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Effect of acaricidal activity of *Solanum nigrum* on *Tetranychus urticae* Koch under laboratory conditions

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Tetranychus urticae **is an economically important pest for different commercial crops and is traditionally controlled with synthetic chemical products that are usually toxic. Therefore, alternative strategies are implemented to allow producers to meet the phytosanitary requirements demanded by quality seals. One of these alternatives is the use of plant extracts. The aim of this study was to determine the effect of median lethal dose of** ***Solanum nigrum*** **extracts on the mortality and fertility of** ***T. urticae*** **females under laboratory conditions. Liquid chromatography-mass spectrometry (LCMS)-based chemical characterization of the ethanol extract of** ***S. nigrum*** **leaves was also conducted. The immersion technique and direct application on rose leaves were used to evaluate the effects of seven doses (1 to 1000 µg/mL) of the ethanol extract of** ***S. nigrum*** **leaves on** ***T. urticae*** **females under laboratory conditions. The mortality and oviposition of spider mites were recorded at 24, 48 and 72 h. The effects of seven concentrations of ethanol extract were evaluated on phytophagous females. The LC_{50} value was 279.69±20.59 µg/mL after 72 h. The test extract also caused a reduction in oviposition. The main components in the leaf extract of** ***S. nigrum*** **were found to be flavonoid and alkaloid-related compounds. The ethanol extract of leaves of** ***S. nigrum*** **showed high potential as an effective management strategy against** ***T. urticae*** **under laboratory conditions.**

Key words: Spider mites, LC_{50}, rose crop, ethanol extracts, liquid chromatography-mass spectrometry (LC-MS).

INTRODUCTION

Tetranychus urticae is a cosmopolitan and polyphagous pest that attacks many economically important crops worldwide. This phytophage causes damage to extensive horticultural crops, such as cotton, corn, sorghum, ornamentals, citrus, grapes and fruit (Moraes and Flechtmann, 2008). These spider mites may cause

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uniform leaves discoloration, because sap sucking possibly leads to whitening or yellowing and drying thereof. When mites are numerous they produce a net that covers infested areas and spreads from leaf to leaf, to cover the whole plant (Helle and Sabelis, 1985).

Rose crop is associated with different phytosanitary problems, such as spider mite (*T. urticae*). The management of this crop pest accounts for nearly 30% of the total costs of chemical products used on roses per year. Because acaricides are used as the main method of regulating the populations of *T. urticae*, this pest has generated populations that are resistant to some of these products (Asocolflores, 2013).

Concern about the use of chemicals for pest control, which can lead to environmental pollution and harmful residue in commodities, as well as insecticide and acaricide resistance, has boosted the use of alternative methods, such as cultural control, ethological control, biological control and plant extracts (Oliveira et al., 2007). The use of plant extracts may be an alternative and environmentally safe strategy of pest management considered as a natural component; they are usually more selective than synthetic chemical pesticides (Oliveira et al., 2007). Plant extracts can also be compatible with biological control and they are therefore considered a component of integrated pest management (IPM) (Pérez et al., 2007). The chemistry of natural plant products has been analyzed in the search for new sources of useful agrochemical agents for the treatment of various pests and diseases that affect cash crops (Scott et al., 2005; Garcez et al., 2009).

As part of this research on acaricidal products, a plant extract (characterized by a variety of secondary metabolites having biological activity) was used. Some plant extracts are effective acaricides and insecticides due to the presence of molecules that possess insecticidal activity, such as flavonoids in the extract of *Solanum nigrum*, which is used in commercial crops to manage agricultural pests; thrips, *Frankliniella occidentalis*, mite, *T. urticae* and aphid, *Shizaphis graminum*. However, the effect has not been generated in the developmental stages of the pests, and its efficiency has been reported (Scott et al., 2005; Garcez et al., 2009; Muthuvel et al., 2014; Chermenskaya et al., 2010; Moreira et al., 2007).

To offer farmers an alternative medium-term strategy for the management of *T. urticae*, this study determined the median lethal dose and its effect on the mortality and fecundity of *T. urticae* females exposed to different concentrations of the plant extract of *S. nigrum* (Solanaceae) under laboratory conditions.

MATERIALS AND METHODS

Sources of biological material

Adult *T. urticae* used in the tests were collected from a colony that is maintained under greenhouse conditions (28.5 ± 1°C and 73.0 ± 0.22% relative humidity [RH]) and renewed every month, with wild individuals. Populations of 5000 individuals were placed under laboratory conditions to begin a new cohort and to begin with laboratory experiments for females than 2 days old.

