

COMPARISON AND CHARACTERIZATION OF GARLIC (*Allium sativum* L.) BULBS EXTRACTS AND THEIR EFFECT ON MORTALITY AND REPELLENCY OF *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE)

Carlos Augusto Hincapié Ll.^{1*}, Gloria Eugenia López P.¹, y Ricardo Torres Ch.²

ABSTRACT

Tetranychus urticae Koch is a pest that causes great economic losses because it attacks a wide range of host plants. Also, some populations have developed resistance against commercial acaricides. This work compares different extracts from garlic (*Allium sativum* L.) bulbs measuring their toxicity and repellency effects on *T. urticae*. Extracts were obtained using as solvents CO₂ in supercritical conditions (CSC), ethanol and petroleum ether using Soxhlet and soaking methods and soaking in water. The supercritical fluid extraction technique allows for obtaining extracts at low temperature using high pressures, avoiding compound degradation and making possible solvent separation by exposing the extract at room temperature. Mites were bred on bean plants (*Phaseolus vulgaris* L.) in a laboratory environment in Laureles Campus, Universidad Pontificia Bolivariana, Medellín, Colombia. Mortality was evaluated putting 10 mites in *P. vulgaris* 3 cm diameter leaf discs previously submersed in the extract. The lowest mean lethal concentrations (LC₅₀) were obtained with the CSC method (8118.8, 5410.5, 2820.6 mg kg⁻¹ at 24, 48 and 72 h, respectively). Extracts were characterized using gas chromatography/mass spectrometry (GC/MS), finding vinyl dithiins (63.11%), diallyl disulfide (10.88%) and diallyl trisulfide (10.4%) as the main compounds in the CSC extract. The main conclusion is that, in comparison with other techniques of extraction from garlic bulbs used in this study, supercritical fluids allow for obtaining extracts with a higher concentration of biologically active compounds against *T. urticae*.

Key words: two-spotted spider mite, plant extracts, miticidal effect, Liliaceae.

INTRODUCTION

The mite *T. urticae* belongs to the Aracnide Class, the Sub-Class Acari, Acariform Order and Tetranychidae Family. Traditionally this pest has been controlled using synthetic chemical acaricides with a level of residuality and permanence that constitute a barrier to the commercialization of agricultural products and cause detrimental effects to environment and human health. *T. urticae* is highly polyphagous species. To date 3877 host species have been reported around the world (Migeon and Dorkeld, 2007). Some populations of *T. urticae* submitted to the pressure of selection have developed resistance to the chemical products used for their control, such as fenbutatin oxide, hexythiazox and abamectin (Beers *et al.*, 1998), bifenthrin, λ-cyhalothrin and dimethoate (Yang *et al.*, 2001), organophosphates (Stumpf *et al.*, 2001; Anazawa *et al.*, 2003), imidacloprid (James and Price, 2002), metil-paration and metomil (Tsagkarakou *et al.*, 2002), tebufenpirad (Herron and Rophail, 2002) and clorfenapir and etoxazole (Uesugi *et al.*, 2002). The resistance development makes it necessary to use greater quantities of products, more applications or more potent active ingredients. Within an Integrated Pest Management Program, botanic extracts with acaricide and/or repellent properties could be used, given that they have very short residual effects and can be compatible with biological control (Chiasson *et al.*, 2004). Besides, these extracts degrade more rapidly than chemical products, which imply less possibility of finding them in plants, as well as in the soil, air and water, although they require a greater number of applications.

¹ Universidad Pontificia Bolivariana, Facultad de Ingeniería Agroindustrial, Grupo de Investigaciones Agroindustriales (GRAIN), A.A. 56006, Medellín, Colombia. E-mail: carlos.hincapie@upb.edu.co * Corresponding author.

² Universidad Nacional de Colombia, Magister en Ciencias Químicas, Calle 59A No 63-20 Medellín, Colombia. E-mail: rtorres@epm.net.co The summary of this work was presented at the 33rd Congress of the Colombian Entomology Society (SOCOLEN) in Manizales, Colombia on 28 July 2006.

