Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae)

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Abstract
The acaricidal activity of clove (*Eugenia caryophyllata*) bud oil compounds (acetyleugenol, β-caryophyllene, eugenol, α-humulene), and congeners of eugenol (isoeugenol, methyleugenol) against adult *Tyrophagus putrescentiae* was examined using impregnated fabric disc and fumigation methods, and compared with that of benzyl benzoate. Responses varied according to compound and dose. LD_{50} values indicated that the compound most toxic to *T. putrescentiae* adults was methyleugenol (1.18 µg/cm²) followed by isoeugenol (8.21 µg/cm²), benzyl benzoate (8.85 µg/cm²), β-caryophyllene (11.77 µg/cm²), eugenol (12.11 µg/cm²), and α-humulene (12.90 µg/cm²). Very low activity was observed with acetyleugenol (28.72 µg/cm²). These results indicate that hydrophobicity of the four phenylpropenes (acetyleugenol, eugenol, isoeugenol, methyleugenol) plays a crucial role in *T. putrescentiae* toxicity. The typical poisoning symptom of the test compounds was a similar death symptom of the forelegs extended forward together, leading to death without knockdown, whereas benzyl benzoate caused death following uncoordinated behavior. In fumigation tests with adult *T. putrescentiae*, all four phenylpropenes were more effective against the mites in closed containers than in open ones, indicating that the mode of delivery of these compounds was largely due to action in the vapor phase. The clove bud oil compounds as well as isoeugenol and methyleugenol merit further study as potential storage mite control agents or as lead compounds.

Key words: Natural acaricide; natural fumigant; Tyrophagus putrescentiae; Eugenia caryophyllata; mode of action

INTRODUCTION
The copra mite, *Tyrophagus putrescentiae* (Schrank), is one of the most important storage mites because of its cosmopolitan occurrence and abundance in a large number of food grains and stored foods, especially those with high fat and protein content (Hughes, 1976; Sinha, 1979). This mite species causes serious economic losses (Zdarkova, 1991) as well as a reduction of nutrient contents and seed viability (Krantz, 1955). Additionally, *T. putrescentiae* is known to be the etiological agent of allergic diseases among farmers and workers handling heavily infested stored products (Hughes, 1976), and causes acute enteritis (Hughes, 1976) and systemic anaphylaxis (Matsumoto et al., 1966) when contaminated food is ingested. *Tyrophagus putrescentiae* also acts as a carrier of bacteria and toxigenic fungi such as *Aspergillus* spp. and *Penicillium* spp. in stored grain kept under warm and moist conditions (van Bronswijk and Sinha, 1973; Franzolin et al., 1999).

Control of this mite is dependent upon the use of chemical methods such as fumigation treatments, spraying with organophosphates, or treatments with benzyl benzoate. However, repeated use of chemicals has sometimes resulted in the development of resistance (Wilkin, 1979; Stables, 1984), has undesirable effects on non-target organisms, and fosters environmental and human health concerns (Hays and Laws, 1991). These problems have highlighted the need for the development of new strategies for selective storage mite control.

Plant essential oils may be an alternative source of compounds for storage mite control because they constitute a rich source of bioactive chemicals. Because of this, much effort has been focused on plant essential oils as potential sources of commercial pest control agents or as lead compounds (Isman, 1999). It has been reported that clove (*Eugenia caryophyllata* Thunberg) bud oil has insecticidal activity against *Tribolium castaneum* (Herbst)

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and *Sitophilus zeamais* (Motsch.) (Ho et al., 1994). It contains various compounds such as acetyl-eugenol, benzaldehyde, benzyl acetate, benzyl alcohol, \( \beta \)-caryophyllene, chavicol, eugenol, \( \alpha \)-humulene, \( m \)-methoxybenzaldehyde, methyl-\( n \)-amylketone, methyl salicylate, \( \alpha \)-ylangene (Deyama and Horiguchi, 1971). Little work has been done with respect to managing storage mites.

