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Research Article

Evaluation of larvicidal activity of essential oils of *Ocimum sanctum* against *Aedes aegypti*

N. KIRUBAKARAN[1], M. S. UMASHANKAR[1*], L. ANURADHA[2], S. SHANTHI[2] and S. SANKAR[3]

[1]*Department of Pharmaceutics, SRM College of Pharmacy, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, Chengalpattu – 603203, Tamil Nadu, India*

[2]*Directorate of Public Health, Theynampet, Chennai – 600006, Tamil Nadu, India*

[3]*Drugs Testing Laboratory, Theynampet, Chennai – 600006, Tamil Nadu, India*

**Corresponding author E-mail:* umashans@srmist.edu.in

ABSTRACT: Mosquitoes are among the most vexing bloodsucking insects that torment the public. They transmit diseases globally and contribute to significant mortality annually. Mosquito control remains a major challenge under current environmental conditions. Traditional Indian medicine systems, such as Ayurveda and Siddha, have long utilized medicinal plants with insecticidal properties. This study evaluates the larvicidal potential of *Ocimum sanctum* (Basil) essential oil against *Aedes aegypti* larvae. Essential oils of *O. sanctum* were used for the larvicidal study and its chemical composition studies, were carried out using Fourier Transform Infra-Red Spectroscopy (FTIR), and Gas Chromatographic techniques. Third to early fourth instar larvae of *Aedes aegypti* of 30 larvae with serial dilution of essential oils, *viz.,* 100 µL, 500 µL, 1000 µL and 2000 µL, were used. The mortality rate was observed for each replicate after 0, 1 h, 4 h, 20 h, 24 h, and 48 h of treatment. The FTIR and GC-MS analyses identified major functional groups and phytoconstituents in the essential oil like methyl chavicol (estragole), linalool, eugenol, methyl cinnamate,1,8-cineole, etc. The results of the mosquitocidal activity of EOs exhibited LC 50 values @126 µL and LC 90@ 762 µL after 24 h's time limit. This study concludes that the FTIR and GC fingerprints revealed major chemical components of *O. sanctum* Eos highlighting the potential natural bio-pesticide properties against *Aedes aegypti* which can contribute in controlling vector-borne diseases by targeting mosquitoes at the larval stage.

KEYWORDS: *Aedes aegypti,* basil oil, *Ocimum sanctum,* toxicology

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INTRODUCTION

Vector-borne diseases are illnesses caused by pathogens (viruses, bacteria, or parasites) that are transmitted to humans and other animals through vectors — living organisms that carry and transmit infectious pathogens between humans or from animals to humans. The most common vectors are blood-feeding arthropods, such as mosquitoes, ticks, fleas, and sand-flies. Mosquitoes are responsible for transmitting several vector-borne diseases, which are illnesses caused by pathogens and parasites. Here are the major mosquito-borne diseases like malaria (mosquito vector: Anopheles species (mainly female), dengue fever, zika virus, chikungunya (mosquito fever: *Aedes aegypti*, *Aedes albopictus*), yellow fever (mosquito vector: *Aedes aegypti*), *etc.* (Sakthivadivel *et al.*, 2020). Controlling mosquito-borne diseases is a major public health priority in many parts of the world, especially in tropical and subtropical regions. Various management methods of mosquitoes are chemical control, biological control, *etc*. (Sritabutra *et al.*, 2011). Use of insect repellent (containing DEET, Picaridin, or IR3535) is one of the controlling method (Kalita *et al.*, 2013). Exploration of the herbal preparations that exhibit larvicidal and repellent properties against mosquitoes is essential (Hazarika, 2018). In indigenous medicine, plant-based repellents have been utilised for generations as a personal defence measure against host-seeking mosquitoes. Knowledge on indigenous mosquito repellent plants as a useful tool for developing herbal larvicidals (Maia & Moore, 2011) and their toxicological profile helps to utilise herbal essential oils as a preventive aspect of vector-borne diseases. So, a study has been taken up to evaluate the efficacy of *Ocimum sanctum* against mosquito, *Aedes aegypti*.

MATERIALS AND METHODS

Collection and authentication of plant herbs

The herbs for the study were collected from Kanchee puram District of Tamil Nadu and authenticated by Dr. K. N. Sunil Kumar, Research Officer, Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, India. After identification, the *Ocimum* plant material was

processed for the extraction of Essential oils (Authentication Reference No: 532.24052301-13) (Khare, 2015).

Extraction of essential oils of *O. sanctum*

The essential oils from Tulsi leaves (Figure 1) were extracted by dissolving 50 g of Tulsi leaves in 400 ml of water using a Clevenger apparatus by hydro-steam distillation for 6-7 h. The essential oil layer was then separated from the aqueous phase using n-hexane in a separating funnel. This extraction process was repeated multiple times, and the pooled samples were dried at a low temperature and stored in a refrigerator at 4°C.

GC fingerprint studies

GC has a high sensitivity for detecting practically all volatile chemical substances, which is especially true for FID detection and GC–MS. Furthermore, capillary columns' high selectivity allows for the separation of many volatile compounds in a short period. It is, however, inconvenient to analyse polar, non-volatile, and heat-labile component samples. The samples must be gasified through time-consuming sample pretreatments like derivatization, but the ingredients in most herbal medicines are highly polar compounds, limiting the use of gas chromatography for chemical identification and authentication.

