Indian Journal of Entomology Online published Ref. No. e20366 DoI.: 10.5958/0974-8172.2021.00171.1

EFFECT OF NANOEMULSIFIED *EUCALYPTUS GLOBULUS* OIL ON DEVELOPMENT, EMERGENCE AND SURVIVAL OF *AEDES AEGYPTI* L.

Navkiran Kaur and D K Kocher*

Department of Zoology, Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: dkocher2002@pau.edu (corresponding author)

ABSTRACT

***Aedes aegypti* L., is a serious global threat to human health as a vector for spreading several deadly diseases. Plant extracts provide an efficient and ecofriendly alternate to manage this menace. The sublethal concentrations i.e. 50 and 60 ppm of nanoemulsified eucalyptus oil evaluated against 4th instar (L4) *Ae. aegypti* larvae resulted in 16.67± 1.67 and 8.33± 1.67% larval survival followed by a significant delay in duration of various developmental stages and decrease in % pupal formation and their survival. Thus, decline in % emergence along with significant reduced body size and longevity were observed in comparison to control and vehicle-control emerged adults. These results reveal the potential of such nanoemulsified oils to be used as phytochemical agents for the sustainable control of *Ae. aegypti*.**

Key words: *Aedes aegypti*, eucalyptus oil, nanoemulsified oil, plant extracts, larvae, emergence, development, duration, pupae, aberrations, survival, mosquito control

Transmission of dengue virus by *Aedes aegypti* L. mosquito is one of the major global issue (Tare et al., 2004) and every year 50-100 million dengue infections occur worldwide (WHO, 2019). Dengue is endemic in all states and union territories of India and according to the recent report of National Vector Borne Disease Control Programme, 1,36,422 dengue cases have been reported during 2019 (NVBDCP, 2019). *Ae. aegypti* has the ability to easily adapt and multiply in new areas resulting in the wide spreading of associated diseases like dengue, chikungunya, yellow fever and Zika. This mosquito bites and feeds during daylight hours and its population is abundant in small temporary fresh water collections lying in peri-domestic areas like desert coolers, earthen pots, road side ditches etc. (Kaur et al., 2016). To control mosquitoes, targeting the larvae is a better approach, as these are confined to a limited breeding habitat and thus easy to handle (Pearis and Cranshaw, 2010). Current approaches of spraying synthetic insecticides to control adult *Ae. aegypti* in urban and rural areas pose risk to human and environmental health and often results in mosquito resistance, making these insecticides ineffective and unsustainable (Amer and Mehlhorn, 2006). Hazards of toxic pesticides makes it an urgent requirement to search for ecofriendly and more effective measures. For this, plant-based extracts and essential oils, are the best alternatives, due to their amazing insecticidal potential (Pavela, 2008; Ghosh et al., 2012). As botanicals are non-toxic, economic, biodegradable and show broad-spectrum target-specific activities against different species of vector mosquitoes, these can act as potential alternative source in controlling mosquito larvae and hence the associated diseases.

Eucalyptus is one among the most significant plants belonging to family Myrtaceae with significant larvicidal and repellent actions against mosquitoes (Traboulsi et al., 2005; Kocher and Riat, 2017; Kaur et al., 2019). At present, interest has been devoted to the development of nano-sized herbal emulsions for enhancing the homogenous dispersion and efficiency of essential oils, so as to ensure biosafety (Duarte et al., 2015). In view of the potential of eucalyptus oil, the present study evaluated the effect of nanoemulsified eucalyptus oil on the development and adult emergence, along with mortality and any kind of morphological aberrations of larvae/ pupae during development and various parameters of emerged male and female *Ae. aegypti.*

MATERIALS AND METHODS

Water samples were collected during July to November 2017 from different peri-domestic water collections like desert coolers, earthen pots and roadside ditches in urban zones of Ludhiana district, Punjab (India) using plastic dippers. From the collected water samples, *Ae. aegypti* larvae were recognized and separated out from other types of mosquito larvae (if present) on the basis of their morphological features following the standard keys (Becker et al., 2010; Andrew and Bar, 2013). Stable nanoemulsified oil was formulated using *Eucalyptus globulus* oil and non-ionic surfactant (Tween-20) by mixing in the ratio 1:2. In a previous study, 70 ppm of the prepared stable nanoemulsified *E. globulus* was reported as lethal concentration against 4th instar larvae of *Ae. aegypti* (Kaur et al*.,* 2019). Therefore, two sublethal concentrations ie 50 and 60 ppm of this stable nanoemulsified oil were selected. Twenty, *Ae*. *aegypti* larvae at 4th instar were exposed to 50 and 60 ppm of the prepared nanoemulsified *E. globulus* oil along with a vehicle-control set (having Tween-20 in de-chlorinated water in same ratio as treatment set) and a control set (having 250 ml de-chlorinated water only). All the sets were run in triplicate and larvae were adequately fed with mixture of dog biscuits and yeast properly ground in the ratio of 3:1 (2 mg/100 ml) (Mavundza et al., 2013). The experimental beakers were kept in B O D. incubator at 26± 2 °C. When 4th instar larvae (L4) moulted into pupae, the mouth of beakers were covered with muslin cloth along with tightly bound rubber to avoid the escape of adult mosquitoes. After emergence, the beakers (containing emerged adults) were placed in mosquito rearing cages and fed with 10% sugar solution kept on cotton swabs.

