Evaluation of ovicidal activity of plant extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae)

Evaluación de actividad ovicida de extractos vegetales en *Tetranychus urticae* Koch (Acari: Tetranychidae)

Avaliação da atividade ovicida de extratos vegetais em *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract

The ovicidal activity of ethanol and hexane extracts of *Azadirachta indica* A. Juss., *Trichilia havanensis* Jacq., *Roldana ehrenbergiana* (Klatt) H. Rob. & Brettell, *Argemone mexicana* L., *Schinus molle* L. y *Gliricidia sepium* (Jacq.) Kunth ex Walp. was evaluated in the laboratory on *Tetranychus urticae* Koch (Acari: Tetranychidae). The extracts (treatments) were applied by spraying at a concentration of 1,000 mg.L^{-1} to a known number of eggs (age <18h) on leaf discs of bean (*Phaseolus vulgaris* L.) (10 leaf discs per treatment), and the percentage of egg mortality was recorded after six days of the treatment. The corrected mortality (percentage) was obtained for each test unit, and a simple ANOVA, followed by multiple comparison of means by the Tukey method (α=0.05), were used to find statistically significant differences between treatment effects. The ethanol and hexane extracts of *T. havanensis* seeds caused an average corrected egg mortality of 77.7±3.5 and 58.0±3.4 %, respectively, which was significantly higher than the egg mortality caused by the other extracts. The ethanol extracts of the other plant species caused a corrected egg mortality, which ranged from 8.8±9.6 to 42.9±8.3 %, while the corrected mortality of the hexane extracts ranged from 0.2±2.3 to 30.1±4.0 %. The results show that the ethanol extract of *T. havanensis* seeds has good potential for the development of products with biological activity for the management of *T. urticae*.

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Resumen

Se evaluó en laboratorio la actividad ovicida de extractos etanólicos y hexánicos de *Azadirachta indica* A. Juss., *Trichilia havanensis* Jacq., *Roldana ehrenbergiana* (Klatt) H. Rob. & Brettell, *Argemone mexicana* L., *Schinus molle* L. y *Gliricidia sepium* (Jacq.) Kunth ex Walp. en *Tetranychus urticae* Koch (Acari: Tetranychidae). Los extractos (tratamientos) se aplicaron por aspersión a una concentración de 1.000 mg.L^{-1} a un número conocido de huevos (edad <18h) de *T. urticae* sobre discos foliares de frijol (*Phaseolus vulgaris* L.) (10 discos foliares por tratamiento) y a los seis días se registró el porcentaje de mortalidad de huevos. Se obtuvo la mortalidad corregida (porcentaje) para cada unidad de ensayo y se aplicó un ANOVA simple seguido de la comparación múltiple de medias por el método de Tukey (α=0,05). Los extractos etanólico y hexánico de semillas de *T. havanensis* ocasionaron mortalidad corregida de huevos de 77,7±3,5 y 58,0±3,4 %, respectivamente. Los extractos etanólicos de las otras especies vegetales ocasionaron mortalidad corregida de huevos de 8,8±9,6 a 42,9±8,3 %, mientras que la mortalidad corregida de los extractos hexánicos varió de 0,2±2,3 a 30,1±4,0 %. Los resultados muestran que el extracto etanólico de semillas de *T. havanensis* tiene buen potencial para el desarrollo de productos con actividad biológica para el manejo de *T. urticae*.

Palabras clave: *Trichilia havanensis*, extractos de plantas, araña roja, araña de dos manchas, mortalidad corregida.

