Insecticidal Activity of Essential Oils on *Triatoma infestans*

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Sixty-three essential oils isolated from Bolivian plants were tested on *Triatoma infestans* for ovicidal and larvicidal properties. Three types of test were used: topical application on insects, nymphs on impregnated paper and eggs on impregnated paper. Twenty oils showed an interesting activity on nymphs and eggs when the impregnated paper tests were used. These tests proved to be the most sensitive and were therefore chosen for studying the action of a dozen terpenes present in those active essential oils. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

Parasitic diseases represent a serious health problem in most developing countries. Chagas' disease, caused by *Trypanosoma cruzi*, affects at present 16 to 18 million people in Latin America (WHO, 1991).

Chagas' disease is transmitted to humans by some Triatominae species which may differ from one region to another. However, *Triatoma infestans* is the most widespread, extending from the arid Peruvian highlands to the very dry northeast Brazilian regions, including the temperate plains of Argentina (Schofield, 1994).

In the absence of an effective treatment for Chagas' disease, the eradication of the Triatominae in the infected regions is one of the best methods to prevent the disease from spreading, particularly when combined with improved housing and sanitary education.

At present, synthetic pyrethroids (e.g. deltamethrin) are among the most effective insecticides against Triatominae (Casabe *et al.*, 1988) but the cost of vector control is beyond the financial resources of some countries. In addition, with the possible development of resistance, the search for new natural insecticide compounds derived from plants represents an important scientific challenge.

In Bolivia, many local plants are used by rural people against plant pests and insects. 'Muña blanca' (*Mynthostachys andina*) is used to control potato worms and external livestock parasites; the branches of 'muña negra' (*Hedeoma mandoniana*) serve as brooms for floor cleaning, which helps to rid houses of lice and fleas; 'suico' or 'wakataya' (*Tagetes minuta*) and 't'hola' (name given to different *Baccharis*) are used to eliminate intestinal worms; 'ch'illca' (*Parastrephia lucida*) has acaricidal properties and is used to heal alpaca scabies (*Sarcoptes scabiei*) in the highlands. All these plants have an aromatic character and contain essential oils that could be responsible for the observed activities.

Saxena and Koul (1978) reviewed the use of essential oils for insect control. Palevitch and Craker (1994) mention the insecticidal activity of aromatic plants such as *Pimpinella anisum*, *Matricaria recutita*, *Anethum graveolens*, *Eucalyptus globulus*, *Tanacetum parthenium*, *Tanacetum vulgare*, *Allium sativum*, *Mentha pulegium*, *Rosmarinus officinalis*, *Ruta graveolens*, *Salvia officinalis* and *Citrus aurantium*. Oil extracted from various parts of *Tagetes minuta* (Asteraceae) is useful in the tropics as a blowfly dressing for livestock, and its high level of effectiveness may warrant its commercialization as a mosquito larvicide (Klocke, 1989).

Triatominae seem to be sensitive to some essential oils. The essential oil of *Melia azederach* (paraíso) slightly increases the mortality rate of *T. infestans* nymphs treated with topical applications (Arias and Schmeda-Hirschmann, 1988). The German cockroach, which could be as resistant as the Triatominae, is also sensitive to essential oils such as those derived from *Tridax procumbens* and *Cyathocline lyrata* (Pathak and Dixit, 1988), or to monoterpenes (components of essential oils) such as D-limonene, linalool and pulegone (Coats *et al.*, 1991).

Few screenings for active components have been carried out with essential oils in the search for insecticides; however, terpenoids make good candidates as most of them are pleasant smelling and non-toxic to humans and other mammals.

This article describes the methodology employed to detect the insecticidal effects on *T. infestans* of 63 essential oils obtained from Bolivian plants and 12 active plant terpenes.

