Efficacy and Persistence of Rosemary Oil as an Acaricide Against Twospotted Spider Mite (Acari: Tetranychidae) on Greenhouse Tomato

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ABSTRACT Efficacy of rosemary, *Rosmarinus officinalis* L., essential oil was assessed against twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), as well as effects on the tomato, *Lycopersicum esculatum* Mill., host plant and biocontrol agents. Laboratory bioassay results indicated that pure rosemary oil and EcoTrol (a rosemary oil-based pesticide) caused complete mortality of spider mites at concentrations that are not phytotoxic to the host plant. The predatory mite *Phytoseiulus persimilis* Athias-Henriot is less susceptible to rosemary oil and EcoTrol than twospotted spider mite both in the laboratory and the greenhouse. Rosemary oil repels spider mites and can affect oviposition behavior. Moreover, rosemary oil and rosemary oil-based pesticides are nonpersistent in the environment, and their lethal and sublethal effects fade within 1 or 2 d. EcoTrol is safe to tomato foliage, flowers, and fruit even at double the recommended label rate. A greenhouse trial indicated that a single application of EcoTrol at its recommended label rate could reduce a twospotted spider mite population by 52%. At that rate, EcoTrol did not cause any mortality in *P. persimilis* nor did it affect their eggs. In general, EcoTrol was found to be a suitable option for small-scale integrated pest management programs for controlling twospotted spider mites on greenhouse tomato plants.

KEY WORDS *Tetranychus urticae*, *Phytoseiulus persimilis*, rosemary oil, EcoTrol, acaricidal activity

Twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), occurs on numerous major food crop and ornamental plants. Among 1,200 species of spider mites known in the world (Zhang 2003), it is the most polyphagous species and has been reported from >150 host plants of economic value (Jeppson et al. 1975). The greatest problem with this mite is its ability to rapidly evolve resistance to pesticides (Cranham and Helle 1985). Spider mites have evolved resistance to >80 acaricides to date, and resistance has been reported from >60 countries (DARP 2004). Spider mites and especially twospotted spider mites have been a priority pest in British Columbia greenhouses for several years. In Canada, pest management in the greenhouse vegetable industry is largely based on biological control. A survey conducted in 2002 indicated that 93% of tomato, *Lycopersicum esculatum* Mill., growers (165 growers) use biological control agents for insect and mite control (Murphy et al. 2002). The problem with biocontrol agents is their limited efficacy against higher populations of spider mites and their susceptibility to most pesticides.

The predatory mite *Phytoseiulus persimilis* Athias-Henriot has been studied extensively with respect to its potential for biological control of tetranychid mites on vegetables and ornamentals in greenhouses (McMurtry 1982, Van Lenteren and Woets 1988). *P. persimilis* is a selective predator that is able to rapidly suppress spider mites (Laing and Osborn 1974, Frises and Gilstrap 1982). Because juvenile development and reproduction of *P. persimilis* depends on the availability of spider mites as prey (Kennet and Hamai 1980), it often disappears from the greenhouse after reducing pest mite populations and thus provides short-term control. Studies of functional responses of *P. persimilis* to different densities of spider mites suggested that they might not provide acceptable control for higher populations of prey (Everson 1979). Thus, use of this predatory mite for controlling TSSM in greenhouses may only be effective when combined with other strategies (Zhang and Sanderson 1995). As an example, Nicetic et al. (2001) found that a combination of petroleum spray oil and the *P. persimilis* can be used to control twospotted spider mite on greenhouse roses (*Rosa* spp.).

Plant essential oils are obtained through steam distillation of herbs and medicinal plants (Yatagai 1997). Most of these oils are environmentally nonpersistent, and nontoxic to humans (with some exceptions) (Roe 1965, Cockayne and Gawkrodger 1997, Hjorther et al.

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1997), fish (with some exceptions), and wildlife (Kumar et al. 2000, Wager-Page and Mason 1997). Rosemary, *Rosmarinus officinalis* L., oil has been traditionally used as a medicine for colic, nervous disorders, and painful menstruation (Abu-Amer et al. 1999). Recent studies revealed that rosemary oil is also an effective antibacterial agent (Managena and Muyima 1999, Burt 2004). Rosemary oil is relatively effective against certain insect and mite pests. The aromatic vapor of rosemary has ovicidal and larvicidal effects on several stored product pests (Tunc et al. 2000, Papachristos and Stampoulos 2004) and twospotted spider mite (Choi et al. 2004) as a fumigant. The oil can have sublethal effects as well (e.g., acting as a repellent to onion thrips, *Thrips tabaci* Lindeman (Koschier and Sedy 2003). Choi et al. (2004) tested 53 essential oils against *T. urticae* and *P. persimilis* as a fumigant. Caraway seed, citronella, lemon, eucalyptus, pennyroyal, and peppermint oil were found to be highly toxic to both mite species. Rosemary oil also was found to be toxic to the predaceous mites *Amblyseius barkeri* Hughes, *A. zaheri,* and *Typhlodromus athiasae* Porath (Momen and Amer 1999).

