

Are vegetable adjuvants increasing the efficiency of the aqueous extract of *Sarcomphalus joazeiro* on *Tetranychus ludeni*?

Josias Jordão Andrade ALVES, Cláudia Helena Cysneiros MATOS, Carlos Romero Ferreira de OLIVEIRA, Vanessa Luana da Conceição PEREIRA, Cinara Wanderléa Félix BEZERRA, Kelem Silva FONSECA
Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, Pernambuco, Brazil

Abstract

This study aimed to evaluate the effects of using coconut, soy, canola, and sunflower vegetable oils as adjuvants on the efficiency of the aqueous extract of *Sarcomphalus joazeiro* (Mart.) Hauenschild in the management of the mite, *Tetranychus ludeni* Zacher. The lethal concentrations (LC₅₀ and LC₉₀) of the extract with the adjuvants (E + A) were tested for toxicity, residual effect, oviposition deterrence, and egg viability in *T. ludeni* females. The curative and preventive effects of E + A were evaluated. Soluble phenols in the extract were identified and quantified using UHPLC+. All tested adjuvants were observed to increase the efficiency of the *S. joazeiro* extracts against *T. ludeni*. LC₅₀ was found to be more efficient in the curative test when sunflower and coconut oils were used, whereas LC₉₀ caused 100% mortality of the mites in both tests. Adjuvants increased the residual effect of the extract and reduced the viability of *T. ludeni* eggs. The lowest number of hatched eggs was observed for the LC₅₀ of extracts with either canola and sunflower oil and for the LC₉₀ of the extract with soybean oil. In the curative test for LC₅₀, the greatest reduction in oviposition was seen in treatments with sunflower, canola, and soybean oils; meanwhile, in the preventive test, deterrence was greater in treatments with sunflower oil compared to that seen with other oils. Gallic acid, a phenolic compound, was quantified to be 6.68 mg/mL at LC₅₀ and 6.77 mg/mL at LC₉₀, whereas the total amount of soluble phenols was 36.84 and 38.82 mg gallic acid equivalents 100 g⁻¹ DW at LC₅₀ and LC₉₀, respectively. Adjuvant vegetable oils increase the efficiency of the aqueous extract of *S. joazeiro*; thus, their use is a viable alternative for the management of *T. ludeni*.

Key words: botanical acaricide, Tetranychidae, spreader-stickers.

Introduction

The spider mite *Tetranychus ludeni* Zacher (Acari Tetranychidae) is a pest commonly associated with cotton (*Gossypium hirsutum*) (Moraes and Flechtmann, 2008). The control of *T. ludeni* is achieved using synthetic acaricides in the semiarid region of northeastern Brazil (Reddy, 2001; Dimetry *et al.*, 2009; Lucini *et al.*, 2010; Ferraz *et al.*, 2017).

Despite the short-term efficiency of synthetic acaricides, the recurrent and often indiscriminate use of these products has led to the development of resistance in mite populations, thus reducing their control efficiency (Escudero and Ferragut, 2005; Singh, 2010; Venzon *et al.*, 2010; Rogers and Dewdney, 2017; PPDB, 2018). Furthermore, synthetic acaricides may cause environmental pollution, leave residue, and have low selectivity, which can lead to the elimination of natural pest enemies and, consequently, ecological imbalance in agroecosystems (Barrêto *et al.*, 2010; Roubos *et al.*, 2014; Bueno *et al.*, 2018). Additionally, these products can be toxic to other organisms, such as aquatic animals, birds, mammals, and humans (Nicolopoulou-stamati *et al.*, 2016).

The problems arising from the use of pesticides and the search for pesticide-free food has resulted in the need for sustainable agricultural systems that ensure environmental and human safety (Michereff *et al.*, 2012; Dara, 2019; Saad *et al.*, 2021).

Plant materials (leaves, stems, bark, and roots) have been studied to identify alternative products to synthetic chemical control. In this context, the use of natural products (oils and extracts) has drawn attention from researchers as a promising alternative (Schwan-Estrada *et*

al., 2008; Bettiol and Morandi, 2009; Tripathi *et al.*, 2009; Farouk and Osman, 2011; 2012; Marangoni *et al.*, 2013; Santos *et al.*, 2013).

The use of plant extracts to control pest mites has been shown to be efficient in some studies (Hincapié *et al.*, 2008; Kumral *et al.*, 2010; Xavier *et al.*, 2015; Ferraz *et al.*, 2017; Nascimento *et al.*, 2018; Seifi *et al.*, 2018). These substances can act in different ways: causing increased mortality; repellence; inhibition of feeding, reduction of oviposition, fecundity, and fertility of females; and sterility in adults (Dequech *et al.*, 2008).

Ferraz *et al.* (2017) reported that *Sarcomphalus joazeiro* (Mart.) Hauenschild (Basionym: *Ziziphus joazeiro* Mart.) extract reduced the population of *T. ludeni* to acceptable levels without causing phytotoxicity to the crop. However, plant extracts usually exhibit rapid degradation and low residual effect (Khater, 2012; Senthil-Nathan, 2013). In this context, the present study evaluated whether the addition of adjuvant vegetable oils to *S. joazeiro* extract increased its efficiency in controlling *T. ludeni*.

S. joazeiro is a common plant in the Brazilian semiarid region, and its native distribution also includes Bolivia, northeastern Argentina, Honduras, and Paraguay. It is a shrub or tree that grows well in seasonally dry tropical biomes (Sutherland, 2008; Zuloaga *et al.*, 2008; Jørgensen *et al.*, 2013; Lima *et al.*, 2020).

Adjuvants are commonly added to insecticide/acaricide and herbicide solutions to improve their activity. In addition to increasing the effectiveness, retention time, and residual effect of plant extracts, adjuvants may also reduce surface tension and drop spreading (Scherhag *et al.*, 2005; Gauvrit *et al.*, 2007; Xu *et al.*, 2010; Cunha *et al.*,

2016); and can reduce product volatilization (Houbraken *et al.*, 2015) while having little or no phytotoxicity to plants (Chuah *et al.*, 2013).

