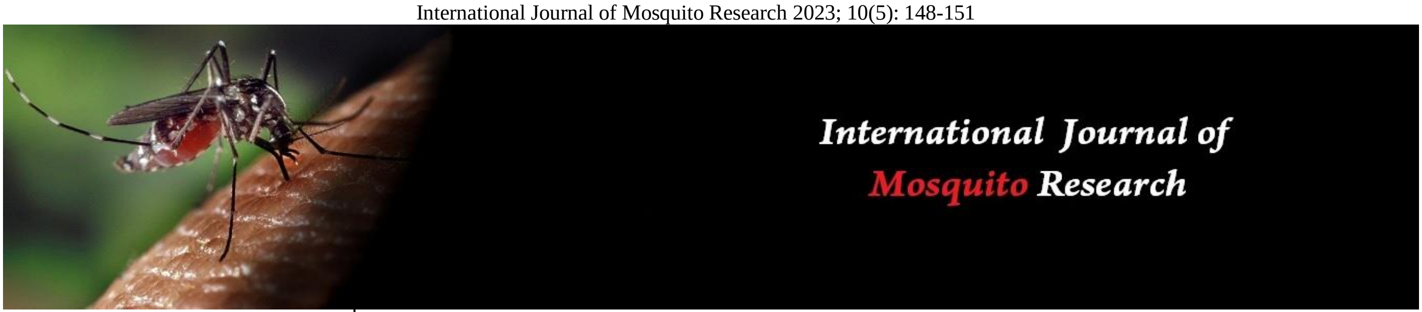


ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2023; 10(5): 148-151

https://www.dipterajournal.com
Received: 05-08-2023
Accepted: 16-09-2023

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# Assessing the effectiveness of bio essential oils on ecologically distinct populations of *Aedes albopictus*

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**DOI:** https://doi.org/10.22271/23487941.2023.v10.i5b.708

**Abstract**
*Aedes albopictus*, a potential vector of dengue and a significant global arbovirus carrier, has seen its geographical distribution expand due to trade and climate change. The accumulation of waste materials in metropolitan areas has provided breeding grounds for this mosquito species. Vector control is crucial in preventing disease transmission since widespread vaccination for vector-borne illnesses, such as dengue, remains inaccessible. However, the indiscriminate use of insecticides in proximity to human habitats has led to the development of insecticide resistance in Aedes albopictus. This study aimed to evaluate the susceptibility of *Aedes albopictus* strains from Barmer and Kota regions of Rajasthan to bio-essential oils, including pine, eucalyptus, and infusion of pine and eucalyptus oils. The results showed that Barmer strain exhibited greater susceptibility to these oils compared to the Kota strain. Pine oil was found to be effective in reducing larval populations, with a 1.19-fold higher susceptibility in the Barmer strain (LC50: 31.458 mg/l) than the Kota strain (LC50: 37.723 mg/l). Eucalyptus oil displayed LC50: 45.920 mg/l for Kota strain while Barmer strain had LC50: 36.763 mg/l. Similarly, infusion of pine and eucalyptus oils had greater efficacy in Barmer strain (LC50: 28.032 mg/l) than the Kota strain (32.197 mg/l). This research emphasizes the importance of tailored vector control measures based on local susceptibility patterns and highlights the potential of plant-based larvicides in disease management. These findings suggest that the Kota region may require more robust vector control strategies than the Barmer region.

**Keywords:** *Aedes albopictus*, dengue, insecticides, susceptibility, vector control

## 1. Introduction

*Aedes albopictus* (Diptera: Culicidae) is the potential vector species of dengue and the most important vector-borne parvovirus in the world. This species was initially considered an endemic species in Southeast Asia. However, its geographical distribution has been dramatically spread due to trade and climate change which opened new ecological niches globally [1]. This species of mosquito is closely associated with the human habitat and lay their eggs in water found around the houses. Their population is more densely populated in metropolitan areas because to the accumulation of tyres and plastic waste, which offers additional breeding grounds. In comparison to the *Aedes aegypti*, *Aedes albopictus* is the more exophilic species [1]. Vector control is the only and best method generally available to control and prevent disease transmission because vaccinations for most vector-borne illnesses, including dengue, are not widely accessible. The routine application of chemical larvicides to breeding sites is the most mainly accepted and commonly used method for controlling the immature stages of *Aedes* species. The NVBDCP data that is currently available indicates that 346 fatalities and 1,93,245 dengue cases have been reported in 2022 (https://ncvbdc.mohfw.gov.in).

