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Research Paper

Acaricidal and repellent activity of plant essential oil-derived terpenes and the effect of binary mixtures against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

Realtionships between toxicity and repellent activity of plant essential oils and their major constituents to insects and mites remain unclear thus far. In the present study, the acaricidal and repellent activity of twenty terpenoid compounds commonly occurring in plant essential oils are evaluated against adults of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Significant differences in toxicity were found for some compounds based on the application volume applied to leaf discs, and between bean and cabbage leaf discs. There was only a weak correlation between toxicity and repellent activity of the compounds. A lack of significant difference in evaporation of selected compounds, as determined by an ultra-fast gas chromatograph, failed to explain differences in toxicity between bean and cabbage leaves. Regarding repellent activity of binary mixtures of selected compounds, several synergistic and antagonistic interactions were observed, but no notable trend was apparent. Only vanillin significantly enhanced the repellency of all compounds tested including carvacrol, thymol, *trans*-cinnamaldehyde and α-terpineol.

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important agricultural arthropod pests, not only owing to its wide range of host plants, but also to its resistance to many pesticides (Miresmailli et al., 2006). Plant essential oils are considered to be good alternatives to conventional pesticides because of their numerous bioactivities including insecticidal, repellent, feeding and oviposition deterrent activities to insects and other arthropod pests (Regnault-Roger et al., 2012). Moreover, due to their minimal environmental persistence and toxicity to humans and mammals (Chae et al., 2014; Isman, 2006), with a few exceptions, they are considered safer than many synthetic pesticides.

Most plant essential oils, obtained *via* steam or hydrodistillation, are largely composed of highly volatile monoterpenoid or sometimes, sesquiterpenoid compounds. Those volatiles can be quantitatively monitored *via* an ultra-fast portable gas chromatograph (GC), the zNose™ (Watkins and Wijesundera, 2006), a hand-held GC using a surface acoustic wave (SAW) sensor for detecting volatile compounds. A reliable correlation between analyses obtained with the zNose™ and with a conventional GC–MS was established in a previous report from our laboratory (Miresmailli et al., 2010).

In the present study, the relationship between toxicity and repellency of plant essential oil-derived compounds was examined, and the effects of carrier solution volume applied and host plant foliage was explored. In addition, effects of binary mixtures of compounds on repellent activity were examined.

2. Material and methods

2.1. Test compounds

Pure standards of the test compounds were purchased from Arylessence Inc. (Marietta, GA, USA, methyl salicylate and terpinene-4-ol), Berje Inc. (Carteret, NJ, USA, menthone), Thermo Fisher Scientific (Waltham, MA, USA, 3-carene, *p*-cymene, α-terpineol, and vanillin), and Sigma-Aldrich (St Louis, MO, USA, borneol, camphor, carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, citronellal, eugenol, geranic acid, limonene, linalool, menthol, α-pinene, and thymol). All the compounds were of the highest purity available (from 95 to 99%, except geranic acid, 85%). A positive control, pyrethrum extract (50% pyrethrins) was obtained from Prentiss Incorporated (Spartanburg, SC, USA).

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2.2. Mites and plants

The colony of *T. urticae* was maintained for more than 5 years on bean plants without exposure to any pesticide, in an isolated section of the insectary at the University of British Columbia. Plants infested with mites were kept at 24 ± 2 °C and 45–60% relative humidity (RH) under a 16:8 h LD photoperiod.

Seeds of bean (cv. Bush Blue Lake 274) and cabbage (cv. Stonehead) were obtained from Stocks Seeds Canada (Thorold, ON, Canada), and the plants were grown under the same conditions as noted above in potting soil. Bean plants ten to twelve days old were provided to the mite colony at least three times a week to maintain a healthy colony, and leaves from two-week-old bean plants and five-six-week-old cabbage plants were used in the present study.

2.3. Bioassays

2.3.1. Acute toxicity of individual compounds

A leaf disc (22 mm diameter) was cut from a bean or cabbage plant by using a cork borer, and placed on an agar (2%) medium in a disposable Petri dish (50 mm diameter) (Pavela, 2015a). An individual test compound dissolved in acetone was applied *via* a leaf painting method using a micropipette (Miresmailli et al., 2006), and ten adult mites per replicate with three replications were introduced onto the leaf disc after the solvent had evaporated. Each Petri dish was tightly covered with the lid, and then incubated under the same conditions noted above. Mortality was examined after 24 h, and mites which were unresponsive when prodded with a small paint brush were considered dead. Acetone alone was used as a negative control, and pyrethrum extract (50% pyrethrins) as a positive control. No mortality was observed in the negative controls.

To examine acute toxicity of test compounds, three different application methods were used in the present study; assay 1:20 μL of test compounds in acetone at 10 mg/mL were applied to a bean leaf disc, assay 2:50 μL of test compounds in acetone at 4 mg/mL on a bean leaf disc, and assay 3:20 μL of test compounds in acetone at 10 mg/mL on a cabbage leaf disc. All three applications had the same loading of dose to each leaf disc (0.2 mg/disc).

