

# Shelf life of essential oil of *Acantholippia deserticola* (Phil. ex F. Phil.) Moldenke and its acaricidal effect on two-spotted mite [*Tetranychus urticae* Koch (Acari: Tetranychidae)] under laboratory conditions

*Vida de anaquel del aceite esencial de Acantholippia deserticola (Phil. ex F. Phil.) Moldenke y su efecto acaricida sobre la araña bimaclada [Tetranychus urticae Koch (Acari: Tetranychidae)] bajo condiciones de laboratorio*

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## ABSTRACT

The acaricidal effect of two formulations of essential oil of “rika-rika” [*Acantholippia deserticola* Moldenke (Verbenaceae)] of 3 and 6 years of shelf life were evaluated on females of two-spotted spider mite [*Tetranychus urticae* (Acari: Tetranychidae)] registering the repellency and effects on the oviposition. The bean leaf discs [*Phaseolus vulgaris* Linneo (Fabaceae)] were covered with the formulation using the immersion technique under laboratory conditions (23.31 ± 2.82 °C, 49.70 ± 1.42% RH and 12:12 L:D photoperiod). The mortality of *T. urticae* was evaluated at 24, 48 and 72 hours post-application. The variables evaluated were: percentage of mortality by residual contact, percentage of repellency and number of eggs laid. Only the formulation at 1% (v/v) with 3 years shelf life reached a mortality of 50% after 24 hours of evaluation. The concentrations at 0.3% and 0.7% (v/v) with 3 years shelf life reached this value after 72 hours. None of the concentrations with 6 years shelf life reached values above 50% of mortality. A maximum mortality of 85.71% was registered in the concentration at 1% (v/v) with 3-year shelf life. The 3-year shelf life formulation showed repellent activity for the three periods evaluated (24, 48 and 72 hours), with values above 90% after 72 hours post-application. The oviposition test revealed that the concentrations of 3 years shelf life had an inhibitory effect on the oviposition of the two-spotted spider. These results suggest a considerable acaricidal activity of the essential oil of *A. deserticola* with a 3-year shelf life on adult females of *T. urticae*.

**Keywords:** Formulation, repellency, oviposition.

## RESUMEN

El efecto acaricida de dos formulaciones del aceite esencial de *Acantholippia deserticola* Moldenke (Verbenaceae) (“rika-rika”) de 3 y 6 años de vida de anaquel fueron evaluados sobre hembras de *Tetranychus urticae* (Acari: Tetranychidae) (araña bimaclada) registrando la repelencia y los efectos sobre la oviposición. Los discos de hojas de poroto [*Phaseolus vulgaris* Linneo (Fabaceae)] fueron cubiertos con la formulación utilizando la técnica de inmersión en condiciones de laboratorio (23,31 ± 2,82 °C, 49,70 ± 1,42% de humedad relativa y fotoperíodo de 12:12 luz: oscuridad). Se evaluó la mortalidad de *T. urticae* a las 24, 48 y 72 horas después de la aplicación. Las variables evaluadas fueron: porcentaje de mortalidad por contacto residual, porcentaje de repelencia y número de huevos puestos. Sólo la formulación al 1% (v/v) con una vida útil de 3 años alcanzó una mortalidad del 50% a las 24 horas de la evaluación. Las concentraciones al 0,3% y 0,7% (v/v) con 3 años de vida de anaquel alcanzaron este valor a las 72 horas. Ninguna de las concentraciones con una vida útil de 6 años alcanzó valores superiores al 50% de mortalidad. Se produjo una mortalidad máxima del 85,71% por la concentración al 1% (v/v) con una formulación de vida útil de 3 años. La formulación de vida útil de 3 años mostró actividad repelente para los tres períodos evaluados (24, 48 y 72 horas), con valores superiores al 90% a las 72 horas después de la aplicación. La prueba de oviposición reveló que las concentraciones de vida de anaquel de 3 años tuvieron un efecto inhibitorio sobre la oviposición de la araña bimaclada. Estos resultados sugieren una considerable actividad acaricida del aceite esencial de *A. deserticola* sobre hembras adultas de *T. urticae*, con una vida de anaquel de 3 años.

