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Acaricidal Properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) Essential Oils Obtained by Three Methods of Extraction

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ABSTRACT Essential oils of *Artemisia absinthium* L. and *Tanacetum vulgare* L. were extracted by three methods, a microwave assisted process (MAP), distillation in water (DW) and direct steam distillation (DSD), and tested for their relative toxicity as contact acaricides to the twospotted spider mite, *Tetranychus urticae* Koch. All three extracts of *A. absinthium* and of *T. vulgare* were lethal to the spider mite but to variable degrees. The LC_{50} obtained from the DSD oil of *A. absinthium* was significantly lower (0.04 mg/cm^2) than that of the MAP (0.13 mg/cm^2) and DW (0.13 mg/cm^2) oil of this plant species. DSD and DW extracts of *T. vulgare* were more toxic (75.6 and 60.4% mite mortality, respectively, at 4% concentration) to the spider mite than the MAP extract (16.7% mite mortality at 4% concentration). Chromatographic analysis indicated differences in composition between the more toxic DSD oil of *A. absinthium* and the other two extracts of this plant, indicating that a sesquiterpene ($C_{15}H_{24}$) compound present in the DSD oil and absent in the other two may enhance the toxicity of the DSD oil. Chemical analysis of the *T. vulgare* extracts indicated that β-thujone is by far the major compound of the oil (>87.6%) and probably contributes significantly to the acaricidal activity of the oil.

KEY WORDS twospotted spider mite, *Artemisia absinthium*, *Tanacetum vulgare*, acaricide, essential oil extracts

PLANTS NATIVE TO North America have generally been overlooked as sources of biologically active compounds (Berenbaum 1989) and most studies report the compounds' activity on insects. However, phytophagous mites are important pests of several crops and alternatives to synthetic acaricides are urgently needed. There are few published reports of the acaricidal properties of botanical pesticides. In this respect, Mansour et al. (1987) reported the effects of neem extracts on the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) and on their predators, *Phytoseiulus persimilis* Athias-Henriot and *Chiracanthium mildei* (L.) Koch. The toxic effects of neem were also reported by Sundaram and Sloane (1995) and more recently El Gangaihi et al. (1996) showed the effects of thyme oil and thyme on spider mites.

The genus *Artemisia* is a rich source of plant-derived pesticides (Duke et al. 1988). Oil of *A. absinthium* has been found to repel fleas and flies (Erichsen-Brown 1979, cited by Duke 1985, pp. 474–567) and mosquitoes (Morton 1981) and to kill house flies (Kaul et al. 1978). The chemical composition of *A. absinthium* has not been fully characterized. Nevertheless, the following constituents have been identified: the terpenes limonene, myrcene (Vostrowsky et al. 1981, Tucker and Maciarello 1993), the terpene-like ketones α- and β-thujone (Vostrowsky et al. 1981, Chialva et al. 1983, Tateo and Riva 1991, Tucker and Maciarello 1993), the sesquiterpene, caryophellene (Tucker and Marciarello 1993), and sabinyl acetate and chrysanthenyl acetate (Chialva et al. 1983, Tucker and Marciarello 1993).

Tanacetum vulgare or common tansy has been used as an insecticide as well as an insect repellent by native Americans after its introduction to North America in the 18th century (Duke 1985). Anti-feedant effects of tansy extracts were reported for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Hough-Goldstein 1990), and the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Larocque et al. 1999), and earlier, the repellency of tansy oil was determined for the Colorado potato beetle (Panasiuk 1984). Several terpenoids have been identified in the oil of tansy: α-pinene, α-terpinene, γ-terpinene and carvone (Panasiuk 1984), dihydrocarvone (Panasiuk 1984, Collin et al. 1993), artemisia ketone and chrysanthenyl acetate (Hendricks et al. 1990), borneol (Schearer 1984, Collin et al. 1993), β-thujone (Panasiuk 1984, Schearer 1984 Collin et al. 1993), chrysanthenone (Collin et al. 1993), and camphor (Schearer 1984, Hendricks et al. 1990, Collin et al. 1993).

Pesticides made from plant extracts are notorious for variable toxicity to the target species even when they are made from the same plant species. Several factors such as phenological age of the plant (Jackson and Hay 1994), percent humidity of the harvested

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Table 1. Major compounds present in *A. absinthium* oil extracted by MAP, DW, and DSD

Compounds present	K.I.a[a]	K.I.p[b]	Relative amount of compounds, %		
			MAP	DW	DSD
Sabinene	—	1101	2.4	4.5	0
α-thujone	1086	1380	1.4	2.9	0
β-thujone	1091	1399	11.5	32.1	12.3
$C_{10}H_{16}O$	1113	1439	54.1	36.1	49.1
Unknown #1	1465	1832	2.1	2.1	3.4
$C_{15}H_{24}$	1485	1868	0	0	4.2

—, Unknown.
[a] Kovat's Index on apolar DB-1 column. Kovat's index (KI) is a qualitative index used in chromatography. Each compounds has a specific combination of KIs dependent on the columns used. This combination makes it possible to identify the compounds of an essential oil analyzed by chromatographic equipment.
[b] Kovat's Index on polar Supelcowax column.

