Indian Journal of Entomology 86(1): 147-152 (2024) DoI. No.: 10.55446/IJE.2023.607

LARVICIDAL POTENTIAL AND RETENTION EFFICACY PERIOD OF CITRONELLA LEAF OIL AGAINST *AEDES AEGYPTI* L.

Simranjeet Kaur[1], Devinder Kaur Kocher[1]*, Arshkamaljot Kaur[1] and Rajender Kumar[1]

[1]Department of Zoology, College of Basic Sciences and Humanities; [1]School of Organic Farming, College of Agriculture, Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: dkocher2002@pau.edu (corresponding author): ORCID ID 0000-0002-1897-8897

ABSTRACT

The mosquito vector *Aedes aegypti* L., plays an important role in transmission of diseases including dengue, chikungunya and zika fever. In this study, citronella leaf oil extracted by hydro-distillation technique has been evaluated for its efficacy against this vector. Citronella leaf oil (100, 125, 150, 175 and 200 ppm) when tested, 150 ppm was found to be the effective larvicidal concentration against 4th instar larvae. LC_{50} and LC_{90} toxicity values were observed to be 107.10 and 141.59 ppm, respectively. GC-MS analysis indicated the presence of β-citronellal, geraniol, elemol and β-citronellol as the major components, which might be causing the larval mortality. Retention efficacy of the oil was found to be up to 84 hr in terms of larval killing and during this retention period, a significant delay in development (L4 to adult) was also observed. No effect of storage was observed on its larvicidal efficacy even after six months.

Key words: *Aedes aegypti* L., *Cymbopogon*, development period, essential oils, larvicidal potential, mosquito control, retention efficacy, β-citronellal, geraniol, elemol and β-citronellol

Mosquitoes belonging to the genera *Aedes*, *Anopheles* and *Culex* are common carriers of various diseases such as chikungunya, dengue fever, filariasis, malaria, yellow fever and Japanese encephalitis. According to the National Vector Control Programme, India recorded 123,106 cases of dengue and 82,671 cases of chikungunya in 2021 (NVBDCP, 2021). These are important vectors transmitting parasites and diseases (WHO, 2019). *Aedes aegypti* is a freshwater mosquito that prefers to deposit its eggs in man-made enclosures and its larvae are frequently found in small temporary water collections including desert coolers, containers and earthen pots lying in the peridomestic areas (Kaur et al., 2016). Controlling the mosquito vector is the most effective way to reduce the associated life-threatening diseases, as there is no treatment or vaccine available (Phillips, 2008). Usually insecticides like carbamates, organochlorines, pyrethroids and organophosphates are used, which raise severe concerns on the environment, non-target species and human health (Sathiyanathan and Umarajan, 2019). There is an urgent need to develop environmentally safe and more effective alternatives. Plant-based products such as various extracts and extracted essential oils (EOs) serve this purpose (Piplani et al., 2019). The genus *Cymbopogon* has about 140 different fragrance grass species and oils extracted from citronella, lemon and eucalyptus have been approved as ecofriendly insecticides by the Environmental Protection Agency (EPA) due to their low toxicity and equivalent efficacy (Katz et al., 2008). Citronella oil repels mosquitoes and citronella leaf extracts function as gastrointestinal venom and respiratory poisons thus, prompting larvae to die. Keeping these in mind, the present study evaluated the larvicidal potential and retention efficacy of oil extracted from citronella leaves against *Ae. aegypti*.

MATERIALS AND METHODS

Water samples were taken from various small fresh water collections like desert coolers, roadside ditches, earthen pots and plastic containers etc lying in peri-domestic areas of urban regions of Ludhiana district of Punjab (India) from June to September, 2021. From these collected water samples, *Ae. aegypti* larvae were identified and separated using standard keys (Becker et al., 2010). Fresh and young citronella leaves were harvested from the herbal garden of Punjab Agricultural University from August to November 2020 and April to August 2021. The collected leaves were brought to laboratory, cleaned, shade dried for 2-3 days and 250 g of leaves were placed in a 5l capacity round bottom flask fitted in Clevenger apparatus for hydro-distillation oil extraction. The flask was half-filled with water and heated for roughly 4-5 hr on a heating mantle. The temperature was initially set at 100°C for 30 min until boiling occurred and then maintained at 60°C. Oil

vapours generated during the heating process were condensed with cold water steam and collected in the recuperating channel. The oil was carefully collected in a clean glass vial and kept in the refrigerator at 4°C. The oil content/yield (%) of extracted citronella leaf oil was calculated by the formula given below:

$$\text{Oil content (\%)} = \frac{\text{Volume of oil collected after distillation (ml)}}{\text{Weight of withered citronella leaves taken initially (g)}} \times 100$$