Method: Agilent GC instruments were used for the study. Initial Temperature 60°C for 2 min and 10°C per min gradual increase and 280°C hold. Internal Temperature 260°C, 1 µl injection volume, flow 1.5 ml per min, and helium gas used with column HP5 with 60 mm dimension. The Peak components compared with the NIST library.

Mosquito culture

Larvae (*Aedes aegypti*) were collected from Chennai metropolitan city stagnant water areas and confirmed in the CMRC (Central Malarial Research Centre), DMS Campus, Thenampet, Chennai. The collected larvae were impregnated with the medium of DE-chlorinated water for sustainability. The larvicidal study was performed at 27±2°C and 60-70% RH under 14:10 h light and dark cycles. Brewer's yeast, dog biscuits, and algae collected from ponds are the diet for the larvae (Kamaraj *et al.*, 2010). The Guidelines for laboratory and field testing of mosquito Larvicidal as per the WHO protocol were used for the study reference (Suter *et al.*, 2017). In addition, Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides are also considered for the study (World Health Organization, 2024).

Larvicidal bioassay

In the preliminary screening with the laboratory trial, the stock solution with different serial dilutions was prepared. The larvicidal activity was assessed by the procedure of the WHO (Guidelines for testing of larvicides). The larva study was carried out 5 times using the method of the WHO protocol. The study was repeated for each oil concentration for accuracy and reproducibility data. The mortality was studied after 0, 1 h, 4 h, 20 h, 24 h, and 48 h. The count of dead larvae was done by needles that were pricked in the neck or siphon. The control was set up with the same conditions, only with dechlorinated water (without adding any essential oil) and reagents. Solubilizer: Polysorbate 80, 2 ml (Tween 80 used for the study). Each contained 0.5 L of de-chlorinated water and 30 numbers of larvae. Stock concentrations were prepared by diluting the plant oil extracts with distilled water and drops of Tween 80 (Polyoxyethylene Sorbitan Monooleate) to obtain volumes v/v), respectively. Serial dilutions of essential oils, like 100 µL, 500 µL, 1000 µL, and 2000 µL used for the study. For each concentration, 5 replicates with a total of 150 larvae are utilized for the experiment. The numbers of dead larvae were counted after 0, 1 h, 4 h, 20 h, 24 h, and 48 h of exposure (Rahuman *et al.*, 2000; Gubler *et al.*, 2014; Aljameeli *et al.*, 2022).

FTIR studies

FTIR performed using a Shimadzu Two FT-IR Spectrometer using (ATR mode) in the 500-4000 cm^{-1} region with 36 scans and 4 cm^{-1} resolution with a data interval of 0.5 cm.



Figure 1. Leaves of *O. sanctum*.

RESULTS AND DISCUSSION

The GC-MS study of the essential oils of *O. sanctum*

GC-MS chromatogram of the essential oils of *O. sanctum* showed major peaks of benzene, 1, 2-dimethoxy-4-(2-propenyl), isocaryophyllene, and eugenol, which were the major components in the EOs and identified with NIST library, indicating the presence of phytocomponents.

Larvicidal results of *O. sanctum* against *Aedes aegypti* mosquito species:

Determination of the percentage yield of basil oil

The percentage of Tulsi oil was 0.18%. 0.09 g yield for 50 g of plant material of Tulsi leaves, and the same process was repeated 6 times for reproducibility.

FTIR study

FTIR of 500-4000 cm^{-1} (Table 1) presented. The major peaks of 3072-2843cm^{-1} lead to C-H alkanes (stretch). Peaks of 1610 cm^{-1} and 1452 cm^{-1} lead to C=C aromatic (stretch). The bands observed in the range 1298-995 cm^{-1} lead to C-O ether (stretch). The band associated with 1452 cm^{-1} belongs to CH_2 alkane (bend).

GC fingerprint of basil oil

The GC Fingerprint of Tulsi Oil is represented in Figure 3. The GC spectrum was compared with the NIST Database and identified Molecules were benzene, caryophyllene, eugenol, *etc*.

Table 1. FTIR spectrum investigations

FTIR Interpretations	
Band Range (Experimental)	Band assignment
3072-2843 cm^{-1}	C-H alkanes (stretch)
1610cm-1 and 1452 cm^{-1}	C=C aromatic(stretch)
1298-995 cm^{-1}	C-O ether (stretch)
1452 cm^{-1}	CH_2 alkane (bend)