**Palabras claves:** Formulación, repelencia, oviposición.

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## Introduction

The mites of the Tetranychidae family correspond to important pests for many crops. One of the most economically important species is *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae), a polyphagous species that attacks more than 1,000 plant species (Ximénez-Embún *et al.*, 2017).

The carmine form of the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), formally known as *Tetranychus cinnabarinus* (Boisduval) (Auger *et al.*, 2013), is one of the most important pests of crops and ornamental plants worldwide.

In Chile, the two-spotted mite is widely distributed and its hosts plant are melon (*Cucumis melo* (Linnaeus)), bean (*Phaseolus vulgaris* (Linnaeus)), alfalfa (*Medicago sativa* (Linnaeus)), peach (*Prunus persica* (Linnaeus) Batsch), cherry tree (*Prunus cerasus* (Linnaeus)), strawberry (*Fragaria x ananassa* (Duchesne)) and carnation (*Dianthus caryophyllus* (Linnaeus)) (Klein and Waterhouse, 2000).

*Tetranychus urticae* are located on the underside of the leaves, increasing their population in warm seasons. Precisely, the environmental conditions of northern Chile, of dryness and heat are very favorable for the development of this phytophagous mite (Peralta and Tello, 2011).

The control of *T. urticae* in ornamentals is exclusively with agrochemicals, but the main problem generated with this control technique is a rapid resistance due to their high fertility and fecundity, inbreeding, parthenogenetic reproduction (pseudo-arrhenotoky) and a short life cycle that produces many generations per year (Lu *et al.*, 2017).

The most used chemicals worldwide are fenazaquin, chlorpyrifos, abamectin and last generation acaricides such as spiroticlofen belonging to the new family of spirocyclic tetroneic acids, ketoenoles (Demaeght *et al.*, 2013).

The indiscriminate use of conventional pesticides, mainly of broad spectrum, has caused serious health problems for human and imbalance in the agroecosystem. The consequences of these chemical applications are the development of pest resistance, environmental pollution, outbreaks of new pests, elimination of beneficial insects, increasing chemical accumulation in the trophic chain and intoxication of workers and consumers (Darçın and Darçın, 2017). This problem has

increased the interest of farmers and researchers for the search of new chemical control alternatives such botanical extracts (Jallow *et al.*, 2017).

Plants have developed different defense strategies against herbivorous, including chemical direct defense mechanisms producing constitutively secondary metabolites such as alkaloids, non protein amino acids, amines, cyanogenic glycosides, glucosinolates, terpenoids, and phenolics (Mithöfer and Boland, 2012). Several studies show the effects of essential oils against mosquitoes, including growth inhibition, larvicide, adulticide, repellents and oviposition deterrent effects (Guo *et al.*, 2017).

In northern Chile, there is a large number of plants species that are used by people for heal different diseases, one of those plants is *Acantholippia deserticola* (Phil. Ex F. Phil) Moldenke commonly known as “rika-rika”; this species grows in arid regions of northern Chile and Bolivia and northeast and south of Argentina in sandy and stony soils between 2,300 and 3,500 meters above sea level (m.a.s.l.) (Morales *et al.*, 2008).

Rojo *et al.* (2006) registered 22 compounds in *A. deserticola* oil by gas chromatography/mass spectrometry (GC/MS) representing 99.31% of the total oil, where the  $\beta$ - thujones compound represent 77.9%, whereas 10.5% corresponds to  $\alpha$ -thujones. Sampietro *et al.* (2016) identified 28 compounds from oil of leaves of *A. deserticola*, with 66.4% of  $\beta$ -thujones, but  $\alpha$ - thujones was not detected.

The toxicity of both  $\alpha$ - and  $\beta$ - thujones compound is due to stimulation of the central nervous system by antagonizing the ionotropic receptors of  $\gamma$ -aminobutyric acid (GABA), which decreases Cl-currents by reducing inhibition; provoking a convulsive effect in mammals and probably their insecticidal/acaricidal activity (Olsen, 2006).

