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LARVICIDAL AND PUPICIDAL ACTIVITIES OF SELECTED PLANT ESSENTIAL OILS AGAINST IMPORTANT VECTOR MOSQUITOES: DENGUE VECTOR, *AEDES AEGYPTI* (L.), MALARIAL VECTOR *ANOPHELES STEPHENSI* (LISTON), FILARIASIS VECTOR, *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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ABSTRACT

The larvicidal and pupicidal potential of five different essential oils (*Citriodora oil, Lemon grass, Gaultheria* oil, *Citronella oil, Clove oil*) were tested against the fourth-instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24-h exposure. All the tested oils showed moderate to good larvicidal and pupicidal activities. However, the maximum larval mortality was detected in oil of *Citriodora* against *Cx. quinquefasciatus* (LC_{50} 13.47, LC_{95} 191.80 ppm) and followed by *Lemon grass* and *Gaultheria* oil. The maximum pupal mortality was observed in oil of *Citriodora* against *Cx. quinquefasciatus* (LC_{50} 54.21, LC_{95} 439.73) followed by *Lemon grass* of the selected oils and encourages further effort to investigate the bioactive compounds in those oils that might possess good larvicidal and pupicidal properties when it will be isolated in pure form.

KEYWORDS: Larvicidal, pupicidal, essential oils, mosquito vectors.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are among the most serious insect pests of medical importance. They are vectors of various disease agents some of which cause millions of cases of illnesses and deaths in human and animal each year. Among these diseases, malaria,

yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at endemic and epidemic areas in many countries (WHO, 1991, Lerdthusnee, *et al.*, 1995 and Madani, *et al.*, 2003). The yellow fever mosquito, *Ae. aegypti* is found throughout subtropical and tropical areas of the world and considered the major vector for the transmission of dengue and yellow fever. It is a largely diurnal-biting species (Chadee, 1988) that apparently uses chemical and visual cues to locate its host (Kawada, Takemura, Arikawa, & Takagi, 2005). *An.stephensi* are major malaria vectors in India. With an annual incidence of 300-500 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world's population lives in areas where malaria is endemic (Wernsdorfer and Wernsdorfer, 2003). Malaria remains an important public health parasitic disease in both tropical and subtropical countries in Africa where, it is mostly seasonal with its major incidence occurring in the rainy season (Oesterholt *et al*., 2006). Despite decades of control efforts, malaria continues to be a major public health concern throughout the world. It is estimated that there are 300-500 million new cases every year, with 1.5 to 2.7 million deaths worldwide particularly in Africa (WHO, 1992) where about 90% of the global cases are recorded (Breman *et al*., 2004). Children under five years and pregnant women are affected most (WHO, 2008). Carvalho *et al.*, 2003 reported the larvicidal activity of the essential oil from *Lippia sidoides*against *Ae. aegypti*. Cheng *et al*., 2003 reported the bioactivity of fourteen essential plant oils against the yellow fever mosquito larvae of *Ae. aegypti and* all essential oils screened was found to be effective. *Cx. quinquefasciatus is* a medium sized brown mosquito that exists throughout the tropics and the lower latitudes of temperate regions. Adults vary from 3.96 to 4.25 mm in length (Lima *et al*., 2003). *Cx. quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al., 2003). Neem oil showed larvicidal activity against *Cx quinquefasciatus* (Shanmugasundaram *et al*., 2001).

Essential oils of many plants have showed larivicidal activity against various mosquito species (Kumar, A. and G.P. Dutta, 1987 and Samuel, *et al*., 2011*)*. The use of herbal products is one of the best alternatives for mosquito control. Several experiments have been observed that the larvicidal properties of plant essential oils against *Anopheles* mosquitoes. Essential oils extracted from *Azadirachta indica* (Okumu, F.O., *et al* 2007) and leaves and rhizomes of *Curcuma longa* (Anatoliy Viktorovich Molodchik, 2013) demonstrated larvicidal activity against *Anopheles gambiae*. Essential oils of *Eucalyptus camaldulensis* (T.M.

Walker, *et al.*, 2008), *Plectranthus amboinicus* (Senthilkumar, A. and V. Venkatesalu, 2010), *Zanthoxylum armatum* (Yadavc, *et al*., 2007) *Eucalyptus tereticornis* (Nathan, S.S., 2007) and *Tagetes patula* (Dharmagadda, V.S.S., *et al* 2005) demonstrated larvicidal activity against *Anopheles stephensi* present studies.

