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Full Length Research Article

Influence of Container Colour and Leaf Infusion on the Ovipositional Preference of *Aedes albopictus* Skuse 1894 (Diptera: Culicidae)

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Abstract

Oviposition studies using ovitraps are proven advantages in vector surveillance, because they are used to monitor mosquito species as well as pre and post density counts, and has been used to identify areas with high concentrations of vector breeding based on egg density index from unexposed breeding sites and surrounding areas. In the present study, the ovipositional preference of *Aedes albopictus* in different containers (ovitraps), viz., new coconut shell (C_1), used coconut shell (C_2), new black coloured plastic cup (C_3), and used black coloured plastic cup (C_4) was assessed in three different types of experiment sets viz., (i) in the above mentioned containers (E_1), (ii) rubber leaf infusion placed inside the containers (E_2), and (iii) rubber leaf infusion + *Bacillus thuringiensis* placed inside the containers (E_3). Further, different treatments ranging from T_1 to T_8 were set up with varied concentrations of rubber leaf infusions with presence and absence of *Bacillus thuringiensis* was also experimented. The results revealed that maximum eggs were oviposited in C_4, and amongst the experimental set up, maximum number of eggs were oviposited in E_2. Further, T_4 (Container holding rain water + 10% rubber leaf infusion with *Bacillus thuringiensis*) recorded the maximum number of eggs oviposited. The present study demonstrated that the black coloured containers and the rubber leaf infusions attracted *Aedes albopictus* and stimulated its ovipositional preference.

Key words: *Aedes albopictus*, Ovipositional preference, Colour, Rubber leaf infusions

Oviposition choice is a well-studied aspect governed by environmental factors, limiting oviposition behaviour, controlled by a complex of responses, such as water, surface area, water depth, temperature and light intensity, and it effects a potential venue for species-specific surveillance and control. *Aedes albopictus* are container breeders in varying degrees of water. Oviposition traps is a possibility to indirectly estimate the vector population and this technique is recognized by WHO as it can attract female *Aedes* to oviposit [1-2], and are used as a surveillance or monitoring tool in the field [3]. Thus, specificity of ovitraps are proven advantages in vector surveillance [1], because they are used to monitor mosquito species pre and post treatment density counts [4], and has been used to identify areas with high concentrations of vector breeding based on egg density index [5] from unexposed breeding sites and surrounding areas [6]. Keeping in view of the above-mentioned factors, the present work was under taken to determine the ovipositional preference of *Aedes albopictus*.

MATERIALS AND METHODS

The experimental study was conducted in rubber plantations at Ittakaveli, Kulasekharam, 35 Km away from Kanyakumari district, Tamil Nadu, India. The study site was selected based on the adult density of *Aedes albopictus*. Oviposition experiments were conducted in 4 different containers (ovitraps), viz., new coconut shell (C_1), used coconut shell (C_2), new black coloured plastic cup (C_3), and used black coloured plastic cup (C_4). Each container (12cm length and 9.5cm width) was coated internally with filter paper to half the water level so as to provide a moistened surface for *Aedes albopictus* to lay eggs. The term 'used' denotes those previously used for collecting latex in rubber plantations. Three different types of experiment sets were performed to determine the ovipositional preference of *Aedes albopictus*, viz., (i) in the above-mentioned containers (E_1), (ii) rubber leaf infusion (prepared from dried rubber leaves collected from rubber plantations by fermenting 100g of rubber leaves in 1L water for 10 days) placed inside the four containers (E_2), and (iii) rubber leaf infusion + *Bacillus thuringiensis* placed inside the four containers (E_3).

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Further, different treatments were set up with varied concentrations of the rubber leaf infusions with presence and absence of *Bacillus thuringiensis* was also experimented which are as follows:

T_1: Container holding rain water without *Bacillus thuringiensis*,
T_2: Container holding rain water + *Bacillus thuringiensis*,
T_3: Container holding rain water + 10% rubber leaf infusion without *Bacillus thuringiensis*,
T_4: Container holding rain water + 10% rubber leaf infusion with *Bacillus thuringiensis*,
T_5: Container holding rain water + 20% rubber leaf infusion without *Bacillus thuringiensis*,
T_6: Container holding rain water + 20% rubber leaf infusion with *Bacillus thuringiensis*,
T_7: Container holding rain water + 30% rubber leaf infusion without *Bacillus thuringiensis*,
T_8: Container holding rain water + 30% rubber leaf infusion with *Bacillus thuringiensis*.

The containers of each experimental study were observed for the number of eggs laid, which were counted and subjected for further data analysis. Five experimental trials were performed with five replicates per trial.

RESULTS AND DISCUSSION

The mean number of eggs oviposited in all the four containers for E_1, E_2 and E_3 are presented in (Table 1). Maximum number of eggs were oviposited in C_4, and amongst the experimental set up, maximum number of eggs were oviposited in E_2 and E_3. The number of eggs laid in different treatments ranging from T_1 to T_8 are presented in (Fig 1). T_4 recorded the maximum number of eggs oviposited and T_2 the minimum.

Oviposition by cues is a complex of responses resulting in a well-defined spatial distribution of a population [7-8]. 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]. Chemosensory cues are used for location of resources may be influenced by the presence of microbial fauna often interacting with plant material [10] or plant odours [11-13]. Visual cues from the oviposition site including colour and optical density of water, texture, temperature and moisture of the oviposition substrate, attract the gravid female from the distance and subsequently olfactory cues direct the female towards the specific oviposition site. Responses to visual, chemical and tactile stimuli have been widely studied in *Aedes* females [14-16].