The composition of freshly extracted citronella leaf oil was determined using Gas Chromatography-Mass Spectrometry analysis from Central University of Punjab, Bathinda, using the GCMS-QP2010 Ultra programme, with helium as carrier gas flowing at a rate of 1ml/min, split ratio 10:0, injection temperature 250°C and oven temperature programming from 40 to 250°C. A capillary column Rtx-5MS was used in the GC (30 mtr) and 1 µl of oil diluted in 1 ml methanol was injected. For detecting the larvicidal potential of extracted citronella leaf oil, preliminary testing was carried out against 4th instar *Ae. aegypti* larvae by randomly selecting its higher and lower concentrations. Then on this basis, five different concentrations of citronella leaf oil @100, 125, 150, 175 and 200 ppm were prepared by mixing its required amount in 1 ml of DMSO (dimethyl sulphoxide- a non-polar and non-toxic emulsifying agent) so as to make total volume 250 ml by the addition of dechlorinated water in plastic beakers. Dechlorinated water was prepared by storing tap water it in opened buckets for 24 hr. Twenty 4th instar *Ae. aegypti* larvae were taken from water samples and treated with five concentrations of citronella oil. A control set (having 250 ml dechlorinated water) and a vehicle-control set (having 1 ml DMSO and 249 ml dechlorinated water) with twenty 4th instar larvae in each beaker were also run simultaneously. All of the experimental sets (treated, control and vehicle-control) were performed in triplicate in the plastic beakers with 250 ml capacity and these beakers were properly covered with muslin cloth. Dog biscuits and crushed yeast in 3:1 ratio (2 mg/ 100 g) were given to all larvae, and larval mortality was recorded after 3, 6, 9, 12, 24, 36 and 48 hr. Larvae not responding or moving after being agitated with a brush were considered dead and were counted in each set. The minimum concentration of the oil that resulted in highest larval mortality within lesser time duration was considered as the effective concentration and that concentration was used for further experimental purpose. After 24 hr of post-exposure, LC_{50} and LC_{90} values were calculated using the log concentration-mortality regression by log probit technique (Finney, 1971) with POLO software (Robertson et al., 1980).

Retention efficacy period was determined by exposing the freshly added twenty *Ae. aegypti* 4th instar larvae in the left over tested solution of the effective concentration after removing all the dead larvae from the beakers of dose-response bioassay (performed earlier). A dechlorinated water control set and a vehicle-control set, were also run in triplicate with their respective treatment sets. After 3, 6, 9, 12, 24, 36 and 48 hr, dead larvae (if any) were counted and replaced with the same number of fresh larvae so as to have total number of larvae as twenty (which were taken initially in the experimental set up). The survived larvae were subsequently monitored to evaluate the retention efficacy on the development duration from 4th larval instar to adult emergence. A control set along with a vehicle-control set was also run simultaneously in triplicate. When larvae got transformed into pupae, the muslin cloth was removed from the beakers and then these beakers were placed in rearing cages. Emerged mosquitoes were fed on sugary juice of deseeded water-soak raisins kept in sterilized petri plate (already kept inside the cages). A moist cotton swab was placed on top of each cage to provide water. In treatment, control and vehicle-control sets, the time taken for each transformation (i.e. from 4th larval instar to pupa and pupa to adult) was recorded, along with recording of adult emergence. To know the effect of storage on the larvicidal efficacy of EO, extracted oil was stored in clean vials wrapped with aluminum foil at 4°C for its usage after 2, 4 and 6 months. Already determined effective larvicidal concentration of oil against *Ae. aegypti* was tested again for freshly prepared and stored oil (2, 4 and 6 months old). Data obtained was statistically analyzed by comparing the larval mortality (during larvicidal bioassay) and developmental duration (during retention efficacy experiments) by one way ANOVA (Duncan multiple range test). The larvicidal potential data of stored citronella oil treated sets was statistically analyzed similarly.

