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Acantholippia deserticola essential oil as a natural pesticide against agricultural plagues woolly whitefly [*Aleurothrixus floccosus* (Maskell)], Chilean false red mite (*Brevipalpus chilensis* Baker) and two-spotted mite (*Tetranychus urticae* Koch)

[Aceite esencial de *Acantholippia deserticola* como pesticida natural contra plagas agrícolas como mosquita blanca algodonosa [*Aleurothrixus floccosus* (Maskell)], falsa arañita de la vid (*Brevipalpus chilensis* Baker) y arañita bimaculada (*Tetranychus urticae* Koch]

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Abstract: Different concentrations of essential oil obtained from *Acantholippia deserticola* (Phil.ex F. Phil.) Moldenke were assessed against Chilean agricultural pests like *Aleurothrixus floccosus* (Maskell), *Brevipalpus chilensis* Baker, and *Tetranychus urticae* Koch. The bioassays were carried out under laboratory conditions and both direct and residual applications were done through Potter precision spray tower test. The oil was obtained by steam distillation containing a rich fraction of α and β-thujones (88.4%) and it shows marked toxic effects against pests. Indeed, a mortality of 82% and 89% was observed in both *B. chilensis* and *T. urticae* after 48 h whereas in *A. floccosus* over 97% of mortality was seen after 7 days. These results open the possibility to use essential oil from *Acantholippia deserticola* as natural pesticide.

Keywords: *Acantholippia desertícola,* thujone, natural pesticide, agricultural primary pests

Resumen: Se evaluó el efecto a diferentes concentraciones de una solución de aceite esencial de *Acantholippia deserticola* sobre plagas agrícolas en Chile, tales como *Aleurothrixus floccosus* (Maskell), *Brevipalpus chilensis* Baker y *Tetranychus urticae* Koch. Los bioensayos fueron realizados mediante una torre de Potter en condiciones de laboratorio y las aplicaciones fueron directas y residuales. El aceite se obtuvo por hidrodestilación, el cual contenía una gran cantidad de α and β-tuyonas (88.4%), mostrando marcados efectos tóxicos para A. floccosus, con un 97% de mortalidad después de 7 d y, para *B. chilensis*, y *T. urticae*, con una mortalidad de 82% y 89% respectivamente, después de 48 h. Estos resultados abren la posibilidad de usar aceite esencial de Acantholippia deserticola como pesticida natural.

Palabras clave: *Acantholippia deserticola*, tuyonas, pesticida natural, plagas primarias agrícolas

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INTRODUCTION

Synthetic insecticides are important tools extensively employed in pest control. Nevertheless, their excessive sprays lead to severe and negative consequences such as toxicity towards farmers, consumers and wild animals; interruption of natural control and pollination; water pollution, and acquisition of pest resistance to these products (Ebadollahi, 2013; Waterfield and Zilberman, 2012). The problems caused by overusing synthetic insecticides have forced farmers and researchers to look for less hazardous alternatives such as those used for many years in developing countries (Henao, 1999; Mareggiani, 2001; Tang *et al*., 2013). It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually (Mohan *et al*., 2011). Thus, more effective, environmentally friendly and safe to human health. It is acknowledged that many essential oils isolated from various plants can exert toxic activity against insect species (Ebadollahi *et al*., 2013). The search of new compounds and control measures with minimal risk to both human health and the environment is of utmost importance from a preventive health perspective (WHO, 1999).

Plants have developed different defense strategies against predators, including chemical defense mechanisms (Broussalis *et al.*, 1999). An increasing number of studies show a wide variety of effects by essential oils against mosquitoes including growth inhibition, larvicide, adulticide, repellents and oviposition deterrents activities (Catiglioni *et al.*, 2002; Carvalho *et al.*, 2003; Cantrell *et al*., 2012). On the other hand, because their efficacy is still lower than that of currently used synthetic pesticides, few natural products have reached the market (Cavalcanti *et al.*, 2004). However, there is a renewed interest in plant products as sources of new insect controlling agents, due to their biodegradation to nontoxic compounds. This minimizes the accumulation of harmful residues, leading to more environmentally friendly pesticides as compared to synthetic compounds (Cavalcanti *et al.*, 2004; Ansari *et al.*, 2005). In this context, essential oils derived from more than 75 plant species have been evaluated for fumigant toxicity against stored product insects so far (Chiasson *et al.*, 2001).