MATERIALS AND METHODS

Plant collection and essential oil extraction. Plants showing an aromatic character were collected in different regions of Bolivia and their essential oils obtained from fresh material by steam distillation. Each sample was analysed by gas

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Table 1. Toxicity of essential oils on Triatominae observed with three types of test

Plant family	Scientific name	Common name	Plant part	Collect. place	Topical application		Impregnated paper (larvae)		Impregnated paper (eggs)
ANACARDIACEAE	*Schinus molle*	mulli	Seed	Cochabamba	2 Kd	3†	—	—	—
	Schinus polygamus	luyu luyu	Leaf	Yuraj Molino	—	—	—	—	—
	Schinus pearcei	terebinto	Leaf	Cochabamba	1† & 1 Kd	—	1 Kd	—	—
APIACEAE	*Coriandrum sativum*	coriandro	Seed	Cochabamba	—	—	4† & 1 Kd	5†	ovicide (6†/10) & larvicide (4†/4)
	Eryngium sp.	quilquiña del monte	Aerial part	Chapare	—	—	—	—	ovicide (10†/10)
	Pimpinella anisum	anis	Seed	La Paz	3 Kd	3 Kd	4†	5†	ovicide (7†/10) & larvicide (3†/3)
							1† & 1 Kd		
ASTERACEAE	*Acanthostyles buniifolium*	romerillo	Aerial part	Totora	—	—	—	—	—
	Acanthostyles buniifolium	romerillo	Aerial part	Tiraque C	—	—	—	—	—
	Baccharis dracunculifolia	thola	Aerial part	Molino	2†	1† & 1 Kd	—	—	—
	Baccharis dracunculifolia	thola	Aerial part	Tiraque C	1†	—	—	—	—
	Baccharis genistelloides		Aerial part	Monte punku	—	—	—	—	—
	Baccharis latifolia	chilca negra	Aerial part	Corani	1†	—	1†	—	larvicide (Kd) (8/10) **& (3/10)**
	Baccharis latifolia	chilca negra	Aerial part	Tiraque C	2†	2†	—	—	—
	Baccharis pentandlii		Leaf	Corani	2†	—	—	—	—
	Baccharis salicifolia	thola alta	Leaf	Corani	—	—	—	—	larvicide (8†/10)
	Baccharis sp.		Aerial part	Candelaria	—	—	—	—	ovicide (6†/10) & larvicide (4†/4)
	Baccharis sp.		Aerial part	Corani	—	—	3 Kd	—	larvicide (10†/10)
							2 Kd		**larvicide (10†/10)**
	Chenopodium ambrosioides	paico	Aerial part	Cochabamba	—	2†	5†	5†	—
	Chrysanthemum parthenium	pelitre	Aerial part	Colomi	—	—	3†	3† & 1 Kd	larvicide (10†/10)
	Coniza rurigena	waytcha	Aerial part	Cochabamba	3 Kd	1†	—	—	—
	Coreopsis fasciculata	misuka	Aerial part	Tiraque C	—	—	2† & 1 Kd	2† & 1 Kd	—
	Gnaphalium gaudichaudianum	wira wira	Aerial part	Monte Punku	—	—	—	—	—
	Gnaphalium gaudichaudianum	wira wira	Aerial part	Tiraque C	2†	1† & 1 Kd	—	—	—
	Gynoxis sp.		Aerial part	Cochabamba	—	—	—	—	—
	Matricaria chamomilla	manzanilla	Flower	Montecillo	3†	—	1†	—	larvicide (Kd) (10/10)
	Parastrephia lepidophyla	thola	Aerial part	La Paz	—	—	—	—	larvicide (4†/6)
	Pluchea fastigiata	uri uri	Aerial part	Cochabamba	—	—	—	—	larvicide (3†/6)
	Porophyllum ruderale	quilquiña	Aerial part	Cochabamba	—	—	1†	—	—
	Senecio adenophylloides		Aerial part	Cochabamba	—	—	—	—	larvicide (Kd) (5/7)
									larvicide (Kd) (4/5)
	Senecio hebeatus	akhana	Aerial part	La Paz	—	—	—	—	ovicide (7†/10) & larvicide (3†/3)

