

*Full Length Research Paper*

# Toxicity of essential oil compounds against *Exorista sorbillans* (Diptera: Tachinidae), a parasitoid of silkworm

Khanikor, B. and Bora, D.\*

Department of Life Sciences, Dibrugarh University, Assam-786004, India.

Accepted 4 November, 2011

Essential oils of *Ageratum conyzoides* and *Ocimum* species are potential candidates for management of *Exorista sorbillans* (Wiedemann) (Diptera: Culicidae), a serious pest of silkworm. Considering that the pure compounds in essential oil may exhibit efficacy against the parasitoid, contact and topical toxicity of 22 essential oil compounds found in plants like *A. conyzoides*, different chemotypes of *Ocimum* species, *Cymbopogon citratus* and *Eucalyptus globulus* were assessed against *Exorista sorbillans*. The compounds under study were terpenes and coumarin. Most of the compounds were toxic to *E. sorbillans* but their toxicity varied with the bioassay method. The LC<sub>50</sub> values of 11 compounds effective as contact fumigant, namely citral, geraniol, nerol, carvacrol, eugenol, isoeugenol, l-citronellol, carvone, coumarin,  $\alpha$ -caryophyllene and terpinen-4-ol were 110.23, 124.11, 135.75, 194.53, 207.25, 266.40, 267.24, 299.23, 364.70, 533.02 and 1213.43 ppm, respectively. Topical application showed a different order of toxicity of the compounds. The compounds,  $\alpha$ -pinene, 1,8-cineole,  $\gamma$ -terpinene,  $\beta$ -pinene, S-limonene and D-limonene which did not possess much efficacy as contact-fumigant were highly effective on topical application. We recommend the compounds found effective in this study for further studies to develop viable control agent against *E. sorbillans*, which is lacking at present.

**Key words:** Essential oil, monoterpenes, coumarin, *Exorista sorbillans*, silkworm.

## INTRODUCTION

In the recent years essential oil compounds are gaining importance because of their wide spectrum of biological action, novel mode of action and eco-friendly nature (Abdelgaleil et al., 2009). They are byproducts of plant metabolism and are commonly referred to as volatile plant secondary metabolites. Most essential oils include monoterpenes with 10 carbon atoms, sesquiterpenes with 15 carbon atoms and rarely diterpenes or higher terpenes. Monoterpenes like eugenol, citral, thymol and 1,8-cineole have been reported to have antidiabetic, anticarcinogenic, anesthetic and analgesic properties (Fang and Lipton, 1987; Ortiz de Urbina et al., 1989; Calacabrini et al., 2004; Pisano et al., 2007). Many of the monoterpenes are widely used in food, flavor, fragrance

and cosmetic industry (Bickers et al., 2003; EPSA, 2006; Belsito, 2008; Koul et al., 2008; Traboulsi et al., 2002; Traboulsi et al., 2007). Monoterpene hydrocarbons like alpha-pinene, beta-pinene, limonene, terpinene, and oxygenated monoterpene like 1,8 cineole, fenchone, menthone, carvacrol, thymol, eugenol, linalool, terpineol etc., have been reported effective against Dipteran, Coleopteran, Dictyopteran and Hemipteran insects (Obeng-Ofori and Reichmuth, 1997; Regnault-Roger and Hamraoui, 2000; Tarelli et al., 2009; Wang et al., 2009; Chang et al., 2009; Kaufman et al., 2010; Mann et al., 2009; Alzogaray et al., 2011). Coumarins, primarily benzo- $\alpha$ -pyrone derivatives, are also known for their insecticidal activities (Gallo et al., 1996; Deshmukh et al., 2008).

Uzi fly, *Exorista sorbillans* Wiedemann, is an endoparasitoid of silkworm larvae. The fly infestation has been reported as major constraint of silk production in many silk producing countries like India, China, Japan,

\*Corresponding author. E-mail: dipsikhabora03@yahoo.com.  
Tel: 91-373-2370250. Fax: 91-373-2370323.

South Korea, Bangladesh and Vietnam, etc. (O'Hara, 1992). The maggots reside inside the body of silkworm larva and thereby either kill the mature larva or render silk cocoons unreelable. Use of synthetic pesticide is not advisable in sericulture field because of their probable adverse effect on silkworms and other beneficial organisms. Different control measures have been attempted to check uzi fly infestation (Kumar et al., 1996; Singh and Maheshwari, 2002; Sarma and Khound, 2004; Shekhar et al., 2007; Sahu et al., 2008), yet the problem is of major concern for sericulture industry. Literatures on the use of essential oil to control the fly are scanty. The authors of this work earlier reported essential oils of *Ageratum conyzoides* and *Ocimum sanctum* as potential candidate plant against uzi fly (Bora and Khanikor, 2011). *A. conyzoides* contains a complex mixture of 213 compounds out of which 43 compounds have been identified (Moreira et al., 2007). Moreover, 75 compounds at concentration of 0.01 to 56.1% have been identified from essential oil of *O. sanctum* (Jirovetz et al., 2003).