Although essential oil-based pesticides are considered reduced-risk pesticides, phytotoxicity to greenhouse crops constitutes one possible obstacle for their use in practice. In a few cases, essential oil-treated plants have become attractive to plant-damaging insects and phytotoxic effects on cultivated plants have been observed. Ibrahim et al. (2001) reported phytotoxicity in limonene-treated plants, whereas Chiasson et al. (2004) did not observe any phytotoxicity among lettuce, roses, and tomatoes that were treated with a *Chenopodium*-based pesticide. The active ingredients in pesticides do not necessarily cause phytotoxicity. Plant damage can result from the solvents in a pesticide formulation, impurities in the spray water, use of more pesticide than prescribed on the label, or poorly mixed spray emulsion. Although low levels of phytotoxicity might not be a physiological threat for plant, cosmetic damage can reduce marketability of the product.

In this study, lethal and sublethal effects of rosemary oil and three rosemary oil-based pesticides were evaluated against twospotted spider mite and *P. persimilis.* In addition, phytotoxic effects of three rosemary oil-based pesticides to foliage, fruit, and flowers of greenhouse tomato plants have been investigated.

Materials and Methods

Chemicals and Pesticides. Pure *R. officinalis* essential oil (Intarome TO, lot no. 0213142MB-100%); the three commercial pesticides Hexacide (5% rosemary oil), EcoTrol (10% rosemary oil), and Sporan (17.6% rosemary oil); and a blank formulation of EcoTrol were obtained from EcoSMART Technologies Inc. (Franklin, TN).

Spider Mites. Spider mites originated from a research colony maintained on tomato plants for >5 yr without any pesticide exposure at Agriculture and Agri-Food Canada (Agassiz, British Columbia, Canada). These mites were reared on 3-wk-old vine tomato plants.

Predatory Mites. Predatory mites were purchased from Applied Bionomics (Sidney, British Columbia, Canada). Predatory mites were transferred to a spider mite colony maintained on tomato plants caged in the greenhouse.

Plant Material. Three-week-old 'Clarance' tomato plants provided by Houweling's Nurseries (Delta, British Columbia, Canada) were transferred to plastic pots containing a mixture of regular peat (50%), fine bark (25%), and pumice (25%) provided by West Creek Farms (Langley, British Columbia, Canada) in a greenhouse at the University of British Columbia.

Growing Conditions. Plants infested with mites were kept inside isolated cages within the greenhouse at 24 ± 6°C and 40–60% RH under natural daylight. Plants were irrigated three times per week, two times with water and one time with water-soluble fertilizer (Peters EXCEL 15-5-15 Cal-Mag) (The Scotts Company, Marysville, OH). Adult female mites were transferred to clean plants, allowed to oviposit for 48 h, and then removed from the plant. Development of these eggs resulted in a cohort of evenly aged mites that were used for all bioassays.

Contact Toxicities. A leaf disc painting method was used for calculating the LC_{50} of rosemary oil and the three pesticides. Tests were conducted in disposable plastic petri dishes (3 cm in diameter). Mites were treated with six nominal concentrations (2.5, 5, 10, 20, 40, and 80 ml/liter) of the essential oil or the commercial pesticides and the blank formulation (for EcoTrol), by using a spreader sticker adjuvant (60 mg/liter Latron B-1956) (Rohm and Haas, Philadelphia, PA) diluted in distilled water. Leaf discs (3 cm in diameter) were cut from leaves of greenhouse-grown plants by using a cork borer. A 20-μl aliquot of each concentration was painted on the under side of the leaf disc with a micropipette giving concentrations of ≅6.25, 12.50, 25, 50, 100, and 200 $\mu g/cm^2$. After drying at room temperature for 5 min, each disc was placed in the bottom of a petri dish atop a 3-cm-diameter disc of Whatman no. 1 filter paper wetted with 50 μl of distilled water. Five adult female twospotted spider mites were introduced into each petri dish and the covered dishes were placed in a growth chamber at 26 ± 2°C, 55–60% RH, and a photoperiod of 16:8 (L:D) h. Mortality was determined under a dissecting microscope 24 h after treatment. Mites were considered dead if appendages did not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone. All treatments were replicated five times.