Observations recorded in treated, vehicle-control and control sets were; % larval survival, % pupal formation (out of the survived larvae) and % emergence of adults (male and female mosquitoes emerged out of the survived pupae), time taken during different developmental stages (from 4th instar larvae to pupa formation and pupa to adult formation), adult body size of emerged male and female mosquitoes (total body length along with measurement of different body parts like; proboscis, wings and abdomen) and longevity of adult male and female mosquitoes in terms of days. Data were statistically analyzed with SPSS statistical software version 16 by comparing treated sets with control and vehicle-control sets using ANOVA (Duncan multiple range test, p=0.05).

RESULTS AND DISCUSSION

Effect of sublethal concentrations: The nanoemulsified *E. globulus* oil when evaluated with 20 nos. of 4th instar *Ae. aegypti* larvae revealed that in control and vehicle-control sets, 100% survival was observed along with 100% emergence of pupae and adults. However, exposure of larvae to sublethal concentrations significantly reduced % larval survival to 16.67± 1.67 and 8.33± 1.67% after treatment with 50 and 60 ppm, respectively. As all the survived larvae got transformed into pupae, % pupal recovery was same as the survived larvae in all of the experimental sets (Table 1). However, these treatments further resulted in non-significant pupal mortality, along with certain morphological deformities (Fig. 1). Adult emergence also got significantly reduced, as only 13.33± 1.67 and 5.00± 2.89% adults emerged, respectively in 50 and 60 ppm treated sets (due to larval and pupal mortality during development). Secondary metabolites of many plant species show effect on growth and development in various life stages of mosquitoes like inhibiting moulting, morphological abnormalities and mortality specially during molting process (Shaalan et al., 2005). Plant extracts also show a juvenile hormone analogue action, producing some morphogenetic abnormalities in treated larvae like larval-pupal intermediates, decolorized and extended pupae and incomplete emerged adults (Sujatha et al., 1998). Thus, it might be possible that properties of phytochemicals from eucalyptus extracts have produced morphological abnormalities that resulted in failure/ less number of pupal survival and moulting to adults. Abnormalities at pupal stage of Ae. albopictus are known- for eg., Zuharah et al. (2016) after exposure to LC_{50} dose of Ipomoea cairica extract. Similarly, Thakur and Kocher (2018) also observed the influence of mixed crustaceans on mortality of developmental stages of *Ae. aegypti* and significant decline in % survival of pupae and emergence of the adults.

Considering the effect of treatment on development period, treatment of *Ae. aegypti* larvae with 50 and 60 ppm of nanoemulsfied *E. globules* oil resulted in delaying of their overall development. In control and vehicle-control sets total time taken from L4 larval stage till the emergence of adult was found to be 4.67± 0.44 and 5.17± 0.17 days, respectively; however, it got significantly delayed to 6.63± 0.33 and 6.83± 0.44 days, after exposure to 50 and 60 ppm, respectively (Table 1). In comparison to control and vehicle-control sets nanoemulsified eucalyptus oil treatment showed significant hampering effect specifically during the development of L4 stage to pupa, however a non-significant effect was observed in the time taken from pupae to emergence of adults (Table 1). Sutiningsih et al. (2017) also observed delay in *Ae. aegypti* larval development after their exposure to a biolarvicide extracted from leaves of *Bruceine* spp. Inhibition effect on development of *Ae. aegypti* larvae by treatment with seaweed extracts is known earlier (Yu et al*.,* 2015). The

Table 1. Effect of sublethal concentrations of nanoemulsified *E globulus* oil on survival, emergence and duration of developmental stages of *A.aegypti*