MATERIALS AND METHODS

Plant Oils

Plant Oils *Citriodora* (*Corymbia citriodora*), *Lemongras* (*Cymbopogon citratus*), *Gaultheria* (*Gaultheria procumbens*), *Citronella* (*Cymbopogon nardus*) and *Clove* (*Syzygium aromaticum*) were purchased from Tamil Nadu Government Co-Operative Super Market, Cherring cross, Udhagamandalam, The Nilgiris, Tamil Nadu, India and collected oils were used for bioassay against Mosquito vectors.

Vector rearing

The mosquito larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were collected from National centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Tamilnadu, India. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Larvicidal bioassay

The larvicidal activity of selected plants oils were evaluated as per the protocol previously described WHO, (2005) Based on the wide range and narrow range tests, all oils tested ranging 30-200ppm were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of selected mosquito species. The plants oils were dissolved in 2 drop twin 20 and then diluted in 100ml of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 2 drop tween 20 in 100ml of dechlorinated water. The larvae of test species (10) were introduced in 250-ml plastic cups containing 100ml of aqueous medium (100ml of dechlorinated + 2 drop tween 20) and the required amount of chemical compositions was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using probit analysis (Finney, 1971). The average mortality

data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics chi-square values were calculated by using the software using statistical package of social science (SPSS) version 16.0 for windows, significance level was set at $p \leq 0.05$.

Pupicidal bioassay

The pupicidal activity of plant oils assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments will be prepared and they will be tested against the pupae of *Ae. Aegypti*, *An. stephensi* and *Cx. quinquefasciatus* Tween 20(emulsifier) in water will be treated as control. The pupae of these mosquito species (10 pupae) will be introduced in 250-ml plastic cups containing 100 ml of aqueous medium (100 ml of dechlorinated water + 2 drops of twin 20) and the required amount of plant extract will be added. The pupal mortality will be observed and recorded after 24 h of post treatment. For each experiment, five replicates will be maintained at a time. The percentage of mortality will be calculated by using Abbott's formula (Abbott, 1925).

RESULTS

As discussed in materials and methods, the results of relative toxicity of five essential oils against *Ae. Aegypti*, *An. stephensi* and *Cx. quinquefasciatus* after 24 hours of treatment are presented in Table 1, 2 and 3. It was evident from table that all the tested essential oils demonstrated significant larvicidal and pupicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus.* All the tested oils showed moderate to good larvicidal and pupicidal activities. The larval mortality was detected in oils against *Ae. aegypt*, *An. stephensi and Cx. quinquefasciatus.* The *Ae. aegypti* highest larval mortality was found in *Citriodora oil* 95.2% (LC_{50} 48.46 and LC_{95} 373.43) followed by *Lemon grass oil* 92.6% (LC_{50} 74.22 and LC_{95} 383.51), *Gaultheria* oil 90.8% (LC_{50} 57.0 and LC_{95} 532.23), *Citronella oil* 90.4% (LC_{50} 66.99 and LC_{95} 517.79), *Clove oil* 74.8% *(*LC_{50} 137.87 and LC_{95} 1114.64). The *Ae. aegypti* highest pupal mortality was found in *Citronella oil* 90.8% (LC_{50} 66.59 and LC_{95} 555.96) followed by *Lemon grass oil* 65.8% (LC_{50} 159.80 and LC_{95} 2243.88), *Gaultheria* oil 73.8% (LC_{50} 111.31 and LC_{95} 1236.57), *Citronella oil* 70.2% (LC_{50} 121.75 and LC_{95} 1715.32), *Clove oil* 61.8% (LC_{50} 184.41 and LC_{95} 2556.0). The *An. stephensi* highest larval mortality was found in *Citriodora oil* 94.8% (LC_{50} 33.84 and LC_{95} 389.92) followed by *Lemon grass oil* 61.4% (LC_{50} 175.39 and LC_{95} 2966.04), *Gaultheria* oil 65.2% (LC_{50} 186.43 and LC_{95} 1418.85), *Citronella oil* 83.4% (LC_{50} 102.73 and LC_{95} 656.53), *Clove oil* 52.4% (LC_{50} 256.66 and LC_{95} 7293.91) The *An. stephensi* highest pupal mortality was found in *Citriodora oil*

