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Evaluation of leaf infusions mediating oviposition in *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae)

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Abstract
Oviposition choice is a well-studied aspect of mosquito life cycle and effects a potential venue for species-specific surveillance and control. In the absence of standardized controlled release lures of known composition, infusions remained the most practical means for baiting gravid female traps. This technique has been further improved by adding plant leaf infusions in different strengths to enhance its attractiveness to ovipositing mosquitoes. In the present study, the ovipositional rate of *Aedes aegypti* in ovitraps treated with infusion of *Azadirachta indica*, *Hevea brasiliensis* and *Ocimum tenuiflorum* leaves was assessed. The results revealed that in all the three plant infusions, there was reduction in the number of eggs laid as the concentration increased and the average number of eggs laid in ovitraps with leaf infusions of *Azadirachta indica*, *Hevea brasiliensis* and *Ocimum tenuiflorum* at concentrations of 30, 50 and 100% were 76.2 ±6.02, 43.6 ±9.09 and 17.8 ±3.97; 80.2 ±8.46, 42.0 ±9.33 and 17.6 ±2.86; 62.0 ±2.81, 49.4 ±2.42 and 21.8 ±4.08 respectively. The present study demonstrated the potential of leaf infusions in stimulating oviposition by mosquitoes in ovitraps. Moreover, leaf infusions represent a new alternative for use in oviposition traps and may expand its usage in homes and consequently assist in the monitoring and management of *Aedes aegypti* population.

Keywords: *Aedes aegypti*, ovipositional rate, *Azadirachta indica*, *Hevea brasiliensis*, *Ocimum tenuiflorum*, leaf infusions

1. Introduction
Epidemiologically, gravid females are the most important component of mosquito population and are targeted in mosquito reduction programs and in active surveillance of disease for early detection of epidemic events. Mosquito ovitraps are a current trend as integral components of surveillance as they contribute to monitoring of mosquito population. *Aedes aegypti*, the dengue vector breeds in assortment of domestic and artificial containers. This container dwelling mosquito provide themselves as useful study organisms for researches because of their ecological and medical importance. Oviposition by cues is a complex of responses resulting in a well-defined spatial distribution of a population [1, 2]. Oviposition choice is a well-studied aspect of the mosquito life cycle and effects a potential venue for species-specific surveillance and control. In recent years, ovitrap surveys for monitoring the *Aedes aegypti* population have found greater acceptability, as they have been found to be sensitive even at times when vector densities were at low levels [3-5]. These traps enabled workers to establish indices of ovitrap positivity and egg density to indicate, respectively, the extent and intensity of the vector prevalence. In the absence of standardized controlled release lures of known composition, infusions remained the most practical means for baiting gravid female traps [6]. This technique has been further improved by adding plant leaf infusions in different strengths to enhance its attractiveness to ovipositing mosquitoes [7, 8]. This method not only yielded higher number of eggs, but its attractiveness remained unchanged despite seasonal variations [7]. Keeping in view of the above mentioned factors, the present work was under taken to evaluate the ovipositional rate of *Aedes aegypti* in ovitraps treated with infusion of *Azadirachta indica*, *Hevea brasiliensis* and *Ocimum tenuiflorum* leaves.

2. Materials and methods

The experimental study was conducted from June to July, 2019 at Poovancode which is situated 30km away from Nagercoil, Kanyakumari district, Tamil Nadu, India. This study site was selected based on the adult density of *Aedes aegypti*. The ovitraps consisted of one litre transparent round plastic containers of 12cm length and 9.5cm width. The oviposition substrata was 12x2cm strip of a 9mm plywood wooden paddle covered by 9x2cm strip of Whatmam No. 1 filter paper fixed by a rubber band at one end and then paced vertically inside the container. The organic infusion of plant leaves was used as oviposition attractant (250mL/trap). The leaves of each plant were cut into small pieces and were used for the preparation of infusion after shade drying for 2-3 days. Infusions prepared from 150g of each dried leaves were placed in bottles containing distilled water (2L) for seven days in anaerobic condition. The resulted infusion was then diluted in distilled water to 30, 50 and 100% concentrations. Distilled water infusion served as control. All ovitraps were thoroughly rinsed with de-ionized water to remove any organic matter before replacing it with fresh infusion as and when necessary during the study period. Five experimental trials were performed in the infusions of each plant leaf to observe ovipositional rate of *Aedes aegypti* for a duration of seven days. Data obtained were subjected to statistical analysis wherein comparison between the best concentrations and oviposition rate between different infusions were performed.

