



## Varun Tyagi<sup>1\*</sup>, Ruchi Yadav<sup>2</sup> and Vijay Veer<sup>1</sup>

<sup>1</sup>Medical Entomology Division, Defence Research Laboratory, Tezpur- 784001, Assam, India

<sup>2</sup>Pharmacology & Toxicology Division, Defence Research & Development Establishment, Gwalior-474002, Madhya Pradesh, India

<sup>3</sup>Defence Research Laboratory, Tezpur- 784001, Assam, India

**Dates:** Received: 12 December, 2016; Accepted: 29 December, 2016; Published: 30 December, 2016

\*Corresponding author: Dr. Varun Tyagi, Research Associate, Medical Entomology Division, Defence Research Laboratory, Tezpur- 784001, Assam, India, Tel: 7086834166; E-mail: varundr1@rediffmail.com

**Keywords:** Essential oils; *Aedes albopictus*: Larvicidal activity

<https://www.peertechz.com>

## Research Article

# Laboratory Evaluation of Certain Essential Oils for Their Larvicidal Activity against *Aedes Albopictus*, Vector of Dengue and Chikungunya

## Abstract

Mosquito borne diseases including malaria, filariasis, dengue, chikungunya and various forms of encephalitis impose enormous menace to human as well as animals. These diseases cause large number of mortality and morbidity across the world. The principal strategy for combating these diseases is the vector control including the use of larvicide against the immature stages of mosquitoes. Use of essential oils as mosquito repellent has been recommended by various studies but their role as larvicide is studied by only a few workers. In the present study, we have evaluated certain essential oils namely; Amyris, Black pepper, Cinnamon, Dill, Jasmine, Juniper and Thyme oils, against *Aedes albopictus* for their larvicidal activity. These essential oils have shown significant larvicidal activity. These oils were shown to have LC<sub>50</sub> values in the range of 10.5 to 62.7 ppm against larvae *Ae. albopictus*. Out of these seven oils the Dill oil was found to show the least LC<sub>50</sub> value against *Ae. albopictus*.

## Introduction

Mosquito-borne diseases cause significant human health problems, and their incidence has increased significantly within last two decades. These diseases profoundly restrict socioeconomic status and development in countries with highest rates of infection, many of which are located in the tropics and subtropics. Vector-borne infectious diseases, such as malaria, filaria, dengue fever, chikunguniya yellow fever, and various forms of viral encephalitis, share a major fraction of the global infectious disease burden. Indeed, nearly half of the world's population is infected with one or other type of vector-borne pathogen [1,2]. Estimates from the world health organization indicate that three mosquito borne diseases malaria, filarial and dengue are among the leading cause of morbidity and mortality in developing countries around the world. Dengue fever virus, particularly its hemorrhagic form, is a threat to more than 2.5 billion people, with an annual incidence in the tens of millions and about 24,000 deaths per year.

Numerous approaches have been developed to control the mosquitoes, in which the mosquito control at larval stage is considered as an efficient approach in the integrated vector management. Existing mosquito control methods are based on synthetic insecticides. Synthetic insecticides are the first line of

action due to their quick action, but continuous use of synthetic insecticides may be lead to the development of resistance in vectors and adverse effect on environment. These factors have created a need for search of simply biodegradable alternative insecticides. The use of plant extracts for vector control has several appealing features as they are biodegradable, less hazardous and rich stock house of chemicals of diverse biological activity. Earlier works of several authors revealed that botanicals can have strong larvicidal [3-9] oviposition deterrent and ovicidal activity [10-14]. In the present study essential oils of seven plants were tested against third instar larvae of *Ae. albopictus*. The study of larvicidal activity is useful for identification of effective essential oils for controlling mosquito borne diseases.

## Materials and Methods

### Mosquitoes

*Ae. albopictus* mosquitoes colony was maintained in our laboratory at 27±2°C temperature, 12:12 light dark photoperiod and 70±5 % relative humidity. Larvae of *Ae. albopictus* were maintained in plastic tray by providing yeast powder as larval food. Adult mosquitoes were reared in wooden cages (30 x 30 x 30 inches) and were provided cotton soaked with 10% sugar solution. *Ae. albopictus* females were offered blood, once in a week.

## Essential oils

Seven essential oils were obtained from Fragrance and Flavour Development Center (FFDC), Kannuj, Uttar Pradesh (Table 1).