The plant material of *S. nigrum* (commonly named as *Hierbamora*) used to prepare the plant extracts was not previously treated with chemicals and was collected in Aguazul town (Casanare Department, Colombia; Coordinates: 5.183629-72.530031) in a forest at the foothill of the west Andean mountains. The plant material consists of the oldest leaves of three wild plants without infections or wounds, that is, healthy leaves (915 g of dried leaves). The identification of the collected plants was conducted in collaboration with the Colombian National Herbarium of the Natural Sciences Institute (National University of Colombia). A voucher specimen was deposited under the collection code COL355705.

Extraction process

After the taxonomic identification of collected specimens of *S. nigrum*, the plant material (leaves) was dried, ground, and subjected to extraction by maceration (for 7 days) using 96% ethanol with daily removal of the solvent. For this process, rotavapor was used to evaporate the solvent.

Reverse phase-liquid chromatography-diode array detection-electrospray ionization-mass spectrometry (RP-LC-DAD-ESI-MS) analysis

The ethanol extract was analyzed by liquid chromatography using a Shimadzu LC-MS QP2020 system (Shimadzu Corp., Nakagyo-ku, Kyoto, Japan). It was done at the Bioorganic Chemistry laboratory, University Militar Nueva Granada in order to define the chemotype of *S. nigrum* leaves used in the biological assays. The separation of the components of the extracts was performed in a C-18 standard Premier column (4.6 mm × 150 mm, 5 um) using the liquid chromatography-mass spectrometry (LC-MS) system consisting of a separation module equipped with a photodiode array detector (DAD), electrospray ionization (ESI), and a detector with a quadrupole mass analyzer. Flow was 0.7 ml/min and the mobile phases were conducted with trifluoroacetic acid (TFA) and 0.005% acetonitrile (ACN). The concentration used was 1.0 µg/mL in absolute ethanol, and 10 µL of this solution was injected into the LC system.

The mass spectrometry method was established by Timóteo et al. (2014), Proestos et al. (2006), and Fraser et al. (2014) and used with the following modifications: a scan with positive and negative ionization mode was performed with an acquisition time of 2 to 33 min, 50 to 800 m/z mass range, 1667 µ/s scan speed, 0.5 event time, 1.5 L/min nebulizer gas flow, 350°C as the interface temperature and DL, and 450°C as the block temperature. The drying gas flow was 9 L/s. The analysis was monitored at wavelengths between 270 to 330 nm.

The tentative identification of the major and minor metabolites in the test extract was performed by the analysis of mass spectra on the LC-MS data, complemented by comparison with literature data. This identification was also supported by the database included in the search engine MassBank Project (free distribution).

Evaluation of the effect of the extract of leaves of *S. nigrum* on females of *T. urticae*

The assay was performed in the biological control laboratory (23.6 ± 0.2°C and RH of 60 ± 2%), located in Colombia. A completely randomized design was used to evaluate the effect of the doses of the extract, consisting of seven concentrations of the extract, an

Table 1. Description of treatments used under laboratory conditions on *T. urticae* females.

Treatment	Final dilution concentration (µg/mL)
Absolute control (No application)	-
Positive control (distilled water + commercial acaricide with Chlorfenapyr 24% active ingredient)	-
S. nigrum [1]	1
S. nigrum [5]	5
S. nigrum [10]	10
S. nigrum [50]	50
S. nigrum [100]	100
S. nigrum [600]	600
S. nigrum [1000]	1000

absolute control (without application) and a positive control (distilled water + commercial acaricide with Chlorfenapyr 24% active ingredient) (Table 1). Each experiment was repeated ten times and the experiment was repeated fifteen times in time (or evaluations of fifteen batches).

Proposed methodology was based on Cahill et al. (1996) with a modification of the experimental unit employed, which consisted of placing a bean leaf disc with a swab placed around it in a Petri dish of 6 cm in diameter. The size of the bean leaf discs was 4 cm in diameter and around it was placed cotton moistened with sterile distilled water.

The experimental unit was sealed with stretch film. Twenty 2-day old females were placed on the abaxial surface of the bean leaf. Following this, all experimental units were maintained in the laboratory (23.6 ± 0.02°C and 58.6 ± 3% RH) for data collection.

