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Bioefficacy of *Cinnamomum tamala* essential oil against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae

Parul, SP Singh and Lalit Mohan

Abstract
Mosquitoes are responsible for causing millions of deaths annually by transmitting dengue, malaria, lymphatic filariasis, chikungunya, etc. Plant-derived insecticides may serve as a suitable alternative for managing these nuisance-creating vectors. The objective of the present study was to find out the bioefficacy of essential oil from *Cinnamomum tamala* leaves against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. The essential oil was extracted from the fresh leaves by the hydrodistillation method and different oil concentrations were applied against the 3rd instar larvae of the mosquito species. The bioassay results showed different responses between the species. The essential oil showed higher efficacy as a larvicidal agent against *Culex quinquefasciatus* (LC_{50} = 52.9 and LC_{90} = 147.5 ppm after 24 hrs and LC_{50} = 30.5 and LC_{90} = 81.7 ppm after 48 hrs. of exposure), *Aedes aegypti* (LC_{50} = 65.1 and LC_{90}= 239.3 ppm after 24hrs, LC_{50} = 34.2 and LC_{90} = 111.6 ppm after 48 hrs. of exposure) and *Anopheles stephensi* (LC_{50} = 85.6 and LC_{90}= 235.7 ppm after 24 hrs. and LC_{50}= 53.6 and LC_{90} = 145.6 ppm 48 hrs. of exposure) respectively. Therefore, it can be inferred that the essential oil derived from *Cinnamomum tamala* leaves exhibits larvicidal potentiality which could further be used in mosquito larval management.

Keywords: *Anopheles*, *Aedes*, *Culex*, *C. tamala*, essential oil, larvicide, mosquito vectors

Introduction
The prevalence of mosquito-borne diseases is one of the world's most important health problems. Mosquitoes are responsible for transmitting various infectious diseases causing millions of deaths every year. Mosquito species, *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* are among the important vectors of a wide range of atrocious diseases like dengue, malaria, lymphatic filariasis, chikungunya, etc. The World Health Organization blames global warming for the expanding range of mosquitoes that are amenable to vector-borne diseases causing millions of people to be at risk. It is estimated by WHO nearly 15,000 deaths per year at all ages occur only in the Indian Peninsula [1]. *Anopheles stephensi* is the primary vector of malaria in India and other West Asian countries; Malaria is one of the most prevalent diseases in the tropical world. It causes 2.7 million deaths worldwide, with 200 to 450 million infections annually. The vector-borne diseases remain endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health [2]. Since the last few years, dengue fever has become the major public health concern in tropical and subtropical regions of the world. The freshwater mosquito, *Aedes* disseminates dengue largely during the wet season in India. The incidence of dengue infections estimated by the World Health Organization is about 390 million cases annually of which 96 million are supposed to be manifested clinically. As per WHO reports, approximately 3900 million individuals, inhabiting over 128 endemic countries, are likely to beat risk of dengue. In India, official records of the Union Health Ministry reveal a massive increase in dengue infections every year [3]. Filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries and lymphatic filariasis is found to be more endemic in the Indian subcontinent. Six *Culex* species are involved in the transmission of Japanese encephalitis in India [4]. It is reported that the *Culex quinquefasciatus* infects more than 100 million individuals worldwide annually [5] and the development of synthetic insecticide resistance in vector population led to be thinking in vector control

