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PEST MANAGEMENT

Toxicity of Ethanolic Extracts from *Lippia origanoides* and *Gliricidia sepium* to *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae)

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Abstract

Botanical compounds with insecticidal and acaricidal activities have been used in pest management with different levels of success. Toxicity of ethanolic extracts obtained from wild oregano (*Lippia origanoides*) and gliricidia (*Gliricidia sepium*) to *Tetranychus cinnabarinus* (Boisduval) were evaluated. Mite population was collected from black bean plants growing in Urachiche Municipality, Yaracuy State, Venezuela. Ethanolic extracts of wild oregano and gliricidia leaves were evaluated at different concentrations (5, 10, 15, and 20%) using the leaf disk immersion technique. The presence of alkaloids, flavonoids, phenols and tannins, essential oils and saponins was verified in the plant material used in our study. *Tetranychus cinnabarinus* oviposition decreased at a rate of 43.7% or 57% when 5% oregano or gliricidia extracts were used, respectively. Also, 10% oregano or gliricidia extracts caused 42.2% or 72.5% of mortality to *T. cinnabarinus*, respectively. Ethanolic extracts showed acaricidal effects on *T. cinnabarinus*, as evidenced by maximum mortality (96.6% and 100% caused by wild oregano and gliricidia, respectively) when used at a concentration of 20%. Our results showed that gliricidia and wild oregano are promising for the management of *T. cinnabarinus*, although their field efficacy remains to be evaluated.

Introduction

The pesticide activity from secondary metabolites occurring in plants is based on plant-pest or plant-pathogen interactions (Hilker & Meiners 2002, Praslička & Huszár 2004), hence most of these natural substances show insecticidal, nematicidal, fungicidal and bactericidal properties. Previous studies have proved growth inhibition in ticks (Alvarez *et al* 2008) and in the brown avocado mite, *Oligonychus punicae* (Hirts) (Vásquez *et al* 2008). Thus, plant-derived molecules with acaricidal activity have emerged as an ecological strategy for pest management (Castiglioni *et al* 2002, Araújo *et al* 2010, Cavalcanti *et al* 2010).

As botanical pesticides can provide mite control at a low cost, and with a low risk to human and to the environment, their recommendation as a control strategy of tetranychids have been increased (Gencsoylu 2007). Powders and ethanolic or aqueous extracts obtained from different plant species have been used for population pest management. There are several plant species which were investigated and shown to produce compounds which can cause mortality in several *Tetranychus* species either by contact on by systemic exposure (Chiasson *et al* 2001, Shi *et al* 2004). In the case of *T. urticae*, 67 plant species belonging to six subfamilies from Lamiaceae were shown to produce compounds with acaricidal properties, from which *Plecthratus* was the genus to show the highest diversity of species (14) producing highly toxic compounds against tetranychids (Rasikari *et al* 2005).

The carmine spider mite *Tetranychus cinnabarinus* (Boisduval) is reported in more than 130 plant species of economic importance, including vegetables, fruit-trees, and ornamentals (Bolland *et al* 1998, Guo *et al* 1998). Synthetic acaricides have been used as the main strategy for *Tetranychus* population management resulting in an increased cost for production and environmental impacts (Argüelles *et al* 2006). Intensive pesticide use has raised resistance development to dicofol, amitraz, propargite, and even to the newly synthesized molecules such a hexythiazox, pyridaben, and tebufenpyrad as for *Tetrancyhus urticae* Koch (Ramasubramanian *et al* 2005) and to abamectin as for *T. cinnabarinus* (Lin *et al* 2009).

Wild oregano (*Lippia origanoides*) and gliricidia (*Gliricidia sepium*) are commonly found in arid environmental conditions in Lara State, Venezuela, representing a valuable source to obtain extracts with pesticidal activity. Biological activity of essential oils from oregano in controlling several microorganisms such as *Staphylococus aureus* (MRSA), *S. aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Candida albicans* and *C. tropicales* was already demonstrated (Dos Santos & Arimatera 2004), but no data is available on its potential or of gliricidia to produce molecules with pesticide activity.

The carmine spider mite is an important pest of various economically important crops often requiring acaricidal treatment (Çakmak *et al* 2005), although the chemical spraying have frequently failed to properly control this pest (Guo *et al* 1998). Thus, the toxicity of ethanolic extracts of *L. origanoides* and *G. sepium* was evaluated in this study based on biological parameters of *T. cinnabarinus*, aiming to provide an ecological alternative control method against this mite species.

