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Larvicidal and pupicidal efficacy of plant oils against *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae)

Manickkam Jayakumar, Subramanian Arivoli, Rajasingh Raveen and Samuel Tennyson

Abstract
Mosquitoes are responsible for the transmission of diseases, such as malaria, filariasis, Japanese encephalitis, dengue, chikungunya, Zika virus disease, etc. causing millions of deaths every year. Repeated use of synthetic insecticides for mosquito control has disrupted the environment. This has necessitated the search for alternative approach for mosquito control programme using natural products of plant origin especially plant oils. Therefore in the present study, the bioactivity of plant oils *viz*., aniseed, camphor, citronella, eucalyptus, geranium, lavender, lemon, rosemary, vetiver and wintergreen were tested at concentrations of 125, 250, 500 and 1000mg/L against the third instar larvae and pupae of *Culex quinquefasciatus* in the laboratory. Results revealed varying degree of toxicity by plant oils on the larvae and pupae of *Culex quinquefasciatus*. Among the plant oils tested, eucalyptus showed the highest activity whose LC_{50} value was 186.77 and 206.08mg/L against the larvae and pupae of *Culex quinquefasciatus* followed by vetiver with 470.37 and 239.39mg/L respectively. One hundred per cent larval mortality was recorded in eucalyptus oil at 1000mg/L after 24 hours of exposure. The order of the larvicidal efficacy of plant oils after 24 hours were eucalyptus > vetiver > camphor > lemon > citronella > lavender > aniseed > wintergreen > rosemary > geranium whereas for pupicidal efficacy, it was eucalyptus > vetiver > geranium > lemon > camphor > wintergreen > rosemary > aniseed > citronella > lavender oil.

Keywords: Plant oils, *Culex quinquefasciatus*, larvicidal and pupicidal activity.

Introduction
Mosquitoes are the most important single group of insects in terms of public health importance as they transmit a number of diseases, causing millions of death every year [1]. Man has suffered from the activities of mosquito since time immemorial and it is ranked as man's most important insect pest. Blood feeding female mosquitoes are responsible for the transmission of diseases, such as malaria, filariasis, Japanese encephalitis, dengue, chikungunya, Zika virus disease, etc. [2, 3]. Control of mosquito-borne diseases is becoming increasingly difficult because of the increasing resistance of mosquitoes to insecticides [4]. The WHO [5] expert committee felt the resistance in vectors was probably the "biggest single obstacle in the struggle against vector-borne diseases". Repeated use of synthetic insecticides for mosquito control has disrupted the environment, undesirable effect on non-target organisms, and human health concerns [6]. This has necessitated the search for alternative approach for mosquito control programme *viz*., natural products of plant origin. Botanical insecticides are target specific, easily biodegradable and are potentially suitable for use in integrated vector control management [7].

Plant oils have been used for centuries as fumigants and topical formulations applied to exposed skin and clothing as recorded in writings by ancient Greek, Roman and Indian scholars [8]. Plant oils in general have been recognized as important natural resources of insecticides and used as larvicidal and pupicidal agents. Biopesticide with plant origins have been given new importance in recent years for their use against several insect species. Essential oils are defined as, any volatile oil(s) that have strong aromatic components which provides distinctive odour, flavour or scent to a plant [9]. A wide variety of plant oils have been used as toxicants with ovicidal, larvicidal, pupicidal and adulticidal activities to sub-lethal effects including repellent action [10-13]. Plant oils are valuable natural products which have received much attention as potentially bioactive phytocompounds against insects and have

shown activity against larvae of various mosquito species [14-28]. Therefore, the present work was carried out to study the larvicidal and pupicidal efficacy of plant oils *viz.*, aniseed, camphor, citronella, eucalyptus, geranium, lavender, lemon, rosemary, vetiver and wintergreen against *Culex quinquefasciatus*.

Materials and Methods

Plant oils

Plant oils *viz*., aniseed, camphor, citronella, eucalyptus, geranium, lavender, lemon, rosemary, vetiver and wintergreen used for the present study were obtained from Government recognized aromatic oil store, Chennai, Tamil Nadu, India (Table 1).