## Larval bioassay

Larvae of *Ae. albopictus* were collected from stock culture. Larval bioassay was carried out as per WHO [15,6] procedure in four replications for each concentration. Twenty, early third instar larvae of *Ae. albopictus* were inoculated in glass beakers (250 ml) containing 100 ml tap water. Different doses of essential oils ranging from 10, 20, 50 and 100 ppm in acetone were prepared. Larvae of mosquito were treated with 1 ml of test solution for each concentration and controls were treated with acetone. Larval mortality was recorded after 24 hrs, of exposure. To determine the lethal concentration (LC<sub>50</sub>) for each essential oil, data were analyzed by probit analysis [16] using POLO PC software.

## Results and Discussion

In the present study, different concentrations of the essential oils viz. 10, 20, 50 and 100 ppm on the larvicidal activity against *Ae. albopictus* were shown in (Table 1, Figure 1) and the data revealed that the highest larval mortality with LC<sub>50</sub> value was 10.52 ppm observed in Dill oil, whereas the lowest mortality with LC<sub>50</sub> value was 62.78 ppm in Black Pepper oil. LC<sub>50</sub> value of other essential oils against *Ae. albopictus* was also

observed, the LC<sub>50</sub> value of Amyris oil, Thyme oil, Jasmine oil, Juniper oil and Cinnamon oil was 39.8, 45.58, 49.99, 53.22 and 60.31 ppm respectively (Table 2, Figure 1). Out of these seven oils the Dill oil was found to show the least LC<sub>50</sub> against *Ae. albopictus*.

In the present study results showed that the mortality of the mosquito larvae increased as the doses of the sample were increased. Another study has showed that, the plant oil formulation was used for larvicidal activity against different mosquito species [17]. A recent study prove that *Vernonia cinerea* and *C. viminalis* leaf extracts have the potential to be used as larvicide and *P. juliflora* as an oviposition-deterrent for the control of *Ae. albopictus* mosquito [5]. In another study results indicate that leaf extracts of three invasive weeds- *Vernonia cinerea*, *Prosopis juliflora* and, *Cassia tora* can be an ecofriendly larvicide for *An. stephensi* [3]. Besides this repellent activity of essential oils against different mosquito species have evaluated by many researchers [18–21]. Plant extract might have complex blend of biocidal active compounds, including flavonoids, phenolics, terpenoids and alkaloids which may jointly or separately contribute to mortality and late growth of mosquito larvae. Other study reported that acetone extracts have maximum amount of phenols and flavonols, while extracts of methanol have flavones, terpenoids, tannins and polyphenols [22], which contains larvicidal activity of mosquito. Mode of action and site of effect for larvicidal phytochemicals has received slight attention. Ray et al., [23] and David et al., [24] found that botanical derivatives primarily affect the

**Table 1:** List of essential oils obtained from different plant sources used for the larvicidal activity against *Ae. albopictus* (Source of oils : Fragrance and Flavour Development Center, Kannuj ,U.P, India).

S.No.	Name of Essential Oils	Botanical Name	Common Name	Origin of Plant	Habitat	Color of Oil	Odor of Oil	Extraction	Part used	Uses
1	Amyris	<i>Amyris balsamifera</i> Linn.	West Indian Sandalwood	Haitai, Jamaica	North and South America	Viscous pale yellow liquid	Sandalwood odor	Steam distillation	Wood	Antiseptic, Antiaging, Antistress, balsamic, sedative. It also acts as muscle relaxant, soothing agent
2	Black pepper	<i>Piper nigrum</i> Linn	Black pepper (kali mirch)	India	India	Pale yellow liquid	Slightly sweet.	Steam distillation	Seed	Antiseptic, Anticholerin, Antiasthmatic, Fever, Cough.
3	Cinnamon	<i>Cinnamomum zeylanicum</i> Linn	Cinnamon (Dalchini)	Sri Lanka	Western Ghats of India	Yellow and brown	woody, spicy odor	Steam distillation	Bark	Antioxidant, Antiseptic, Constipation, Gastric Irritation.
4	Dill	<i>Anethum graveolens</i> Linn	Dill (Sowa)	Hungary	Cultivated in all over India	Pale yellow to yellow	Spicy caraway odor	Steam distillation	Seed	Antiseptic, Stomachic, Low blood pressure,
5	Jasmine	<i>Jasminum grandiflorum</i> Linn	Jasmine (Chameli)	India, South Asia	North-Western Himalayas of Persia and India	Reddish Brown	Exotic and warm odor	Hydro distillation	Flower	Dry skin, Coughs, Disorders of the chest
6	Juniper	<i>Juniperus communis</i> Linn.	Juniperd (Aaraar)	India	Western Himalayas	pale yellow	Pine-needle like odor	Steam distillation	Fruit	Antiseptic, Obesity, Urinary, Antiseptic, Digestive
7	Thyme	<i>Thymus serpyllum</i>	Thyme (Banajwain)	Europe and North Africa	Cultivated in temperate Himalayas of Asia	Yellow color	Pungent odor	Steam distillation	Leaves	Antiseptic, Bronchitis, Coughs and Common cold, Diarrhea.