Essential oil of *Cymbopogon citratus* and *Eucalyptus globulus* acted as repellent for *E. sorbillans* (Prakash et al., 2010). However, no study has so far been done to control uzi fly by using essential oil compounds. Therefore, the present work was undertaken to study the effect of twenty two essential oil compounds on *E. sorbillans*. Of the selected 22 compounds, 18 were monoterpenes, two sesquiterpenes, one diterpene and one C9 compound known as coumarin.

## MATERIALS AND METHODS

### Chemicals

Essential oil compounds were purchased from Sigma-Aldrich Chemicals Co., Steinheim, Germany (Table 1).

### Test Insects

All experiments were conducted in laboratory using third day old adults of Uzi fly, *E. sorbillans* (Wiedemann) (Diptera: Tachinidae). Matured maggots of uzi fly were collected from the Government Sericulture Farm, Assam, India and kept in wire-netted wooden box (size: 30 cm × 30 cm). The newly emerged adult flies were maintained on 20% honey in a well ventilated room under natural light and temperature conditions.

### Contact toxicity assay

The insecticidal activity of 22 essential oil compounds against adult uzi fly was evaluated by contact residual film technique (Marcon, 1997) with modification. 1.5 ml of 1000 ppm concentration of each compound prepared in acetone as solvent was applied on 110 mm Whatman No. 1 filter paper covering the base and rim of a Petri dish. The treated Petri plates were allowed to dry for 5 min in room temperature (19 to 32°C, relative humidity 56 to 82%) and then were covered by 110 mm diameter glass funnel. The stem of the funnel was wrapped with nylon net for subjecting the test insects to treatment in an aerated system. Only acetone was applied for

control group against each test concentration. Three replications, each containing 10 insects were maintained for each treatment. Mortality of flies was recorded at an interval of 1, 3, 6, 24, 30 and 48 h of treatment.

In addition, LC<sub>50</sub> value was determined for those compounds which caused an average of 50% mortality at 1000 ppm concentration. Three replications each containing 10 insects were maintained for each treatment. LC<sub>50</sub> values were calculated using SPSS (16 version) software. In each case, percent mortality was corrected by using Abbot's (1925) correction formula given as:

$$\text{Corrected (\%)} = \frac{1 - n \text{ in T after treatment}}{n \text{ in Co after treatment}} \times 100$$

where, n is = insect population, T = treated and Co = control.

### Topical assay

Comparative toxicity of 22 oil compounds was evaluated by using topical application method. 1 µL of each compound was applied with micropipette (Himedia) on thorax of third day old adult of *E. sorbillans*. The time required to cause 100% mortality denoted as lethal time (LT) was recorded up to 48 h. LT was constantly recorded till 6 h of treatment and later at 24, 30 and 48 h. Three replications were maintained for each compound.

## RESULTS

### Contact toxicity

In the toxicity study carried out by using 1000 ppm of 22 essential oil compounds against *E. sorbillans*, mortality was recorded at a time interval of 1, 3, 6, 24, 30 and 48 h and presented in Table 2. At 1 h of exposure period, eugenol, terpinen-4-ol and citral caused 6.67 ± 3.34, 16.67 ± 6.67 and 100 ± 0% mortality, respectively. At 3 h of exposure period, eugenol, terpinen-4-ol, nerol, geraniol, carvacrol and citral caused 20 ± 0, 53.33 ± 3.34, 56.67 ± 3.34, 73.33 ± 6.67, 80 ± 0 and 100 ± 0% mortality, respectively. At 6 h of treatment, coumarin, isoeugenol, eugenol, terpinen-4-ol, carvacrol and carvone caused 6.67 ± 3.34, 20 ± 5.78, 26.67 ± 6.67, 53.33 ± 3.34, 80 ± 0 and 93.33 ± 6.67% mortality, respectively, while nerol, geraniol and citral caused 100% mortality of the flies. At 24 h of exposure period, carvacrol, carvone, citral, eugenol, geraniol and nerol caused 100% mortality while thymol, farnesol, isoeugenol, terpinen-4-ol, 1-citronellol, coumarin and α-humulene caused 17.5 ± 2.50, 43.33 ± 3.34, 66.67 ± 16.69, 66.67 ± 16.69, 73.33 ± 3.76, 83.33 ± 3.34 and 90 ± 5.78% mortality, respectively. Moreover, at 30 h of exposure period, 100% mortality of flies was caused by carvacrol, carvone, citral, eugenol, geraniol, and nerol. Other compounds like thymol, farnesol, terpinen-4-ol, 1-citronellol, isoeugenol, α-humulene and coumarin caused 17.50 ± 2.50, 46.67 ± 3.34, 66.67 ± 16.69, 73.33 ± 3.76, 75 ± 14.45, 90 ± 5.78 and 96.67 ± 3.34% mortality,