Oil conc. (ppm)	Parameters (%)						Developmental period (days)		
	Larval survival after 48 hr of exposure (n=20)	Pupal formation (out of survived larvae)	Pupal mortality (out of formed pupae)	Total adult emergence (n=20)	Males (out of emerged adults)	Females (out of emerged adults)	L4-Pupa	Pupa-adult	Total (L4-Adult)
0 (Control)	100± 0.00^{c}	100± 0.00^{c}	0.00^{a}	100± 0.00^{c}	51.67±4.41^{a}	48.33± 4.41^{a}	3.33± 1.67^{a}	0.17^{a}	4.67± 0.44^{a}
0 (Vehicle-Control)	100± 0.00^{c}	100± 0.00^{c}	0.00^{a}	100± 0.00^{c}	51.34± 4.42^{a}	48.66± 4.39^{a}	3.17± 1.67^{a}	0.16^{a}	5.17± 0.17^{a}
50	16.67± 1.67^{b}	16.67± 1.67^{b}	19.33± 9.95^{a}	13.33± 1.67^{b}	50.00± 9.82^{a}	50.00± 9.82^{a}	4.50± 0.29^{b}	0.17^{a}	6.63± 0.33^{b}
60	8.33± 1.67^{a}	8.33± 1.67^{a}	21.03± 2.03^{a}	5.00± 2.89^{a}	47.67± 8.37^{a}	52.33± 9.33^{a}	4.83± 0.17^{b}	0.29^{a}	6.83± 0.44^{b}

'n' represents total number of L4 larvae taken initially; Values Mean± SD; Figures followed DMRT)

(a) (b)

Fig. 1. Morphological changes in pupa moulted from larvae exposed with sublethal concentration (50 ppm) of nanoemulsified eucalyptus oil (control on left side (a) and treated on right side (b)) (4x)

mechanism by which eucalyptus oil suppresses larval growth and development is likely due to its inhibitory effect on feeding, and acting like an antifeedant. Such antifeedant compounds do not directly kill, expel or entrap insects, but inhibit feeding activity and decrease the growth rate of larvae (Dono et al., 2002).

When effect on body size and longevity of emerged *Ae. aegypti* was studied it was observed that treatment with sublethal concentrations of nanoemulsified eucalyptus oil resulted in significant reduction of the total body length. Males of control and vehicle-control sets were found to have 2.53± 0.03 and 2.51± 0.02 mm body length, which got significantly reduced to 2.33± 0.01 and 2.21± 0.01 mm, respectively after treatment with 50 and 60 ppm of nanoemulsified eucalyptus oil. Similarly, in emerged females the exposure of 50 and 60 ppm resulted in significant reduction of their body length. Even reduction in other body parts like size of proboscis, wing length and abdomen size was observed in both the sexes (Table 2; Fig. 2). The longevity of emerged *Ae. Aegypti* when observed, under laboratory conditions, male and female mosquitoes emerged from the control and vehicle-control sets, were having similar lifespan. But, those emerged from the treated sets (50 and 60 ppm of nanoemulsified eucalyptus oil) showed a significantly shorter lifespan; female survived for more days than males in all experimental sets. Body size is a parameter related to the survival of mosquitoes directly or indirectly. Of the various body parts, size of wing is of great significance as reduction in wing length is a morphological abnormality caused by plant phytochemicals in *Ae. aegypti* (Rohani et al., 2001). Wing length is also known to be directly related to body size, as smaller the wing length, smaller the body size; and the small sized *Ae. aegypti* with shorter wings are unable to fly long distance migration and exhibit reduced blood feeding success, thus most likely mosquito tends to die before reaching hosts due

Table 2. Effect of sublethal concentrations of nanoemulsified *E. globulus* on body measurements and longevity of adult *Ae. aegypti*

Oil conc. (ppm)	Body measurements of emerged mosquitoes (mm) Proboscis	Wing	Abdomen	Total body length	Adult longevity (Number of days adult survived)
Male					
0 (Control)	0.87± 0.17^{c}	1.89± 0.01^{b}	1.66± 0.12^{c}	2.53± 0.03^{c}	14.33± 0.33^{b}
0 (Vehicle-Control)	0.85± 0.01^{c}	1.89± 0.01^{b}	1.65± 0.01^{c}	2.51± 0.02^{c}	13.00± 0.58^{b}
50	0.77± 0.01^{b}	1.53± 0.02^{a}	1.55± 0.15^{a}	2.33± 0.01^{b}	11.00± 0.58^{a}
60	0.61± 0.01^{a}	1.47± 0.02^{a}	1.59± 0.01^{b}	2.21± 0.01^{a}	10.67± 0.66 ^{a}
Female					
0 (Control)	1.21± 0.01^{b}	2.26± 0.02^{c}	2.23± 0.01^{c}	3.45± 0.01^{c}	23.67± 0.88^{c}
0 (Vehicle-control)	1.24± 0.01^{b}	2.26± 0.01^{c}	2.21± 0.18^{c}	3.44± 0.02^{c}	23.00± 0.58^{bc}
50	0.86± 0.01^{a}	1.82± 0.02^{b}	1.86± 0.01^{b}	2.71± 0.02^{b}	21.00± 0.57^{b}
60	0.83± 0.01^{a}	1.75± 0.02^{a}	1.75± 0.01^{a}	2.59± 0.01^{a}	17.33± 0.67^{a}

