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Survival and oviposition of *Tetranychus urticae* Koch (Acari: Tetranychiidae) under exposure to unfractionated botanical extracts

Supervivencia y oviposición de *Tetranychus urticae* Koch (Acari: Tetranychiidae) bajo exposición a extractos botánicos crudos

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ABSTRACT

Chemically synthesized acaricides are widely used to control *Tetranychus urticae* Koch (Acari: Tetranychidae), a major agricultural pest that causes significant crop damage. However, the excessive use of synthetic acaricides has led to the emergence of resistant mite populations, complicating pest management. This challenge has driven the search for alternative strategies, including cultural and biological control, which have shown promise. Another potential alternative is botanical extracts, which may be effective even at sublethal doses. This study aimed to evaluate the effects of 10 botanical extracts on the mortality and oviposition of *Tetranychus urticae* adults under laboratory conditions. The extracts were tested at a single mean dose (0.06% w/v). The ethanol crude extract of *Nectandra amazonum* exhibited the highest corrected mortality (14.4%) at 96 hours. Significant oviposition alterations were observed throughout the bioassay, with notable effects at 24 and 96 hours ($P<0.05$). LC-MS analysis of the extracts identified 42 major compounds, including flavonoids, alkaloids, and terpenes, which are likely responsible for the observed effects. These findings indicate that the tested botanical extracts significantly affect *Tetranychus urticae* reproductive capacity, supporting their potential role in integrated pest management strategies.

Keywords:
Botanicals
Ornamental plants
Pest Control
Phytophagous mite

RESUMEN

Aunque los acaricidas sintetizados químicamente han sido una estrategia ampliamente utilizada para controlar el ácaro *Tetranychus urticae* Koch (Acari: Tetranychidae), una plaga económicamente importante debido a su daño significativo causado en cultivos de flores y ornamentales, la utilización indiscriminada de acaricidas sintéticos ha generado poblaciones de ácaros resistentes a tratamientos químicos. Por lo tanto, el control de este fitófago parece ser más desafiante. Este hecho ha llevado a la búsqueda de otras estrategias, como el control cultural y biológico, que han demostrado ser prometedores. Otra alternativa potencial se basa en el uso de extractos botánicos, con efectos incluso a dosis subletales. El propósito de esta investigación fue evaluar el efecto de 10 extractos botánicos sobre la mortalidad y oviposición de *Tetranychus urticae* en condiciones de laboratorio. Los extractos se probaron con una dosis media única (0,06% p/v). La mayor mortalidad corregida (14,4%) fue obtenida con el extracto etanólico crudo de *Nectandra amazonum* a 96 horas. Así mismo, se observaron alteraciones principales en la oviposición a los diferentes tiempos a lo largo de la duración del bioensayo, que implicaron efectos significativamente diferentes después de 24 y 96 horas ($P<0,05$). El análisis de la composición química de los extractos mediante LC/MS reveló la presencia de 42 compuestos principales que incluyeron flavonoides, alcaloides y terpenos, que podrían ser responsables de los efectos observados en *Tetranychus urticae*. Los resultados indican que los extractos evaluados alteran la capacidad de reproducción de *Tetranychus urticae* como un efecto subletal relevante, que podría aprovecharse para controlar las poblaciones de este fitófago en combinación con otras estrategias de manejo de plagas.

Palabras clave:
Productos botánicos
Plantas ornamentales
Control de Plagas
Ácaro fitófago

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Tetranychus urticae Koch (Acari: Tetranychiidae), commonly known as the red spider mite or two-spotted spider mite, is one of the most significant economic pests worldwide (Assouguem et al. 2022b). This phytophagous mite has a broad host range, including flower crops such as roses (Chacón-Hernández et al. 2020b), carnations (Chauhan et al. 2011), and chrysanthemums (Siebert et al. 2020), as well as fruit crops like citrus species (Assouguem et al. 2022a), strawberries, cucurbits, and vegetables such as tomatoes and beans (Assouguem et al. 2022b). Its feeding activity causes aesthetic and physiological damage, significantly reducing the commercial value of crops and their final products.

The red spider mite feeds on foliage, stems, and fruits by piercing plant tissues with its stylophore and sucking the sap, primarily from the undersides of leaves. This feeding behavior produces characteristic stippling on the upper leaf surface. The mite's life cycle consists of four stages: egg, larva, nymph (protonymph and deutonymph), and adult. Females lay eggs on the undersides of leaves, which hatch in approximately 6–7 days at an average temperature of 20 °C. The larvae, initially creamy-colored and lacking dorsal and ventral spots, develop into nymphs after 3 days. Under optimal conditions (21–25 °C and 55% relative humidity in greenhouses), *T. urticae* populations can rapidly increase and complete its life cycle (Daza Vallejos et al. 2010).

Despite the availability of various control strategies, chemically synthesized acaricides remain the most effective and widely used method for managing *T. urticae*. However, their excessive and indiscriminate use has led to the development of resistant mite populations, posing significant challenges for pest control. Synthetic acaricides such as chlorfenapyr, acequinocyl, and fenazaquin achieve nearly 100% efficacy by inhibiting mitochondrial respiration, disrupting growth, and exerting neurotoxic effects (Dekeyser 2005). Other compounds, including abamectins, hexythiazox, and propargite, exhibit high toxicity against the nymph and adult stages of *T. urticae* (Singh et al. 2017). However, prolonged exposure to these acaricides promotes the selection of resistant populations, further complicating pest management (Van Leeuwen et al. 2010).