Resumo

A atividade ovicida de extratos etanólicos e hexânicos de *Azadirachta indica* A. Juss., *Trichilia havanensis* Jacq., *Roldana ehrenbergiana* (Klatt) H. Rob. & Brettell, *Argemone mexicana* L., *Schinus molle* L. y *Gliricidia sepium* (Jacq.) Kunth ex Walp. em *Tetranychus urticae* Koch (Acari: Tetranychidae) foi avaliada em laboratório. Os extratos (tratamentos) foram aplicados por pulverização na concentração de 1.000 mg.L^{-1} em um número conhecido de ovos (idade <18h) de *T. urticae* disponibilizados em discos foliares de feijão (*Phaseolus vulgaris* L.) (10 discos foliares por tratamento) e, após seis dias, a porcentagem de mortalidade de ovos foi registrada. A mortalidade corrigida (porcentagem) foi obtida para cada unidade de teste, que foi analisada estatisticamente por meio de ANOVA simples, seguida de comparação múltipla de médias pelo método de Tukey (α=0,05). Os extratos etanólicos e hexânicos das sementes de *T. havanensis* causaram mortalidade média corrigida de ovos de 77,7±3,5 e 58,0±3,4 %, respectivamente, que foi significativamente maior que a mortalidade de ovos dos demais extratos. Os extratos etanólicos das demais espécies vegetais causaram mortalidade corrigida de ovos de 8,8±9,6 a 42,9±8,3 %, enquanto a mortalidade corrigida dos extratos hexânicos variou de 0,2±2,3 a 30,1±4,0 %. Os resultados mostram que o extrato etanólico de sementes de *T. havanensis* apresenta bom potencial para o desenvolvimento de produtos com atividade biológica para o manejo de *T. urticae*.

Palavras-chave: *Trichilia havanensis*, extratos de plantas, aranha vermelha, ácaro rajado, mortalidade corrigida.

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae), commonly called "two-spotted spider" or "red spider", has a wide worldwide distribution, is one of the main pests in agriculture and affects more than 1200 species of host plants; among them, fruit trees, oilseeds, vegetables, ornamental and medicinal plants, and more than 150 plants of economic importance in Mexico (Souza-Pimentel *et al*., 2016). It causes damage to plants by feeding on the content of epidermal and parenchymal cells of stems and leaves; reducing its efficiency, as well as the yields and quality of the crops (León *et al*., 2014). For its control, synthetic acaricidal chemical products are used that affect the health of agricultural workers and consumers, have a negative impact on the environment and have caused toxicological problems and the development of resistance to acaricides (Cerna *et al*., 2009; Villegas-Elizalde *et al*., 2010).

Faced with this problem, it is necessary to develop alternative strategies to the use of synthetic chemical products for crop protection, with the possibility of being incorporated into agroecological pest management, where priority is given to cultural, biological strategies and the use of extracts or vegetable oils, among others (Altieri and Nicholls, 2018). Plants produce secondary metabolites that help them defend themselves against herbivores (Sierra *et al*., 2018). These biomolecules affect the physiology, growth and development of insects and mites, since they act as growth regulators, feeding inhibitors, repellents, oviposition inhibitors, molt inhibitors and chitin formation (Erdogan and Sever, 2017). These plant properties have been evaluated in insects, mites (Souza de Jesus *et al*., 2020), nematodes (Sepúlveda-Vázquez *et al*., 2018) and bacteria (Cuervo *et al*., 2019). However, no references to studies on the ovicidal effect of *Azadirachta indica* A. Juss (Meliaceae) "nim" extracts were found, *Trichilia havanensis* Jacq. (Meliaceae) "jar branch", *Roldana ehrenbergiana* (Klatt.) H. Robinson & Brettell. (Asteraceae) "dog grass", *Argemone mexicana* L. (Papaveraceae) "Chicalote", *Schinus molle* L. (Anacardiaceae) "Pirul" and *Gliricidia sepium* (Jacq.) Kunth ex Walp. (Fabaceae) "Cocuite" or "Mata rata" in *T. urticae*.

Therefore, the objective of this work was to evaluate the effect of ethanolic and hexanic extracts of the plant species mentioned above, on the viability of *T. urticae* eggs.

Materials and methods

Obtaining plant extracts

To obtain the extracts, *G. sepium* bark collected in Zolonquiapa, Izúcar de Matamoros, Puebla was used; stems, leaves, flowers and fruits of *A. mexicana*, and leaves of *S. molle* from San Pedro Zacachimalpa, Puebla; seeds of *T. havanensis* from Cuetzalan del Progreso, Puebla; seeds of *A. indica* from Acayucan, Veracruz and stems, leaves, flowers and fruits of *R. enrenbergiana* from Santa Cruz Alpuyeca, Puebla. The plant material was left to dry in the shade at room temperature (18 ± 5 °C) for 20 days and was crushed in an industrial blender (PDH® industrial 5 L stainless steel 1 HP PD14) until obtaining homogeneous particles that were passed through a sieve (Mont-Inox®) No. 16 with 1 mm opening. The powdered material was placed in duly labeled brown paper bags and stored in a dry place for later use.