3. Results

The number of eggs laid in the control ovitrap in the five trials were 126, 73, 90, 103 and 87 respectively and the mean value was 95.8 ±10.0. The respective number of eggs laid at concentrations of 30, 50 and 100% in *Azadirachta indica* leaf infusions were 97, 83, 78, 69 and 54; 53, 42, 70, 33 and 20; 17, 26, 13, 18 and15. In *Hevea brasiliensis*, it was 105, 54, 86, 81 and 75; 28, 37, 72, 49 and 24; 25, 21, 14, 10 and 18 and for *Ocimum tenuiflorum*, it was 48, 72, 83, 78 and 29; 43, 37, 59, 66 and 42; 23, 16, 27, 23 and 20. The average number of eggs laid at concentrations of 30, 50 and 100% in *Azadirachta indica*, *Hevea brasiliensis* and *Ocimum tenuiflorum* leaf infusions was found to be 76.2 ±6.02, 43.6 ±9.09 and 17.8 ±3.97; 80.2 ±8.46, 42.0 ±9.33 and 17.6 ±2.86; 62.0 ±2.81, 49.4 ±2.42 and 21.8 ±4.08 respectively (Figure 1). Data subjected to statistical analysis revealed that in all the three leaf infusions experimented, more number of eggs were found to be laid in 30% concentration and the egg count was low at the highest concentration. Oneway ANOVA indicated significant difference in all three infusions at P=0.05 level and F value for *Azadirachta indica*, *Hevea brasiliensis* and *Ocimum tenuiflorum* were 0.241, 0.255 and 0.221 respectively.