In India, Mittal *et al.* (2004) [2] reviewed the resistance status of different species of mosquitoes such as *Anopheles dirus*, *An. fluviatilis*, *An. minimus* etc. and other anophelines as well as *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. vishnui* etc. against various insecticides that indicated development of insecticide resistant to chlorinated and organo – phosphorous insecticides in Bangladesh, Bhutan, India and Nepal.

Apart from this, Raghavendra and Subbarao, (2002) [3] reviewed the resistance status of *An. stephensi* and *An. culicifacies* which showed the development of triple & quadruple insecticide resistance in these species, respectively, against DDT, HCH, malathion and deltamethrin from many parts of India.

One approach to controlling *Aedes albopictus* populations is the use of insecticides, and in recent years, there has been increasing interest in the development of environmentally friendly and sustainable alternatives [4]. Essential oils derived from plants, such as pine oil and eucalyptus oil, have demonstrated larvicidal properties against mosquito species [4, 5]. These oils are considered safe for the environment and human health, making them attractive options for mosquito control in different ecological settings [4, 5].

Pine oil, extracted from the Pinus genus, is known for its historical use in repelling insects. Eucalyptus oil, derived from Eucalyptus species, possesses aromatic and medicinal properties. Both oils have shown potential for mosquito larval control in various studies [6-9].

However, there is no record on susceptibility status of *Aedes albopictus* from Barmer and Kota area against insecticides so far. Therefore, in context of this, the present study evaluated the susceptibility status of *Aedes albopictus* strain collected from Barmer and Kota areas of Rajasthan against bio-essential oils (pine, eucalyptus, and infusion of pine + eucalyptus). We believe that the finding of this study would be helpful in better planning of control operations from diseases transmission in those areas.

## 2. Materials and Methods

### 2.1 Mosquito collection

In September 2020, a research study was conducted in the regions of Kota and Barmer in Rajasthan, India. The study involved the collection of *Aedes albopictus* mosquito larvae from cement tanks located in the yards of houses in these regions. Specifically, larvae were collected from 21 houses in Kota and 15 houses in Barmer.

The collected mosquito larvae were then transported to the Department of Zoology at Career Point University in Kota, Rajasthan, for further experimentation.

### 2.2 Mosquito rearing and larvicides

The collected *Aedes albopictus* mosquito larvae were reared under controlled laboratory conditions at 26±2 °C, 65±5% relative humidity (RH), and light: dark (LD) 12:12 h photoperiod. The larvae were reared in plastic containers with a capacity of 1 litre, measuring 18 cm in length and 7 cm in diameter. These containers were filled with 500 mL of dechlorinated water, and approximately 100 mosquito larvae were placed in each container. To provide nourishment for the larvae, dried Brewer's yeast was added to the water at a rate of 15 mg per 500 mL daily. The water in which the larvae were reared was refreshed on alternate days to maintain a clean and suitable environment. Third instar larvae of *Aedes albopictus* were used for the susceptibility experiment for both (Kota and Barmer) the strain. For larval bioassay pine, eucalyptus and infusion of pine + eucalyptus was used. Pine and eucalyptus bio essential (BEO) were obtained by local supplier, Barlani & Sarandhana Industries, Jhalawar, Rajasthan.