**Table 1.** Chemical characters of essential oil compounds evaluated for toxicity to *Exorista sorbillans*.

| Name  | Chemical structure | Molecular formula                              | Group                          | Purity (%) |
|---|--------------------|--|--------------------------------|------------|
| Carvacrol or<br>5-isopropyl-2-methylphenol <sup>I</sup>                         |                    | C <sub>10</sub> H <sub>14</sub> O              | Monoterpene phenol             | 99         |
| Carvone or<br>2-Methyl-5-(1-methylethenyl)-2-cyclohexenone                      |                    | C <sub>10</sub> H <sub>14</sub> O              | Monoterpene ketone             | 99         |
| Citral or<br>3,7-dimethylocta-2,6-dienal  |                    | C <sub>10</sub> H <sub>16</sub> O              | Monoterpene aldehyde (acyclic) | 95         |
| l-Citronellol or<br>3,7-Dimethyloct-6-en-1-ol                                   |                    | C <sub>10</sub> H <sub>20</sub> O              | Monoterpene alcohol (acyclic)  | 98         |
| Coumarin<br>Or 2H-chromen-2-one   |                    | C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>   | Benzopyrone                    | 99         |
| Eucalyptol or<br>1.8-cineole<br>Or<br>1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane |                    | C <sub>10</sub> H <sub>18</sub> O              | Monoterpene Ether (bicyclic)   | 99         |
| Eugenol or<br>4-Allyl-2-methoxyphenol   |                    | C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> | Monoterpene alcohols (cyclic)  | 99         |
| Farnesol or<br>(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol                 |                    | C <sub>15</sub> H <sub>26</sub> O              | Sesquiterpene (acyclic)        | 99         |

Table 1. Contd.

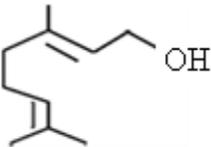
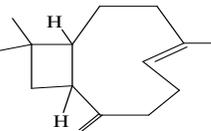
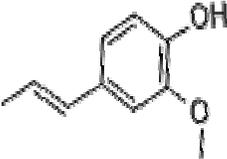
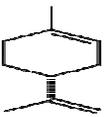
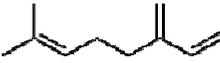
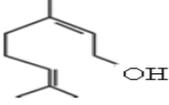
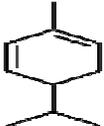
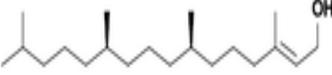
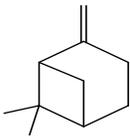
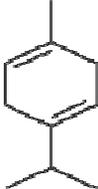
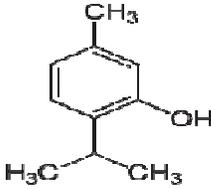
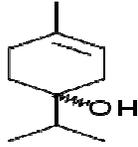
| Name  | Chemical structure   | Molecular formula                              | Group  | Purity (%) |
|---|--|--|--|------------|
| Geraniol or<br>3,7-Dimethylocta-2,6-dien-1-ol                                       |     | C <sub>10</sub> H <sub>18</sub> O              | Monoterpene<br>alcohols (acyclic)                  | 98         |
| $\alpha$ -Humulene or<br>(1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-<br>1,4,8-triene |     | C <sub>15</sub> H <sub>24</sub>                | Sesquiterpene<br>(Cyclic)                          | 99         |
| Isoeugenol or<br>2-methoxy-4-(prop-1-en-1-yl)phenol                                 |     | C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> | Monoterpene<br>alcohols (cyclic),<br>phenylpropene | 98         |
| D-Limonene<br>1-methyl-4-(1-methylethenyl)-cyclohexene                              |    | C <sub>10</sub> H <sub>16</sub>                | Monoterpene<br>hydrocarbon<br>(Cyclic)             | 99         |
| S-Limonene<br>(S)-4-Isopropenyl-1-methylcyclohexene                                 |   | C <sub>10</sub> H <sub>16</sub>                | Monoterpene<br>hydrocarbon<br>(Cyclic)             | 99         |
| $\beta$ -Myrcene or<br>7-Methyl-3-methylene-1,6-octadiene                           |   | C <sub>10</sub> H <sub>16</sub>                | Monoterpene<br>hydrocarbon<br>(Acyclic)            | 99         |
| Nerol or<br>(Z)-3,7-dimethyl-2,6-octadien-1-ol                                      |   | C <sub>10</sub> H <sub>18</sub> O              | Monoterpene<br>alcohols<br>(Acyclic)               | 97         |
| R- $\alpha$ -Phellandrene<br>2-Methyl-5-(1-methylethyl)-1,3-cyclohexadiene          |   | C <sub>10</sub> H <sub>16</sub>                | Monoterpene<br>Hydrocarbon<br>(Cyclic)             | 99         |
| Phytol or<br>(2E,7R,11R)-3,7,11,15-<br>tetramethyl-2-hexadecen-1-ol                 |  | C <sub>20</sub> H <sub>40</sub> O              | Diterpene  | 97         |

