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Evaluating the Efficacy of Thyme, Camphor, and Eucalyptus Essential Oils as Larvicides Against *Aedes Aegypti* Mosquitoes

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Abstract:- Dengue fever is a significant public health issue in India, affecting millions of people annually. The virus is transmitted by infected Aedes mosquitoes, and India is among the countries most affected by dengue fever, with an estimated 100 million new cases each year worldwide. Plant essential oils (EOs) are the most preferred phytochemicals to control mosquito larvae. In the present study, the larvicidal efficacy of Thyme, Camphor, and Eucalyptus EOs was investigated against the 4th instar larva of *Aedes aegypti.* Thyme, Eucalyptus, and Camphor EOs were procured from a local organic shop in Hyderabad. 0.5%, 1%, 2%, and 3% oils were prepared using Tween 80 as an emulsifier. A control solution was prepared excluding essential oils. All three tested EOs were proved to be efficient against the 4th instar larvae of *Ae. aegypti.* However, thyme EO showed better larvicidal efficacy than the remaining two EOs. Thyme, Eucalyptus, and Camphor EOs have the potential to be used as effective larvicides against *Ae. aegypti.*

Keywords:- *Biopesticides, Mosquito Larvicides, Biological Control, Dengue Vector Control.*

I. INTRODUCTION

Dengue fever is a significant public health issue in India, affecting millions of people annually. The virus is transmitted by infected Aedes mosquitoes, and India is among the countries most affected by dengue fever, with an estimated 100 million new cases each year worldwide. In India, the first Dengue-like epidemic illness was recorded in 1780 in Madras, and the first confirmed viral Dengue fever was recorded in Calcutta in 1963-1964 (Gupta et al., 2012). In India, 404 species and subspecies of mosquitoes belonging to 50 genera and 2 subfamilies have been identified (Tyagi et al., 2015). These mosquitoes serve as vectors for various parasites that pose a threat to public health. Malaria was once a major concern in India, with 1.6 million cases and 1100 deaths reported in 2009 (Das et al., 2012). However, the number of malaria cases has declined in recent years, and the incidence of Japanese encephalitis (JE) and dengue fever has increased rapidly, with a high case-fatality rate (Dev et al., 2015). In a study conducted in Tamilnadu, India aimed to identify the breeding sites of container-borne mosquito larvae and the dengue vector (Aedes species) in the area, Aedes aegypti (45%) and Aedes vittatus (45%) were the two dominant mosquito species in the area (Rajesh et al., 2013).

Using synthetic pesticides to control mosquito larvae exert negative effects on the environment and human health. Plant extracts have been shown to be a promising alternative to pesticides (Regnault-Roger, 1997). The use of plant extracts to control mosquito larvae dates back to 1933 when Campbell et al. reported the larvicidal properties of plant alkaloids such as nicotine, anabasine, methyl anabasine, and lupinine extracted from the Russian weed Anabasis aphylla against larvae of *Culex pipiens, Culex territans,* and *Culex quinquefasciatus.* Haller (1940) discovered that extracts from Amur cork-tree fruit (*Phellodendron amurense*) had a similar effect on mosquitoes. Wilcoxon et al. (1940) identified filipin, a phloroglucinol propyl ketone toxic to *Cx. quinquefasciatus,* as a toxic constituent of extracts from the male fern *Aspidium filixmas*. Hartzell and Wilcoxon (1941) conducted a comprehensive study on the toxicity of extracts from 150 plant species such as *Amomum krevanh, Carthamus tinctorius, Coriandrum sativum, Eugenia caryophyllata, Illicium vernum, Kaempferia galangal, Murraya paniculata, Myristica fragrans, Ocimum gratissimum,* and *Spilanthes acmella,* for their larvicidal potential against *C. quinquefasciatus*.

Plant essential oils (EOs) are the most preferred phytochemicals to control mosquito larvae. Dias, et al. (2014) reviewed the larvicidal efficacy of 361 EOs from 269 plant species against *Aedes aegypti* larvae. They found that more than 60% of these EOs were active, with the most active ones having effective concentrations comparable to those of temephos, a commonly used insecticide for mosquito control. The most active compounds in these EOs were phenylpropanoids, oxygenated sesquiterpenes, and monoterpene hydrocarbons.

In a study conducted by Lucia (2007), turpentine oil was found to be more effective against the larvae of *Ae. aegypti* than *Eucalyptus grandis* EO, where the latter also gave considerable results. Both EOs were found to possess α-pinene as the principal compound which was thought to be responsible for the larvicidal efficacy. Four EOs from the flora of the Brazilian Legal Amazon were found to be effective against *Ae. aegypti* larvae with lethal concentration

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(LC50) values ranging from 230 to 292 mg/L after 24 hours of exposure. These oils were mainly composed of sesquiterpene hydrocarbons or oxygenated monoterpenes (Dias, et al. 2015). In the present study, the larvicidal efficacy of Thyme, Camphor, and Eucalyptus EOs was investigated against the 4th instar larva of *Ae. aegypti.*

II. MATERIALS AND METHODS

The early instar larvae of *Ae. aegypti* were collected from Sangareddy town. They were identified by Prof. M. Madhavi, Department of Zoology, Osmania University, Hyderabad, Telangana, India. The mosquito larvae were reared on dog biscuits and yeast powder in glass troughs. The fourth instars were used for the larvicidal bioassay. Thyme, Eucalyptus, and Camphor EOs were procured from a local organic shop in Hyderabad. 0.5%, 1%, 2%, and 3% oils were prepared using Tween 80 as an emulsifier. A control solution was prepared excluding essential oils.