This paper describes a laboratory study in which we examined the acaricidal activity of clove bud oil compounds (acetyleneugenol, \( \beta \)-caryophyllene, eugenol, \( \alpha \)-humulene) and congeners of eugenol (isoeugenol, methyleugenol) against adult *T. putrescentiae*, and investigated their acaricidal route of action. The structure-acaricidal activity relationships of the phenylpropenes (acetyleneugenol, eugenol, isoeugenol, methyleugenol) are also discussed.

**MATERIALS AND METHODS**

**Chemicals.** Benzyl benzoate, \( \beta \)-caryophyllene, and \( \alpha \)-humulene were purchased from Aldrich (Milwaukee, WI, USA). Acetyleneugenol, eugenol, isoeugenol, and methyleugenol were supplied by Wako (Osaka, Japan), Sigma (St. Louis, MO, USA), Fluka (Buchs, Swiss), and Merck (Mohenbrunn, Germany), respectively. All other chemicals were of reagent grade.

**Mites.** Cultures of *T. putrescentiae* were maintained in the laboratory for six years without exposure to any acaricide. Mites were reared in plastic containers (12.5 \( \times \) 10.5 \( \times \) 5.0 cm) containing 25 g of sterilized diet (fry feed No. 1/dried yeast, 1:1 by weight) at 25 \( \pm \) 1°C and 75% RH in continuous darkness. Fry feed was purchased from Korea Special Feed Meal Co. Ltd., Inchon, Korea.

**Chromatographic analysis of clove bud oil.** The essential oil of clove bud was purchased from Jin Aromatics, Anyang, Kyunggi Province, Korea. Chromatographic analyses were performed using a Hewlett-Packard 6890 series gas chromatograph, equipped with a splitless injector and a flame ionization detection system. Analytes were separated with a DB-WAX column (J&W Scientific, Folsom, CA, USA), 60 m \( \times \) 0.25 mm ID, with a film thickness 0.25 \( \mu \)m. The temperature program used for the analysis was as follows: initial temperature at 50°C, held for 10 min ramped at 2°C/min to 200°C, held for 15 min. Helium was used as the carrier (1 ml/min). The detector gases were hydrogen and air and their flow-rates were regulated at 500 and 45 ml/min, respectively. The detector temperature was set at 250°C and the injector temperature at 210°C.

GC-MS experiments were performed on a GC (HP 6890)-Mass spectrometer (JMS-600W, JEOL, Japan). The capillary column and temperature conditions for the GC-MS analysis were the same as described above. Helium was used as the carrier gas at a flow of 1 ml/min. The interface was kept at 230°C and mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 50 to 800 amu for every 0.7 s. The compounds of clove bud oil were identified by comparisons of the mass spectra of each peak with those of authentic samples in a Mass spectra library (The Wiley Registry of Mass Spectral Data, 6th ed.) and confirmed by comparison of retention times obtained by GC with those of authentic samples.

**Bioassay.** An impregnated fabric disc bioassay was used for acaricidal activity of test materials. Amounts (50.9, 32.5, 25.5, 12.7, 6.4, 3.2, 1.6, 0.8, and 0.4 \( \mu \)g/cm²) of each test material dissolved in 100 \( \mu \)l of ethanol were applied to discs of black cotton fabric (5 cm diameter). Control fabric discs received 100 \( \mu \)l of ethanol. After drying in a fume hood for 1 min, each disc was placed in the bottom of a petri dish (5 cm diameter \( \times \) 1.2 cm). Then groups of 25 adults (7–10 days old) were placed in each petri dish and covered with a lid.

In a separate experiment, susceptibility of adult *T. putrescentiae* to the test compounds in the vapor phase was investigated according to the method of Kwon and Ahn (2002). Briefly, groups of 25 adults (7–10 days old) were placed in the bottom of a petri dish (5 cm diameter \( \times \) 1.2 cm) and covered with a lid with a fine wire sieve (4.7 cm diameter) attached to the center hole (4.5 cm diameter). Each fabric disc (5 cm diameter), treated with 25.5 \( \mu \)g/cm² of the test compound dissolved in 100 \( \mu \)l of ethanol, was placed over the wire sieve. This prevented direct contact of test adults with the test compound. Each petri dish was then either covered with another lid (method A) or left uncovered (method B). Control fabric discs received 100 \( \mu \)l of ethanol.