### 2.3 Development of larvicides and bioassays

The larval mortality bioassays were carried out according to the test method of larval susceptibility as suggested by the World Health Organization [10]. The Emulsifiable concentrate (EC) were prepared for BEOs i.e., pine, eucalyptus, and mix (Pine + Eucalyptus) oils. The active oil (pine and eucalyptus) was comprised of 50% (V/V) of the total formulation which was mixed with carrier (refined oil), surfactants, stabilizers, and anti-oxidants in certain proportions. An 1% stock solution and serial dilution of BEOs were performed in distilled water. Each bioassay tested 4-5 doses ranging from 20 – 80 ppm (BEOs). The control cups were not treated with any chemical for all larvicide evaluation. The 3rd instar larvae of *Ae. albopictus* from Kota and Barmer city were used for evaluation of efficacy of above larvicides. During the experiment, larvae were kept under standard laboratory conditions at 26±1 °C, 65±5% relative humidity (RH) and light: Dark (LD) 12:12 h photoperiod. The experiment was conducted with three replicate and repeated for three times. (Fig 1).

**Fig 1:** Susceptibility experiment against Bio essential oils

### 2.4 Data analysis

The data of various larvicidal bioassays from different replicates were pooled dose wise. Larvicidal effect was recorded 24 h after the treatment. Data obtained from each dose larvicidal bioassay were subject to probit analysis using PASW Statistics V. 18 software. The transformed probit – mortality was estimated against log10 transformed dose: LC50, LC90 and LC99 values, and slope was generated. Chi square value, degree of freedom (DF) and Pearson Goodness-of-Fit test (p) values were also estimated for all the larvicides against *Aedes albopictus* larvae from Kota and Barmer region. The mortality in control was below 5%, hence, no correction factor was used to adjust the treatment mortality.

## 3. Results and Discussions

The results indicated that Barmer strain of *Aedes albopictus* showed more susceptibility against all three bio-essential oils pine, eucalyptus, infusion oil (pine + eucalyptus) in comparison to the Kota strain after 24 hours exposure.
The LC50 value of Barmer strain (31.458 mg/l) against pine oil ranged from 30.014 mg/l to 32.886 mg/l whereas Kota strain (37.723 mg/l) showed ranges from 35.849 to 39.633 mg/l. This indicates the 1.19 folds more susceptibility in Barmer strain than the Kota strain (Table 1).
Furthermore, Kota strain showed LC50: 45.920 mg/l against eucalyptus with ranged from 43.095 to 48.795 mg/l while Barmer strain had LC50: 36.763 mg/l with 34.704 – 38.676 mg/l ranges. This showed the wide range (36.763- 45.920 mg/l) of LC50 indicating increase in resistance from 1.24 folds in comparison to the Barmer strain (Table 1).
Efficacy of infusion oil composition of pine and eucalyptus bio-essential oils was found significantly different for Kota and Barmer strain. Barmer strain has LC50: 28.032 mg/l with ranged from 26.311 – 29.675 mg/l whereas Kota strain had LC50: 32.197 mg/l (30.151 - 34.203 mg/l). This showed the 1.14-fold more efficacy in Barmer strain (Table 1).
A key tactic for reducing the spread of vector-borne diseases including chikungunya, dengue, and zika is the management of the mosquito larval population. The management of vectors depends critically on understanding the processes by which mosquito vectors resist various chemical classes [11]. Active community participation, health education, medication administration, environmental sanitation, and vector suppression have all been recommended by WHO, (2005) [10] as ways to reduce the disease caused by *Aedes albopictus* as well as vector transmission at various levels.
The tree *Pinus longifolia*, also known as pine, belongs to the Pinaceae family and produces an oil that has long been used to ward against mosquito bites. In some rural areas of India, it is also utilized as an herbal medication. In the present study, Barmer strain of *Ae. albopictus* has shown 31.458 mg/l of $LC_{50}$ value against pine oil which is 1.19 times less than the Kota strain ($LC_{50}$: 37.723 mg/l). In the previous study, Ansari *et al.*, (2005) [6] also used pine oil as larvicidal and repellent activity. They improved the efficacy of pine oil after diluting with acetone for the treatments showed $LC_{50}$ as 82.1, 85.7 and 112.6 ppm against *Ae. Aegypti*, *Cx. quinquefasciatus* and *An. stephensi*, respectively. In another study, Mukhtar *et al.*, (2015) [7] reported pine oil efficacy against *Ae. Aegypti* larvae with 0.089 ml of $LC_{50}$ value after 24 hours of exposure.
Eucalyptus oils are obtained by hydro distillation of the leaves of *Eucalyptus* and have an aroma characteristic. Larvicidal bioassay showed that eucalyptus showed an $LC_{50}$ of 36.763 ppm for Barmer strain and 45.920 ppm for Kota strain. This range (36.763 – 45.920 ppm) of eucalyptus oil is higher than the finding reported by Lucia *et al.*, (2007) [8]. Lucia *et al.*, (2007) [8] reported $LC_{50}$ of 32.4 ppm against *Ae. Aegypti* larvae. Similarly, Kocher and Riat (2019) [9] also used *Eucalyptus globulus* oil against 4th instar larvae of *Anopheles stephensi* in India. They applied four different treatments i.e., 50, 60, 70, 80, 90, 100 mg/l and found 46.11 ppm and 69.33 ppm was the $LC_{50}$ and $LC_{90}$ respectively.
Besides the use of individual oil formulation, we have also used the combination of pine and eucalyptus oil (1:1) to estimate and compare the efficacy with individual oil application. The present study observed the 28.032 ppm (Barmer) and 32.197 ppm (Kota) of $LC_{50}$ value. This indicates the combination of pine and eucalyptus produced higher mortality in comparison to the eucalyptus ($LC_{50}$: 36.763 – 45.920 ppm) and pine ($LC_{50}$: 31.458 – 37.723 ppm). These findings would be helpful in making efficient plant based larvicides.