strategies [6]. The recent WHO report confirmed that malaria vectors developed resistance to the four commonly used synthetic insecticides: pyrethroids, organochlorines, carbamates, and organophosphates worldwide [7]. Since mosquitoes develop resistance to chemical insecticides, researchers have been diverted their attention to search out the alternative control strategies [8]. One of the ideal methods for controlling mosquito infestation is by preventing mosquito breeding sites to bring interruption in disease transmission and the control of mosquitoes at the larval immature stage, as this stage is aquatic and much easier to control the mosquito population as compared with the adult. The use of plant-based insecticides has been provided with effective and eco-friendly tools against mosquitoes as they have been reported to be non-toxic to mammals, biodegradable, target specific. The essential oils extracted from various parts of plants have been found good properties of larvicidal, ovicidal, insect growth hormone regulators, and deterrent agents against mosquitoes. The larvicidal properties of indigenous plants have also been documented in many parts of India along with the repellent and adulticidal activities [9]. Traditionally, plant essential oils and their derivatives were used to kill mosquitoes and other household and agricultural pests [10, 11]. Nowadays, we believe that organic, plant-based insecticides that rely on plant's natural defences against mosquitoes may not only be cost-effective and inexpensive for protecting health but also safer and more eco-friendly. *Cinnamomum tamala* (Tezpatta) belonging to *Lauraceae* family is a tree native to India, Bangladesh, Nepal, Bhutan, and China. It has aromatic leaves called tezpatta which are used for culinary, cosmetic product and medicinal purposes. In the rural area, *C. tamala* leaves have been used to protect stored grain pests and insect infestation since time immemorial. Researchers have evaluated for repellent, insecticidal, feeding inhibitory, oviposition inhibitory and acetyl cholinesterase enzyme inhibitory activities in rice weevil, *Sitophilus oryzae* [12]. Due to its aroma, the leaves are kept in clothes and chewed to disguise bad mouth odor. In Punjab, the leaves are used in rheumatism and as a stimulant in colic and diarrhoea. The leaves yield 1.2% of essential oil with light pale-yellow colour and clove-like peppery odor and resemble the oil of Ceylon cinnamon leaves [13, 14]. The present investigation was carried out to explore the relative larvicidal efficacy of *C. tamala* (leaves) essential oil against mosquito vectors, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

Materials and Methods

Collection of plant material and extraction of essential oil

The leaves of *C. tamala* (Figure1) were collected from the Bishnupur (Nadia) West Bengal horticulture area. It is also known as an Indian bay leaf, tezpatta tree and belongs to the family Lauraceae. The plant is native to India, Bangladesh, Nepal, Bhutan, and China. *C. tamala* is an evergreen tree that can reach up to 20m (66ft) the leaves are 4-5 inches long and variable in breadth, rarely alternate, shining above, rarely elliptical and obtuse. The tiny, greenish-yellow, insignificant flowers are arranged in drooping axillary panicles. Indian bey leaf is a spice used almost exclusively in the kitchens of Northern India. The bark is also sometimes used for cooking, although it is regarded as inferior to true cinnamon or cassia. Its leaves have a clove-like aromatic with a hint of peppery taste; they are used for culinary and medicinal purposes and could be used as an adjunct therapy in diabetes [15].

Fig 1: Leaves of *C. Tamala* (bay leaf) collected from the Bishnupur (Nadia) West Bengal.

Extraction of plant essential oil

The collected leaves were washed in running tap water to remove extra impurities like dust and other particles and were cut into small pieces and subjected to steam distillation for 5-6 hours in the Clevenger type apparatus (Figure2). Steam distillation is the most popular method used to extract and isolate essential oils from plants for use in natural products. This happens when the steam vaporizes the plant material's volatile compounds, which eventually go through a condensation and collection process. The steam distillation processed in a large glass container, having the plant material with water. Through an inlet, steam is injected through the plant material containing the desired oils, releasing the plant's aromatic molecules and turning them into vapor. The

vaporized plant compounds travel to the condensation flask or the condenser. Here, two separate pipes make it possible for hot water to exit and for cold water to enter the condenser. This makes the vapor cool back into liquid form. The aromatic liquid by-product drops from the condenser collects inside a nozzle underneath it, which is called a separator. Because water and oil do not mix, the essential oil floats on top of the water. From here, it is siphoned off and collected the essential oil in a glass vial. After manual collection of the essential oil, traces of remaining moisture in the oil was removed over anhydrous sodium sulphate. The isolated oil was stored under refrigeration for further use and the yield of oil (w/v) was calculated from the weight of fresh leaves used.

