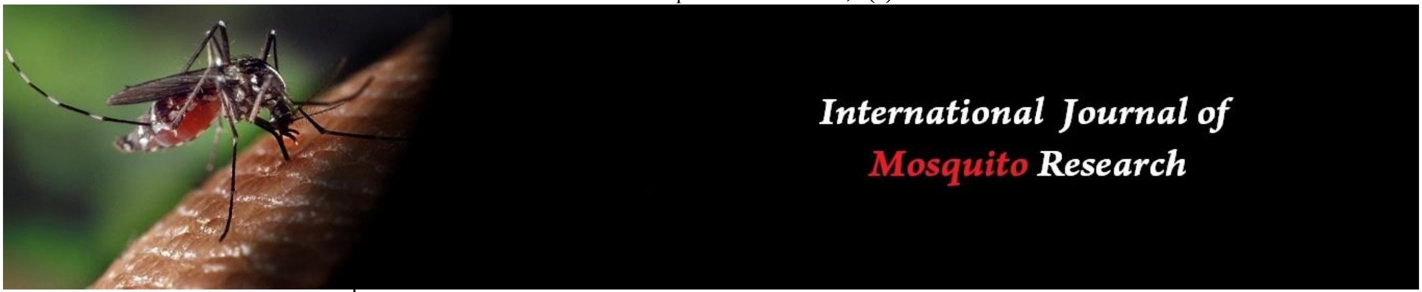

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Surveillance of dengue vector *Aedes aegypti* using infusion baited ovitraps at Ernakulam in Kerala, South India

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Abstract
We used ovitraps, to monitor *Aedes aegypti* at Ernakulam district in Kerala, South India in 2017-18. The average proportion of ovitraps with mosquitoes – the ovitrap positivity index (OPI) and the Egg density index (EDI) were 96.3 and 29.41 respectively. This study provides the reason for the optimization of ovitraps as monitoring tools for *Aedes* sp.; supporting the use of infusion baited ovitraps with cumin seeds and sugar and specific coloured containers to increase the attractiveness of adult mosquito females to lay eggs. Our survey also indicated that air temperature and precipitation are related to an increase in egg count in ovitraps. Due to the direct impacts on offspring survival and development, the choice of oviposition site by mosquitoes should be considered while designing vector control programs.

Keywords: *Aedes aegypti*, dengue, ovitrap, Kerala

1. Introduction
Dengue is an infectious disease caused by an arbovirus of the genus Flavivirus and family Flaviviridae that infects humans through the bite of *Aedes aegypti* Linnaeus (Diptera: Culicidae) female mosquito [1]. It is also transmitted by *Aedes albopictus* Skuse (Diptera: Culicidae). This is a disease of concern as there is no specific antiviral drugs or vaccine. Kerala is a place where there is a large prevalence of dengue cases [2]. The Directorate of health services of Kerala has reported 21,993 dengue fever cases and 165 deaths in 2017 and 3,834 cases and 32 death cases in 2018. Ernakulam district in the state, regarded as the industrial hub with 3.2 million people, has reported 494 dengue cases in 2017 (2.25% of total cases in Kerala) and 177 cases in the year 2018 (4.34% of total cases in the state). In the past 5 years, no deaths due to dengue were recorded in the district. But in 2019, the number of dengue cases spiked to 423 from 177 in 2018. While one person died in 2019, no deaths were recorded in 2018 [1, 3-4].
In a tropical region like Kerala, the dengue outbreak is mostly seasonal. Monsoon lashing over the state for six months from June-November provides a conducive environment for the breeding of these disease-causing mosquitoes. An increase in vector population can also be attributed to unplanned urbanization and climate change. Another possible reason is the habit of storing water in large containers and stagnant rainwater collected in discarded containers [5]. Extensive breeding was reported in containers that were discarded in the open which fills up during the monsoon season [6, 7]. *A. aegypti* also oviposit in tree holes where a small amount of clean water and organic matter is present [8].
The monitoring of *Aedes aegypti* can be done through ovitraps, where the female mosquito lays eggs. Ovitraps are useful in assessing the breeding and dispersal of local *A.aegypti* populations [9]. The content and colour of ovitraps attract or deter gravid female mosquitoes [10, 11]. However, there is only limited information about the potential of using household substances for preparing infusions in ovitraps to enhance the efficiency of these traps. Our objective was to test under field conditions whether some small modifications in the ovitrap could change the egg catching capability of these traps. In the absence of a vaccine against the dengue virus, the control of *Aedes* mosquito is the only tool for limiting the disease. The involvement of the community and their role in vector control is particularly important. Thus, mapping the distribution of *Aedes aegypti* is essential for public health planning and prevention of dengue.