The larvicidal experiments were conducted in the Department of Zoology, tara Government Degree and PG College, Sangareddy. Clean plastic bottles were used for the larvicidal bioassays. Three replicates were made for each concentration, along with three control replicates in each experiment. 20 larvae of *Ae. aegypti* were introduced into each replicate and the number of dead larvae was observed for three days after every 12 hours of exposure. Abbott's formula was used to calculate corrected mortality percentages.

Corrected mortality = (% test mortality - % control mortality) / (100 - control mortality x 100).

III. RESULTS & DISCUSSION

The results of the present study are given in Table No. 1 and Figure No.1. All three tested EOs were proved to be efficient against the 4th instar larvae of *Ae. aegypti.* However, thyme EO showed better larvicidal efficacy than the remaining two EOs. In all three bioassays, concentration dependant results were observed. At the maximum tested concentration of 3%, Thyme EO yielded 100% mortality, whereas 94.12% and 88.24% mortalities were observed with Camphor EO and Eucalyptus EO, respectively. At the least tested concentration of 0.5%, Thyme and Camphor EOs showed more than 50% mortality. At the same concentration, Eucalyptus EO efficacy also was close to 50% (47.06%).

The results of the study clearly showed that all three tested EOs are effective against the 4th instar larvae of *Ae. aegypti.* The same results were obtained in other studies also. The EOs of *Syzygium aromaticum* and *Croton nepetaefolius* showed significant larvicidal activity against both *Ae. aegypti* and *Ae. albopictus*, with LC50 values ranging from 32.7 to 81.7 ppm (Rodrigues, et al. 2019). The EOs of Piper betel, a plant native to Indonesia, exhibited promising larvicidal activity against third-instar larvae of *Ae. aegypti,* with LC50 values of 13.1 ppm and 11.2 ppm after 24 and 48 hours of exposure, respectively, against *Ae. aegypti* mosquitoes (Wahyuni, 2012). The leaf and bark essential oils of *Cryptomeria japonica* have the potential as larvicides against *Ae. aegypti* and could be useful in the search for new natural larvicidal compounds (Cheng, et al. 2003).

IV. CONCLUSION

In the present study, the EOs of Thyme, Camphor, and Eucalyptus showed efficient larvicidal properties against the 4th instar larvae of *Ae. aegypti.* At 3% concentration, thyme EO yielded 100% mortality. Camphor and Eucalyptus EOs also gave significant results at the same concentration. at 3% concentration. To know which phytochemicals are responsible for this larvicidal efficacy, further studies are required.

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Table 1. Larvicidal efficacy of different essential oils against the 4th instar larva of *Ae. aegypti*

Conc.	Thyme Oil	Camphor Oil	Eucalyptus Oil
0	0 ± 2.00	0 ± 2.00	0 ± 2.00
0.50%	66.67 ± 3.53	52.94 ± 2.83	47.06 ± 3.33
1%	72.22 ± 3.46	64.71 ± 3.53	58.82 ± 3.53
2%	83.33 ± 1.33	76.47 ± 1.76	70.59 ± 3.46
3%	100 - 1.76	94.12 ± 2.40	88.24 ± 2.00

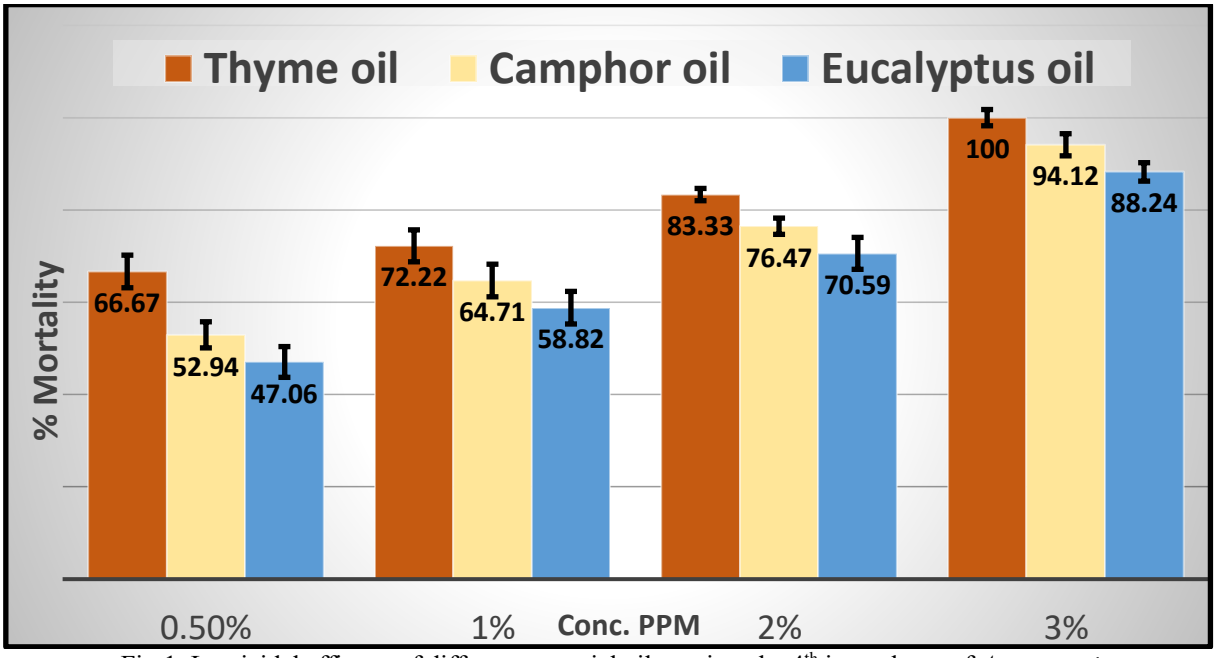

Fig 1. Larvicidal efficacy of different essential oils against the 4th instar larva of *Ae. aegypti*