Different studies support the toxic effects of thujones compound present in essential oils of different plant species against a variety of arthropods: toxic and repellents effect of *Artemisia sieberi* Besser (Asteraceae) oil on the mite *Dermanyssus gallinae* (De Geer, 1778) (Acari: Dermanyssidae) (Tabari *et al.*, 2017); insecticidal/acaricidal activity of *A. deserticola* oil on *Planococcus citri* Risso, 1813 (Hemiptera: Pseudococcidae), *Aleurothrixus floccosus* Maskell, 1896 (Hemiptera: Aleyrodidae), *T. urticae* and *Brevipalpus chilensis* Baker, 1949 (Acari:

Tenuipalpidae) (Benites *et al.*, 2014); acaricidal properties of essential oils of the asteraceae *Artemisia absinthium* L. and *Tanacetum vulgare* L. on adults of *T. urticae* (Chiasson *et al.*, 2001).

Considering the importance of thujones compound from *A. deserticola* as promising acaricide/insecticide molecules, we propose to evaluate the shelf life of a formulation at different concentrations of the essential oil of *A. deserticola* on the red form of *T. urticae* under laboratory conditions.

## Materials and Methods

### Plants

The *A. deserticola* plants were collected in two dates: first collection in July 2011 and second in July 2013. These collections were made in the same area (Colchane, Región de Tarapacá, Chile; 19°16'22.50 " S and 68°37'40.42 " W, about 3,720 m.a.s.l.). Only the aerial part of plant was collected and transferred to a chamber at low temperature in the Natural Products Laboratory of the Faculty of Health Sciences of the Arturo Prat University, Iquique, Chile.

### Acarological material

For testing the shelf life of the essential oil of *A. deserticola*, we worked with *T. urticae* adults. The mites were obtained from a carnation (*Dianthus caryophyllus* L.) crop, in 'La Tirana' town (Province of Tamarugal, Chile; 20°21'12.87 "S and 69° 39'12.82" W, about 1,006 m.a.s.l.) in January 2016 and reared in laboratories of the Faculty of Natural Resources and Renewable until testing. For its massive reproduction, *P. vulgaris* Linneo var. "Canario" were planted weekly, in polyethylene containers (15x15 cm).

### Oil isolation of *Acantholippia deserticola*

The aerial part of plant was dried at room temperature a week approximately. Once dried, the leaves were separated, which were submitted to a hydro-distillation for 3 hours using Clevenger-type apparatus. The oil was protected from direct light and maintained at 4 °C until the moment to make the formulated. The yield of essential oil of *A. deserticola* was calculated based on the mass from dry plant material.

To determine the weight of plant material and the volume of the essential oil obtained, a standard-sized plant of *A. deserticola* (without roots) was measured, and the shoots were pruned. The leaves were dried at room temperature and weighed on an analytical balance, and then the volume of essential oil was determined according to the methodology described above.

### Elaboration of the formulated

The formulated was prepared according to Zarria *et al.* (2010), in the Laboratory of the Natural Products Institute of the Faculty of Health Sciences of the Arturo Prat University. 250 mL of the formulations were prepared for different concentrations (0.3, 0.7 and 1.0% w/v). Two formulations were prepared: one from plants collected in 2011 (shelf-life: 6 years) and other from plants collected in 2014 (shelf-life: 3 years).

### Bioassays

Leaf disks (diameter = 2 cm) were cut with a punch. They were immersed for 10 seconds in each concentration (treatment) of the essential oil (0.3, 0.7 and 1% w/v), in 2 mL of solution for 30 s and dried at room temperature. Once dry, the leaf discs were placed on wet cotton into a Petri dish (diameter = 10 cm). The edges of each leaf disc were covered with a fine line of glue (Point Sticky Glue®, Point Chile) to prevent the escape of adult mites. Finally, using a fine brush, 10 adult females were transferred to the surface of each leaf disk. As a negative control, water was used, whereas as a positive control the chemical abamectin ( $2.4 \times 10^{-5}$  g in 2 mL of the solution). The experimental unit was a leaf disc with 10 individuals and each treatment was repeated 10 times. All the tests were carried out under conditions at  $23.31 \pm 2.82$  °C of temperature,  $49.70 \pm 1.42\%$  of RH and a photoperiod of 12:12 L: D.

### Residual contact mortality test on adults of *Tetranychus uticae*

Eggs laid by approximately 50 females during a period of 12 hours were collected in plastic boxes. These boxes were kept in the same conditions until the larvae hatched. This cohort was reared until when the adults of *T. urticae* were obtained.