Material and Methods

Plant extracts

Extracts of *L. origanoides* and *G. sepium* were obtained from natural populations (Marcano & Hasegawa 2002). Mature leaves were dried and macerated and soaked in 96% ethanol for one to two weeks. The liquid was filtered using a four-layered gauzed and concentrated in a rotary vacuum drier system (roto-vapor Brinkmam^{MR}, Mod. RE111) at 100°C. The concentrated crude extract obtained after water and ethanol evaporation was recovered from the rotary drier system and stored at 8°C until qualitative determination of secondary metabolites and evaluation of the acaricidal properties.

Secondary metabolites

Qualitative determination of secondary metabolites (alkaloids, essential oils, polyphenol and tannin, saponins, antraquinones and flavonoids) in ethanolic extract followed the methods in Marcano & Hasegawa (2002). After dehydration, the ethanolic extract was mixed with 2.5 ml 10% hydrochloric acid and 5 ml chloroform in a separatory funnel to determine alkaloids. This procedure yielded three phases, with the weak-basic alkaloids being collected in the first phase. The intermediary phase was alkalinized with ammonium hydroxide (NH_4OH) and re-extracted with chloroform in order to obtain the basic alkaloids. The third phase aimed to detect the quaternary ammonium salts. One microlitre aliquot from each of phases was placed on a silica-gel cromatoplate and treated with Meyer's reagent. Alkaloids presence was confirmed by formation of a brown-reddish coloration. Results were confirmed by adding Dragendorff's reagent.

Essential oils were determined by organoleptic procedures using characteristic odor as indicative (Marcano & Hasegawa 2002). Total polyphenols and tannins were evidenced by characteristic brown coloration in ethanolic extract when added 1% FeCl. The extract was evaporated and added 1% agar in a 1% NaCl solution. The presence of polyphenols and tannins was indicated by the formation of a precipitate (Marcano & Hasegawa 2002).

Saponins were evidenced by the formation of a spume which remained more than 20 min when ethanolic extract was strongly shaken with distilled water (Marcano & Hasegawa 2002). For the determination of the presence of antraquinone, the ethanolic extract was neutralized with 5 ml 0.5% KOH, acidified with 0.5 ml acetic acid (pH 4.8) and then agitated with 5 ml benzene. Finally, NH_4OH was added and antraquinone presence was indicated by the development of a reddish coloration.

Flavonoids were detected by a reddish coloration after 20 min 0.5 ml 10% HCl and magnesium (0.35 g) were added to the ethanolic extract. Flavonoids were confirmed by the yellow color development of sample spots in 1% $AlCl_3$-treated filter papers, when observed under 450 nm UVL.

Mite collection and rearing

Mites were collected on 40 d-old black bean plants *Phaseolus vulgaris* growing under irrigation conditions during a dry season in Fundo Zamorano Bella Vista, Urachiche Municipality, Yaracuy State, Venezuela. In the laboratory, females and males were mounted on slides for microscope observations using the Hoyer´s medium. Taxonomical identification was made by using the taxonomical keys of Gutierrez (1985), and species identification was confirmed by comparisons with voucher specimens from the Acarological Collection from Universidad Central de Venezuela, especially by comparing the morphology of the aedeagus (Ochoa *et al* 1994).

A laboratory mite culture was initiated with 100 females and males from a field population using rearing units or arenas (Helle & Overmeer 1985) maintained at 27 ± 2ºC, 70 ± 10% RH and 12:12 photoperiod. Males and females were transferred to *P. vulgaris* leaf disks (6 cm diameter) on each rearing unit (9 cm diameter and 1.5 cm high). They were maintained for 24h period to lay eggs. After this period, males and females were discarded and the eggs were reared until adult emergence.

Toxicity of ethanolic extracts to T. cinnabarinus

Toxicity of ethanolic extracts to *T. cinnabarinus* was evaluated following Cowles *et al* (2000). Leaf disks (6 cm diameter) were soaked during five seconds in ethanolic extract (5, 10, 15, and 20%) from both plant species evaluated or into distilled water as a control treatment (0%). Afterwards, the excess of liquid was eliminated and leaves were left to dry at room temperature (25 ± 2 ºC) during 0.5h, before being offered as a substratum into the rearing units. Then, 10 1 d-old females were randomly selected from the laboratory culture and placed on each of the rearing units containing leaf disks from the respective plant species and extract concentration. Ten replicates were run for each treatment. The evaluations were conducted at 24, 48 and 72h after treatment to determine female mortality and oviposition. A female was considered dead when not responding to touch.