RESULTS AND DISCUSSION

Citronella leaf oil extracted in Clevenger apparatus by hydro-distillation technique was transparent in colour having pleasant floral smell and found to be insoluble in water but soluble in organic solvents and showed an average yield of 1.05± 0.43% (v/w). Cassel

and Vargas (2006) have also reported approximately 1% oil content from fresh citronella leaves following hydro-distillation method of extraction. The GC-MS analysis revealed 57 peaks in the extracted oil, indicating the presence of 57 compounds and out of these the most prevalent were β-citronellal (27.48%) followed by Geraniol (24.10%) >elemol (13.42%) >β-citronellol (9.06%) >geranyl acetate (4.07%) >viridiflorol (3.82 %) >2,6-dimethyl 2,6 octadiene (2.67%) >limonene (1.98%) >N,N-dimethylacetamide (DMA) (1.69%) >2,4disopropyl methyl (1.59%) as given in Table 1. Exposure of 4th instar *Ae. aegypti* larvae to 100 ppm oil resulted in 20.00± 5.00% larval mortality after 3 hr, this increased with exposure time and after 48 hr to 56.66± 7.63%. No more mortality was observed after 48 hr and the larvae that remained got converted into the pupae stage. With increase in concentration to 125 and 150 ppm, similar mortality trend was found. The exposure of larvae to 175 ppm oil resulted in 100% mortality within 48 hr, but when larvae were exposed to 200 ppm, they died completely within 6 hr (Table 2). Citronella leaf oil @ 150 ppm was found to be statistically effective larvicidal concentration, since it resulted in maximum mortality (100%) of *Ae. aegypti* with the minimal dose of oil (150 ppm) and within lesser duration of treatment (36 hr).

Table 1. Major compounds reported in extracted fresh citronella leaf oil analyzed by GC-MS

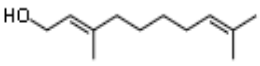
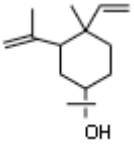
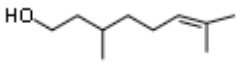
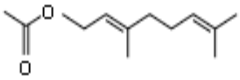
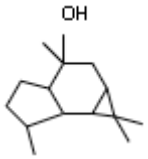
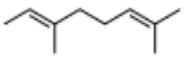
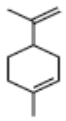
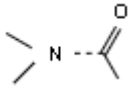
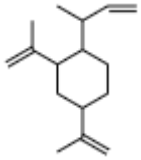
Sl. No.	Peak No	Retention time (min)	Area (%)	Molecular formula	Name of compound IUPAC name (Common name)	Structure
1	10	19.839	27.48	$C_{10}H_{18}O$	3,7-dimethyloct-6-enal (β-citronellal)	
2	17	23.708	24.10	$C_{10}H_{18}O$	3,7-dimethylocta-trans-2,6-dien-1-ol (Geraniol)	
3	37	33.729	13.42	$C_{15}H_{26}O$	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl) (Elemol)	
4	15	22.647	9.06	$C_{10}H_{20}O$	3,7-Dimethyloct-6-en-1-ol (β-citronellol)	
5	25	27.947	4.07	$C_{12}H_{20}O_2$	3,7-dimethyl-2,6-octadiene-1-ol acetate (Geranyl acetate)	
6	38	34.539	3.82	$C_{15}H_{26}O$	1,1,4,7-Tetramethyl decahydro-1H-cyclopropanE-azulen-4-ol (Viridiflorol)	
7	22	26.945	2.67	$C_{10}H_{18}$	2,6 dimethyl 2,6 octadiene	
8	3	14.628	1.98	$C_{10}H_{16}$	1-methyl-4-(1-methylethenyl) cyclohexene (Limonene)	
9	1	8.132	1.69	C_4H_9NO	N,N-dimethylacetamide (DMA)	
10	27	28.376	1.59	$C_{15}H_{24}$	2,4disopropyl methyl-1-vinyl cyclohexane (Elemene)	

Table 2. Effect of concentrations of citronella leaf oil on 4th instar larvae of *Aedes aegypti*