Table 1: Details of plant oils used in the study

Common Name	Scientific Name	Family
Aniseed	*Pimpinella anisum*	Apiaceae
Camphor	*Cinnamomum zeylanicum*	Lauraceae
Citronella	*Cymbopogon nardus*	Poaceae
Eucalyptus	*Eucalyptus globulus*	Myrtaceae
Geranium	*Pelargonium graveolens*	Geraniaceae
Lavender	*Lavandula officinalis*	Lamiaceae
Lemon	*Citrus aurantium*	Rutaceae
Rosemary	*Rosmarinus officinalis*	Lamiaceae
Vetiver	*Vetiveria zizanioides*	Poaceae
Wintergreen	*Gaultheria fragrantissima*	Ericaceae

Test mosquitoes

Culex immatures collected from various places in Chennai, Tamil Nadu, India were transported to the laboratory where, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto species level and confirmed before rearing. Cyclic generations of *Culex quinquefasciatus* were maintained separately in mosquito cages (2'x2'x2') in an insectary with a mean room temperature of 27±2°C and a relative humidity of 70-80%. The adult mosquitoes were fed on ten per cent glucose solution in water. The eggs laid in ovitraps placed inside the mosquito cage were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside another mosquito cage for adult emergence.

Larvicidal and pupicidal bioassay

Standard WHO [29] protocol with minor modifications was adopted for the present study. The tests were conducted in glass beakers. *Culex quinquefasciatus* immatures particularly early third instar larvae and freshly emerged pupae from laboratory colonized mosquitoes of F_1 generation were used for the study. Bioassay at test concentrations of 125, 250, 500 and 1000mg/L of plant oil each was assessed for both larvicidal and pupicidal bioassay. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of plant oil each. Twenty five healthy larvae were released into each 500mL glass beaker containing 250mL of water and test concentration for larvicidal. Mortality was observed 24, 48 and 72 hours after treatment. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of Dimethyl sulfoxide (DMSO) to distilled water. Distilled water served as untreated control. Likewise, the same methodology was adopted for pupicidal bioassay and mortality was observed after 24 hours treatment. The larval and pupal per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula [30]. Data from all replicates were pooled for statistical analysis. LC_{50} and LC_{90} values were calculated using SPSS software by probit analysis [31].

$$\text{Larval per cent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

$$\text{Pupal per cent mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100$$

$$\text{Abbott's formula} = \frac{\text{Percentage of test mortality} - \text{Percentage of control mortality}}{100 - \text{Percentage of control mortality}} \times 100$$

Results and Discussion

All plant oils tested at various concentrations were found to exhibit varying degree of activity against the larvae and pupae of *Culex quinquefasciatus* (Table 2; Figure 1). No larval and pupal mortality was observed in treated and untreated control. Among the plant oils tested, eucalyptus showed the highest activity whose LC_{50} value was 186.77 and 206.08mg/L against the larvae and pupae of *Culex quinquefasciatus* followed by vetiver with 470.37 and 239.39mg/L respectively after 24 hours (Table 2; Figure 2). Eight out of ten plant oils showed more than 50% larval mortality at 1000mg/L concentration while six plant oils showed more than 50% pupal mortality. One hundred per cent larval mortality was recorded in eucalyptus oil at 1000mg/L after 24 hours of exposure. The order of the larvicidal efficacy of plant oils after 24 hours were eucalyptus > vetiver > camphor > lemon > citronella > lavender > aniseed > wintergreen > rosemary > geranium. For pupicidal efficacy, it was eucalyptus > vetiver > geranium > lemon > camphor > wintergreen > rosemary > aniseed > citronella > lavender oil.