2.3.2. Repellent activity of individual compounds

To evaluate the repellent activity of the test compounds, a bridge assay method was designed (Fig. 1). A heavily mite-infested bean leaf was cut from the plant and transferred into a Petri dish (7 cm diameter), then held overnight before the repellent assay. The edge of the agar medium (2%) in a 50 mm diameter Petri dish was scraped and filled with deionized water (3 mL) to prevent mites from escaping, and either a bean or a cabbage leaf disc was placed on the center of the medium. Twenty μL of test solution at 10 mg/mL was painted onto a bean or a cabbage leaf disc as mentioned above (assays 1 and 3, in 2.3.1). A control leaf disc received 20 μL of acetone alone. Two Petri dishes (treatment and control) were interconnected by a curved plastic 'bridge' (1 cm width, 5 cm long) with a small patch of paper adhesive tape attached at the apex. Carrier solvents on the treated leaf discs were allowed to evaporate for 1 h, and then ten adult mites per replicate with three replications were introduced onto the middle of the bridge (on the paper tape). The bridge was removed when all mites had migrated to the leaf discs, and repellent activity was determined using the equation below;

$$\text{Percent repellency}(\%) = \frac{(N_c - N_t)}{(N_c + N_t)} \times 100$$

where N_c is the number of mites on the control leaf disc, while N_t is the number of mites on the treated leaf disc (You et al., 2015).

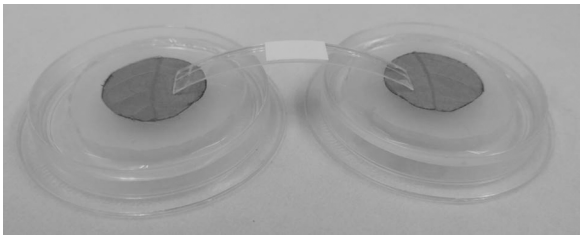

Fig. 1. A bridge assay method for repellent activity. The two leaf discs (treated and control) were connected by a plastic bridge, and the edges of each agar medium was scraped and filled with water to prevent the mites escaping. The mites were introduced onto the middle of the bridge, which was removed once all of the mites have moved onto either of the leaf discs.

2.4. RC_{50} determination of selected compounds and effects of binary mixtures

Four of the most active compounds (based on toxicity) were selected, including carvacrol, *trans*-cinnamaldehyde, α-terpineol and thymol, and their RC_{20} and RC_{50} (repellent concentration) values were determined by Probit analysis using the repellent activity of six to ten different concentrations in the bridge assay.

To investigate effects of combining compounds, a series of binary mixtures was prepared at their RC_{20} concentrations of the four compounds (1:1, w:w), and their repellent activity was examined using the same method above. To determine the interaction of combinations, an expected repellent activity was calculated by the equation;

$$E = O_a + O_b(1 - O_a)$$

where E is expected repellent effect, and O_a and O_b are observed repellency of individual compounds (Hummelbrunner and Isman, 2001). The combination effect was determined to be synergistic or antagonistic ($\chi^2 > 3.84$), or additive ($\chi^2 < 3.84$) by the formula;

$$\chi^2 = (O_m - E)^2/E$$

where O_m is observed repellent effect of the combination; $\chi 2$ with degree of freedom = 1 and $\alpha = 0.05$ is 3.84. The interaction was determined as synergistic when O_m was greater than E, and antagonistic when O_m was lesser than E.

From the combination test, vanillin showed notable synergy with all four compounds. To validate this synergistic effect of vanillin, RC_{50} values of the binary mixtures were examined in the same manner as well.

2.5. Headspace analysis of volatile compounds using zNose™

Four of the test compounds which showed notable differences in the acute toxicity and repellent activity between the leaves of bean and cabbage (camphor, 1,8-cineole, limonene and linalool) were selected, and their evaporation was examined on filter paper as well as from leaves of both plants. A retention index for each compound was predetermined by directly introducing the pure compounds in a septum-capped GC vial. A filter paper disc (22 mm diameter) was placed in a 20 mL glass vial, and then the filter paper was treated with 20 μL of test compound solution at 10 mg/mL in acetone. The same size of leaf disc was placed on an agar medium (2%, 3 mL) in a 20 mL glass vial, and the same amount of test compound was applied onto either a bean or cabbage leaf disc. The treated filter paper or leaf discs were introduced to the zNose™ without closing the lid. The distance between the leaf disc and a bent 4-cm stainless needle at the inlet was 3 cm.

The conditions for the zNose™ operation followed a previous study with a minor modification of the temperature setting (Miresmailli et al., 2010). Sampling for volatile compounds was set for 10s, and the temperatures of the inlet, valve and initial column were 200, 165, and 40 °C, respectively. The temperature of the column was increased to 200 °C at a rate of 10 °C/s, with helium as the carrier at a speed of 3 cc/m. The system was switched into a data acquisition mode for 10s, and