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BIO-EFFICACY OF PUPICIDAL ACTIVITY OF SOME PLANT ESSENTIAL OILS ON *CULEX QUINQUEFASCIATUS* AND *ANOPHELES STEPHENSI*

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ABSTRACT

Bio-efficacy of pupicidal potential of some essential oils from seven plants (Pimpinella anisum, Cinnamomum veerum, Myrtus caryophyllus, Citrus sinensis, Thymus vulgaris, Ocimum sanctum and Vetiveria zizanioides) was tested against the pupae of Culex quinquefasciatus and Anopheles stephensi. The pupal susceptibility test were carried out using WHO standard method. In the pupicidal assay at five different concentrations viz., 31.25, 62.5, 125, 250, and 500ppm concentrations were used andthe mortality was observed after 24-h exposure. All the tested essential oils showed moderate to good pupicidal activity. However, the maximum pupal mortality was registered at 500 ppm concentration as 100 ± 0.00% for Cx. quinquefasciatus and An. stephensi respectively. The Pupal mortality was resigered as of aniseed (87.5 ± 062%), tulsi (85.0 ± 0.72%) and cinnamom (82.2 ± 0.42%) at 500 ppm for Cx. quinquefasciatus respectively. The efficacy results of pupal mortality of aniseed (85.2 ± 023%), tulsi (84.1 ± 0.23%) and cinnamom (80.1 ± 0.51%) at 500 ppm for An. stephensi respectively. The LC_{50} and LC_{90} of clove oil (106.3 and 313.3 ppm), tulsi (133.6 and 539.3 ppm) and cinnamon (141.0 and 575.5 ppm) against Cx. quinquefasciatus after 24 h respectively, and LC_{50} values of 110.5, 144.2 and 150.1 ppm and LC_{90} values of 310.4, 502.3 and 603.0 ppm against An. stephensi after 24 h of treatment, respectively. The results suggest that the essential oils have potential to be used as a pupicidal activity an ideal eco-friendly approach for the control of filarial and malaria vectors, Cx. quinquefasciatus and An. stephensi as target species in vector borne diseases control programs.

Keywords: Bioefficacy, Pupicidal, Essential oils, Dose response assay, Lethal concentrations determination, *Culex quinquefasciatus, Anopheles stephensi.*

1. INTRODUCTION

Mosquito-borne diseases still remain a major health problem in both human and veterinary sectors. Diseases transmitted by mosquitoes such as malaria, dengue hemorrhagic fever, Japanese encephalitis, yellow fever and filaria (Yang *et al.*, 2002). *Anopheles stephensi* and *Culex*

quinquefasciatus are the potential vectors of malaria and filariasis, respectively. Worldwide, these vectors are responsible for the transmission of 500 and 100 million clinical cases of malaria and filariasis diseases per annum, respectively. Therefore, they can be called as to as global vectors (Das, 2007). To control those vectors, chemical insecticides such as Temephos, S-methoprene, Monomolecular films, Spinosad, Bti,etc. have been used to reduce mosquito larvae or pupae and as such prevent diseases (Brattsten and Hamilton, 2012).

In most urban and rural areas of the country, mosquito populations are menacing throughout the year, except for some attenuation during summer and winter. Mosquitoes control by means of chemicals is an easy way, which gives immediate control. But the mosquito problem has increased ever before. The main reason for this increased problem is because of the indiscriminate use of chemical insecticides. Mosquitoes rapidly develop resistance to many insecticides. Majority of the chemical pesticides are harmful to man and animal, because some of them are not easily degradable and they have toxic effects (Ghosh, 1991). Although effective, their repeated use has disrupted natural biological control systems and has led to outbreaks of insect species, which sometimes resulted in the widespread development of resistance, had undesirable effects on nontarget organisms, and fostered environmental and human health concerns (Yang *et al*., 2002). Though several plants from different families have been reported for insecticidal activity only a few botanicals like neem based insecticides have moved from the laboratory to field use, which might be due to the light and heat stability of neem compounds compared to synthetic insecticides (Green *et al*., 1991).