Subsequently, groups of 10 adult females were placed on leaf disks previously washed with distilled water and its edges delimited with a fine line of glue (Point StickyGlue®) to prevent the escape of mites. The experiment was repeated 10 times for each concentration. The mortality of the females of *T. urticae* was quantified at 24, 48 and 72 h after the application, registering as dead those individuals without mobility when they were stimulated with a fine brush.

### Repellency test

Bean leaf discs (diameter = 2 cm) were used. The discs were cut so that the mid-rib of the leaf was in the middle of the leaf disc. Each disc was submerged only halfway (midrib of the leaf) in the different treatments and allowed to dry at room temperature. Only one concentration corresponding to 0.7% (w/v) of the essential oil of *A. deserticola* was applied. Subsequently, the discs were placed into Petri dishes (diameter = 10 cm).

After the application, 10 adult females were placed in the central venation of the leaf disk (choice test). The repellent effect was calculated by the percentage of adults who stayed out of the applied half, in three evaluation periods: 24, 48 and 72 h post-application. A leaf disc with 10 adult females corresponded to an experimental unit.

### Effect on oviposition

For this test, the same methodology applied in the residual contact mortality test was used. Once the treatments were applied, glue was applied to the border of the leaf discs; each of them had a female and a male of *T. urticae*. Each treatment was repeated 10 times. The same controls were used. The number of eggs laid per female was evaluated up to the third day after application.

### Design and statistical analysis

For all experiments, a completely randomized design was used. Natural mortality was corrected using Abbott's formula (Abbott, 1925). The data of percentages and counts due to not complying with the normality assumption were analyzed through a non-parametric analysis of variance (Kruskal-Wallis), with a level of significance of  $\alpha = 0.05$ . To compare two means, the non-parametric Wilcoxon

test and the paired t-test were used, both with a significance of 5%. The statistical analyzes were performed with the InfoStat program, version 2018.

## Results and discussion

### Mortality test

In the formulated of 3 years shelf life, at 24, 48 and 72 h post-application, there were significant differences ( $p < 0.0001$ ) between the treatments (Table 1). The treatment at 1.0% (w/v) concentration was the only one that reached over 50% mortality after 24 h, compared to the concentrations at 0.3 and 0.7% (w/v) that reached this value at 72 h post-application. Only in the last evaluation, the three concentrations reached mortality percentages statistically equal to the chemical control (98.57%). The extracts of *A. deserticola* of 6 years of storage did not show significant differences among treatments at 0.3, 0.7 and 1% (w/v). Although the mortality percentages were greater than 72 h post-application, they did not exceed 50%.

Comparing the mortality produced between the formulations from 3 and 6 years shelf life at 72 h post-application (Figure 1), there were significant differences in the concentrations at 0.7% ( $W = 143.00$ ,  $p < 0.05$ ) and 1% ( $W = 86.50$ ,  $p < 0.05$ ).

Benites *et al.* (2014) evaluated the essential oil of *A. deserticola* on *T. urticae*, with the lowest concentration at 2% (w/v) obtaining a mortality of 10.44% at 24 h post-application, and with the highest concentration at 5% (w/v) reached a mortality of 82.18%; whereas, in the present study after 24 h and a concentration of 0.3% (w/v) the formulation of 3 years shelf life reached a mortality of 23.65%.

Chiasson *et al.* (2001) studied the essential oil of *Tanacetum vulgare* L. (Asteraceae) on adult of *T. urticae*, which contained 91.1% of  $\beta$ -thujone, using a concentration at 4% v/v after 48 hours post-application obtained a mortality of 75.6%.

Immaraju (1998) indicated that the storage time of a formula based on the active ingredient azadirachtin showed a stability of 1 year; then, losing between 3-5% of the content of azadirachtin at room temperature and with low relative humidity. In addition, Jadev *et al.* (2006) evaluated the systemic and persistent effect of Neem formulations against nematodes of root knots and its shelf life; finding that after 8 months of storage with exposure to direct light the efficiency decreased, thus for

Table 1. Mortality (%) of *Tetranychus urticae* adults by indirect application of different concentrations of the essential oil of *Acantholippia deserticola* of 3 and 6 years shelf life.