Given the challenges posed by resistance development and the environmental and health concerns associated with synthetic acaricides, alternative management strategies are increasingly being explored. These approaches consider the biological characteristics of *T. urticae* and incorporate rotation schemes to enhance susceptibility to control measures (Sudo et al. 2018). Among these alternatives, plant-derived extracts offer a promising strategy for developing biopesticides for plant pest management (Khursheed et al. 2022). The advantages of using plant extracts include their rapid degradation, minimal environmental persistence, reduced risk to natural enemies, and safer handling for farmers (Kaur et al. 2023). Particularly, several studies have demonstrated the acaricidal effects of plant extracts against *T. urticae*. A recent review compiled data on 458 botanical extracts from 67 plant families with potential acaricidal properties (Rincón et al. 2019). For instance, Al-Alawi (2014) reported that three plant extracts induced over 50% mortality in *T. urticae* deutonymphs and adults, with *Ruta chalepensis* L. (Rutaceae) being the most effective, causing 65 and 53% mortality in deutonymphs and adults, respectively. Additionally, *Matricaria chamomilla* aqueous extracts and *Pimpinella anisum* hydroethanolic extracts achieved mortality rates of 83% after 120 h and 75% after 24 h in female mites (Tabet et al. 2018). Extracts from wild tomato (*Lycopersicon* sp.) have also demonstrated acaricidal action and repellent properties (Antonious and Snyder 2006).

The anti-mite effects of botanical extracts are attributed to their diverse bioactive specialized metabolites, which are concentrated during extract preparation (Jakubowska et al. 2022). These complex mixtures containing bioactive metabolites can induce single and/or concomitant detrimental effects on mites (Numa et al. 2018), which can ensure a low occurrence of resistance. Moreover, botanical extracts with sublethal effects may play a crucial role in integrated pest management (IPM) programs. Expanding the search for new bioactive botanicals against mites remains a priority in bioprospecting efforts to enhance current knowledge and develop sustainable pest control solutions. Thus, as part of ongoing research on bioacaricides, this study aimed to evaluate the effects of 10 bioactive botanical extracts on the mortality and oviposition of *T. urticae* females under laboratory conditions. The findings may contribute to the development of IPM strategies that incorporate botanical extracts for sustainable and effective *T. urticae* management.

MATERIALS AND METHODS

Plant materials

Plant material for extract preparation was collected from various locations in the Cundinamarca, Casanare, and Meta departments, specifically in Villa Pinzón (5.195608, -73.612221), Aguazul (5.200453, -72.587894), and Puerto López (4.055872, -72.993582), respectively. The Colombian National Herbarium assisted with plant identification, and a voucher specimen for each accession was independently deposited under the corresponding collection number (CN). A total of 10 plant species were collected, and their leaves were selected as test materials based on previous reports of their bioactivity. The selected species and their corresponding CNs were as follows: *Baccharis latifolia* (COL64911), *Genista monspessulana* (COL55658), *Nectandra amazonum* (COL269178), *Galipea trifoliata* (COL71165), *Solanum nigrum* ecotype-1 (collected in Cundinamarca; COL37524), *Solanum nigrum* ecotype-2 (collected in Casanare; COL355705), *Tithonia diversifolia* (COL80466), *Ulex europaeus* (COL60248), *Virola elongata* (COL401848), and *Virola peruviana* (COL369292).

Tetranychus urticae mites

Forty-eight-hour-old adult *T. urticae* females (preovipositing) used in bioassays were obtained from a breeding stock maintained under greenhouse conditions. To avoid overlapping generations and ensure age uniformity, the breeding was conducted in batches, as detailed in Rincón et al. (2024). The stock was fed a mixture of *Fragaria vesca*, *Lupinus bogotensis*, *Alstroemeria pelegrina*, and *Phaseolus vulgaris* in equal proportions to create a heterogeneous feeding environment. Previous studies have indicated that *T. urticae* behavior and susceptibility to chemical agents can vary depending on the host plant (Miresmailli et al. 2006). Mites were maintained under controlled greenhouse conditions (17±2 °C and 70±2% relative humidity) and randomly selected for bioassays to ensure a heterogeneous population.

Crude extracts preparation

Leaves (100 g) from each plant species were used for crude extract preparation. The samples were individually ground under nitrogen (N_2) and dried via lyophilization. Extraction was performed using 96% ethanol by maceration (2:1 ethanol-to-dry leaves ratio, v/w) for 7 days. Daily solvent removal under reduced pressure with a rotary evaporator yielded crude extracts with 10–15% efficiency. The crude extracts were stored at 4 °C without further fractionation until chemical analysis and bioassays.

High-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) analysis

Ethanolic extracts were analyzed using a Shimadzu 8030 liquid chromatography (LC) system (Shimadzu Corp., Nakagyo-ku, Kyoto, Japan) equipped with a photodiode array (PDA) detector, electrospray ionization (ESI), and a mass spectrometry (MS) detector with a triple quadrupole mass analyzer. Separation was performed using a Premier C18 standard column (4.6 × 150 mm, 5 µm) with gradient elution. The mobile phase consisted of 0.005% formic acid and acetonitrile mixtures at a flow rate of 0.7 mL min^{-1}. A 5 µL aliquot of each plant extract solution (2.5 mg mL^{-1} in absolute ethanol) was injected into the LC system. The mass spectrometry method included scanning in both positive and negative ionization modes with an acquisition time of 2–33 min, a mass range of 50–800 m/z, a scan speed of 1,667 μs^{-1}, an event time of 0.5 s, a nebulizer gas flow rate of 1.5 L min^{-1}, an interface temperature of 350 °C, a desolvation line (DL) temperature of 450 °C, a block temperature of 450 °C, and a drying gas flow rate of 9 L s^{-1}. The analysis was monitored at wavelengths between 270 and 330 nm. Metabolite annotation and level 3 putative identification of major and minor metabolites in the test extracts were performed by analyzing their mass spectra, quasimolecular ions, and fragment ions, using the MassBank Project database (www.massbank.jp) for comparison.