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Some plants with acaricide and/or repellent effect against the mite are: *A. sativum* in an aqueous solution (Boyd and Alverson, 2000), *Artemisia absinthium* L. and *Tanacetum vulgare* L. (Chiasson *et al.*, 2001), *Piper longum* L. (Park *et al.*, 2002), *Trichilia pallida* Swartz and *Melia azedarach* L. (Castiglioni *et al.*, 2002), *Azadirachta indica* A. Juss. (Castiglioni *et al.*, 2002; Knapp and Kashenge, 2003; Chiasson *et al.*, 2004); *Satureja hortensis* L., *Ocimum basilicum* L. and *Thymus vulgaris* L. (Aslan *et al.*, 2004); *Carum carvi* L., *Cymbopogon nardus* (L.) Rendle, *Eucalyptus citriodora* Hook, *Mentha pulegium* L., *Mentha piperita* L., *Mentha spicata* L. (Choi *et al.*, 2004), *Chenopodium ambrosioides* L. var. *ambrosioides* (Chiasson *et al.*, 2004). Some organisms on which the insecticide or repellent action of garlic have been tested are: *Acanthoscelides obtectus* Say (Regnault-Roger and Hamraoui, 1993); *Tribolium castaneum* and *Sitophilus zeamais* (Ho *et al.*, 1996; Chiam *et al.*, 1999; Huang *et al.*, 2000); *Cydia pomonella* L. (Landolt *et al.*, 1999); *Lipaphis erysimi* Kaltenbach and *Dysdercus cingulatus* (Bandyopadhyay *et al.*, 2001; Banerjee *et al.*, 2004); *Meloidogyne incognita* (Chitwood, 2002); *Reticulitermes santonensis* and *Reticulitermes grassei* (Auger *et al.*, 2004); and *Attagenus unicolor japonicus* (Han *et al.*, 2006).

The traditional extraction methods can cause interactions between solvents and extracts, producing modifications in the original structure of the compounds. Besides, there is the risk of chemical residues that affects the ecological behavior of the extract. Extraction with supercritical fluids is a highly beneficial alternative to remove these problems. A supercritical fluid is any substance in conditions of pressure and temperature higher than its critical point; it is a quasi-state with intermediate properties between a liquid and a gas. Supercritical CO₂ presents the best extraction conditions, with less interaction with the extract and low environmental impact (McClain, 2000).

The objective of this study was to compare the effects of different extract obtention methods from bulbs of *A. sativum* (supercritical CO₂, water, ethanol and petroleum ether as solvents) on *T. urticae*. Each extract was characterized through CG/MS to identify the compounds and determine which have possibilities of biological action on mites.

MATERIALS AND METHODS

The study was done in 2006 at the Universidad Pontificia Bolivariana (Campus Laureles), Medellín (6°14' N,

75°35' W), Antioquia, Colombia, at an average altitude of 1450 m.a.s.l. and an average temperature of 22 ± 1 °C (IDEAM, 1999). The initial colony of mites was supplied by the Universidad Militar Nueva Granada, Cajicá Campus, Cundinamarca, Colombia. To maintain the colony and carry out the tests, bean plants cv. Cargamanto, were seeded under laboratory conditions, so that germination and growth occurred in isolation. A tray was seeded weekly with approximately 12 bean seeds. When the plants were 4 weeks old, they were inoculated with leaves infested with mites. The plants were replaced every 4 weeks. The plants were irrigated daily and organic fertilizer was applied at seeding. Pest control was done manually in order to not use synthetic chemical products and thus avoid introducing error factors to the experiment.

Garlic bulbs from a cultivar known as "China white" were obtained in the local market, an option that has been taken by other researchers (Kuo and Ho, 1992; Calvey *et al.*, 1994; Abu-Lafi *et al.*, 2004; Kimbaris *et al.*, 2006; Han *et al.*, 2006). Subsequently, the bulbs were selected, cleaned, cut and mashed manually in the laboratory. The product obtained was used for the extractions. For the extraction with supercritical CO₂ the gas (UN 2817, Agafano, Medellín, Colombia) was used as the solvent under supercritical conditions in an extractor (SFE1000, Thar Technologies, Pittsburgh, USA) located in the private company Colflavor S.A., Envigado, Antioquia. The temperature in the extractor was 40 °C and the pressure and CO₂ flow were 25.33 MPa and 30 g min⁻¹, respectively (Ryback *et al.*, 2004). The extraction time was 1.5 h. For extraction using the Soxhlet method, 100 g of sample was used, and petroleum ether 35-60 and ethanol at 99.7% were used as solvents, for 8 h. For soaking at room temperature, 100 g of sample in a hermetically sealed container was submitted to agitation for 24 h, using the same solvents as used with Soxhlet. Finally, the extractions were filtered and the solvents separated at low temperature in a rotary evaporator (Waterbath-B-480, Büchi Labortechnik AG, Flawil, Switzerland). Soaking was also carried out in water. Unlike the previously described methods, in the latter case, the concentration was determined making a mass balance. To prepare each treatment, the solvents were removed exhaustively to avoid their incidence in the tests. The extracts prepared were: supercritical CO₂ (CSC), soaking with petroleum ether (MEP), Soxhlet with ethanol (SAE), Soxhlet with petroleum ether (SEP), soaking with ethanol (MAE), and soaking with water (MA). Before use the extracts were stored in a refrigerator in hermetically sealed amber-colored

containers and injected with highly purified nitrogen gas (Agafano, Medellín, Colombia).