Treatments	3 years shelf life			6 years shelf life		
	24 h	48 h	72 h	24 h	48 h	72 h
	Average Mortality <sup>1</sup> ( $\pm$ EE <sup>2</sup> )					
0.3% w/v	23.65 $\pm$ 6.18 bc	42.65 $\pm$ 8.92 b	68.00 $\pm$ 9.61 a	15.52 $\pm$ 3.46 b	29.01 $\pm$ 5.26 b	43.73 $\pm$ 9.81 b
0,7% w/v	17.62 $\pm$ 7.75 c	47.00 $\pm$ 8.66 b	85.00 $\pm$ 6.31 a	9.15 $\pm$ 3.31 bc	20.58 $\pm$ 3.22 b	45.44 $\pm$ 9.42 b
1% w/v	54.17 $\pm$ 15.02 ab	78.57 $\pm$ 10.10 ab	85.71 $\pm$ 14.29 a	17.45 $\pm$ 4.76 b	30.42 $\pm$ 8.24 b	42.14 $\pm$ 11.38 b
Water	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 b	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 c
Abamectin	86.28 $\pm$ 2.18 a	94.68 $\pm$ 2.23 a	98.57 $\pm$ 1.43 a	86.28 $\pm$ 2.18 a	93.68 $\pm$ 2.19 a	98.57 $\pm$ 1.43 a
	ANOVA					
KW	26.83	31.43	25.03	30.62	33.74	29.53
f.d. <sup>3</sup>	4	4	4	4	4	4
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

<sup>1</sup> Means with different letters within each column are statistically different according to the Kruskal-Wallis test ( $P < 0.05$ ).

<sup>2</sup> S.E.: Standard error.

<sup>3</sup> f.d.: freedom degrees.

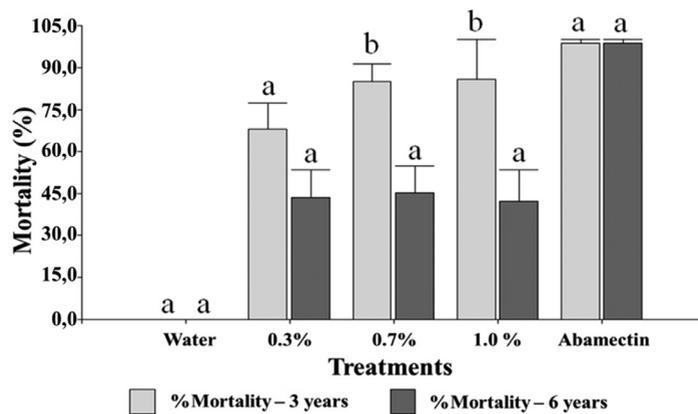


Figure 1. Mortality (%) of *Tetranychus urticae* adults by indirect application of different concentrations of the essential oil of *Acantholippia deserticola* of 3 and 6 years shelf life. Means with different letters within each evaluation time are statistically different according to the Wilcoxon test ( $P < 0.05$ ).

increasing its shelf life should be stored in dark with low temperatures or under normal storage conditions (in darkness and at room temperature). In the present study, the incidence of light had no any effect on the microemulsion of the essential oil of *A. deserticola* since the oil was storage in amber bottles completely hermetic and without filtration of light.

Likewise, Balasubramanian *et al.* (2008) evaluated different forms of storage based on Ayurvedic principles, indicating that the storage time of some biopesticides, according to their

physical composition persist between 1 to 3 years. In that regard, we found that the acaricidal properties were maintained in *A. deserticola* microemulsion with 3 years shelf life, unlike to 6 years shelf life. Therefore, the *A. deserticola* formulation loses the biological activity after 3 years shelf life.

#### Repellency test with formulations of 3 years shelf life

According to Table 2, the control treatment (water) did not show significant differences

Table 2. Distribution of *Tetranychus urticae* adults in treated and untreated areas with essential oil of *Acantholippia deserticola*.

Concentration	24 h		48 h		72 h	
	Treated area	Untreated area	Treated area	Untreated area	Treated area	Untreated area
	% (means <sup>1</sup> ) ± S.E. <sup>2</sup>					
Water	50.00 ± 3.16 a	50.00 ± 3.16 a	56.00 ± 5.10 a	44.00 ± 5.10 a	54.00 ± 6.00 a	46.00 ± 6.00 a
0.7%	2.00 ± 2.00 b	98.00 ± 2.00 a	10.00 ± 5.48 b	90.00 ± 5.48 a	8.00 ± 3.74 b	92.00 ± 3.74 a

<sup>1</sup> Means with different letters within each column are statistically different according to the paired t-Student test ( $P < 0.05$ ).