Table 2. Major compounds present in *T. vulgare* oil extracted by MAP, DW, and DSD

Compounds present	K.I.a[a]	K.I.p[b]	Relative amount of compounds, % with each extraction method[c]		
			MAP	DW	DSD
α-thujone	1,083.4	1,378	0.4	0.9	1.6
β-thujone	1,094	1,400	92.2	87.6	91.1
camphor	1,118	1,622	1.1	1.0	0
terpinen-4-ol	1,158.3	1,559	0	0.6	0
α-cubebene	1,460	1,663	0	5.1	0

[a] Kovat's Index on apolar DB-1 column.
[b] Kovat's Index on polar Supelcowax column.
[c] On apolar DB-1 column.

material (Chialva et al. 1983, Tateo and Riva 1991), plant parts chosen for extraction (Chialva et al. 1983, Jackson and Hay 1994), and the method of extraction (Perez-Souto et al. 1992) have been identified as possible sources of variation for the chemical composition and toxicity of the extracts.

This study demonstrates the acaricidal properties of extracts obtained from *A. absinthium* and *T. vulgare.* Furthermore, it shows how the extraction technique may quantitatively and qualitatively affect the constituents of the extract and, consequently, its acaricidal properties toward the twospotted spider mite.

Materials and Methods

Extraction of Essential Oils. Whole plants of *A. absinthium* and *T. vulgare* were harvested in full bloom in the fall of 1993 from a cultivated plot at the Agriculture and Agri-Food Canada experimental farm at L'Acadie (45° 18′ N, 73° 20′ W), Quebec, Canada. A microwave assisted process (MAP) and two variants of steam distillation, distillation in water (DW), and direct steam distillation (DSD) (Duerbeck 1993), were used to extract the fresh plant material.

The MAP process uses microwaves to excite water molecules in the plant tissues causing plant cells to rupture and release the essential oils trapped in the extracellular tissues of the plant (Bélanger et al. 1991). Whole plant parts were shredded and 20 g were immersed in 100 ml of hexane and irradiated at 2,450 Mhz for 90 s at an intensity of 675 W.

The DW and DSD distillations were carried out according to Duerbeck (1993). A 380-liter distillator with a capacity for processing ≈20 kg of fresh plant material was used. During the DW process, plant material was completely immersed in water, heated, and boiled by a steam coil located at the base of the still body. In the DSD extraction method, the plant material was supported within the still body and packed uniformly and loosely to provide for the smooth passage of steam through it. Steam was produced by an external generator and allowed to diffuse through the plant material from the bottom of the tank. The rate of entry of the steam was set at 400 ml/min or 24 liters/h. With both methods, the oil constituents released from the plant material were combined with the water vapor and allowed to cool in a condenser to separate into two components, oil and water.

Chromatographic Studies. The essential oils and extracts were analyzed by capillary gas chromatography (GC) using a Varian 6000 series Vista chromatograph equipped with two flame ionization detectors. Peak areas were computed by a Varian DS 654 integrator (Varian, Palo Alto, CA). SPB-1 (30 m × 0.25 mm ∅, 0.25 μm) and Supelcowax (30 m, 0.25 mm ∅, 0.25 μm) fused silica columns were used with helium as a carrier gas at a velocity of 30 cm/s (1.5 ml/min). The oven temperature was programmed to increase in increments of 2°C/min from 40°C to 240°C and the injector and detector temperatures were set at 230°C and 250°C, respectively.

Bioassays. The procedure described below was followed for each plant species. Four concentrations of each extract, i.e., DW and DSD and three concentrations of MAP extract were tested on *T. urticae* taken from a wild colony reared in the laboratory of the Horticultural Research and Development Center, Saint-Jean-sur-Richelieu. Emulsions were made by preparing a 300-ml stock solution of emulsifier (0.32% of Alkamul EL-620), denatured ethanol (9%) and microfiltered water. Emulsifiers and alcohols are common constituents of essential oil formulations. Microfiltered water was used for the control (0%). Forty, 80, 160, and 320 μl of oil were completed with the stock solution to 4 ml to give the 1, 2, 4, and 8% solutions,

Table 3. Percent adult *Tetranychus urticae* mortality 48 h after treatments with *A. absinthium* oil extracted by MAP, DW, and DSD

Extraction method	Concentration of oil, %				
	0 (Control)	1	2	4	8
MAP	5.3	15.7[a]	19.5	52.7	—[b]
DW	2.8	20.5	28.2	51.1	65.6
DSD	2.6	42.1	71.3	83.2	92.8

n, 270 mites for each concentration except where specified.
[a] *n*, = 180 mites.
[b] Quantity of MAP oil was insufficient for assays at this concentration.