Adjuvants comprise a wide variety of compounds that increase the efficiency of products. They are characterized as wetting, adhesive, surfactant, and evaporation-reducing agents (Curran *et al.*, 1999). Most adjuvants found on the market are synthetic and have low economic viability.

Farmers use oils as adjuvants to increase the concentration of the oil phase in spray solutions as the oils help prevent evaporation of the sprays, thus providing the product with more time to penetrate the leaves (Tu and Randall, 2003). Some oils also modify the cuticular waxes of leaves, increasing the penetration of the product into the plant (Tu and Randall, 2003). However, there is relatively little published information on the use of adjuvant vegetable oils associated with plant extracts as an alternative for pest control (Jha *et al.*, 2010; Chuah *et al.*, 2013; Wang *et al.*, 2018).

The following hypotheses were tested: 1) adjuvant vegetable oils increase the efficiency of *S. joazeiro* aqueous extract in controlling *T. ludeni*; 2) adjuvant vegetable oils prolong the residual effect of *S. joazeiro* aqueous extract and reduce the reproductive capacity of *T. ludeni*.

Materials and methods

Mites

T. ludeni individuals were obtained from cultures established for more than two years on jack bean leaves (*Canavalia ensiformis*) in laboratory under controlled conditions (27 ± 2 °C, $70 \pm 10\%$ relative humidity, and a photoperiod of 12 hours).

Extract preparation

Leaves of *S. joazeiro* were collected in the early morning from Caatinga, Pernambuco, Brazil. The plants were in a vegetative state when sampled. Once in the laboratory, the plant material was disinfected with active chlorine (0.05%) for 20 minutes and then washed with distilled water (Vieira *et al.*, 2006). Subsequently, they were left to dry at room temperature (~ 27 °C) for 2 hours before being packed in Kraft paper bags for oven-drying under forced air circulation at 50 °C for 48 hours (Vieira *et al.*, 2006). After drying, the leaves were ground with a crusher and blended to obtain the dry extract.

Estimated lethal concentrations of *S. joazeiro* extract for *T. ludeni* (Ferraz *et al.*, 2017) were used, with $LC_{50} = 3.54\%$ and $LC_{90} = 8.81\%$, which were the concentrations required to kill 50% and 90% of the insect population, respectively. These concentrations were obtained from a standardized stock solution prepared using 500 mL of water and 100 g of dry extract (Santos, 2018). The solution was stored in a hermetically sealed glass covered with aluminium foil and placed in a refrigerator at 8 ± 2 °C for 24 hours.

For LC_{50} and LC_{90} of the *S. joazeiro* extract, 1.77 and 4.41 mL of the stock solution were used, respectively, and the final volume was made up to 100 mL with distilled water.

Treatments used and experimental conditions

The treatments used in the present study were based on previous results obtained by Ferraz *et al.* (2017) who tested the aqueous extract of *S. joazeiro* (AESJ) on *T. ludeni* using distilled water as a control and confirmed the acaricidal effect on this mite.

To increase the acaricidal effect, fixation, and persistence of the extract for use in the management of *T. ludeni*, adjuvant vegetable oils were selected for evaluation in this study. AESJ was used as the control because the intention was to determine whether the adjuvant oils would enhance the effect of the aqueous extract.

Thus, we compared the treatments using adjuvant vegetable oils + aqueous extract with the control as described below.

T1 = LC_{50} or LC_{90} of the AESJ (control).

T2 = LC_{50} or LC_{90} of the AESJ + 1.5% coconut oil.

T3 = LC_{50} or LC_{90} of the AESJ + 1.5% soybean oil.

T4 = LC_{50} or LC_{90} of the AESJ + 1.5% canola oil.

T5 = LC_{50} or LC_{90} of the AESJ + 1.5% sunflower oil.

The isolated actions of the oils were evaluated in preliminary laboratory tests and showed no significant effects on this mite. The adjuvant percentage of 1.5% was chosen based on the studies by Ferreira *et al.* (2010) and Mendonça *et al.* (2007).

All experiments were performed in a completely randomized design with five treatments (LC_{50} or LC_{90} of the extract + adjuvant oils and control) and 10 replicates, totalling 50 experimental units for each lethal concentration. The experimental units were maintained in Biochemical oxygen demand (BOD) chambers (27 ± 2 °C, $70 \pm 10\%$ relative humidity, and a photoperiod of 12 hours) to standardize the environmental variables during the experiments.

Tests

Toxicity against *T. ludeni* adult females

Cotton leaf disks (3 cm \varnothing) were transferred individually to petri dishes (5 cm \varnothing) containing foam, which were covered with paper filter and surrounded by absorbent cotton moistened with distilled water. Treatments were applied using a manual sprayer that sprayed 2 mL (approximately 0.28 mL cm^{-2}) (Ferraz *et al.*, 2017) of the extract with or without the adjuvants, for each estimated lethal concentration for *T. ludeni* (LC_{50} and LC_{90}). For the curative tests, 10 adult *T. ludeni* females were placed in each arena, and the extract was subsequently sprayed according to the established treatments. For the preventive tests, the arenas were sprayed with the treatments and 10 female mites were released into the arenas after 30 minutes. For both the tests, the mortality of the mites was evaluated after 48 hours.

Egg viability and oviposition deterrence in adult females of *T. ludeni*

Egg viability was evaluated in cotton leaf disk arenas similar to those used in the toxicity tests.

To evaluate the ovicidal effect, 10 *T. ludeni* eggs (age: 48 hours) were placed in the arenas, and the LC_{50} (3.54%) or LC_{90} (8.81%) of the AESJ + adjuvants were sprayed. The number of larvae that emerged in each treatment was

observed for four days (Erdogan *et al.*, 2012).

In the oviposition deterrence test, the experimental arenas and the method of application of treatments (curative and preventive) were similar to those used in other tests. Ten adult females of *T. ludeni* were used per arena in each test. The degree of deterrence was evaluated by the number of eggs present after 48 hours in each treatment (Roobakkumar *et al.*, 2010).

