EFFECTS OF EUCALYPTOL ON HOUSE FLY (DIPTERA: MUSCIDAE) AND BLOW FLY (DIPTERA: CALLIPHORIDAE)

Kabkaew L. SUKONTASON, Noppawan BOONCHU, Kom SUKONTASON & Wej CHOOCHOTE

SUMMARY

The effects of eucalyptol were evaluated against the house fly, *Musca domestica* L., and blow fly, *Chrysomya megacephala* (F.). The bioassay of adults, using topical application, indicated that *M. domestica* males were more susceptible than females, with the LD$_{50}$ being 118 and 177 µg/fly, respectively. A higher LD$_{50}$ of *C. megacephala* was obtained; 197 µg/fly for males and 221 µg/fly for females. Living flies of both species yielded a shorter life span after being treated with eucalyptol. The bioassay of larvae, using the dipping method on the third instar, showed that *M. domestica* was more susceptible than *C. megacephala*, with their LC$_{50}$ being 101 and 642 µg/µl, respectively. The emergence of adults, which had been treated with eucalyptol in larvae, decreased only in *M. domestica*. Having the volatile property, fumigation or impregnated paper test of eucalyptol or the efficacy of repellence or attractiveness merits further investigations to enhance bio-insecticidal efficacy.

KEYWORDS: *Musca domestica*; *Chrysomya megacephala*; Eucalyptol; Bio-insecticide.

INTRODUCTION

The common house fly, *Musca domestica* L., and blow fly, *Chrysomya megacephala* (F.), are medically important insects; being mechanical carriers of several pathogens (e.g., virus, bacteria, protozoa, helminth eggs) that may cause illness and disease in humans or annoyance to humans and agronomic livestock. The economic loss in livestock business resulting from fly annoyance and/or myiasis caused by these species has been recorded.

A conventional method for fly control in the short-term is the use of insecticides. Nevertheless, the widespread and massive applications of chemical insecticides frequently produce the risk of developing insect resistance and insecticidal residual for humans and the environment. Accordingly, bio-insecticides, a botanical type based on natural compounds from plants, are expected to be a possible application as selective, efficacious and toxicologically safe insecticides. Several reports have shown the efficacy of natural compounds on insects. As for flies, the majority have been found to assess crude extracts from many botanical sources. Regarding this, assessment of the pure active compound extracted from plants against flies is of interest.

Eucalyptol or 1,8-cineole, the major component of eucalyptol oil and other plants, is a monoterpenoid substances. Eucalyptol is safe for humans, and has been used as medicine and aromatherapy for a long time. According to the primarily lipophilic property, several monoterpenoids have been reported as having toxic capability to herbivore insects, and were therefore considered as a potential, alternative biopesticide. Toxicity of eucalyptol on several species of medical or economical insects (e.g., triatomine bug, red flour beetle, lessor grain borer, rice weevil, sawtoothed grain beetle, house fly, Hessian fly, German cockroach, and stored food mite) have been recorded. We therefore examined the effect of eucalyptol on *M. domestica* and *C. megacephala* to provide the knowledge for natural products in fly control.

MATERIALS AND METHODS

Collection and maintenance of flies: The colonies of *M. domestica* and *C. megacephala* originated from adults collected from markets in Muang district, Chiang Mai province, northern Thailand, using a sweeping net. They were transferred into a small cage (16×16×16 cm) and transported to the Department of Parasitology, Faculty of Medicine, Chiang Mai University, for identification and colonization. Before identification, the flies were anesthetized by placing the cages in a refrigerator set at 4 °C for 15 min. The anesthetized flies were identified following the taxonomic keys. *M. domestica* and *C. megacephala* were segregated into separate cages (30x30x30 cm) screened with black cloth. Adults of both species were reared with two kinds of food; (I) mixture of 10% (w/v) sugar solution at 985 ml and multivitamin syrup at 15 ml; and (II) fresh pork liver as both a food source and oviposition site. Furthermore, *M. domestica* flies were provided with a combination of rice polish, chaff and water at a weight ratio of 2:1:1, and 40 g of this mixture was placed on a 9 cm glass plate, as a supplementary food and oviposition site. Small pieces of fresh pork liver were changed daily,
were studied further for their life span.

caps. The flies in each group were re-anesthetized using CO2 fumigation and then the bottles were tightly closed immediately with their lids. The flies were separated into males or females. Each sex was randomly divided into seven groups of 20 flies per group and transferred into each small cage (16×16×16 cm) using a transparent test-tube. This cage was then covered with a transparent plastic bag in order to anesthetize the flies by fumigation with 5 L of CO2 for 3 min. The flies were transferred into each rearing cage and provided with adult food. A bowl containing each concentration of eucalyptol was tightly covered with the lid until they were used for the dipping method. For the experiment, the larvae of each group were wrapped in a voile cloth and gently dipped into eucalyptol solution, whereas those of the controls were dipped in absolute ethanol. After being dipped for exactly 30 sec, the larvae were transferred to the rearing box containing food. The mortality of each larva was assessed at 24 h by touching each one with a paint brush (no. 0), and those not responding were considered dead. The dipping experiments were carried out in three replications. The LD50, LD95 and LD99 of toxicity was determined based on mortality data at 24 h assessments, and Probit analysis (Harvard Programming; Hg1, 2) was used in analyzing the dosage-mortality response. Living larvae were studied further for their adult emergence after being treated with eucalyptol.