Concentration (ppm)	% mortality (Mean± S.D) (n=20)							Range of mortality (within hours)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
100	20.00±5.00^{b} (3-5)	25.00± 5.00^{b} (4-6)	28.33±7.63^{b} (4-7)	30.00±5.00^{b} (5-7)	35.00±5.00^{b} (6-8)	45.00± 5.00^{b} (8-10)	56.66± 7.63^{b} (10-13)	3-48
125	36.66±2.88^{c} (6-8)	56.66± 7.63^{c} (10-13)	65.00±5.00^{c} (12-14)	73.33±5.77^{c} (14-16)	81.66±7.63^{c} (15-18)	85.00± 5.00^{c} (16-18)	90.00± 8.66^{c} (17-20)	3-48
150	65.00±5.00^{d} (12-14)	73.33± 7.63^{d} (13-16)	81.66±5.77^{d} (15-17)	88.33±5.77^{d} (17-19)	91.66±2.88^{d} (18-19)	96.66± 2.88^{d} (19-20)	100.00±0.00^{d} (20)	3-48
175	80.00±5.00^{e} (15-17)	90.00± 5.00^{e} (17-19)	93.33±2.88^{e} (18-19)	95.00±5.00^{d} (18-20)	98.33±2.88^{e} (19-20)	100.00±0.00^{d} (20)	–	3-36
200	91.66±5.77^{f} (17-19)	100.00±0.00^{f} (20)	–	–	–	–	–	3-6
0 (Control)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0
0 (Vehicle-control)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0.00± 0.00^{a} (0)	0

Toxicity values

Toxicity Value (ppm)	Fiducial limits		χ^2
	Lower limit (ppm)	Upper limit (ppm)	
LC_{50}=107.10	100.50	112.41	1.95
LC_{90}=141.59	134.44	152.09	1.95

n represents number 4th instar larvae taken; Figures in parentheses range in no. of dead larvae from the start of experiment till that period; Figures followed with different superscripts indicate significant difference ($p<0.05$, DMRT)

GC-MS analysis of the prepared citronella leaf oil showed the presence of β-citronellal (27.48%) in maximum amount along with other major components like geraniol, elemol and β-citronellol (Table 1). These compounds could be responsible for the larval mortality of *Ae. aegypti* (Katiyar et al., 2011). LC_{50} and LC_{90} toxicity values for 4th instar larvae were based on the record of deaths up to 24 hr after being exposed. Manimaram et al., (2012) observed LC_{50} for larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* as 47.61, 91.23 and 47.21 ppm, respectively; with oil of *C. citratus*, good LC_{90} at 69 ppm on third and fourth *Ae. aegypti* larvae was observed (Cavalcanti et al., 2004).

When *Ae. aegypti* 4th instar larvae were exposed to the leftover effective concentration @ 150 ppm, 100% mortality was observed up to 6 hr; and thereafter showed a declining trend and no larval mortality was observed after 48 hr. Larval mortality was statistically significant after 9, 12, 24 and 36 hr revealing that prepared oil showed the retention efficacy up to a period of 36 hr. However no larval mortality was observed in the control and vehicle-control sets (Table 3). Thus, the total retention efficacy was 84 hr (48 hr spent during larvicidal bioassay + 36 hr spent during the retention efficacy experiment). Knowing the retention efficacy period is critical for vector control since it reveals the minimal interval between treatments required to sustain the pesticide's persistence against the targeted vector (Roseli et al., 2007). In field studies, neemarin, a commercial neem extract product, was shown to substantially lower the frequency of *An. stephensi* and *Cx. quinquefasciatus* larvae at recommended doses of 1 and 2 l/ ha with a 7-day estimated residual impact. Amer and Mehlhorn (2006) assessed the durability of the larvicidal action of 13 essential oils and observed 100% mortality in three among these (*C. camphora, Thymus serpyllum* and *C. limon*) and up to three weeks, these oils were completely effective. Exposure of *Ae. aegypti* 4th instar larvae to leftover effective concentration (150 ppm) resulted in a substantial delaying of development of L4 to pupa and a non-significant delay in pupae to adult development, taking 4.66± 0.28 and 1.83± 0.28 days, respectively. However, it took significantly longer duration in the treated set i.e. 6.49± 0.56 days (Table 3). Citronella leaf oil, on the other hand, had no effect on adult emergence. Sutiningsih et al. (2017) also showed that biolarvicide derived from *Bruceine* spp. leaves slow down the development of *Ae. aegypti* larvae. EO derived from *Pseudocalymma alliaceum* had larvicidal action, while aqueous, ethanol and methanol extracts were found to hinder normal growth and development of *Cx.*

Table 3. Larvicidal retention efficacy of effective concentration (150 ppm) against *Aedes aegypti*

Experimental sets	% mortality (Means S.D) (n=20)							Retention activity time (hours)
	3hr	6 hr	9 hr	12 hr	24 hr	36 hr	48 hr	
Treated citronella leaf oil (150 ppm)	100.00± 0.00^{b}	100.00± 0.00^{b}	98.33± 2.88^{b}	88.33± 5.77^{b}	73.33± 5.77^{b}	58.33± 7.63^{b}	0.00± 0.00^{a}	36
Control	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0
Vehicle-control	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0