Table 1 Ovipositional preference of *Aedes albopictus* in different experimental sets

Season	C_1	C_2	C_3	C_4	F value
			E_1		
Summer	8.1 ±3.0	13.5 ±2.9	8.8 ±3.1	17.1 ±3.0	11.41*
Monsoon	12.6 ±3.6	18.6 ±2.8	16.6 ±3.0	25.8 ±4.2	15.80*
			E_2		
Summer	12.3 ±3.2	19.8 ±6.9	17.1 ±3.1	38.3 ±2.8	19.09*
Monsoon	16.5 ±3.7	24.5 ±3.6	20.0 ±2.7	37.6 ±2.9	47.17*
			E_3		
Summer	12.3 ±2.9	19.8 ±2.4	14.6 ±2.9	26.5 ±3.2	28.02*
Monsoon	16.1 ±3.1	27.3 ±1.7	20.0 ±2.3	37.1 ±2.4	79.50*

Values expressed as Mean ±S.D.; *Values significant @ P<0.05 level

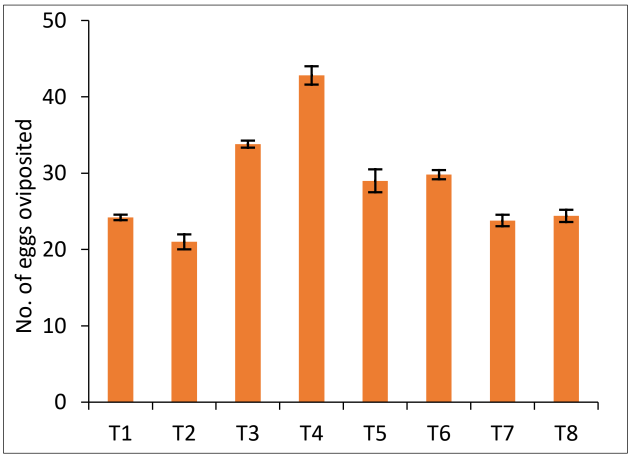

Fig 1 Ovipositional preference of *Aedes albopictus* in different treatments

Diurnally active mosquitoes are believed to have better developed colour sensitivity than crepuscular nocturnally active species. *Aedes* mosquitoes are active diurnal mosquitoes and rely more on optical cues like the contrast between dark container openings and water surface (specular) reflections for selection of resting and oviposition sites than active nocturnal mosquito species [17]. Colour preference by *Aedes* females are primarily based on a greater attraction to dark surfaces [18]. In the present study, *Aedes albopictus* preferred the containers which were black coloured. Beckel [19] stated that black surfaces have been found to be most attractive in oviposition site selection. Colton *et al*. [20] reported that *Aedes* mosquitoes oviposited maximum number of eggs on black ovitraps, and the same was observed in the present study too. Further, Marin *et al*. [21] also showed that black coloured ovitraps attracted ovipositing *Aedes aegypti* mosquitoes to oviposit their eggs. Hence, the present study revealed that the colour of the ovitraps played an important role in attracting the ovipositing females of *Aedes* species, especially *albopictus* and due attention can be given while considering the colour of the ovitraps, to be used for different objectives of further investigations. Different coloured containers have different effects on the oviposition of mosquitoes regarding their colour and major habitats. Black and red colours are considered to be the most attractive colours for oviposition of mosquitoes [22]. Coloured ovipositional substrates or containers have been used by numerous authors to examine the role of colour as an ovipositional attractant. Bates [23] examined the use of background colour as an ovipositional attractant in *Anopheles atroparvus* by placing different coloured papers (black, yellow, and white) in the bottom of ovipositional pans and found that the pans with black paper were most frequently selected by ovipositing mosquitoes. Gubler [24] placed clear glass egg traps over circular discs of black, brown or white paper and determined that the black background was preferred by *Aedes albopictus* and *Aedes* *polynesiensis*. Frank [25] compared white, green, blue, and black, artificial bromeliad flower ovipositional sites and reported that *Aedes aegypti* was attracted to the black ones.

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], [26]. The attractiveness of organic infusions is influenced by the process of bacterial growth, with subsequent metabolite production [27-29]. Organic infusions have successfully been used in ovitraps for surveying populations of *Aedes* species, 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* females has been reported. Organic infusions, commonly developed from a range of fermented plant materials, are frequently used to increase the attraction of gravid mosquitoes to ovitraps. Infusions release volatile chemicals which act as chemical cues for gravid mosquitoes and help in selection of oviposition sites. Sumodan [30] 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 ovipositional preference of *Aedes albopictus* in the present study, and thereby indicate rubber leaf infusions to be more attractive, which may be attributed to the fact that leaf infusions contain 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 [33-34] of organic matter. In the present study, maximum eggs were oviposited in 10% rubber leaf infusion and not in 20 and 30%. 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 [24]. The next factor is the duration of fermentation, because it is the stage at which the leaves are used may produce different levels of chemical cues. Sant'ana *et al*. [35] 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. Optimally attractive infusions for *Aedes* species require fermentation periods of different lengths depending on the plant species, and in the present study, the rubber leaf infusions were fermented for 10 days.

CONCLUSION

Manipulating the oviposition behaviour of mosquito is a useful tool in determining the preference for oviposition sites by the gravid females as a vital strategy in ovipositional studies. The present study demonstrated the importance of colour of container, and the role and influence of rubber leaf infusions in stimulating ovipositional preference of *Aedes albopictus* mosquitoes.

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