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Does colour of ovitrap influence the ovipositional preference of *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae)

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Abstract

Oviposition traps or ovitraps indirectly estimate the vector population as it as a simple, inexpensive and sensitive tool for monitoring oviposition and vector indices. In the present study, ovitraps painted with five colours (red, black, green, blue and orange) were used to find out the effect of different colours on the oviposition response of *Aedes aegypti* females. An organic infusion of 50% rubber leaves concentration was used as oviposition attractant. The mean number of eggs oviposited in red, black, green, blue and orange coloured ovitraps were found to be 72.0 ±7.1, 105.8 ±12.9, 51.2 ±6.9, 45.8 ±16.3 and 33.7 ±10.7 respectively and it was observed that *Aedes aegypti* females oviposited maximum number of eggs in the black ovitrap followed by red. The present study revealed that the colour of ovitraps played a vital role in attracting the ovipositing females of *Aedes aegypti* and due attention can be given while considering the colour of the ovitraps, and attractants to be used for different objectives of further investigations as continued laboratory and field investigations need to be conducted to better understand the ovipositional behavior of mosquitoes in their natural habitat.

Keywords: Ovitraps, colour, oviposition, *Aedes aegypti*

1. Introduction

Dengue, a mosquito-borne disease of human is caused *Aedes aegypti* and *Aedes albopictus* mosquitoes which are the known vectors [1, 2]. Rapid responses to dengue outbreaks are needed in order to control the spread of the virus as prevention of dengue fever and its more severe forms is of primary importance in the absence of vaccines [3]. Currently, vector control remains the key strategy in dengue prevention and control [4], as it reduces or interrupts the dengue virus transmission. One of the strategies in vector control is mosquito surveillance [5]. The life cycle of mosquito is disturbed if oviposition is prevented and thereby, population growth can be reduced. Hence, understanding the oviposition behaviour of mosquitoes may not only give a new insight about their life history, but also lead to more refined dengue surveillance and control practices. *Aedes aegypti* are container breeders in varying degrees of water. Several tools and methods have been designed for vector surveillance to establish the minimum threshold of vector density (vector indices). The use of oviposition traps or ovitraps is a possibility to indirectly estimate the vector population and this technique is recognized by WHO as it can attract female *Aedes* to oviposit [6, 7]. Ovitraps are used as a surveillance or monitoring tool in the field [8]. Lenhart *et al.* [7] recommended the ovitrap as a simple, inexpensive and sensitive tool for both monitoring oviposition and collecting large quantities of *Aedes aegypti* eggs. Thus, simplicity, specificity and effectivity of ovitraps are proven advantages of this technique as a surveillance tool [6]. The ovitrap technique has been also used to monitor *Aedes aegypti* pre and post treatment density counts [9] and has been used to identify areas with high concentrations of vector breeding based on egg density index [10] from unexposed breeding sites and surrounding areas [11]. Extrinsic environmental factors limiting oviposition behaviour is controlled by a complex of responses, such as water, surface area, water depth, temperature and light intensity. Places where people store water for a longer period in different colour containers generally support and enhance the mosquito breeding and the colour of the container may play an important role in container choice among gravid females. Keeping in view of this aspect, the present study was planned and since ovitraps in the recent years, have been considered for the surveillance of *Aedes* mosquitoes, as an important surveillance tool, different coloured ovitraps have been used to study the colour preference of gravid females of *Aedes aegypti*.

2. Materials and Methods

The experimental study was conducted from September to November, 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 ovitraps were painted with five colours (red, black, green, blue and orange) to find out the effect of different colours on the oviposition response of *Aedes aegypti* females. An organic infusion of 50% rubber leaves concentration was used as oviposition attractant (250mL/trap). The rubber leaf infusion stock solution was prepared by adding 30g of rubber leaves to 7.5L of tap water and was kept for seven days. The leaves were cut into small pieces and were used for the preparation of infusion after shade drying for 2-3 days. All ovitraps were thoroughly rinsed with de-ionized water to remove any organic matter before replacing with fresh infusion and paddle. The experimental set up was kept undisturbed and six trials were performed.

3. Results

The number of eggs oviposited in each coloured ovitrap was noted. The respective number of eggs oviposited in red, black, green, blue and orange coloured ovitraps in the six trials were 73, 80, 65, 74, 62 and 78; 91, 100, 112, 98, 106 and 128; 62, 50, 46, 55, 42 and 52; 34, 65, 40, 32, 68 and 36; 31, 52, 23, 40, 26 and 30. The mean number of eggs oviposited in red, black, green, blue and orange coloured ovitraps were 72.0 ±7.1, 105.8 ±12.9, 51.2 ±6.9, 45.8 ±16.3 and 33.7 ±10.7 respectively (Figure 1). It was observed that *Aedes aegypti* females oviposited maximum number of eggs in the black ovitrap followed by red and the values were statistically significant at P=0.05 level.