Table 1. Contd.

| Name   | Chemical structure  | Molecular formula                 | Group                                 | Purity (%) |
|--|---|-----------------------------------|---------------------------------------|------------|
| $\alpha$ -Pinene or<br>(1 <i>S</i> ,5 <i>S</i> )-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene | <br>(+)- $\alpha$ -p nene    (-)- $\alpha$ -p nene | C <sub>10</sub> H <sub>16</sub>   | Monoterpene hydrocarbon<br>(Bicyclic) | 98         |
| $\beta$ -Pinene  |    | C <sub>10</sub> H <sub>16</sub>   | Monoterpene hydrocarbon<br>(Bicyclic) | 98         |
| $\gamma$ -Terpinene or<br>4-methyl-1-(1-methylethyl)-1,4-cyclohexadiene                  |    | C <sub>10</sub> H <sub>16</sub>   | Monoterpene Hydrocarbon<br>(Cyclic)   | 99         |
| Thymol or<br>2-Isopropyl-5-methylpheno   |   | C <sub>10</sub> H <sub>14</sub> O | Monoterpene phenol<br>(Cyclic)        | 98         |
| Terpinen-4-ol or<br>(-)-4-isopropyl-1-methyl-1-cyclohexen-4-ol                           |    | C <sub>10</sub> H <sub>18</sub> O | Monoterpene alcohols<br>(Cyclic)      | 98         |

respectively at 30 h. At 48 h of exposure, 100% mortality of flies was caused by carvacrol, carvone, citral, coumarin, eugenol, geraniol and nerol. Other compounds namely R-( $\alpha$ )-phellandrene, S-limonene, thymol,  $\alpha$ -pinene,  $\beta$ -pinene, D-limonene, 1-citronellol, isoeugenol, farnesol, terpinen-4-ol and  $\alpha$ -humulene caused  $3.3 \pm 3.3$ ,  $26.67 \pm 6.67$ ,  $38.89 \pm 5.56$ ,  $40 \pm 0$ ,  $53.33 \pm 6.67$ ,  $53.33 \pm 6.67$ ,  $73.33 \pm 3.76$ ,  $75 \pm 14.45$ ,  $76.67 \pm 3.34$ ,  $80 \pm 10.01$  and  $90 \pm 5.78\%$  mortality, respectively. Some compounds viz. eucalyptol,  $\beta$ -myrcene, phytol,  $\gamma$ -terpinene had no lethal effect till 48 h of treatment.

#### Determination of sublethal concentrations of certain compounds on *E. sorbillans*

The pure terpenoid compounds which caused more than 50% mortality of flies at 24 h were further tested for determining their sublethal concentrations. Results indicate that the aliphatic aldehyde citral (LC<sub>50</sub> = 110.23 ppm), and

acyclic monoterpene alcohols geraniol (LC<sub>50</sub>=124.11 ppm) and nerol (LC<sub>50</sub> = 135.75 ppm) were most highly toxic. Aromatic phenols; carvacrol (LC<sub>50</sub>=194.53 ppm), eugenol (LC<sub>50</sub> = 207.25) and isoeugenol (LC<sub>50</sub>=266.40 ppm), acyclic monoterpene alcohol; 1-citronellol (LC<sub>50</sub>=267.24 ppm), monocyclic ketone; carvone (LC<sub>50</sub>=299.23 ppm), and benzo- $\alpha$ -pyrone derivatives; coumarin (LC<sub>50</sub>=364.70 ppm) were moderately toxic, while the least toxic compounds were sesquiterpene;  $\alpha$ -humulene (LC<sub>50</sub>=533.02 ppm) and monocyclic alcohol; terpinen-4-ol (LC<sub>50</sub>=1213.43 ppm) (Table 3).