2. Materials and Methods

This study was conducted from July 2017- March 2018 in urban peridomicile areas (5 m from the house) at Aluva (10.1077° N, 76.3593° E) in Ernakulam district of Kerala, South India. The population of the dengue vector was monitored using ovitraps - traps used for collecting mosquito eggs. Ovitraps consisted of a plastic container with 10 cm height and 8.5 cm diameter and a wooden paddle of 12.5 cm long and 2.5 cm wide used as a substratum for oviposition of mosquitoes. Dry white towel was wrapped on to the paddle to identify mosquito eggs when laid on the surface (Fig.1). To study the effect of infusion based ovitraps, substances such as Cumin (4g), sugar (4g), vinegar (20ml), and salt (4g) in 100 ml of water were provided in triplicates in 300ml ovitrap bowls for oviposition. Ovitrap with dechlorinated tap water was kept as control. Three different coloured ovitraps- black (control), red and transparent were selected for the study. These traps were used to find out the effect of colour on the oviposition response of *Aedes* mosquito. The traps were checked daily for egg counts. The temperature and humidity of the area were also noted (Table 1). To prevent the hatching of adult mosquitoes, containers that had larvae were overturned and destroyed. After three days of each trial, the cloth of the paddle was removed and disposed of properly. The ovitraps were also cleaned. Each ovitraps was then refilled with water and a new paddle was used each time.

Table 1: Mean air temperature and humidity recorded in the ovitrap study sites from July 2017- March 2018

Month	Mean	
	Temperature (◦C)	Humidity (%)
July	26	85
August	26.2	85
September	26.2	80
October	26.6	84
November	27.1	80
December	26.6	74
January	32	64
February	32	63
March	33	70

864 ovitraps were installed, Egg density index (EDI) and Ovitrap positivity index (OPI) were calculated to identify the periods of higher and lower reproduction of females[12]. Ovitrap positivity Index (OPI)= NPT/NPE X 100; NPT= Number of Positive traps and NTE= Number of Traps examined. Egg density Index (EDI)= NE/NPT; NE= Number of Eggs, NPT= Number of positive traps. To compare the oviposition response using different coloured ovitraps and to compare the egg counts obtained using different infusions; an independent One-Way ANOVA test was used. Spearman correlation analysis was used to check the influence of temperature and humidity on egg count. P<0.05 represented a significant difference. All the statistical analysis was done with XLSTAT 2020.3.1 Software.