Residual effect of the extract

Cotton plants (40 days old) were sprayed with the pre-established treatments. To ensure uniformity in the application of the treatments, hydrosensitive paper cards (26 × 76 mm) were hung in different positions between the plant leaves before spraying, enabling the evaluation of the distribution of the drops applied in each treatment (Debortoli *et al.*, 2012). After application of the extract + adjuvant, leaf samples were collected at different time intervals (3 hours; 1, 2, and 4 days) (Filho *et al.*, 2015). In the laboratory, leaf disks (3 cm \varnothing) for each treatment were placed individually in petri dishes and 10 adult females of *T. ludeni* were placed on each disk (Matos, 2006; Ferraz, 2011). The mortality was evaluated after 48 hours, and the mites that did not move vigorously after a light touch with a fine brush were considered dead (Abbott, 1925).

Chemical composition of the extract of *S. joazeiro*

Phenolic compounds were identified via Ultra High Performance Liquid Chromatography (UHPLC).

LC₅₀ and LC₉₀ samples were injected into the UHPLC+ *Thermo Scientific Ultimate* to identify and quantify soluble phenols in the AESJ. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 μ m), and the control was implemented using the Chromeleon Chromatography Management System software. We used the isocratic method that comprises a mobile phase of acidified water, 2% acetic acid (phase A), and pure methanol (phase B); flow rate: 1 mL/min; wavelength λ = 270 nm; injection volume: 20 μ L; and run

time: 10 minutes. Gallic acid at concentrations 0, 0.05, 0.10, 0.15, 0.20, and 0.25 mg/ml was used to determine the standard curve.

Total phenolic compounds

Total phenolic compounds were quantified according to the methodology adapted by Reyes *et al.* (2007). The stock solution of the extract was left to rest for 24 hours in the dark at 4 °C. Next, 11 mL of LC₅₀ and LC₉₀ samples were centrifuged at 9,000 × g at 2 °C for 23 minutes. Subsequently, 150 μ L of the AESJ, 150 μ L of the Folin Ciocalteu reagent (0.25 N), and 2,400 μ L of distilled water were pipetted into a Falcon tube. The mixture was homogenized using a tube shaker for 3 minutes. Subsequently, 300 μ L of sodium carbonate (1 M) was pipetted and the solution was kept in the dark for 2 hours. The blank solution was obtained by replacing the supernatant with 150 μ L methanol. Finally, absorbance was recorded using a spectrophotometer (Libra S8, Biochrom Cambridge, England) at 725 nm.

Statistical analysis

For all the tests, data normality and homoscedasticity were assessed using the Shapiro-Wilk test and Levene test, respectively. The data were then subjected to an analysis of variance. Means were compared using Tukey's test at 5% probability level using SAS Studio 9.4 (SAS Institute, 2002).

Results

The acaricide effect of *S. joazeiro* extract on *T. ludeni* increased with the addition of adjuvant vegetable oils (figure 1). In preventive tests, the highest average mortality rates of mites were observed for treatments with sunflower, soybean, and canola oil (80, 74, and 71% respectively) at LC₅₀. Significant differences were found between all extract + adjuvant treatments and the extract alone (figure 1A).

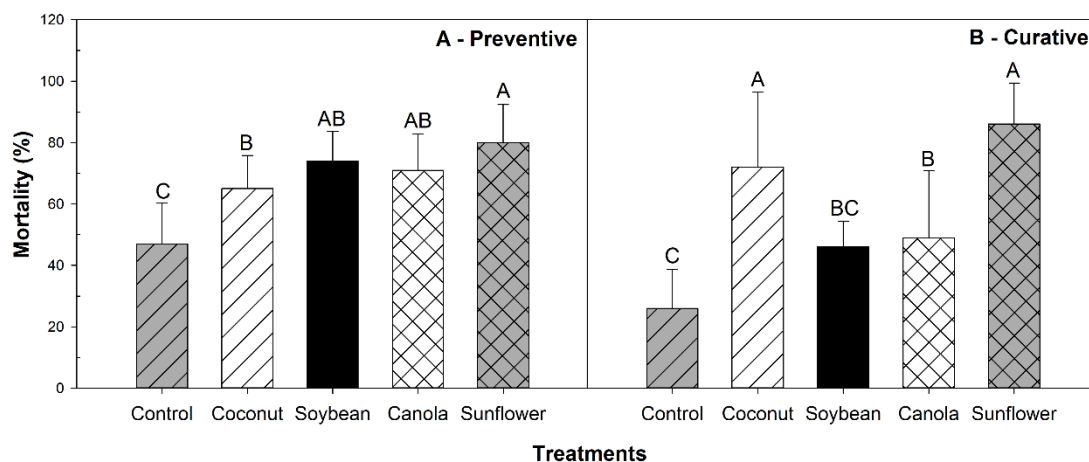


Figure 1. Average mortality (%) of *T. ludeni* adults subjected to different treatments with LC₅₀ of the extract of *S. joazeiro* associated with adjuvant vegetable oils in a 48 hours period: (A) preventive test (F = 11.58, p < 0.0001) and (B) curative test (F = 18.55, p < 0.0001). Data are presented as mean + SE. Bars followed by the same capital letter do not differ significantly, as determined by Tukey's test (p < 0.05).