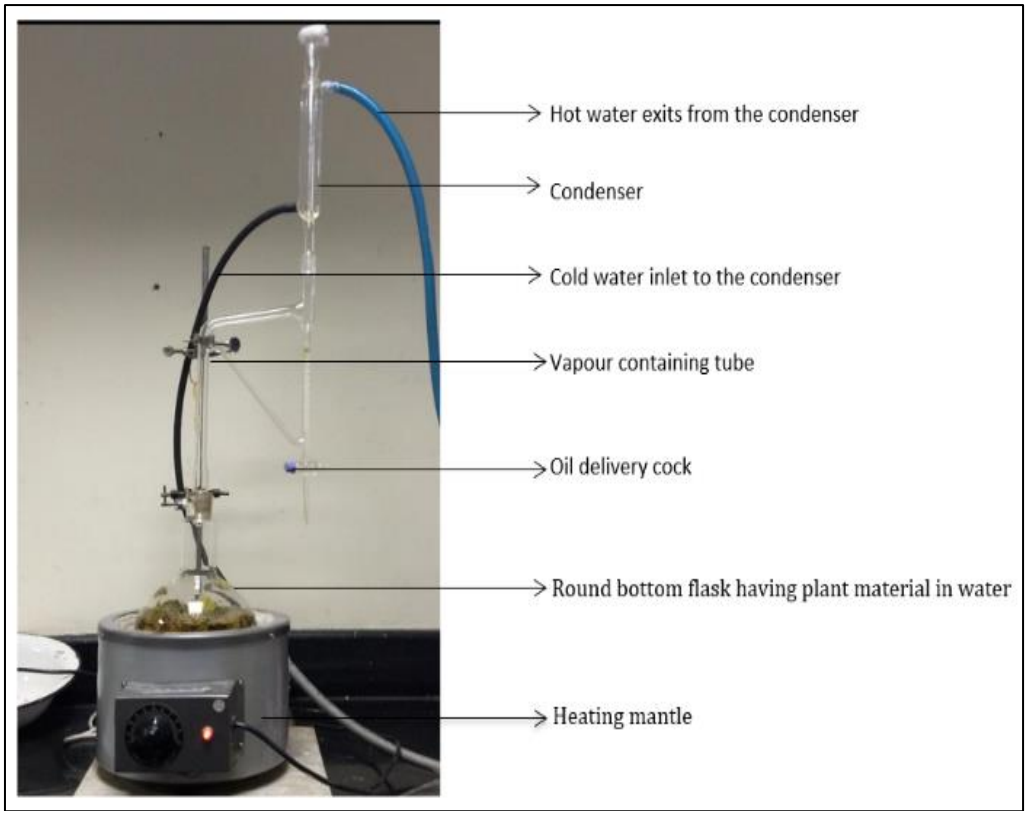

Fig 2: Clevenger apparatus used for essential oil extraction from *C. tamala*.

Maintenance of mosquito colony

The larvicidal efficacy was performed against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. Larvae were collected from the insectary of the National Institute of Malaria Research, New Delhi (NIMR), and transported to the laboratory. The mosquito speciesselected were reared independently in the laboratory, maintained continuously at 27±2 ^{0}C and 75±5% relative humidity (RH) under a photoperiod of 14:10 hours (light/ dark). The eggs were immersed in de-chlorinated tap water in enamel basins of 30 cm diameter. The hatched larvae were fed with fine biscuit powder and brewer's yeast. The transformed pupae were separated manually with a glass dropper into a 500 ml beaker with water and introduced into adult cages of 12 ×12 × 12 in for adult emergence. For egg maturation, periodic blood meals were provided to female mosquitoes by keeping restrained albino rats in the cages. The moist filter paper was kept in a beaker in the cages for mosquitoes to lay eggs on it. Eggs laid on the filter paper were immersed in larval basins containing water for the maintenance of the colony [16].

Preparation of the stocks and test concentrations

The stock solution of *C. tamala* essential oil was prepared by dissolving of essential oil in ethyl alcohol and the procedure was followed for all experiments. The different six desired test concentrations were prepared by adding 1 ml of stock solution to 199 ml of water in 250 ml capacity of glass beakers. Twenty, 3rdinstar larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to the prepared independent series of test concentrations in each case. All experiments were set in triplicate and the control experiments were run parallel with each replicate.