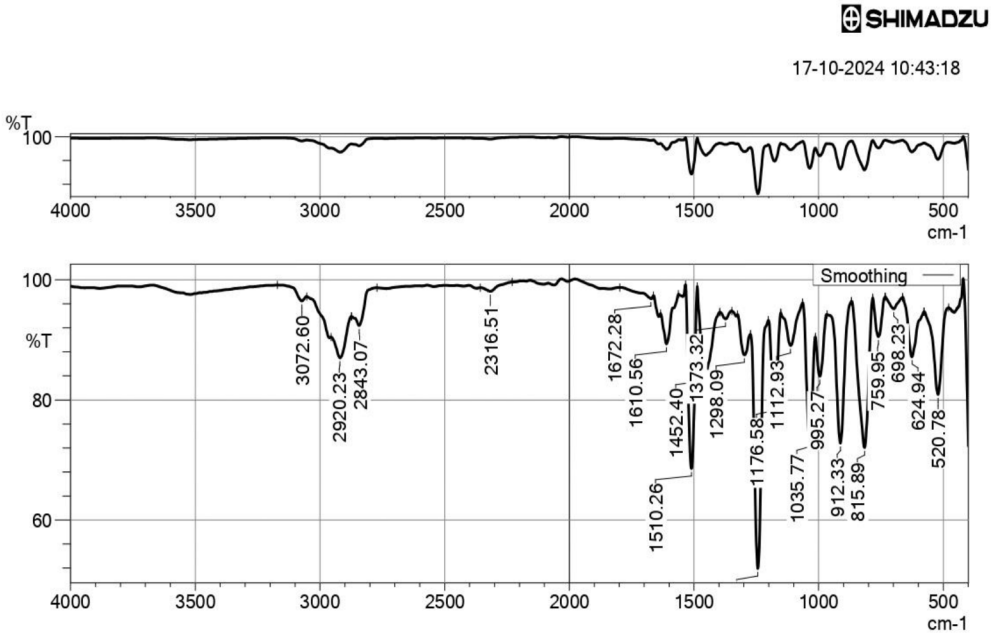


Figure 2. FTIR spectrum of essential oils of *O. sanctum.*

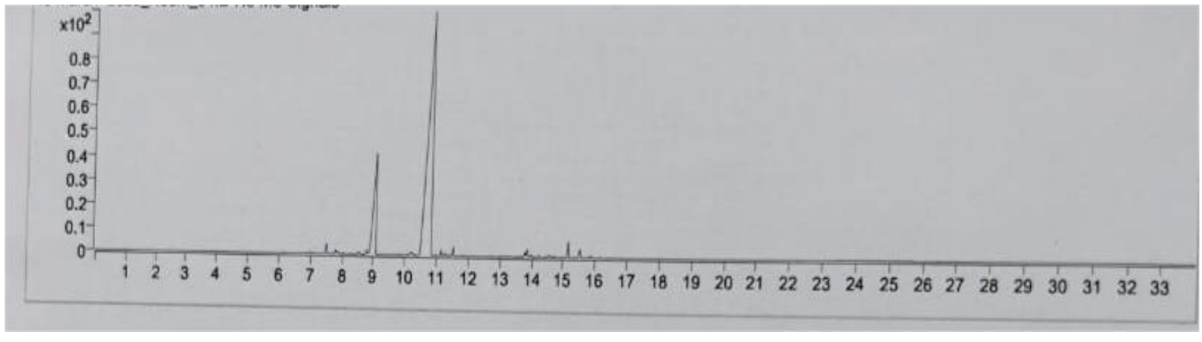


Figure 3. GC fingerprint of Tulsi oil.

Table 2. Larvicidal potential of *O. sanctum* oil

Larvicidal potential of *O. sanctum* oil									
S. No.	**Time (hour)**	**No larvae at mortality (average of 5 replicates) ± SD**	**% inhibition ± SD**	**No larvae at mortality (average of 5 replicates) ± SD**	**% inhibition ± SD**	**No larvae at mortality (average of 5 replicates) ± SD**	**% inhibition± SD**	**No larvae at mortality (average of 5 replicates) ± SD**	**% inhibition ± SD**
		100 µL		500 µL		1000 µL		2000 µL	
1	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	1	6 ± 0.71	20 ± 2.36	16 ± 0.71	53.33 ±2.36	22.2± 0.45	74 ± 1.49	30± 0	100 ± 0
3	4	14 ± 0.71	48 ± 1.83	23.6 ±0.55	78.67 ±1.83	30 ± 0	100 ± 0	30 ± 0	100 ± 0
4	20	30 ±0	100 ± 0	30±0	100 ± 0	30±0	100 ± 0	30±0	100 ± 0
5	24	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0
6	48	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0

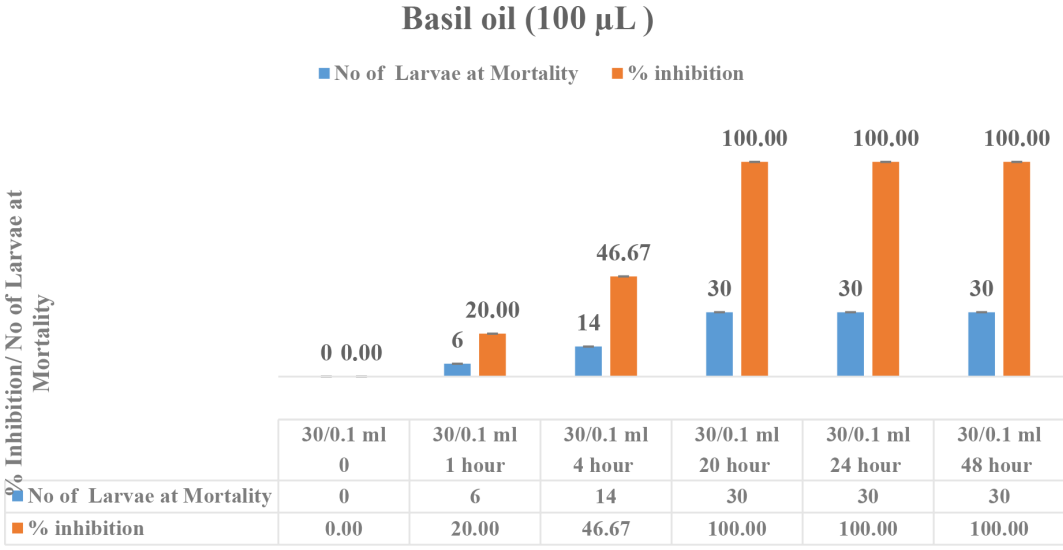


Figure 4. Larvicidal potential of Basil oil (100 µL).