Many studies have shown the bioactivity of essential oils against mosquitoes as growth inhibitors and/or larvicides, adulticides, repellents, or oviposition deterrents (Sukumar *et al*., 1991; Carvalho *et al*., 2003; Cavalcanti *et al*., 2004; Ansari *et al*., 2005). Essential oils from a large number of plants, including *Ocimum* spp, *Cymbopogon* spp), *Eucalyptus maculate citriodon Pelargonium citrosum*, *Artemisia vulgaris*, *Lantana camara*, *Mentha piperita*,*Vitexr otundifolia*, *Curcuma* spp. (Pitasawat *et al*., 2003), *Conyza newii*, *Plectranthus marrubioides*, *Tetradenia riparia*, *Tarchonanthus camphoratus*, *Lippia javanica* and *L. ukambensis* (Omolo *et al*., 2004), have been demonstrated to exhibit good repellent properties against vector mosquitoes. *Eucalyptus tereticornis* (Myrtaceae) has long been recognized for its insecticidal properties; especially its mosquito repellent activity (Traboulsi *et al*., 2005) but has yet to be extensively analyzed. The plant derived products have received increased attention from scientists and nowadays more than 2000 plant species have already been screened as potent insecticides providing possible lead candidates to replace synthetic chemical insecticides for controlling mosquito in aquatic stages (larvae and pupae) (Anupam *et al*., 2012).

Essential oils are natural volatile substances found in a variety of plants. When isolated from plants, essential oils are not usually extracted as chemically pure substances, but consist of mixtures of many compounds. Essential oils were the first preservatives used by man, originally in their natural state within plant tissues and then as oils obtained by water distillation. Essential

oils composed by isoprenoid compounds, mainly mono- and sesquiterpenes are the carriers of the smell found in the aromatic plants (Franzios *et al*., 1997). Research on the use of plant essential oils to control mosquitoes and their applications has increased in recent years. This is especially true for the use of natural products based on plant oils (EOs) as pupal and larval mortality were observed in different essential oils (Ramar *et al*., 2013a). At present, evaluation of essential oils against mosquitoes and isolation, identification and development of natural products from them are under the focus of numerous research programmes around the globe. So far only few insecticides of plant origin have reached the market. Recently a number of plant volatiles have been evaluated for their actions against mosquito larvae (Ramar *et al*., 2013c; 2014). There is a renewed interest in plant essential oils products as sources of new insect controlling agents, because they may be biodegradable to nontoxic compounds, thus minimizing the accumulation of harmful residues, leading them to be more environmentally friendly compared to synthetic compounds (Choochote *et al*., 2005). In this context, a survey of the literature on insecticidal properties of essential oils from the recent year onwards observed that essential oils from about 90 plant genera belonging to 38 plant families were reported to have toxic properties against mosquito larvae and pupae (Mann and Kaufman, 2012; Gokulakrishnan *et al*., 2013). In previous works showed the richness of the plant materials in volatile constituents quantitatively and qualitatively (Choochote *et al*., 2005; Praveen *et al*., 2012). Therefore, the aim of this present work was to evaluate the pupicidal potential of some effective essential oils against *Cx. quinquefasciatus* and *An.stephensi* of early pupae.

2. MATERIAL & METHODS

2.1. Botanical Essential Oils

The plant oils (POs) were procured from commercial producers of plant oils and aromatic substances were used in this study. The seven plant essential oils (PEOs) were selected on the basis of preliminary study (Ramar *et al*., 2013a): Aniseed(*Pimpinella anisum* Linn.), Cinnamon(*Cinnamomum veerum*), Clove(*Myrtus caryophyllus*), Orange(*Citrus sinensis*), Thyme(*Thymus vulgaris*), Tulsi(*Ocimum sanctum*) and Vetiver(*Vetiveria zizanioides*).

2.2. Rearing of Test Organism

The test organism *Cx. quinquefasciatus* and *An.stephensi* was reared continuously from several generations in the Entomology Research Institute, Loyola College, Chennai-34, India. They were free of exposure to pathogens and insecticides and maintained at 25 ± 2°C and 60-80% relative humidity. The larvae were fed on dog biscuits and yeast powder in a ratio 3:2 until moulting to become pupae, pupae was transferred into a mosquito cage. The pupae were transferred from culture trays to glass beakers containing tap water and placed in screened cages (45 x45 x 45 cm), where adults emerged. The cage was made up of metal frames and covered with a muslin cloth. The emergent adults were fed with 10% glucose solution dipped in a piece of cotton.