Essential oils such as citronella, calamus, thymus, and eucalyptus have been found promising in killing mosquito larva [32-34]. The results of the present study were comparable with the 24 hour LC_{50} values of recent studies. Samuel *et al*. [27] screened six plant oils (lemon grass, palmarosa, geranium, tulsi, rosemary and mentha) against larvae of *Aedes aegypti* and found that palmarosa exhibited the highest activity and LC_{50} value was 13.96ppm. Youssif and Shaalan [35] tested the cinnamon, white camphor and wintergreen oil for activity against the larvae of *Aedes caspius* and LC_{50} values were 58.41, 42.98 and 81.32mg/L respectively. Pugazhvendan and Elumali [36] indicated camphor, clove and eucalyptus oil to exhibit 70, 100 and 100% activity against the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* respectively. Manzoor *et al*. [37] reported larvicidal activity in the essential oil from *Ocimum basilicum* against *Aedes aegypti* and *Culex quinquefasciatus* with LC_{50} values of 75.35 and 92.30ppm respectively and also from *Acorus calamus*, *Mentha arvensis*, *Saussurea lappa* and *Cymbopogon citratus* against *Aedes aegypti* with LC_{50} values of 99.41, 114.33, 128.89 and 136.28ppm respectively and against *Culex quinquefasciatus* as 107.81, 112.18, 141.43 and 148.54ppm respectively. Samuel *et al*. [28] tested ten

plant oils (cedar wood, citronella, clove, eucalyptus, lemon grass, orange, nutmeg, palmarosa, pine and tulsi) and found orange oil to exhibit the highest larvicidal activity with LC_{50} values of 85.93ppm, followed by palmarosa (88.78), tulsi (92.48) and nutmeg (93.62) against the larvae of *Aedes aegypti*. Liu *et al*. [38] indicated the essential oil of *Illicium henryi* to exhibit larvicidal activity against *Aedes albopictus* and LC_{50} value was 35.43µg/mL. El-Akhal *et al*. [39] reported the essential oil of *Thymus vulgaris* and *Origanum majorana* to possess activity against larvae of *Anopheles labranchiae* with LC_{50} values of 351.63 and 107.13µg/mL respectively. Nasir *et al*. [40] reported the LC_{50} values (in ppm) of oils of eucalyptus (161 and 799), neem (117 and 709), peppermint (346 and 715), basil (659 and 219) and ginger (152 and 777) against the larvae and pupae of *Aedes aegypti* respectively. Ramar *et al*. [41] screened twenty two essential oils for toxicity against the pupae of *Culex quinquefasciatus* and found seven of them *viz*., aniseed, cinnamon, clove, orange, thyme, tulsi and vetiver to be promising as they provided 100% mortality at 1000ppm at 24 hours. Ramar *et al*. [42] reported the bioefficacy of pupicidal potential with the LC_{50} values (in ppm) of some essential oils against *Culex quinquefasciatus* and *Anopheles stephensi viz*., clove (106.3 and 110.5), tulsi (133.6 and 144.2) and cinnamon (141.0 and 150.1) after 24 hours respectively.

Essential oil from various plants has been found to be toxic against different mosquito species in the field of vector control [43, 44]. Essential oils are presumed to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects [43]. Ruiz *et al*. [45] found a significant vacuolation, swollen nuclei and elongated epithelial cells of the mosquito larvae treated with essential oil. These epithelial cells were disrupted at the apical region with vesicle formation, lysis and leakage of cytoplasm material into the gut lumen. In addition, it is also known that the mosquito larvae and pupae breathe through spiracles located on the eighth abdominal segment and therefore must come to the surface frequently to breathe. The oils block the spiracles, resulting in blockage of respiratory siphons (asphyxiation) and death [46, 47]. Further, Rattan [48] reviewed the mechanism of action of essential oils on the body of insects and documented several physiological disruptions, such as inhibition of acetylcholinesterase, disruption of the molecular events of morphogenesis and alteration in the behaviour and memory of cholinergic system. Of these, the most important activity is the inhibition of acetylcholinesterase activity as it is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway. The mode of action of essential oils was investigated by Corbet *et al*. [49] reported the susceptibility of mosquito larvae and pupae to surface materials entering their tracheal system. Further, he observed that the essential oils increased the tendency to tracheal flooding and chemical toxicity. Suryanarayanamurthy *et al*. [50] in his study reported that pupal death might be due to multiple mechanisms of action including the oil blocking the moulting of pupae to adult.

Larviciding is an effective method to reduce mosquito densities before they emerge as adults and synthetic insecticides have been widely used for this purpose [51]. The larvicides may best be used in small breeding places, where the water is stagnant [52] because it is easier to kill larvae in stagnant water than to kill adult mosquitoes. Plant oils may be of potential benefit for mosquito control, since they have a rich source of bioactive compounds that may be biodegradable into nontoxic products and are potentially suitable for use in integrated management programs for mosquito control [53], as they have been observed to possess mosquito larvicidal and pupicidal properties [54-58]. It is evident from the present study that plant oils have larvicidal and pupicidal activity against *Culex quinquefasciatus* and the results have identified additional plant oils as promising larvicides/pupicides. Moreover, these results could be useful in the search for more selective and biodegradable larvicidal/pupicidal natural compounds.