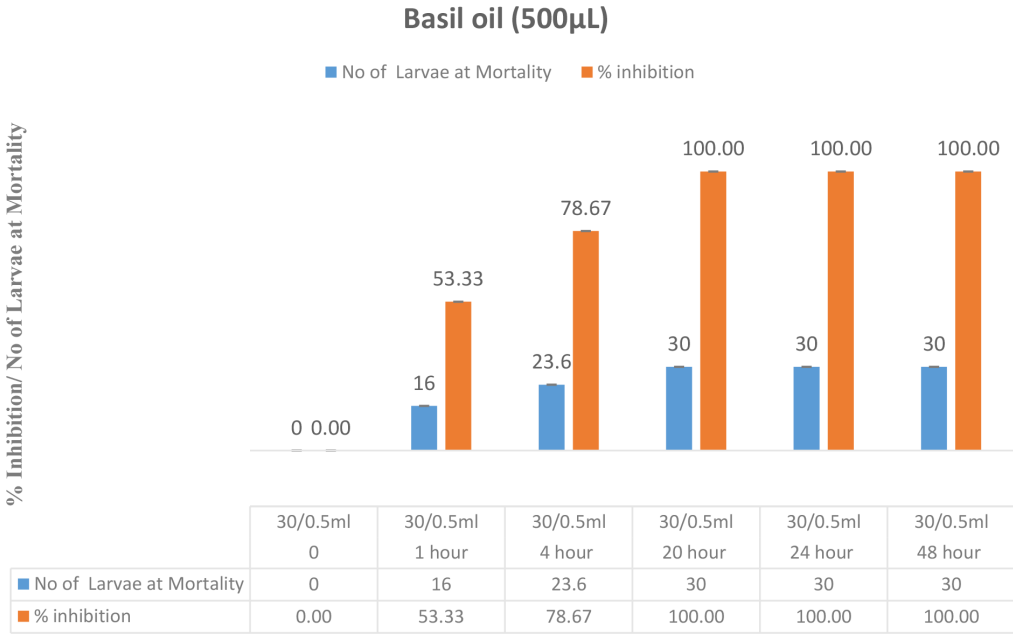
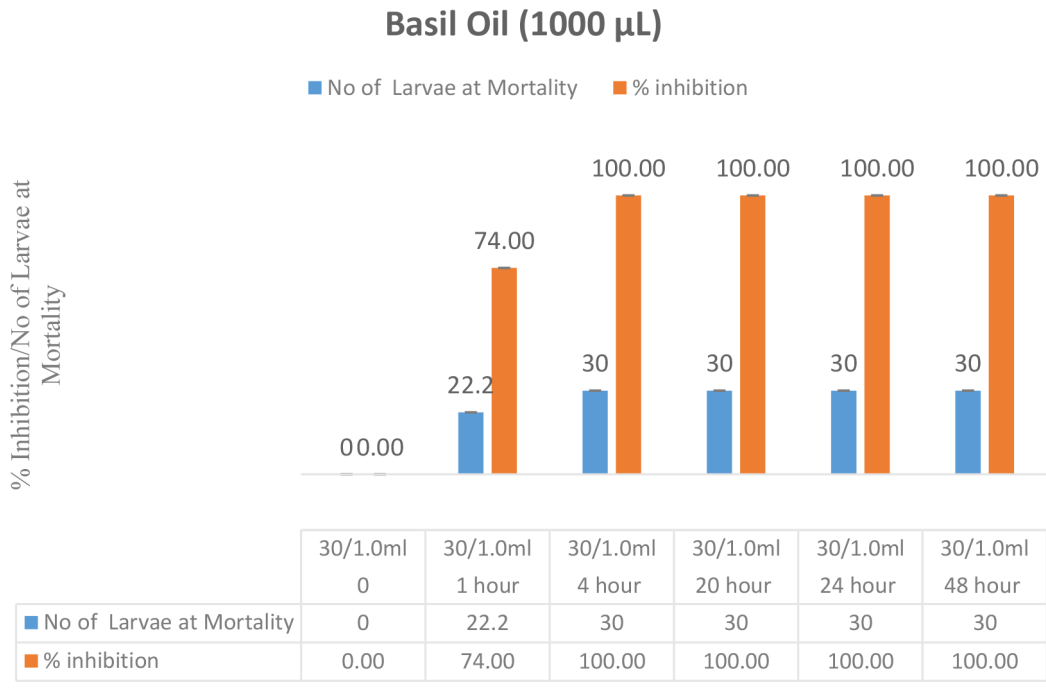
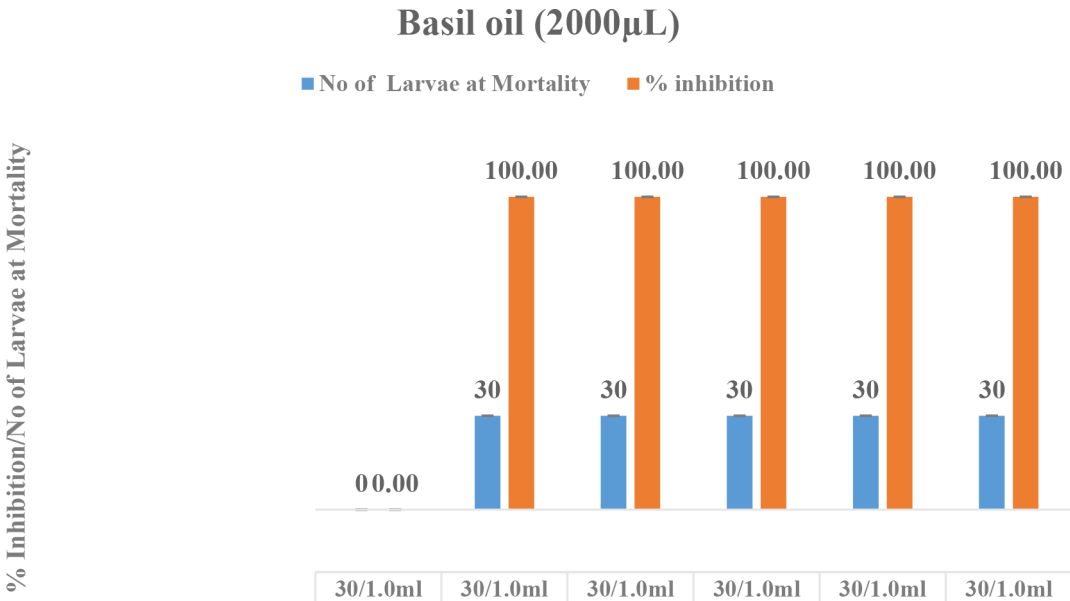