<sup>2</sup> S.E.: Standard error.

between both treated and untreated areas in the three evaluation periods, discarding the influence of water in the distribution of mites and the mortality due to the humidity by evaporation of water in laboratory.

The results obtained in the concentration at 0.7% (w/v) showed significant differences for the three evaluation periods in the distribution of adults on leaf discs, concentrating in untreated area with percentages  $\geq 90\%$  (24 h:  $t = 24.0$ ,  $p < 0.0001$ ; 48 h:  $t = 4.9$ ,  $p < 0.01$ ; 72 h:  $t = 6.0$ ,  $p < 0.01$ ).

Tello *et al.* (2014) showed that *T. urticae* presented a repellence percentage of 94% with extracts of *Lampaya medicinalis* F. Phil (Verbenaceae) and *Baccharis tola* Phil. (Asteraceae) at 3% concentration after 24 h. However, these percentages decreased at 72 h post-application, with 66% and 84% of repellency for the extracts of *L. medicinalis* and *B. tola*, respectively. In the present study, the percentage of repellency generated by the essential oil at 0.7% w/v of *A. deserticola* was 98% after 24 h, and remained above 90% after 72 h of exposure.

Harder *et al.* (2014) conducted a repellency study using extracts of *Chenopodium quinoa* Willd. (Chenopodiaceae) with 4% of concentration on *T. urticae* and exposition during 24 h, they obtained 100% repellency; in addition, this percentage of repellency decreased at 76.24% after 72 h of exposition. However, the essential oil of *A. deserticola* presented a lower percentage of repellency (98%), although remained above 90% after 72 h of exposition.

### Effect of essential oil on the oviposition of *Tetranychus urticae*

There were significant differences between the concentrations evaluated at 24, 48 and 72 h

with formulations of 3 and 6 years of storage (Table 3). The concentration at 1% (w/v) had the lowest number of eggs after 72 h of application. The three concentrations of *A. deserticola* did not show significant differences with the chemical control (Abamectin) in different evaluation times.

The formulated of *A. deserticola* with 6 years of storage showed significant differences after 24, 48 and 72 h of exposition. The treatments of 0.3 and 0.7% w/v did not show significant differences for all evaluations. The concentration at 1% w/v presented the highest average number of eggs. The average of eggs laid by *T. urticae* females exposed to different concentrations of *A. deserticola* microemulsion was lower using formulated with 3 years shelf life, compared to concentrations of 6 years of storage. Likewise, Balasubramanian *et al.* (2008) indicates that the plant extracts can be stored up to 3 years maintaining the biological activity intact, corroborating the results obtained in the present study, where the highest average of eggs laid by *T. urticae* females was registered in formulated with 6 years shelf life.

The number of eggs laid was increased with the exposition time in all treatments and formulations, except in the treatment at 1% w/v with 3 years shelf life; seemingly this was caused by the low residual power of these extracts and environmental conditions. The chemical control registered a low number of eggs laid; whereas, the water control showed the highest number of eggs laid.

Comparing the formulated of 3 and 6 years shelf life, there were significant differences in three concentrations evaluated after 72 h of exposition: 0.3% ( $W = 55.00$ ,  $p < 0.001$ ), 0.7% ( $W = 55.00$ ,  $p < 0.001$ ) and 1.0% ( $W = 65.00$ ,  $p < 0.05$ ) (Figure 2).

Castiglioni *et al.* (2002) evaluated aqueous extracts of Meliaceae plants on total oviposition

Table 3. Number of eggs laid by adult females of *Tetranychus urticae* exposed to three concentrations of *Acantholippia deserticola* oil of 3 and 6 years shelf life.