An electronic microsprayer was developed to measure the direct contact toxicity of the toxicants to test organism mimicking spraying practices inside greenhouses. A mechanical switch was installed on an airbrush (Badger 200NH, Badger Airbrush Co., Franklin Park, IL) linked to a solenoid controlled electronically by a digital timer. The digital timer was designed and developed by Maryam Antikchi (Department of Engineering Physics, University of British Columbia).

The timer was calibrated in five stages based on the amount of aliquot sprayed. In stage 1, it delivers 20 ± 5 μg, and in subsequent stages this amount increases up to 100 μg. To reduce errors and increase the accuracy of the tests, the timer was calibrated once before each experiment.

Toxicity of rosemary oil to the predatory mite *P. persimilis* was measured using a leaf disc painting method (as described above) and the direct contact method. In leaf disc painting bioassays, almost 50 twospotted spider mite eggs were placed onto each painted leaf disc (3 cm in diameter) as a food source for predators. Adult predatory mites were used for bioassays. Tomato leaves containing both spider mites and predatory mites were placed inside a petri dish (10 cm in diameter) on top of an ice pack inside a Styrofoam box to immobilize the predators for a short period (2 min) before the bioassay. Each treatment was replicated five times.

Direct contact toxicity of the commercial pesticides Hexacide (7.5 ml/liter), EcoTrol (7.5 ml/liter) or Sporan (7.5 ml/ liter) against predatory mites and spider mites were measured using the electronic microsprayer. Five adult predatory mites were transferred to a leaf disc (3 cm in diameter) containing ≈50 spider mite eggs and then sprayed. Five adult female spider mites were put on another leaf disc and then sprayed. Controls were sprayed with carrier solvent alone (60 mg/liter Latron B-1956). All treatments were kept inside a growth chamber at the same conditions described above. Mortality was measured 24 h after treatment. Mites were considered dead if they did not move their appendages when prodded with a paintbrush. Mortality in control groups was corrected by Abbott's formula (Abbott 1925). Each treatment was replicated five times.

Residual Toxicities. Residual toxicity of three rosemary oil-based pesticides against TSSM and predatory mites was measured. Three-week-old tomato plants were sprayed individually with Hexacide (7.5 ml/liter), EcoTrol (7.5 ml/liter), or Sporan (7.5 ml/liter) (each plant received ≈80 ± 10 g of sprayed material). A spreader sticker adjuvant (60 mg/liter Latron B-1956) diluted in distilled water was used as the carrier solvent. Control plants were sprayed with carrier solvent alone (n = 5 plants for each treatment). Treated and untreated plants were then placed randomly on a greenhouse table. Leaf discs (3 cm in diameter) were cut from each individual plant 1 and 24 h after spraying. Leaf discs were placed inside a petri dish as described above. Five adult female twospotted spider mites or five adult predatory mites plus ≈50 spider mite eggs (as a food source for predators) were placed on the sprayed surface of the leaf disc. Covered petri dishes were held inside a growth chamber for 24 h at 26 ± 2°C, 55–60% RH, and a photoperiod of 16:8 (L:D) h. Mortality was determined under a dissecting microscope 24 h after treatment. Predators or mites were considered dead if appendages did not move when prodded with a fine paintbrush. All treatments were replicated five times.

Choice Test Bioassay. Choice test bioassays were conducted for twospotted spider mites by using rosemary oil. Two leaf discs (3 cm in diameter) were cut from tomato plants and placed on top of a wetted Whatman no. 7 filter paper placed inside a disposable plastic petri dish (10 cm in diameter). One leaf disc was painted with a 20-μl aliquot of rosemary oil (10 ml/liter; ≅25 μg/cm^2) dissolved in a spreader sticker adjuvant (60 mg/liter Latron B-1956) diluted in distilled water as the carrier solvent, and the other leaf disc was treated with the carrier solvent alone. Thirty female adult mites were placed in the middle of the petri dish between the two leaf discs. The number of mites found on each leaf disc was counted under a dissecting microscope after 1, 12, 24, and 48 h. Numbers of eggs on treated and nontreated leaf discs were counted at the end of experiment at 48 h. All treatments were replicated 10 times.

Translaminar Activity. Five-week-old tomato plants were sprayed from above with Hexacide (7.5 ml/liter), EcoTrol (7.5 ml/liter), or Sporan (7.5 ml/ liter) by using a spreader sticker adjuvant (60 mg/liter Latron B-1956) diluted in distilled water as the carrier solvent (each plant received ≈80 ± 10 g of sprayed material). Leaf discs (3 cm) were cut from tomato plants and were placed on top of a wetted filter paper disc (as described above) with either the upper surface (sprayed) or undersurface (not sprayed) facing up. Five adult female spider mites were introduced into each petri dish, and the covered dishes were placed in a growth chamber at 26 ± 2°C, 55–60% RH, and a photoperiod 16:8 (L:D) h. Mortality was determined under a dissecting microscope 24 h after treatment as described above.