Figure 5. Larvicidal potential of Basil oil (500µL).



	30/1.0ml 0	30/1.0ml 1 hour	30/1.0ml 4 hour	30/1.0ml 20 hour	30/1.0ml 24 hour	30/1.0ml 48 hour
No of Larvae at Mortality	0	22.2	30	30	30	30
% inhibition	0.00	74.00	100.00	100.00	100.00	100.00

Figure 6. Larvicidal potential of Basil oil (1000 µL).



	30/1.0ml 0	30/1.0ml 1 hour	30/1.0ml 4 hour	30/1.0ml 20 hour	30/1.0ml 24 hour	30/1.0ml 48 hour
No of Larvae at Mortality	0	30	30	30	30	30
% inhibition	0.00	100.00	100.00	100.00	100.00	100.00

Figure 7. Larvicidal potential of Basil oil (2000µL).

Larvicidal potential of essential oils of *O. sanctum*

The essential oils, 100 µL observed the inhibition of 20 ± 2.36%, 48 ± 1.83%, 100 ± 0, 100 ± 0%, and 100 ± 0% at 1 h, 4 h, 20 h, 24 h, and 48 h, respectively. 500 µL observed that inhibition of 53.33 ± 2.36%, 78.67 ± 1.83%, 100 ± 0%, 100 ± 0%, 100 ± 0% at 1 h, 4 h, 20 h, 48 h, respectively. 1000 µL observed that inhibition of 74 ± 1.49%, 100 ± 0%, 100 ± 0%, 100 ± 0%, and 100 ± 0% at 1 h, 4 h, 20 h, 24 h, and 48 h, respectively. 2000 µL observed that inhibition of 100 ± 0%, 100 ± 0%, 100 ± 0%, 100 ± 0%, 100 ± 0% at 1 h, 4 h, 20 h, 24 h, 48 h, respectively. *The results of the* mosquitocidal activity of EOs oils exhibited LC 50 values @126 µL and LC 90@ 762 µL after 24 h's time limit.

Some previous studies, like Cheng *et al.*, (2018), compared the essential oils from the leaves of eight