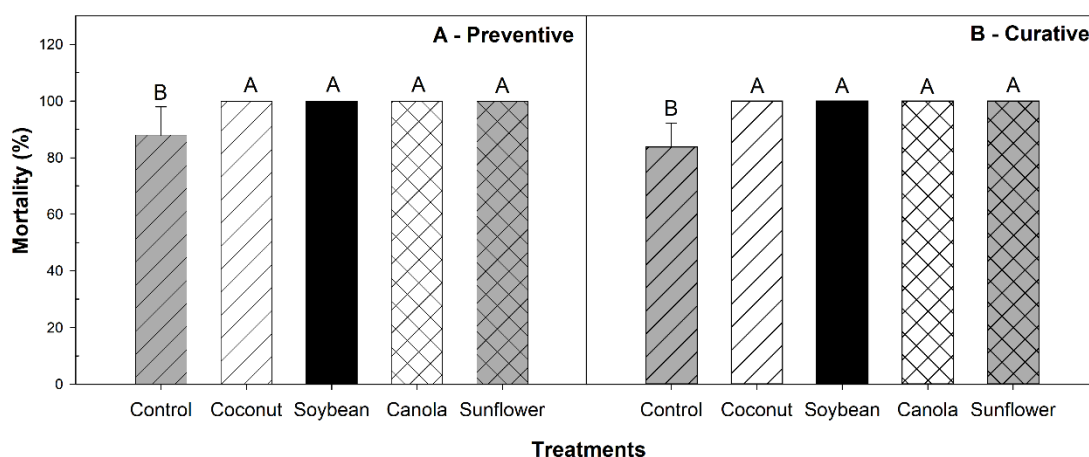


Figure 2. Average mortality (%) of *T. ludeni* adults subjected to different treatments with LC₉₀ of the extract of *S. joazeiro* associated with adjuvant vegetable oils in a 48 hours period: (A) preventive test ($F = 11.17$, $p < 0.0001$) and (B) curative test ($F = 32.21$, $p < 0.0001$). Data are presented as mean + SE. Bars followed by the same capital letter do not differ significantly, as determined by Tukey's test ($p < 0.05$).

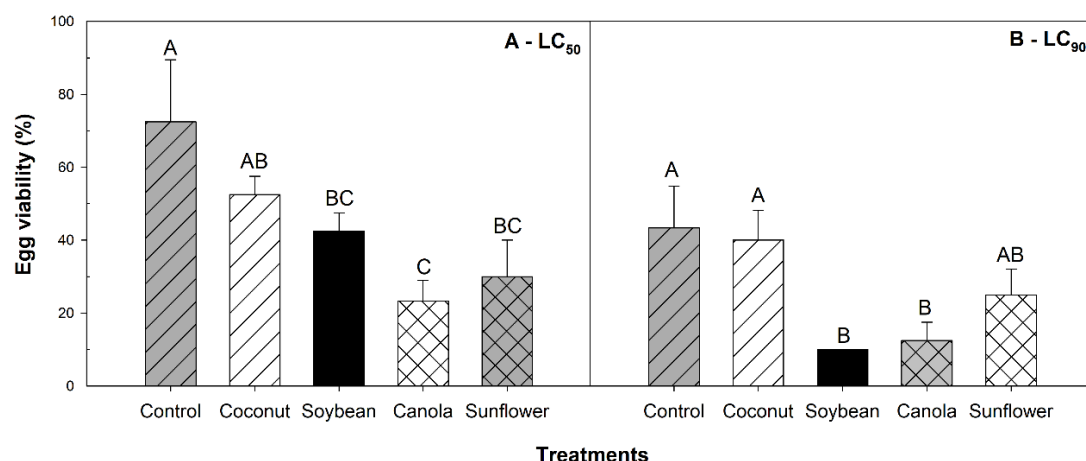


Figure 3. Viability of *T. ludeni* eggs subjected to LC₅₀ (A) ($F = 13.43$, $p < 0.0001$) and LC₉₀ (B) ($F = 14.80$, $p < 0.0001$) of the AESJ associated with adjuvant vegetable in a 96 hours period. Data are presented as mean + SE. Different uppercase letters on the bars indicate a significant difference between treatments, as determined by Tukey's test ($p < 0.05$).

The curative test for LC₅₀, showed significantly higher acaricide activity in the treatments with sunflower oil (86%) and coconut oil (72%) (figure 1B). The association of the extract with sunflower oil caused a 60% increase in mortality compared to that seen with the extract alone in the curative test (figure 1B).

LC₉₀ caused 100% mortality of mites in all treatments, both in the curative and preventive tests, differing significantly from the control (figure 2).

Adjuvant oils associated with the extract reduced the viability of *T. ludeni* eggs compared to the control. For LC₅₀, the lowest number of hatched eggs was observed in treatments with canola, sunflower, and soybean oil (23, 30, and 42 eggs, respectively). Treatments with coconut oil did not differ significantly from treatments with soybean and sunflower oils (figure 3A). A similar behaviour was observed in LC₉₀, with a higher reduction upon association with soybean, canola, and sunflower oils (10,

12, and 25 eggs, respectively) and coconut oil (40 eggs), thereby not differing significantly from sunflower oil (figure 3B).

Moreover, adjuvants significantly reduced *T. ludeni* oviposition compared to the control (figure 4). In the curative test using LC₅₀, sunflower, canola, and soybean oils obtained the best results, with a maximum of six eggs laid by the mite; meanwhile, this number exceeded 40 eggs in the control. The effect of coconut oil + AESJ was similar to that of soy + AESJ (figure 4A). In the preventive test, the lowest oviposition rates were observed in the treatments with sunflower, soy, and coconut oil (4.20; 6 and 7.66 eggs, respectively), whereas canola oil (14.8 eggs) showed a result statistically similar to soy and coconut oils (figure 4B). The oviposition deterrence test was not performed for LC₉₀, because the toxicity test showed that the association in this concentration causes 100% mortality of the mites.

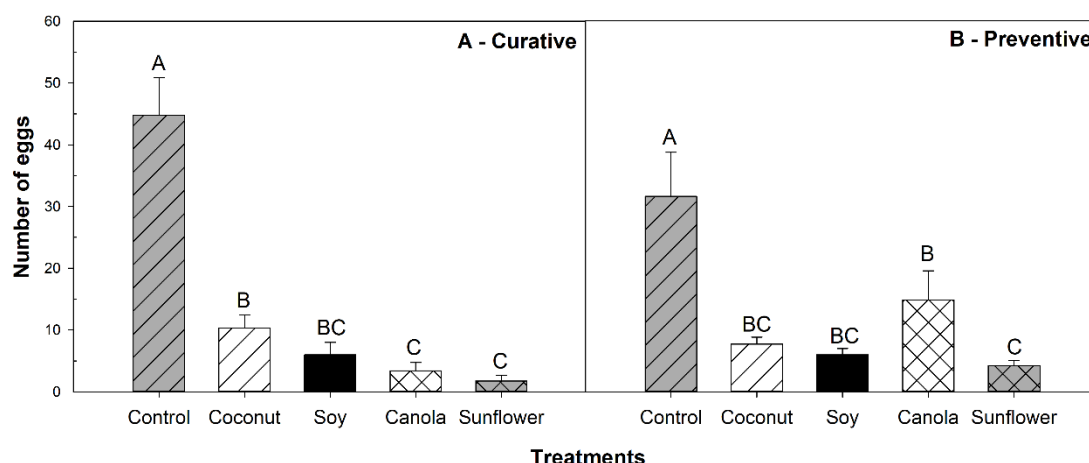


Figure 4. Oviposition deterrence of *T. ludeni* in curative (A) ($F = 162.35$, $p < 0.0001$) and preventive (B) ($F = 30.90$, $p < 0.0001$) tests with LC_{50} of the extract of *S. joazeiro* associated with adjuvant vegetable oils in a 96 hours period. Data are presented as mean + SE. Different uppercase letters on the bars indicate a significant difference between treatments, as determined by Tukey's test ($p < 0.05$).