Efficacy Test. A 2 by 2 factorial design was used with two factors (pesticide or predator) and two levels (absence or presence) in each factor. Treatments were randomly assigned inside cells of cages. Data were analyzed by two-way analysis of variance (ANOVA) (SPSS Inc., Chicago, IL). Two tomato plants were placed inside each cell (total of 40 plants). Eight extra plants were randomly placed inside some cells as indicators to estimate initial density of mites before pesticide application or predator release. Three HOBO data-loggers (Onset, Contoocook, NH) were randomly installed inside cells to collect temperature and moisture data at 10-min intervals.

Approximately 15–20 adult twospotted spider mites were placed on each plant. After 1 wk, extra plants were removed from the cells, and numbers of spider mites on their foliage was counted under a stereomicroscope. Based on the initial density of the spider mites, *P. persimilis* was introduced to one-half of the treatments at 1:20 predators to prey. Two hours after releasing the predators, treatments that were designated to receive pesticide were sprayed with EcoTrol at 7.5 ml/liter (=label rate; water was used as carrier solvent as recommended by manufacturer). After 7 d, tomato plants were fully harvested and placed inside paper bags. Bags then were put inside a cold room (4°C) to prevent further development of mites during data collection. Numbers of spider mites and preda-

Table 1. Toxicity of rosemary oil and three rosemary oil-based pesticides to twospotted spider mite and predatory mite

Toxicant	Organism	*n*	Slope (± SE)	95% CL	LC_{50} (ml/liter)	LC_{50} ($\mu g/cm^2$)	χ^2
Rosemary	TSSM	5	2.2 ± 0.3	10.05–17.78	13.19	33.09	2.13
Hexacide	TSSM	5	2.2 ± 0.4	2.36–5.46	4.01	10.05	2.58
EcoTrol	TSSM	5	3.0 ± 0.5	4.03–7.06	5.51	13.79	1.35
Ec-Blank	TSSM	5	4.3 ± 0.6	57.52–165.5	82.14	220.38	0.90
Sporan	TSSM	5	3.0 ± 0.8	7.56–15.91	11.44	28.70	1.62
Rosemary	PP	5	3.4 ± 0.5	13.53–20.71	16.62	41.73	3.16

Ec-Blank, EcoTrol blank formulation without rosemary oil; PP, *Phytoseiulus persimilis;* and TSSM, twospotted spider mite.
[a] *n*, number of replicates for each tested concentration (total of six concentrations).

tory mites were counted on all foliage under a stereomicroscope. The single apical leaflet at the end of each leaf was selected as a subsample for counting the number of spider mite and predatory mite eggs.

Phytotoxicity Test. Pesticides were tested at three concentrations (one-half of label rate [3.75 ml/liter], label rate [7.5 ml/liter], and double label rate [15 ml/liter]). Five plants of equal age (4 wk old) were used for each concentration. Control plants were sprayed with carrier solvent (water) alone. Sprayed plants were randomly placed on a greenhouse bench.

Cuttings made from stems containing flowers or fruits were transferred to new pots placed inside a steam room for one week. Plants were then transferred to regular benches where they remained for 1 wk before the experiment. Fruits and flowers of the same size and age were sprayed with the same pesticides at the same concentrations.

To determine the phytotoxic effect of pesticides on foliage, medium-sized leaflets were selected as subsamples. Damage was defined in five grades from grade 0 (no damage) to grade 5 (completely burned leaflet). Five leaflets of the same size from each plant were selected randomly from all tested plants for damage assessment. Damage to flowers was defined as no damage (grade 0), minor burning sign on petals (grade 1), and major burning sign or flower abortion due to severe damage (grade 2). Damage to fruits was defined as no damage (grade 0) or any particular burning sign, which might cause cosmetic damage to fruit (grade 1). Effects were recorded 24, 48, and 72 h after spraying. Five flowers and five fruits from each plant were used for damage assessment.

Data Analysis. Mortality observations were analyzed using the SPSS program, version 11.5, for ANOVA. Tukey's test was used to compare means. Probit analysis was used to determine LC_{50}, by using the Environmental Protection Agency probit analysis program, version 1.5. Abbott's formula (Abbott 1925) was used to correct mortality in controls.

Results

Lethal Concentration$_{50}$. The LC_{50} of pure rosemary oil was 13.19 ml/liter (≅33.09 $\mu g/cm^2$) for spider mites (Table 1). Hexacide (containing 5% rosemary oil) and EcoTrol (containing 10% rosemary oil) were found to be 2 times more active than Sporan (containing 18% rosemary oil). This might be due to difference in formulation. No mortality was observed in control mites treated with carrier solvent alone. Unformulated rosemary oil seemed less toxic to *P. persimilis* than to spider mites (Table 1), although the difference was not significant.