Treated and control mites were held at the same
conditions used for colony maintenance. Mortality was determined 24 h after treatment under a binocular microscope (20×). Mites were considered dead if appendages did not move when the mite was prodded with a pin. All treatments were replicated three times. The LD$_{50}$ values were calculated by probit analysis (SAS Institute, 1990).

RESULTS

Chemical constituents of clove bud oil

According to the analysis results of clove bud oil, three major and one minor constituents were identified by comparison of mass spectral data and retention times of authentic compounds (Fig. 1). The clove bud oil was mainly composed of eugenol (Rt, 64.49 min; 68.96%), acetyleneugenol (Rt, 69.19 min; 20.19%), and $\beta$-caryophyllene (Rt, 35.02 min; 10.07%), and also contained $\alpha$-humulene (Rt, 39.19 min; 0.78%) as a minor component. The result essentially coincided with the data of Deyama and Horiguchi (1971). The peaks which appeared regularly from ca. 55 min to 90 min were octadecanoic silanate derived from the capillary column.

Contact activity with treated fabric disc

When clove bud oil was bioassayed, significant differences were observed in toxicity to adult mites (Table 1). After 24 h of exposure the essential oil gave 100 and 17% mortality against adult *T. putrescentiae* at 12.7 and 6.4 $\mu$g/cm$^2$, respectively.

The toxicity of clove bud oil compounds (acetyleneugenol, $\beta$-caryophyllene, eugenol, $\alpha$-humulene) and congeners of eugenol (isoeugenol, methyleugenol) against adult *T. putrescentiae* was compared with that of the commonly used benzylo

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Acaricidal activity of clove bud oil against adult <em>T. putrescentiae</em> using the impregnated fabric bioassay</th>
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</thead>
<tbody>
<tr>
<td>Dose ($\mu$g/cm$^2$)</td>
<td>Mortality, % (mean±SE)$^a$</td>
</tr>
<tr>
<td>25.5</td>
<td>100±0.0</td>
</tr>
<tr>
<td>12.7</td>
<td>100±0.0</td>
</tr>
<tr>
<td>6.4</td>
<td>17±2.7</td>
</tr>
<tr>
<td>3.2</td>
<td>3±2.7</td>
</tr>
</tbody>
</table>

$^a$ Exposed for 24 h.

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<tr>
<th>Table 2.</th>
<th>Toxicity of eugenol congeners and acaricides against adult <em>T. putrescentiae</em> using the impregnated fabric disc bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound$^d$</td>
<td>Slope ($\pm$SE)</td>
</tr>
<tr>
<td>Acetyleneugenol$^d$</td>
<td>11.31±1.44</td>
</tr>
<tr>
<td>Eugenol$^d$</td>
<td>4.18±0.46</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>6.76±0.81</td>
</tr>
<tr>
<td>Methyleugenol</td>
<td>7.68±0.76</td>
</tr>
<tr>
<td>$\beta$-Caryophyllene$^d$</td>
<td>4.85±0.43</td>
</tr>
<tr>
<td>$\alpha$-Humulene$^d$</td>
<td>4.10±0.34</td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td>3.26±0.43</td>
</tr>
</tbody>
</table>

$^a$ Exposed for 24 h.

$^b$ Confidence limit.

$^c$ Relative toxicity, LD$_{50}$ value of benzyl benzoate/LD$_{50}$ value of each chemical.

$^d$ Clove bud oil compounds identified in this study.

benzoate which served as a standard for comparison in toxicity tests (Table 2). Responses varied according to compound. On the basis of 24-h LD$_{50}$ values, the compound most toxic to adult *T. putrescentiae* was methyleugenol followed by isoeugenol, benzyl benzoate, $\beta$-caryophyllene, eugenol, and $\alpha$-humulene. Very low activity was observed with acetyleneugenol. Methyleugenol was
7.5 times more toxic than benzyl benzoate. Isoeugenol and benzyl benzoate were equitoxic. There was no mortality in the untreated controls.