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Table 1. continued

Plant family	Scientific name	Common name	Plant part	Collect. place	Topical application		Impregnated paper (larvae)		Impregnated paper (eggs)
	Tagetes pusilla	pampa anis	Aerial part	Chapare	1†	—	5†	5†	larvicide (10†/10)
	Tagetes pusilla	pampa anis	Aerial part	La Paz	1 Kd	—	2† & 3 Kd	5†	ovicide (10†/10)
	Tagetes minuta	suico	Aerial part	Tusko pujillo	—	—	—	—	—
	Tagetes minuta	wakataya	Aerial part	La Paz	1†	—	2†	1†	ovicide (10†/10)
BUDDLEJACEAE	*Buddleja sp.*		Aerial part	Tiraque C	2† & 3 Kd	1†	—	—	—
	Buddleja tucumanensis		Aerial part	Totora	1†	1†	—	—	—
CAPPARACEAE	*Cleoma albida*		Aerial part	Chapare	—	—	—	—	—
	Cleoma espinosa		Aerial part	Cochabamba	4 Kd	—	1 Kd	—	—
LAMIACEAE	*Hedeoma mandoniana*	muña negra	Aerial part	Larati	—	3†	4†	4†	ovicide (7†/10) & larvicide (Kd) (3/3)
	Hedeoma mandoniana	muña negra	Aerial part	Tiraque C	1 Kd	—	3†	3†	—
	Lepechinia floribunda		Aerial part	Montecillo	—	—	—	—	—
	Lepechinia graveolens	rakha rakha	Aerial part	Tiraque C	—	—	—	—	—
	Lepechinia meyeni		Aerial part	Tiraque C	—	—	2†	2†	—
	Mentha arvensis	menta	Aerial part	Chapare	—	—	5†	5†	ovicide (10†/10)
	Myntostachys andina	muña blanca	Aerial part	Larati	2 Kd	1†	1 Kd	4† & 1 Kd	ovicide (10†/10)
	Myntostachys andina	muña blanca	Aerial part	Tiraque C	8†	1†	3†	1†	—
	Mynthostachys mollis	mintostachis	Aerial part	La Paz	1 Kd	—	1†	2† & 2 Kd	ovicide (10†/10)
	Ocimum basilicum	albahaca	Aerial part	Chapare	—	—	1†	—	ovicide (5†/10) & larvicide (5†/5)
	Rosmarinus officinalis	romero	Aerial part	La Paz	—	—	1†	—	larvicide (3†/9)
	Salvia hankei		Aerial part	Montecillo	2†	1† & 1 Kd	—	—	—
	Satureja boliviana	khoa	Aerial part	La Paz	—	—	—	—	ovicide (7†/10) & larvicide (2†/3)
	Satureja sp.		Aerial part	Tiraque C	4† & 4 Kd	4† & 3 Kd	5†	5†	—
MYRTACEAE	*Eucalyptus citriodora*		Leaf	Chapare	—	—	—	—	ovicide (7†/10) & larvicide (3†/3)
	Eucalyptus globulus	eucalipto	Leaf	Cochabamba	—	—	3† & 2 Kd	4 Kd	—
PINACEAE	*Pinus radiata*	pino	Leaf	Quillacollo	—	—	—	—	—
POACEAE	*Cymbopogon citratus*	pasto cedron	Aerial part	Chapare	—	—	—	—	—
	Cymbopogon citratus	paja cedron	Aerial part	La Paz	—	—	—	—	ovicide (6†/10) & larvicide (4†/4)
	Vetiveria zizanioides	vetiver	Root	Chapare	—	—	3† & 2 Kd	—	—
UMBELLIFERAE	*Foeniculum vulgare*	hinojo	Seed	Montecillo	1†	3 Kd	2† & 3 Kd **1†**	5† **1 Kd**	ovicide (9†/10)
VERBENACEAE	*Aloysia gratissima*	kutu kutu	Leaf	Cochabamba	2†	4†	1†	3† & 1 Kd	—
	Lantana sp.		Aerial part	Montecillo	2† & 1 Kd	2† & 2 Kd	—	—	—
	Lippia boliviana	toronjil del monte	Aerial part	Chapare	—	—	—	—	—
ZINGIBERACEAE	*Custos albiflora*		Root	Chapare	—	—	2†	1 †	—

†, dead insect or egg; Kd, insect knocked down.
Results correspond to 1 mg/insect for topical application and 2 mg/cm² for impregnated papers. Results shown in bold correspond to a concentration of 200 μg/cm² for impregnated papers.

chromatography (GC) due to the possible variability of essential oil composition depending on collection place and date. A Shimadzu chromatograph model GC-14B, equipped with a DB-225 (Supelco Inc.) capillary column (30 m), was used for this analysis.