Values Mean± S.D; Figures followed with different superscripts indicate significant difference with respect to control and vehicle-control sets ($p<0.05$, DMRT).

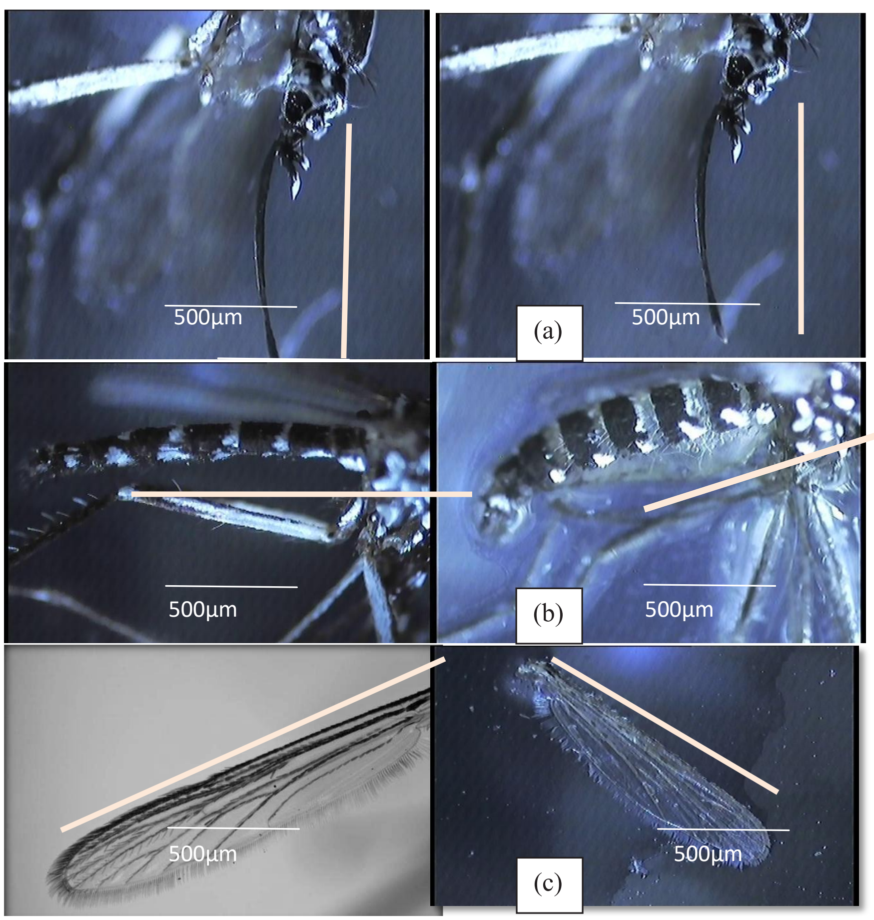

Fig. 2. Changes in length of various body parts of adult *Ae. aegypti* female due to treatment with sublethal concentration (50 ppm) of nanoemulsified eucalyptus oil (control on left side and treated on right side) (4x) (a) Proboscis; (b) Abdomen; (c) Wing

to the lack of substantial energy (Dieng et al*.,* 2006). Thus, indicating that the body size/ wing length of *Ae. aegypti* positively correlates with feeding and flight capabilities, survival and in turn their pathogenicity (Zuharah et al*.,* 2016). So, treatment with sublethal concentrations of nanoemulsified eucalyptus oil in the present study may have resulted in reduction of total body length, in turn decreased longevity and survival of the adult mosquitoes. Such nanoemulsified plant-based products on one side have shown effective larvicidal potential (Kaur et al., 2019), while on the other hand they can indirectly play an important role in delaying of the overall development, decrease in % pupal formation and their survival and overall reduction in % adult emergence along with reducing adult body size and life span of emerged mosquitoes. Thus, any alteration in these parameters which are responsible for the survival and spreading *of Ae. aegypti* can be manipulated to be used for *Ae. aegypti* related control programmes in future.