Based on direct contact toxicity, the three commercial pesticides at their recommended label rate produced no mortality among predatory mites, indicating that twospotted spider mites are more susceptible to rosemary oil-based pesticides. No mortality was observed in control mites (Fig. 1).

Residual Toxicity. Residues of all three pesticides were found to be moderately toxic to spider mites within the first hour after spraying. However, toxicity decreased significantly after 24 h. There was a significant difference in the toxicity of residues over time [$F(7, 32) = 18.836; P < 0.05$]. This result clearly shows that rosemary oil is not persistent in the environment due to its volatile nature (Fig. 2). No mortality was observed among controls.

Residues of three commercial pesticides did not show statistically significant toxicity to *P. persimilis* (Fig. 3). Although slight changes in the toxicity of the residues over time were observed, no statistically significant differences were found among pesticides [$F(7, 32) = 1.026; P > 0.05$ ($P = 0.394$)].

Choice Test. Rosemary oil has a significant deterrent effect on mites, but this effect declined over time. There was a significant interaction between the location of mites and time [$F(3,72) = 81.203; P < 0.05$], and treatment had a significant main effect [$F(1,72) = 985.289; P < 0.05$]. During the first 12 h, mites aggre-

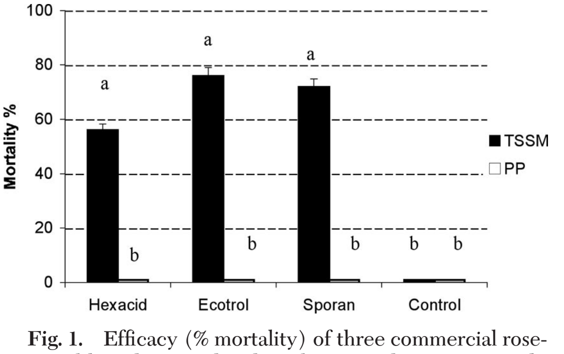

Fig. 1. Efficacy (% mortality) of three commercial rosemary oil-based pesticides directly sprayed on *P. persimilis* (PP) and *T. urticae* (TSSM) on tomato plants. Bars represent means ± SE; $n = 5$ replicates with five adult mites per replicate. Bars marked with the same letter do not differ significantly [Tukey: $F(7, 32) = 36.196; P > 0.05$].

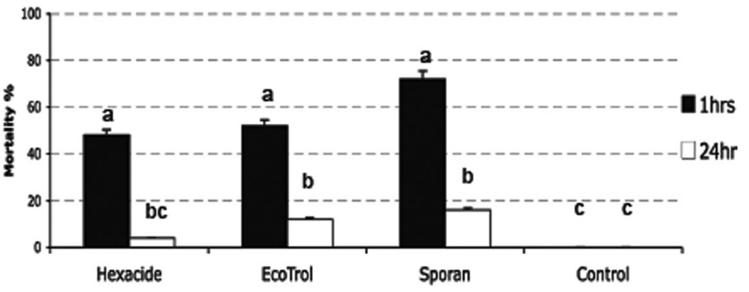

Fig. 2. Efficacy (% mortality) via residual toxicity of three rosemary oil-based pesticide to twospotted spider mite on tomato plants. Bars represent means ± SE; $n = 5$ replicates with five adult female mites per replicate. Bars marked with the same letter do not differ significantly [Tukey: $F(7, 32) = 18.836$; $P > 0.05$].

gated more on the control disc or at locations far from the treated disc within the test arena. After 24 h, they started to occur on both discs (Fig. 4), but even after 48 h, there were significantly more mites on the control discs.

Mites laid greater number of eggs on the control discs than on treated discs. Although they started to oviposit on both treated and control discs after 12 h, the final numbers of eggs after 2 d was significantly higher on control discs.

Translaminar Activity. No mortality was observed among mites that were placed on the unsprayed surface of the leaf discs (Fig. 5), whereas significant toxicity was observed among mites that were placed on the sprayed surface, indicating that rosemary oil lacks translaminar activity.

Efficacy Test. Approximately 100 ± 8 mites were found on each plant (i.e., on eight extra plants randomly placed inside cages) before spraying pesticide or releasing predators. Temperature within cages during the experiment was 24 ± 6°C, and relative humidity was 60 ± 15%. Numbers of mites in the blocks treated with pesticide or predator was significantly decreased compared with controls (Table 2), but no significant interaction between the two factors was observed (Table 3).

