Compounds of minor proportion (<2 %)	21.77
Total	95.78

main constituents are esters and aromatic compounds such as pentanoic acid and p-xylene (Perumal *et al*., 2020). In the case of *Trichilia gigliana* Vahl (Meliaceae), several terpenes and esters have been reported such as hexadecanoic acid ethyl ester or octadecane, 2-methyl, which are also present in the *T. havanensis* extract used (Lucie *et al*., 2016).

Mortality evaluation in laboratory tests

The average mortality percentage was less than 5 % and no significant difference was observed between the median mortality of the control treatments consisting of distilled water (A) and distilled water mixed with surfatol (S), control A, and control S (Mann and Whitney, $p > 0.05$). Therefore, the analyzes were carried out with the original mortality data, and the repetitions of the two controls were joined to consider a single control.

Determination of egg mortality

The *T. havanensis* seeds ethanolic extract at 1500 mg•L^{-1} was the treatment with the highest ovicidal activity in *T. urticae*, with a median egg mortality of 45.0 %, which was significantly higher than the mortality of the other treatments and of the control (Table 3). The ovicidal effect on *T. urticae* has been previously reported in ethanolic extracts of *T. havanensis* (Rodríguez-Cabrera *et al*., 2022). Also, the extract from the seeds of *Swietenia humilis* Zucc. (Meliaceae), belonging to the same family as *T. havanensis* was effective on females and the viability of *T. urticae* eggs at concentrations of 1 % (Maldonado-Michel *et al*., 2022). The ovicidal effect can be attributed to the compounds obtained from the plant extract, such as thymol. The lipophilicity of this compound allows it to easily cross the cuticle and enter inside the egg. It can cause an alteration of the cell function and can interact with the cholinergic system which affects the development of the mite (Jukic *et al*., 2007).

Table 3. Median mortality (M) of *Tetranychus urticae* eggs treated with *Citrus sinensis* and *Trichilia havanensis* extracts.
Tabla 3. Medianas de mortalidad (M) de huevecillos de *T. ruticae* tratados con los extractos de *C. sinensis* y *Trichilia havanensis.*

Treatments	*C. sinenis* M [LQ, UQ]* (%)	*T. havanensis* M [LQ, UQ] (%)
Control	00.0 [00.0, 07.2] a**	00.0 [00.0, 07.2] a
Hydrolate	16.0 [06.7, 18.7] bc	08.8 [00.0, 26.7] ab
500 mg•L^{-1}	11.2 [00.0, 14.3] b	22.0 [18.2, 31.2] b
1000 mg•L^{-1}	14.4 [08.3, 25.0] bc	18.9 [11.8, 24.0] b
1500 mg•L^{-1}	17.5 [11.1, 26.7] c	45.0 [37.5, 55.6] c

*LQ = Lower quartile, UQ = Upper quartile.
**Different letters indicate significant differences between treatment medians (p<0.05).

The extract and the hydrolate from the *C. sinensis* peel caused egg mortality between 11.2 % and 17.5 %, with a significant difference compared to the mortality of the control treatment. The highest mortality treatments were with extracts at 1000 and 1500 mg•L^{-1}, and the hydrolate, with a median mortality of 14.4 %, 17.5 %, and 16.0 %, respectively, without difference (p>0.05) between them. Unfortunately, no information was found in the scientific literature on the ovicidal effect of the *C. sinensis* plant extract on *T. urticae* or on other mite species. However, its ovicidal effect has been proven in other arthropods such as *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and *Dermestes maculatus* Deg (Coleoptera: Dermestidae) at concentrations lower than 1 % (Don-Padro, 1996).

Determination of mortality in larvae neonate

Table 4 shows the median mortality caused by the treatments with the two species extracts and the result of the comparison of the medians, where the highest larvicidal activity was caused by the concentrations of 1000 and 1500 mg•L^{-1} of the *C. sinensis* extract, with a mortality of 40.0 %. Some studies demonstrate the effect of limonene at 80 % has a mortality greater than 50 % at this stage of the mite (Golec *et al*., 2020). In other mites, such as *Tetranychus kanzawai* Kishida (Acari: Tertranychidae), the limonene present in the *Plectranthus tomentosea* Forssk (Lemiaceae) extract has a larvicidal effect of 80 % at concentrations of 50 mg•L^{-1} 48 h after its application (Sun *et al*., 2022).