The extracts were obtained by the Soxhlet extraction method (Sierra *et al*., 2018), based on 100 to 180 g of powdered plant material (depending on availability) and 800 mL of 96 % ethanol

(Merck Emsure® Reag Ph Eur) or hexane (Merck Emsure® ACS). The extracts were concentrated in a rotary evaporator (Sev® A402-2) at 200 rpm at constant temperature (<40 °C) and stored in amber glass bottles at 4 °C. The ethanolic extracts of *T. havanensis* and *A. indica* seeds presented two phases that were separated in a centrifuge (Hermle® Z326K) at 6000 rpm for 5 minutes, obtaining a supernatant of oily consistency and a precipitate, which were preserved and evaluated separately.

Breeding of *T. urticae*

Adults present in damaged leaves (chlorotic spots and presence of cobwebs) of bean (*Phaseolus vulgaris* L.), rose (*Rosa* spp.), tomato (*Solanum lycopersicum* L.) and sweet cucumber (*Solanum muricatum* Aiton) crops were collected without treatment of insecticides and/or acaricides in the municipalities of Atlixco and Tepeojuma, Puebla, Mexico. The plant material with the mites was placed in duly labeled polypropylene containers, which were transferred to the Laboratorio de Manejo Agroecológico de Plagas, Centro de Agroecología, Instituto de Ciencias of the Benemérita Universidad Autónoma de Puebla, where the species was confirmed with the help of keys and verification of the taxonomic characteristics of *T. urticae* (Ferragut and Santonja, 1989). The colonies were established under controlled conditions of temperature (26 ± 1 °C), relative humidity (50 ± 5 %) and photoperiod (12:12 h Light: Darkness), on bean plants (*Phaseolus vulgaris* L.) contained in acrylic transparent boxes 30x47x20 cm (high, long and wide, respectively), covered with tricot fabric, from which the adults necessary for the development of the bioassays were obtained.

Bioassays

The bioassays were carried out in the laboratorio de Manejo Agroecológico de Plagas, Centro de Agroecología, Instituto de Ciencias of the Benemérita Universidad Autónoma de Puebla, México.

The experimental unit consisted of a bean leaf disc of 2 cm in diameter with a certain number of eggs depending on the laying of two females of *T. urticae* that had been placed in it, for a period of 18 h, as indicated next. The leaf disc was placed in a 60x15 mm Petri dish with the bottom covered with two layers of filter paper moistened to saturation with distilled water. Leaf disc turgidity was maintained by placing the Petri dish inside an 8x9 cm polypropylene container with distilled water in the bottom. A piece of absorbent cloth was used that was attached to the Petri dish with a clip to maintain the flow of water between the filter paper of the Petri dish and the water reservoir of the polypropylene container.

Leaf discs were obtained from bean plants grown in pots arranged in a greenhouse. Using a camel hair brush and a stereoscopic microscope (Zeigen®), 5 adults (2 females and 3 males) of *T. urticae* were placed on the underside of each leaf disc. Females were allowed to oviposit for a period of 18 hours to obtain as many eggs as possible on each disk. After this time, the adults were removed, the eggs laid on each disc were counted and the treatments (plant extracts) were applied at a concentration of 1,000 mg.L^{-1}, using 10 foliar discs containing between 8 and 20 eggs each. The average number of eggs per leaf disc in the trials was 9.4 with a standard deviation of 2.5 eggs.

To obtain the ethanolic extracts at a concentration of 1,000 mg.L^{-1}, 25 mg of the extract were weighed on an analytical balance (Ohaus® PX124 Pioneer™) and 25 mL of ethanol were added. The extract with the solvent was placed in a 50 mL rigid polypropylene container, which was passed through a laboratory shaker (Sunburst®) for 10 minutes to obtain a homogeneous mixture. The mixture was transferred to a manual sprayer for subsequent application to the test units. The treatments consisted of the seven plant ethanolic extracts and two controls (distilled water and ethanol alone).