87.8% (LC_{50} 50.0 and LC_{95} 729.72) followed by *Lemon grass oil* 72.2% (LC_{50} 110.89 and LC_{95} 2260.80), *Gaultheria oil* 71.8% (LC_{50} 94.12 and LC_{95} 2696.07), *Citronella oil* 81.6% (LC_{50} 71.44 and LC_{95} 1231.88), *Clove oil* 61.8% (LC_{50} 177.25 and LC_{95} 3311.26) The *Cx. quinquefasciatus* highest larval mortality was found in *Citriodora oil* 98% (LC_{50} 13.47 and LC_{95} 191.80), followed by *Lemon grass oil* 57.2% (LC_{50} 187.73 and LC_{95} 11272.61), *Gaultheria* oil 90.6% (LC_{50} 20.87 and LC_{95} 609.15), *Citronella oil* 93.6% (LC_{50} 28.36 and LC_{95} 383.96), *Clove oil* 51.6% (LC_{50} 264.01 and LC_{95} 10308.17) The *Cx. quinquefasciatus* highest pupal mortality was found in *Citriodora oil* 91.6% (LC_{50} 54.21 and LC_{95} 439.73), followed by *Lemon grass oil* 67.6% (LC_{50} 113.99 and LC_{95} 3348.37), *Gaultheria* oil 58.2% (LC_{50} 77.60 and LC_{95} 1132.09), *Citronella oil* 90.2% (LC_{50} 72.18 and LC_{95} 494.57) *Clove oil* 52.6% (LC_{50} 260.57 and LC_{95} 10883.51) respectively. Chi- square value was significant at $p<0.05$ level.

In conclusion, our findings showed that oils of *Citriodora oil*, *Lemon grassoil*, *Gaultheria* oil, *Citronella oil* and *Clove oil* can be developed as ecofriendly larvicides. Also our results open the possibility for further investigations of the efficacy of larvicidal and pupicidal properties of natural product in essential oils.

Table 1: Larvicidal activity of essential oils against 4th instar larvae.

Essential oils	Concentration (ppm) % of larval mortality					LC_{50} (ppm)	95% Confidence Limit (ppm)		LC_{95} (ppm)	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
	50 (ppm)	100 (ppm)	150 (ppm)	200 (ppm)	250 (ppm)		LCL	UCL		LCL	UCL	
Larvicidal activity of essential oils against 4th instar larvae of *Ae. aegypti*.												
Citriodora oil	55.4 ± 2.6	66 .8± 1.6	78.2 ±1.9	87.2±2.2	95.2±2.3	48.46	36.56	64.25	373.43	258.48	539.49	6.080
Lemon grass	38.2±1.4	59.4±1.6	68.8±2.1	85.8±2.2	92.6±2.7	74.22	63.30	87.23	383.51	288.12	510.48	5.536
Gaultheria oil	51.2±3.9	60.8±3.1	71.2 ±3.8	81.2±1.6	90.8±1.9	57.00	43.63	74.46	532.23	336.13	842.74	4.089
Citronella oil	46.0±1.5	54.4±2.9	72.8 ±3.1	80.2±2.6	90.4±2.7	66.99	54.04	83.03	517.79	342.97	781.72	6.259
Clove oil	24.0±2.9	38.2±1.4	48.2 ±2.8	57.8±2.3	74.8±3.9	137.87	119.27	159.37	1114.64	622.81	1994.84	4.237
Larvicidal activity of essential oils against 4thinstar larvae of *An. stephensi.*												
Citriodora oil	62.8± 3.1	74.4± 3.5	82.2 ±2.2	86.6±2.3	94.8±2.3	33.84	21.70	52.76	389.92	247.60	614.04	3.104
Lemon grass	25.6±1.1	33.4±1.9	45.4±2.7	51.8±2.4	61.4±3.9	175.39	141.54	217.34	2966.04	985.52	8926.67	1.487
Gaultheria oil	19.0±1.2	25.6±2.3	35.4 ±3.5	54.2±2.5	65.2±3.9	186.43	158.60	219.14	1418.85	752.78	2674.25	6.915
Citronella oil	31.6±2.0	41.0 ±2.2	58.2 ±1.6	74.0±3.1	83.4±3.2	102.73	89.25	118.23	656.53	437.40	985.44	6.643
Clove oil	23.6±2.7	29.4±1.1	35.8 ±3.7	46.0±1.5	52.4±3.2	256.66	180.37	365.22	7293.91	1341.51	3965.44	1.790
Larvicidal activity of essential oils against 4thinstar larvae of *Cx. quinquefasciatus.*												
Citriodora oil	80.6± 2.4	87.6± 1.1	92.8 ±2.5	94.6±3.2	98.0±2.7	13.47	5.29	34.31	191.80	131.63	279.47	1.362
Lemon grass	31.8±2.4	38.2±2.5	43.2±2.2	51.2±2.5	57.2±3.1	187.73	135.51	260.06	11272.61	1231.75	103163.13	1.007
Gaultheria oil	66.8±1.9	78.6±2.4	81.2 ±2.3	85.6±2.8	90.6±0.8	20.87	9.19	47.39	609.15	278.56	1332.07	0.796
Citronella oil	65.2±3.5	78.6 ±2.9	82.6 ±3.1	88.8±1.9	93.6±2.0	28.36	16.58	48.48	383.96	235.35	626.40	1.332
Clove oil	24.4±2.9	31.4±1.6	37.4 ±2.7	44.8±2.5	51.6±2.1	264.01	177.86	391.90	10308.17	1417.49	74962.04	0.911