Table 2. Larvicidal and pupicidal activity of plant oils against *Culex quinquefasciatus*

Concentration (mg/L)	Plant oils									
	Aniseed	**Camphor**	**Citronella**	**Eucalyptus**	**Geranium**	**Lavender**	**Lemon**	**Rosemary**	**Vetiver**	**Wintergreen**
Larvicidal (24 hours)										
125	0.00 ±0.00 (0.00)	11.00 ±0.44 (44.00)	1.33 ±0.05 (5.32)	12.00 ±0.48 (48.00)	3.33 ±0.13 (13.32)	0.33 ±0.01 (1.32)	0.33 ±0.01 (1.32)	0.00 ±0.00 (0.00)	12.00 ±0.48 (48.00)	3.33 ±0.13 (13.32)
250	1.67 ±0.07 (6.68)	12.33 ±0.49 (49.32)	3.67 ±0.15 (14.68)	21.67 ±0.87 (86.68)	3.67 ±0.15 (14.68)	2.33 ±0.09 (9.32)	12.33 ±0.49 (49.32)	2.00 ±0.08 (8.00)	12.00 ±0.48 (48.00)	4.00 ±0.16 (16.00)
500	3.00 ±0.12 (12.00)	13.67 ±0.55 (54.68)	8.67 ±0.35 (34.68)	22.67 ±0.91 (90.68)	4.33 ±0.17 (17.32)	2.67 ±0.11 (10.68)	13.33 ±0.53 (53.32)	3.67 ±0.15 (14.68)	17.00 ±0.68 (68.00)	4.67 ±0.19 (18.68)
1000	5.00 ±0.20 (20.00)	14.33 ±0.57 (57.32)	11.00 ±0.44 (44.00)	25.00 ±0.00 (100.00)	4.67 ±0.19 (18.68)	5.67 ±0.23 (22.68)	13.67 ±0.55 (54.68)	3.67 ±0.15 (14.68)	18.00 ±0.72 (72.00)	5.33 ±0.21 (21.32)
LC_{50}	1469.43	624.52	955.43	186.77	1817.61	1447.13	715.18	1677.53	470.37	1651.19
LC_{90}	2313.39	1510.43	1654.18	362.30	3295.94	2322.78	1398.40	2708.80	1131.14	3005.75
Larvicidal (48 hours)										
125	1.33 ±0.05 (5.32)	12.33 ±0.49 (49.32)	3.67 ±0.15 (14.68)	20.67 ±0.83 (82.68)	5.67 ±0.23 (22.68)	3.67 ±0.15 (14.68)	3.00 ±0.12 (12.00)	4.33 ±0.17 (17.32)	15.67 ±0.63 (62.68)	5.33 ±0.21 (21.32)
250	4.67 ±0.19 (18.68)	13.00 ±0.52 (52.00)	6.67 ±0.27 (26.68)	24.33 ±0.97 (97.32)	5.67 ±0.23 (22.68)	4.67 ±0.19 (18.68)	14.00 ±0.56 (56.00)	5.67 ±0.23 (22.68)	16.33 ±0.65 (65.32)	8.33 ±0.33 (33.32)
500	6.67 ±0.27 (26.68)	13.67 ±0.55 (54.68)	12.33 ±0.49 (49.32)	24.67 ±0.99 (98.68)	8.00 ±0.32 (32.00)	5.67 ±0.23 (22.68)	16.33 ±0.66 (65.32)	10.00 ±0.40 (40.00)	22.00 ±0.88 (88.00)	9.00 ±0.36 (36.00)
1000	8.67 ±0.35 (34.68)	16.33 ±0.66 (65.32)	13.00 ±0.52 (52.00)	25.00 ±0.00 (100.00)	8.33 ±0.33 (33.32)	7.33 ±0.29 (29.32)	16.67 ±0.67 (66.68)	11.00 ±0.44 (44.00)	23.67 ±0.95 (94.68)	14.33 ±0.57 (57.32)
LC_{50}	1124.40	545.42	600.53	106.40	1173.38	1322.58	560.20	925.92	243.11	778.71
LC_{90}	1974.12	1343.23	1258.59	194.34	2311.82	2439.11	1169.73	1770.29	583.99	1531.04