Bioassay

Essential oil of *C. tamala* was diluted in ethyl alcohol (V/V) to obtain the stock solutions of desired strength. Different test concentrations were prepared from these stock solutions for the exposure of target mosquito larvae. A set of twenty, 3rd instar larvae of each species was exposed to each test concentration after acclimatization to lab conditions. The experiments were conducted separately in 250 mL glass beakers containing 1 mL of the test concentration and 199 mL water in triplicates. Twenty, 3rd instar larvae of *An. stephensi*, *A. aegypti* and *Cx. quinquefasciatus* were exposed independently for each test concentration along with control. Controls were conducted for each series of test concentrations by adding 1 ml of ethyl alcohol in 199 ml of water in triplicates. All experiments were performed according to the standard WHO protocol (World Health Organisation 2005) at 27$\pm$2 °C and 75$\pm$5% relative humidity (RH).Yeast powder was provided for larval feeding at 0 and 24 h and mortality was observed at 24 and 48 h after exposure. The moribund

and dead larvae in all three replicates were combined and expressed as a percent larval mortality for each concentration. Dead larvae were defined as those that failed to move after probing with a needle, while moribund larvae were defined as those incapable of rising to the surface within a reasonable period or not demonstrating characteristic diving reactions when the water was disturbed. Replicates with 20% mortality in the controls were discarded and repeated. Mortality values ranging 5-20% in the controls were corrected using Abbott's formula [17], as follows:

Corrected mortality % = [(T-C) /(100 – C)] ×100

Where T is the percent mortality in the test concentrations and C is the percent mortality in the control.

Data analysis

The data collected after 24 and 48 hrs. of the exposure period were used to calculate corrected percent mortality and proceeded further for relevant statistical analysis. The LC_{50} and LC_{90}were calculated by Probit analysis [18] and other statistical parameters were calculated by using the software developed by [19].

Results

The results of relative efficacy of *C. tamala* leaves essential oil against mosquito vectors *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae are depicting in Table 1 and Figure 3a & 3b. The LC_{50} values of *C. tamala* essential oil against *An. stephensi* were 85.6 ppm (103.4 and 67.72 ppm as upper and lower fiducial limits) and 53.6 ppm (65.82 and 40.42 ppm as upper and lower fiducial limits) after 24 and 48 h. of exposure, respectively. The LC_{90} values were 235.7 ppm (306.4 and 163.9 ppm as upper and lower fiducial limits) and 145.6 ppm (184.3 and 106.8 ppm as upper and lower fiducial limits) after 24 and 48 h. of exposure, accordingly. The LC_{50} values for the *Ae. aegypti* were 65.1 ppm (82.8 and 47.43 ppm as upper and lower fiducial limits) and 34.2ppm (45.32 and 23.63 ppm as upper and lower fiducial limits) after 24 and 48 h. of exposure respectively, and the LC_{90} values were 239.3 ppm (346.9 and 131.5 ppm as upper and lower fiducial limits) and 111.6 ppm (147.8 and 74.8 ppm as upper and lower fiducial limits) after 24 and 48 h. of exposure, respectively. In case of *Cx. quinquefasciatus*, the LC_{50} values were 52.9 ppm (64.98 and 39.58ppm as upper and lower fiducial limits) and 30.5 ppm (38.92 and 21.48 ppm as upper and lower fiducial limits) after 24 and 48 h. of exposure, respectively, and LC_{90} values were 147.5 ppm (193.8 and 101.2 ppm as upper and lower fiducial limits) and 81.7 ppm (105.7 and 56.6 ppm as upper and lower fiducial limits) after 24 and 48 h of exposure, accordingly.

Table 1: Larvicidal efficacy of *C. tamala* (leaves) essential oil against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

Larval species	Exposure period	Regression equation	Chi-square (X^2)	LC_{50} ± SE (fiducial limits) (ppm)	Relative toxicity	LC_{90}±SE (fiducial limits) (ppm)	Relative toxicity
An.stephensi	24	Y=2.923x-3.561	6.562	85.6±9.15 103.4-67.72	1	235.7±36.36 306.4-163.9	0.984
	48	Y=2.933x-2.999	8.284	53.6 ± 6.42 65.82 – 40.42	1	145.6 ± 19.72 184.3 – 106.8	1
Ae. aegypti	24	Y=2.268x-1.379	6.007	65.1 ± 8.97 82.8- 47.43	0.760	239.3±54.67 346.9 – 131.5	1
	48	Y=2.522x-1.417	3.313	34.2±5.55 45.32 – 23.63	0.638	111.6 ± 18.63 147.8 – 74.8	0.766
Cx. quinquefasciatus	24	Y=2.843x-2.721	3.675	52.9 ± 6.41 64.98 – 39.58	0.617	147.5 ± 23.23 193.8 – 101.2	0.616
	48	Y=2.988x-2.402	2.893	30.5 ± 4.49 38.92 – 21.48	0.569	81.7 ± 12.49 105.7 – 56.6	0.561