Table 4. Probit analysis of adult *Tetranychus urticae* mortalities 48 h after treatments with *A. absinthium* oil extracted by MAP, DW, and DSD

Extraction method	*n*	Intercept ± SEM	Slope ± SEM	*t* ratio*	LC_{50} (mg/cm^2)	99% CL of LC_{50}
MAP	900	2.12 ± 0.31	2.44 ± 0.30	8.06	0.134	0.096–0.280
DW	1260	1.72 ± 0.15	1.94 ± 0.16	11.78	0.130	0.081–0.205
DSD	1260	2.93 ± 0.18	2.15 ± 0.16	13.65	0.043	0.028–0.057

*, *t* values > 1.96 are significant at $P = 0.01$.

respectively. The 8% concentrations of the MAP extracts for both *A. absinthium* and *T. vulgare* were not prepared because of insufficient quantities of the oils.

Thirty adult female mites were placed on their dorsum with a camel's-hair brush on a double-sided adhesive tape glued to a 9-cm petri dish (after Anonymous 1968). Three dishes were prepared for each concentration of the oil extracted by the three methods and the control (water), for a total of 90 mites per extraction method per treatment-day. One milliliter of each preparation and of microfiltered water for the control was added with a Gilson Pipetman P-1000 (Gilson Medical Electronics (France), S.A., Villiers-le-Bel, France) to the reservoir of the spray nozzle of a Potter Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England.) mounted on a stand and connected to a pressure gauge set at 3 psi (20.7 kPa). Petri dishes were weighed before and immediately after each application and, on average, 205 mg (±42; $n = 50$) of solution was deposited on each dish, representing 2.1 (1%), 4.1 (2%), 8.2 (4%), and 16.4 mg/cm^2 (8%) of oil deposited with each concentration. The entire procedure was repeated twice (1 and 2% of *A. absinthium* MAP and 4% of *T. vulgare* MAP solutions) for a total of 180 mites or three times (the remaining MAP and all DW and DSD solutions of both plant species) for a total of 270 mites.

Mite mortality was assessed 48 h after treatment. Mites that failed to respond to probing with a fine camel's-hair brush with movements of the legs, proboscis, or abdomen were considered dead. Results were subjected to Probit analysis using the POLO computer program (LeOra Software 1987). Percentage mortalities were recorded with corresponding weighed dose (mg/cm^2) to take into consideration variability in application rate. The significance of differences in LC_{50} values was determined by comparing the 99% confidence intervals computed by POLO (LeOra Software 1987).

Results

Chemical Analysis of the Essential Oil Extracts. Chromatographic analysis of the oils of *A. absinthium* extracted by the three methods indicated differences in chemical composition between the oils (Table 1). Both sabinene and α-thujone were absent in the DSD oil and present in the MAP and DW oils. A sesquiterpene compound ($C_{15}H_{24}$) was present in DSD at 4.2% and absent in MAP and DW.

In *T. vulgare* extracts, β-thujone was the major component of all three extraction techniques (MAP: 92.2%; DW: 87.6%; DSD:91.9%) (Table 2). Terpinen-4-ol and α-cubebene were present in the DW extract and absent in the other two.

Bioassay Results. After 48 h, all three extracts (MAP, DW, and DSD) of *A. absinthium* were lethal to *T. urticae* (Table 3). However, there were differences in the degree of toxicity of the extracts to the twospotted spider mite. For example, at 4% concentration, oil extracted by the MAP and the DW methods caused 52.7 and 51.1% mite mortality, respectively, whereas oil extracted by DSD resulted in 83.2% mortality. The LC_{50} of the oil extracted by DSD was lower (0.043 mg/cm^2) than those obtained for oil extracted by MAP (0.134 mg/cm^2) and by DW (0.130 mg/cm^2) (Table 4). *T. vulgare* extracts obtained by DW and DSD had greater acaricidal effect than the extract obtained by the MAP process (Table 5). At 4% concentration, the oil extracted by the DW and DSD methods caused 60.4 and 75.6% mortality, respectively, whereas the oil extracted by MAP gave 16.7% mortality.

