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Use of different coloured ovitraps in the surveillance of *Aedes* mosquitoes in an arid-urban area of western Rajasthan, India

Rina Kumawat[1], Karam V. Singh[2], S.K. Bansal[2] & Himmat Singh[3]

[1]*Epidemiologist, Office of CMHO, Jodhpur;* [2]*Desert Medicine Research Centre (ICMR), Jodhpur;* [3]*National Institute of Malaria Research, New Delhi, India*

ABSTRACT

Background & objectives: In the absence of any commercially available dengue vaccine or antiviral therapy, controlling the primary mosquito vector, *Aedes aegypti*, is currently the only means to prevent dengue outbreaks. Ovitraps are being employed as a sensitive method for detecting the presence of *Ae. aegypti*. Size, colour, material, paddle and use of oviposition attractant have been reported as important factors influencing the efficacy of ovitraps.

Methods: For the study of coloured ovitraps baited with grass infusion, 24 households were randomly selected in a locality and five ovitraps/premises were installed. Weekly ovitrap surveys were conducted during the study for four times. Ovitrap data were analyzed on the basis of its location, day-wise percent positivity, presence of eggs laid and inference of different ovitrap parameters were drawn.

Results: Grass infusion, used in the ovitraps has highest attractiveness on Day 6 in comparison to other days. Percent positivity of different coloured ovitraps revealed that red coloured ovitraps have highest positivity (92.7%), followed by black and orange (91.7% each), green (76.3%) and transparent (45.8%). The individual colour-wise ovitrap positivity revealed that the highest ovitrap positivity on Day 1 was recorded for black colour (21.8%), which on D2 and D3, in case of red colour (37.5 and 26%). However, on D4, D5 and D6 highest number of positive ovitraps was recorded in orange coloured (12.5, 21.9 and 31.3%, respectively) and on D7 highest number of positive ovitraps were recorded in transparent (21.9%), followed by green coloured ovitrap (20.8%). The overall location-wise ovitraps positivity revealed that highest positivity was recorded among the ovitraps installed inside bathrooms (92.0%), followed by bedrooms (85.0%), lobby (66.7%) and stores (48.3%).

Interpretation & conclusion: The study revealed that for immediate detection of the presence of gravid females in a particular locality during epidemics, the black coloured ovitraps may be useful, however, in normal situations for surveillance of the vector species, the orange coloured ovitraps might be more suitable. Bathrooms were found to be the highest positive location for pitching ovitraps.

Key words *Aedes aegypti*; coloured ovitraps; *Cynodon dactylon*; surveillance

INTRODUCTION

Dengue, a mosquito-borne flavivirus infection of humans is caused by four serologically distinct viruses, namely dengue virus-1, 2, 3 and 4 and is mainly transmitted by *Aedes* mosquitoes. Dengue and its severe manifestations—dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the serious public health problems in the tropics[1–2]. In India, dengue virus infections have been frequently encountered in epidemic proportions in several states[3–6].

Aedes aegypti, the main dengue vector in many dengue-endemic countries including India, is highly adapted household-container breeder[7]. Vision plays a principle role in adult mosquito biology, including location of hosts, food sources, mates, resting sites, and oviposition sites[8]. Large number of studies has 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[9]. 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[10]. For diurnally active artificial-container breeders, such as *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), vision undoubtedly influences oviposition site selection; however, very little is known of the degree to which the visual parameters of colour and contrast influence oviposition site selection of either species. Diurnally active mosquitoes are believed to have better developed colour sensitivity than crepuscular or nocturnally active species[8]. Ovitraps are being employed as a sensitive method in detecting the presence of *Ae. aegypti*[11]. They are considered more sensitive than larval surveys where the population density is low and larval surveys are largely unproductive[12-13]. Several improvements and modifications have been made in order to enhance the efficacy of ovitraps. Size, colour, material, paddle and oviposition attractant have been reported to be the important factors that influence the efficacy of the ovitrap[14].