2.3. Pupicidal Activity

The pupicidal activity of the selected plant essential oils was assessed by WHO protocol (World Health Organization (WHO), 2005). The selected plant oils were studied in dose response bioassay was done by the standard procedure. Based on the preliminary results, effective oils were further tested at five different concentrations viz, 31.25, 62.5,125, 250 and 500 ppm. Twenty newly formed pupae of *Cx. quinquefasciatus* were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1.0 ml of desired concentrations. Five replicates for each concentration were maintained. A control was set up with 1.0 ml of Tweem80 (0.01%) dissolved in 249 ml of dechlorinated water. Mortality rates of pupae were recorded after 24 h of treatment. In recording the percentage mortalities for each concentration, the moribund and dead pupae in five replicates were combined. The mouth of each bowl containing pupae was covered with muslin cloth to prevent the escape of any emerged adult mosquitoes. The mortality, was corrected by using Abbotts formula (Abbott, 1925)

$$\text{Percent mortality} = \frac{\text{Number of pupae dead}}{\text{Number of pupae released}} \times 100$$

$$\text{Corrected mortality (\%)} = \frac{\text{Percent mortality in test - percent mortality in control}}{100 - \text{Percent mortality in control}} \times 100$$

2.4. Data Processing

Statistical analysis was performed using SPSS software package, version 15. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Duncan, 1957). All the results were expressed as mean ± SE of five replicates in each treatment. *P*-values 0.05 were considered as significant. The median lethal concentration (LC_{50}) and LC_{90} values and 95% of lower and upper confidence limits (UCL) were calculated by using probit analysis (Finney, 1971).

3. RESULTS

3.1. Percentage Pupicidal

The percentage mortality results of mosquito pupicidal activity of essential oils against early pupae of *Cx. quinquefasciatus* and *An. stephensi* after 24 h treatment are summarised in (Tables 2 & 3). The preliminary screening of plant oils showed that seven oils viz., aniseed, cinamom, clove, orange, thyme, tulasi and vetiver oils were the effective oils against pupae of *Cx. quinquefasciatus* and *An. stephensi*. Among the seven oils the clove oil showed very potent effect at 500 ppm concentration as 100% pupal mortality of *Cx. quinquefasciatus* and *An. stephensi* were recorded. In Other treatments not showed 100% pupal mortality at 500ppm concentration on *Cx. quinquefasciatus* and *An. stephensi*. The results of pupal mortality for aniseed (85.2 ± 023%), tulsi

(84.1 ± 0.23%) and cinnamom (80.1 ± 0.51%) at 500 ppm for *An. stephensi* respectively. The least effective treatment was noted in orange oil which recorded only 7.50 ± 0.32% pupicidal activity. From these results, *Cx. quinquefasciatus*was also the most susceptible mosquito species in this study achieving pupal mortality for aniseed (87.5 ± 062%), tulsi (85.0 ± 0.72%) and cinnamom (82.2 ± 0.42%) at 500 ppm respectively and orange oil causing (8.75 ±0.42%) mortality at the lowest concentration of was 31.25 ppm. The *An. stephensi* which was came in second position in term of susceptibility; clove oil (26.3± 0.21%) mortality was achieved with the concentration of 31.25 ppm and completely suppressed the exposed pupae in 500 ppm getting (100 ± 0.00%) mortality. The pupal mortality due to all the oils was found to be directly propotional to the concentration and also type of the oil. When the concentration increased and the level of pupal mortality also increased. The results were clearly indicates that dose dependent activity.

3.2. Determination Median Lethal Concentrations (LC_{50} and LC_{90})

The data presented in Tables 2 and 4 reveal that the most effective essential oils against the *Cx. quinquefasciatus* and *An. stephensi* pupae of LC_{50} and LC_{90} values were observed after 24 h of exposure. The efficacy results of clove oil were followed by that the tulsi and cinnamon, which had LC_{50} values of 106.3, 133.6 and 141.0 ppm and LC_{90} values of 313.3, 539.3 and 575.5 ppm against *Cx. quinquefasciatus* after 24 h, respectively, and LC_{50} values of 110.5, 144.2 and 150.1 ppm and LC_{90} values of 310.4, 502.3 and 603.0 ppm against *An. stephensi* after 24 h of treatment, respectively. The LC_{50} values of the clove oil were lower than those of the tulsi and cinnamon, despite of which species was evaluated or at the time of experiment. In addition, the potentiality of each of the tested oils increased as the concentration of the material for which the pupal mortality was increased. The toxicity results of the essential oils against early pupal stages of *Cx quinquefasciatus* and *An. stephensi* after 24 h of exposure clearly indicated that the oil was toxic against all the two mosquito vector species.