Acantholippia deserticola (Phil) Moldelke is a species of herbaceous plant of the Verbenaceae genus, popularly named “rika-rika” in Chilean folk medicine. The local inhabitants have used this medicinal plant to treat various gastrointestinal, cardiovascular and central nervous system (CNS) conditions (Villagran *et al*., 2003). Recently we reported its sedative, anxiolytic and antidepressant activities on the rat central nervous system (Benites *et al*., 2013). We also reported the chemical composition of *A. deserticola* essential oil (Rojo *et al.*, 2006). Twenty-two compounds representing 98.9% of the total oil were identified and α- and β-thujone were characterized as the main constituents, namely 10.5% and 77.9%.

Essential oils containing diverse composition of thujones have a wide variety of biological effects, including fungicidal and antimicrobial activity (Farzaneh *et al*., 2006, Hayouni *et al*., 2008), and neurotoxic insecticide and pesticide as well (Macedo *et al.*, 1997; Ratra *et al.*, 2001).

Over the past half century a number of disruptive insect pests have become serious problems in several parts of the world, including Chile. The arthropods *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae), *Brevipalpus chilensis* Baker (Acari: Tenuipalpidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae), bring about significant losses in primary agriculture production in Chile (Klein and Waterhouse, 2000).

The aim of this study was to determine *in vitro* the insecticidal potential of *A. deserticola* essential oil against *A. floccosus*, *B. chilensis* and *T. urticae*, commonly named “woolly whitefly”, “Chilean false red mite” and “two-spotted mite” respectively.

MATERIALS AND METHODS

Plant material and oil isolation

Acantholippia deserticola was collected near Colchane at 3500 m.a.l.s, in September 2006, 1st region of Chile. The plant material was identified and authenticated by Professor Roberto Rodriguez, Concepcion University. A voucher specimen (# 158057) is deposited in the Herbarium of the Concepcion University.

The aerial parts of the plant (leaves and flowers) were submitted to hydro-distillation for 3 hours using Clevenger-type apparatus. The oil was protected from direct light and stored at 4 ◦C until its use.

Experimental conditions and used plagues

The bioassays were carried out at the Instituto Nacional de Investigación Agropecuaria (INIA), Centro Regional de Investigación (CRI), La Cruz, Valparaíso (Chile), using the following plagues:

- *A. floccosus* are tiny flying insects that derive their name from the mealy white wax covering their wings and body. *A. floccosus* affects the host plant by the suction of sap from the infested young leaves. High infestations can be detrimental to young plants. Indirect damage is also caused by the large amount of honeydew produced by the nymphs.

- *T. urticae* is a highly polyphagous species that causes great economic losses because it attacks a wide range of host plants. Recently about 3800 host species have been reported around the world (Hincapie *et al*., 2008).

- *Brevipalpus* is a large, widespread genus, including over 65 species of very small flat mites. *B. chilensis* is a native pest of quarantine significance, which is a serious economic problem in grapes (*Vitis vinifera*), lemons (*Citrus limon*), kiwifruits (*Actinidia deliciosa*), persimmons (*Diospyros kaki*), and various flowers and ornamentals (González, 1989).

Individuals of *A. floccosus, B. chilensis* and *T. urticae* were obtained from crianzas kept in *Citrus limon*, *Phaseolus vulgaris* y *Ligustrum lucidum* plants respectively, they were reared in a controlled environment (temperature of 26 ± 2° C; 80 ± 15% of relative humidity; and 8h/16h daily night-day cycle). These species have remained for more than five years without exposure to pesticides. The *A. deserticola* essential oil solutions were prepared as previously reported (Benites *et al.,* 2013) and solved in 65% methanol. The applications were direct and residual performed through Potter precision spray tower test (Makers Burkards Manufacturing Co. Ltd., Rickmansworth, U.K.) under a pressure of 55 kPa.