Larvicidal (72 hours)										
125	6.67 ±0.27 (26.68)	13.67 ±0.55 (54.68)	6.00 ±0.24 (24.00)	20.67 ±0.83 (82.68)	6.00 ±0.24 (24.00)	5.33 ±0.21 (21.32)	9.33 ±0.37 (37.32)	6.00 ±0.24 (24.00)	17.00 ±0.68 (68.00)	8.67 ±0.36 (34.68)
250	8.33 ±0.33 (33.32)	13.67 ±0.55 (54.68)	10.67 ±0.43 (42.68)	24.33 ±0.97 (97.32)	7.00 ±0.28 (28.00)	8.00 ±0.32 (32.00)	14.67 ±0.59 (58.68)	6.67 ±0.27 (26.68)	18.67 ±0.75 (74.68)	9.67 ±0.39 (38.68)
500	11.00 ±0.44 (44.00)	15.67 ±0.63 (62.68)	14.67 ±0.59 (58.68)	24.67 ±0.99 (98.68)	10.67 ±0.43 (42.68)	8.33 ±0.33 (33.32)	18.67 ±0.75 (74.68)	15.00 ±0.60 (60.00)	23.00 ±0.92 (92.00)	14.00 ±0.56 (56.00)
1000	13.00 ±0.52 (52.00)	16.33 ±0.65 (65.32)	16.33 ±0.65 (65.32)	25.00 ±0.00 (100.00)	11.00 ±0.44 (44.00)	11.00 ±0.44 (44.00)	19.67 ±0.79 (78.68)	16.33 ±0.65 (65.32)	24.00 ±0.96 (96.00)	17.00 ±0.68 (68.00)
LC_{50}	789.28	496.35	600.53	106.40	902.86	950.82	413.20	632.23	203.43	649.00
LC_{90}	1628.60	1297.14	1258.59	194.34	1826.29	1896.44	962.53	1255.56	497.51	1254.83
Pupicidal										
125	3.33 ±1.53 (13.32)	2.67 ±0.58 (10.68)	5.00 ±2.00 (20.00)	17.00 ±1.00 (68.00)	4.00 ±1.73 (16.00)	0.67 ±1.15 (2.68)	4.67 ±0.58 (18.68)	2.67 ±1.15 (10.68)	14.67 ±3.51 (58.68)	2.33 ±0.58 (9.32)
250	7.33 ±2.31 (29.32)	4.33 ±2.08 (17.32)	5.67 ±2.89 (22.68)	18.67 ±4.04 (74.68)	7.00 ±2.65 (28.00)	3.33 ±1.53 (13.32)	6.00 ±2.00 (24.00)	5.00 ±1.00 (20.00)	17.33 ±2.52 (69.32)	5.33 ±2.25 (21.32)
500	7.67 ±2.52 (30.68)	11.00 ±1.00 (44.00)	6.33 ±1.53 (25.32)	21.00 ±3.61 (84.00)	11.67 ±2.52 (46.68)	6.33 ±1.53 (25.32)	12.00 ±2.00 (48.00)	9.00 ±1.00 (36.00)	20.67 ±2.08 (82.68)	11.00 ±1.00 (44.00)
1000	12.33 ±2.52 (49.32)	16.00 ±2.00 (64.00)	10.67 ±3.06 (42.68)	24.67 ±0.58 (98.68)	18.00 ±2.00 (72.00)	8.33 ±1.53 (33.32)	17.33 ±2.08 (69.32)	14.00 ±1.00 (56.00)	24.33 ±0.58 (97.32)	15.67 ±2.08 (62.68)
LC_{50}	898.49	735.96	1035.69	206.08	642.78	1142.12	660.68	824.33	239.39	738.87
LC_{90}	1685.26	1303.30	1960.30	474.78	1196.53	1929.48	1237.36	1473.76	542.88	1321.24

Values are mean of three replicates of three trials ±standard deviation; Values in parenthesis denote per cent larval mortality.
LC_{50}: Lethal concentration that kills 50% of the exposed larvae; LC_{90}: Lethal concentration that kills 90% of the exposed larvae.