The application of the plant extract was performed as follows: initially, a bean leaf disc k was immersed in solutions of the ethanoic extracts, exposed to a gentle stream of air to remove excess moisture and then placed in the Petri dish. Finally, a direct application of the extract was made on *T. urticae* females present in the experimental unit.

Direct application of the extract was performed on individuals with an airbrush at a height of 20 cm from the experimental unit, with 96 droplets/cm^2 and a pressure of 20 to 30 PSI. Mortality and fecundity of *T. urticae* at 24, 48, and 72 h were recorded with the help of a stereoscope. Thus, the mortality of *T. urticae* individuals was confirmed by a smooth movement not greater than the length of insect body after soft contact with a fine haired brush (Ponte Teles et al., 2011).

Data analysis

Using the obtained data, daily corrected mortality was calculated for each of the three days of the trial, in three replicates conducted over time, using Abbott's formula (Abbott, 1925):

$$\text{Corrected mortality (\%)} = \frac{(\text{\% Mortality in the treatment} - \text{\% Control mortality}) \times 100}{100 - \text{\% Control mortality}}$$

Fertility data *per capita* at 24, 48, and 72 h and the corrected mortality of *T. urticae* females at 72 h were transformed by the function $y = arsin\sqrt{p}$, where "*p*" is the value of the mortality ratio and "*y*" is the transformed value. Analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to assess significant differences between the treatments. Analyses were performed in the statistical language R version 3.1.2 (R Core Team, 2013). Logistic regression models were fitted using the generalized linear model technique assuming a binomial distribution and using the logit link function to determine the median lethal dose (LC_{50}) at different times of evaluation. The analyses were performed in the statistical language R, version 3.1.2 (Grainge and Ahmesds, 1988).

RESULTS AND DISCUSSION

Acaricidal effect of ethanol extract of leaves of *S. nigrum* on females of *T. urticae*

The mortality that occurred in females of *T. urticae* in the absolute control (without application) was 0, 0, and 4.5% at 24, 48, and 72 h, respectively, before correcting the mortality using Abbott's formula.

The extract of *S. nigrum* leaves generated mortality in the adults of *T. urticae* at concentrations of 600 and 1000 µg/mL at 72 h. Concentrations of 1, 5, 10, and 50 µg/mL induced low mortality rates (below 25%) (Table 2). Statistically, significant differences ($p = 2.2 \times 10^{-16}$) were obtained between the corrected mortalities (Table 2), which showed that mortality effects at concentrations of 600 and 1000 µg/ml did not differ from those of the positive control. Therefore, the ethanol extract in the two highest concentrations could be a promising natural miticide.

According to the results presented in Table 2, the highest mortality occurred at 72 h for the highest concentrations of extract applied (600 and 1000 µg/mL). This outcome agrees with the results of some authors who mention that higher concentrations of plant extracts increased the mortality of adult insects and arthropods, such as *Tetranychus cinnabarinus* and *T. urticae* (Alves et al., 2002; Castagnoli et al., 2005; Roobakkumar et al., 2010; Sivira et al., 2011).

Table 3 shows that the median lethal dose decreases with an increase in the exposure time of adults of *T. urticae* to the ethanol extract, because a residual effect becomes more effective when treatment is presented, whereas a lower LC_{50} value indicates a better mortality generating ability. This is consistent with the results

Table 2. Corrected mortality rates of *T. urticae* females 72 h after applying ethanolic extract of *S. nigrum* leaves under laboratory conditions (mean ± standard error) (23.6 ± 0.2°C and 60 ± 2% RH).

Treatment	Dose (µg/mL)	Corrected mortality[*,b]
Positive Control[a]	-	97.50±1.12[a]
S. nigrum [1]	1	5.83±2.01[d]
S. nigrum [5]	5	14.17±2.71[cd]
S. nigrum [10]	10	15.83±3.52[cd]
S. nigrum [50]	50	23.33±4.41[c]
S. nigrum [100]	100	47.67±5.58[b]
S. nigrum [600]	600	85.00±4.28[a]
S. nigrum [1000]	1000	95.00±1.83[a]

*Values obtained after 72 h of exposure. [a]Distilled water + commercial acaricide (active ingredient Chlorfenapyr 24%). Applied dose: 0.4 cm^3/mL. [b]Treatments followed by the same letter showed no statistically significant difference according to the Tukey test.