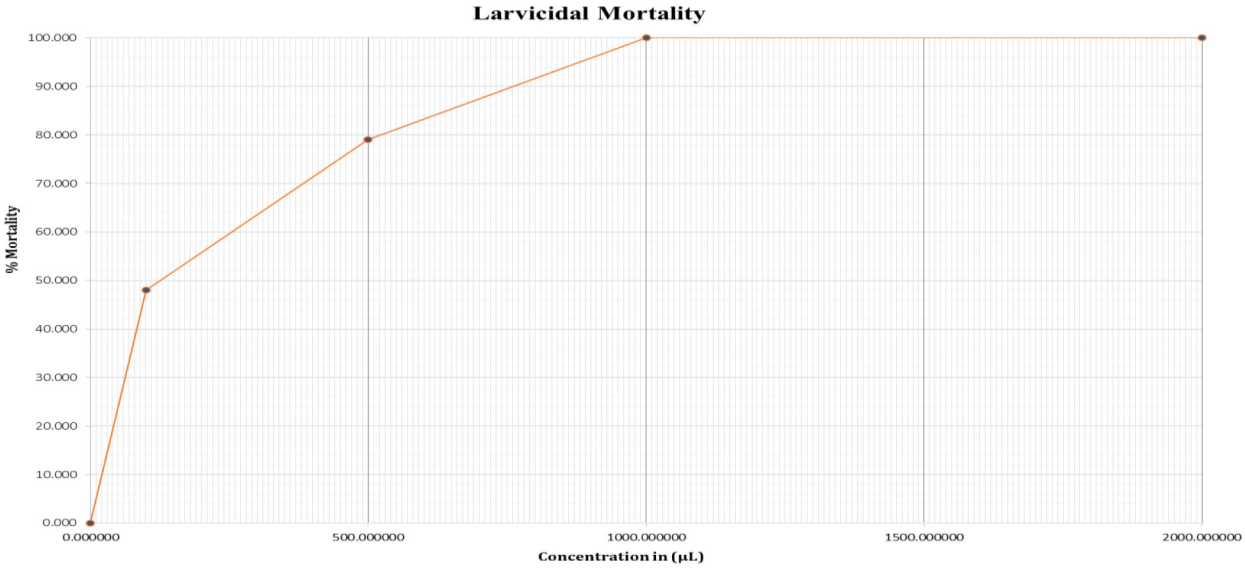


Figure 8. % of mortality vs concentrations.

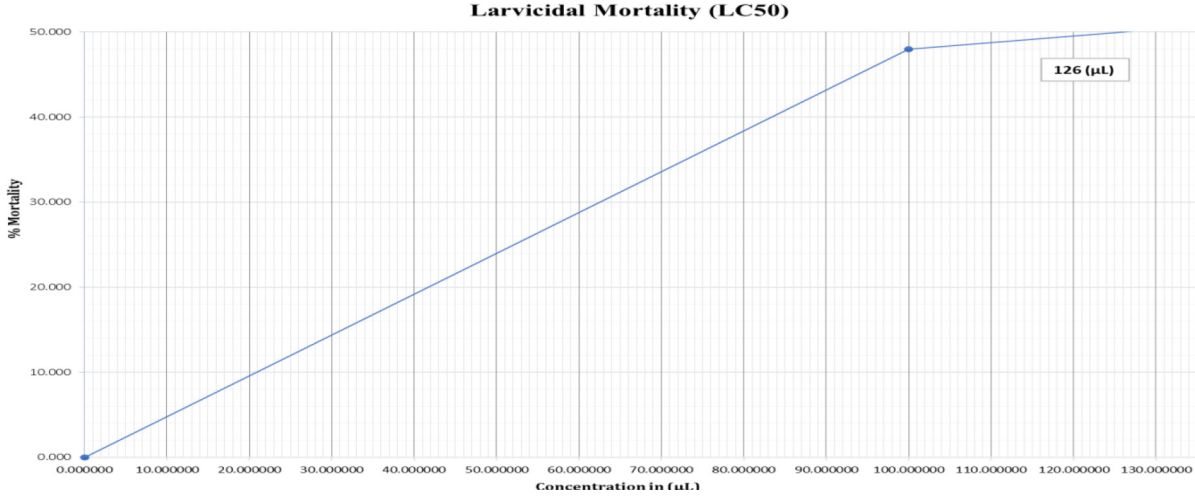


Figure 9. Determination of LC_{50}.

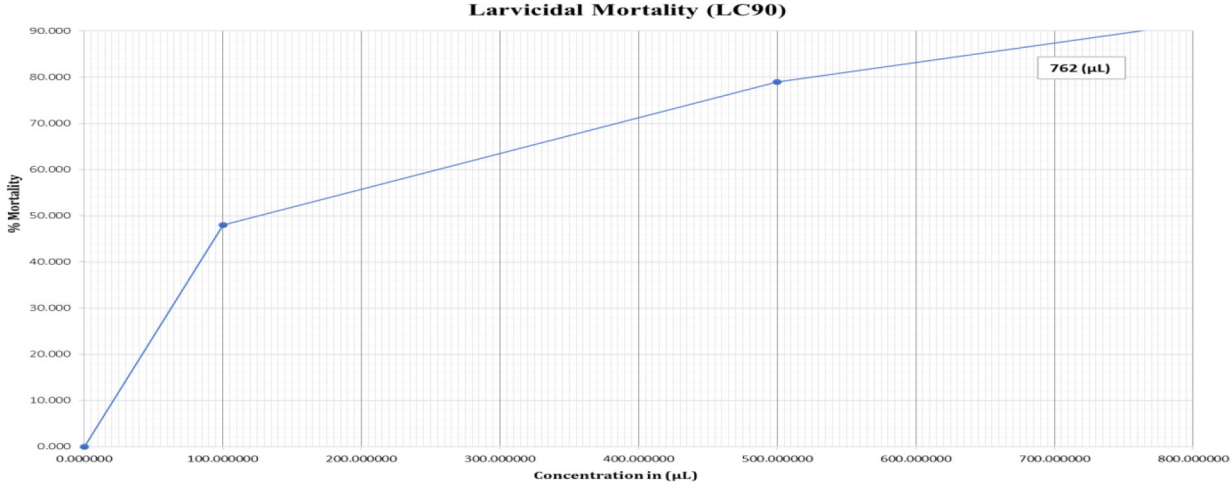


Figure 10. Determination of LC_{90}.

provenances of indigenous cinnamon (*Cinnamomum osmophloeum* an excellent inhibitory effect against the *Ae. Aegypti*. Similarly (Zhu *et al.*, 2006, 2008) reported the larvicidal activity of cinnamon oil along with other essential oils. Amer and Mehlhorn (2006) documented the larvicidal properties of *Lippia citriodora* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*.