Table 4. Median mortality (M) of neonate larvae of *Tetranychus urticae* treated with *Citrus sinensis* and *Trichilia havanensis* extracts.
Tabla 4. Medianas de mortalidad (M) de larvas neonatas de *T. ruticae* tratados con los extractos de *Citrus sinensis* y *Trichilia havanensis.*

Treatments	*C. sinensis* M [LQ, UQ] * (%)	*T. havanensis* M [LQ, UQ] (%)
Contro	l00.0 [00.0, 05.0] a**	00.0 [00.0, 05.0] a
Hydrolate	30.0 [20.0, 30.0] b	20.0 [10.0, 20.0] c
500 mg•L^{-1}	20.0 [20.0, 30.0] b	15.0 [10.0, 20.0] b
1000 mg•L^{-1}	40.0 [30.0, 50.0] c	15.0 [10.0, 20.0] b
1500 mg•L^{-1}	40.0 [30.0, 40.0] c	20.0 [10.0, 30.0] c

*LQ = Lower quartile, UQ = Upper quartile.
**Different letters indicate significant differences between treatment medians (p<0.05)

For *T. havanensis*, the highest larvicidal activity was obtained with the hydrolate and the concentration of 1500 mg•L^{-1} of the ethanolic extract of seeds, with a mortality of 20 % in both treatments.

Determination of female adult mortality

The highest activity was caused by concentrations of 1000 and 1500 mg•L^{-1} of the aqueous extract of *C. sinensis*, with a mortality of 45 and 50 %, respectively (Table 5).

The highest activity of the *T. havanensis* seeds ethanolic extract was obtained at concentrations of 1000 and 1500 mg•L^{-1} with 30 and 35 % mortality, respectively, without a significant difference between them.

The extract of *C. sinensis* showed a higher mortality than the *T. havanensis* extract in female adults. Previous reports evaluated the mortality of *T. urticae* adults in concentrations of 1250, 2500, 5000, and 10 000 mg•L^{-1} of a *C. sinensis* extract. The most effective treatment was at a concentration of 10 000 mg•L^{-1} (Hassan *et al*., 2022). Likewise, the lethality of extract of *C. sinensis* in adults of *T. urticae* with different concentrations were evaluated, and the results obtained demonstrate the acaricidal activity of the *C. sinensis* extract at an 8 % concentration due to the limonene as responsible for the mortality of *T. urticae* (Born *et al*., 2018; Asmaa and Amal, 2021).

Table 5. Median mortality (M) of adult females of *Tetranychus urticae* treated with *Citrus sinensis* and *Trichilia havanensis* extracts.
Tabla 5. Medianas de mortalidad (M) de hembras adultas de T*etranychus urticae* tratados con los extractos de *Citrus sinensis* y *Trichilia havanensis.*

Treatments	*C. sinensis* M [LQ, UQ]* (%)	*T. havanensis* M [LQ, UQ] (%)
Control	00.0 [00.0, 10.0] a**	00.0 [00.0, 10.0] a
Hydrolate	10.0 [00.0, 20.0] a	05.0 [00.0, 10.0] a
500 mg•L^{-1}	20.0 [10.0, 30.0] b	10.0 [00.0, 10.0] a
1000 mg•L^{-1}	45.0 [40.0, 60.0] c	30.0 [30.0, 40.0] b
1500 mg•L^{-1}	50.0 [50.0, 50.0] c	35.0 [30.0, 40.0] b

*LQ = Lower quartile, UQ = Upper quartile.
**Different letters indicate significant differences between treatment medians (p<0.05)

Laboratory repellency bioassays

Determination of repellency in larvae neonate

The repellency results in larvae are shown in Table 6, where the treatments with the highest repellent activity were the concentrations of 1000 and 1500 mg•L^{-1} of the *C. sinensis* extract with repellency indices of 20 and 30 %. This extract's concentration of 500 mg•L^{-1} caused 20 % attracting activity for the larvae. In comparison, the hydrolate caused a low repellency (10 %) that was not significantly different from that observed with 500 mg L^{-1} of the extract.

The hydrolate and the different concentrations of the *T. havanensis* seeds ethanolic extract did not cause a repellent effect on the *T. urticae* larvae. The highest repellent activity was 20 % with the extract at 1000 mg L^{-1}, an activity that was not significantly different from that observed with the other treatments.

Table 6. Median repellency (M) in neonate larvae of *Tetranychus urticae* treated with *Citrus sinensis* and *Trichilia havanensis* extracts.
Tabla 6. Medianas de repelencia (M) en larvas neonatas de T*richilia havanensis* tratados con los extractos de *Citrus sinensis* y *Trichilia havanensis*.

Treatments	*C. sinensis*	*T. havanensis*
	M [LQ, UQ]* (%)	M [LQ, UQ] (%)
Hydrolate	10.0 [-60.0, 40.0] a	-20.0 [-40.0, 20.0] a
500 mg•L^{-1}	-20.0 [-20.0, 00.0] a	00.0 [-20.0, 20.0] ab
1000 mg•L^{-1}	20.0 [00.0, 20.0] ab	20.0 [00.0, 20.0] ab
1500 mg•L^{-1}	30.0 [00.0, 40.0] b	00.0 [00.0, 20.0] b

*LQ = Lower quartile, UQ = Upper quartile.
**Different letters indicate significant differences between treatment medians ($p<0.05$)

Determination of repellency in adult female

The results of the repellency test with adult females are presented in Table 7, where the *C. sinensis* extract show high repellent activity at the concentration of 1000 mg•L^{-1} with an index of 60 %, equal to the repellency caused by 1500 mg•L^{-1} of the extract.