Table 3. LC_{50} values for ethanol extract of leaves of *S. nigrum* on adult females of *T. urticae* under laboratory conditions (23.6 ± 0.2°C and 60 ± 2% RH).

Hours after application	LC_{50} (µg/mL) (Confidence interval, 95%)	
	LC_{50} ± standard error	Lower and upper limit of CI at 95%
24	450.57±26.43	(398.76-502.38)
48	407.47±25.17	(358.13-456.80)
72	279.69±20.59	(239.34-320.04)

CI: Confidence Interval.

Table 4. Activity of ethanol extract of *S. nigrum* leaves on the fertility of adult *T. urticae* under laboratory conditions (23.6 ± 0.2°C and 60 ± 2% RH) over three days.

Concentration (µg/mL)	Per capita fecundity (eggs / day / female)[a]		
	24 h	48 h	72 h
Positive Control[b]	0.00±0.00	0.00±0.00	0.10±0.00
0	0.71±0.08[a]	0.76±0.10[a]	1.02±0.05[a]
1	0.51±0.08[ab]	0.52±0.09[ab]	0.74±0.07[b]
5	0.37±0.08[bc]	0.38± 0.10[bc]	0.59±0.08[bc]
10	0.40±0.07[abc]	0.42±0.05[bc]	0.36±0.06[cd]
50	0.24±0.07[bc]	0.26±0.10[bc]	0.37±0.03[cd]
100	0.18±0.04[c]	0.20±0.03[c]	0.21±0.05[d]
600	0.21±0.07[bc]	0.22±0.07[bc]	0.19±0.03[d]
1000	0.12±0.05[c]	0.12±0.05[c]	0.12±0.04[d]

[a]Treatments followed by the same letter are not significantly different at α = 0.05. [b]Distilled water + commercial acaricide (active ingredient Chlorfenapyr 24%). Applied dose: 0.4 cm^3/mL.

reported by Alves et al. (2002), Castagnoli et al. (2005), Hincapié et al. (2008), Roobakkumar et al. (2010), Sivira et al. (2011), Shi et al. (2008), and Teles et al. (2007), who found that increasing the exposure time of the product on the phytophage can generate toxic interference in biochemical and physiological functions, causing a decrease in the LC_{50} value.

Significant differences in fecundity (Table 4) were observed at the different evaluation times with the seven concentrations of the extract compared to those of the control (p = 3.92×10^{-06} at 24 h; p = 2.44×10^{-06} at 48 h; and p = 3.73×10^{14} at 72 h).

However, at 24 and 48 h, no significant difference was observed between the concentration 1 µg/mL and the