Table 1. Residual effect of LC_{50} and LC_{90} of the AESJ associated with adjuvant vegetable oils on *T. ludeni* under laboratory conditions (27 ± 2 °C, $70 \pm 5\%$ relative humidity, and a photoperiod of 12 hours). LC_{50} $F = 27.69$, $p < 0.0001$; LC_{90} $F = 28.67$, $p < 0.0001$.

Treatments	Mortality (%) × time after application			
	3 hours	24 hours	48 hours	96 hours
Control	18 Ca	10 Ca	0 Db	0 Ab
LC_{50} + Coconut	80 Aa	32 Bb	16 Bbc	4 Ac
LC_{50} + Soy	54 Ba	30 Bb	6 Cc	2 Ad
LC_{50} + Canola	72 Aa	48 Aa	14 Bbc	8 Ac
LC_{50} + Sunflower	84 Aa	46 ABb	34 Ab	8 Ac
Control	32 Ca	14 Cb	6 BCcd	2 Cd
LC_{90} + Coconut	66 Aba	48 ABab	42 Ab	12 Bc
LC_{90} + Soy	62 Ba	34 BCb	22 Bb	8 Bc
LC_{90} + Canola	92 Aa	52 Ab	36 Abc	22 Ac
LC_{90} + Sunflower	86 Aa	74 Aa	26 Bb	6 Bc

Means followed by uppercase letters in the column and lowercase letters in the rows do not differ statistically, as determined using Tukey's test at 5% probability.

The adjuvant vegetable oils increased the residual effect of the extract (table 1). By analysing these findings with the results obtained in the LC_{50} , the treatments with sunflower, coconut, and canola oil were found to be the most effective, promoting mortality rates of 84, 80, and 72%, respectively, after 3 hours of application (table 1). Canola, sunflower, and coconut oils caused significantly greater residual effects in the first 24 hours after application (table 1). After 48 hours, the highest mortality rate was observed with sunflower oil (34%), indicating that the compounds present in this oil allowed a greater fixation of the product in the plant, thereby negatively affecting mite survival even after this period. Moreover, the association with adjuvants leads to higher mortality rates than the extract alone, for all periods studied, except for 96 hours (table 1).

In the LC_{90} of the extract, the association with canola, sunflower, and coconut oils maintained the mortality of *T. ludeni* above 60% after 3 hours of application. Even after 24 hours, the efficiency was higher than that

observed when the extract was used alone (above 47%). After 48 hours, coconut (42%) and canola (36%) oils were more efficient than the other treatments, whereas after 96 hours, the association with canola oil was more efficient than the others (table 1).

Several soluble phenols were identified via UHPLC+ in the AESJ, including gallic acid. Gallic acid was detected at concentrations of 6.68 mg/mL and 6.77 mg/mL for LC_{50} and LC_{90} , respectively. Absorbance values were at 229,025 mAU (figure 5) and 632.94 mAU (figure 6), respectively, occurring within 3.34 minutes.

The total amount of soluble phenols was obtained using the standard curve and the results were expressed in mg gallic acid equivalents 100 g^{-1} DW. The gallic acid calibration equation obtained a correlation coefficient $R^2 = 0.9964$.

LC_{90} showed a higher amount of soluble phenols (38.82 mg gallic acid equivalents 100 g^{-1} DW) than LC_{50} (36.84 mg gallic acid equivalents 100 g^{-1} DW) (figure 7). This significant difference was because of their different dilutions (3.51% and 8.81% for LC_{90} and LC_{50} , respectively).