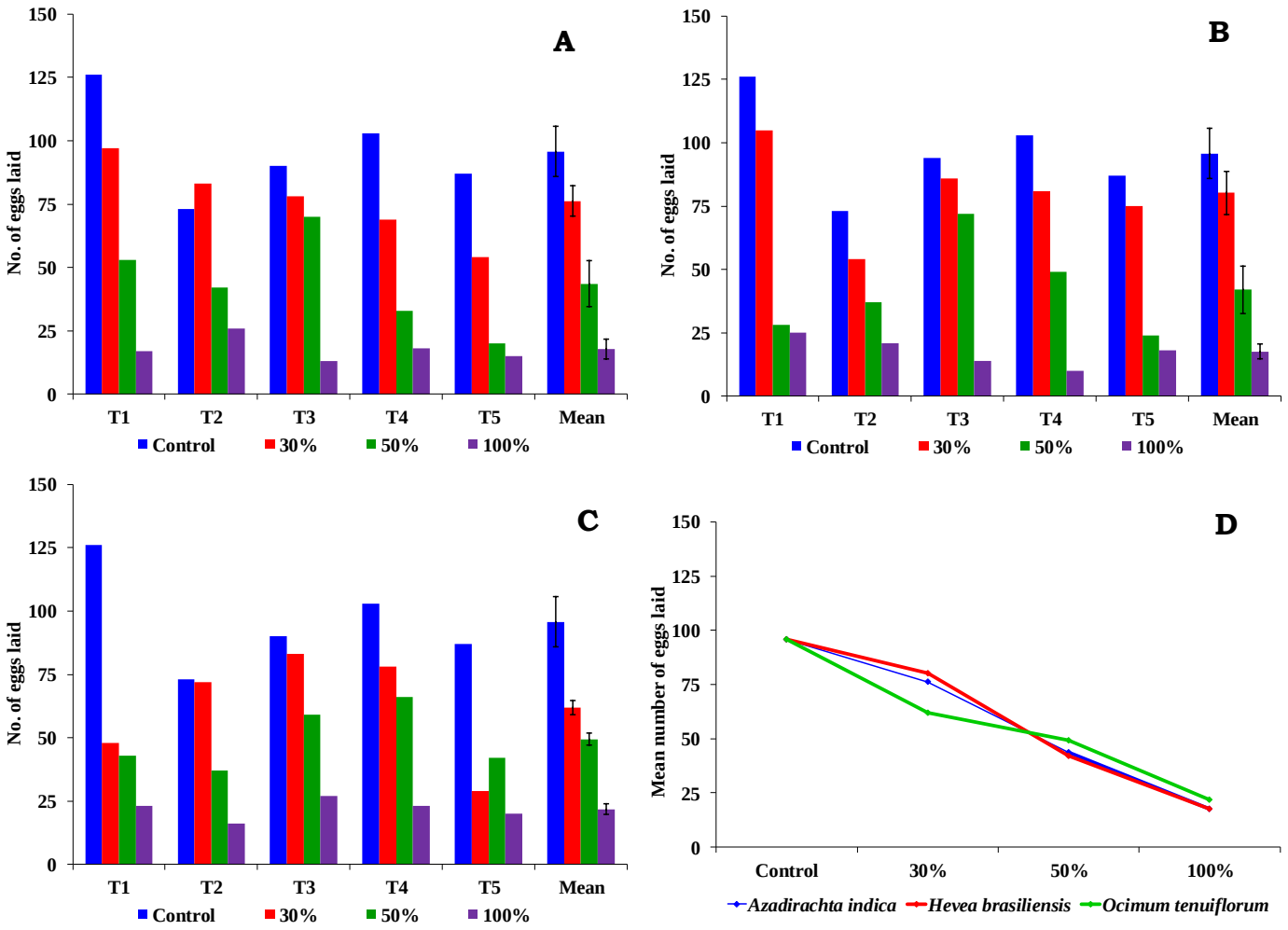

Fig 1: Ovipositional rate of *Aedes aegypti* on leaf infusions. A: *Azadirachta indica*; B: *Hevea brasiliensis*; C: *Ocimum tenuiflorum*; and D: Comparative data

4. Discussion

Oviposition habitat selection is influenced by a diversity of chemical, physical and physiological factors. Once attracted to the oviposition site, gravid females use visual (colour, texture, brightness), and olfactory cues (semiochemicals) to decide the suitability of a potential habitat for egg laying [9]. The association of plants with mosquito oviposition and larval habitats has been characterized for a wide range of mosquito species with plants providing habitat (phytotelmata), air, shelter, or nutrition associated with microbial activity [9-12]. Chemosensory cues used for location of resources may be influenced by the presence of microbial fauna often interacting with plant material [13] or plant odours [14-16]. A wide range of mosquito species, *viz*., *Aedes albopictus*, *Aedes triseriatus* [17], *Aedes aegypti* [3], *Culex nigripalpus* [18] and *Culex quinquefasciatus* [19] are attracted to hay and grass infusions for oviposition. Infusions made from a variety of grasses [3, 7, 8, 20, 21] and from oak leaves [17, 22] have been used in ovitraps for monitoring the egg-laying activity of container-inhabiting *Aedes* mosquitoes in the field. Other organic materials, such as leaves, grass, sod, and pelletized plant-based animals feeds, have been fermented to create infusions that are attractive to gravid *Aedes* mosquitoes [3, 7, 8, 17, 23, 24].