Hexanic extracts at a concentration of 1,000 mg.L^{-1} were obtained by combining 25 mg of each extract, 25 mL of distilled water and 25 µL of Surfatol+6® (30 % polyoxyethylene tridecyl alcohol + 20 % phosphoric acid + diluents and conditioners 49 % + fulvic acid 1 %) as adjuvant. Hexane was not used to dissolve the plant extracts because a preliminary test showed toxicity of this product on *T. urticae* eggs, so it was decided to use distilled water and surfatol+6®. This product allowed an adequate mixture of the hexanic extracts of oily consistency with the distilled water. Each of the containers was placed in an ultrasound tub (Hantec® HNT-UL188) for 30 minutes at high intensity and then placed in a shaker-vibrator (JT-14®) for 10 minutes to obtain a homogeneous mixture. Finally, the mixture of each extract was transferred to a manual sprayer for subsequent application to the test units. The treatments consisted of the six plant hexanic extracts and the controls consisting of surfatol+6® (to rule out a possible effect of this product) and distilled water.

The treatments were applied to the test units (leaf discs with *T. urticae* eggs) by spraying approximately one mL of each solution with the help of a manual atomizer with a capacity of 75 mL and they were standardized at a distance of 10 cm from the outlet of the spray to the leaf disc, with a fine droplet size to ensure a homogeneous spray on the surface of the leaf disc with the *T. urticae* eggs. The treated discs were left to dry at room temperature for 5 minutes on a laboratory table and once dry, they were placed according to the completely randomized design (Infante and Zárate de Lara, 1990) in the insect breeding room, where the conditions of temperature (26 ± 1 °C), relative humidity (50 ± 5%) and photoperiod of 12:12 h (L: O) they were controlled. Six days after the application of the treatments, the number of dead eggs was recorded and later, for each test unit, the percentage of egg mortality and the corrected mortality (%) were obtained according to Abbott (1925), considering that the average mortality of the control treatments in the bioassays was between 5 and 10 %.

Statistical analysis

To rule out a possible effect of the solvents water and ethanol, and the adjuvant Surfatol+6®, t-Student tests ($\alpha = 0.05$) were performed, where the mean mortality of the control treatments consisting of the application of distilled water versus ethanol and the mean mortality of the control (without solvent) versus the water plus surfatol+6® control. The corrected mortality data were processed by means of a one-way analysis of variance (simple ANOVA), after verifying the assumption of homogeneity of variances by the Bartlett method, followed by the multiple comparison test of means by the Tukey method ($\alpha = 0.05$) (Infante and Zárate de Lara, 1990). Statistical calculations and analyzes were performed with the statistical package Statgraphics Centurion version XVI.I (StatPoint Technologies, Inc., 2009).

Results and discussion

Ovicidal activity of ethanolic extracts

The mean egg mortality of the control treatments (water and ethanol) did not present a statistically significant difference ($P>0.05$), so they were averaged to consider a single control for the calculation of corrected mortality. The average mortality percentage was 7.9, which corresponds to the average mortality of the negative control (leaf discs not treated with plant extract).

The ethanolic extracts of *T. havanensis* seeds, precipitate and supernatant, were the ones that showed the highest ovicidal activity in *T. urticae*, with average corrected mortality of eggs of 60.0 and 77.7 %, respectively. The difference between these mortality means was not statistically significant (table 1).

Table 1. Mean mortality (M) and corrected mortality (CM) of *Tetranychus urticae* eggs treated with ethanolic plant extracts.

Vegetable species	M ± SE* (%)	CM ± SE* (%)
Trichilia havanensis (seeds) supernatant	79.5 ± 03.4	77.7 ± 03.5^{a**}
Trichilia havanensis (seeds) precipitated	63.2 ± 04.9	60.0 ± 05.1^{ab}
Argemone mexicana (aerial part)	47.4 ± 07.9	42.9 ± 08.3^{bc}
Azadirachta indica (seeds) supernatant	44.1 ± 08.2	39.3 ± 08.6^{bc}
Roldana ehrenbergiana (aerial part)	5.0 ± 11.9	29.4 ± 12.1^{cd}
Gliricidia sepium (cortex)	33.4 ± 05.1	27.7 ± 05.3^{cd}
Azadirachta indica (seeds) precipitated	31.3 ± 05.9	25.4 ± 06.1^{cd}
Schinus molle (foliage)	16.0 ± 09.1	8.8 ± 09.6^{d}
Negative control (without vegetable extract)	7.9 ± 02.4	

*SE= Standard error.
**Corrected mortality means (CM) followed by the same letter are not significantly different from each other (Tukey's HSD test, P>0.05).