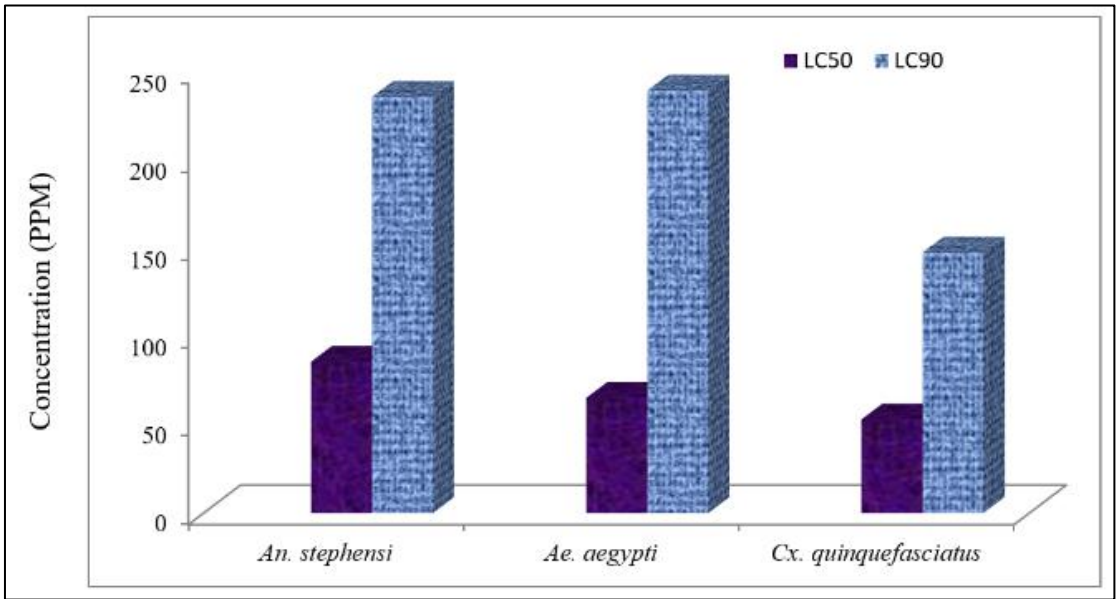

Fig 3a: Larvicidal efficacy of essential oil of *C. tamala* (leaves) against *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*after24 hrs. of exposure.