To determine mortality with CSC extracts, concentration of 2 500, 5 000 and 10 000 mg kg⁻¹ were prepared; as well, concentrations of 10 000, 25 000, 50 000, 100 000, 150 000 and 200 000 mg kg⁻¹ were prepared for the MEP, MAE, SEP and SAE extracts. Concentrations of 68 500, 45 700, 22 800, 11 400 and 4 600 mg kg⁻¹ were prepared for the MA extract. The extract was solved in distilled water. Owing to conditions inherent to the soaking process and the concentration of the solvent, extracts of over 76 800 mg kg⁻¹ were not obtained. Thus 34 treatments were obtained, with three replicates of each one. Concentrations of 1 000, 2 000 and 5 000 mg kg⁻¹ were used with all the extracts for repellence tests. A completely random design was used, with 24 treatments and three replicates of each, 18 treatments (six extracts with three concentrations), the absolute control (distilled water) and the positive control, abamectina 75 mg kg⁻¹ (CATOMBE 1.8 EC, Químicos Oma, Colombia). The concentrations were determined after preliminary tests to establish the ranges of action on the mites.

For the mortality tests eight 3-cm diameter disks of young leaves of bean were immersed in each treatment, and then deposited on Petri plates with filter paper moistened with distilled water. After drying, 10 adult mites were placed in each disk and were covered. Mortality was evaluated at 24, 48 and 72 h and a mite was considered dead when it presented no movement after being poked by a brush bristle (Chiasson *et al.*, 2001; 2004; Castiglioni *et al.*, 2002; Kwon and Ahn, 2002a; 2002b; Kim *et al.*, 2003; 2004a; 2004b; Choi *et al.*, 2004). The percentage of mortality was calculated considering the data obtained from the absolute control and the treatments corrected with Abbott's formula (1925).

For the repellence bio-tests the same procedure was used as for the mortality tests, except that over half of each 3 cm disk a 14 mm leaf disk without treatment was placed, and 10 adult mites were placed on this smaller disk. The mites that remained there were counted at 0.25; 0.5; 0.75; 1; 2; 3 and 4 h (Boyd and Alverson, 2000). The information gathered in the repellence tests was analyzed with repeated measurement using the PROC MIX module from the SAS program (SAS Institute, 1999), with which the variance analysis was carried out. Significant differences were identified with the Minimal Significant Differences test. The mean lethal concentration (LC₅₀) was determined using the

PROBIT module of the SAS program, with the probit method proposed by Bliss (1934) and developed by Finney (1947).

The extracts were re-dissolved into their respective solvents for the chromatographic analysis. The CSC extract was dissolved in dicloromethane (Calvey *et al.*, 1994). For the MA extract, an equal quantity of dicloromethane was added in order to extract the sulphurated compounds of low to medium polarity present in this extract (Kuo and Ho, 1992). The solvents were eliminated before using the mass detector. The characterization was done by CG/MS (6890N Network GC System, Mass selective detector 5973 Network and Injector 7683 Series; Agilent Technologies, Palo Alto, California, USA) in the laboratory of instrumental analysis of the Universidad Nacional de Colombia, Medellín Campus. An HP 5 fused silica capillary column was used (30 m x 0,25 mm, film thickness of 0.25 µm) (Abu-Lafi *et al.*, 2004; Kimbaris *et al.*, 2006). For detection it was used ionization energy of 70 eV. The temperatures of the injector and the detector were fixed at 220 and 290 °C, respectively. An helium flow used as a gas drag was 1 mL min⁻¹ (Kimbaris *et al.*, 2006). The oven was programmed to begin at 40 °C for 2 min, increasing 2 °C every 2 min (Block, 1992) until 300 °C, which was maintained for 20 min (Abu-Lafi *et al.*, 2004). The compounds were identified with the NIST 98 library incorporated into the equipment.