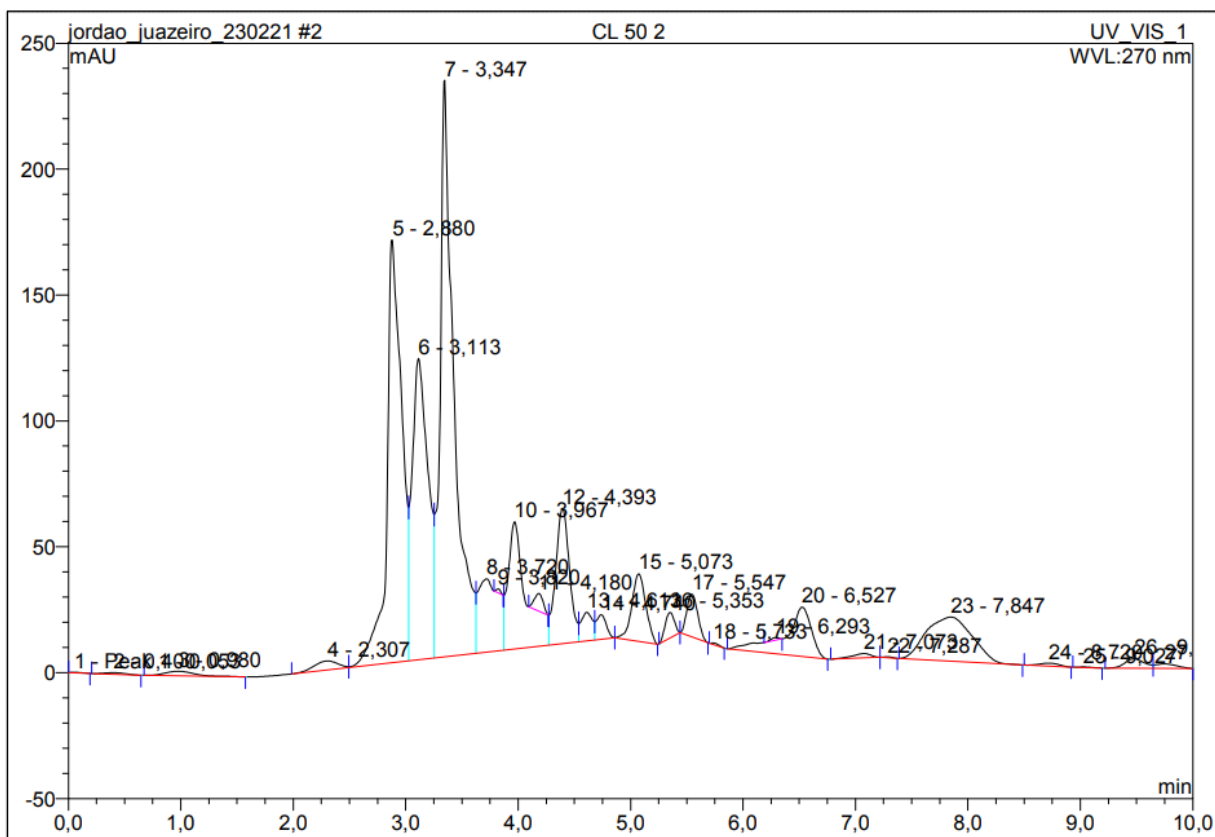


Figure 5. Chromatographic retention profile of LC₅₀ fractions and compounds of the AESJ.

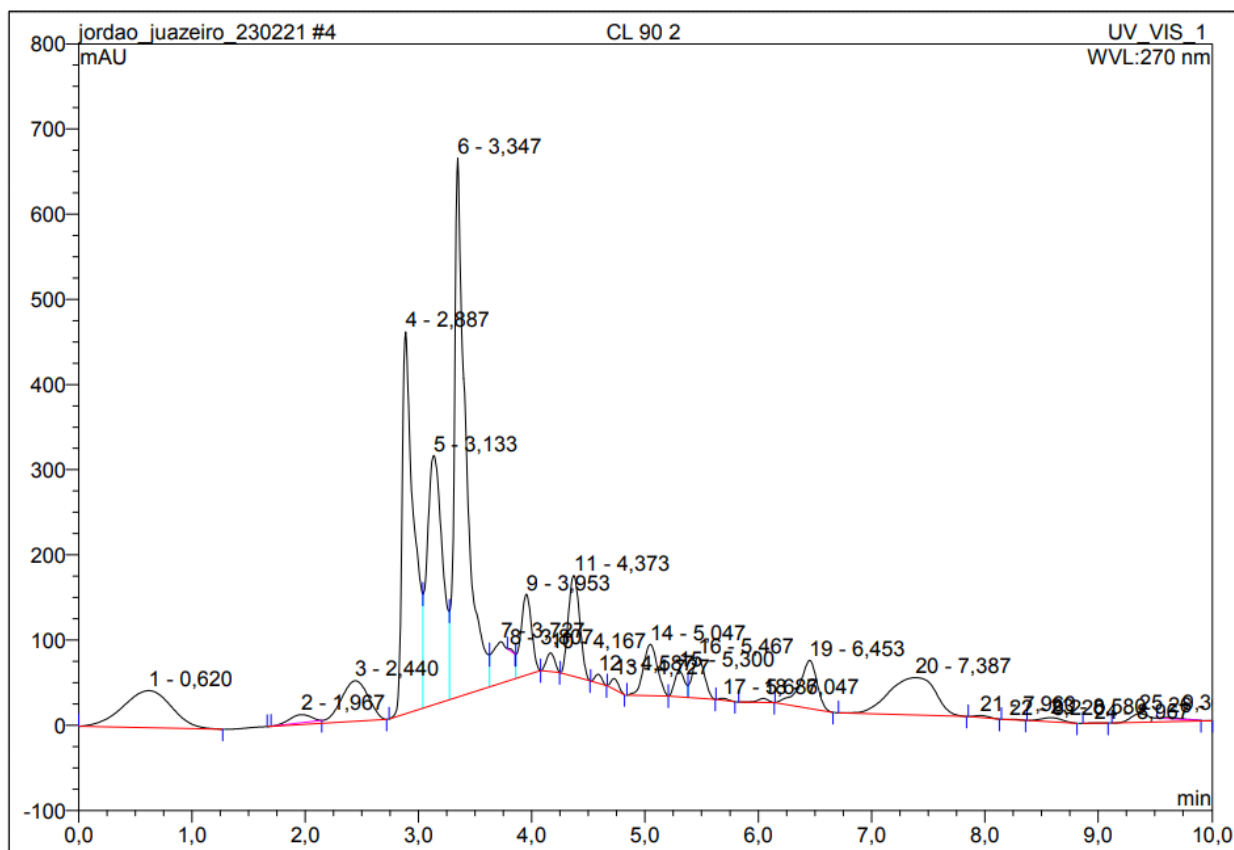


Figure 6. Chromatographic retention profile of LC₉₀ fractions and compounds of the AESJ.

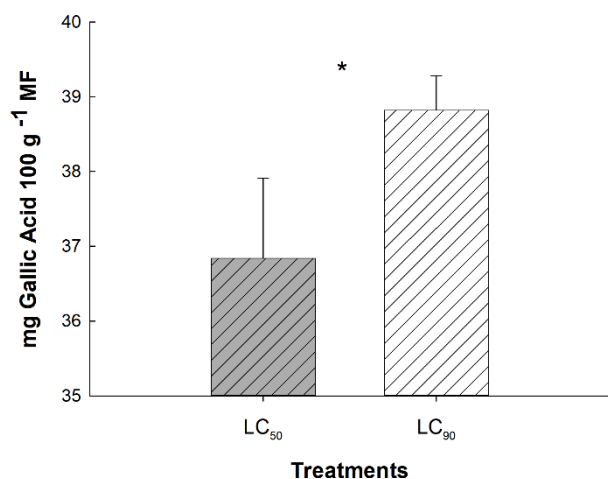


Figure 7. Absorbance determined using the Folin Ciocalteu spectrophotometric analysis at 725 nm in mg gallic acid equivalents 100 g⁻¹ DW of the AESJ for LC₅₀ and LC₉₀. The asterisk * indicates a significant difference between treatments, as determined by analysis of variance ($p < 0.05$).