#### Determination of LT of some standard compounds for *E. sorbillans*

The toxicity study carried out to determine LT of the compound through topical application presented in Table 4 shows that 100% mortality was observed within five minutes in case of monoterpene hydrocarbon;  $\alpha$ -pinene

**Table 2.** Effect of essential oil compounds on percent mortality of *E. sorbillans* at different time interval. (% mortality  $\pm$  standard error) (contact residual film bioassay).

| S/N | Name of the compound      | Contact time (h) |                  |                  |                   |                   |                  |
|-----|---------------------------|------------------|------------------|------------------|-------------------|-------------------|------------------|
|     |                           | 1                | 3                | 6                | 24                | 30                | 48               |
| 1.  | Carvacrol                 | 0 $\pm$ 0        | 80 $\pm$ 0       | 80 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0      |
| 2.  | Carvone                   | 0 $\pm$ 0        | 0 $\pm$ 0        | 93.33 $\pm$ 6.67 | 100 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0      |
| 3.  | Citral                    | 100 $\pm$ 0      | 100 $\pm$ 0      | 100 $\pm$ 0      | 100 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0      |
| 4.  | 1-Citronellol             | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 73.33 $\pm$ 3.76  | 73.33 $\pm$ 3.76  | 73.33 $\pm$ 3.76 |
| 5.  | Coumarin                  | 0 $\pm$ 0        | 0 $\pm$ 0        | 6.67 $\pm$ 3.34  | 83.33 $\pm$ 3.34  | 96.67 $\pm$ 3.34  | 100 $\pm$ 0      |
| 6.  | Eucalyptol                | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 0 $\pm$ 0        |
| 7.  | Eugenol                   | 6.67 $\pm$ 3.34  | 20 $\pm$ 0       | 26.67 $\pm$ 6.67 | 100 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0      |
| 8.  | Farnesol                  | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 43.33 $\pm$ 3.34  | 46.67 $\pm$ 3.34  | 76.67 $\pm$ 3.34 |
| 9.  | Geraniol                  | 0 $\pm$ 0        | 73.33 $\pm$ 6.67 | 100 $\pm$ 0      | 100 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0      |
| 10. | $\alpha$ -Humulene        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 90 $\pm$ 5.78     | 90 $\pm$ 5.78     | 90 $\pm$ 5.78    |
| 11. | Isoeugenol                | 0 $\pm$ 0        | 0 $\pm$ 0        | 20 $\pm$ 5.78    | 66.67 $\pm$ 16.68 | 75 $\pm$ 14.45    | 75 $\pm$ 14.45   |
| 12. | D-Limonene                | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 53.33 $\pm$ 6.67 |
| 13. | S-Limonene                | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 26.67 $\pm$ 6.67 |
| 14. | $\beta$ -Myecene          | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 0 $\pm$ 0        |
| 15. | Nerol                     | 0 $\pm$ 0        | 56.67 $\pm$ 3.34 | 100 $\pm$ 0      | 100 $\pm$ 0       | 100 $\pm$ 0       | 100 $\pm$ 0      |
| 16. | R- $\alpha$ -Phellandrene | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 3.3 $\pm$ 3.3    |
| 17. | Phytol                    | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 0 $\pm$ 0        |
| 18. | $\alpha$ -Pinene          | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 40 $\pm$ 0       |
| 19. | $\beta$ -Pinene           | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 53.33 $\pm$ 6.67 |
| 20. | $\gamma$ -Terpinene       | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0         | 0 $\pm$ 0         | 0 $\pm$ 0        |
| 21. | Terpinen-4-ol             | 16.67 $\pm$ 6.67 | 53.33 $\pm$ 3.34 | 53.33 $\pm$ 3.34 | 66.67 $\pm$ 16.69 | 66.67 $\pm$ 16.69 | 80 $\pm$ 10.01   |
| 22. | Thymol                    | 0 $\pm$ 0        | 0 $\pm$ 0        | 0 $\pm$ 0        | 17.5 $\pm$ 2.50   | 17.50 $\pm$ 2.50  | 38.89 $\pm$ 5.56 |