Poisoning symptom

Acetyleugenol, β-caryophyllene, eugenol, α-humulene, isoeugenol, and methyleugenol resulted in a similar death symptom of the forelegs extended forward together without knockdown, whereas benzyl benzoate caused death following uncoordinated behavior. Loss of glossiness of the cuticle was only observed in the mites treated with methyleugenol but not in those treated with other compounds.

Acaricidal route of action

The response of adult *T. putrescentiae* to vapors of acetyleugenol, eugenol, isoeugenol, and methyleugenol varied with the treatment method (Table 3). After 24 h of exposure to 25.5 μg/cm², there was a significant difference in acaricidal activity of eugenol between exposure in a closed container (method A), which resulted in 100% mortality, and exposure in an open container (method B), which resulted in 21% mortality against adult mites. Similar differences in the response of adult *T. putrescentiae* to acetyleugenol, isoeugenol, and methyleugenol in treatments A and B were observed. There was no mortality in the untreated controls.

DISCUSSION

Plant essential oils are potential candidates for storage mite control because some of them are selective, have little or no harmful effects on non-target organisms and the environment, and may be applied to stored products in the same way as conventional acaricides (Isman, 1999). Many plant essential oils and phytochemicals are known to possess acaricidal activity against storage mites. The acaricidal constituents of many essential oils are mainly monoterprenoids. The reported naturally occurring acaricidal compounds against *T. putrescentiae* include benzaldehyde, d-carvone, l-carvone, and methyl salicylate (Watanabe et al., 1989); eucalyptol, fenchone, linalool, menthone, and pulegone (Perrucci, 1995; Sanchez-Ramos and Castanera, 2001); cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde (Kim et al., 2003); and butylidene phthalide (Kwon and Ahn, 2002). In the present study, clove bud oil revealed potent acaricidal activity against adult *T. putrescentiae*. The acaricidal constituents of the oil were identified as acetyleugenol, β-caryophyllene, eugenol, and α-humulene. β-Caryophyllene, eugenol, and α-humulene were slightly less active than benzyl benzoate. However, the acaricidal activity was more pronounced with eugenol congeners methyl-eugenol and isoeugenol than with benzyl benzoate against adult *T. putrescentiae*. Eugenol possesses attractant effects for adult *Diabrotica barberi* (Smith and Lawrence) (Ladd et al., 1983; Lampman and Metcalf, 1988) and insecticidal activity against *T. castaneum* and *S. zeamais* (Ho et al., 1994), while methyleugenol has potent attractant effects for male *Dacus dorsalis* (Hendel) (DeMilo et al., 1994) and insecticidal activity against *T. castaneum* and *S. zeamais* (Ho et al., 1994). Isoeugenol has insecticidal activity against *T. castaneum* and *S. zeamais* (Ho et al., 1994).

Structure-activity relationships of plant oil compounds against arthropod pests have been well studied. Rice and Coats (1994) and Tsao et al. (1995) attempted to enhance the potency of monoterpenes and phenols through derivatization of the hydroxyl group. They stated that enhanced bioactivity of the derivatives appeared to result from increased vapor pressure, leading to greater fumigant action, as well as increased lipophilicity, leading to better penetration in the insect’s body. Kim et al. (2003) studied the structure-activity relationship between cinnamaldehyde and its 11 congeners and acaricidal activity against adult *T. putrescentiae*: hydrophobicity appears to play a crucial role in toxicity but a conjugated double bond

