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# Acaricidal and repellent effects of myrtacean essential oils and their major constituents against *Tetranychus urticae* (Tetranychidae)

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ABSTRACT

Nineteen plant essential oils (EOs) extracted from the family Myrtaceae growing in Australia were screened for their acaricidal and repellent activities against two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), in the laboratory by dipping method and choice- and no-choice tests. Acaricidal bioassays showed that five EOs of *Callistemon viminalis*, *Eucalyptus bicostata*, *Eucalyptus maidenii*, *Eucalyptus sideroxylm* and *Eucalyptus approximans* significantly increased the mortality of female adult mites and decreased the total number of eggs. In a choice test, *Callistemon sieberi*, *E. bicostata*, *Eucalyptus ovata*, *E. sideroxylm*, *Eucalyptus mannifera*, *Eucalyptus dives*, *Eucalyptus elata*, *Eucalyptus condonocarpa*, *Kunzea ericoides*, *Melaleuca armillaris* and *Melaleuca fulgens* demonstrated good repellency to the mite. In another test, *C. sieberi*, *E. bicostata*, *E. mannifera*, *E. condonocarpa*, *K. ericoides*, *M. armillaris*, and *M. fulgens* EOs decreased the egg production of the females significantly. In the acaricidal and repellent tests, *E. bicostata* and *E. sideroxylon* EOs showed both acaricidal and repellent effects (choice test) and decreased the number of eggs on treated bean leaves. The gas chromatograph/mass spectroscopy analyses revealed that the major components of *E. bicostata* and *E. sideroxylon* were 1,8-cineole, limonene, and α-pinene. The 1,8-cineole and limonene showed significant repellent effects on the mites, resulting in reduced numbers of eggs in the choice test. Hence, EOs of *E. bicostata* and *E. sideroxylon* and limonene and 1,8-cineole may be potential agents to be used in the sustainable management of *T. urticae*.


## Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most important pests regarding fruits, vegetables, and ornamental plants (Ho, 2000; Takafuji et al., 2000; Raworth, 2001), causing damage to around 1200 plant species (Zhang, 2003). The mites have evolved a resistance to more than 80 acaricides from more than 60 countries (Michigan State University, 2005). As a result, the two-spotted spider mite imposes a great expense on greenhouse growers worldwide in terms of damage and control costs and is therefore considered a serious pest in greenhouse production (Miresmailli et al., 2006).

To control *T. urticae* with conventional acaricides is particularly difficult because of its ability to rapidly develop resistance to the particular acaricides used (Martison et al., 1991; Kim et al., 1999; Badawy et al., 2010). Subsequently, continuation of conventional acaricide use against the mite can cause serious adverse effects against humans and the environment, as well as non-target organisms, including insects and mites that prey on the pest (Kumral et al., 2010). It is therefore important to reduce the use of synthetic acaricides and to alternate them with products having a different mode of action to achieve the sustainable management of *T. urticae* (Isman, 2000; Choi, et al., 2003; Lee et al., 2003).

Essential oils are one of the promising candidates to achieve environmentally friendly pest management by modifying the toxicity to specific target species. In search of alternatives to conventional pesticides, essential oils (EOs) extracted from aromatic plants have been widely investigated. Their toxicities, and arresting and repellent effects on stored-product insects and greenhouse pests have been of special interest during the last decade (Tunc et al., 2000; Tripathi et al., 2000; Choi et al., 2003, 2004; Aslan et al., 2004). Several EOs have shown acaricidal, repellent, and oviposition deterrent activities against different species of spider mites, including the two-spotted spider mite. For example, acaricidal effects of *Datura* spp. extracts against the flat mite *Brevipalpus phoenicis* Geijskes (Acari: Tenuipalpidae), the two-spotted spider mite, and the coconut eriophyid mite *Aceria guerreronis* Keifer (Acari: Eriopyhidae) have been reported (Guirado et al., 2001; Thevan et al., 2005; Kumral et al., 2010). Leaf and seed extracts from *Datura metel* L. have a repellent effect on cassava red mites (Zang et al., 2006). Two EOs from rosemary and sweet marjoram showed repellent and oviposition deterrent activity against the two-spotted spider mite (Momen et al., 2001). Also the negative impacts of some extracts from

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plants, such as *Azadirachta indica* A. Juss (meliaceae), *Capsicum annuum* L. (Solanaceae), *Melodinus suaveolens* Hance (Apocynaceae), *Pterospermum heterophyllum* Hance (Sterculiaceae) and *Haworthia angustifolia* Haw. (Asphodelaceae), were demonstrated on the oviposition deterrent activity of mites (Martinez-Villar et al., 2005; Zang et al., 2006). Even though many plant EOs have been proven to be effective against the two-spotted spider mite, as mentioned above, the effects of Australian Myrtacean EOs on these mites have not been studied.

In this study, EOs from 19 Myrtacean species growing in Australia were evaluated in terms of their acaricidal, repellent, and oviposition deterrent activities in the laboratory against female adult two-spotted spider mites. The major chemical components of the EOs with effective acaricidal activity were further tested for their repellency against these mites.

## Materials and methods

### *Mites*

The acaricide-susceptible two-spotted spider mite was obtained from Gyeongnam Agricultural Research and Extension Services in 2007. The colony was maintained at 25 ± 2 °C, 60 ± 10% relative humidity, and 16:8 h light:dark in the laboratory on kidney bean (*Phaseolus vulgaris* var. *humilis*) plants without any exposure to any pesticides until they were used in experiments. The fresh un-infested kidney bean plants bearing three or four leaves were placed between the bean plants infested with two-spotted spider mites for 24 to 48 h. During this time, adults would move on to the un-infested plants, and the mites moved to fresh bean leaves were used in subsequent experiments.