Insects. *T. infestans* were raised in the laboratory under controlled temperature (28°C±1°C) and relative humidity (80% RH±10%).

Chemicals. Deltamethrin was used from a K-Othrin solution (E.C. 25, Roussel Uclaf). Pyrethrins I and II were isolated from a pyrethrum extract (Programa Agroquímico). The other pure compounds tested came from different sources as follows: E-nerolidol, 1-8 cineole (Programa Agroquímico), limonene, L-menthol and linalool (Merck), menthyl acetate, L-menthone, E-anethol and pulegone (Aldrich), isomenthol and α,β-thujone (Fluka) and piperitone (Laseve, Canada).

Biological tests. All the tests were conducted during all the experiments under controlled temperature (27°C±1°C) and relative humidity (80%RH±10%).

Topical application on insects. The essential oils were used as ethanol solutions with concentrations of 2% and 20% (v:v). 1 µL of each solution was applied directly over the abdomen of ten fourth instar nymphs of *T. infestans*. After observing daily for a week, the nymphs were treated again with 5 µL of the same solution. Two sets of controls were utilized. One control group was treated with ethanol only, while the other was not treated at all. The effect of the application (knock-down or mortality) was observed for another week and compared with the controls. Insects were considered to be knocked-down if they laid on their backs and were unable to right themselves when disturbed. This experiment was carried out in duplicate on different days.

Nymphs on impregnated paper. 200 µL of each ethanol solution of essential oils (2% and 20%, v:v) were deposited over filter paper disks having an area of 20 cm^2, resulting in concentrations of 2 and 0.2 mg/cm^2 on the paper (taking the density of the essential oils to be 1). The disks were dried at room temperature (about 25°C) for 5 min and placed in Petri dishes, then five fourth instar nymphs of *T. infestans* were introduced in each dish. Insect control groups were treated in the same way but dosed only with ethanol. The effect (knock-down or mortality) was observed daily for 4 days and compared with controls. This experiment was carried out in duplicate on different days.

With the pure compounds, the experiment was carried out in triplicate on different days using nine ethanol solutions with the following concentrations: 20; 15; 12.5; 10; 7.5; 5; 3.5; 2 and 1% corresponding to 2; 1.5; 1.25; 1; 0.75; 0.5; 0.35; 0.2 and 0.1 mg/cm2 on the paper. The results were compared with controls treated with deltamethrin and a mixture of natural pyrethrins I and II under the same conditions.

Eggs on impregnated paper. 200 µL of ethanol solutions of essential oils (2% and 20%, v:v) were deposited over filter paper disks having an area of 20 cm^2. The disks were dried at room temperature (about 25°C) for 5 min and placed in Petri dishes. Ten eggs of *T. infestans*, selected for their age (about 1 week) according to the characteristic cream colour, were introduced in each dish. Two sets of controls were utilized. One set of control eggs was placed over a paper disk impregnated with ethanol only while the other was deposited over a paper disk not treated at all. The hatching of the larvae and the effect on them (knock-down or mortality) were observed every day until the control eggs completed hatching. This experiment was carried out in duplicate on different days.

With the pure compounds, the experiment was carried out in triplicate on different days using nine ethanol solutions with the following concentrations: 20; 15; 12.5; 10; 7.5; 5; 3.5; 2 and 1% corresponding to 2, 1.5; 1.25; 1; 0.75; 0.5; 0.35; 0.2 and 0.1 mg/cm^2 on the paper. The results were compared with controls treated with deltamethrin and a mixture of natural pyrethrins I and II respectively.

Statistical analysis. The lethal doses LD_{50} were calculated with the INRA-INSA 'Toxicologie' analysis software which uses the probit method developed by Bliss (1935).