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 2: Pupicidal activity of essential oils against Ae. aegypti.

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC_{50} (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC_{95} (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Citriodora oil									
Control	95.2 ± 3.1	0± 0.0	66.59	53.30	83.18	555.96	356.46	867.13	7.261
50	51.2 ± 2.2	46.4 ± 1.8							
100	41.8 ± 2.2	57.2 ± 2.6							
150	31.6 ± 2.3	67.6 ± 2.0							
200	18.6 ± 1.3	80.2 ± 2.6							
250	8.4 ± 1.1	90.8 ± 3.3							
Control	92.4 ± 3.7	0± 0.0	159.80	132.21	193.16	2243.88	874.49	5757.61	2.338
50	72.8 ± 2.4	26.2 ± 2.5							
100	63.6 ± 3.0	34.8 ± 3.1							
150	52.4 ± 2.0	46.2 ± 2.3							
200	42.6 ± 3.7	53.4 ± 1.3							
250	33.6 ± 2.6	65.8 ± 2.4							
***Gaultheria* oil**									
Control	91.4 ± 2.6	0± 0.0	130.01	111.31	151.86	1236.57	646.20	2366.30	3.664
50	63.8 ± 2.9	27.8 ±2.2							
100	54.2 ± 3.7	38.2 ±2.0							
150	41.6 ± 2.6	50.2 ± 2.2							
200	32.4 ± 3.2	61.0 ±2.3							
250	21.4 ± 1.6	73.8 ± 3.0							
Citronella oil									
Control	96.2 ± 1.3	0± 0.0	121.75	101.53	146.00	1715.32	735.20	4002.04	1.213
50	64.2 ± 2.8	31.2 ± 2.1							
100	54.2 ± 3.4	43.2 ± 2.1							
150	42.8 ± 2.7	51.8 ± 2.5							

200	**36.6 ± 2.0**	**62.2 ± 2.8**							
250	**24.4 ± 2.8**	**70.2 ± 1.6**							
Clove oil									
Control	**93.6 ± 2.6**	**0± 0.0**	**184.41**	**149.93**	**226.82**	**2556.00**	**951.06**	**6869.29**	**2.330**
50	**73.2 ± 2.3**	**23.4 ± 2.8**							
100	**66.8 ± 1.9**	**32.6 ± 1.6**							
150	**57.8 ± 1.6**	**40.4 ± 1.5**							
200	**46.8 ± 2.7**	**51.6 ± 2.1**							
250	**37.2 ± 1.6**	**61.8 ± 2.2**							

Value represents mean ± S.D. of five replications. *Mortality of the pupae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 3: Pupicidal activity of essential oils against An. stephensi.

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC_{50} (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC_{95} (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Citriodora oil									
Control	**94.6 ± 3.1**	**0± 0.0**	**50.0**	**35.03**	**71.36**	**729.72**	**385.33**	**1381.90**	**3.170**
50	**41.4 ± 1.6**	**53.4 ± 3.4**							
100	**34.4 ± 2.3**	**62.4 ± 3.7**							
150	**26.4 ± 2.5**	**71.2 ± 1.9**							
200	**18.4 ± 1.1**	**80.6 ± 1.5**							
250	**10.8± 2.2**	**87.8 ± 2.2**							
Lemon grass oil									
Control	**91.6 ± 3.0**	**0± 0.0**	**110.89**	**89.62**	**137.22**	**2260.80**	**785.33**	**6508.36**	**3.333**
50	**61.8 ± 2.4**	**37.2 ± 2.1**							
100	**55.6± 1.8**	**42.8± 3.1**							