Bioassay

Experiments were conducted under laboratory conditions (20±0.2 °C temperature and 55±2% relative humidity). A completely randomized design was used to evaluate the effects of the ethanolic extracts. Absolute (no application), relative (70% ethanol), and positive (Sunfire® commercial acaricide containing 24% chlorfenapyr as the active ingredient) controls were included, along with the 10 botanical crude extracts: *B. latifolia* (Bl), *G. monspessulana* (Gm), *N. amazonum* (Na), *G. trifoliata* (Gt), *S. nigrum* ecotype-1 (Sn1), *S. nigrum* ecotype-2 (Sn2), *T. diversifolia* (Td), *U. europaeus* (Eu), *V. elongata* (Ve), and *V. peruviana* (Vp). Each trial included three replicates per treatment, and each experiment was replicated three times. All test extracts and positive controls were applied at the same concentration (0.06% w/v). Previous research has

successfully demonstrated the direct effects of leaf extracts from various botanical species at this concentration (Numa et al. 2018).

Each experimental unit consisted of a 9-cm Petri dish containing a treated bean leaf disk surrounded by moistened cotton. Bean leaves were immersed in test solutions (0.06% w/v) for 1 min, air-dried for 15 min to remove excess moisture, and placed inside the Petri dish. Twenty *T. urticae* females were then randomly selected from the breeding stock and placed on the abaxial surface of the leaf. Since mites were placed on pre-treated leaves, mortality was expected to result primarily from ingestion or feeding deterrence. To ensure contact toxicity, an additional application of extract solutions (0.06% w/v) was administered over the mites using an airbrush (20 cm height, 96 drops cm^{-2}, 20–30 psi, 1.0 mL). Each experimental unit was sealed with stretch film and placed in a climate-controlled chamber. Mortality and oviposition of *T. urticae* were recorded for up to 96 h, the point at which mortality in the absolute control remained below 10% (Numa et al. 2018).

Data analysis

Daily *T. urticae* mortality data from the three replicates were used to calculate corrected mortality using Abbott's formula (Piepho et al. 2024) for each evaluation day (Equation 1):

$$\text{Corrected mortality}(\%) = \frac{(\text{mortality in treatment}(\%) - \text{mortality in absolute control}(\%)) * 100}{100 - \text{mortality in absolute control}(\%)} \quad (1)$$

The eggs laid by the females placed in each Petri dish were counted under a stereoscope at different evaluation times. Thus, fecundity was calculated as the total number of oviposited eggs per female in each treatment at 24, 48, 72, and 96 h (Equation 2):

$$\text{Total fecundity} = \frac{\text{total number of eggs}}{\text{total number of living females}} \quad (2)$$

Data on corrected mortality and total fecundity at 96 h were analyzed using a generalized linear model (GLM) assuming binomial and Poisson distributions, respectively, using the GLM function in R v4.1.2 (R Core Team 2021).

RESULTS AND DISCUSSION

In this study, mites were intentionally fed on various plants to ensure a generalizable mortality response across different pest populations from a range of host plants. The positive control (Sunfire® at a test concentration of 0.06% w/v) exhibited significantly higher mortality rates (54.6–89.7%) compared to the relative control (0.42–7.6%) from 48 to 96 h (P<0.05) (Table 1). This result aligns with expectations and indicates that the variability in female diet, due to different host plant species, did not significantly alter the response to the evaluated treatments. Unfractionated botanical extracts were prepared using 96% ethanol as the primary extractant due to its availability, ease of recovery, and ability to solubilize and extract a broad range of bioactive specialized metabolites. The bioassay demonstrated that the ethanolic extracts induced mortality and affected oviposition in *T. urticae* females at varying levels at the selected concentration (0.06% w/v). This dose was chosen as a representative value within the concentration range commonly used for biopesticides, such as those derived from *Azadirachta indica* (0.02–0.10% w/v)(Biswas 2013).

The corrected mortality percentages of *T. urticae* females exposed via direct contact with various crude ethanol extracts under laboratory conditions over 24 to 96 h are presented in Table 1. Among the tested extracts, *N. amazonum* exhibited the highest corrected mortality (14.4%) at 96 h, with significant differences from the relative control (P<0.01). *Solanum nigrum*-EC2 showed significantly different mortality rates at 24, 48, and 72 h (P<0.05), whereas *U. europaeus* exhibited its highest mortality at 96 h. Conversely, *S. nigrum*-EC1, *T. diversifolia*, and *V. elongata* displayed low corrected mortality rates, with *S. nigrum*-EC2 reaching a maximum of 8.9% after 96 h. These findings indicate that the test extracts had weak lethal effects (<15%). For *T. diversifolia*, its low impact on *T. urticae* agrees with Pavela et al. (2018), who reported a maximum mortality of 50% at a dose of 150 µg cm^{-3} of a polar extract in acute toxicity assays. The observed low mortality may be attributed to the characteristics and traits of the tested mite population. This observation was