In the desert areas due to shortage of water, the people have tendency to store water for a longer period in different colour containers which generally support and enhance the mosquito breeding, and their colour may play an important role in container choice among gravid females. Keeping in view this aspect, the present studies, have been planned and since ovitrap 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 the gravid females of *Ae. aegypti*.

MATERIAL & METHODS

Study area

Jodhpur City, located in the western part of Rajasthan was selected for carrying out the experiments. Jodhpur comes under arid zone of the Rajasthan state and represents a part of Great Indian Thar Desert. Extreme of heat in summer and cold in winter are the characteristics of this city. The temperature varies from 49°C in summer to 1°C in winter. The rainy days are limited to maximum 15 in a year with an average rainfall of 302 mm. The scarcity of water and food remains throughout the year. So the common people store large amount of available water in the man-made artificial containers for rather prolonged periods, which in turn serve as potential breeding sites for the dengue vectors.

The locality Bamba Mohalla was selected for ovitrap study as the earlier studies also showed that the area has most prominent and potential breeding sites favourable for the dengue vectors[15]. Bamba locality is situated in the old city area of Jodhpur with thickly populated human dwellings. Both experimental houses as well as control houses were randomly selected in the same locality. The study was conducted in March and April which was considered as transmission period of dengue in this part of desert[15].

Data collection

In all, 24 houses were randomly selected for the experiment of coloured ovitrap studies; in each experimental household five different coloured ovitraps were placed. In the experimental houses, ovitraps were fixed for seven days. Numbers of eggs laid in each ovitrap in each house were recorded for seven days on daily basis, keeping in consideration of presence of eggs larvae and pupae, the data were started collecting from the following day of installation, which is designated as Day 1 in the text. The experiment was repeated for four times in the same houses of the locality. In each household, the transparent ovitrap was considered as the control ovitrap. The number of eggs laid in each ovitrap was counted and compared with the control ovitrap (transparent) in each household. Since, the variations of colour in ovitraps were same in each house hold, therefore, only 24 houses were selected randomly so that representation of different house types can be included in the locality.

Different locations were also selected as a variation in the study for the installation of ovitraps in each household so as to find most suitable installation site of ovitrap also. After a week, the paddles were removed and *Ae. aegypti* eggs on the walls of the ovitrap were gently dislodged and the water was filtered using a fine strainer. The total number of eggs on the paddle and on the walls was counted. Each ovitrap was then refilled with grass-infusion and a new paddle was used each time. During the study, some eggs were allowed to hatch and develop into adults, in the laboratory, for their species identification.

Ovitrap preparation

The ovitraps consisted of one litre transparent round plastic containers of 12 cm tall by 9.5 cm wide. The oviposition substrate was a 12×2 cm strip of a 9 mm plywood wooden paddle covered by 9×2 cm strip of Whatman 42 filter paper fixed by a rubber band at one end and then placed vertically inside the container. The ovitraps were painted with four colours—black, green, orange and red, besides one, *i.e.* transparent. These traps were used to find out the effect of different colours on the oviposition response of *Ae. aegypti* females. A grass infusion of *Cyndon dactylon* (Fig. 1) of 50% concentration was used as oviposition attractant (250 ml/ trap)[16]. The grass infusion stock solution was prepared by adding 31.25 g of grass to 7.5 liters of tap water and was kept for seven days[17]. The grass without seeds was plucked and cleaned to remove the soil and only its green portion was used for the preparation of infusion after drying in shade for 2–3 days. The time of ovitrap laying was between 0900 and 1200 hrs, the time of lowest oviposition activity of *Ae. aegypti*[18]. All ovitraps were thoroughly rinsed with de-ionized water to remove any organic matter before replacing with fresh infusion and paddle. Later to it fresh 500 ml grass infusion was added. In each house, five different coloured ovitraps were laid together at different locations, *i.e.* bedrooms, bath-

Fig. 1: Cynodon dactylon grass used in infusion.

rooms, store, lobby, etc. Each coloured ovitrap was given an identification number for households and a separate number for its location specific placement. Mosquito eggs were counted and identified to species-wise based on their shape, luster, size, and colour. To prevent misidentification, 25% of all eggs were reared to adults under laboratory conditions and were identified.