Laboratory and field studies have demonstrated that *Aedes albopictus* lays significantly more eggs in ovitraps containing white oak leaves [17], maple leaves [25], guinea grass [21], and Bermuda grass [26] than in water only controls. Gravid trap studies using red oak leaf baited infusions have also reported greater captures of adult *Aedes albopictus* compared with standard hay infusions [27]. Although these studies have shown enhanced oviposition by *Aedes albopictus* using plant infusions, there is limited information on attractiveness of infusions utilizing leaf substrates to mosquitoes in general and *Aedes aegypti* in particular. Ovitraps with rubber leaf infusions recorded maximum number of eggs than ordinary tap water. Sumodan [28] found rubber plantations as potential breeding ground for *Aedes* mosquitoes as it provides a canopy and dense vegetation for its survival. This information corroborates with the oviposition rates of *Aedes aegypti* in the present study and thereby indicate rubber leaf infusions to be more attractive than ordinary water. Different oviposition rates in the present study confirm these concepts. Santos *et al.* [29] and Trexler *et al*. [30] reported that higher oviposition rates were found for cashew leaf and grass infusions and they differed significantly from distilled water, thereby lending support to the hypothesis tested by other authors that leaf infusions are more attractive to *Aedes aegypti* than water [3]. The present study found that the ovipositional rate of gravid *Aedes aegypti* females was high in low concentrations and vice-versa. Reiter *et al*. [7] also recorded significantly more *Aedes aegypti* eggs in ovitraps containing a lower concentration of hay infusion when compared to a higher concentration. This may be attributed to the fact that leaf infusions contains a complex mixture of compounds affecting not only mosquito oviposition performance, but oviposition site selection also by gravid females [31]. The potential attraction of infusions is highly influenced by the type [32], and concentration [7, 23] of organic matter. There are a number of variables that may alter the degree of infusion attractiveness. Protein concentration and bacteria levels are known to transform an infusion from an attractant to a repellent [33]. The next factor that may change the attractiveness of an infusion is the duration of fermentation. Sant'ana *et al*. [21] demonstrated that *Aedes albopictus* females were most attracted to guinea grass infusions fermented for 15 to 20 days compared with those fermented for 30 days. Finally, the stage at which the leaves are used may produce different levels of chemical cues. Sant'ana *et al*. [21] also demonstrated that *Aedes albopictus* deposited more eggs in infusions made from fresh guinea grass leaves than from dried leaves. Optimally attractive infusions for *Aedes aegypti* and *Aedes albopictus* required fermentation periods of different lengths depending on the plant species. Similar results were reported by Isoe *et al*. [34] for response of *Culex quinquefasciatus* and *Culex tarsalis* to Bermuda grass infusion. *Culex quinquefasciatus* continued to respond significantly to Bermuda grass infusions that were fermented over a longer period of time compared to *Culex tarsalis*, which preferred infusions that were fermented over a shorter period. Sant'ana *et al*. [21] found that *Aedes* mosquitoes exhibited the highest oviposition responses to 15-20 day old infusions produced from the grass thereby stating that ovitraps containing younger or older infusions received lower numbers of eggs.

Plant extracts contain various chemicals, particularly hydrocarbons and fatty acids that are directly and indirectly involved in the oviposition process of mosquitoes and have been reported to play a major role [35,36]. A variety of plant species and plant-associated materials have been used to produce organic infusions for investigating the oviposition behavior of mosquitoes or for monitoring oviposition activity in the field. Organic infusions have successfully been used in ovitraps for surveying populations of *Aedes aegypti* [7], and its application as oviposition attractants may serve as potential control measures. The use of organic and plant infusions as attractants in ovitraps for gravid *Aedes aegypti* females has been extensively reported [23, 29, 30]. Organic infusions, commonly developed from a range of fermented plant material to animal waste products, are frequently used to increase the attraction of gravid mosquitoes to ovitraps and gravid traps [37]. Infusions release volatile chemicals which act as chemical cues for gravid mosquito and help in selection of oviposition sites. The attractiveness of organic infusions are influenced by the process of bacterial growth, with subsequent secondary metabolite production [19, 38]. The significant variation in oviposition found within and between the different infusions tested may be also affected by specific active compounds in the infusions and considered another source of attractant. It should be noted that attraction of gravid females to odorants that emanate from a plant infusion might not result in increased oviposition because volatile chemicals that attract females may not necessarily function also as oviposition stimulants. However, some species of cultivable bacteria in bamboo leaf infusion produce metabolites that attract gravid females and also stimulate them to lay eggs [39]. It has been reported that plant materials, near the water resources get fermented and released various types of volatile chemicals which influence the female gravid mosquitoes for oviposition [40]. The metabolic products formed by the microbial decomposition of organic matter present in water, attract the gravid mosquitoes, by communicating to female with reference to suitability of site and food availability for its next progeny [21, 39]. Additional laboratory or field studies on the chemical and microbial properties of plant leaf infusion could serve as a stimulant for selecting traps as egg laying sites. Therefore, as suggested by Santos *et al.* [29], leaf infusion represents a new alternative for use in oviposition traps and

offers the advantage of having a squalid odour which may expand its usage in homes and consequently assist in the monitoring and management of *Aedes aegypti* population.

5. Conclusion

The present study demonstrates the potential of plant infusions in stimulating oviposition by *Aedes aegypti* mosquitoes in ovitraps. Manipulating the oviposition behaviour of mosquito is a useful tool in determining the preference for oviposition sites by the gravid females and further application of leaf infusions as an attractant with combination of some lethal substance for control of other container breeding mosquito species could be used as a vital strategy in integrated pest management.

6. References

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