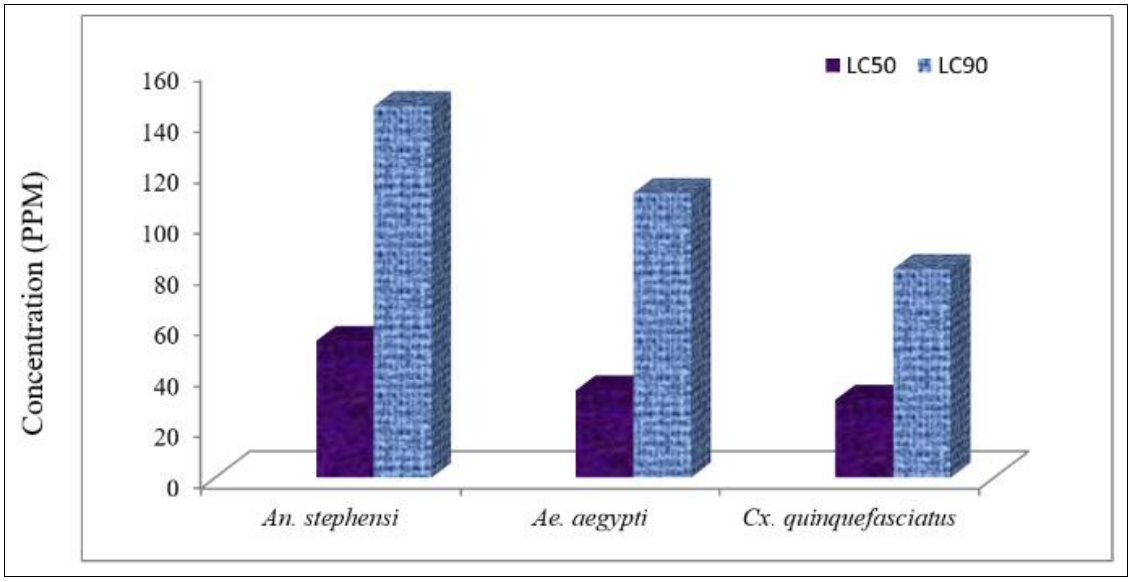

Fig 3b: Larvicidal efficacy of essential oil of *C. tamala* (leaves) against *An. stephensi, Ae. aegypti, Cx. quinquefasciatus* after 48 hrs. of exposure.

Discussion

Studies focusing on the investigation of larvicides from the plant essential oils have been considered as an important strategy for controlling agricultural pests and vectors of medical and veterinary importance [20, 21]. Keeping in view of this, the present study was targeted to evaluate larvicidal activity of the essential oil from the fresh leaves of *C. tamala* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The findings revealed variation in the larvicidal potentiality of the essential oil in the target mosquito species and *Cx. quinquefasciatus* was observed the most susceptible followed by *Ae. aegypti* and *An. stephensi*, respectively. The differences in essential oil toxicity against different mosquito species may be due to the larval natural habitat in which they grow as *Cx. quinquefasciatus* breeds in dirty-stagnant water while *Ae. aegypti* is a container breeder and *An. Stephensi* breeds in fresh water. The results revealed that the toxicity variation may also be due the impact of different constituents of essential oil tested in qualitative and quantitative variations of the components. The findings also support the fact that the plant derivatives possess target specificity. The researchers considered the environmental safety to be of paramount importance along with the efficacy of an insecticide. Phytochemicals may serve the purpose as these are relatively safe, cost effective, and easily available, therefore, technocrats shown a provocative interest to find out larvicidal compounds from plant sources [22].*C. tamala* essential oil components also have been screened for their role in pest management programmes. Linalool and linalyl acetate displayed significant fumigant toxicity to the rice weevils [23].

The present study revealed the significant larvicidal activity of *C. tamala* leaf essential oil against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} 85.6, 65.10 and 52.90 ppm after 24 hrs of treatment, respectively. *Ae. aegypti* is considered one of the main targets since it has great dispersal capacity of DENV, ZIKV, CHIKV viruses [24] where as *An. stephensi* and *Cx. quinquefasciatus*are considered as primary vectors of malaria and filariasis. The results of the present communication exhibit the potential of essential oil as a means of biological control for mosquitoes, which are vectors of diseases with high incidence in the tropical and subtropical countries and constitute a serious public health problem. Further, they are easily biodegradable; ecofriendly does not leave residues in the environment. Thus, the plant essential oil tested required further detailed research regarding the chemical nature and possible risk assessments against non-targets that will be a step towards the development of an efficient vector management strategy through phytolarvicide. Many workers have reported different compositions of essential oils and extract obtained from different plants [25, 26, 27] and concluded that the plants exhibit significant variation in terms of both the number and percentage composition of the various components in the essential oil and this appears to be a characteristics of a particular plant. The larvicidal activity of *Nigella sativa L.* (seed) essential oil against early fourth instar larvae of *Ae. aegypti*, *An. stephensi*, and *Cx.quinquefasciatus* has been evaluated and considered that the essential oil obtained from *N. sativa* is inexpensive and has efficient larvicidal potential of natural source [28]. Plant derivatives have been shown to be effective against mosquitoes in a safe manner and the screening of 11 local plants was conducted for larvicidal activity against early fourth instar larvaeof *An. arabiensis* and *Ae. aegypti* and mentioned that plant-based insecticides are efficient, biodegradable as well as suitable and adaptive to local conditions and have the widespread insecticidal property [29]. Larvicidal potential of the wood, seed, bark, flower, fruit, and leaves of *Amyris balsamifera*, *Piper nigrum*, *Cinnamomum zeylanicum*, *Anethum graveolens*, *Jasminum grandiflorum*, *Juniperus communis*, and *thymus serpyllum* has been evaluated against *Aedes albopictus* mosquito vector and *A. graveolens* (seed) was observed the most effective among all these plants [30]. The efficiency of phytochemicals as mosquito larvicides may vary greatly depending on the species of plant part used, age of plant parts, solvent used during extraction, and temperature as well as upon the vector species of mosquito [31]. Larvicidal activity of *Coccinia grandis* leaf essential oil was tested against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* and *An. stephensi* was observed the most susceptible mosquito species [32]. Larvicidal activity of *Zanthoxylum acanthopodium* (Bokaytimbur) essential oil was tested against *An. anthropophagus* and *An. sinensis* and proceeded the oil to