Data analysis

For comparison with the weekly *Ae. aegypti* larval and adult surveys, the total number of eggs counted during each week, three indices were calculated as follows:

$$\text{Prevalence} = \frac{\text{Total positive ovitraps}}{\text{Total ovitraps installed}}$$

$$\text{Intensity} = \frac{\text{Total no. of } \textit{Ae. aegypti} \text{ eggs collected}}{\text{Total positive ovitraps}}$$

$$\text{Mean egg density} = \text{Prevalence} \times \text{Intensity}$$

Various entomological indices were calculated to know correlation among them. Chi-square (χ^2) was calculated for selection of appropriate coloured ovitrap day-wise, location-wise, immature stages-wise and overall percent positivity. All levels of statistical significance were determined minimum at $p = 0.01$ and $p = 0.001$ by using a statistical programme with student *t*-test.

RESULTS

The observations on the percent positivity of different coloured ovitraps revealed that red coloured ovitraps have highest positivity (92.7%), followed by black and orange (91.7% each), green (76.3%) and transparent (45.8%). Day-wise percent positivity of ovitraps revealed that highest percentage of positive ovitraps was found on Day 6 (15.21%), followed by Day 2 (14.58%), Day 7 (12.71%), Day 3 (12.08%), Day 5 (10%), and least on Day 1 (7.9%). It indicates that maximum number of gravid females in the study area were present on Day 6 and minimum on Day 1 (Table 1).

On Day 1 highest number of positive ovitraps were recorded in black coloured (21.9%) while on Days 2 and 3 highest number of positive ovitraps were recorded in red coloured (37.5 and 26%, respectively). On Days 4, 5 and 6 highest number of positive ovitraps were recorded in orange colour (12.5, 21.9 and 31.3%, respectively) and on Day 7 in transparent (21.9%), followed by green colored ovitrap (20.8%) (Table 1). To find the difference in the positivity in each type of ovitraps day-wise the chi-square test was applied with the expectation of equal number of positive ovitraps each day.

It was found that on Day 1 transparent and green coloured ovitraps were significantly less positive ($\chi^2 = 10.9$; $p \geq 0.001$ each) and black coloured ovitraps were significantly highly positive ($\chi^2 = 9.30$; $p \geq 0.01$). On Day 2 red and black coloured ovitraps were found more positive which was significantly high in comparison to other ovitraps ($\chi^2 = 57.6$ and 26.7; $p \geq 0.001$). On Day 3 red coloured ovitraps were highly positive ($\chi^2 = 18.2$; $p \geq 0.001$), whereas transparent ovitraps were found to be significantly less positive ($\chi^2 = 9.0$; $p \geq 0.01$). On Day 4 the variability in the positivity of ovitraps did not differ much. On Day 5 orange coloured ovitraps were found significantly less positive ($\chi^2 = 9.30$; $p \geq 0.01$), whereas on Day 6 orange ($\chi^2 = 33.3$; $p \geq 0.001$) and green ($\chi^2 = 18.2$; $p \geq 0.001$) coloured ovitraps exhibited significantly high positivity (Table 1). On Day 7 the transparent ovitraps found highly positive ($\chi^2 = 9.3$; $p \geq 0.01$). The day-wise variability observed in positiveness of ovitraps was also found statistically significant ($\chi^2 = 25.13$; $p \geq 0.001$ and at df = 6).

Each experiment was repeated four times and the collected data were pooled for number of eggs laid in different coloured ovitraps. The results showed that black coloured ovitraps fetched highest percentage of eggs (37.78%) which was found significantly higher ($\chi^2 = 1664$; $p \geq 0.001$) than green, orange, red and transparent coloured ovitraps, whereas least egg percentage was found in the transparent ovitraps (4.33%—Table 2).