150	**38.4± 1.1**	**53.2 ± 1.6**							
200	**31.8 ± 2.4**	**61.6 ± 2.7**							
250	**24.8 ± 2.2**	**72.2± 2.2**							
***Gaultheria* oil**									
Control	**91.4 ± 2.8**	**0± 0.0**	**94.12**	**72.47**	**122.23**	**2696.07**	**772.61**	**9408.16**	**1.201**
50	**58.6 ± 1.5**	**39.8 ±1.0**							
100	**50.4 ± 3.9**	**48.8 ±1.3**							
150	**41.2± 1.6**	**57.2± 2.4**							
200	**35.6± 1.3**	**62.6 ±2.8**							
250	**23.8± 3.0**	**71.8± 3.5**							
Citronella oil									
Control	**88.4 ± 1.1**	**0± 0.0**	**71.44**	**54.03**	**94.45**	**1231.88**	**545.22**	**2783.33**	**3.426**
50	**51.6 ± 2.8**	**45.4 ± 3.7**							
100	**42.4 ± 2.0**	**54.6± 2.1**							
150	**38.4 ± 1.1**	**61.2± 2.1**							
200	**26.8 ± 1.7**	**72.2 ± 2.7**							
250	**18.8 ± 1.3**	**81.6± 3.8**							
Clove oil									
Control	**89.8 ± 3.1**	**0± 0.0**	**177.25**	**141.73**	**221.68**	**3311.26**	**1017.50**	**10775.81**	**2.092**
50	**71.6 ± 1.5**	**26.6 ± 1.5**							
100	**62.6 ± 2.1**	**33.8 ± 1.4**							
150	**55.6 ± 2.8**	**43.4 ± 1.5**							
200	**43.6 ± 3.0**	**51.8 ± 3.0**							
250	**32.8 ± 2.2**	**61.8 ± 1.7**							

Value represents mean ± S.D. of five replications. *Mortality of the pupae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 4: Pupicidal activity of essential oils against Cx. quinquefaciatus.

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC_{50} (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC_{95} (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Citriodora oil									
Control	92.2 ± 1.9	0± 0.0	54.21	41.70	70.47	439.73	296.10	653.02	3.528
50	44.6 ± 3.1	51.6± 2.3							
100	33.6 ± 2.6	62.2± 2.8							
150	19.8 ± 1.9	77.2± 2.1							
200	12.4± 2.0	85.2± 2.6							
250	5.8± 1.7	91.6 ± 2.7							
Lemon grass oil									
Control	91.8 ± 1.4	0± 0.0	113.99	90.05	144.28	3348.37	874.47	12821.01	0.969
50	58.8 ± 2.7	36.6 ± 1.9							
100	55.2 ± 2.9	44.6 ± 2.9							
150	40.4 ± 1.1	54.2 ± 3.0							
200	36.8 ± 2.5	59.8 ± 1.9							
250	30.8 ± 1.6	67.6 ± 2.5							
***Gaultheria* oil**									
Control	93.6± 1.1	0± 0.0	77.60	60.67	99.24	1132.09	538.55	2379.81	1.661
50	70.2± 1.7	28.8 ±1.3							
100	63.6± 3.0	30.8 ±1.3							
150	53.8 ± 2.3	38.4± 1.1							
200	41.2 ± 1.9	42.2 ±1.7							
250	32.4 ± 2.8	58.2 ± 1.9							
Citronella oil									
Control	89.4 ± 2.4	0± 0.0	72.18	59.75	87.21	494.57	339.54	720.38	4.509
50	57.6 ± 3.2	41.2 ± 1.7							
100	39.6± 1.1	58.2 ± 2.1							
150	24.2 ± 1.4	67.4 ± 2.3							
200	13.4± 2.5	81.2 ± 1.9							

250	**8.6 ± 2.9**	**90.2±2.8**							
Clove oil									
Control	**90.8 ± 2.2**	**0± 0.0**	**260.57**	**175.16**	**387.61**	**10883.51**	**1407.96**	**84129.24**	**1.547**
50	**69.6 ± 2.0**	**25.6 ± 3.5**							
100	**66.6 ± 3.9**	**31.2 ± 2.3**							
150	**62.6 ± 2.4**	**37.2 ± 2.4**							
200	**52.8 ± 2.6**	**45.2 ± 3.2**							
250	**35.6 ± 2.7**	**52.6 ± 2.9**							

Value represents mean ± S.D. of five replications. *Mortality of the pupae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

DISCUSSION

The genus *Gaultheria* (Ericaceae) is widely distributed around the Pacific Ocean, westwards to western slopes of the Himalayas and the southern areas of India (Mabberiey, D.J.1997). Most *Gaultheria* species growing in Southwest China are regarded as traditional herbal medicines. Parts of plants in this genus are used by nine minority nationalities for the treatment of wind-damp, as well as relieving pain. Additionally, *G. procumbens* is used as a folk remedy in America and Canada, and *G. fragrantissima* is employed in India (Simon., et al 1984).