RESULTS AND DISCUSSION

Mortality

The CSC extract was the most toxic, followed by MEP (Table 1). Mortalities of less than 40% were obtained with MA in the majority of the cases, because of which it registered LC₃₀; it should be noted that the concentration of the extract could not be increased because it is not obtained in pure form but diluted in water, at a concentration that does not exceed on average 76 800 mg kg⁻¹. The extracts that showed greater mortality were obtained under conditions of low temperatures, which suggests that these conditions favor obtaining compounds with greater acaricide effect than those that can be obtained at higher temperatures. The solvent polarity also appears to influence the results, given that the CO₂ and petroleum ether are non-polar solvents. These two extracts have similar percentages of diallyl sulfide (CSC: 23.058% and MEP: 24.842%) and disulfide (CSC: 12.537% and MEP: 14.798%), but present major differences in vinyl dithiins (CSC: 63.11% and MEP: 10.173%). With regard to the disulfides, the two

Table 1. Mean lethal concentration (LC₅₀) on *Tetranychus urticae* from garlic bulb extract in each evaluated time.

Extraction method	Time					
	24 h		48 h		72 h	
	CL ₅₀	CI a 95%	LC ₅₀	CI a 95%	LC ₅₀	CI a 95%
	mg kg ⁻¹		mg kg ⁻¹		mg kg ⁻¹	
CSC	8 118.8	(5 815.1-17 868.3)	5 410.5	(3 715.3-8 514.3)	2 820.6	(282.1-4 425.6)
MEP	26 794.6	(17 087.9-37 012.8)	17 179.5	(7 978.6-26 683.7)	17 665.0	(9 969.7-25 480.9)
SAE	117 838.4	(87 073.3-177 283.5)	82 080.6	(53 166.7-142 646.5)	47 993.6	(3 612.5-195 831.5)
SEP	208 759.5	(--)	209 557.4	(--)	97 872.9	(--)
MAE	231 321.0	(140 026.2-669 253.3)	176 537.4	(102 478.7-586 922.1)	71 134.6	(--)
MA ^a	67 296.9	(26 566.8-103 380)	55 912.5	(--)	12 130.8	(179.6-27 863.4)

^a CL₃₀ is registered because 50% mortality was not reached in any of the tests, nor 40% in some.

CI: confidence interval; (--): because of the behavior of the data, the probit did not provide a confidence interval.

CSC: supercritical CO₂; MEP: soaking with petroleum ether; SAE: Soxhlet with ethanol; SEP: Soxhlet with petroleum ether; MAE: soaking with ethanol; MA: soaking with water.

extracts have higher percentages than the other extracts (Table 2 and 3). With STATGRAPHICS PLUS V.5.0 (StatPoint Inc., 1994) the correlation was determined between the relative percentage of disulfides and LC₅₀ at 24 ($r^2 = 79.4192$ and $p = 0.0424$), 48 ($r^2 = 73.9096$ and $p = 0.0617$) and 72 h ($r^2 = 75.3829$ and $p = 0.0563$). Aqueous soaking was not considered for this analysis because was not obtained LC₅₀ with it.

Repellence

Through the comparison of covariance structures, using goodness of fit tests or information criteria that are generated for each structure, the type of behavior of the data was identified, and it was found that it had a compound symmetrical structure. The Schwartz's Bayesian information criterion (BIC) (Correa, 2004) was used because it was the most accurate. Subsequently, a variance analysis was carried out, finding significant differences between the means of the treatments and significance in the first order interaction between the treatments and the time ($F = 1.31$; $df = 114$; $P = 0.0427$). As interaction was significant, it was not adequate to do individual analysis of main effects (Correa, 2004). The most repellent extract was that obtained by soaking with petroleum ether (MEP) (Table 2).

The compounds found in greater quantity in the MEP extract are methyl 2-propanol disulfide, dimethyl trisulfide, diallyl disulfide, 2-ethylidene[1,3]dithiane, and di-2-propenyl trisulfide and 3-vinyl-1,2-dithiocyclohex-5-ene (Table 3). These could be responsible for the repellent effect owing to their volatility and strong odor. The MA extract has a low percentage of such compounds and the CSC has only 1.657% methyl

2-propanol disulfide and trisulfide, but an appreciable concentration of diallyl disulfide (10.88%). These two extracts were the least repellent. These results are better than those obtained by Boyd and Alverson (2000), who used concentrations of 100, 50, 20 and 10% of Garlic Barrier AG (a commercial insect repellent described as aqueous garlic [Garlic Barrier, 1999]), an aqueous garlic juice produced in the laboratory and a mixture of mineral oil and garlic powder. Significant repellence was observed with the commercial product at 100% in all the times, and for 30 and 45 min at 50%. This suggests that this type of extract should be used in almost pure form in order to have any effect.

From the statistical point of view, the use of any extract as a repellent of *T. urticae* in the concentrations used in this study is the same (with the exception of the aqueous and the CSC extracts at 1000 and 2000 mg kg⁻¹). As well, it was found that there is a statistically significant relationship between the percentage of acyclic sulphurated compounds and maximum repellence obtained at 4 h ($r^2 = 87.2046$ and $p = 0.0064$). This correlation suggests that acyclic compounds present in the extracts are, in great part, responsible for the repellence of mites. To define a possible commercial product or to carry out further tests it will be necessary to analyze factors such as the performance of the extraction, solvent cost, saving of energy and processing time, among others.