4. DISCUSSION

In the present study volatile oils (VOs) registered pupicidal activity. Plenty of literature is available with regard to bioefficacy of volatile oils against vector mosquitoes. Different volatile oils showed differential toxicity against different life stages of mosquito. Sharma and Subramaniam (1999) reported the larvicidal activity of *Bilumca eriantha*, *Calotropis procera* and *Catharanthus reseus* leaves, petioles and bark against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Oil of *Mentha pipertia* was evaluated for its larvicidal activity against the third instar larvae of *Aedes*, *Anopheles* and *Culex* (Ansari *et al*., 2000). Massoud and Labib (2000) obtained from the stem of *Commiphora molmol* proved to have insecticidal activity against *Culex pipiens* and *Aedes caspius* larvae. Carvalho *et al.* (2003) reported the larvicidal activity of the essential oil from *Lippia sidoides* cham, against *Aedes aegypti* Linn. Thymol, an alkylated phenol derivative and one of the major components of *Lippia sidoides* essential oil, was identified as the

active principle responsible for the larvicidal action, causing 100 percent larval mortality at the lowest tested concentration of 0.017 % (w/v). Cheng *et al.* (2003) reported the bioactivity of the selected essential oils against the larvae of *Aedes aegypti*. Cavalcanti *et al.* (2004) reported the Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. are medicinal plants used to prepare a wide range of herbal products including nonprescription drugs and herbal teas (Juliani *et al.*, 2006).

The results of the present study are compared with earlier reports. The bioactivity of ten plant oils, Cedar wood (*Cedrus atlantica* (Endl.) Carrière), Citronella (*Cymbopogon nardus* (Linn.) W. Watson), Clove (*Myrtus caryophyllum* Spreng), Eucalyptus (*Eucalyptus globulus* Labill. (Myrtaceae)), Lemon grass (*Cymbopogon flexuosus* (Steud) Wats), Orange (*Citrus sinensis* (Linn.), Nutmeg (*Myristica fragrans* Houtt.), Palmarosa (*Cymbopogon martini* Roxb.), Pine (*Pinus radiate* D. Don) and Tulsi (*Ocimum sanctum* Tulsi) were tested against the 3rd instar larvae of *Ae. aegypti*. Larval mortality was observed after 24 h. Among the plant oils tested, orange oil exhibited the highest larvicidal activity with LC50 of 85.93 ppm, followed by Palmarosa with 88.78 ppm, Tulsi with 92.48 ppm and Nutmeg oil with 93.62 ppm (Tennyson *et al.*, 2013). The highest larvicidal activity was observed in the essential oil of *O. basilicum* against *Ae. aegypti* and *Cx. quinquefasciatus* with LC50 values of 75.35 ppm and 92.30 ppm, respectively (Manzoor *et al.*, 2013).

Shaalan *et al.* (2005) conculeded that essential oils such as citronella, calamus, thymus, and eucalyptus are promising as mosquito larvicides. Senthil (2007) has recorded the larvicidal and pupicida activity of essential oil extracted from the forest redgum, *Eucalyptus tereticornis* Sm. (Myrtaceae) against mosquito vector *Anopheles stephensi*. Ramar *et al.* (2013b) studied the larvicidal and pupicidal properties of *Crotonsparciflorus* Linn. Tewtrakul *et al.* (1998) found the rhizomes of *Zingiber zerumbet* had larvicidal and pupicidal activities. Kamaraj *et al.* (2010) reported the hexane extract of *Z. zerumbet* had larval activity against *Cx. quinquefasciatus* and had potential to be used as an eco-friendly agent to control *Cx. quinquefasciatus*. These phytoconstituents may be responsible for the mosquito larvicidal and pupicidal potentials claimed in this study.*quinquefasciatus* Say under laboratory conditions. Essential oil from various plants has been found to be toxic against different mosquito species in the field of vector control (Mann and Kaufman, 2012).