ACKNOWLEDGEMENTS

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

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.

Andrew J, Bar A. 2013. Morphology and morphometry of *Aedes aegypti* adult mosquito. Annual Research and Review in Biology 3(1): 52-69.

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.

Dieng H, Boots M, Eshita Y. 2006. Some insights into the concept of body size in mosquitoes. House Household Insect Pests 28: 47-62.

Dono D, Ismayana S, Idar P D, Muslikha I. 2002. Status and biochemical resistance of *Crocidolomia pavonana* (F.) (Lepidoptera: Crambidae) to organophosphate insecticide and its sensitivity to botanical insecticide. Journal of Entomology Indonesia 7: 9-27.

Duarte J L, Jesus R R A, Anna E M F M O, Rodrigo A S C, Adriana M F, Raimundo N P S, Deborah Q F, Jose C T C, Caio P F. 2015. Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. Revista Brasileira de Farmacognosia 25: 189-192.

Ghosh A, Chowdhury N, Chandra G. 2012. Plant extracts as potential mosquito larvicides. Indian Journal of Medical Research 135: 581-598.

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.

Kaur N, Kocher D K, Sidhu A. 2019. Synthesis and testing of *Eucalyptus globulosa* oil based nanoemulsion for its larvicidal potential against *Aedes aegypti.* African Entomology 27(2): 433-438.

Kocher D K, Riat A K. 2017. Larvicidal potential of *Eucalyptus globulus* oil against *Aedes* and *Culex* mosquitoes. Journal of Insect Science 30(1): 5-9.

Mavundza E J, Maharaj R, Chukwujekwujekwu J C, Finnie J F, Staden J V. 2013. Larvicidal activity against *Anopheles arabiensis* of 10 South African plants that are traditionally used as mosquito repellents. South African Journal of Botany 88: 86-89.

NVBDCP. 2019. National vector borne disease control program. http:// nvbdcp.gov.in/ dengu1.html, http://nvbdcp. /gov.in

Pavela R. 2008. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitology Research 102: 555-559.

Pearis F B, Cranshaw W S. 2010. Mosquito management.www.ext. colostate.edu

Rohani A, Wan-loy C, Han-lim L, Siew-moi P. 2001. Effect of four chlorophytes on larval survival, development and adult body size of the mosquito *Aedes aegypti*. Journal of Applied Phycology 13: 369-374.

Shaalan E A S, Canyon D, Younes M W F, Abdel-wahab H, Mansour A H. 2005. A review of botanical phytochemicals with mosquitocidal potential. Environment International 8: 1149-1166.

Sujatha C H, Vasuki V, Mariappan T, Kalyanasundaran M, Das P K. 1988. Evaluation of plant extracts for biological activity against mosquitoes. Integrated Pest Control 30: 398-401.

Sutiningsih D M, Satoto T B T, Martono E. 2017. Inhibitory effects of bruceine a biolarvicide on growth and development of *Aedes aegypti* larvae. Journal of Entomology 14(2): 104-111.

Tare V, Deshpande S, Sharma R N. 2004. Susceptibility of two different strains of *Aedes aegypti* (Diptera: Culicidae) to plant oils. Journal of Economic Entomology 97: 1734-1736.

Thakur A, Kocher D K. 2018. Influence of antagonistic crustaceans on population of *Aedes aegypti* larvae under simulated conditions. Haya: Saudi Journal of Life Sciences 3(6): 465-468.

Traboulsi A F, El-haj S, Tueni M, Taoubi K, Nader N A, Mrad A. 2005. Repellency and toxicity of aromatic plant extracts against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). Pest Managment Science 61: 597-604.

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

Yu K X, Wong C L, Ahmad R, Jantan I. 2015. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in *Aedes aegypti* (Diptera: Culicidae). Asian Pacific Journal of Tropical Medicine 8(12): 1006-1012.

Zuharah W F, Ahbirami R, Dieng H, Thiagaletchumi M, Fadzly N. 2016. Evaluation of sublethal effects of *Ipomoea cairica* Linn. extract on life history traits of dengue vectors. Revista do Instituto de Medicina Tropical de Sao Paulo 58: 44.

(Manuscript Received: September, 2020; Revised: January, 2021;
Accepted: January, 2021; Online Published: August, 2021)
Online published (Preview) in www.entosocindia.org Ref. No. e20366