Compounds identified and their influence on mortality and repellence

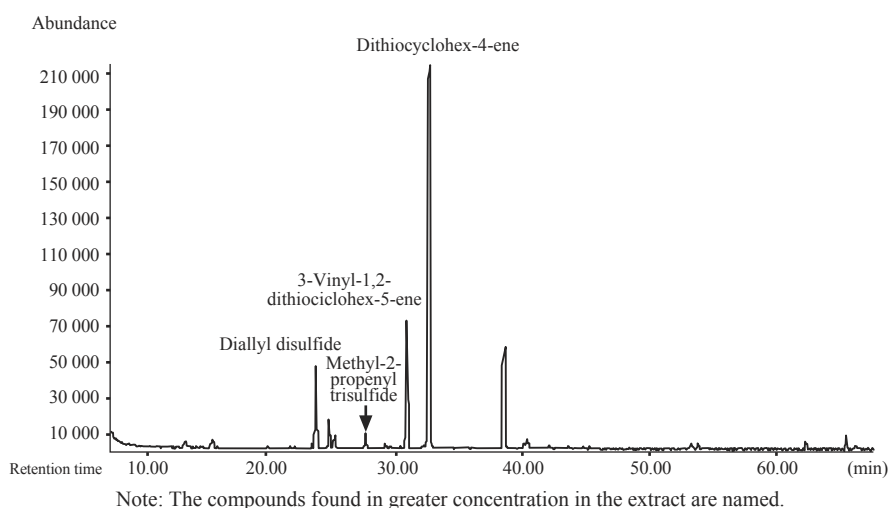
A number of important sulphurated compounds were identified in the majority of the extracts between 10 and 63 min (retention time) (Figures 1 and 2). After

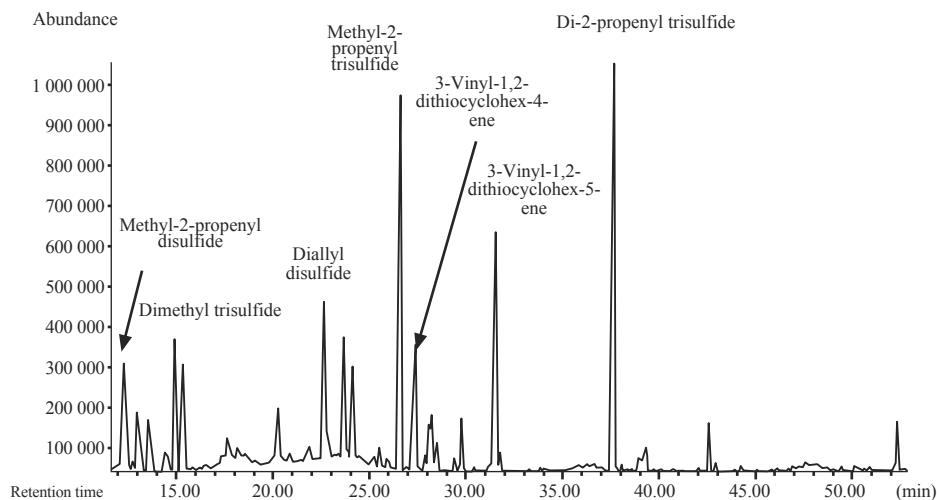
Table 2. Repellence percentages on *Tetranychus urticae* of each garlic bulb extract in each evaluated time.