The experiments were carried out in a completely randomized design with treatment arranged in a divided plot in time, with the plant extract as the main plot and concentrations as the subplots. Oviposition rate was calculated as the mean number of eggs laid per female per day. Data were corrected for control mortality (Abbott 1925). Linear regression analysis was performed to describe oviposition rate or cumulative mortality against metabolite concentration using Statistix version 8.0

Results and Discussion

Secondary metabolites

The groups of secondary metabolites were similar in extracts from wild oregano (*L. origanoides*) and gliricidia (*G. sepium*) (Table 1). Conversely to our results, alkaloids and saponins and lower concentrations of phenols were not found in gliricidia by Galindo *et al* (1989). These variations could be related to environmental conditions in which plants were cultivated, which could evoke secondary metabolites production as an adaptive strategy (Zhao *et al* 2005). Also, genetic polymorphism in plant taxa could result in differences in chemical composition in secondary metabolites (Izco 1997).

Toxicity of ethanolic extracts to T. cinnabarinus

Significant differences were detected in *T. cinnabarinus* oviposition when this mite was exposed to the different ethanolic extract concentrations and plant species ($P < 0.01$). According to regression lines correlating extract concentration and oviposition rates, higher oviposition rates were observed when leaves were treated with 5% and 10% gliricidia extracts compared to wild oregano at the same concentrations (Fig 1). However, similar oviposition rates were observed at 15% and 20%, regardless the plant species. The lowest concentration of the oregano crude extract (5%) caused 43.7% reduction in the oviposition of *T. cinnabarinus*, with a little higher reduction (57%) being induced by the gliricidia extract. No oviposition was observed when leaf discs were treated with 20% ethanolic extract solutions.

Tetranychus cinnabarinus mortality increased with concentration of oregano and gliricidia ethanolic extracts (Fig 2). Mortality was 45.7, 55.9, 69.5, and 96.6% higher than control when wild oregano extract was applied at 5, 10, 15, and 20%, respectively. Meanwhile, similar gliricidia extract concentrations caused 41.7, 80.6, 88.9, and 100% mortality.

Botanical products with activity against mites have been shown promising results in field or in laboratory conditions. High mortality levels (> 78.8%) for *T. urticae*, *T. cinnabarinus* and *T. viennensis* were observed with extracts of *Kochia scoparia* (Shi *et al* 2006) and

Table 1 Secondary metabolites ("+" = present; "-" = absent) in ethanolic extracts from oregano (*Lippia origanoides*) leaves and gliricidia (*Gliricidia sepium*) leaves and flowers.

Plant species	Alkaloids			Flavonoid	Phenol and tannins	Essential oils	Saponins
	WB	B	AQS				
L. origanoides	+	+	+	+	+	+	+
G. sepium	+	+	+	+	+	+	+

Weak-basic alkaloids (WB); basic (B) and ammonium quaternary salts (AQS).

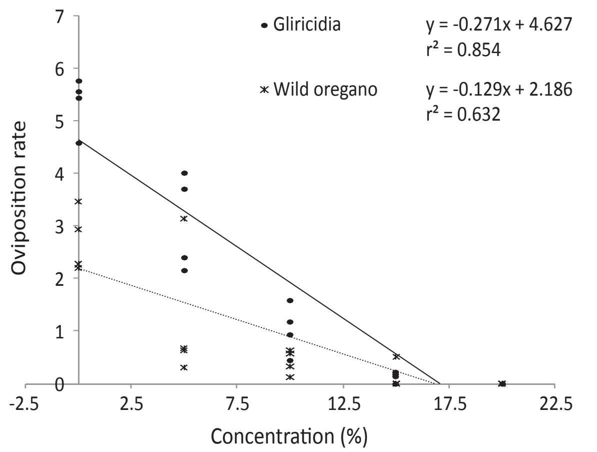

Fig 1 Oviposition rate of *Tetranychus cinnabarinus* females caged on leaf discs treated with different concentrations of ethanolic extracts from oregano and gliricidia (P < 0.001).

for *Mononychellus tanajoa* (Bondar) treated with neem extracts (Gonçalves *et al* 2001a, b). *Tetranychus urticae* females treated with leaf and fruit extracts from *Capparis aegyptia* showed high mortality and reduction in oviposition rate 15 days after applications (Hussein *et al* 2006). More recent studies have devoted to identify the active ingredients in plant extracts causing mite mortality. The metil-palmitate (MP), a fatty acid ester from *Juglans regia* seeds showed to be efficient, causing between 62.3% and 79.0% mortality of *T. cinnabarinus* and *Tetranychus viennensis* Zacher at 1 mg/ml MP, in a 24h interval (Wang *et al* 2007, Wang *et al* 2009). Furthermore, although higher MP concentration (10 mg/ml MP) caused 97.9% mortality to *T. cinnabarinus* adults, it showed lower efficacy (57.2%) when used on eggs.