Discussion

The results obtained in this study clearly demonstrate the increase in the efficiency of the AESJ after the addition of the adjuvant oils. The extract concentrations used (LC₅₀ and LC₉₀) and the mode of application (curative and preventive) were also decisive in the variation of the toxicity on *T. ludeni*. This becomes more evident when analysing the toxicity of LC₉₀ of the *S. joazeiro* extract associated with adjuvants, which allowed for a 20% increase in action of the extract, causing mortality of 100% of the mites. In the preventive test, adjuvant oils may have increased fixation of the extract to the plant, resulting in increased ingestion by the mite as they fed on the leaf, and thereby potentiating toxicity by ingestion. In the curative test, adjuvants may have acted directly on the mites' cuticle, enabling greater penetration of the extract and consequently potentiating toxicity by contact.

The efficiency of a product is considered satisfactory when it has a mortality rate above 60% and excellent when it exceeds 80% (Potenza *et al.*, 2006). Therefore, the efficiency of LC₉₀ of the *S. joazeiro* extract associated with adjuvants observed in the present study was satisfactory in all treatments, both preventively and curatively. LC₅₀, in contrast, showed satisfactory efficiency for all treatments in the preventive test; and in the curative test, only the association with coconut and sunflower oils resulted in a mortality rate of above 60%.

Some authors have already reported the acaricidal effect of the AESJ used alone on Tetranychidae mites. Siqueira *et al.* (2014) found a repellent and toxic effect on the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari Tetranychidae). Ferraz *et al.* (2017) evaluated its toxicity to the spider mite, *T. ludeni*; the extract was classified as a repellent as it exerted an effect on female mites even 120 hours after spraying, while not having a phytotoxic effect on cotton plants.

The main advantage of using natural substances, such

as plant extracts, is that pests have not yet been able to inactivate these generation of compounds, in addition to being easily degraded in the environment, having lower toxicity to humans, and a broader mode of action (Santos *et al.*, 2013).

Studies on the use of adjuvant oils are usually conducted with mineral oils associated with synthetic products. We found only one report that used a mineral oil associated with an insecticide for the control of a mite, *Panonychus ulmi* (Koch) (Acari Tetranychidae) (Mineiro *et al.*, 2015), reinforcing the novelty of the present study.

Robinson and Nelson (1975) highlighted the importance of evaluating the potential of vegetable oils as adjuvants and an alternative for mineral oils. The authors discussed the viability of these oils as they are extracted from oilseed crops, which are a renewable resource and produced annually, in contrast to mineral oils, which are sourced from non-renewable fossil reserves. Furthermore, they can be more or equally effective as mineral oils, depending on the product to which they are associated, the agricultural crop, and the pests for which they are used (Peterson, 1988).

Spreader-stickers used as adjuvants in the application of insecticides are non-ionic surfactants. The high potential of their association is because of the formation of a protective barrier that reduces losses due to volatilization, photodegradation, hydrolysis, drift, and rainwater washing of the leaves (Somerville *et al.*, 2011; Oliveira *et al.*, 2013). Moreover, the oils degrade part of the leaf cuticle (cutin) and allow the entry of the extract directly into the plant (Hull, 1970), as they have non-polar properties (similar to waxes and lipids that make up the leaf cuticle) that promote better penetration of applied products (Gunstone, 2011). In this sense, thin oil layers resulting from the adjuvants were observed on cotton leaves, which might explain the action of these products on *T. ludeni*. It was observed, for example, that the mite lost part of its mobility in treatments with adjuvant oils, being attached to this protective layer. This might have caused greater intake of the extract and prevented its escape from the leaves.

Another important factor is the mode of action of adjuvant oils that depends on the development stage of *T. ludeni*. It is known that the toxicity of plant extracts depends on the qualitative and quantitative compounds present in the plant (Medeiros, 1990; Papanastasiou *et al.*, 2017), which act differently depending on the development stage of the mite. The mode of action in eggs is direct contact; meanwhile, in adult females, in addition to direct contact, ingestion of the product deposited on the leaf may occur. Furthermore, the egg represents a sessile stage that is under the direct action of the product; the activity of the mobile stages during the application of the product or the place where they are applied also influence the mode of action.

It is possible that the variation in the viability of *T. ludeni* eggs treated with *S. joazeiro* extract and the different adjuvants occurred due to the penetration of toxic bioactive compounds into the eggs, as discussed by Dittrich and Streibert (1969) and Gonçalves *et al.* (2001). Dittrich and Streibert (1969) pointed out that spider mite eggs have a breathing mechanism wherein the stigmas and

chorion connect and are attached to a porous region of the intermediate membrane where gas exchange with the atmosphere probably occurs. Therefore, these structures could be important routes for the penetration of plant extract bioactive compounds.

In general, the low persistence of natural products is due to their rapid degradation in the environment after application, whether by light, heat, broad spectrum of action, or derivation of natural resources (Gardiano *et al.*, 2009), which may be even quicker in aqueous extracts (Gonçalves *et al.*, 2001). This leads to the need of frequent reapplication of these products, which reinforces the importance of adjuvants to prolong the useful life of these products.

These considerations support the findings of the present study because the residual effect of the AESJ is prolonged when it is associated with adjuvant vegetable oils. This was already expected, as the association primarily helps to reduce volatilization, prolonging the active life of the product on the surface of plants (Somerville *et al.*, 2011). Despite the low residual effect found in the present study (48 hours), the data obtained are important to determine application intervals and improve pest control in the field.

In the present study, soybean, canola, and sunflower oils showed the lowest percentages of egg viability (LC₅₀ and LC₉₀) of *T. ludeni*, thereby being more effective in controlling the mite. The inferior result of coconut oil can be explained by its poor oiliness compared to other oils; that is, it is not as adhesive and cohesive to cotton leaves (Matharage *et al.*, 2013).