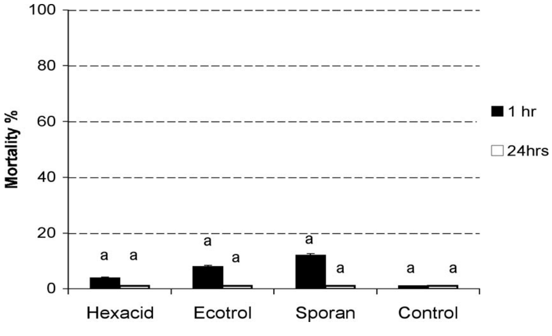

Fig. 3. Efficacy (% mortality) via residual toxicity of three rosemary oil-based pesticides at their recommended label rate to *P. persimilis* on tomato plants. Bars represent means ± SE; $n = 5$ replicates with five adult mites per replicate. Bars marked with the same letter do not differ significantly [Tukey: $F(7, 32) = 1.026$; $P > 0.05$ ($P = 0.394$)].

Numbers of twospotted spider mite eggs were not significantly affected by pesticide or predators (Table 4), although predators alone seemed to suppress spider mite eggs. No interaction was found between the two factors (Table 5)

No significant difference was found among the number of predators [$F(1, 6) = 0.00$; $P > 0.05$ ($P = 1.00$)] or their eggs [$F (1, 6) = 1.720$; $P > 0.05$ ($P = 0.238$)] on blocks sprayed with pesticide compared with those not sprayed.

Phytotoxicity. Hexacide and EcoTrol were not phytotoxic to foliage, flowers, or fruit (grade 0). Sporan (containing 18% rosemary oil) caused first grade damage (burning signs on less than one-fifth of leaflet) to 12% of foliage at the recommended label rate and second grade (burning signs on between one-fifth to two-fifths of the leaflet) damage to 32% of leaflets after 24 h. No additional damage was found at day 2 or day 3 of data collection. Sporan caused second grade damage (some petals of flowers demonstrated minor burning sings) in 40% of tested flowers at its label rate, second grade damage (complete burning or flower abortion) in 56% of flowers, and third grade damage (three-fifths of flowers demonstrated burning signs) in 36% of flowers at double the label rate after 24 h. No additional damage was found at day 2 or day 3 of data collection. None of the pesticides were found to be phytotoxic to fruit.

Discussion

Our results clearly indicate that rosemary oil can be considered as an acaricide against the twospotted spider mite, causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants.

Rosemary oil was found to be toxic to spider mites as a contact toxicant, which also might have fumigant toxic effect due to its chemical composition. Choi et al. (2004) evaluated the toxicity of 53 essential oils including rosemary against eggs and adults of twospotted spider mite as fumigants. Rosemary oil was not very toxic (mortality <60%) compared with oils of caraway seed, citronella java, lemon, eucalyptus, pennyroyal, and peppermint. However, Sampson et al.

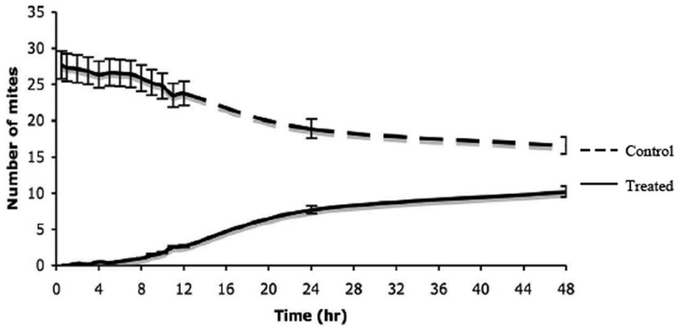

Fig. 4. Number of twospotted spider mites staying on leaf discs when given a choice between a treated and nontreated leaf discs with rosemary oil at 1%. Error bars representing standard error, $n = 10$ replicates with 30 adult spider mites per replicate.

(2005) tested 23 essential oils, including rosemary oil, against turnip aphids, *Lipaphis erysimi* (Kaltenbach) and found most acted as contact toxicants, causing mortality in aphids after 1 h. In another study, Sim et al. (2006) examined both direct contact and vapor phase toxicity of 44 plant essential oils, including rosemary against almond moth, *Cadra cautella* (Walker) larvae. They found rosemary oil very effective both as contact and fumigant toxicant. Tunc et al. (2000) found that vapors of some essential oils were toxic to the cotton aphid and twospotted spider mite.