Fig 1: Cloth paddle with eggs of *Aedes aegypti* mosquito

3. Results & Discussion

A total of 864 ovitraps were observed during the study period, of which 798 (92.36%) were found to be positive. Ovitrap positivity and Egg density index has been widely used to monitor the breeding habitat of the *Aedes* mosquito. For the entire study habitat, OPI and EDI were found to be 96.3 and 29.41 respectively. Both indices were high during the monsoon month of November and low during the summer month of March (Fig. 2). High values of OPI indicates the region as a high-risk area for dengue outbreak [13]. The use of ovitraps with infusions enhanced the effect of the trap, attracting female mosquitoes whereas certain substances served as deterrents [9, 11, 14]. Maximum number of eggs were noted in ovitraps with cumin solution (OPI=100%, EDI=56.26), followed by sugar solution (OPI=100%, EDI=52.06). Ovitraps with vinegar and salt solution showed 0% oviposition exhibiting the deterrent effect (Fig. 3). One-way ANOVA analysis confirmed the significant differences in egg count in response to different infusions in the ovitrap (R^2= 0.81, F= 195.6, P<0.05). Studies conducted by Tilak, *et al*. [11] observed positive oviposition in ovitraps baited with Cumin seeds and deterrence in traps baited with fenugreek seeds, curry leaves, hibiscus, radish. Deterrence shown by gravid *Aedes* to ovitraps baited with vinegar and salt is well documented and this study too supports the findings. Reze, *et al*. [15] proved the efficiency of copper-deposited ovitraps in killing the larvae of *Aedes* sp. mosquito. We can increase the effectiveness of these simple larvicidal tools by using household substances which served as attractants in the ovitraps [16, 17]. The environmental safety of larvicidal components should be considered while incorporating them in ovitraps. Further studies should be conducted to document the active ingredients of these infusions which could be used as deterrents in water containers/pots. Previous studies have demonstrated that comparatively much greater egg yield was reported on cotton fabric as substrate[8] than using wooden paddles alone [18].

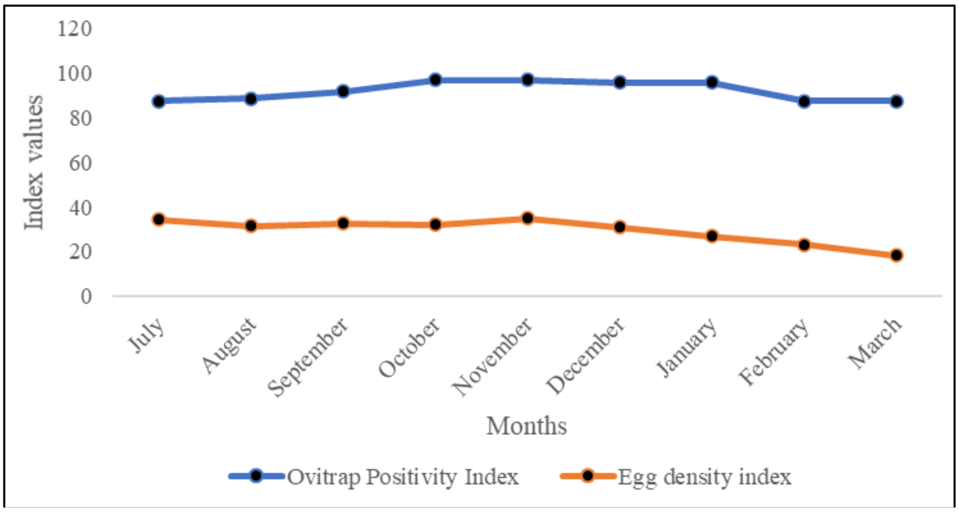

Fig 2: Monthly variation in the values of Ovitrap positivity index (OPI) and Egg density index (EDI)