The association of AESJ with adjuvant vegetable oils of coconut, soy, canola, and sunflower produced active compounds against *T. ludeni*, reducing egg hatching and controlling the level of infestation on cotton. Secondary compounds present in plants have significant potential for pest management (Noman *et al.*, 2021). Studies have reported that these compounds have repellent and ovicidal toxins that prevent the oviposition and feeding of pests (Isman, 2006).

The ovicide effect is one of the most important properties of an acaricide. This is because by reducing or preventing the hatching of larvae, pests are controlled in their initial stage of development, reducing the number of injuries caused by mites in high populations (Oliveira, 2013).

Moreover, deterrent substances promote a decrease in oviposition due to the presence of several volatile compounds (Kumari and Kaushik, 2016). The deterrence indices obtained in this study indicate that the association of AESJ with coconut, soy, canola, and sunflower adjuvant oils are deterrent for the oviposition of *T. ludeni*.

The deterrent effect of the AESJ on *T. ludeni* was evaluated by Ferraz *et al.* (2017), which observed a reduction of up to 46.92% in the oviposition of this mite. According to Siqueira *et al.* (2014), who evaluated the effect of plant extracts on the mite *M. tanajoa*, the reduction in oviposition can be caused by factors such as the decrease in the feeding activity (phage-inhibitory effect), which leads to a reduction in mite fertility.

Potential effects of oviposition deterrence were evaluated by applying *Pongamia pinnata* (L.) oil on whitefly

(Pavela and Herda, 2007). Roobakkumar *et al.* (2010) found oviposition deterrence to range from 80 to 100%, when they evaluated the effect of extracts of *Azadirachta indica* (A. Juss), *P. pinnata*, and *Allium sativum* (L.) on the mite *Oligonychus coffeae* (Nietner).

The chromatographic analysis of the AESJ revealed phenolic compounds, such as gallic acid (GA), with a higher concentration in LC₉₀. These compounds are widely distributed in plant tissues and are often associated with allelopathic and development-inhibiting phenomena (Rabaioli and Silva, 2016). The acaricidal effect on *T. ludeni* can be attributed to the presence of GA in the AESJ and other phenols such as caffeic acid (CA), which were identified in preliminary studies (Bezerra *et al.*, 2021).

GA or 3,4,5-trihydroxybenzoic acid is an intermediate organic compound in the secondary metabolism of plants that can be obtained through hydrolysable tannins (Sousa *et al.*, 2013). Phenolic acids are known as hydroxybenzoates. These are a diverse class of compounds present in plants, which when under attack from herbivores, activate an induced response and increase the content of soluble phenols. Accordingly, GA along with other hydroxybenzoates causes a reduction in the conversion efficiency of biomass assimilated by insects, in addition to having an inhibiting effect on digestion (Punia *et al.*, 2021).

CA is a polyphenol synthesized during secondary metabolism of plants, which has been reported in some species of fruits, coffee, potatoes, and carrots (Espíndola *et al.*, 2019). CA is present in the plant cell wall and essential for the defence mechanism of plants against pests and predators. It has an inhibitory effect on the development of bacteria, fungi, and insects (Tosovic, 2017). Furthermore, Joshi *et al.* (2014) observed that CA inhibits the detoxification enzymes of *Helicoverpa armigera* (Hubner), thereby intensifying insecticidal effects on this pest and further demonstrating the importance of exploring CA for the development of effective dietary pesticides.

The potential acaricidal effect observed can be explained not only by the presence of GA and CA in the AESJ and the association with adjuvant vegetable oils, but also by the presence of other compounds, such as catechin, chlorogenic acid, ellagic acid, epicatechin, rutin, quercetin, and isoquercitrin (Brito *et al.*, 2015). Flavonoids and saponins have repellent activity and are known to affect pest growth and reproduction, increase mortality rates, and decrease insect digestibility (Sousa *et al.*, 2013; Panche *et al.*, 2016).

Studies have demonstrated an effect of GA on *Meloidogyne incognita* (Kofoid et White) (Nguyen *et al.*, 2013b), an antifungal effect on *Fusarium solani* (Nguyen *et al.*, 2013a), and an insecticidal effect on *Spodoptera litura* (F.) larvae, melon fly, and *Bactrocera cucurbitae* (Coquillett) larvae (Punia *et al.*, 2021). GA also exhibits microbial activity, inhibiting the growth of *Campylobacter jejuni* and *Campylobacter coli* strains (Sarjit *et al.*, 2015) and acting against *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *C. jejuni* (Nohynek *et al.*, 2006; Chanwitheesuk *et al.*, 2007; Alkan *et al.*, 2011; Li *et al.*, 2015).

Conclusions

The hypotheses proposed for this study were confirmed. The use of adjuvant vegetable oils associated with the AESJ increased its control efficiency on the mite *T. ludeni*.

The extract associated with the tested adjuvants exhibited toxicity to adult mites, deterred oviposition, reduced egg viability and increased their residual effect. Sunflower oil was the most persistent adjuvant tested throughout the period evaluated.

S. joazeiro extract was most effective at LC₉₀ in all tests performed and showed 100% toxicity to *T. ludeni* when in association with the adjuvants tested.

Canola and sunflower adjuvants and *S. joazeiro* extract at LC₅₀ significantly reduced the fecundity of female mites. Soybean and canola adjuvants and *S. joazeiro* extract at LC₉₀ were the most effective in reducing *T. ludeni* egg viability.

Sunflower oil was the most persistent adjuvant tested throughout the evaluated period.

All the adjuvants used in this study increased the efficiency of the extract; thus, these results indicate that farmers may choose an adjuvant that is associated with the AESJ and exhibits the lowest cost of acquisition among all the oils tested.

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Authors' addresses: Cláudia Helena Cysneiros MATOS (corresponding author: claudia.matos@ufrpe.br), Josias Jordão Andrade ALVES, Carlos Romero Ferreira de OLIVEIRA, Vanessa Luana da Conceição PEREIRA, Kelem Silva FONSECA, Cinara Wanderléa Félix BEZERRA, Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, Serra Talhada, Pernambuco, Brazil.

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