Figures followed with different subscripts indicate significant difference ($p<0.05$, DMRT) on development and emergence

Experimental set	Duration of developmental period in days (Mean± S.D)			Emergence (%)	
	L4-Pupa	Pupa-Adult	L4-Adult	L4-Pupa	Pupa-Adult
Treated (150 ppm)	4.66± 0.28^{b} (4.5-5)	1.83± 0.28^{b} (1.5-2)	6.49± 0.56^{b} (6-7)	100.00± 0.00^{a}	100.00± 0.00^{a}
Control	3.33± 0.57^{a} (3-4)	1.00± 0.28^{a} (1-1.5)	4.33± 0.85^{a} (4-5.5)	100.00± 0.00^{a}	100.00± 0.00^{a}
Vehicle-Control	3.66± 0.28^{a} (3.5-4)	1.33± 0.28^{a} (1-1.5)	4.99± 0.56^{a} (4.5-5.5)	100.00± 0.00^{a}	100.00± 0.00^{a}

Values are Mean± S.D; Figures in parentheses represents range in days; Figures in subscripts indicate significant difference ($p<0.05$, DMRT)

Table 4. Effect of storage on larvicidal potential of citronella leaf oil (150 ppm) against *Aedes aegypti*

Storage of oil (months)	% mortality upto (Mean± S.D) (n=20)							Range of mortality (within hours)
	3 hr	6 hr	9 hr	12 hr	24 hr	36 hr	48 hr	
0 (fresh)	61.66± 7.63^{a} (11-14)	68.33± 7.63^{a} (12-15)	73.33± 5.77^{a} (14-17)	86.66± 2.88 (17-18)	93.33± 2.88^{a} (18-19)	98.33± 2.88^{a} (19-20)	100.00± 0.00^{a} (20)	3-48
2	60.00± 5.00^{a} (11-13)	68.33± 5.77^{a} (13-15)	71.66± 2.88^{a} (14-15)	85.00± 5.00^{a} (16-18)	90.00± 5.00^{a} (17-19)	95.00± 5.00^{a} (19-20)	100.00± 0.00^{a} (20)	3-48
4	63.33± 7.63^{a} (11-14)	66.66± 7.63^{a} (12-15)	70.00± 5.00^{a} (13-15)	83.33± 7.63^{a} (15-18)	88.00± 7.63^{a} (16-19)	93.33± 5.77^{a} (18-20)	100.00± 0.00^{a} (20)	3-48
6	65.00± 5.00^{a} (13-14)	66.66± 7.63^{a} (12-15)	70.00± 5.00^{a} (13-15)	78.33± 7.63^{a} (14-17)	90.00± 5.00^{a} (17-19)	91.66± 7.63^{a} (17-20)	100.00± 0.00^{a} (20)	3-48

n represents number of 4th instar larvae taken; Figures in subscripts indicate significant difference ($p<0.05$, DMRT)

quinquefasciatus larvae thus, extending and delaying larval and pupal duration (Granados-Echegoyen et al., 2014). Plant based EOs have growth regulating effects on a number of insects, slowing or stopping their growth at various phases of development (Regnault-Roger 2013). When 4th instar *Ae. aegypti* larvae were exposed to the effective concentration (150 ppm) of citronella leaf oil stored for two, four and six months, it resulted in 100% larval mortality up to 48 hr, which was statistically non-significant with respect to that of freshly extracted oil (Table 4). Thus, storage has no effect on the larvicidal efficiency if the oil is stored properly. The oils of *E. globulus* and *A. vera* when preserved in dark bottles, resulted in no significant variations in their larvicidal efficacy after storage (Misharina et al., 2003). Dua et al., (2009) also investigated the effect of storing neem oil at room temperature for 18 months and observed no significant variations in LC_{50} and LC_{90} values before and after storage. Santos et al., (2014) tested fresh and stored *Croton rhamnifolioides* essential oil against *Ae. aegypti* and found that the larvicidal efficacy remained unaltered even after three years.

ACKNOWLEDGEMENTS

The authors thank the Head, Department of Zoology, for providing facilities and Department of Science and Technology, Government of India, New Delhi, for providing infrastructural facilities under FIST grant.

AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Amer A, Mehlhorn H. 2006. Larvicidal effects of various essential oils against *Aedes*, *Anopheles* and *Culex* larvae (Dipetra, Culicidae). Parasitology Research 99: 466-472.