Emergence of adults after being dipped with eucalyptol: Living larvae were reared in each rearing box after being dipped in each concentration of eucalyptol or absolute ethanol, and the maintenance of the larvae was conducted in the same manner as previously described until emergence. Once emergence occurred, the adult flies were counted. The Chi Square test was used to compare the emergence of treated and control groups.

RESULTS

Assessment of eucalyptol toxicity: The eucalyptol toxicities against adult M. domestica and C. megacephala, evaluated by LD50, LD95 and LD99 values, are presented in Table 1. Regarding M. domestica, all were dead after they had been treated with eucalyptol 0.902 g/ml. Males were more susceptible than females in all concentrations used. The LD50 and LD99 of males were 118 and 460 µg/fly, respectively, whereas those of females were 177 and 500 µg/fly, respectively. No significant difference was found between males and females, based on the overlapping of 95% confidence interval at LD50. As for C. megacephala, toxicity of eucalyptol against males was slightly lower than females. The LD50 and LD99 of males were 197 and 380 µg/fly, respectively, while in females, they were 221 and 422 µg/fly, respectively. No significant difference in mortality between males and females was detected.

Life span of living flies after being topically tested with eucalyptol: After being tested with the same concentration of eucalyptol, all living flies were pooled together and maintained within the same cage. Food was provided, and the mortality of flies was investigated daily. All flies in the rearing cages were maintained under the same conditions including quality and quantity of food. Replacement of food was performed as previously described until all the flies were dead. The life span of the flies in each group was summarized and analyzed by comparing with controls using the Mann Whitney U test.

Dipping method for the bioassay test of the larvae: The third instar, M. domestica and C. megacephala, used in this experiment were 3-day-old after hatching from the same egg batch. Each species was randomized into 12 groups (10 larvae/group) and reared in separate rearing boxes. Eucalyptol solutions were immediately prepared in a ceramic bowl by the serially 2-fold dilution method using absolute ethanol as the solvent, and the concentration was the same as that in the adult experiments. A bowl containing each concentration of eucalyptol was tightly covered with the lid until they were used for the dipping method. For the experiment, the larvae of each group were wrapped in a voile cloth and gently dipped into eucalyptol solution, whereas those of the controls were dipped in absolute ethanol. After being dipped for exactly 30 sec, the larvae were transferred to the rearing box containing food. The mortality of each larva was assessed at 24 h by touching each one with a paint brush (no. 0), and those not responding were considered dead. The dipping experiments were carried out in three replications. The LD50, LD95 and LD99 of toxicity was determined based on mortality data at 24 h assessments, and Probit analysis (Harvard Programming; Hg1, 2) was used in analyzing the dosage-mortality response. Living larvae were studied further for their adult emergence after being treated with eucalyptol.

Life span of living flies after being topically tested with eucalyptol: After being tested with the same concentration of eucalyptol, all living flies were pooled together and maintained within the same cage. Food was provided, and the mortality of flies was investigated daily. All flies in the rearing cages were maintained under the same conditions including quality and quantity of food. Replacement of food was performed as previously described until all the flies were dead. The life span of the flies in each group was summarized and analyzed by comparing with controls using the Mann Whitney U test.

Control flies were divided into 2 groups; treated with absolute ethanol and untreated. Both groups were anesthetized twice in a similar manner to that of the treated groups. After being tested, the flies in all groups were transferred into each rearing cage and provided with adult food. Mortality in each group was assessed at 24 h periods after exposure by softly stimulating each fly with the tip of a pen. Those flies that showed no response were considered dead. The experiments were carried out in three replications. The lethal dose (LD) of toxicity (LD50, LD95 and LD99) was determined based on mortality data at 24 h assessments, and Probit analysis (Harvard Programming; Hg1, 2) was used for analyzing the dosage-mortality response. Later, all living flies, both treated and controls, were studied further for their life span.
Table 1

Toxicity of eucalyptol against adult *Musca domestica* and *Chrysomya megacephala* using topical application