The location-wise positivity of the ovitraps revealed that highest positivity was recorded among the ovitraps installed inside bathrooms (92%), followed by bedrooms (85%), lobby (66.7%), and stores (48.3%—Table 3). The highest positivity of all coloured ovitraps, except trans-

Table 1. Day-wise percent positivity of individual ovitraps recorded during the study

Colour type	Day-wise percent positive ovitraps							Individual % positive (n = 96)	Total % positive (n = 480)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
Transparent	0*	2.08	1.04*	3.13	7.29	10.42	21.88*	45.84	9.17
Black	21.88†	29.17†	19.79	9.38	4.17	4.17	3.13	91.69	18.33
Green	0*	1.04	8.33	7.29	12.5	26.04†	20.83	76.03	15.21
Orange	3.13	3.13	5.21	12.5	21.88*	31.25†	14.58	91.68	18.33
Red	14.58	37.5†	26.04†	3.13	4.17	4.17	3.13	92.72	18.54
Total ovitraps positive/day (n = 480)	7.92	14.58	12.08	7.08	10	15.21	12.71		79.58

*Represents test of significance at p >0.01; †Represents test of significance at p >0.001 (χ^2 calculated on original values).

Table 2. Day-wise egg laying percentage of individual coloured ovitraps recorded during the study

Colour type	Total no. of eggs	Day-wise egg laying (%)							Percentage of eggs
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Transparent	456*	0	5.26	6.58	20.61	30.04	19.52	17.98	4.33
Black	3976*	33.48	37.63	21.1	5.28	1.26	0.98	0.28	37.78
Green	1094	0	3.02	30.16	21.02	18.65	20.02	7.13	10.4
Orange	1562	12.74	6.91	10.37	18.05	22.54	24.46	4.93	14.84
Red	3435*	18.43	53.28	24.48	1.08	1.31	1.19	0.23	32.64

*Chi-square test significant at $p \geq 0.001$.

Table 3. Location-wise positivity of different coloured ovitraps observed in the study area

Colour type	Different locations of traps installed														
	Bathrooms			Bedrooms			Lobby			Store			Total		
	No. installed	No. positive	% Positivity	No. installed	No. positive	% positivity	No. installed	No. positive	% positivity	No. installed	No. positive	% Positivity	No. installed	No. positive	% Positivity
Transparent	20	12	60	52	32	61.5	12	0	0	12	0	0	96	44	45.8
Black	20	20	100	52	51	98.1	12	12	100	12	5	41.7	96	88	91.7
Green	20	20	100	52	41	78.9	12	8	66.7	12	4	33.3	96	73	76
Orange	20	20	100	52	48	92.3	12	8	66.7	12	12	100	96	88	91.7
Red	20	20	100	52	49	94.2	12	12	100	12	8	66.7	96	89	92.7
Total	100	82	92	260	221	85	60	40	66.7	60	29	48.3	480	382	79.6

Table 4. Observations on trap positivity and egg collection with respect to different colours

Coloured ovitrap	Total ovitraps installed	Total positive ovitraps	Prevalence	Total eggs in positive ovitrap	Intensity	Mean egg density	Relative percentage of eggs in different ovitraps
Transparent	96	48	0.50	456	9.5	4.75	4.3
Black	96	84	0.88	3976	47.3	41.6	37.8
Green	96	68	0.71	1094	16.1	11.4	10.4
Orange	96	88	0.92	1562	17.8	16.4	14.8
Red	96	84	0.88	3435	40.9	36	32.6

parent one, was found in bathrooms, followed by bedrooms. The black, green and red coloured ovitraps were found least positive when installed inside stores (41.7, 33.3 and 66.7%, respectively), whereas orange coloured ovitraps inside lobby (66.7%) (Table 3). The transparent ovitraps were not found positive inside lobby and stores . The overall percent positivity was found highest in case of red coloured ovitraps (92.7%), followed by black and orange (91.7% each), green (76%) and least in transparent ovitraps (45.8%) and the difference between transparent and orange coloured ovitraps was found to be statistically significant ($\chi^2 = 7.2$; $p \geq 0.01$) and between transparent and black or red colored ovitraps was also found to be more statistically significant ($\chi^2 = 15.8$; $p \leq 0.001$).