**Table 3.** LC<sub>50</sub> values of essential oil compounds against *E. sorbillans* (Contact residual film bioassay).

| S/N | Name of the compound | LC <sub>50</sub> ppm | Z-value | X <sup>2</sup> -value | d.f. | Significance | 95% Confidence interval | Regression equation |
|-----|----------------------|----------------------|---------|-----------------------|------|--------------|-------------------------|---------------------|
| 1.  | Carvacrol            | 194.53               | 19.80   | 70.26                 | 16   | 0            | 4.585 - 5.592           | Y = -4.57 + 4.18X   |
| 2.  | Carvone              | 299.23               | 26.75   | 7311.07               | 16   | 0            | 2.009 - 2.326           | Y = -1.24 + 2.52X   |
| 3.  | Citral               | 110.23               | 25.99   | 269.34                | 16   | 0            | 1.906 - 2.217           | Y = 0.78 + 2.07X    |
| 4.  | 1 - Citronellol      | 267.24               | 18.86   | 93.94                 | 16   | 0            | 0.988 - 1.218           | Y = 2.32 + 1.10X    |
| 5.  | Coumarin             | 364.70               | 21.23   | 75.56                 | 16   | 0            | 1.597 - 1.921           | Y = 0.491 + 1.76X   |
| 6.  | Eugenol              | 207.25               | 24.87   | 565.12                | 16   | 0            | 2.631 - 3.081           | Y = -1.88 + 2.97X   |
| 7.  | Geraniol             | 124.11               | 22.49   | 240.54                | 16   | 0            | 3.837 - 4.570           | Y = -3.35 + 3.99X   |
| 8.  | $\alpha$ - Humulene  | 533.02               | 17.40   | 295.20                | 16   | 0            | 0.896 - 1.124           | Y = 2.24 + 1.01X    |
| 9.  | Isoeugenol           | 266.40               | 10.12   | 484.89                | 16   | 0            | 0.380 - 0.562           | Y = 3.86 + 0.47X    |
| 10. | Nerol                | 135.75               | 25.75   | 556.38                | 16   | 0            | 2.128 - 2.479           | Y = 0.307 + 2.33X   |
| 11. | Terpinen-4-ol        | 1213.43              | 15.13   | 241.07                | 16   | 0            | 0.791 - 1.027           | Y = 2.19 + 0.91X    |

d.f., Degree of freedom.

(LT = 1.41  $\pm$  0.36 min) and bicyclic alcohol; eucalyptol (LT = 2.58  $\pm$  1.26 min). Although  $\alpha$ -pinene (1.41  $\pm$  0.36 min), eucalyptol (2.58  $\pm$  1.26 min),  $\gamma$ -terpinene (5.83  $\pm$

0.72 min),  $\beta$ -pinene (7.67  $\pm$  0.88 min) and S-limonene (11.33  $\pm$  1.45 min) did not cause 50% mortality within 24 h when applied by using contact residual film technique,