Extraction method	Concentr. mg kg ⁻¹	Repellence for each observation time (h)						
		0.25	0.5	0.75	1	2	3	4
		%						
CSC	1000	36.67a	30.00a	26.67a	26.67a	30.00a	23.33a	16.67a
	2000	36.67a	33.33a	36.67a	33.33a	30.00a	26.67a	26.67ab
	5000	83.33c	70.00cd	63.33bc	66.67bcde	66.67bcdef	66.67cdef	63.33def
MEP	1000	90.00c	90.00de	90.00cde	90.00ef	86.70ef	90.00f	90.00fgh
	2000	100.00c	100.00e	100.00e	100.00f	93.30f	93.30f	96.70h
	5000	96.70c	93.30de	90.00cde	90.00ef	86.70ef	90.00f	83.30efgh
SAE	1000	96.70c	93.30de	96.70de	93.30ef	93.30f	93.30f	90.00fgh
	2000	93.30c	86.70de	90.00cde	76.70cdef	66.70bcdef	70.00def	70.00defgh
	5000	86.70c	76.70de	86.70cde	86.70def	83.30ef	86.70ef	83.30efgh
SEP	1000	83.30c	76.70de	76.70bcde	76.70cdef	76.70def	76.70def	76.70defgh
	2000	96.70c	90.00de	90.00cde	93.30ef	83.30ef	76.70def	80.00efgh
	5000	93.30c	93.30de	93.30de	90.00ef	93.30f	90.00f	93.30gh
MAE	1000	83.30c	83.30de	83.30cde	80.00cdef	80.00ef	80.00ef	73.30defgh
	2000	83.30c	80.00de	76.70bcde	86.70def	73.30cdef	70.00def	73.30defgh
	5000	80.00c	80.00de	76.70bcde	76.70cdef	70.00cdef	73.30def	63.30def
MA	1000	43.30a	40.00ab	0.00a	40.00ab	40.00ab	40.00abc	33.30abc
	2000	53.30ab	53.30abc	53.30ab	53.30abc	50.00abcd	50.00abcd	50.00bcd
	5000	76.70bc	70.00cd	70.00bcd	70.00cde	63.30abcde	60.00bcde	56.70cde
Absolute control	0	73.30bc	66.70bcd	63.30abc	53.30bcd	46.70abc	33.30ab	23.30ab
Positive control (abamectin)	750	86.67c	73.33cde	70.00bcd	70.00cde	66.67bcdef	66.67cdef	66.67defg

Data followed by the same letter does not have a significant difference according to the minimum significant difference test ($P < 0.05$).

CSC: supercritical CO₂; MEP: soaking with petroleum ether; SAE: Soxhlet with ethanol; SEP: Soxhlet with petroleum ether; MAE: soaking with ethanol; MA: soaking with water.

**Figure 1.** Chromatogram with peaks that indicate identified compounds from supercritical CO₂ extract.



Note: The compounds found in greater concentration in the extract are named.

Figure 2. Chromatogram with peaks that indicate identified compounds from petroleum ether soaking extract.

Table 3. Comparison between most found compounds in each extract.

Name of the compound	Peak area with each extraction method					
	CSC	MEP	SAE	SEP	MAE	MA
Methyl 2-propanol disulfide	1.66	8.20	Nd	1.18	2.48	0.92
Dimethyl trisulfide	Nd	5.08	1.15	7.89	2.51	0.87
Diallyl disulfide	10.88	6.60	7.25	5.06	5.01	2.23
2-Ethylidene[1,3]ditiene	Nd	5.09	Nd	6.91	2.33	0.41
Methyl 2-propanol trisulfide	1.68	14.57	9.48	16.75	6.02	3.66
3-Vinyl-1,2-dithiocyclohex-4-ene	47.78	1.67	Nd	8.19	8.39	10.80
3-Vinyl-1,2-dithiocyclohex-5-ene	15.33	8.50	Nd	Nd	6.80	22.72
Unknown ^a	Nd	Nd	32.16	Nd	Nd	Nd
Di-2-propenyl trisulfide (isomer) ^b	Nd	Nd	Nd	Nd	12.50	Nd
Di-2-propenyl trisulfide	10.40	16.76	19.82	16.39	11.71	5.96
Diallyl tetrasulfide	1.78	1.48	4.83	0.87	1.92	0.95
Benzeneacetaldehyde	Nd	2.89	4.70	2.80	1.29	Nd
Total disulfide ^c	12.54	14.80	7.25	6.25	7.49	3.15
Total trisulfide ^d	12.08	36.40	30.45	41.03	32.74	10.50
Total vinyl dithiins ^e	63.11	10.17	Nd	8.19	15.19	33.52
Total diallyl sulfide ^f	23.06	24.84	31.91	22.33	31.14	9.14
Total acyclic sulfides ^g	26.40	52.68	42.53	48.15	29.65	14.60

CSC: supercritical CO₂; MEP: soaking with petroleum ether; SAE: Soxhlet with ethanol; SEP: Soxhlet with petroleum ether; MAE: soaking with ethanol; MA: soaking with water.

Nd: not detected by the equipment.