Based on our results, wild oregano and gliricidia ethanolic extracts seems to be a promising alternative for pest management, which could be readily available for small growers. Thus, this natural based product could be included among the practices of carmine mite control by farmers, reducing the dependence on synthetic pesticides, and the risks of environmental pollution. Although in our study alkaloids, flavonoids, phenols and tannins, saponins and essential oils showed to be present, both in wild oregano and gliricidia ethanolic extracts, more comprehensive studies should be addressed in order to evaluate the concentration of each of these secondary metabolites in order to determine their potential use in pest control.

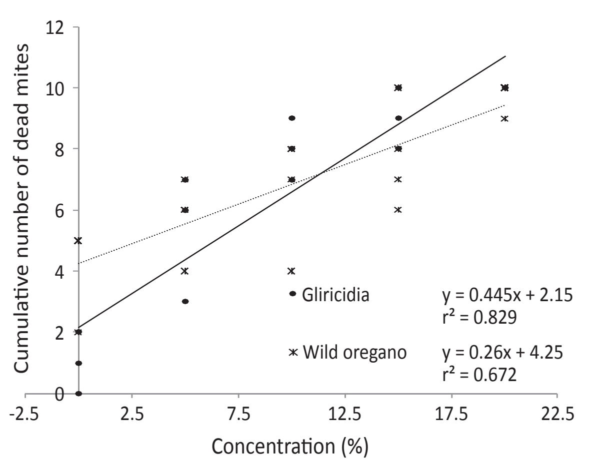

Fig 2 Cumulative mortality (from 0 to 5 days) of *Tetranychus cinnabarinus* females caged on leaf discs treated with different concentrations of ethanolic extracts from oregano and gliricidia (P < 0.001).

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265-267.

Alvarez V, Loaiza J, Bonilla R, Barrios M (2008) Control *in vitro* de garrapatas (*Boophilus microplus*; Acari: Ixodidae) mediante extractos vegetales. Rev Biol Tropl 56: 291-302.

Araújo Jr CP, Camara CA, Neves IA, Ribeiro N de C, Gomes CA, Moraes MM de, Botelho P de S (2010) Acaricidal activity against *Tetranychus urticae* and chemical composition of peel essential oils of three Citrus species cultivated in NE Brazil. Nat Prod Commun 5: 471-476.

Argüelles A, Plazas N, Táutiva D, Cantor F, Bustos A, Rodríguez D (2006) Evaluación de un método a campo abierto para la producción de *Tetranychus urticae* Koch (Acari: Tetranychidae) como suministro de presas para *Phytoseiulus persimilis* Athias. Rev Fac Cien Básicas 2: 117-122.

Bolland H, Gutierrez J, Fletchmann C (1998) World catalogue of the spider mite family (Acari: Tetranychidae). Koninklijke Brill NV, Leiden.

Çakmak Ü, Başpinar H, Madanlar N (2005) Control of the carmine spider mite *Tetranychus cinnabarinus* Boisduval by the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) in protected strawberries in Aydin, Turkey. Turk J Agric 2: 259-265

Castiglioni E, Vendramim J, Tamai M (2002) Evaluación del efecto tóxico de extractos acuosos y derivados de meliáceas sobre *Tetranychus urticae* Koch (Acari: Tetranychidae). Agrociencia. 6: 75-82.

Cavalcanti SC, Niculau Edos S, Blank AF, Câmara CA, Araújo IN, Alves PB (2010) Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). Bioresour Technol 101: 829-832.

Chiasson H, Belanger A, Bostanian N, Vincent C, Poliquin A (2001) Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by tree methods of extraction. J Econ Entomol 94: 167-170.

Cowles R, Cowles E, McDermott A, Ramoutar D (2000) "Inert" formulation ingredients with activity: toxicity of trisiloxane surfactant solutions to two spotted spider mites (Acari: Tetranychidae). J Econ Entomol 2: 180-188.

Dos Santos F, Arimatera J (2004) Composition and biological of essential oils from *Lippia origanoides* HBK. J Essential Oil Res 16: 504-506.

Galindo W, Rosales M, Murgueitio E, Larrahondo J (1989) Sustancias antinutricionales en las hojas de guamo, nacedero y matarratón. Livest Res Rural Develop 1: 36-47

Gencsoylu I (2007) Effects of the *Asphedolus aestivus* Brot. as a botanical acaricide against *Tetranychus cinnabarinus* Boisd. (Acari: Tetranychidae). Int J Agric Res 2: 189-192.