evident from the results obtained for a reference active extract derived from *S. nigrum*-EC2 (collected in Casanare, Colombia) (Numa et al. 2016). This extract was previously tested on a homogeneous *T. urticae* population composed of susceptible individuals, yielding high corrected mortality (85.0±4.3% after 72 h) (Numa et al. 2016). However, when tested on the current heterogeneous population under identical exposure conditions, mortality was markedly lower (5.9±1.6%). To rule out chemical composition differences as a cause of these discrepancies, LC-MS analyses were performed on the botanical extracts, confirming identical chemical profiles. Thus, the differential mortality between assays can be attributed to variations in *T. urticae* populations. In this study, *S. nigrum* ecotype 1 (EC1) was included among the tested botanicals to compare the effects of extracts from the same species growing in different locations. The extract from *S. nigrum*-EC1 (collected in Cundinamarca, Colombia) exhibited lower mortality compared to that of *S. nigrum*-EC2. This difference is likely due to variations in chemical composition, with ecotype 2 containing higher levels of certain metabolites, particularly alkaloidal triterpenes.

Table 1. Corrected mortality percentage of *Tetranychus urticae* adults exposed to crude ethanol extracts under laboratory conditions from 24 to 96 h.

Treatment	Corrected Mortality (%)			
	24 h	48 h	72 h	96 h
Relative Control[a]	0.42±0.08	1.53±1.6	1.7±1.6	7.6±3.3
Positive Control[b]	54.6±6.3**	71.3±5.7**	86.4±4.3**	89.7±3.8**
Baccharis latifolia	2.08±18	3.34±2.3	3.90±2.4	6.21±3.1
Genista monspessulana	2.92±2.1	1.65±1.6	2.91±2.1	7.50±3.3
Nectandra amazonum	0.83±1.2	1.66±1.6	3.46±2.3	14.4±2.3**
Galipea trifoliata	0.83±1.2	2.49±7.84	1.68±1.6	3.05±2.2
Solanum nigrum-EC1[c]	2.08±2.6	2.07±2.9	1.61±3.0	3.92±3.6
Solanum nigrum-EC2[d]	4.40±1.8	5.60±1.84	5.90±1.6	8.90±2.5
Tithonia diversifolia	2.92±2.1	2.92±9.80	1.62±0.8	2.19±1.9
Ulex europaeus	0.83±1.2	2.92±2.1	3.49±2.3	7.97±3.4
Virola elongata	2.08±1.2	1.24±0.4	2.22±0.8	4.39±2.6
Virola peruviana	1.25±1.4	2.93±2.1	3.01±2.2	6.16±3.0

[a]70% ethanol; [b]Commercial acaricide (24% Chlorfenapyr as active ingredient); [c]ecotype 1, collected in Cundinamarca, Colombia; [d]ecotype 2, collected in Casanare, Colombia. Laboratory conditions: 19±0.2 °C and 60±2% relative humidity. Data expressed as mean ± standard error of the mean (SEM). *Significant differences (P<0.05) regarding the relative control, **highly significant differences regarding the relative control (P<0.01).

Despite the low impact on *T. urticae* mortality, the main effect of test botanicals was observed on reproductive alterations. Females from positive control (Sunfire®) increased oviposition at 72 and 96 h of exposure (Table 2). A similar effect was observed for the *N. amazonum*-derived extract, which was the most active botanical in terms of mite mortality. This response can be attributed to physiological stress, leading to hormonal changes that accelerate reproduction. A similar phenomenon has recently been reported in the brown planthooper *Nilaparvata lugens*, where exposure to certain insecticides has been associated with increased juvenile hormone levels, responsible for stimulating egg development and oviposition (Gao et al. 2025).

Significantly lower oviposition rates were recorded for *S. nigrum* (ecotype-2) at 96 h (P<0.05) and *T. diversifolia*, *V elongata*, and *V. peruviana* at 24 h. Additionally, *U. europaeus* induced an oviposition reduction below one egg per day per female at 96 h (Table 2). These extracts exhibited a sublethal effect by reducing *T. urticae* oviposition, consistent with findings for other plant extracts. Pavela et al. (2018) reported that polar extracts from *T. diversifolia*, despite their low direct toxicity, strongly

inhibited oviposition. This effect was attributed to the presence of tagitinin C and A, which act as repellents and antifeedants. In the present study, tagitinin C and A were not detected in the *T. diversifolia* extract; however, a structurally related compound, tagitinin D, was identified. This finding suggests that sesquiterpene lactones such as tagitinins may play a role as bioactive metabolites in the genus *Tithonia*, which has been previously associated with plant defense mechanisms (Chagas-Paula et al. 2012).

Table 2. Effects of crude ethanol extracts on the *Tetranychus urticae* female oviposition under laboratory conditions from 24 to 96 h.