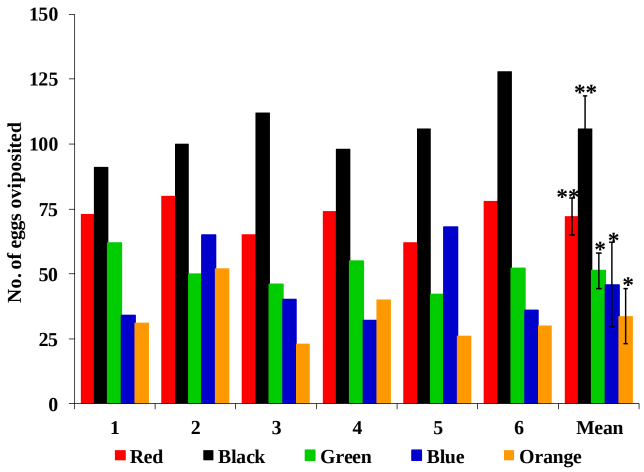

Fig 1: Ovipositional preference of coloured ovitraps by *Aedes aegypti*

4. Discussion

An important aspect of mosquito ecology is to find out the factors that limit species-specific larvae to specific type of oviposition medium and selection of oviposition site is one of the most important behavioural components of mosquito survival. *Aedes aegypti*, the main dengue vector in many dengue endemic countries including India, is a highly adapted household container breeder. A proper understanding of oviposition behaviour may provide insights about life history of *Aedes aegypti* which helps in dengue surveillance. Oviposition activity of mosquitoes on a water body is a complex behaviour mediated by several factors involving physiological exigency, physical (visual, tactile) and chemical cues [12, 13]. Other factors reported to affect oviposition of gravid female mosquitoes include light intensity, size and color of container, depth, turbidity, temperature, pH and dissolved oxygen [14, 15]. Vision plays a principle role in adult mosquito biology, including locations of hosts, food, source, mates, resting sites and oviposition sites. Large number of studies have examined the visual parameters of shape, size, contrast, light intensity, texture and colour attraction to host seeking mosquitoes, while few studies have explored that which of these parameters are attractive to gravid adult females. Most oviposition attraction studies seek to uncover specific odours generated from microbial agents that are responsible for attracting gravid females to a potential oviposition site, however site selection is also dependent on tactile and visual cues with vision possibly as important as olfactory cues in site selection among some mosquito species. A female mosquito chooses oviposition site by a combination of visual and chemical cues. Size, colour, paddle material and oviposition attractant have been reported to be the important factors that influence the efficacy of ovitraps. 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 aegypti* females [16-18]. Colour preference of *Aedes aegypti* females are primarily based on a greater attraction to dark surfaces [19]. Black surfaces have been found to be most attractive in oviposition site selection [20]. Other studies have documented the attraction to a black-white contrast [19, 21]. However, Muir *et al*. [16] suggested that *Aedes aegypti* and *Aedes albopictus* females [22-24] prefer red and black breeding areas, while colors such as blue, yellow and white are unattractive to them. These findings were consistent with the results of the current study.