The corrected mortality means of the ethanolic extracts of *A. mexicana*, *R. ehrenbergiana*, *G. sepium*, *A. indica* supernatant and *A. indica* precipitate were significantly higher than the mean mortality of the control treatment (P<0.05) with values from 25.4 to 42.9 %, showing no statistically significant difference between them. While the ethanolic extract of *S. molle* caused corrected mortality of eggs that does not differ statistically from the mortality of the control.

Ovicidal activity of hexanic extracts

No significant difference was observed between the corrected mortality means of white control eggs (untreated leaf discs) and the application of distilled water plus surfatol+6®; with a general average of 5.7 %; therefore, the repetitions of both controls were considered for the calculation of corrected mortality.

Table 2 shows the corrected mortality means and the result of the multiple comparison of means, where the hexanic extract of *T. havanensis* seeds caused the highest activity with 58.0 % corrected mortality of eggs.

The hexanic extracts of *A. mexicana*, *G. sepium* and *A. indica* also caused corrected mortality significantly higher than the mortality of the control treatment, with no significant difference between their mortality means. This group of extracts presented low ovicidal activity in *T. urticae* with corrected mortality averages between 17.6 and 30.1 % while the hexanic extract of *S. molle* did not cause significant ovicidal activity.

Table 2. Mean mortality (M) and corrected mortality (CM) of *Tetranychus urticae* eggs treated with hexanic plant extracts.

Vegetable species	M ± SE* (%)	CM ± SE* (%)
Trichilia havanensis (seeds)	60.4 ± 3.2	58.0 ± 3.4^{a**}
Azadirachta indica (seeds)	34.1 ± 3.8	30.1 ± 4.0^{b}
Gliricidia sepium (cortex)	22.5 ± 3.2	17.8 ± 3.4^{bc}
Argemone mexicana (aerial part)	22.3 ± 2.1	17.6 ± 2.2^{bc}
Roldana ehrenbergiana (aerial part)	17.6 ± 3.2	12.6 ± 3.5^{c}
Schinus molle (foliage)	5.9 ± 2.2	0.2 ± 2.3^{d}
Negative control (without vegetable extract)	5.7 ± 1.9	

*SE= Standard error.
**Corrected mortality means (CM) followed by the same letter are not significantly different from each other (Tukey's HSD test, P>0.05).

According to Castiglioni *et al*. (2002) and Vieira *et al*. (2014), plants of the genus *Trichilia* biosynthesize limonoids, terpenes, diterpernes, triterpenes, coumarins and flavonoids, with a potential pesticidal effect. Its biological activity has been evaluated in different pest species, such as in larvae of *Spodoptera littoralis* (Boisduval) (López-Olguín *et al*., 1997), larvae of *Spodoptera litura* (Fabricius) (Wheeler and Isman, 2000), adults of *T. urticae* (Castiglioni *et al*., 2002), eggs of *Ceratitis capitata* (Wied.) (López-Olguín *et al*., 2002), larvae of *Spodoptera exigua* (Hübner) (De la Torre-Anzures *et al*., 2017) and larvae and adults of *Copitarsia decolora* (Guenée) (García-Gómez *et al*., 2018).

De la Torre-Anzures *et al*. (2017) observed mortality of neonatal larvae of *S. exigua* from the concentration of 100 mg.L^{-1} of an acetone extract of *T. havanensis* seeds in solid state (resin) and the supernatant oil, with mortality of 75 % at a concentration of 10,000 mg.L^{-1}, while García-Gómez *et al*. (2018) evaluated the effect of the hexanic extract from the bark of *T. havanensis* on larvae, pupae and accumulated mortality of *C. decolora* and observed that it presents toxicity in larvae from a concentration of 1,000 mg.L^{-1}, with a mortality of 48 % in larvae and 16 % in pupae, having a cumulative mortality of 64 %. This activity is attributed to limonoids with a furan ring that are known to have antifeedant, repellent, toxic and physiological effects on insects (Ortego *et al*., 1999, Vieira *et al*., 2014). According to Isman (2000), the acaricidal activity of plant extracts is due to the presence of various chemical compounds with more than one mode of action.