Treatment	Per capita fecundity (eggs/day/female)			
	24 h	48 h	72 h	96 h
Relative Control[a]	1.44±0.20	1.62±0.35	1.36±0.34	1.15±0.15
Positive Control[b]	1.08±0.17	1.83±0.34	1.87±0.50*	2.15±0.74**
Baccharis latifolia	1.18±0.21	1.69±0.34	2.15±0.46**	1.16±0.22
Genista monspessulana	1.42±0.21	1.51±0.19	1.30±0.28	1.83±0.40
Nectandra amazonum	1.40±0.11	1.64±0.24	2.02±0.27	1.35±0.14
Galipea trifoliata	1.01±0.15	1.23±0.21	1.47±0.41	1.29±0.22
Solanum nigrum-EC1[c]	1.15±0.12	1.11±0.24	1.00±0.19	1.34±0.32
Solanum nigrum-EC2[d]	1.30±0.10	1.60±0.30	1.60±0.40	0.60±0.10**
Tithonia diversifolia	0.92±0.14**	1.14±0.24	1.08±0.21	1.13±0.17
Ulex europaeus	1.07±0.18	1.32±0.29	1.32±0.32	0.87±0.23**
Virola elongata	0.84±0.13**	1.15±0.13	1.00±0.15	1.01±0.17
Virola peruviana	0.85±0.12**	1.34±0.20	0.99±0.18*	1.38±0.29

[a]70% ethanol; [b]Commercial acaricide (24% Chlorfenapyr as active ingredient); [c]ecotype 1, collected in Cundinamarca, Colombia; [d]ecotype 2, collected in Casanare, Colombia. Laboratory conditions: 19±0.2 °C and 60±2% relative humidity. Data expressed as mean ± standard error of the mean (SEM) (n=30). *Significant differences (P<0.05) regarding the relative control, **highly significant differences regarding the relative control (P<0.01).

Several studies have investigated the oviposition-deterring effects of plant by-products on *T. urticae*, demonstrating that phenolic- and terpene-rich botanicals significantly impact mite reproduction (Rincón et al. 2019). In this context, flavonoids and phenolics (from *V. elongata*, *V. peruviana*, and *U. europaeus*), alkaloidal triterpenes (from *S. nigrum*-EC2), and sesquiterpenes (from *T. diversifolia*) may be the active phytochemicals responsible for the observed reductions in oviposition. Similar effects have been reported by Chacón-Hernández et al. (2020b), who found that exposure to *Magnolia tamaulipana* powder extract led to a concentration-dependent reduction in *T. urticae* oviposition, with the effect increasing between 5 and 1,000 µg mL^{-1}. Additionally, ethanolic extracts of *Lippia origanoides* and *Gliricidia sepium* at a concentration of 5% v/v inhibited oviposition in *Tetranychus cinnabarinus* by 43.7–57.0% after 72 h of exposure (Sivira et al. 2011). This oviposition-inhibitory effect may have two possible explanations: 1) specialized metabolites produced by plants could directly impair ovarian function (Dimetry et al. 2003), or 2) these compounds may induce incomplete or temporary sterility, leading to reduced oviposition (Hosny et al. 2010). Further research is needed to elucidate the physiological mechanisms underlying these sublethal effects.

Although preference tests were not conducted in this study, previous experiments suggest that botanicals can modify oviposition behavior, as females often avoid locating and ovipositing on treated surfaces (Keskin et al. 2020). This response could be mediated by specific phytoconstituents, such as volatile compounds, present in botanicals (Keskin et al. 2020). In multiple-choice tests, Hata et al. (2020) demonstrated that aromatic plants with low acceptance by *T. urticae* females also induced significantly lower oviposition rates per day.

Similarly, Zhang et al. (2008) reported that an extract derived from *Artemisia annua* leaves significantly reduced *T. cinnabarinus* oviposition. Kumral et al. (2010) further demonstrated the effectiveness of *Datura stramonium* L. seed- and leaf-derived extracts against *T. urticae*, not only as an acaricide but also as a repellent, oviposition deterrent, and hatch rate reducer after 24 h of exposure. These findings highlight the potential efficacy of compounds in the tested extracts for disrupting *T. urticae* reproduction.

LC/MS-based chemical characterization of the unfractionated extracts used in the bioassay detected 42 main compounds (annotated at level three based on their mass spectral data, Table 3), comprising phenolics, flavonoids, alkaloids, and terpene-related metabolites. Some of these compounds shared retention times in certain extracts, while most were detected only in a specific extract, reflecting the taxonomic variability of the tested botanicals (Figure 1, Table 3). Thus, this comparative LC/MS-based analysis provides an overview of the number and semiquantitative levels of metabolites present in a single extract, particularly those derived from leaves, which exhibit highly varied phytoconstituent profiles. These plant-derived metabolites can act as toxic agents or oviposition disruptors for many pests (Numa et al. 2015), causing various single or concomitant effects on mites (Numa et al. 2018). This complexity presents a challenge in identifying the specific compounds responsible for the observed acaricidal activity. However, existing records on the toxicity of certain metabolites can guide further investigation. For instance, by-products from *Eugenia langsdorffii* and *Ocotea* spp. contain high concentrations of sesquiterpenes, primarily found in leaves, which exhibit strong toxic effects on *T. urticae*, attributed to these terpene-related compounds (de Moraes et al. 2017).