The prevalence was calculated as 0.9 for orange coloured ovitraps, whereas the intensity, mean egg density and relative percentage of eggs results exhibited higher values in case of black ovitraps (Table 4), which indicated that the orange coloured ovitraps having grass infusion, as attractant, can be used for the detection of the presence of the vector species, but for quantification or correlation purposes with adults, the black coloured ovitraps may be preferred.

DISCUSSION

Epidemiologically, gravid females are the most important component of the mosquito population and are targeted in mosquito control programmes in active surveillance of disease for the early detection of epidemic events. Field mosquitoes are also collected using the ovitrap technique, the cheapest and easiest method for collecting *Aedes* mosquitoes[19]. Fay and Perry[20] were first to use ovitraps for *Ae. aegypti* (Linn.) surveillance, and Fay and Eliason demonstrated that the ovitrap was in some aspects superior to larval surveys[21]. Ovitraps were also shown to be useful sampling devices in determining *Ae. aegypti* distribution and seasonal population fluctuation[22]. Ovitraps are relatively easy to construct and are sensitive in detecting the presence of gravid females, even at low population densities, making them ideal for surveillance and control of vector species[23].

The studies have demonstrated the advantages in time and labour savings and increased sensitivity in using artificial oviposition devices as compared to various adult traps for surveillance of *Ae. aegypti*[22–24]. The addition of insecticides or insect growth regulators to the traps renders them lethal ovitraps[25]. Behavioural choices of female mosquitoes are clearly affected by colour, either as a substrate or as light. Snow surmised that *Ae. aegypti* were most sensitive to green-orange light (470–610 nm) and thus avoided those colours while seeking more cryptic oviposition sites[26]. Earlier studies have also exhibited that black containers are the most attractive coloured targets for male[27] and female[28] mosquitoes.

Similarly, as observed during present studies, Yap[29] also found that ovitraps which are black in colour are more attractive to mosquitoes. Yap[19] in another study in Malaysia found that gravid *Ae. albopictus* in rural habitats oviposited more in red and black ovitraps than in blue, yellow, green, white, and plain (unpainted) ovitraps. Later, in the laboratory studies, gravid females of *Ae. albopictus* laid significantly more eggs in black, red, and blue ovitraps than in green, yellow, white, or clear (unpainted glass) ovitraps[30], which correlated well with Snow's[26] findings for colour preferences of gravid *Ae. aegypti.* Yap *et al*[30] also reported preference for dark coloured glass jars, especially black, blue, and red ones over light coloured jars by *Ae. albopictus* mosquitoes during their study.

Hoel *et al*[31] tested five choices of colours against *Ae. albopictus* in Florida and based on the mean eggs collected, the choices of colours were black > blue > checkered > orange > striped and white. Like our studies, they also found preference of black colour more than the other competing colours and also observed the positive response of gravid *Ae. albopictus* to black and orange targets and concluded that orange is perceived as an attractive stimulus. As concluded by Hoel *et al*[31] that traditionally used black ovitraps do produce superior results, in our study too, the black coloured ovitraps proved to be fastest attracting colour for ovipositing females of *Ae. aegypti*. Burkett and Butler[32] demonstrated that orange lighted targets attract host seeking *Ae. albopictus,* and Muir *et al*[28] also reported that *Ae. aegypti* can detect orange light based on the electoretinographic examination and also revealed spectral sensitivity ranging from UV to orange coloured light.

The study reveals that the colour of the ovitraps plays an important role in attracting the ovipositing females of *Ae. aegypti* species and due attention can be given while considering the colour of the ovitraps, to be used for different objectives of the investigations. The ovitraps of black colour were found to be appropriate. The grass infusion of *Cyandon dactylon* due to its easy availability can be a very useful 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.

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Correspondence to: Dr Karam V. Singh, Scientist 'F', Vector Biology Laboratory, Desert Medicine Research Centre (ICMR), New Pali Road, Jodhpur–342 005, India.
E-mail: karamvs@gmail.com

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