Probit analysis of mortality data obtained from bioassays with the DW and DSD methods were compared (Table 6) and results indicate a similar degree of toxicity for the two oils, LC_{50} of 0.054 mg/cm^2 for DW and 0.046 mg/cm^2 for DSD. Mortality values obtained with the MAP oil (Table 5) did not increase in toxicity from a lower to a higher concentration and LC_{50} values were inconclusive. This may be due to the physical properties of the MAP extract. During this process, organic compounds such as waxes and resins are often released from plant cells along with the essential oils. These products and other constituents of the oils may not be adequately mixed by the Alkamuls-EL620 emulsifier, resulting in a heterogeneous emulsion.

Table 5. Percent adult *Tetranychus urticae* mortality 48 h after treatments with *T. vulgare* oil extracted by MAP, DW, and DSD

Extraction method	Concentration of oil, %				
	0 (Control)	1	2	4	8
MAP	6.3	17.8	11.1	16.7[a]	—[b]
DW	5.6	48.1	64.9	60.4	89.3
DSD	5.0	52.9	64.1	75.6	95.6

$n = 270$ mites for each concentration except where specified.
[a] n, 180 mites.
[b] Quantity of MAP oil was insufficient for assays at this concentration.

Table 6. Probit analysis of adult *T. urticae* mortalities 48 h after treatments with *T. vulgare* oil extracted by DW and DSD

Extraction method	*n*	Intercept ± SEM	Slope ± SEM	*t* ratio*	LC_{50} (mg/cm^2)	99% CL of LC_{50}
DW	1,350	1.81 ± 0.15	1.42 ± 0.14	10.08	0.054	0.013–0.088
DSD	1,350	2.50 ± 0.17	1.86 ± 0.15	12.43	0.046	0.022–0.066

*, *t* values > 1.96 are significant at $P = 0.01$.

Discussion

Artemisia absinthium oil extracted by DSD was more effective in controlling the spider mite than the oils extracted by the two other methods. A sesquiterpene ($C_{15}H_{24}$), present at 4.2% in DSD and absent in the other two extracts (Table 1), may be responsible for the higher degree in biological activity. However, identification of the unknown $C_{15}H_{24}$ compound in *A. absinthium*, and bioassays with individual compounds obtained from the three extraction methods, will be necessary for the complete identification of the active ingredients found in *A. absinthium* oil. The identification of active ingredients can be complex because certain plant compounds may have either a synergistic or antagonistic effect on the biological activity of the extract. Dubey and Kishore (1987) demonstrated that combinations of oils of *Lippia alba* (Mill.), *Ocimum canum* Sims., and *Chenopodium ambrosioides* L. were more fungitoxic to the mycelial growth of *Rhizoctonia solani* Kuhn than the individual oils, suggesting a synergistic effect between compounds of the different oils. Essential oils of oregano plants *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart, *Coridothymus capitatus* (L.) Reichenb., and *Satureja thymbra* L., as well as the compounds thymol and carvacrol present in the three extracts, were tested for insecticidal and genotoxic activities on *Drosophila melanogaster* Meigen (Karpouhtsis et al. 1998). The toxic effect of carvacrol and thymol did not correspond with their relative presence in the oils and mixtures of the two compounds at levels resembling their content in the three extracts showed that the toxicity of carvacrol was reduced in the presence of thymol, suggesting an antagonistic effect.

The similarity in biological response between the oil of tansy extracted by DW and DSD suggests that terpinen-4-ol and α-cubebene (present in DW and absent in DSD) contribute little to the acaricidal activity of the oil extracted by DW. Because of the considerably high percentage of β-thujone in all three extracts (from 87.6 to 92.2%), this component is likely to be the main active ingredient with negligible activity attributable to the other constituents. This would explain the similar results obtained from DW extracts at 4% concentration (60.4% mortality and 87.6% β-thujone), and DSD extracts (75.5% mortality and 91.9% β-thujone). As mentioned previously a heterogeneous MAP emulsion may account for inconsistent results obtained with this oil.

The results of this study indicate that the natural oils extracted from *A. absinthium* and *T. vulgare* have acaricidal properties. The results also indicate that different extraction techniques qualitatively and quantitatively affect the constituents of a natural oil as a pesticide. This phenomenon has been recognized before by Bélanger et al. (1996) who observed from 5- to 15-fold variations in thujone-like compounds from the oil of western red cedar, *Thuja plicata* Donn., extracted by variations of the MAP process rather than by DW. Therefore, several extraction techniques available should be evaluated to produce a more effective natural pesticide. DSD is at present the most widely accepted method for the production of essential oils on a commercial scale and should be considered for large-scale production of a biologically active oil because, besides producing oil of greater toxicity as shown in this study, it is less expensive and yields are comparable to that of the other extraction methods (H.C. and A.B., unpublished data). Identification of the different constituents and their role in the toxicity of the final product will help in determining the optimal extraction technique as well as other factors involved (e.g., percent humidity and phenology of harvested plant material) to ensure a constant and optimal performance of the ultimate pesticide product.

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