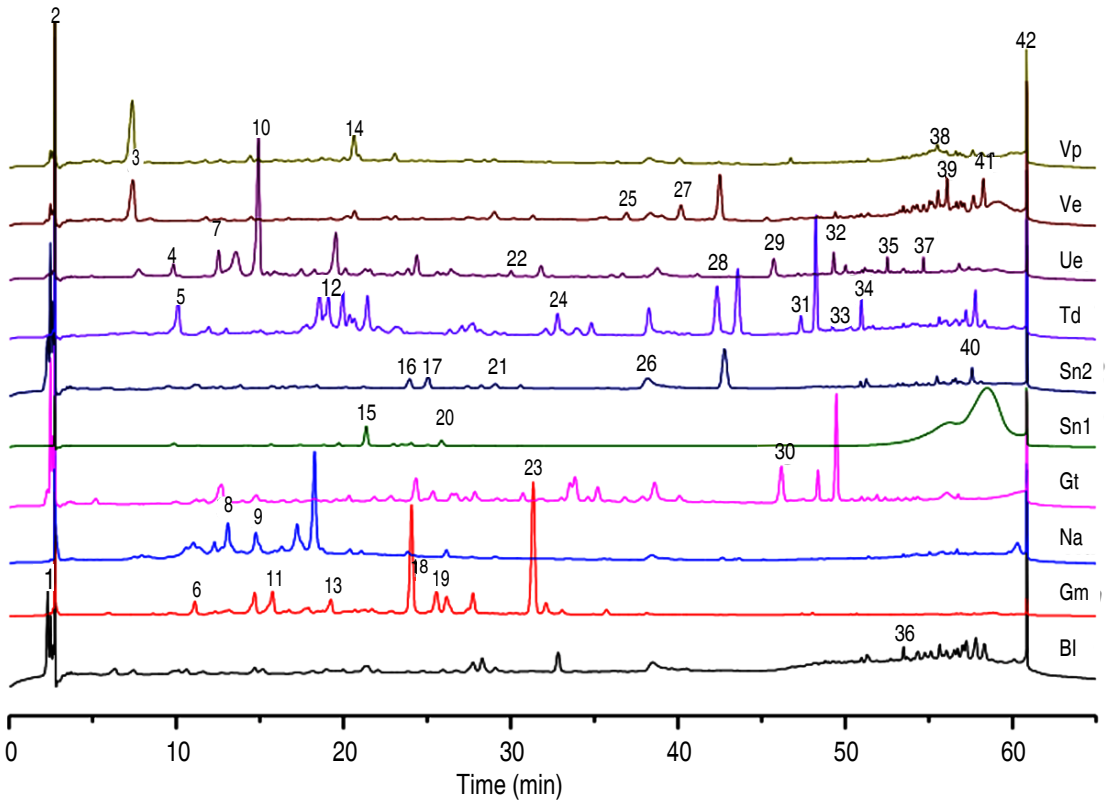

Figure 1. RP-LC-ESI-MS-derived profiles of test extracts: *Baccharis latiflolia* (Bl), *Genista monspessulana* (Gm), *Nectandra amazonun* (Na), *Galipea trifoliata* (Gt), *Solanum nigrum*-EC1 (Sn1), *Solanum nigrum*-EC2 (Sn2), *Tithonia diversifolia* (Td), *Ulex europaeus* (Ue), *Virola elongata* (Ve), *Virola peruviana* (Vp).

Table 3. Chemical composition of test ethanol extracts.

# [a]	R_t (min)	Name	Ct[b]	Molecular Formula	*m/z* [M+H$^+$]	Detection in plant samples[c]									
						1	2	3	4	5	6	7	8	9	10
1	2.46	hispidulin sulfate	f	$C_{16}H_{12}O_9S$	381.03						x				
	2.51	hexahydro-hydroxy-methyl-(methylethyl)-naphthalenecarboxaldehyde	t	$C_{15}H_{22}O_2$	235.52	x									
2	2.67	alpinumisoflavone (or isomers)	f	$C_{20}H_{16}O_5$	337.13		x								
	2.73	dimethoxyflavone	f	$C_{17}H_{14}O_4$	282.95										x
	2.73	monatin	a	$C_{14}H_{16}N_2O_5$	293.11						x				
	2.82	*N*-methylcytisine	a	$C_{12}H_{16}N_2O$	205.55		x								
	2.83	scopolin (or isomers)	p	$C_{16}H_{18}O_9$	353.14							x			
	2.85	glaziovine	a	$C_{18}H_{19}NO_3$	297.24			x							
3	7.43	juruenolide F	p	$C_{25}H_{38}O_5$	391.45									x	
4	9.88	methyltectorigenin apiofuranosylglucopyranoside	f	$C_{28}H_{32}O_{15}$	609. 21								x		
5	10.23	tagitinin D	t	$C_{19}H_{28}O_6$	353.12							x			
6	11.22	dehydrolupanine	a	$C_{15}H_{22}N_2O$	247.45		x								
7	12.53	luteolin rutinoside (or isomers)	f	$C_{27}H_{30}O_{15}$	595.12								x		
8	13.33	reticuline (or isomers)	a	$C_{19}H_{23}NO_4$	329.16			x							
9	14.67	unknown	-		446.21		x								
	14.74	methoxyflindersiamine (or isomer)	a	$C_{14}H_{11}NO_5$	274.51				x						
	14.77	norlirioferine (or isomers)	a	$C_{19}H_{21}NO_4$	327.14			x							
10	14.91	methylgenistein glucoside	f	$C_{22}H_{22}O_{10}$	447.23								x		
11	15.84	*N*-acetylcytisine	a	$C_{13}H_{16}N_2O_2$	431.42		x								
12	18.31	rhombifoline (or isomers)	a	$C_{15}H_{20}N_2O$	245.05								x		
	18.43	corydine (or isomers)	a	$C_{20}H_{23}NO_4$	341.35			x							
13	19.22	cinegalline	a	$C_{23}H_{30}N_2O_6$	429.25		x								
	19.54	genistein glucoside	f	$C_{21}H_{20}O_{10}$	433.21										
	20.18	formononetin rutinoside	f	$C_{28}H_{32}O_{13}$	577.16								x		
14	20.50	yangambin	p	$C_{24}H_{30}O_8$	446.92										x
	20.52	surinamensin or dimethoxylariciresinol	p	$C_{22}H_{28}O_6$	388.11										x
	20.65	unknown	-		265.54									x	
15	21.50	isorhamnetin glucopyranoside	f	$C_{28}H_{32}O_{17}$	641.23					x					
16	24.05	ajmaline	a	$C_{20}H_{26}N_2O_2$	327.21						x				
	24.13	methylgenistein (or isomer)	f	$C_{16}H_{12}O_5$	285.32		x								
	24.33	evoxanthine	a	$C_{16}H_{13}NO_4$	284.35				x						
	24.38	methylgenistein (or isomer)	a	$C_{16}H_{12}O_5$	285.05								x		
17	25.13	calligonine	a	$C_{12}H_{14}N_2$	187.12						x				
18	26.65	daidzein (or isomer)	f	$C_{15}H_{10}O_4$	255.35		x								
19	27.82	luteolin isomer	f	$C_{15}H_{10}O_6$	285.12		x								
20	28.01	solasodoside A	tr	$C_{51}H_{82}O_{21}$	1031.12					x					
	28.90	abutiloside A	a	$C_{49}H_{83}NO_{17}$	958.45					x					