Treatment	3 years shelf life			6 years shelf life		
	24 h	48 h	72 h	24 h	48 h	72 h
N° Eggs after application						
0.3% w/v	3.30 ± 1.17 b	3.60 ± 1.32 b	3.90 ± 1.44 b	36.78 ± 7.14 b	49.40 ± 6.71 b	47.30 ± 7.26 bc
0.7% w/v	2.00 ± 1.41 b	2.20 ± 1.38 b	2.20 ± 1.38 b	30.30 ± 6.20 b	50.70 ± 10.70 b	58.80 ± 11.21 b
1% w/v	1.00 ± 0.56 b	1.40 ± 0.62 b	1.00 ± 0.56 b	7.30 ± 3.14 c	14.50 ± 5.17 c	17.60 ± 5.94 cd
Water	99.00 ± 5.73 a	131.00 ± 12.3 a	141.40 ± 15.42 a	99.00 ± 5.73 a	131.00 ± 12.30 a	141.40 ± 15.42 a
Abamectin	0.70 ± 0.26 b	0.90 ± 0.23 b	0.90 ± 0.23 b	0.70 ± 0.26 c	0.90 ± 0.23 c	0.90 ± 0.23 d
ANOVA						
K.W.	27.26	26.29	27.20	39.73	39.73	39.54
f.d. <sup>3</sup>	4	4	4	4	4	4
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

<sup>1</sup> Means with different letters within each column are statistically different according to the Kruskal-Wallis test ( $P < 0.05$ ).

<sup>2</sup> S.E.: Standard error.

<sup>3</sup> f.d.: freedom degrees.

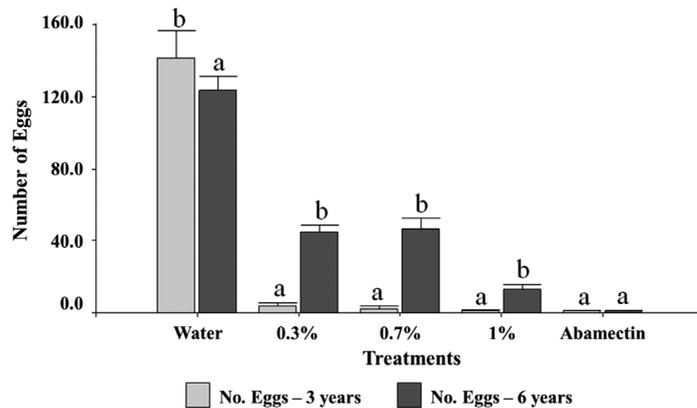


Figure 2. Number of eggs laid by *Tetranychus urticae* females treated by indirect application of different concentrations of the essential oil of *Acantholippia deserticola* of 3 and 6 years shelf life. Means with different letters within each evaluation time are statistically different according to the Wilcoxon test ( $P < 0.05$ ).

of eggs laid by *T. urticae*, registering 900 eggs laid by females treated with extracts of *Trichilia pallida* Sw. (Meliaceae) shoots, and between 600-700 eggs laid by females treated with leaves and seeds of Neem at 5%. In that regard, our results were lower using *A. deserticola* extracts of 3 years shelf life, in comparison to Castiglioni *et al.* (2002); it seems the relative humidity would have influence since in this study (58%) was lower than that Castiglioni *et al.* (2002) (98%). Finally, it should be mentioned that increasing the concentration of *A. deserticola* extracts, the number of eggs laid decreases.

## Conclusions

Only the essential oil of *A. deserticola* of 3 years shelf life shows acaricidal activity on adult females of *T. urticae*, and reducing the eggs laid considerably.

The *A. deserticola* extracts of 3 years shelf life (storage) presents acaricidal properties by indirect contact on adult females of *T. urticae*. The concentration at 1% w/v reached mortalities higher than 50% after 24 h of exposition. The other concentrations reached those values after 72 h of exposition.

On the other hand, the essential oil of *A. deserticola* of 6 years shelf life does not present acaricidal properties because none of the tested concentrations reach mortalities higher than 50%. The concentration at 0.7% w/v reached 50% of mortality after 72 h of exposition, without significant differences with the water control.

In relation to repellency, the concentration at 0.7% w/v of the essential oil of *A. deserticola* registered activity on *T. urticae* females, registering significant differences between the treated and

untreated zone. After 24 h of exposition a 98% of individuals in the untreated area was registered, and over time this value decreases until 92% showing the repellent property of *A. deserticola* oil.

With respect to oviposition, the females of *T. urticae* present differences in the number of eggs laid when were exposed to formulations of 3 and 6 years shelf life. The essential oil of *A. deserticola* of 3 years shelf life maintains its acaricidal properties, presenting adult mortality over 50% and significantly affecting the oviposition of females.

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