Predatory mites *P. persimilis* are less susceptible to rosemary oil than twospotted spider mite (Table 1). When both mites were directly sprayed with different pesticides containing rosemary oil, no mortality was found among predators, whereas up to 60% mortality was observed in twospotted spider mite (Fig. 1). This difference in toxicity level between spider mites and predators might be due to differential metabolism of rosemary oil-based pesticides in predatory and phytophagous mites. Little is known about the mode and site of action of rosemary oil and other plant essential oils in mites. The octopaminergic nervous system is considered to be the site of action of certain essential oils in the American cockroach, *Periplaneta americana* (L.) (Enan 2001), and fruit fly (Enan 2005), but this may not be the case for twospotted spider mite and rosemary oil, and there is a possibility that the essential oils have more than one site of action because they are complex mixtures.

As Dekeyser (2005) mentioned, new pesticides should be safer toward nontarget organisms and have shorter environmental persistence than existing products. Our results clearly indicate that rosemary oil-based pesticides are not environmentally persistent. In all experiments, toxicity of residues significantly declined after 24 h.

Essential oils are mixture of odorous and volatile compounds that can easily break down in the environment (Isman 2000). Many environmental factors affect the breakdown of essential oils, most importantly, temperature and light.

Limited residual toxicity is an important advantage for these pesticides. Growers can apply them closer to harvest time. It is also important to have a safer environment for biocontrol agents with fewer pesticide residues. However, quick breakdown of essential oils in the environment reduces the risk of pesticide resistance in the pest population.

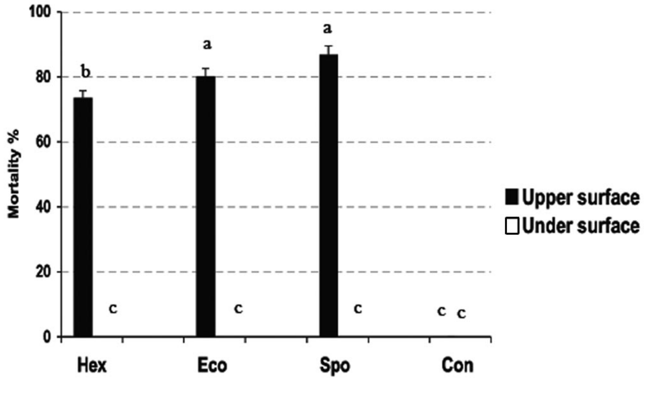

Fig. 5. Efficacy (% mortality) of three rosemary oil-based pesticides applied at their label rate on tomato leaves when exposed to twospotted spider mites that were placed either on upper surface or under surface of the leaf discs. Bars representing means ± SE, $n = 5$ replicates with five adult female mites per replicate. Bars marked with the same letter do not differ significantly [Tukey: $F(3, 16) = 36.196$; $P > 0.05$)].

Table 2. Average number of spider mites on each block

Pesticide	Predator	Mean TSSM	SD	Mean LogTSSM	SD LogTSSM	*n*
Absent	Absent	183.38	65.67	5.16	0.36	4
	Present	47.75	4.17	3.86	0.09	4
	Total	115.56	84.33	4.51	0.74	8
Present	Absent	87.87	29.41	4.43	0.32	4
	Present	42.50	21.42	3.64	0.53	4
	Total	65.18	33.99	4.04	0.58	8
Total	Absent	135.62	69.46	4.80	0.50	8
	Present	45.12	14.56	3.75	0.37	8
	Total	90.37	67.33	4.27	0.69	16

LogTSSM, log-transformed number of spider mites; and TSSM, twospotted spider mite.

Table 4. Average number of spider mite's eggs on each block

Pesticide	Predator	Mean egg	SD	Mean LogEggs	SD LogEggs	*n*
Absent	Absent	159.75	90.93	4.92	0.66	4
	Present	54.87	22.26	3.91	0.56	4
	Total	107.31	83.05	4.41	0.78	8
Present	Absent	73.87	35.34	4.23	0.42	4
	Present	75.50	44.43	4.10	0.87	4
	Total	74.69	37.17	4.17	0.64	8
Total	Absent	116.81	78.65	4.57	0.63	8
	Present	65.19	34.35	4.00	0.69	8
	Total	91.00	64.40	4.29	0.70	16

LogEggs, log-transformed number of spider mite eggs.

In addition to pesticidal properties, sublethal effects (repellent, deterrent, and antifeedant) of many plant essential oils have been reported against several pests (Isman 2000). According to our choice tests results, rosemary oil is significantly repellent to twospotted spider mite. It repelled mites for ≈6 h and then mites gradually started to move toward treated discs. However, mites preferred untreated leaves for oviposition.

Repellent effects of rosemary oil cannot be considered as a stand-alone control method, but they can be combined with other methods to improve pest management strategies. For example, rosemary oil application might be combined with trap plants as a "push-pull" tactic to repel pests from major host plants and attract them to trap plants.

In our study, the efficacy of EcoTrol (10% rosemary oil) at its recommended label rate (7.5 ml/liter for spider mites on tomato) was evaluated against twospotted spider mite on greenhouse tomato plants, both individually and in combination with predatory mite *P. persimilis* under greenhouse conditions.