find out their chemical composition by gas chromatography and mass spectroscopy and found estragole (15.46%) and eucalyptol (10.94%) the main compounds among the 63 compounds noticed and considered that there is some testimony indicating that essential oils often prove to be more effective than their components [33].

It is apparent from the present findings that the essential oil of the plant, *C. tamala* exhibits potential larvicidal activity against mosquito vectors (*An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*). The study not only predicts with greater or lesser accuracy in the larvicidal effectiveness of essential oil but may also give an indication for further study. The essential oil tested may contribute greatly in efficient, ecofriendly and cost-effective mosquito larval management through the development of essential oil-based formulation. It deserves thorough larvicidal activity to find out the components of the essential oil that determine the significant increase in their effectiveness.

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References

1. Shukla D, Wijayapala S, Vankar PS. Effective mosquito repellent from plant based formulation. International Journal of Mosquito Research 2018;5(1):19-24.
2. Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research 2011;109(2):353-36.
3. Sharma A, Kumar S, Tripathi P. Evaluation of the larvicidal efficacy of five indigenous weed against an Indian strain of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). Journal of Parasitology Research 2018, 1-8.
4. Selvaraj G, Alagarmalai J. Larvicidal properties of *Psychotria octosulcata* (WA Talbot.) (Rubiaceae) crude extracts on human vector mosquitoes *Aedes aegypti* (Linn.), *Culex quinquefasciatus* (Say.) and *Anopheles stephensi* Liston. Journal of Entomology and Zoology Studies 2018;6(1):1190-1195.
5. Govindarajan M, Sivakumar R. Repellent properties of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) plant leaf extracts against three important vector mosquitoes. Asian Pacific Journal of Tropical Biomedicine 2012;2(8):602-607.
6. Indusree OG, Chandana JS, Ajitha VS. Larvicidal Efficacies of Certain Plant extracts against Fourth Instar Larvae of *Aedes albopictus* Skuse. International Journal of Scientific Research in biological Sciences 2019;6(1):2347-7520.
7. World Health Organization. World Malaria Report. Geneva, Switzerland 2008.
8. Ersino W, Teressa H, Alemayo T. Larvicidal activity of *Juniperus procera* extracts against Anopheles mosquito in vitro, North Western Ethiopia. Journal of Medicinal Plants Research 2020;14(9):445-450.
9. Zhu J, Zeng X. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. Journal of the American Mosquito Control Association 2006;22(3):515-522.
10. Maurya P, Sharma P, Mohan L, Batabyal L, Srivastava CN. Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *Anopheles stephensi* and *Culex quinquefasciatus*. Journal of Asia-Pacific Entomology 2009;12(2):113-115.
11. Ayvaz A, Sagdic O, Karaborklu S, Ozturk I. Insecticidal activity of the essential oils from different plants against three stored-product insects. Journal of Insect Science 2010;10(21):1-13.
12. Chaubey MK. Insecticidal activities of *Cinnamomum tamala* (Lauraceae) essential oil against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). International Journal of Entomological Research 2016;4(3):91-98.
13. Ahmed A, Choudhary MI, Farooq A, Demirci B, Demirci F, Can Başer KH. Essential oil constituents of the spice *Cinnamomum tamala* (Ham.) Neesand Eberm. Flavour and Fragrance Journal 2000;15(6):388-390.
14. Lohani H, Singh SK, Bhandari Ujjwal, Haider S, Zafar Gwari, Garima Chauhan. Nirpendra Chemical polymorphism in *Cinnamomum tamala* (Buch-Ham) Nees. And Eberm. growing in Uttarakhand Himalaya (India). Journal of Chemical and Pharmaceutical Research 2015;7(8):67-71.
15. Gupta K, Pharma M. Antimalarial Activity of *Cinnamomum tamala*. International Journal of Medical Science in Clinical Research and Review 2018;1(01):1-10.
16. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP 2005;13:10-11.
17. Abbot WS. A method of computing of the effectiveness of an insecticide. Journal of Economic Entomology 1925;18:265-267.
18. Finney DJ. Probit Analysis, 3rd edition. Cambridge University Press, Cambridge 1971.
19. Reddy PJ, Krishna D, Murthy US, Jamil K. A microcomputer FORTRAN program for rapid determination of lethal concentration of biocides in mosquito control. Bioinformatics 1992;8(3):209-213.
20. Benelli G, Rajeswary M, Vijayan P, Senthilmurugan S, Alharbi NS, Kadaikunnan S *et al*. *Boswellia ovalifoliolata* (Burseraceae) essential oil as an eco-friendly larvicide? Toxicity against six mosquito vectors of public health importance, non-target mosquito fishes, backswimmers, and water bugs. Environmental Science and Pollution Research 2018;25(11):10264-10271.
21. Lopez V, Pavela R, Gomez-Rincon C, Les F, Bartolucci F, Galiffa V *et al*. Efficacy of *Origanum syriacum* essential oil against the mosquito vector *Culex quinquefasciatus* and the gastrointestinal parasite Anisakis simplex, with insights on acetylcholinesterase inhibition. Molecules 2019;24(14):2563.
22. Kumar D, Chawla R, Dhamodaram P, Balakrishnan N. Larvicidal activity of *Cassia occidentalis* (Linn.) against the larvae of bancroftian filariasis vector mosquito *Culex quinquefasciatus*. Journal of Parasitology Research 2014;236838.5.
23. Chaubey MK. Insecticidal activities of *Cinnamomum tamala* (Lauraceae) essential oil against *Sitophilus oryzae*