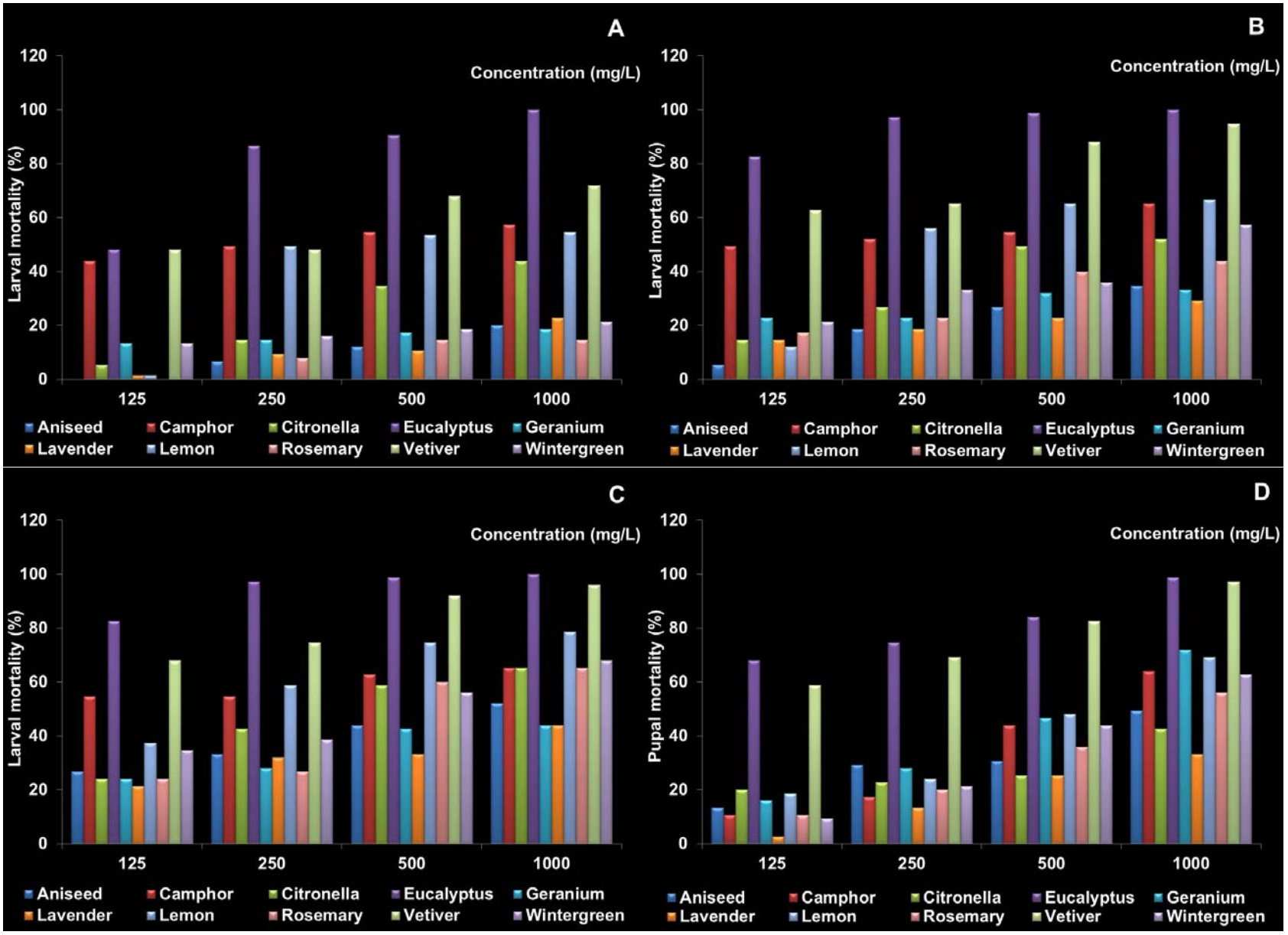

Fig 1: Efficacy of plant oils on *Culex quinquefasciatus* larvae (A: 24 hours; B: 48 hours; C: 72 hours) and pupae (D)