The ethanolic extract of *A. mexicana* caused 42.9 % corrected mortality of *T. urticae* eggs. While Carrillo-Rodríguez *et al*. (2011) recorded with this same extract corrected mortality of adults of *T. urticae* greater than 50 %. These results are similar to those obtained in this research in terms of biological activity; however, higher mortality was observed in adults than the mortality of *T. urticae* eggs obtained in this work, which could be due to the different concentration of the extracts evaluated in these studies or to the different susceptibility of the different stages of development. Granados-Echegoyen *et al*. (2019) reported that the insecticidal

activity of *A. mexicana* could be due to the alkaloids, anthraquinones, flavonoids and terpenoids found in the crude extracts of different parts of the plant.

A. indica extracts have been extensively studied for their insecticidal effect. Encina Romero *et al*. (2011) found that the aqueous extract of neem leaves at 1 % caused a mortality of 37.8 % at 72 h after exposure in adult mites of the genus *Tetranychus*. This mortality was similar to the corrected mortality of eggs obtained in this investigation (39.3 %) with the supernatant ethanolic extract of neem seeds at a concentration ten times lower. The difference could be due to a greater susceptibility of the eggs to the activity of the extracts and a higher concentration of active compounds in the seeds, compared to the neem leaves.

On the other hand, the hexanic extract of *A. indica* seeds showed moderate ovicidal activity with corrected mortality of *T. urticae* eggs of 30 %. In other arthropods, neem has been shown to have similar biological activity, as observed by Barrientos *et al*. (2018), who found that the hexanic extract of *A. indica* seeds caused a mortality of 47.9, 37.5 and 27.1 % of the *Meccus pallidipennis* (Stal) bug nymphs because of topical application of concentrations of 80, 60 and 30 % of the extract, respectively. The main biomolecule of *A. indica* is azadirachtin, which affects the physiology and life cycle of organisms (Esparza-Díaz *et al*., 2010).

No references to studies on the biological activity of ethanolic and hexanic extracts of *G. sepium* and *R. ehrenbergiana* on phytophagous mites were found; however, its effects have been evaluated on other pest organisms. Martin de la Guardia *et al*. (2003) observed that the ethanolic extract of *G. sepium* has antifeedant activity of 40 % in larvae of *Pieris phileta* (Fabricius) and *Plutella xylostrella* (L.). In this work, the ethanolic extract of *G. sepium* bark caused 27.7 % of corrected mortality of *T. urticae* eggs, while the hexane extract of *G. sepium* showed a corrected mortality of 17.8 % of eggs. Santacoloma and Granados (2012), suggest that the activity of *G. sepium* is due to the coumarins, tannins, lignins, phenols and saponins that it contains in leaves and stems.

In this study, the ethanolic extract of aerial parts of *R. ehrenbergiana* caused corrected mortality of *T. urticae* eggs of 29.4 %. No information was found on the activity of extracts of this plant in mites, but the ethanolic extract of the root, at concentrations of 10 and 20 %, caused mortality of 83 % and 99 %, respectively, in larvae of *Culex quinquefasciatus* (Say) (García, 2009). The discrepancy could be due to the species treated or the plant tissue used and the concentrations tested.

The ethanolic and hexanic extracts of *S. molle* were the ones that presented the lowest biological activity in *T. urticae* eggs with values of 8.8 % and 0 % corrected mortality, respectively. Topuz *et al*. (2018) also did not observed an effect of an essential oil from *S. molle* leaves applied as a fumigant on *T. urticae* eggs.

Conclusion

The seed extracts of *T. havanensis*, especially the ethanolic ones, have potential for the development of products for the management of *T. urticae*, and thus, to contribute to the agroecological management of crops. The extracts of the other plants did not show ovicidal activity with potential for the management of *T. urticae*.

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