**Table 1:** Probity mortality and log dose (mg/l) of Kota and Barmer strain of *Aedes albopictus against* various bio essential oils

| Bio essential oils | Population | LC50 | LC90 | LC99 | $\chi^2$ | *DF* | *P* | Slope |
|---|---|---|---|---|---|---|---|---|
| Pine | Barmer | 31.458 (30.014 – 32.886) | 53.920 (50.690 – 58.035) | 83.664 (75.724 – 94.633) | 39.090 | 43 | 0.642 | Y = 5.476X-8.202 |
| | Kota | 37.723 (35.849 – 39.633) | 73.305 (67.755 – 80.638) | 125.997 (110.819 – 147.828) | 42.371 | 43 | 0.498 | Y = 4.442X-7.003 |
| Eucalyptus | Barmer | 36.763 (34.704 – 38.676) | 65.153 (60.737 – 71.190) | 103.884 (91.958 - 122.033) | 15.945 | 34 | 0.996 | Y = 5.157X-8.072 |
| | Kota | 45.920 (43.095 - 48.795) | 101.671 (90.056 - 119.862) | 194.368 (157.833- 259.510) | 5.270 | 34 | 1.00 | Y = 3.712X-6.170 |
| Infusion Pine + Eucalyptus | Barmer | 28.032 (26.311 – 29.675) | 45.566 (42.366 - 49.952) | 67.709 (60.230 - 79.213) | 70.629 | 43 | 0.005 | Y = 6.074X-8.793 |
| | Kota | 32.197 (30.151 - 34.203) | 56.397 (51.974 - 62.491) | 89.068 (78.101 - 105.957) | 72.149 | 43 | 0.004 | Y = 5.264X-7.938 |

## 4. Conclusion

The susceptibility experiment showed more resistance in Kota strain against BEOs (pine, eucalyptus, and pine + eucalyptus infusion) than the Barmer strain. This indicated the Kota region needs better vector control practices than the Barmer region.

## 5. Conflict of interest

All the authors declare no conflict of interest.

## 6. Author contribution

SC -experimentation, data acquisition, analysis, designing and writing original draft
SJ - conception, designing and writing original draft

## 7. Acknowledgment

The author thanks the Director, Career Point Uni versity, Kota for approval and for providing the facility required for the study. The author acknowledges the staff members of Career Point University, Kota.

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