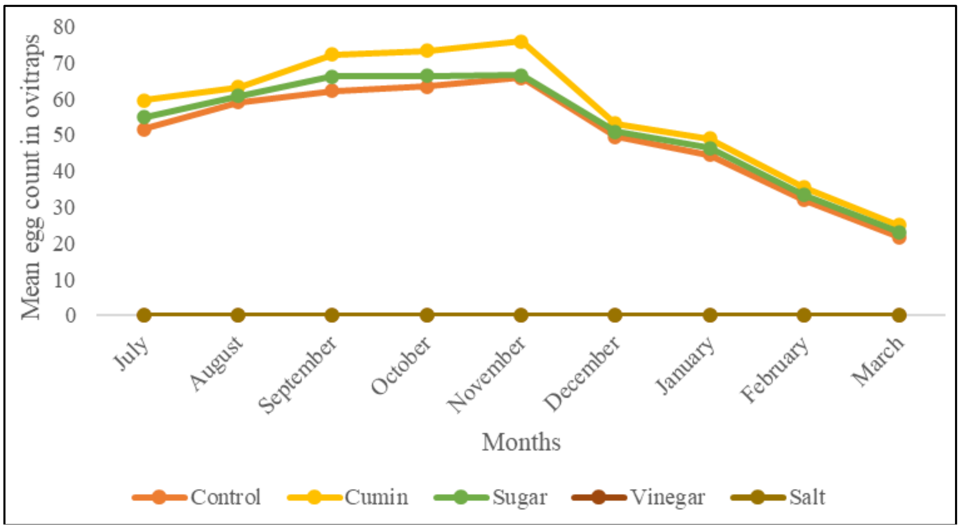

Fig 3: Egg count of *Aedes aegypti* in infusion baited ovitraps

When the results from coloured containers were subjected to ANOVA analysis, they showed significant difference (R^2= 0.46, F= 44.96, P<0.05). Red coloured containers showed an OPI of 98.14%, followed by black (94.4%) and transparent (43.5%). Studies done by Kumavat, *et al.* [16] in Rajasthan households in North India also supports these facts. The efficiency of different coloured ovitraps is summarized in Fig. 4.

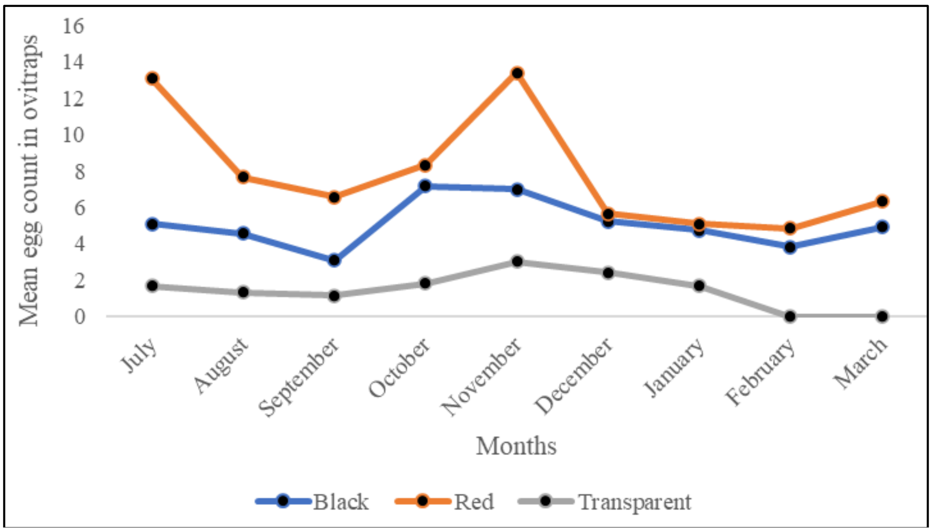

Fig 4: Egg count of *Aedes aegypti* in different coloured ovitraps

Air temperature and precipitation play an important role in the maturation of eggs in adults [19, 20]. We did Pearson's correlation analysis to find out the effect of air temperature and humidity on the number of eggs found in the ovitraps. Results showed that there existed a positive correlation between egg numbers and humidity (r= 0.637, *P*<0.05) whereas with an increase in temperature number of eggs observed in the ovitraps showed a sharp decline (r= -0.792, *P*<0.05). Many studies have reported a strong and positive correlation between rainfall and temperature with the increase in the number of eggs and larvae [7], whereas Ho, *et al*. [21] reported no direct relationship.

4. Conclusion
Efforts to prevent the outbreak of dengue largely depend on vector control. As ovitraps provide a remarkably simple and cost-effective method to examine these mosquitoes, incorporating factors that modify the effectiveness of these ovitraps should be considered. This study recommends the use of infusions such as sugar and cumin in the ovitraps to increase the sensitivity of these traps. Further research is needed to identify the volatile compounds which acted as attractants in these infusions. We also found that dark colour containers when used as ovitraps helps in collecting more *A. aegypti* eggs.

5. Acknowledgements
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