Becker N, Petric D, Zgomba M, Boase C, Dahl C, Madon M, Kaiser A. 2010. Mosquitoes and their Control (2nd ed). Springer Publication. pp. 9-40.

Cassel E, Vargas R M. 2006. Experiments and modeling of the *Cymbopogon winterianus* essential oil extraction by steam distillation. Journal of the Mexican Chemical Society 50: 126-129.

Cavalcanti E S B, Morais S M D, Lima M A A, Santana E W P. 2004. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. Memórias do Instituto Oswaldo Cruz 99: 541-544.

Dua V K, Pandey A C, Raghavendra K, Gupta A, Sharma T, Dash A P. 2009. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. Malaria Journal 8: 1-6.

Finney D J (1971) Probit analysis, III edn. Cambridge: Cambridge University Press. pp. 68-72.

Granados-Echegoyen C, Perez-Pacheco R, Soto-Hernandez M, Ruiz-Vega J, Lagunez-Rivera L, Alonoso-Hernandez, Gato-Armas R. 2014. Inhibition of the growth and development of mosquito larvae of Culex quinquefasciatus (Diptera: Culicidae) treated with extract from leaves of Pseudocalymma alliaceum (Bignonaceae). Asian Pacific Journal of Tropical Medicine 7(8): 594-601.

Katiyar R, Gupta S, Yadav K R. 2011. *Cymbopogon winterianus*: An Important Species for Essential Java Citronella Oil and Medicinal Value. National Conference on Forest Biodiversity: Earth's Living Treasure, Kanpur. pp. 115-118.

Katz T M, Miller J H, Hebert A A. 2008. Insect repellents: historical perspectives and new developments. Journal of the American Academy of Dermatology 58: 865-871.

Kaur K, Jamwal S, Kocher D K. 2016. Monitoring of mosquito larvae from temporary water collections of Ludhiana, Punjab. Indian Journal of Ecology 43(1): 357-358.

Manimaran A, Cruz M M J J, Muthu C, Vincent S, Ignacimuthu S. 2012. Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston). Scientific Research 3: 855-862.

Misharina T A, Polshkov A N, Ruchkina E L, Medvedeva I B. 2003. Changes in the composition of the essential oil of marjoram during storage. Applied Biochemistry and Microbiology 39: 311-316.

NVBDCP. 2021. Dengue situation in India. *https://nvbdcp.gov.in/index4.php? lang= 1&level=0&linkid=431&lid=3715*

Phillips M. 2008. Dengue reborn: Widespread resurgence of a resilient vector. Environmental Health Perspectives 116: 382-388.

Piplani M, Bhagwat D P, Singhvi G, Sankaranarayanan M, Balana-Fouce R, Vats T, Chander S. 2019. Plant-based larvicidal agents: an overview from 2000 to 2018. Experimental Parasitology 199: 92-103.

Regnault-Roger C. 2013. Essential oils in insect control. In: Ramawat K G, Mérillon J M, editors. Natural products. New York. pp. 4088-102.

Robertson J L, Russel R M, Savin N. 1980. POLO: a user's guide to probit or logit analysis. Pacific South-West forest and range experiment station, Berkeley, USA.

Roseli L C S, Arnaldo S F, Antonio E F A, Durval B R V, Marinete M P. 2007. Evaluation of the residual effect of pyrethroids on *Anopheles* in the Brazilian Amazon. Revista Saúde Pública 41(2): 276-283.

Santos G K N, Dutra K A, Lira C S, Lima B N, Napoleão T H, Paiva P M G, Maranhão C A, Brandão S S F, Navarro D M A F. 2014. Effects of *Croton rhamnifolioides* Essential oil on *Aedes aegypti* oviposition, larval toxicity and trypsin activity. Molecules 19: 16573-16587.

Sathiyanathan M, Umarajan K M. 2019. Larvicidal activity of endophytic fungi isolated from selected medicinal plants on *Aedes aegypti*. Journal of Pharmacognosy and Phytochemistry 8(2): 247-253.

Sutiningsih D, Mustofa M, Satoto T B, Martono E. 2017 Inhibitory effects of bruceine A biolarvicide on growth and development of *Aedes aegypti* larvae. Journal of Entomology 14: 104-111.

WHO. 2019. Dengue and severe dengue. https://www.who.int/news-room/fact-sheets/ detail/dengue-and-severe-dengue

(Manuscript Received: September, 2022; Revised: December, 2022;
Accepted: December, 2022; Online Published: January, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e22607