Toxicity tests

***Aleurothrixus floccosus* bioassays** (FAO, 1979).

***(a)* Direct and residual applications of *A. deserticola* essential oil against *A. floccosus* second instar nymphs.**

Citrus leaves were collected in CRI-La Cruz without exposure to insecticide and lightly infested with woolly whitefly nymphs. Assays were done by using 20 nymphs of 2nd instar per leave. Citrus leaves were placed on Petri dish (diameter 10 cm) and sprinkle with *A. deserticola* essential oil solution at concentrations of 0; 2; 5 and 10% v/v. Mortality was observed at 1st, 4th and 7th days.

***(b)* Residual applications of *Acantholippia deserticola* essential oil against *Aleurothrixus floccosus* adults.**

Adult individuals of *A. floccosus* were collected with a vacuum cleaner from lemon tree located in the CRI-La Cruz. Petri dishes (diameter 5 cm) were sprinkle with *A. deserticola* essential oil solution at four concentrations (0; 0.25; 1 and 2% v/v) and dried at room temperature. Afterwards, woolly whitefly adults were introduced and adult mortality was observed until 17 hours post-application.

The two assays were done in six replicates using 20 nymphs of 2nd instar per experimental unit.

***Brevipalpus chilensis* and *Tetranychus urticae* bioassays** (Knight *et al.*,1990; Chiasson *et al.,* 2004).

Direct and residual applications of A. deserticola essential oil against B. chilensis and T. urticae adults.

Two assays were carried out against adult plagues to determine first the toxicity (lethal action) of the essential oil and thereafter its lethal concentration (LC_{50} and LC_{90}). The Chilean false red mite and two-spotted mite individual adults were isolated from leaves of *Ligustrum vulgare* Linnaeus (Oleaceae) and *Phaseolus vulgaris* Linnaeus (Fabaceae) without exposure to insecticide and reared in a greenhouse. The leaves were washed with distilled water and dried at room temperature. Then, they were cut into circles (2 cm diameter) and their boards were lined with sticker. For each treatment, ten of these circles were placed on two Petri dish (five discs per Petri dish) having a wet cotton support. Then, Petri dishes were placed on a container covered with a wet sponge. In each of these circles, 10 individuals of each species of mite were placed.

Petri dishes containing adult pests were sprayed with *A. deserticola* essential oil solution at concentrations of 0-2-3.5-5% v/v for lethal action and 0-1.5-2.5-3.5-5-7% v/v for lethal concentration (LC_{50} and LC_{90}). Adult mortality was recorded at 24 and 48 h, taking as dead those individuals without mobility when stimulated with a brush. All analysis were performed ten times, using 10 adults per experimental unit.

Statistical analysis

Abbott's formula (Abbott, 1925) was used for correction with respect to the blank control mortality. One-way analysis of variance (ANOVA) of mortality was used to determine the statistical significance compared with the controls; p-values < 0.05 were considered significant. Tukey's test was used to determine which means ($p < 0.05$) differed significantly from one another using PROC GLM (SAS Institute Inc., Cary NC, USA, 2000). The slopes of the regression between mortality (probit) and concentration (log) were analyzed using PROC

PROBIT in SAS (SAS Institute Inc., Cary NC, USA, 2000) and applied according to Flores *et al*., (2007). Significant differences on LC_{50} and LC_{90} were observed based on non-overlapping confidence intervals (Manonmani *et al*., 2011).

RESULTS AND DISCUSSION

This study shows for the first time the effect of *A. deserticola* essential oil as potential pesticide against arthropods such as *A. floccosus*, *B. chilensis* and *T. urticae*. These pests cause great economic losses in Chilean agricultural crops because they attack a wide range of host plants.

Table Nº 1

Percentage of mortality against *Aleurothrixus floccosus* nymphs (stage second) exposure at different concentration of *Acantholippia deserticola* essential oil by means of direct and residual application.