Jantan *et al.* (2003) also found *Zingiber cassumunar* oil to be effective against mosquito larvae with a LC50 value less than 200 µg/ml. Furthermore, the essential oils from *Boesenbergia rotunda*, *Curcuma zedoaria*, *Etlingera littoralis*, *Z. ottensii* and *Z. zerumbet* also exhibited high larvicidal activity against *Ae. aegypti* larvae. Pierre *et al.* (2014) have reported that essential oil of the two plants showed significant larval and pupal toxicities against all the target mosquito species. *Plectranthus glandulosus* caused LC50 values of 2.66, 7.37 and 43.16 ppm against *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* larvae, respectively and 27.22, 22.60 and 104.75 ppm against *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* pupae, respectively. *Callistemon C.rigidus* displayed LC50 values of 66.67, 99.61 and 176.81 ppm against *Ae. aegypti*, *Anopheles*, *An. gambiae* and *Cx.*

quinquefasciatus larvae, respectively and 50.95, 47.63 and 307.19 ppm against *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* pupae, respectively. Moreover, five major components in essential oil from *Pogostemon cablin* (Blanco) Benth. (Lamiaceae) (â-patchoulene, á-guaiene, ã-patchoulene, á-bulnesene and patchouli alcohol) were tested for pupicidal activity against three medically important human vector mosquitoes at 100 mg/L. Among the five compounds tested, patchouli alcohol was found to be the most effective for pupicidal activity provided 28.44, 26.28 and 25.36% against *Ae. aegypti*, *An. stephensi* and *Cx.quinquefasciatus*, respectively (Gokulakrishnan *et al*., 2013). In the present study the essential oils exhibited pupicidal activity against *Cx. quinquefasciatus* and *An.stephensi.*

5. CONCLUSION

In conclusion, the findings of the present study reveal that essential oil from the clove oil can be effectively used as potent mosquito pupicides. Application of these oils could be very useful to reduce the pupae of those vectors borne-diseases in wide variety of breeding sides, especially in the polluted and non polluted water bodies near by human habitats. This would offer an ideal eco-friendly and less expensive method which may replace the conventional chemical, to reduce the problem of these vector borne mosquitoes. In India plants are largely available and being used in traditionally for mosquito magement. Further studies on mode of action, identification of active compounds and field trials are needed to recommend the development of eco-friendly product from this plant based oil for mosquito control.

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Table-1. Pupal mortality of plant essential oils against *Culex quinquefasciatus* after 24 h exposure treatment period

Essential oils	% Pupal mortality (Mean ± SEM)				
	31.25 ppm	**62.5 ppm**	**125 ppm**	**250 ppm**	**500 ppm**
Aniseed	20.0 ± 0.62^{cd}	37.5 ± 0.22^{e}	52.5 ± 0.21^{d}	68.7 ± 0.43	87.5 ± 0.62^{d}
Cinnamon	35.5 ± 0.42^{e}	40.0 ± 0.43^{e}	52.5 ± 0.62^{d}	71.2 ± 0.41^{de}	82.2 ± 0.42^{cd}
Clove	27.5 ± 0.21^{ef}	41.2 ± 0.22^{e}	62.5 ± 0.61^{bc}	77.5 ± 0.23^{e}	100 ± 0.00^{e}
Orange	8.75 ± 0.42^{b}	17.5 ± 0.64^{b}	27.5 ± 0.21^{b}	43.7 ± 0.42^{b}	62.5 ± 0.62^{b}
Thyme	12.5 ± 0.41^{b}	23.7 ± 0.73^{bc}	38.7 ± 0.62^{c}	61.2 ± 0.82^{c}	80.0 ± 0.41^{c}
Tulsi	23.7 ± 0.61^{de}	36.2 ± 0.42^{de}	62.5 ± 0.21^{e}	76.2 ± 0.41^{e}	85.0 ± 0.72^{cd}
Vetiver	11.2 ± 0.42^{b}	30.3 ± 0.42^{cd}	46.2 ± 0.42^{e}	72.5 ± 0.42^{e}	78.7 ± 0.42^{c}
Control (0.01% Tween 80)	3.21 ± 0.00^{a}				

Each value represent mean of five replicates, in a column mean values followed by same letter are not statistically different ($P<0.05$) by DMRT.