**Table 4.** Lethal time (minute) of essential oil compounds (1 $\mu$ L/insect) with respect to *E. sorbillans*.

| S/N | Name of the compound      | 0 - 60min (0 - 1 h) | 61 - 180min (1 - 3 h) | 181 - 360 min (3 - 6 h) | 1440 min (24 h) | 1800 min (30 h) | 2880 min (48 h) |
|-----|---------------------------|---------------------|-----------------------|-------------------------|-----------------|-----------------|-----------------|
| 1.  | Carvacrol                 | 52.67 $\pm$ 8.02    |                       |                         |                 |                 |                 |
| 2.  | Carvone                   | 12 $\pm$ 1.16       |                       |                         |                 |                 |                 |
| 3.  | Citral                    | 0 $\pm$ 0           | 0 $\pm$ 0             | 345 $\pm$ 22.94         |                 |                 |                 |
| 4.  | 1-Citronellol             | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 1440 $\pm$ 0    |                 |                 |
| 5.  | Coumarin                  | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 0 $\pm$ 0       | 0 $\pm$ 0       | 2880 $\pm$ 0    |
| 6.  | Eucalyptol                | 2.58 $\pm$ 1.26     |                       |                         |                 |                 |                 |
| 7.  | Eugenol                   | 46.33 $\pm$ 5.24    |                       |                         |                 |                 |                 |
| 8.  | Farnesol                  | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 1440 $\pm$ 0    |                 |                 |
| 9.  | Geraniol                  | 38.50 $\pm$ 3.18    |                       |                         |                 |                 |                 |
| 10. | $\alpha$ - Humulene       | 48 $\pm$ 4.36       |                       |                         |                 |                 |                 |
| 11. | Isoeugenol                | 0 $\pm$ 0           | 0 $\pm$ 0             | 210 $\pm$ 15            |                 |                 |                 |
| 12. | D-Limonene                | 19.67 $\pm$ 1.77    |                       |                         |                 |                 |                 |
| 13. | S-Limonene                | 11.33 $\pm$ 1.45    |                       |                         |                 |                 |                 |
| 14. | $\beta$ -Myecene          | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 0 $\pm$ 0       | 0 $\pm$ 0       | 2880 $\pm$ 0    |
| 15. | Nerol                     | 25 $\pm$ 2.65       |                       |                         |                 |                 |                 |
| 16. | R- $\alpha$ -Phellandrene | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 0 $\pm$ 0       | 0 $\pm$ 0       | 2880 $\pm$ 0    |
| 17. | Phytol                    | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 1440 $\pm$ 0    |                 |                 |
| 18. | $\alpha$ -Pinene          | 1.41 $\pm$ 0.36     |                       |                         |                 |                 |                 |
| 19. | $\beta$ -Pinene           | 7.67 $\pm$ 0.88     |                       |                         |                 |                 |                 |
| 20. | $\gamma$ -Terpinene       | 5.83 $\pm$ 0.72     |                       |                         |                 |                 |                 |
| 21. | Terpinen-4-ol             | 14.33 $\pm$ 2.61    |                       |                         |                 |                 |                 |
| 22. | Thymol                    | 0 $\pm$ 0           | 0 $\pm$ 0             | 0 $\pm$ 0               | 0 $\pm$ 0       | 0 $\pm$ 0       | 2880 $\pm$ 0    |

they caused 100% mortality within 15 min when applied topically.

## DISCUSSION

As indicated by the results, the toxicity study shows that out of the 22 compounds, three oxygenated monoterpenes namely citral, eugenol and terpinen-4-ol exerted their toxic action within one hour. Alcoholic monoterpenes; nerol, geraniol and carvacrol caused 56 to 80% mortality at 3 h. The toxic action of carvone, coumarin and isoeugenol started appearing after 6 h of exposure, while that of farnesol and thymol appeared at 24 h exposure. All the compounds whose onset time of toxic action was within 24 h of exposure, caused more than 50% mortality during the said period. Except in l-citronellol and alpha-humulene, in all other cases percent mortality increased with time from the onset of toxic action. Oils of lavender, borneol, eucalyptus, pine, thyme and lemongrass along with spearmint oil were earlier attempted to repel the fly (Prakash et al., 2010), but no study has been carried out so far on toxicity of pure essential oil compounds against *E. sorbillans*.

Certain compounds were also tested against other dipteran insects. 19 semiochemicals classified as aliphatic alcohols, terpenoids, ketones and carboxylic esters

showed toxicity against the house and stable flies (Mann et al., 2008). Carvacrol and thymol were found effective against *Drosophila* and *Thecodiplosis japonensis* (Diptera: Cecidomyiidae) (Lee et al., 1997; Karpouhtsis et al., 1998). In case of another dipteran *Lycoriella ingéne* (Diptera: Sciaridae), carvacrol, thymol, terpinen-4-ol and carvone were found effective in fumigation bioassay at  $10 \times 10^{-3}$  mg/L air (Park et al., 2003). Fumigant action of eugenol and geraniol was more effective than citral, citronellol and  $\alpha$ -terpineol in case of *Musca domestica* (Samarasekera, 2006). Moreover, coumarin has been reported as active against *Musca*, *Culex* and *Aedes* in several works (Deshmukh et al., 2008).

## Determination of sublethal concentrations of certain compounds against *E. sorbillans*

As shown by the LC<sub>50</sub> values presented in Table 3, oxygenated monoterpenes exhibited higher contact toxicity against uzi fly. This was in agreement with certain other studies regarding toxic action of pure terpene compounds on dipterans. In studies carried out with different species of mosquitoes, geranyl acetate (LC<sub>50</sub> = 38.51  $\mu$ g cm<sup>-2</sup>) and citronellol (LC<sub>50</sub> = 48.55  $\mu$ g cm<sup>-2</sup>) caused greater toxicity to *Aedes aegypti*, while geraniol (LC<sub>50</sub> = 31.8  $\mu$ g cm<sup>-2</sup>) was effective against *Anopheles*

*quadrinaculatus* (Diptera: Culicidae) (Kaufman et al., 2010). Similarly oxygenated monoterpenes; citronellol, nerol, geraniol, alpha-terpenol and D-carvone was found effective against house fly, *Musca domestica*, and stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) (Mann et al., 2008).