Treatment	1st day	4th day	7th day
	% (media)±SE[1]	% (media)±SE[1]	% (media)±SE[1]
Control	0.00 ± 0.00a	2.50 ± 0.38a	2.50 ± 0.38a
Ethanol 65%	0.00 ± 0.00a	17.50 ± 0.99a	17.50 ± 0.99a
2.0 % v/v e.o[2]	30.83 ± 1.30b	57.50 ± 0.31b	77.50 ± 1.40b
5.0 % v/v e.o[2]	73.33 ± 0.85c	80.00 ± 1.08b	89.16 ± 0.78b,c
10.0 % v/v e.o[2]	92.50 ± 1.07d	94.16 ± 1.09c	97.50 ± 0.55c

Multiple range test using Tukey's test (p<0.05). Within each compound, the same letters denote treatments not significantly different from each other.

n= 120 number of individuals.

[1]SE= Standard error.

[2]e.o= Essential oil of *Acantholippia deserticola.*

In Table Nº 1, it is shown the mortality effect of essential oil against wooly whitefly nymphs. Both, the length of time and the doses of essential oil influenced the mortality of nymphs. The highest nymph mortality was seen at 7th day at 10% *A. deserticola* essential oil concentration. The rapidity of the effect against some pests suggests a neurotoxic mode of action. Indeed, the interference with either the neuromodulator octopamine (Kostyukovsky *et al*., 2002) or with GABA-gated chloride channels (Priestley *et al*., 2003) has been reported for essential oils containing thujone.

Table Nº 2 shows the mortality at 24 and 48 h caused by *A. deserticola* essential oil against two-spotted mite adults when used at concentrations ranging of 2.0, 3.5 and 5.0%. At 48 h, the mortality by 5.0% essential oil was higher than 82%. While the concentrations of essential oil clearly influenced the mortality of mite adults, the length of time did not affect the mortality since similar values for each concentration of essential oil were observed at either 24 h or 48 h. Interestingly, a similar effect against *T. urticae* by using essential oil from *Tanacetum vulgare* has been reported (Chiasson *et al*., 2001). Indeed, at 48 h post-application and at doses of 4 and 8%, this essential oil causes a mortality of 75.6 and 95.6%, respectively. Since this plant (*T. vulgare*) contains 91.1% of β-thujone as a major constituent (a similar amount to that measured in *A. deserticola*), this make possible that the acaricide activity by essential oil from both plants may be triggered by β-thujone.

Table Nº 3 shows the results of tests carried out against *B. chilensis* adults at 24 and 48 h. The analysis of variance shows significant differences between treatments at either 24 h ($F_{3, 36} = 24.99$; $p < 0.001$) or at 48 h ($F_{3, 36} = 49.89$; $p < 0.001$). However, the length of time did not influence the mortality rates of adult *B. chilensis* exposed to different *A. deserticola* essential oil concentrations. From a mechanistic point of view, the toxicity of essential oil may be related to the impairment of critical functions. Indeed, most monoterpenes are cytotoxic to plants and animal tissue, causing a drastic reduction in the number of intact mitochondria and Golgi bodies, impairing respiration and photosynthesis and decreasing cell membrane permeability (Tripathi *et al*., 2000).

Table Nº 2
Percentage of mortality against *Tetranychus urticae* (adults) exposure at different concentration of *Acantholippia deserticola* essential oil by means of direct and residual application

Treatment	n[1]	24 h	n[1]	48 h
		% (media) ± SE[2]		% (media) ± SE[2]
Control	93	1.00 ± 0.32a	92	1.11 ± 0.36a
Ethanol 65%	101	1.00 ± 0.31a	100	2.11 ± 0.44a,b
2.0 % v/v e.o[3]	98	10.44 ± 1.00a	97	14.66 ± 1.13b
3.5 % v/v e.o[3]	100	58.00 ± 2.30b	100	61.00 ± 2.47c
5.0 % v/v e.o[3]	101	80.27 ± 0.91b	101	82.18 ± 0.78c

Multiple range test using Tukey's test ($p < 0.05$). Within each compound, the same letters denote treatments not significantly different from each other.
[1]n = number of individuals.
[2]SE = standard error.
[3]e.o = Essentials oil of *Acantholippia deserticola*.