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method</th>
<th>Mortality, % (mean±SE)</th>
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</thead>
<tbody>
<tr>
<td>Acetyleugenol</td>
<td>A, vapor in close containers</td>
<td>100±0.0</td>
</tr>
<tr>
<td></td>
<td>B, vapor in open containers</td>
<td>19±3.5</td>
</tr>
<tr>
<td>Eugenol</td>
<td>A, vapor in close containers</td>
<td>100±0.0</td>
</tr>
<tr>
<td></td>
<td>B, vapor in open containers</td>
<td>21±2.7</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>A, vapor in close containers</td>
<td>100±0.0</td>
</tr>
<tr>
<td></td>
<td>B, vapor in open containers</td>
<td>24±2.3</td>
</tr>
<tr>
<td>Methyleugenol</td>
<td>A, vapor in close containers</td>
<td>100±0.0</td>
</tr>
<tr>
<td></td>
<td>B, vapor in open containers</td>
<td>23±3.5</td>
</tr>
</tbody>
</table>

*a Exposed for 24 h at a dose of 25.5 μg/cm².*
and a length of CH chain outside the ring appear not to be responsible for the toxicity. Regnault-Roger and Hamraoui (1995) studied the structure-activity relationship between monoterpenoids and fumigant activity against Acanthoscelides obtectus (Say): the oxygenated structures proved to be the most active compounds, especially carvacrol, linalool, and terpineol. In our study, the order of lethal effect against adult *T. putrescentiae* was methyleugenol > eugenol > isoeugenol > acetyleneugenol. These results indicate that the toxicity of the phenylpropenes might be due to their hydrophobicity.

Five types of poisoning symptoms of chemicals against mites have been reported: ① a knockdown-type death (Furuno et al., 1994); ② death related with uncoordinated behavior without knockdown (Kim et al., 2003); ③ death associated with desiccation (Sanchez-Ramos and Castanera, 2001); ④ death related with a characteristic depression of the dorsal surface of the idiosoma (Ignatowicz, 1981); and ⑤ death associated with lethargy (Kwon and Ahn, 2002). In our study, all test compounds resulted in a death symptom of extending the forelegs side by side together without knockdown, resembling those type of ②. It is worth noting that loss of glossiness of cuticle was observed only in the mites treated with methyleugenol.

Volatile compounds from many plant extracts and essential oils are composed of alkanes, alcohols, aldehydes, and terpenoids, especially monoterpenoids, and exhibit fumigant activity (Coats et al., 1991; Isman, 1999; Kim and Ahn, 2001; Kwon and Ahn, 2002; Kim et al., 2003). Fumigant activity against adult *T. putrescentiae* has been reported in cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde (Kim et al., 2003), as well as butylidenephthalide (Kwon and Ahn, 2002). In our study, acetyleneugenol, eugenol, isoeugenol, and methyleugenol were more effective in closed containers than in open ones against adult *T. putrescentiae*. These results indicate that the mode of delivery of these compounds was largely due to action in the vapor phase: they may be toxic by penetrating the storage mite's body via the respiratory system. The acaricidal mode of action of these phenylpropenes may be related to the specific neurotoxic action such as the octopaminergic action, as previously described (Isman, 1999), although this remains to be proven.

Results of this and earlier studies indicate that clove bud oil-derived materials as well as isoegenol and methyleugenol could be useful as fumigants for *T. putrescentiae*. Methyleugenol causes hepatic tumors in mice and rats (Miller et al., 1983), induces intrachromosomal recombination in a yeast assay (Schiestl et al., 1989), and elicits a positive response in a bacterial DNA repair test (Sekizawa and Shibamoto, 1982) and a negative response in the Ames mutagenicity test (Schiestl et al., 1989), although this compound is a food flavoring agent and appears on the Flavor and Extract Manufacture's Generally Regarded as Safe (GRAS) list. Eugenol can be found on the US Food and Drug Administration's GRAS list, and are exempt from toxicity data requirements by the US Environmental Protection Agency, although this compound gives a positive response in the yeast assay and is carcinogenic in mice and rats (Miller et al., 1983). For practical use of these compounds as novel fumigants, further research should be done on safety issues of these compounds for human health and changes in quality such as the color, flavor, odor, and texture of stored products treated with these compounds, and formulations improving the acaricidal potency and stability.

ACKNOWLEDGEMENTS

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