### Determination of lethal time of some standard compounds for *E. sorbillans*

Toxicity of the compounds in terms of lethal time through topical application is presented in Table 4. Looking at both LC<sub>50</sub> and LT values, nerol and geraniol may be considered highly toxic because of its low LC<sub>50</sub> and LT value. The LC<sub>50</sub> value of carvacrol, eugenol and carvone were of medium range and their LT value was low (12 to 52 min). Although contact residual toxicity (LC<sub>50</sub>) of terpinene-4-ol and α-humulene were the least, their LT values were 14.33 ± 2.61 and 48 ± 4.36 min, respectively.

Earlier, 50% knockdown time of *Musca* was reported to be 2.3 min for eucalyptol and 7.5 min for limonene (Tareli et al., 2009). 1,8-cineole (LC<sub>50</sub> = 3.3 mg/dm<sup>3</sup>) was reported as the most active terpene on *M. domestica* followed by γ-terpinene, (4S)(-)-limonene, α-terpinene, and (4R)(+)-limonene with LC<sub>50</sub> of 4, 5, 6.2 and 6.2 mg/dm<sup>3</sup>, respectively (Palacios et al., 2009). Thymol, carvacrol, (1R)(+)-α-pinene and (1S)(-)-α-pinene were reported as the most toxic (LC<sub>50</sub> = 36 to 49 mg L<sup>-1</sup>), while menthone, 1,8-cineole, linalool and terpineol (LC<sub>50</sub> = 156 to 194 mg L<sup>-1</sup>) were less toxic on *Culex pipiens molestus* (Diptera: Culicidae) (Traboulsi et al., 2002). From our findings, on the basis of LC<sub>50</sub> and LT value, the compound considered for evaluation of toxicity may be divided into following groups: i) nerol and geraniol which have high contact and topical toxicity; ii) carvacrol, carvone, eugenol, terpinen-4-ol and α-humulene which have moderate contact toxicity but high topical toxicity; iii) α-pinene, eucalyptol, γ-terpinene, β-pinene, S-limonene and D-limonene which have rapid topical toxicity; iv) coumarin which have only contact toxicity, and v) phytol, β-myrcene and R-(α)-phellandrene which have no contact toxicity and least topical toxicity.

The mechanism of toxic effect of essential oil compound on insect at present is not well known. Insects vary enormously in their response to different essential oil compounds probably either for their chemical structures or mode of entry. Coumarin and mainly furanocoumarins can alter the detoxication capability of an organism by reversibly or irreversibly inhibiting cytochrome P450 detoxication enzymes (Letteron, 1986; Neal and Wu, 1994). Rapid action may indicate neurotoxic mode of action probably through binding with different types of octapamine receptors and interference with octapamine activity (Enan, 2001; Kostyukovsky et al., 2002; Price and Berry, 2006), interference with GABA-gated chloride channels (Koul et al., 2008) or inhibition of acetylcholine esterase (Abdelgaleil et al., 2009; Ryan and Byrne, 1988;

Coats et al., 1999). Rapid action of citral, geraniol and nerol observed during contact bioassay studies, and α-pinene, eucalyptol, γ-terpinene, β-pinene, S-limonene and D-limonene during topical bioassay may indicate their neurotoxic mode of action. While monoterpene hydrocarbons were found more toxic in topical bioassay, mostly oxygenated monoterpenes were effective in contact residual film bioassay. Therefore, the toxicity of the compounds may vary depending on bioassay techniques. That toxicity and efficacy of monoterpenes vary with assay method have been reported in studies with stored product insects (Abdelgaleil et al., 2009; Prakash et al., 2010; Prates et al., 1988). Hence, we concluded that the essential oil compounds recorded to have toxicity in this study may serve as viable control agent against *E. sorbillans*.

### Conclusion

22 essential oil compounds commonly found in *A. conyzoides*, different chemotypes of *Ocimum* species, *C. citratus*, *E. globulus* and others were bioassayed against uzi fly, *E. sorbillans*. The oxygenated monoterpenes like nerol, geranio, carvacrol, carvone, eugenol and terpinene-4-ol were effective on both contact and topical application. While monoterpene hydrocarbons like α-pinene, γ-terpinene, β-pinene, S-limonene and D-limonene were effective on topical application. Moreover, coumarin a major constituent of *A. conyzoides* was effective as contact poison. The compounds found effective in this study may be further used for development of viable control agent against *E. sorbillans*.

### ACKNOWLEDGEMENT

Authors are grateful to University Grants commission, New Delhi, India, for financial support in carrying out the work.

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