Table-2. Lethal concentration of plant essential oils against pupae of *Culex quinquefasciatus* after 24 h exposure

Essential oils	LC_{50} (ppm)	95% CL[b]		LC_{90} (ppm)	95% CL[b]		Chi-square
		LCL	UCL		LCL	UCL	
Aniseed	160.3	39.65	275.2	489.5	345.8	1032.0	10.3
Cinnamon	141.0	23.86	255.4	575.5	398.4	1332.1	7.7
Clove	106.3	42.21	159.8	313.3	236.0	526.9	6.6
Orange	360.8	258.4	606.6	772.9	554.3	1541.5	7.4
Thyme	252.4	122.7	513.4	614	414.6	1756.8	14.4
Tulsi	133.6	#	#	539.3	#	#	30.3
Vetiver	200.0	#	#	540.8	#	#	35.2

Chi-square value significant at $P < 0.05$ level

[b] CL: confidence limits

LCL: lower confidence limits; UCL: upper confidence limits

LC_{50} = Lethal concentration required to kill 50% of the population exposed

LC_{90} = Lethal concentration required to kill 90% of the population exposed

#= Lethal concentrations could not be determined

Table-3. Pupal mortality of plant essential oils against *Anopheles stephensi* after 24 h exposure treatment

Essential oil	% Pupal mortality (Mean ± SEM)				
	31.25 ppm	**62.5 ppm**	**125 ppm**	**250 ppm**	**500 ppm**
Aniseed	19.0 ± 0.23^{c}	35.2 ± 0.32^{e}	50.3 ± 0.41^{d}	63.1 ± 0.82^{d}	85.2 ± 0.23^{d}
Cinnamon	32.3 ± 0.21^{e}	39.0 ± 0.42^{de}	50.2 ± 0.13^{d}	69.1 ± 0.25^{de}	80.1 ± 0.51^{cd}
					Continue
Clove	26.3 ± 0.12^{ef}	40.5 ± 0.72^{e}	60.4 ± 0.52^{de}	76.4 ± 0.13^{e}	100 ± 0.00^{e}
Orange	7.50 ± 0.32^{b}	16.3 ± 0.51^{b}	25.3 ± 0.31^{b}	40.2 ± 0.23^{b}	59.5 ± 0.32^{b}
Thyme	11.3 ± 0.42^{b}	21.5 ± 0.61^{bc}	35.7 ± 0.31^{c}	58.2 ± 0.61^{c}	78.3 ± 0.62^{c}
Tulsi	22.3 ± 0.41^{de}	34.3 ± 0.32^{de}	61.1 ± 0.34^{e}	74.2 ± 0.51^{e}	84.1 ± 0.23^{d}
Vetiver	10.3 ± 0.43^{b}	26.3 ± 0.43^{cd}	42.2 ± 0.42^{e}	69.5 ± 0.41^{de}	74.2 ± 0.34^{c}
Control (0.01% Tween 80)	2.24 ± 0.00^{a}				

Each value represent mean of five replicates, in a column mean values followed by

same letter are not statistically different (P<0.05) by DMRT.

Table-4. Lethal concentration of plant essential oils against pupae of *Anopheles stephensi* after 24 h exposure

Essential oils	LC_{50} (ppm)	95% CL[b]		LC_{90} (ppm)	95% CL[b]		Chi-square
		LCL	UCL		LCL	UCL	
Aniseed	181.9	72.45	297.46	529.1	378.16	1062.5	9.1
Cinnamon	150.1	28.37	247.3	603.0	433.1	1208.3	5.4
Clove	110.5	89.7	130.6	310.4	272.6	365.7	5.1
Orange	387.4	287.2	652.2	807.3	580.0	1594.7	6.9
Thyme	256.1	163.1	407.5	583.2	424.2	1106.4	10.1
Tulsi	144.2	-715.0	453.1	502.3	306.3	8213.0	22.3
Vetiver	234.3	#	#	596.1	#	#	29.2

Chi-square value significant at P < 0.05 level

[b] CL: confidence limits

LCL: lower confidence limits; UCL: upper confidence limits

LC_{50} = Lethal concentration required to kill 50% of the population exposed

LC_{90} = Lethal concentration required to kill 90% of the population exposed

#= Lethal concentrations could not be determined