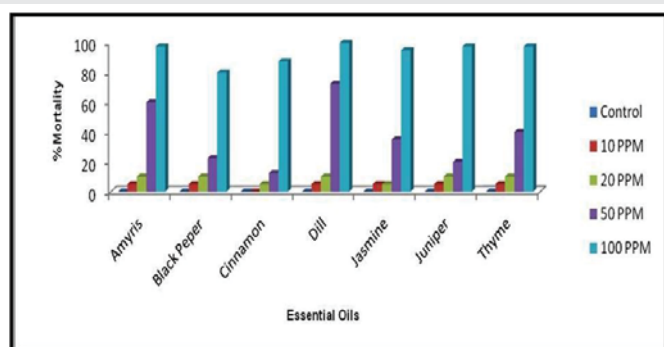


Figure 1: Larvicidal activity of essential oils against *Ae. albopictus*

Table 2: Larval mortality and  $LC_{50}$  value of different essential oils against *Ae. Albopictus*.

S. No.	Essential Oil	Conc (ppm)	<i>Aedes albopictus</i>	
			% Mortality	$LC_{50}$
1	Amyris	10	5	39.8
		20	10	
		50	60	
		100	97.5	
2	Black pepper	10	5	62.78
		20	10	
		50	22.5	
		100	80	
3	Cinnamon	10	0	60.31
		20	5	
		50	12.5	
		100	87.5	
4	Dill	10	5	10.52
		20	10	
		50	72.5	
		100	100	
5	Jasmine	10	5	49.99
		20	5	
		50	35	
		100	95	
6	Juniper	10	5	53.22
		20	10	
		50	20	
		100	97.5	
7	Thyme	10	5	45.58
		20	10	
		50	40	
		100	97.5	

midgut epithelium and secondarily affect the malpighian tubules in mosquito larvae. The efficiency of phytochemicals against mosquito larvae can differ significantly depending on species of plant, plant parts used, age of plant parts, solvent used during extraction as well as upon the available vector species of mosquito. Sukumar et al., [25] have described the existence of variations in the level of effectiveness of phytochemical compounds on the mosquito species in respect of plant parts from which these were extracted, responses in

species and their developmental stages against the particular extract, solvent of extraction, geographical origin of the plant species, photosensitivity of some of the compounds in the plant extract, effect on growth and reproduction. Further, it was observed that many larvae were failed to ecdyze to perfect pupae producing larval-pupal intermediate [26]. The results of this study are very promising in creating new efficient and reasonable approaches to the control of vector mosquitoes.

## Acknowledgement

The authors are thankful Director, DRDE, Gwalior, Madhya Pradesh, India for interest and providing all necessary facility to conduct this research work. Help rendered by staff Vector Management Division while for carrying out the work is deeply acknowledged.

## References

1. CIESIN (Center for International Earth Science Information Network) (2007) Changes in the incidence of vector-borne diseases attributable to climate change.
2. WHO (2004) Global strategic framework for integrated vector management, Geneva. WHO/CDS/CPE/PVC- 1-15. [Link: https://goo.gl/CnJMTc](https://goo.gl/CnJMTc)
3. Tyagi V, Yadav R, Sharma AK, Tyagi V, Yadav S, et al. (2013) Larvicidal activity of leaf extract of some weeds against malaria vector *Anopheles stephensi*. *International Journal of Malaria Research and Reviews* 1: 35-39. [Link: https://goo.gl/WEWQ5S](https://goo.gl/WEWQ5S)
4. Tyagi V, Yadav R, Sukumaran D, Vijay Veer (2015) Larvicidal activity of invasive weed *Prosopis juliflora* against mosquito species *Anopheles subpictus*, *Culex quinquefasciatus* and *Aedes aegypti*. *International Journal of Applied Research* 1: 285-288. [Link: https://goo.gl/XV5FC2](https://goo.gl/XV5FC2)
5. Yadav R, Tyagi V, Tikar SN, Sharma AK, Mendki MJ, et al. (2014) Differential Larval Toxicity and Oviposition Altering Activity of Some Indigenous Plant Extracts against Dengue and Chikungunya Vector *Aedes albopictus*. *J Arthropod-Borne Dis* 8: 174-185. [Link: https://goo.gl/w3xcic](https://goo.gl/w3xcic)
6. Yadav R, Tikar SN, Sharma AK, Tyagi V, Sukumaran D, et al. (2015) Screening of some weeds for larvicidal activity against *Aedes albopictus*, a vector of dengue and chikungunya. *J Vector Borne Dis* 52: 85-94. [Link: https://goo.gl/koL3Ay](https://goo.gl/koL3Ay)
7. Ansari MA, Vasudevan P, Tandon M, Razdan RK (2000) Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. *Bioresource Technology* 71: 267-271. [Link: https://goo.gl/Tx0Zmi](https://goo.gl/Tx0Zmi)
8. Vasudevan P, Madan, Sharma S (1989) Larvicidal Property of castor. *Pesticides II*: 36-39.
9. Anyanwu GIEC, Amaefule, Nguukwem C (2001) larvicidal effects of lemon peel on mosquito larvae. *J Aquatic Sciences* 16: 111-114. [Link: https://goo.gl/DQuKci](https://goo.gl/DQuKci)
10. Millar JG, Chaney JD, Mulla S (1992) Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. *J Am Mosq Cont Assoc* 11-17. [Link: https://goo.gl/zB2q24](https://goo.gl/zB2q24)
11. Su T, Mulla S (1998) Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus*. *J Am Mosq Cont Assoc* 14: 204-209. [Link: https://goo.gl/C01eIA](https://goo.gl/C01eIA)
12. Su T, Mulla S (1999) Oviposition bioassay responses of *Culex tarsalis* and *Culex quinquefasciatus*. *Entomologia Exp Appl* 91: 337-345. [Link: https://goo.gl/i6PaGr](https://goo.gl/i6PaGr)
13. Ritchie SA (2001) Effects of some animal feeds and oviposition substrates