### *Essential oils and synthetic compounds*

Nineteen species of the family Myrtaceae originating from Australia were used. Plant species and plant parts extracted in these experiments are described in Table 1. The methods of extraction are described in Lee et al. (2004). 200 μl of each EO and major component was dissolved in 20 ml of ethanol (97%, Burdik and Jackson, Muskegon, MI, USA), and then mixed with 180 ml of distilled water. The final concentration was a 0.1 % (1 ml/l) solution. Triton X-100 (20 μl) was added to the diluted solution. A mixture of ethanol (20 ml), Triton X-100 (20 μl) and distilled water (180 ml) was used as solvent control. No material was treated in a blank control.

**Table 1**
The species and yields of 19 Australian Myrtacean plant essential oils.

| Essential oil source | Plant part used |
|---|---|
| *Callistemon sieberi* | Leaf and twig |
| *Callistemon viminalis* | Leaf and twig |
| *Eucalyptus approximans* | Leaf |
| *Eucalyptus bicostata* | Leaf |
| *Eucalyptus blakelyi* | Leaf |
| *Eucalyptus condonocarpa* | Leaf |
| *Eucalyptus dives* | Leaf |
| *Eucalyptus elata* | Leaf |
| *Eucalyptus eximia* | Leaf and twig |
| *Eucalyptus maidenii* | Leaf |
| *Eucalyptus mannifera* | Leaf |
| *Eucalyptus. nicholii* | Leaf |
| *Eucalyptus ovate* | Leaf |
| *Eucalyptus sideroxylon* | Leaf |
| *Kunzea ericoides* | Leaf and twig |
| *Melaleuca armillaris* | Leaf and twig |
| *Melaleuca fulgens* | Leaf and twig |
| *Melaleuca linariifolia* | Leaf and twig |
| *Melaleuca thymifolia* | Leaf and twig |

### *Bioassay on acaricidal activity: dipping test*

Nineteen EOs were pre-screened for their acaricidal effects with a 0.1% concentration using the dipping method. Each experimental unit consisted of a kidney bean leaf disk (diameter 30 mm) onto which 30 female *T. urticae* adults were inoculated on wet cotton pads soaked in distilled water in a plastic Petri dish (diameter 60 × 15 mm). The female adults were inoculated on the lower surface of a bean leaf disk with a fine brush. The bean leaf disk with adult mites was dipped in the test solution for 5 s, then, placed upside down, so that the lower surface of the leaf was facing up, on the cotton pad in the Petri-dish, which was then maintained at 24 ± 2 °C, 40–80% relative humidity, and 16:8 h light:dark in the laboratory. The total number of live and dead adult mites was checked 24 h after dipping. The *T. urticae* adults were considered dead if no movement was apparent by probing with the tip of a fine brush. The number of eggs oviposited by the female mites treated with EOs was counted under a dissecting microscope (Stemi 2000, Carl Zeiss, Jena, Germany). These experiments were replicated three times. Using the result of this experiment, five EOs were preselected as more effective than the others. These oils were re-tested for their acaricidal effects using higher concentrations, 0.5% (5 ml/l) and 1% (10 ml/l).

### *Bioassay on repellency: choice test*

Bioassay choice tests similar to those used by Roh et al. (2012) were conducted using all 19 EOs at 0.1% concentration to investigate whether they are repellent to the mites. One of two leaves from the kidney bean plant was dipped in the test solution for 10 s and the other leaf was used as a control blank. After being air-dried, an adhesive (Fujitangle, Fuji Pharmaceutical, Inc., Tokyo, Japan) was painted around the central part of the main stem of the kidney bean plant. Approximately 30 female *T. urticae* adults were inoculated onto the stem just above the adhesive. The plants were arranged randomly in an air-conditioned laboratory to remove the influence of visual cues such as light on the position of the two-spotted spider mites. The number of adult mites on the petioles, on the leaves, treated and un-treated, and on the sticky board (13 × 15 cm) was recorded 24 h after mite inoculation. The number of eggs on both sides of the leaf surface was also counted under a dissecting microscope. This experiment was replicated three times.

### *Bioassay on repellency: no-choice test*

Eleven essential oils selected from the choice test were subjected to the no-choice test, similar to those used by Roh et al. (2012). One of the two leaves from the kidney bean plant was removed by trimming with a razor blade. The remaining leaf was dipped in the 0.1% EO solution for 10 s. A leaf of the control plants was dipped in the solvent control. Approximately 30 female *T. urticae* adults were inoculated on the stem just above the adhesive. The number of adult mites on the stem, petiole, leaf, and sticky board were counted 12 and 24 h after mite inoculation. The number of eggs on both sides of the leaf was counted at the end of the experiment. This was replicated three times.

### *Bioassay on repellency of individual constituents and a blend*

The acaricidal activities of three compounds (limonene, α-pinene, and 1,8-cineole) on *T. urticae* have been reported by many other studies, so we tested only the repellency of each component and from a blend of limonene and 1,8-cineole, two of the three major components of *Eucalyptus bicostata* and *Eucalyptus sideroxylon*, against two-spotted spider mites using both choice and no-choice behavioral tests as described earlier. A solvent control was added to the choice and no-choice tests. This was repeated three times.