^a Considered unknown because of the low probability found in the mass spectrometer; ^b the compound di-2-propenyl trisulfide was identified in two different retention times in the MAE extract, one of these with a probability of 95.5% and the other with 98.8%; it is assumed that this last as indicated compound and the first as an isomer given the very high probability; ^c Methyl 2-propanol disulfide and diallyl disulfide; ^d Dimethyl trisulfide, methyl 2-propanol trisulfide and di-2-propenyl trisulfide; ^e 3-vinyl-1,2-dithiocyclohex-4-ene y 3-vinyl-1,2-dithiocyclohex-5-ene; ^f Diallyl disulfide, di-2-propenyl trisulfide and diallyl tetrasulfide; ^g Diallyl tetrasulfide, di-2-propenyl trisulfide, methyl 2-propanol trisulfide, diallyl disulfide, dimethyl trisulfide, and methyl 2-propanol disulfide.

this time different compounds were obtained that were not of interest for the research, mainly long chain hydrocarbons. The main compounds of CSC extract are 2 vinyl dithiins, which together represent 63.11% (Table 3). These compounds were also identified by Yu *et al.* (1989), Abu-Lafi *et al.* (2004) and Calvo-Gomez *et al.* (2004). Block (1992) proposed that the conversion of alliin in vinyl dithiins, as well as in other compounds, is evident with chromatographic analysis under certain conditions. To verify that the transformation of alliin does not occur during the extractive process, Abu-Lafi *et al.* (2004) used cryogenic extraction techniques and CG/MS and high performance liquid chromatography (HPLC) as analysis methods and still obtained alliin as the predominant compound in all the extracts analyzed, concluding that these compounds are the major sulphurated components of fresh garlic and that during some processes (vapor distillation, frying or bleaching) they decompose and form other compounds. In the extracts obtained with petroleum ether higher relative percentage of trisulfides were found (MEP: 36.407% and SEP: 41.03%) and, in those obtained with ethylic alcohol, somewhat lower percentages were obtained (MAE: 32.743% and SAE: 30.446%); in contrast, in those obtained with water and supercritical CO₂ the trisulfides percentages were substantially lower (CSC: 12.08% and MA: 10.50%) (Table 3). Figure 3 shows the structures of the main compounds found.

Interaction with the solvents seems to be a very influential factor in the formation of compounds within the extracts. Notable is the case of extracts obtained

using petroleum ether and ethylic alcohol, whose relative concentrations of dimethyl trisulfide range from 30.47% to 41.03%, while that in water and CO₂ reached 12.08% as a maximum. Cyclic compounds predominate in the latter extracts. The permanence time of CO₂ with the extract was the same as the extraction process (1.5 h), consequently it vaporized at room temperature, while with the other solvents the permanence time was 8 h (Soxhlet) and 24 h (maceration), plus the lapse between extraction and separation of the solvent. Iberl *et al.* (1990) propose that vinyl dithiins are not directly produced by catalyzed reactions by allinase, but rather by the alliin decomposition produced in solvents less polar than water.

Temperature also appears to influence the type of compounds found in the different extracts. Vinyl dithiins were found mainly in the extracts obtained at low temperatures (except for 8.19% found in one of them in the SEP extracts at approximately 60 °C). Nevertheless, a simple linear correlation between process temperatures and vinyl dithiins percentage in each extract showed that there is no statistically significant relationship among them ($r^2 = 11.7894\%$ and $p = 0.5052$). However, analyzing only the information of the extracts obtained with ethylic alcohol and petroleum ether it was established that there is a statistically significant relationship between temperature and vinyl dithiins percentage ($r^2 = 73.232\%$ and $p = 0.1212$). It is very probable that the greater toxicity of CSC extract is due to the high percentage of vinyl dithiins compared to the other extracts. As well, it was found that the CSC

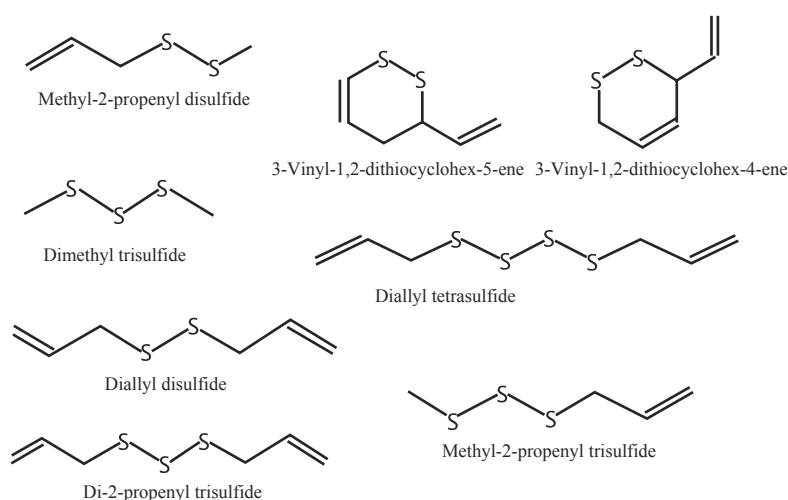


Figure 3. Structures of the main compounds in the extracts.