Advantages of EOs as larvicides

To combat mosquito-borne diseases such as malaria and dengue, the World Health Organization (WHO) (2024) recommends the use of pesticides and larvicides, which are currently employed in 55 countries worldwide. However, the continuous application of synthetic larvicides like malathion and temephos contributes to environmental pollution—affecting soil, surface water, and living organisms—and has led to the development of resistance in various mosquito populations, including *Aedes aegypti*, *Culex pipiens*, and *Anopheles stephensi*. This resistance poses a major challenge to controlling medically important arthropod vectors and significantly increases the risk of re-emergence and outbreaks of vector-borne diseases on a global scale (Soltani *et al.*, 2015; Assis *et al.*, 2012; Arredondo-Jiménez and Valdez-Delgado, 2006; Cheng *et al.*, 2018; Barber *et al.*, 2017; Zhang *et al.*, 2015; National Research Council, 1986). In contrast, certain naturally occurring herbs possess insecticidal and larvicidal properties, making them effective and environmentally friendly alternatives to harmful chemical pesticides. These plant-based agents have gained increased attention in recent years for their potential use in sustainable vector control strategies (Al-Rashidi *et al.*, 2022). Essential oils derived from natural plants are the most important alternative approach to control vector-borne disease for their ease of obtaining and Minimal cost compared to synthetic chemical products, which were available in the market (Hemanga *et al.*, 2018). Moreover, Essential Oils (EOs), being derived from renewable sources and rapidly biodegradable, do not leave harmful residues in the environment. Resistance to larvicides is more likely to develop when a single active compound is used, whereas EOs contain multiple Bioactive components, reducing the likelihood of resistance development in mosquito populations (Araujo *et al.*, 2016; Intirach *et al.*, 2012; Okumu *et al.*, 2007; Buré and Sellier, 2004).

CONCLUSION

This study investigated the potential of essential oils of *O. sanctum*, a siddha plant, for its larvicidal activities against mosquito vectors such as *Ae. aegypti*. The Investigation utilized various analytical techniques such as FTIR and GC-MS to identify the chemical composition of the essential oils. Three major bio-active compounds were identified in the EOs which proved significant larvicidal activities against *Ae. aegypti*. From this study, information is generated to incorporate this larvicidal potential herbal oil into mosquito inhibitory sprays and pharmaceutical topical preparations like creams and gels for the repellent products. In addition, this larvicidal knowledge will help the public health department and municipal corporations as an alternate approach for herbal-based, environmental, eco-friendly pesticides when compared to currently using synthetic and chemical-based pesticides to control the mosquito species against the various kinds of vector-borne diseases.

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REFERENCES

Aljameeli, M. 2022. Larvicidal effects of some essential oils against *Aedes aegypti* (L.), the vector of dengue fever in Saudi Arabia. *Saudi J Biol Sci*, *29*(12): Article 103552. https://doi.org/10.1016/j.sjbs.2022.103552

Al-Rashidi, H. S., Mahyoub, J. A., Alghamdi, K. M., and Al-Otaibi, W. M. 2022. Seagrasses extracts as potential mosquito larvicides in Saudi Arabia. *Saudi J Biol Sci*, *29*(12): Article 103433. https://doi.org/10.1016/j.sjbs.2022.103433

Amer, A., and Mehlhorn, H. 2006. Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera: Culicidae). *Parasitol Res*, *99*(4): 466-472. https://doi.org/10.1007/s00436-006-0182-3

Amer, A., and Mehlhorn, H. 2006. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol Res*, *99*(4): 478-490. https://doi.org/10.1007/s00436-006-0184-1

Araujo, A. F. O., Ribeiro-Paes, J. T., Deus, J. T., Cavalcanti, S. C. H., Nunes, R. S., Alves, P. B., and Macoris, M. L. G. 2016. Larvicidal activity of *Syzygium aromaticum* and *Citrus sinensis* essential oils and their antagonistic effects with temephos. *Mem Inst Oswaldo Cruz*, *111*(7): 443-449. https://doi.org/10.1590/0074-02760160075

Arredondo-Jiménez, J. I., and Valdez-Delgado, K. M. 2006. Effect of Novaluron (Rimon® 10 EC) on

mosquitoes from Chiapas, Mexico. *Med Vet Entomol*, *20*(4): 377-387. https://doi.org/10.1111/j.1365-2915.2006.00656.x

Assis, C. R. D., Linhares, A. G., Oliveira, V. M., França, R. C. P., Carvalho, E. V. M. M., Bezerra, R. S., and de Carvalho, L. B. 2012. Comparative effect of pesticides on brain acetylcholinesterase in tropical fish. *Sci Total Environ*, *441*: 141-150. https://doi.org/10.1016/j.scitotenv.2012.09.058

Barber, B. E., Rajahram, G. S., Grigg, M. J., William, T., and Anstey, N. M. 2017. World Malaria Report: Time to acknowledge *Plasmodium knowlesi* malaria. *Malar J*, *16*(1): Article 135. https://doi.org/10.1186/s12936-017-1787-y

Buré, C. M., and Sellier, N. M. 2004. Analysis of the essential oil of Indonesian patchouli using GC/MS. *J Essent Oil Res*, *16*(1): 17-19. https://doi.org/10.1080/10412905.2004.9698638

Cheng, S. S., Lin, R., Zhang, N., Yuan, S., Zhou, X., Huang, J., Yu, C. 2018. Toxicity of six insecticides to predatory mite *Amblyseius cucumeris*. *Ecotoxicol Environ Saf*, *161*: 715-720. https://doi.org/10.1016/j.ecoenv.2018.06.018

Gubler, D. J., Ooi, E. E., Vasudevan, S., and Farrar, J. 2014. *Dengue and dengue hemorrhagic fever*. CABI, Wallingford.