- on *Aedes* oviposition in ovitraps in Cairns, Australia. *J Am Mosq Control Assoc* 17: 206-208. [Link: https://goo.gl/i76TjI](https://goo.gl/i76TjI)
14. Poonam S, Paily KP, Balaraman K (2002) Oviposition attractance of bacterial culture filtrates – response of *Culex quinquefasciatus*. *Memorias do Instituto Oswaldo Cruz* 97: 359-362. [Link: https://goo.gl/xRSkpc](https://goo.gl/xRSkpc)
15. WHO (1981) Instructions for determining susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC- 81: 807. [Link: https://goo.gl/xHDxTr](https://goo.gl/xHDxTr)
16. Finney DJ (1971) *Probit Analysis*, Cambridge University Press, Cambridge, UK, 1971, III edn.1–333.
17. Elangovan A, Veeraiyan G, Elumalai K, Prakash M (2008) Larvicidal activity of plant oil formulation against three important vector mosquito species. *The Internet Journal of Veterinary Medicine* 6: 1-4. [Link: https://goo.gl/XiPTGi](https://goo.gl/XiPTGi)
18. Tyagi V, Islam J, Agnihotri A, Goswami D, Rabha B, et al. (2016) Repellent efficacy of some essential oils against *Aedes albopictus*. *Journal of Parasitic Diseases: Diagnosis and Therapy* 1: 1-5. [Link: https://goo.gl/PnPBjS](https://goo.gl/PnPBjS)
19. Amer A, Melhorn H (2006) Repellency effect of forty one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol Res*. 99: 478–490. [Link: https://goo.gl/UFQVPU](https://goo.gl/UFQVPU)
20. Barnard D (1999) Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J Med Entomol* 36: 625–629. [Link: https://goo.gl/5G8Qya](https://goo.gl/5G8Qya)
21. Uniyal A, Tikar SN, Singh R, Shukla SV, Agarwal OP, et al. (2015) Synergistic effect of effective oils against *Aedes aegypti* female mosquito, vector of dengue and chikungunya. *Int J Mosquito Res* 2: 29-35. [Link: https://goo.gl/NZZ7LW](https://goo.gl/NZZ7LW)
22. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011) Phytochemical screening and Extraction: A Review. *Int Pharm Sci* 1: 90-106. [Link: https://goo.gl/Gqsq1e](https://goo.gl/Gqsq1e)
23. Rey D, Cuany A, Pautou MP, Meyran JC (1999) Differential sensitivity of mosquito taxa to vegetable tannins. *J Chem Eco* 25: 537-548. [Link: https://goo.gl/0WJa06](https://goo.gl/0WJa06)
24. David JP, Rey D, Pauntou MP, Meyran JC (2000) Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. *J Invert Path* 75: 9-18. [Link: https://goo.gl/pClqz0](https://goo.gl/pClqz0)
25. Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 7: 210-37. [Link: https://goo.gl/YdBSM4](https://goo.gl/YdBSM4)
26. Mwangi RW, Mukiama TK (1988) Evaluation of *Melia volkensii* extract fractions as mosquito larvicides. *J Am Mos Contr Assoc* 4: 442-447. [Link: https://goo.gl/WSJ99K](https://goo.gl/WSJ99K)