Diurnally active mosquitoes are believed to have better developed colour sensitivity than crepuscular nocturnally active species. 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 [25]. Coloured ovipositional substrates or containers have been used by numerous authors to examine the role of colour as an ovipositional attractant. Bates [26] 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 [27] 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 [28] compared white, green, blue, and black, artificial bromeliad flower ovipositional sites and reported that *Aedes aegypti* was most attracted to the black artificial flowers. Dhileepan [29] noted that black and red were the most preferred colours for oviposition of *Culex annulirostris* and *Culex molestus*, whereas yellow and green were the least preferred colors. With black and red being the most preferred colours, and the other colours being among the least preferred, colour preference results of the present study on *Aedes aegypti* confirmed Dhileepan's [29] results. In studying the vision responses of *Aedes aegypti*, Muir *et al*. [16, 17] noted a preference for black and red. They further mentioned that *Aedes* species were unable to see red and would actually assume the colour as black. According to Reiskind and Zarrabi [30], as *Aedes* mosquitoes are active diurnal mosquitoes they 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. Colton *et al*. [31] reported that *Aedes* mosquitoes lay maximum number of eggs on black ovitraps. In the present study, *Aedes aegypti* mosquitoes had the choice of coloured ovitraps for egg laying, and maximum number of eggs was oviposited on black ovitrap followed by red. This revealed that there was no statistical difference between the number of eggs laid on black and red ovitraps. Similar observations were made in different species of mosquitoes by Yanoviak [32]. Most insects are unable to see red colour, therefore, the red ovitraps probably would have appeared dark grey to the mosquitoes. This may conclude that *Aedes aegypti* mosquitoes could not differentiate between these two colours. *Aedes* mosquitoes breed in different types of household containers including coloured flower pots. In a study conducted by Panigrahi *et al*. [33] to find out the preference for coloured ovitraps for oviposition by *Aedes aegypti* females, the green and blue coloured ovitraps were not distinguished by *Aedes aegypti*, as similar number of eggs were oviposited on the ovitraps of these two colours and yellow coloured ovitraps were least preferred which confirms the earlier report of Chua and Chua [34]. In another study, Hoel *et al*. [11] tested five choices of coloured ovitraps against *Aedes albopictus* in Florida and based on the mean number of eggs oviposited, the choice of colours were in the order of black > blue > checkered > orange > striped and white. Similar to the present study, ovipositing *Aedes albopictus* females preferred black colour more than other competing colours and also observed a positive response of gravid *Aedes albopictus* to black and orange targets, and concluded that black was perceived as an attractive stimulus. Hoel *et al*. [11] concluded that traditionally used black ovitraps did produce superior results in their study too, and that the black coloured ovitraps proved to be the fast attracting colour for ovipositing females of *Aedes albopictus*. Yap [22] also found that ovitraps which are black in colour were more attractive to mosquitoes. Yap [23] in Malaysia found that gravid *Aedes albopictus* in rural habitats oviposited more in red and black ovitraps than in blue, yellow, green, white, and plain (unpainted) ovitraps which correlated with Snow's [35] findings for colour preferences of gravid *Aedes aegypti*. Yap *et al*. [24] also reported preference for dark coloured glass jars, especially black, blue, and red ones over light coloured jars by *Aedes albopictus* mosquitoes during their study. The present study also revealed that the colour of the ovitraps played an important role in attracting the ovipositing females of *Aedes aegypti* and due attention can be given while considering the colour of the ovitraps, to be used for different objectives of further investigations.

Mosquito species prefer to oviposit in black containers in comparison to other colours [28, 29], but would still oviposit in other coloured containers. Jones and Schreiber [36] for instance, demonstrated that though *Toxorhynchites splendens* preferred to oviposit in black containers, substantial oviposition was also recorded in green, blue and orange containers. According to Hilburn *et al*. [37] and Jones and Schreiber [36] oviposition in recipients with other colours might increase in the absence of adjacent black containers. In the present study, it was found out that black colour was the most preferred colour for oviposition, after that red was preferred, followed by green, blue and orange. Orange colour ovitrap was the least preferred coloured for oviposition by *Aedes aegypti* mosquitoes. There was non-significant difference between red and black colour. Colour adaptations are helpful in insect survivorship and species fitness [38]. Williams [39], Wilton [40], McDaniel *et al*. [41], Beehler *et al*. [42] and Jones and Schreiber [36] found that black and red colours are not distinguished by mosquitoes, thereby, the number of eggs laid on these two colours would almost be the same. Same was the case with yellow and green colours [25]. It may be to survive the eggs and offsprings from the attack of predators, as the colour of the eggs is usually black. The light colours are specially avoided by mosquitoes as is obvious from the results and previous findings by Gjullin [43]. Ovipositing mosquitoes do not deposit eggs indiscriminately; rather their selection criteria for oviposition are probably related to the chemical nature of the water. It has been reported that ovpositing females normally choose water with the presence of life. This is because several attributes of the water, both physical and chemical, influence hatching success and larval survival. Therefore, the mosquitoes have a strong selection for discrimination of potential oviposition sites based on offspring viability. Gravid females follow visual or olfactory cues to appropriate water collection and are guided by chemical cues and physical factors in the water and the quality of water before deciding to lay their eggs. 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. Sumodan [44] found rubber plantations as potential breeding ground for *Aedes* mosquitoes as it provides a canopy and dense vegetation for its survival. Rubber leaf infusion due to its easy availability served as a useful substrate for attracting gravid female and this combination can be a good vector surveillance tool in case where gravid females are not traceable during the household surveillance and would be helpful in detection of gravid females more appropriately even in the area of low density.

5. Conclusion

Ovitraps provide a very sensitive and economical method of detecting container breeders and it is a useful sampling device in determining *Aedes aegypti* distribution and seasonal abundance. The egg laying preference for coloured ovitraps indicated that the maximum number of eggs was laid in black colour, thereby pointing out that the colour of an ovitrap played an important role in attracting the ovipositing *Aedes aegypti*. In addition, the rubber leaf infusion could also be useful for attracting gravid females and this combination will certainly serve as a good vector surveillance tool especially for *Aedes aegypti*. Additional investigations concerning the role of colour, and other ovipositional cues are warranted. Continued laboratory and field investigations examining colour, and egg attractants need to be conducted to better understand the ovipositional behavior of mosquitoes in their natural habitat.

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