Table 3

# [a]	R_t (min)	Name	Ct[b]	Molecular Formula	*m/z* [M+H$^+$]	Detection in plant samples[c]									
						1	2	3	4	5	6	7	8	9	10
21	29.10	leptomerine	a	$C_{13}H_{15}NO$	201.11						x				
	29.01	grandisin	p	$C_{24}H_{32}O_7$	431.23									x	
	30.05	methylgenistein glucoside	f	$C_{22}H_{22}O_{10}$	445.05								x		
22	30.66	dimethoxyflavone isomer	f	$C_{17}H_{14}O_4$	283.14						x				
	30.73	subaphyllin	p	$C_{14}H_{20}N_2O_3$	264.53				x						
23	31.52	trihydroxyxanthone	p	$C_{15}H_{10}O_5$	271.35		x								
	31.83	resokaempferol	f	$C_{15}H_{10}O_5$	269.21								x		
	32.11	eupafolin	f	$C_{16}H_{12}O_7$	317.18							x			
24	32.96	hispidulin	f	$C_{16}H_{12}O_6$	299.21							x			
	33.00	abutiloside J	a	$C_{48}H_{81}NO_{17}$	943.23					x					
25	36.91	unknown	-		499.31									x	
26	38.28	Hydroxy-methoxy-methyl-formylflavanone isomer	f	$C_{18}H_{16}O_5$	313.11						x				
	38.59	dictamine	a	$C_{12}H_9NO_2$	200.52				x						
27	40.13	unknown	-		223.53									x	
	42.34	nevadensin	f	$C_{18}H_{16}O_7$	345.35							x			
28	42.45	xenognosin B (or isomer)	f	$C_{16}H_{12}O_5$	285.31									x	
	42.84	hydroxy-methoxy-methylflavone	f	$C_{17}H_{14}O_4$	283.12						x				
29	45.71	isowighteone	f	$C_{20}H_{18}O_5$	339.43								x		
30	46.18	tigloyldiderroside	a	$C_{15}H_{15}NO_3$	258.41				x						
31	48.25	homobutein	f	$C_{16}H_{14}O$	287.23							x			
	49.20	abutiloside B	a	$C_{46}H_{77}NO_{17}$	914.21					x					
32	49.31	claussequinone-vestitol	f	$C_{32}H_{28}O_9$	555.22								x		
	49.41	helianyl octanoate	tr	$C_{38}H_{66}O_2$	555.21							x			
	49.47	methoxyflindersiamine (or isomer)	a	$C_{14}H_{11}NO_5$	274.43				x						
33	50.94	hymenoxin	f	$C_{19}H_{18}O_8$	373.14							x			
	50.94	desmethyleucalyptin (or isomer)	f	$C_{18}H_{16}O_5$	313.11						x				
34	51.29	desmethyleucalyptin (or isomer)	f	$C_{18}H_{16}O_5$	313.11						x				
	52.51	onogenin	f	$C_{17}H_{14}O_6$	315.51								x		
35	52.56	limonin	tr	$C_{26}H_{30}O_8$	471.22				x						
	52.57	bisparasin (isomer)	p	$C_{30}H_{28}O_6$	485.25				x						
36	53.64	conycephaloide	t	$C_{20}H_{20}O_5$	339.23	x									
37	54.68	betulin	tr	$C_{30}H_{50}O_2$	443.71								x		
	55.14	unknown	-		255.32										x
38	55.53	artonin U	f	$C_{20}H_{20}O_5$	353.14						x				
	55.58	unknown	-		893.33									x	
39	56.13	xenognosin B (or isomer)	f	$C_{16}H_{12}O_5$	284.06									x	
	56.81	daidzein (or isomer)	f	$C_{15}H_{10}O$	255.41								x		
40	57.63	hydroxysolasodine	a	$C_{27}H_{45}NO_3$	430.33						x				
	57.72	juruenolide E	p	$C_{23}H_{34}O_5$	391.42									x	