Hazarika, H., Tyagi, V., Krishnatreyya, H., Kishor, S., Karmakar, S., Bhattacharyya, D. R., Chattopadhyay, P. 2018. Toxicity of essential oils on *Aedes aegypti*: A vector of chikungunya and dengue fever. *Int J Mosq Res*, *5*(3): 51-57.

Intirach, J., Junkum, A., Tuetun, B., Choochote, W., Chaithong, U., Jitpakdi, A., …, Pitasawat, B. 2012. Chemical constituents and combined larvicidal effects of selected essential oils against *Anopheles cracens* (Diptera: Culicidae). *Psyche - J Entomol*, *2012:* 1-11. https://doi.org/10.1155/2012/591616

Kalita, B., Bora, S., and Sharma, A. K. 2013. Plant essential oils as mosquito repellent - A review. *Int J Res Develop Pharmacy Life Sci*, *2013;* (1).

Kamaraj, C., Rahuman, A. A., Bagavan, A., Elango, G., Rajakumar, G., Zahir, A. A., Jayaseelan, C. 2010. Evaluation of medicinal plant extracts against blood-sucking parasites. *Parasitol Res*, *106*(6): 1403-1412. https://doi.org/10.1007/s00436-010-1816-z

Khare, C. P. 2015. *Ayurvedic pharmacopoeial plant drugs*. CRC Press.

Maia, M. F., and Moore, S. J. 2011. Plant-based insect repellents: A review of their efficacy, development and testing. *Malar J*, *10*(S1): Article S11. https://doi.org/10.1186/1475-2875-10-s1-s11

National Research Council (U.S.). 1986. Committee on strategies for the management of pesticide resistant pest populations. *Pesticide resistance: Strategies and tactics for management*. National Academy Press.

Okumu, F. O., Knols, B. G., and Fillinger, U. 2007. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. *Malar J*, *6*(1): Article 63. https://doi.org/10.1186/1475-2875-6-63

Rahuman, A. A., Gopalakrishnan, G., Saleem, B. G., Arumugam, S., and Himalayan, B. 2000. Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia*, *71*(5): 553-555. https://doi.org/10.1016/s0367-326x(00)00164-7

Sakthivadivel, M., Mukhilan, R. R., Jeevitha, G., Sakthivadivel, J., Narayanan, K., and Tennyson, S. 2020. Knowledge, attitude and practice of vectors and vector-borne diseases with special reference to dengue at metropolitan Chennai, Tamil Nadu, India. *Int J Community Med Public Health*, *7*(6): 2337-2342. https://doi.org/10.18203/2394-6040.ijcmph20202495

Soltani, A., Vatandoost, H., Oshaghi, M. A., Ravasan, N. M., Enayati, A. A., and Asgarian, F. 2015. Resistance mechanisms of *Anopheles stephensi* (Diptera: Culicidae) to temephos. *PubMed*, *9*(1): 71-83.

Sritabutra, D., Soonwera, M., Waltanachanobon, S., and Poungjai, S. 2011. Evaluation of herbal essential oil as repellents against *Aedes aegypti* (L.) and *Anopheles dirus* Peyton and Harrion. *Asian Pac J Trop Biomed*, *1*(1, Suppl): S124-S128. https://doi.org/10.1016/S2221-1691(11)60138-X

Suter, T., Crespo, M. M., de Oliveira, M. F., de Oliveira, T. S. A., de Melo-Santos, M. A. V., de Oliveira, C. M. F., Silva-Filha, M. H. N. L. 2017. Insecticide susceptibility of *Aedes albopictus* and *Ae. aegypti* from Brazil and the Swiss-Italian border region. *Parasit Vectors*, *10*(1): Article 431. https://doi.org/10.1186/s13071-017-2364-5

World Health Organization. 2024. *Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides*. WHO. https://doi.org/WHO/VBC/81.807

Zhang, M., Zeiss, M. R., and Geng, S. 2015. Agricultural pesticide use and food safety: California's model. *J Integr Agric*, *14*(11): 2340-2357. https://doi.org/10.1016/s2095-3119(15)61126-1

Zhu, J., Zeng, X., Liu, T., Qian, K., Han, Y., Xue, S., Zhang, A. 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J Am Mosq Control Assoc*, *22*(3): 515-522. https://doi.org/10.2987/8756-971x(2006)22[515:aralao]2.0.co;2

Zhu, J., Zeng, X., O'Neal, M., Schultz, G., Tucker, B., Coats, J., Xue, R.-D. 2008. Mosquito larvicidal activity of botanical-based mosquito repellents. *J Am Mosq Control Assoc*, *24*(1): 161-168. https://doi.org/10.2987/8756-971x(2008)24[161:mlaobm]2.0.co;2