Dayal reported betulinic and ursolic acids, eucalyptin and b-sitosterol in the leaves. Glabrous leaves may contain oil with 65.5% citronellal, 12.2% citronellol, and 3.6% isopulegol; hairy leaves contain more oil with 86.6–90.1% citronellal, 4.6–6.0% citronellol, and 0.7–0.8% isopulegol, 1-pinene, b-pinene, and isovaleric aldehyde are also recovered (Morton, 1981). Bark contains ca 9% tannin (Watt and Breyer-Brandwijk, 1962). The young leaf is reported to contain citric-, glutaric-, malic-, quinic-, shikimic- (carcinogenic), and succinic-acids (Watt and Breyer Brandwijk, 1962). Leaves and fruits test positive for flavonoids and sterols. Citronellal found in Eucalyptus and Melissa is reported to be mutagenic (Lewis and Elvin-Lewis, 1977).

As the concentration of the Plant oil formulation increases the good larval mortality of mosquito vectors. In the present study, the results of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* clearly indicated that the highest larval mortality was observed at 400 ppm concentration of 97.20, 99.00 and 99.24% respectively. Whereas the lowest mortality of 23.32, 26.33 and 22.46 respectively was noted at 25 ppm concentration. Similarly (Elango, G., et al 2009) reported that the ethyl acetate extract of *E. prostrata* showed LC_{50} value of 78.28 and LC_{90} value of 360.75ppm against *A. subpictus* and LC_{50} 119.89 and LC_{90} 564.85ppm against *Cx. tritaeniorhynchus. Eclipta paniculata* were the most active with a LC_{90} of 17.2 mg/L and LC_{50} of 3.3 mg/L against the larvae of *Ae. fluviatilis* (Macedo, M.E., et al 1997, Nivsarkar., et al 1996) have reported that the secondary plant metabolite alpha-terthienyl derived from the plant family Asteraceae is among the new class of light activated insecticide. Also, trials under tropical conditions indicate a very high level of activity as a Larvicidal to mosquito. In *N. nucifera* synthesized AgNPs against the larvae of *A. subpictus* (LC_{50} 0.69ppm; LC_{90} 2.15ppm) and against the larvae of *C. quinquefasciatus* (LC_{50} 1.10ppm; LC_{90} 3.59ppm), respectively (Santhoshkumar, T., *et al* 2010).

The results of present study are comparable with similar reports of earlier workers. Jeyasankar et al. (2012) who have been reported that ethyl acetate extracts of *phyllanthus emblica* showed highest larval mortality against Ae. Agypti and Cu. quinquefaciatus with LC_{50} and LC_{90} 80.04, 78.89 and 323.53, 502.10 ppm respectively and followed by hexane and diethyl ether extracts was found in leaf extracts against *Ae. agypti* and *Cx. quinquefaciatus* with LC_{50} and LC_{90} values of 111.34, 136.78, 114.77, 82.65 and 617.5, 939.01, 333.50,206.65 ppm respectively.

In the present investigation of the Plant Oil Formulation was tested for its larvicidal activity against 25 numbers of late third instar larvae of *Ae. aegypti*, *An. stephensi* and *Cu. quinquefasciatus*. The effect of different concentration of the Plant oil formulation 25, 50, 100, 200 and 400 ppm on the Larvicidal activity against *Ae. aegypti* LC_{50} 68.18 and LC_{90} 248.37. Larvicidal activity against *An. stephensi* LC_{50} 56.83 and LC_{90} 208.30. Larvicidal activity against *Cx. quinquefasciatus* LC_{50} 70.80 and LC_{90} 234.15(Pugazhvendan and K. Elumalai 2013). Ramar., *et al* (2014) who have been reported that present investigation was designed to determine the maximum larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of *Cleistanthus collinus* against the larvae of *Ae. aegypti*.

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