L. (Coleoptera: Curculionidae). International Journal of Entomological Research 2016;4(3):91-98.
24. Monteiro VVS, Navegantes-Lima KC, de Lemos AB, Da Silva GL, de Souza Gomes R, Reis JF *et al*. *Aedes*–Chikungunya Virus Interaction: Key Role of Vector Midguts Microbiota and Its Saliva in the Host Infection. Frontiers in microbiology 2019;10:492.
25. Sedaghat MM, Dehkordi AS, Abai MR, Khanavi M, Mohtarami F, Abadi YS *et al*. Larvicidal activity of essential oils of Apiaceae plants against malaria vector, *Anopheles stephensi*. Iranian Journal of Arthropod Borne Diseases 2011;5(2):51-59.
26. Manzoor F, Samreen KB, Parveen Z. Larvicidal activity of essential oils against *Aedes aegypti* and *Culex quinquefasciatus* larvae (Diptera: Culicidae) Journal of Animal and Plant Science 2013;23(2):420-424.
27. Liu XC, Liu Q, Zhou L, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Allium macrostemon* Bunge and its selected major constituent compounds against *Aedes albopictus* (Diptera: Culicidae). Parasites and Vectors 2014;7(1):1-5.
28. Raj GA, Chandrasekaran M, Krishnamoorthy S, Jayaraman M, Venkatesalu V. Phytochemical profile and larvicidal properties of seed essential oil from *Nigella sativa* L. (Ranunculaceae), against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research 2015;114(9):3385-3391.
29. Massebo F, Tadesse M, Bekele T, Balkew M, Gebremichael T. Evaluation on larvicidal effects of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera, Culicidae) in Ethiopia. African Journal of Biotechnology 2009;8(17):4183-4188.
30. Tyagi V, Yadav R, Veer V. Laboratory evaluation of certain essential oils for their larvicidal activity against *Aedes Albopictus*, vector of dengue and chikungunya. Global Journal of Zoology 2016;1(1):003-006.
31. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian Journal of Medical Research 2012;135(5):581-598.
32. Mohammed SI, Vishwakarma KS, Maheshwari VL. Evaluation of larvicidal activity of essential oil from leaves of *Coccinia grandis* against three mosquito species. Journal of Arthropod Borne Diseases 2017;11(2):226-235.
33. He Q, Wang W, Zhu L. Larvicidal activity of *Zanthoxylum acanthopodium* essential oil against the malaria mosquitoes, *Anopheles anthropophagus* and *Anopheles sinensis*. Malaria Journal 2018;17(1):1-7.