In the case of *T. havanensis* (Table 7) there was no significant difference in the repellency of larvae and adults of the two-spotted red mite.

The repellency results showed a higher effect in adult females than larvae stage by *C. sinensis*. This is due to the setae present around the body of the adult stage, which lets the mite detect different types of molecules present in the environment (Tuttle *et al*., 1976). However, the repellent effect of the plant extract of *C. sinensis* was shown at concentrations of 1 % with a repellency of 60 % in *T. ruticae* adults, while at a concentration of 2 %, a repellent effect greater than 50 % was observed (Júnior *et al*., 2010; Da Camara *et al*., 2015). Therefore, in the present study, it was possible to determine the repellent activity of the *C. sinensis* extract due to the action of the main limonoids that constitute the plant extract, such as limonene and squalene and possible synergistic activity between them.

On the other side, the extract of *T. havanensis* showed lower mortality and repellence in the larvae and adult stages. This could be due to the proportion of compounds present in the extract which varies depending on the part of the plant, stage of maturity, and the drying process (López-Malo *et al*., 2006). Nevertheless, no registered papers on the use of *T. havanensis* in *T. urticae* were found. Still, in the extract are compounds present in other plants of the meliaceous family, to which acaricidal and repellent properties have been attributed. For example, the ethanolic extract of *Melia azedarach* L. (Meliaceae) affected the development of mite at a concentration of 4 mg•mL^{-1} due to the limonoids present in the extract (Ashrafju *et al*., 2014). On the other hand, the aqueous extract of *Azadirachta indica* A. Juss (Meliaceae) in tomato crops had a 30 % lethal effect on the larval population of *T. urticae* at 8•g.L^{-1} (Augustin *et al*., 2015).

Other studies suggested an acaricidal effect attributed to the secondary metabolite alpha-cubenene, present in extracts of *Artemisia absinthium* L. (Asteraceae), *Tanacetum vulgare* L. (Asteraceae), and *T. havanensis* on the two-spotted red mite in its adult state (Chiasson *et al*., 2001). Besides, there are not enough reports on the repellent activity of the *T. havanensis* extract on mites; recent works evaluated the acaricidal and repellent effect of *Croton spp*. L. (Euphorbiaceae) in adults of *T. urticae*. When carrying out the characterization of the plant, spatulenol (a compound present in the extract of *T. havanensis* used in the present investigation) was identified as the main plant extract compound and responsible for a lethal and repellent effect greater than 50 % in bioassays performed at a concentration of 27.08 µL•mL^{-1} (Da Camara *et al*., 2021). Similarly, the thymol compound was evaluated in adults of *T. urticae* and reported repellent activity close to 100 % with concentrations of 10 µL•mL^{-1} diluted in acetone (Tak *et al*., 2017).

Table 7. Median repellency (M) in adult female of *Tetranychus urticae* treated with *Citrus sinensis* and *Trichilia havanensis* extracts.
Tabla 7. Medianas de repelencia (M) en hembras adultas de *Tetranychus urticae* tratadas con los extractos de *Citrus sinensis* y *Trichilia havanensis*.

Treatments	*C. sinensis*	*T. havanensis*
	M [LQ, UQ] * (%)	M [LQ, UQ] (%)
Hydrolate	- 10.0 [- 20.0, 00.0] a	00.0 [- 20.0, 20.0] a
500 mg•L^{-1}	00.0 [- 20.0, 20.0] a	- 10.0 [- 40.0, 20.0] ab
1000 mg•L^{-1}	60.0 [40.0, 80.0] b	10.0 [00.0, 20.0] ab
1500 mg•L$_{-1}$	60.0 [40.0, 80.0] b	20.0 [00.0, 40.0] b

*LQ = Lower quartile, UQ = Upper quartile.
**Different letters indicate significant differences between treatment medians ($p < 0.05$)

CONCLUSIONS

As shown in the present work, by studying the interaction of the extract in greenhouse or fields, the vegetal extract of *C. sinensis* and *T. havanensis* current mortality and repellency in the different development stages of *T. urticae* could present an agroecological alternative for the management of pest and reduction of the health and environmental impacts derived from the use of acaricides. Besides, although the *T. havanensis* extract showed less repellency and mortality in larvae and adult females, the higher mortality in eggs suggests that the synergic activity of a *C. sinensis* and *T. havanensis* extracts mixture could present an alternative for the management of any development stage of the red mite in the crops.

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INTEREST CONFLICT

The authors declare no conflict of interest related to this publication.

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