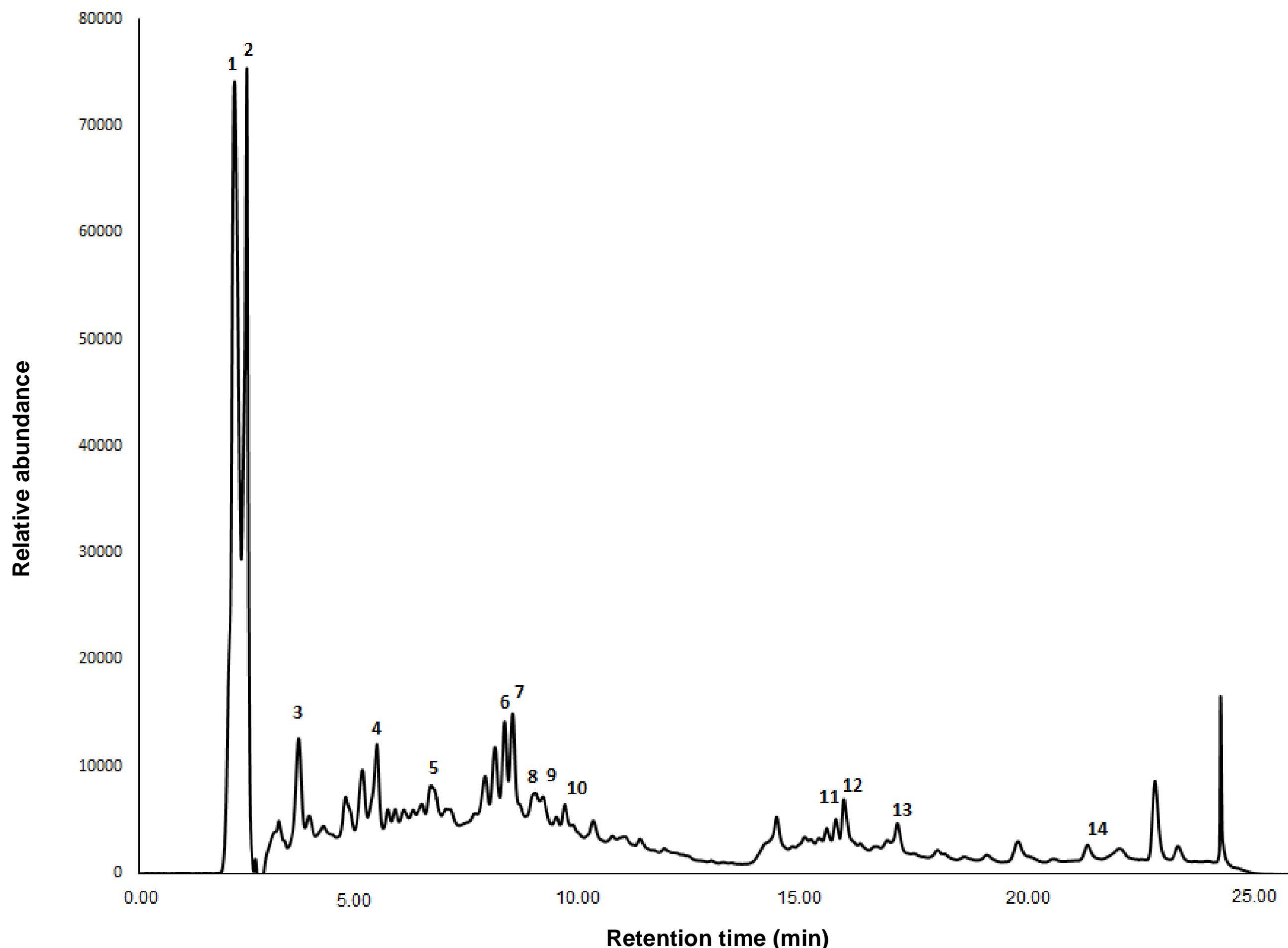

Figure 1. LC-MS-derived profile of the *Solanum nigrum* leaf-derived extract.

absolute control (0 µg/mL). At 72 h, there was no significant difference in reducing fertility between the concentrations of 100, 50, 10, 600, and 1000 µg/ml. Concentrations between 100 and 1000 g/ml generated fertility reductions that were 70% higher than that of the absolute control.

Table 4 shows that in general, there is a reduction in the number of eggs laid per female per day with an increase in the concentration of the ethanolic extract. This is consistent with the results reported by Sivira et al. (2011), who found that there was a reduction in the number of eggs laid per *T. cinnabarinus* female per day with an increase in the concentration of *Lippia origanoides* and *Gliricidia sepium* ethanolic extracts. These results could be considered potential concentrations for product formulation for the management of *T. urticae* females.

RP-HPLC-DAD-ESI-MS analysis on *S. nigrum* leaf extract

Fourteen compounds present in the ethanol extract of the leaves of *S. nigrum* were tentatively identified by the analysis of MS spectra of each component registered in the chromatogram (Figure 1). Table 5 shows that the main compounds were flavonoids (hispidulin-sulfate, dimethoxyflavone, and hydroxy-methoxy-methyl-formylflavanone) and alkaloids (Monatin, Ajmaline and Calligonine).

The chemical profile of the extract of leaves of *S. nigrum* showed four peaks corresponding to the major components related to flavonoids (Figure 1, retention times of 2.28, 8.27, 8.47, 15.23 and 16.86 min). Four additional peaks, corresponding to alkaloids (retention times of 2.59, 3.35, 5.33 and 6.53 min), were also evident.

Additionally, minor compounds were found, such as flavonoids (hydroxy-methoxy-methylflavone, desmethyleucalyptin, sideroxylin, artonin U and dimethoxyflavone) and alkaloids (leptomerine, hydroxysolasodine and solasodine) (Table 5).

The presence of flavonoids in *S. nigrum* leaf extract could explain the high mortality rate and the reduction in oviposition of female *T. urticae.* This is consistent with results reported by Abdel-Aziz et al. (2014) and El-

Table 5. Chemical composition of ethanol extract of *Solanum nigrum* leaves.