Gonçalves M, Oliveira J, Barros R, Leite M (2001a) Extratos aquosos de plantas e o comportamento do ácaro verde da mandioca. Sci Agric 58: 475-479.

Gonçalves M, Oliveira J, Barros R, Torres JB (2001b) Efeito de extratos vegetais sobre estágios imaturos e fêmeas adultas de *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae). Neotrop Entomol 30: 305-309.

González A, Del Pozo E, Galván B, González A, González J (2006) Extractos vegetales y aceites minerales como alternativa de control de mosca blanca (*Bemisia* spp) en berenjena (*Solanum melongena* L.) en el valle de Culiacán, Sinaloa, Mexico. Rev Agric 6: 84-91.

Guo F, Zhang Z, Zhao Z (1998) Pesticide resistance of *Tetranychus cinnabarinus* (Acari: Tetranychidae) in China: a review. Syst Appl Acarol 3: 3-7.

Gutierrez J (1985) Systematics, p.75-90. In Helle W, Sabelis M (eds) Spider mites: their biology, natural enemies and control. Vol 1A. Amsterdam, Elsevier Science Publishers B. V., 405p.

Helle W, Overmeer W (1985) Toxicological test methods, p.391-395. In Helle W, Sabelis M (eds) Spider mites: their biology, natural enemies and control. Vol 1A. Amsterdam, Elsevier Science Publishers B. V., 405p.

Hilker M, Meiners T (2002) Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. Entomol Exp Appl 104: 181-192.

Hussein H, Abou-Elella M, Amer A, Momen F (2006) Repellency and toxicity of extracts from *Capparis aegyptia* L. to *Tetranychus urticae* Koch (Acari: Tetranychidae). Acta Phytopathol Entomol Hung 41: 331-340.

Izco J (1997) Botánica. Madrid, Mc GrawHill, Interamericana de España, 781p.

Lin H, Gao X, Wanga J, Zhao Zh, Liu N (2009) Genetic analysis of abamectin resistance in *Tetranychus cinnabarinus*. Pestic Biochem Physiol 93: 47-52.

Marcano D, Hasegawa M (2002) Fitoquímica orgánica. Caracas, Consejo de Desarrollo Cientifico y Humanistico universidad Central de Venezuela, 588p.

Ochoa R, Aguilar H, Vargas C (1994) Phytophagous mites of Central America: an illustrated guide. Turrialba, CATIE, 234p.

Prasličkа J, Huszár J (2004) Influence of temperature and host plants on the development and fecundity of the spider mite *Tetranychus urticae* (Acarina: Tetranychidae). Plant Prot Sci 40: 141-144.

Ramasubramanian T, Ramaraju K, Regupathy A (2005) Acaricide resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae) – global scenario. J Entomol 2: 33-39.

Rasikari H, Leach D, Waterman P, Spooner-Hart R, Basta A, Banbury L, Forster P (2005) Acaricidal and cytotoxic activities of extracts from selected genera of Australian Lamiaceae. J Econ Entomol 98: 1259-1266.

Shi G, Liu S, Cao H, Zhao L, Li J, Li S (2004) Acaricidal activities of extracts of *Stellera chamaejasme* against *Tetranychus viennensis* (Acari: Tetranychidae). J Econ Entomol 97: 1912-1916.

Shi G, Zhao L, Liu S, Cao H, Clarke R, Sun J (2006) Acaricidal activities of extracts of *Kochia scoparia* against *Tetranychus urticae*, *Tetranychus cinnabarinus*, and *Tetranychus viennensis* (Acari: Tetranychidae). J Econ Entomol 99: 858-863.

Vásquez C, Aponte O, Morales J, Sanabria ME, Garcia G (2008) Biological studies of *Oligonychus punicae* (Acari: Tetranychidae) on grapevine cultivars. Exp Appl Acarol 45: 59-69.

Wang Y, Shi G, Zhao L, Liu S, Yu Q, Clarke R, Sun J (2007) Acaricidal activity of *Juglans regia* leaf extracts on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (Acari: Tetranychidae). J Econ Entomol 100: 1298-1303.

Wang Y, Wang X, Shen J, Zhao L, Clarke R, Sun J, Du Y, Shi G (2009) Methyl palmitate, an acaricidal compound occurring in green walnut husks. J Econ Entomol 102: 196-202.

Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 23: 283-333.