Table Nº 3
Percentage of mortality against *Brevipalpus chilensis* (adults) exposure at different concentration of *Acantholippia deserticola* essential oil by means of direct and residual application

	24 h		48 h	
Treatment	n[1]	% (media) ± SE[2]	n[1]	% (media) ± SE[2]
Ethanol 65%	98	3.00 ± 0.68a	98	5.11 ± 0.72a
2.0 % v/v e.o[3]	100	21.00 ± 1.66b	101	26.00 ± 1.70b
3.5 % v/v e.o[3]	100	69.00 ± 2.02c	99	77.88 ± 2.20c
5.0 % v/v e.o[3]	100	77.00 ± 1.49c	101	89.18 ± 0.85c

Multiple range test using Tukey's test ($p < 0.05$). Within each compound, the same letters denote treatments not significantly different from each other.
[1]n = number of individuals.
[2]SE = Standard error.
[3]e.o = Essential oil of *Acantholippia deserticola*.

Table Nº 4
Lethal concentration values (expressed in v/v) against *Tetranychus urticae* and *Brevipalpus chilensis* plagues adult and 95% confidence intervals.

Time	*Tetranychus urticae*				*Brevipalpus chilensis*			
hours	LC_{50}	95%CI[1]	LC_{90}	95% CI	LC_{50}	95% CI	LC_{90}	95% CI
24	4.1a	2.8 – 6.7	7.5a	5.2 – 39.9	2.9a	2.2 – 3.7	6.6a	4.9 – 12.9
48	3.2a	2.0 – 5.1	6.4a	4.4 – 33.5	2.6a	2.3 – 2.8	7.1a	6.1 – 8.8

[1] Confidence intervals.
[2] Different letters within each column are significantly different at $p < 0.05$ based on not overlapping 95% confidence intervals

Table 4 summarizes the lethal concentration values (95% confidence intervals) obtained when adult individuals of both plagues, namely *T. urticae* and *B. chilensis*, were exposed to *A. deserticola* essential oil. The values were calculated by using Probit analysis.

The estimated LC_{50} values for *T. urticae* were 4.1% (2.8-6.7) and 3.2% (2.0-5.1), and their LC_{90} values were 7.5% (5.2-39.9) and 6.4% (4.4-33.5), at 24h and 48h respectively. On the other hand, LC_{50} values for *B. chilensis* were 2.9% (2.2-3.7) and 2.6% (2.3-2.8), and their LC_{90} values were 6.6% (4.9-12.9) and 7.1% (6.1-8.8) at 24h and 48h respectively. These results indicate that similar concentrations are required at 24 h and 48 h to provoke 50% and 90% mortality. Although the literature reporting the effects of essential oils on insects is scarce, the treatments with various essential oils or their constituents cause similar symptoms suggesting a neurotoxic mode of action (Kostyukovsky *et al*., 2002).

It is noteworthy that no research regarding a pesticide use from *A. deserticola* essential oil has been done and reported up to now. Thujone however, which has been identified as the main constituent of *A. deserticola* essential oil, has been found in several plants like *Artemisia absinthium*, *Thuja occidentalis*, *Salvia officinalis* with several biological activities including insecticidal (Höld *et al*., 2000, Tripathi *et al*., 2000), acaricidal (Chiasson *et al*., 2001; Tsolaskis and Ragusa 2004; Ruffinengo *et al.*, 2005), fungicidal (Mohamed and Geetha, 2004) and bactericidal (Morales *et al*., 2002).

CONCLUSIONS

Results obtained during this study showed marked toxic effects leading to mortality in nymphs and adult individuals of *A. floccosus*, *B. chilensis* and *T. urticae*. Considering the currently use of synthetic insecticides associated with harmful effects, the pesticide activity shown by the crude essential oil from *A. deserticola* is quite promising and may be developed as natural pesticide to control some major plagues. However, prior to the practical application of this essential oil, further studies are necessary to determine its safety human utilization and possible phytotoxicity to crops of economic importance. In addition, it is also required the development of adequate oil formulations reducing costs and improving its efficacy and stability.

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