Retention time (min)	Name	Compound type	Molecular Formula	m/z $[M+H]^+$
2.28	Hispidulin sulfate	Flavonoid	$C_{16}H_{12}O_9S$	381.03
2.59	Monatin	Alkaloid	$C_{14}H_{16}N_2O_5$	293.11
3.35	Ajmaline	Alkaloid	$C_{20}H_{26}N_2O_2$	327.21
5.33	Calligonine	Alkaloid	$C_{12}H_{14}N_2$	187.12
6.53	Leptomerine	Alkaloid	$C_{13}H_{15}NO$	201.11
8.27	Dimethoxyflavone isomer	Flavonoid	$C_{17}H_{14}O_4$	283.10
8.49	Hydroxy-methoxy-methyl-formylflavanone	Flavonoid	$C_{18}H_{16}O_5$	313.11
8.73	Hydroxy-methoxy-methylflavone	Flavonoid	$C_{17}H_{14}O_4$	283.10
8.92	Desmethyleucalyptin	Flavonoid	$C_{18}H_{16}O_5$	313.11
9.97	Sideroxylin	Flavonoid	$C_{18}H_{16}O_5$	313.11
15.22	Artonin U	Flavonoid	$C_{20}H_{20}O_5$	353.14
15.23	Hydroxysolasodine	Alkaloid	$C_{27}H_{45}NO_3$	430.33
16.86	Solanidine	Alkaloid	$C_{27}H_{43}NO$	398.34
21.02	Solasodine	Alkaloid	$C_{27}H_{43}NO_2$	414.34

Gengaihi et al. (2011), who identified that the flavonoids act by inhibiting the production of eggs and by generating mortality in *T. urticae*.

The alkaloids found in the ethanol leaf extract (Table 5) may indicate that the extract has a greater effect as repellent, insecticide, and miticide. One well-documented example of repellent alkaloid compounds is the case of solasodines, which have repellency against flies and ants (Grainge and Ahmeds, 1988). In addition, it has been reported that the alkaloids generate repellency against larvae of *Spodoptera frugiperda* (Lizarazo et al., 2008).

Flavonoids found in this ethanol extract of leaves of *S. nigrum* could have a significant effect on the mortality of females of *T. urticae*, because the flavonoids present in the extracts of these plants are reported as acaricides and insecticides, generating significant mortality on pests as well as modifying the behavior of herbivores (Abut et al., 2014; Muthuvel et al., 2014; Chermenskaya et al., 2010; Moreira et al., 2007).

The metabolites identified in the ethanol extract of *S. nigrum* leaves (Table 5) can afford a preliminary indication of a possible acaricidal activity provided by flavonoids and alkaloids, indicating that this test plant material could be considered an effective method for controlling *T. urticae* adults. In addition to the important findings in this work about the activity of the *S. nigrum*-derived ethanol extract, it is necessary to perform the corresponding compound isolation to determine the effectiveness, by specific tests, of the metabolites that are responsible for the acaricide and repellent activities, with the aim of formulating and using these compounds to manage populations of *T. urticae* under commercial greenhouse conditions.

Although the ethanol extract of leaves of *S. nigrum* showed a high potential for the implementation of this strategy for the management of *T. urticae* females in commercial crops, the development of compatibility tests to know the effects of the extracts evaluated in this study on predatory mites, such as *Phytoseiulus persimilis* and *Neoseiulus californicus*, in terms of population decline, behavioral changes, and even the ability to settle in rose crops is recommended. These predators are usually released as biological control for *T. urticae*, as components of IPM and they have few negative effects on the environment. However, before implementing this in field conditions, it is necessary that laboratory experiments are performed to determine if there is interaction between chemicals rose plants that can reduce the effect of *S. nigrum* extract on *T. urticae* and which does not affect this interaction in the present work performed with bean plants.

Conclusion

The *S. nigrum* leaf-derived extract showed high potential as an effective strategy against *T. urticae* under laboratory conditions, since *S. nigrum* (600 and 1000 μg/mL) caused the highest mortality (over 85%) of *T. urticae*, with an LC_{50} value of 450.57 after 24 h. The test extracts also generated a reduction in oviposition. The main components in the *S. nigrum* leaf-derived extract (flavonoids and alkaloids) would explain the good activity. Incorporating *S. nigrum* extract into the IPM might lead to a reduction of the use of synthetic acaricides and improve the control levels. However, field application of *S. nigrum* and isolated main components need to be tested.

Conflict of interests

The authors have not declared any conflict of interests.

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