RESULTS AND DISCUSSION

Screening of essential oils using three types of test

In order to evaluate the toxicity of 63 essential oils extracted from Bolivian plants on *T. infestans* eggs and larvae, three types of test were used (Table 1). The topical application test and the impregnated paper test on fourth instar nymphs are both recommended by WHO (1994). The impregnated paper test on eggs gives indications of the ovicidal and the larvicidal activities of the oils on hatching first instar larvae.

For the primary screening of essential oils, the three tests were duplicated on different days. The results (Table 1) generally show a good reproducibility. Where this is not the case, analysis of the GC data can be used to determine whether a difference in the chemical composition is responsible. The impregnated paper test is more sensitive than the topical application technique, but requires a rather longer observation period. As the essential oils are made up of volatile compounds, it is possible that, when they are applied directly on the insects, their potency is quickly lost due to the evaporation of the active components. Conversely, a greater amount is applied on the impregnated paper making the Petri dish saturated with vapors which can possibly affect the insects. In the ovicidal test, the larvae take 15 days to hatch, therefore only remnant products will remain in the dish, which may explain why some oils show no larvicidal activity with this test even though they showed toxicity with the two other types of experiments.

Plants whose essential oils have a larvicidal activity on fourth instar nymphs with the impregnated paper test are: *Coriandrum sativum*, *Pimpinella anisum*, *Chenopodium ambrosioides*, *Tagetes pusilla*, *Menta arvensis*, *Satureja sp*, *Foeniculum vulgare*, and to a lesser extent, *Chrysanthemum parthenium*, *Hedeoma mandoniana*, *Mynthostachys andina* and *Eucalyptus globulus*. The essential oil of *Baccharis sp.* showed a high larvicidal activity with young larvae that emerged during the ovicidal test. An ovicidal effect was observed with the essential oils extracted from the following plants: *Eryngium sp., Tagetes pusilla, Tagetes minuta, Mentha arvensis, Mynthostachys andina, Mynthostachys mollis* and *Foeniculum vulgare*.

Terpenes activity on Triatominae larvae and eggs

In order to search for the active components responsible for these larvicidal or ovicidal properties, we studied the main compounds of each oil (Fig. 1) that had shown toxicity towards eggs or fourth instar nymphs when using the impregnated paper method.

The main components of these active essential oils were determined by gas chromatography. These were E-anethol for anisic oils [*Pimpinella anisum* (74%), *Tagetes pusilla* (96%), *Foeniculum vulgare* (66%) and *Coreopsis fascicu-lata* (23%)], limonene (38%) and ascaridol (35%) in *Chenopodium ambrosioides*, pulegone (49%) in *Hedeoma mandoniana* and (53%) in *Mynthostachys andina*, L-menthone (29%) in *Mynthostachys andina* and (73%) in *Mentha arvensis*, 1-8 cineole (63%) in *Eucalyptus globulus*, E-nerolidol (80%) in *Baccharis sp.* and linalool (82%) in *Satureja sp.* Menthyl acetate, L-menthol, isomenthol and piperitone were also present in *Mentha arvensis*. Moreover, α,β-thujone was tested on account of its reputed acute toxicity.

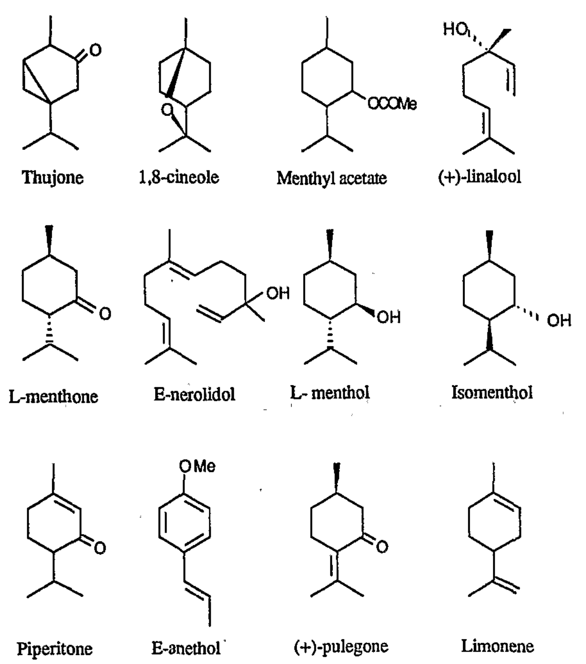

Figure 1. Main essential oil compounds tested on *Triatoma infestans* larvae and eggs.