In our laboratory bioassays, contact toxicity and possible fumigant toxicity of rosemary oil based-pesticides was observed in twospotted spider mite. However, under greenhouse conditions, less mortality was observed. Based on the average number of mites on each block (log-transformed), we can conclude that pesticide application, predator release and the combination of pesticide and predators, suppressed the twospotted spider mite population by 52 ± 16, 74 ± 2, and 76 ± 12%, respectively (Table 2). Several factors may have led to this difference. As mentioned, this product lacks translaminar activity and cannot affect mites that move deep within the canopy or remain on parts of the plant that the pesticide does not reach. In addition, environmental conditions such as temperature and light may accelerate the degradation of the oil and also its volatilization. There was a fluctuation of 12°C in temperature and 30% in relative humidity within the greenhouse during the experiment. This fluctuation might account for differences in efficacy in different blocks and cages. Moreover, it was shown that this product has repellent effects so there might be another scenario. Mites that were not directly hit by the pesticide might move to the nonsprayed parts of the plants, enhancing their survival. However, this should not affect numbers of eggs of spider mites and predatory mites.

Further experiments are needed to clarify the efficacy of this pesticide against spider mites in the greenhouse. According to our results, we conclude that EcoTrol can effectively suppress twospotted spider mite populations on greenhouse tomato, and it is safe for predatory mites and their eggs at the tested concentration.

In accord with the Pest Management Regulatory Agency's (PMRA) efficacy guidelines for plant protection products (PMRA 2003), phytotoxicity tests were performed on foliage, flowers, and fruit. No sign of phytotoxicity was found among tested tomato plants at the recommended label rate.

Certain plant essential oils have recently been used as least-toxic herbicides (Tworkoski 2002, Ghosheh 2005). Unlike essential oil-based insecticides and acaricides, the herbicides contain higher concentrations of

Table 3. Results from analyze of variance of number of spider mites (log transformed) after pesticide and predator treatment

Source	Sum of squares	df	Mean square	*F*	Significance
Corrected model	5.50 (a)	3	1.83	13.98	0.000
Intercept	292.72	1	292.72	2231.31	0.000
Pesticide	0.88	1	0.88	6.74	0.023
Predator	4.35	1	4.35	33.20	0.000
Pesticide × predator	0.26	1	0.26	1.98	0.184
Error	1.57	12	0.13		
Total	299.79	16			
Corrected total	7.07	15			

[a] $R^2 = 0.77$ (adjusted $R^2 = 0.77$).

Table 5. Results from analyze of variance of number of spider mites eggs (log transformed) after pesticide and predator treatment

Source	Sum of squares	df	Mean square	*F*	Significance
Corrected model	2.33 (a)	3	0.78	1.82	0.196
Intercept	294.69	1	294.69	691.13	0.000
Pesticide	0.255	1	0.25	0.58	0.461
Predator	1.29	1	1.29	3.03	0.107
Pesticide × predator	0.79	1	0.79	1.86	0.198
Error	5.12	12	0.42		
Total	302.14	16			
Corrected total	7.45	15			

[a] $R^2 = 0.31$ (adjusted $R^2 = 0.14$).

essential oils. For example, Matran EC, a contact, nonselective, broad spectrum, foliar herbicide developed by EcoSMART Technologies Inc. contains 50% clove oil. It might be possible for EcoTrol to cause phytotoxicity if used at higher concentrations; however, our results show that EcoTrol is safe to tomato (Clarance) even when applied at double the recommended label rate.

Although rosemary oil based-pesticides' effects might vary on different pests or plants and there might be some limitation for their application, they are still competitive with other pesticides and are economically efficient. Some important advantages of essential oil-based pesticides include low mammalian toxicity, safety to terrestrial and aquatic species (with some exception) (Wager-Page and Mason 1997, Kumar et al. 2000), rapid pest mortality owing to their neurotoxic mode of action and low cost, a result of their extensive worldwide use as fragrances and flavorings (Isman 2006).

Moreover, another important characteristic of rosemary oil is its complex chemical composition (Miresmailli et al. 2006). Like other essential oils, natural rosemary oil is a complex mixture of terpenoids. Considering that target site resistance is an important problem for mite control, it is less likely that mites will evolve resistance to a mixture of different active compounds than to an acaricide based on a single active ingredient. It has been reported that green peach aphids, *Myzus persicae* Sulzer, developed resistance to pure azadirachtin (the major ingredient of neem, *Azadirachta indica* A. Juss, insecticide) but not to a refined neem seed extract containing the same absolute amount of azadirachtin but with many other constituents present (Feng and Isman 1995).

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