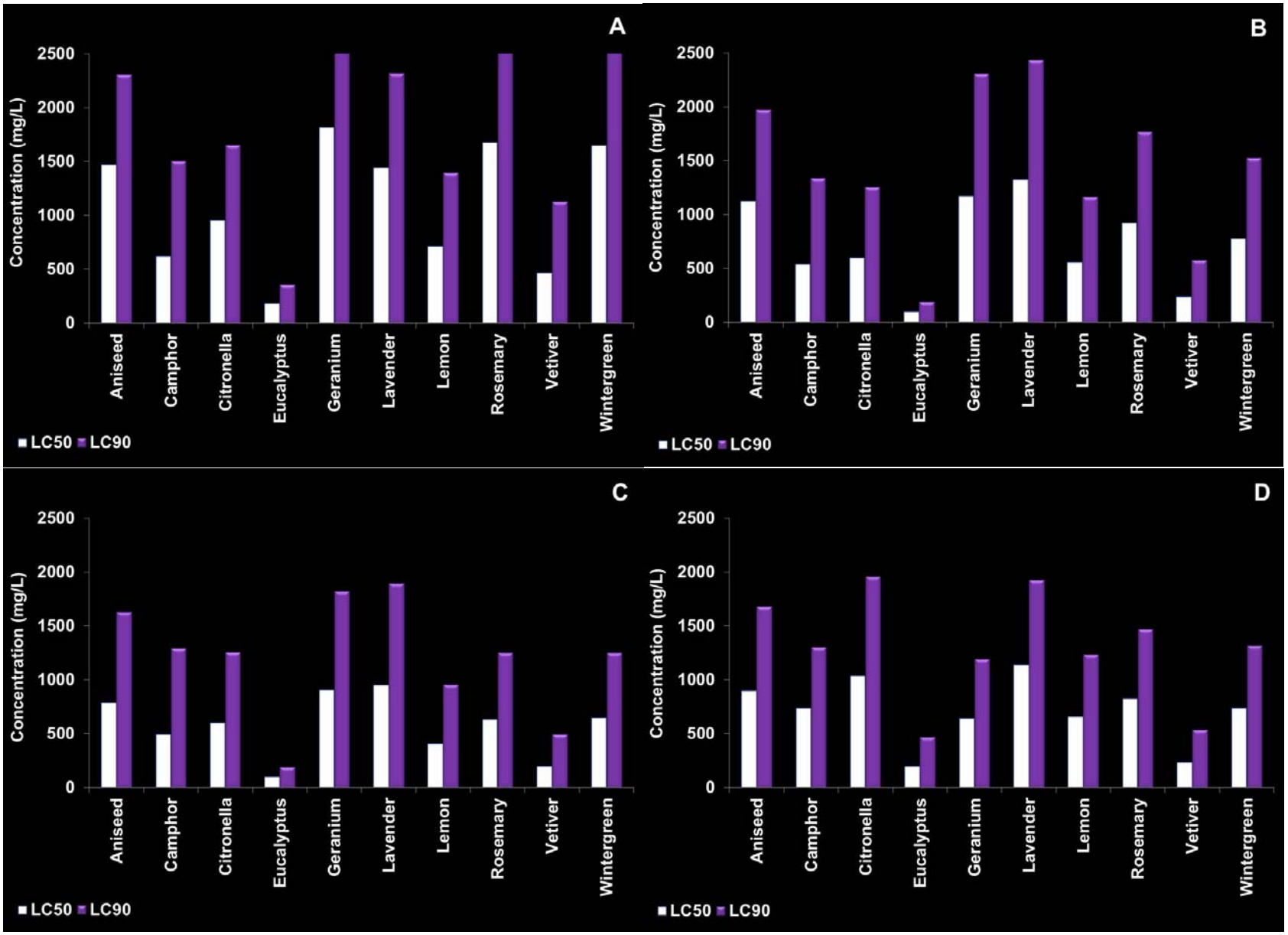

Fig 2: Probit analysis of plant oils on *Culex quinquefasciatus* larvae (A: 24 hours; B: 48 hours; C: 72 hours) and pupae (D)

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