The activity of these terpenes was compared with two controls: deltamethrin and a mixture of natural pyrethrins I and II. The experiments were carried out in triplicate on different days according to the recommendations of WHO for the assessment of insecticidal effects on Triatominae (WHO, 1994).

The results are shown in Table 2. The products can be classified in five groups: (i) products showing no toxicity, such as menthyl acetate, α,β-thujone and limonene; (ii) products revealing an ovicidal activity such as L-menthol, isomenthol, piperitone, linalool and E-nerolidol; (iii) substances demonstrating a weak larvicidal action such as 1-8 cineole and L-menthone; (iv) those showing a medium larvicidal effect like pulegone and piperitone; (v) those revealing a high larvicidal activity such as isomenthol, linalool, E-anethol and E-nerolidol.

Nevertheless, even the substances rated as having 'high activity' fall far short of the results obtained using the control insecticides. It is estimated that deltamethrin has an LD_{50} of 0.1 $\mu g/cm^2$ for the larvicidal test on fourth instar nymphs (i.e. approximately 1/10 000 the concentration of the natural terpenes used in our experiments) resulting in knock-down symptoms (no locomotor activity even when stimulated with a paint brush). The pyrethrin I and II mixture has an LD_{50} only slightly higher (0.5 $\mu g/cm^2$), causing a different form of knock-down (insects remain on their back but wiggle their legs when stimulated with a paint brush).

The larvicidal activities of these different terpenes are not high enough to consider relying solely on them as insecticides. On the other hand, some of them display an ovicidal activity which could be exploited in a combined treatment together with deltamethrin. Further studies on synergistic effects between these terpenes and deltamethrin are under consideration.

Table 2. Toxicity (LD_{50}) of terpenes against Triatominae larvae and eggs with the impregnated paper test

Pure products	Ovicidal effect	Larvicidal effect (1st instar)	Larvicidal effect (4th instar)
Menthyl acetate	>to 2 mg/cm^2	>to 2 mg/cm^2	>to 2 mg/cm^2
L-menthone	>to 2 mg/cm^2	>to 2 mg/cm^2	1.49 mg/cm^2
L-menthol	0.61 mg/cm^2	>to 0.75 mg/cm^2	>to 2 mg/cm^2
Isomenthol	0.76 mg/cm^2	0.5 mg/cm^2	0.29 mg/cm^2
Piperitone	1.65 mg/cm^2	0.96 mg/cm^2	0.65 mg/cm^2
Linalool	0.58 mg/cm^2	0.45 mg/cm^2	0.57 mg/cm^2
Pulegone	>to 2 mg/cm^2	0.75 mg/cm^2 (Kd)	1.07 mg/cm^2
1–8 cineole	>to 2 mg/cm^2	>to 2 mg/cm^2	1.38 mg/cm^2
alpha,beta-thujone	>to 2 mg/cm^2	>to 2 mg/cm^2	>to 2 mg/cm^2
Limonene	>to 2 mg/cm^2	>to 2 mg/cm^2	>to 2 mg/cm^2
E-anethol	>to 2 mg/cm^2	0.83 mg/cm^2	0.26 mg/cm^2
E-nerolidol	0.5 mg/cm^2	0.14 mg/cm^2	1.35 mg/cm^2
Deltamethrin	>to 2 mg/cm^2	0.05 mg/cm^2 (Kd)	0.1 μg/cm^2 (Kd)
Pyrethrins I and II	>to 2 mg/cm^2	0.05 mg/cm^2 (Kd)	0.5 μg/cm^2 (Kd)

Products were tested at 2: 1.5; 1.25; 1; 0.75; 0.5; 0.35; 0.2 and 0.1 mg/cm^2.
Kd, insects knocked down.

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