<table>
<thead>
<tr>
<th>Sex</th>
<th><em>M. domestica</em> LD₅₀ (95% CI)</th>
<th><em>M. domestica</em> LD₉₅ (95% CI)</th>
<th><em>M. domestica</em> LD₉₉ (95% CI)</th>
<th><em>C. megacephala</em> LD₅₀ (95% CI)</th>
<th><em>C. megacephala</em> LD₉₅ (95% CI)</th>
<th><em>C. megacephala</em> LD₉₉ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>118 (91-154)</td>
<td>460 (354-598)</td>
<td>987 (759-1283)</td>
<td>197 (177-218)</td>
<td>380 (323-495)</td>
<td>549 (435-816)</td>
</tr>
<tr>
<td>Female</td>
<td>177 (153-202)</td>
<td>500 (392-754)</td>
<td>895 (623-1682)</td>
<td>221 (199-244)</td>
<td>422 (357-560)</td>
<td>608 (478-924)</td>
</tr>
</tbody>
</table>

Unit of mortality is µg/fly.

Table 2

Life span of living flies [median (range)] after being topically tested with varying concentrations of eucalyptol

<table>
<thead>
<tr>
<th>Concentration of eucalyptol (g/ml)</th>
<th><em>M. domestica</em> (Days)</th>
<th><em>C. megacephala</em> (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0.451</td>
<td>-</td>
<td>2 (2-3) *</td>
</tr>
<tr>
<td>0.226</td>
<td>5 (2-17) *</td>
<td>4 (2-18) *</td>
</tr>
<tr>
<td>0.113</td>
<td>8 (2-19) *</td>
<td>8 (2-20) *</td>
</tr>
<tr>
<td>0.056</td>
<td>13 (3-33) **</td>
<td>17 (5-29) **</td>
</tr>
<tr>
<td>Absolute ethanol</td>
<td>15 (2-30) ***</td>
<td>15.5 (2-33) ***</td>
</tr>
<tr>
<td>Natural control</td>
<td>17 (2-31)</td>
<td>17 (2-31)</td>
</tr>
</tbody>
</table>

`*` Significant difference from control group (absolute ethanol) (Mann Whitney U test, *p* < 0.05); `**` No significant difference from control group (absolute ethanol) (Mann Whitney U test, *p* > 0.05); `***` No significant difference from natural control (Mann Whitney U test, *p* > 0.05).

Table 3

Toxic activity of eucalyptol against the third instar, *M. domestica* and *C. megacephala*, using the dipping method

<table>
<thead>
<tr>
<th>Species</th>
<th><em>M. domestica</em> LD₅₀ (95% CI) µg/µl</th>
<th><em>M. domestica</em> LD₉₅ (95% CI) µg/µl</th>
<th><em>M. domestica</em> LD₉₉ (95% CI) µg/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. domestica</em></td>
<td>101</td>
<td>239</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>(92-111)</td>
<td>(203-305)</td>
<td>(305-558)</td>
</tr>
<tr>
<td><em>C. megacephala</em></td>
<td>642</td>
<td>1539</td>
<td>2511</td>
</tr>
<tr>
<td></td>
<td>(585-710)</td>
<td>(1272-2044)</td>
<td>(1915-3797)</td>
</tr>
</tbody>
</table>

Emergence of adult flies after being dipped with eucalyptol: After the larvae had been dipped, they were reared to determine the success of emergence. As shown in Table 4, all *M. domestica* larvae died when they had been dipped in eucalyptol at 0.902 g/ml, thus no emergence of flies was found. When the larvae had been subjected to eucalyptol at 0.451, 0.226, 0.113 and 0.056 g/ml, emergence was 50, 67, 35 and 60%, respectively. In contrast, the emergence of *C. megacephala* after the larvae had been dipped with eucalyptol at 0.902, 0.451, 0.226, 0.113 and 0.056 g/ml was 100, 93, 90, 93 and 73%, respectively.

**DISCUSSION**

Our study shows that both the adults and larva of *M. domestica* were more significantly susceptible than *C. megacephala* to eucalyptol. The reason for such a difference between species was unknown. It might...
Eucalyptol yielded a moderate larvicide effect (LD₅₀ = 101 µg/µl) on the larvae, *M. domestica*, while it was low against *C. megacephala* (LD₅₀ = 642 µg/µl). The low toxicity in this study was similar to that of LAURENT *et al.* in that the commercially pure compound of eucalyptol (1,8-Cineole) had a weak larvicidal action on the fourth instar nymph of the triatomine bug, *Triatoma infestans* (Klug), when topical application was used.

Although the toxicity of eucalyptol against the adults or larvae of flies varied from low to moderate in this study, some modes might enhance insecticidal toxicity. The small addition of compounds, called synergist, enormously increased toxicity. D-limonene, the component of citrus peel oil extract, was synergized by piperonyl butoxide. When combined, these compounds produced a synergistic ratio of 3.2 and more rapid mortality of the adult cat fleas, *Ctenocephalides felis* (Bouché). Regarding this, a combination of eucalyptol with synergist, either from plant extract or commercial products, is a subject of interest.

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**REFERENCES**


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