Table 3

# [a]	R_t (min)	Name	Ct[b]	Molecular Formula	*m/z* [M+H$^+$]	Detection in plant samples[c]									
						1	2	3	4	5	6	7	8	9	10
41	58.24	formononetin	f	$C_{16}H_{12}O_4$	269.25									x	
	60.84	verrucosin	P	$C_{20}H_{24}O_5$	344.09										x
	60.86	solasodine	a	$C_{27}H_{43}NO$	398.34						x				
42	60.87	ichangensin	tr	$C_{25}H_{32}O_7$	445.28				x						
	60.91	dihydro-(hydroxyphenyl)-pyranocatechinone	f	$C_{24}H_{20}O_8$	436.16			x							
	60.93	methyl-[tetrahydromethylbutadienyl) methylene-oxofuranyl]-butenal (or isomers)	t	$C_{15}H_{18}O_3$	247.44							x			
	60.94	geranyllinalololide (or isomers)	t	$C_{20}H_{30}O_3$	319.55	x									

[a]Numbering according to detection order showed in Figure 1; [b]Ct = Compound type: flavonoid (f), phenolics (p), alkaloid (a), terpenoid (t), triterpene (tr); [c]Compound distribution among test plant-derived extracts: **1**= *Baccharis latiflolia* (Bl), **2**= *Genista monspessulana* (Gm), **3**= *Nectandra amazonun* (Na), **4**= *Galipea trifoliata* (Gt), **5**= *Solanum nigrum* 1 (Sn1), **6**= *Solanum nigrum* 2 (Sn2), **7**= *Tithonia diversifolia* (Td), **8**= *Ulex europaeus* (Ue), **9**= *Virola elongata* (Ve). **10**= *Virola Peruviana* (Vp).

The findings of this study serve as a foundation for developing alternative control strategies targeting *T. urticae* reproduction using test plant extracts, particularly *U. europaeus*, an invasive species in South America. In this context, quinolizidine alkaloids (e.g., rhombifoline) and prenylated isoflavones (e.g., isowighteone) were identified in the *U. europaeus* extract and may contribute to oviposition inhibition (Isman 2020). Indeed, prenylated isoflavones, precursors of rotenoids, have demonstrated acaricidal activity and completely inhibited the oviposition of the female tick *Rhipicephalus appendiculatus* (Van Puyvelde et al. 1987), while matrine-type quinolizidine alkaloids have been shown to affect *T. urticae* oviposition (Marčić and Među 2014). Additionally, nitrogenated triterpenes (e.g., abutiloside J), lignans (e.g., grandisin), and sesquiterpene lactones (e.g., tagitinin D) were detected in extracts with the highest oviposition inhibitory effects, such as those from *S. nigrum*, *V. elongata*, and *T. diversifolia*. These findings warrant further bio-guided fractionation studies to isolate and evaluate the active compounds responsible for anti-mite effects, defining the active principles and their specific roles in *T. urticae* oviposition alteration.

T. urticae susceptibility to acaricidal agents has been primarily assessed through acute contact toxicity assays. However, resistance development is well-documented and is often linked to genetic traits enabling adaptation to acaricide pressure via transposable elements in its genome (Rincón et al. 2019). Resistance mechanisms include reduced target site sensitivity, metabolic detoxification, and decreased penetration, all classified as physiological resistance, while behavioral adaptations remain poorly studied (Adesanya et al. 2019). Developing behavioral manipulation techniques is a promising approach to mitigating resistance and optimizing synthetic insecticide use (de França et al. 2013). From this perspective, botanical extracts from *S. nigrum*, *T. diversifolia*, *V. elongata*, and *U. europaeus* show potential for use as oviposition inhibitors or disruptors. Information on the extraction yield and crop productivity of these plants is essential for assessing the feasibility of incorporating oviposition inhibitors into integrated pest management (IPM) programs for controlling *T. urticae*. Additionally, further studies should evaluate the dose-dependent effectiveness of the most promising extracts under laboratory conditions to validate their potential in *T. urticae* management through targeted field trials. Once high effectiveness is confirmed, standardization processes must be established, including plant selection, cultivation management, and formulation development for field application. A common challenge with botanical products is the inconsistency in efficacy and lack of reproducibility, often due to variations in agronomic practices and extract stability (Ivase et al. 2021). Therefore, caution is advised in generalizing these findings, as physiological and behavioral aspects may influence the magnitude of the observed effects (Thompson and Reddy 2016).

CONCLUSION

This study evaluated the capacity of 10 leaf-derived unfractionated ethanol extracts to alter *T. urticae* survival and oviposition behavior, identifying promising candidates

for disrupting its reproduction. Among the tested extracts, *S. nigrum* EC2 and *U. europaeus* significantly inhibited oviposition after 96 h, while *N. amazonum*, *S. nigrum* EC1, and *T. diversifolia* were most effective in inducing mite mortality. These findings suggest that specific phytochemicals, such as alkaloidal triterpenes, phenolics, and sesquiterpenes, may play crucial roles in these biological activities. The results highlight the importance of understanding phytochemical-pest interactions and emphasize the need for further research to isolate and characterize the active compounds responsible for these effects. Additionally, the observed reduction in *T. urticae* oviposition may be attributed to the sublethal effects of the extracts, potentially leading to decreased population growth and reduced dependence on synthetic pesticides. This research underscores the potential of botanical extracts as sustainable IPM tools, offering an environmentally friendly alternative to conventional chemical controls and contributing to the reduction of pesticide use in commercial agriculture.

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CONFLICT OF INTERESTS

The authors have no conflict of interest.

ETHICS IN RESEARCH AND LEGAL REGULATIONS

The authors declare they have not violated or omitted ethical or legal norms when conducting this study.

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