Source: NIST98 database (National Institute for Standards and Technology, 1998).

extracts had very small quantities of other sulphurated compounds others than vinyl dithiins, di-2-propenyl trisulfide, methyl 2-propanol disulfide and diallyl disulfide and tetrasulfide, resulting in a relatively clean product. These observations are in accordance with Calvey *et al.* (1998) and Ryback *et al.* (2004). The aqueous extract also has a relatively important percentage of vinyl dithiins (33.52%) probably because dicloromethane was used to obtain the extract for chromatographic analysis, a solvent that was also used to dissolve the extract obtained by supercritical CO₂ to carry out the same operations.

Another important compound in the CSC extract was diallyl disulfide (DADS), with the greatest percentage of all the extracts (10.88%). Auger *et al.* (2004) found that the LC₅₀ of this pure substance against *R. santonensis* and *R. grassei* was 3400 and 2500 mg kg⁻¹, respectively; thus suggesting that DADS can also be responsible for acaricide activity. In this same study dimethyl disulfide showed a LC₅₀ against these two termites of 150 and 100 mg kg⁻¹, respectively; this compound was not found in any extraction in the present research, perhaps because of its high volatility, its capture not being possible under the conditions used in this study.

CONCLUSIONS

The garlic bulbs extract obtained with supercritical CO₂ showed the lowest CL₅₀ against *T. urticae* under laboratory conditions in comparison to the other extracts. The high concentration of cyclic compound could be the explanation for this effect.

There are no significant statistical differences between most of extraction methods in terms of repellence against *T. urticae* with the concentrations analyzed in this study (except for the aqueous extract and the CSC at 1000 and 2000 mg kg⁻¹). The concentration of acyclic compounds explains 87.21% of the repellence of each extract on *T. urticae* (with a 99% confidence level).

Extraction with supercritical CO₂ provided higher concentrations of sulphurated compounds, in comparison to the other techniques used in this study, resulting in a relatively clean product.

The results suggest that with extraction methods at high temperatures acyclic compounds are obtained and that processes at room temperature favor the formation of cyclic compounds in greater proportion. The extract

obtained at room temperature using petroleum ether was more toxic than the other extracts (except that obtained with supercritical CO₂) and while it did not show significant differences from the other treatments, it was observed that this extract had a stronger repellent effect.

RESUMEN

Comparación y caracterización de extractos de bulbos de ajo (*Allium sativum* L.) y su efecto en la mortalidad y repelencia de *Tetranychus urticae* Koch (Acari: Tetranychidae). Carlos Augusto Hincapié Ll.¹, Gloria Eugenia López P.¹, y Ricardo Torres Ch.². *Tetranychus urticae* Koch es una plaga que causa grandes pérdidas económicas porque ataca un numeroso grupo de cultivos. Además, algunas poblaciones han desarrollado resistencia a acaricidas comerciales. Este trabajo compara diferentes extractos a partir de bulbos de ajo (*Allium sativum* L.) a través de su toxicidad y repelencia sobre *T. urticae*. Los extractos se obtuvieron usando como solventes CO₂ en condiciones supercríticas (CSC), etanol y éter de petróleo usando el método Soxhlet y maceración y agua usando maceración. La técnica de fluidos supercríticos permite obtener extractos a temperaturas bajas usando altas presiones, evitando la degradación de los compuestos y permitiendo su separación del solvente al exponerlo a temperatura ambiente. El ácaro fue criado en laboratorio sobre plantas de frijol (*Phaseolus vulgaris* L.) en el Campus Laureles de la Universidad Pontificia Bolivariana, Medellín, Colombia. La mortalidad fue evaluada colocando 10 ácaros en un disco de hoja de *P. vulgaris* de 3 cm de diámetro previamente sumergido en el extracto. Las concentraciones letales medias (CL₅₀) más bajas se obtuvieron con el método CSC (8 118,8; 5 410,5; 2 820,6 mg kg⁻¹ a las 24, 48 y 72 h respectivamente). Los extractos se caracterizaron usando cromatografía de gases-espectrometría de masas (GC/MS), obteniendo en el extracto CSC principalmente vinilditiinas (63,11%), dialil disulfuro (10,88%) y di-2-propenil trisulfuro (10,4%). Se concluyó que la extracción con fluidos supercríticos permite obtener, a partir de bulbos de ajo, extractos con mayor concentración de compuestos biológicamente activos contra *T. urticae*, comparada con las otras técnicas usadas en este estudio.

Palabras clave: araña roja bimaclada, extractos de plantas, efecto acaricida, Liliaceae.

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