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# **RESEARCH PAPER**

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# Laboratory and field evaluation of oviposition color preference in mosquitoes

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# Abstract

Survival of the offsprings is the top most priority of all the insects, including mosquitoes. To ensure it, mosquitoes usually oviposit at some particular sites. Characteristics of these sites are sensed by some cues, including chemical and visual cues. In the present study oviposition color preference of certain mosquito species was determined by placing different colored ovitraps in the laboratory as well as in the field. Eggs laid in those ovitraps were counted and reared till the emergence of adults in the Biosystematics laboratory of PMAS Arid Agriculture University Rawalpindi. Black and red were the most preferred ovitraps, 17.66 and 13.33 eggs were deposited respectively, while blue was least preferred, 2.06 eggs were laid. Yellow and green were almost equally preferred after black and red ovitraps. The oviposition color preferences were found almost same in both laboratory and field conditions. These results of color preference are helpful in preparing ovitraps for mosquitoes, hence can be used as a tool in the management and control of mosquitoes.

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#### Introduction

Mosquitoes are the vector of many lethal diseases, spread epidemics and cause a lot of mortalities in humans. Mosquitoes prefer to live in different types of habitats. To survive and increase their population, mosquitoes choose some particular site for oviposition (Rey and O'Connell, 2014). There are some cues on the basis of which mosquitoes search the oviposition site. These cues include the larval food, chemical and visual cues. To sense chemicals in the water, mosquitoes usually taste the water. Not only these cues, but a phenomenon called as skipoviposition is also used in which, mosquitoes lay their eggs at multiple sites. Optical cues are also used by mosquitoes; not only for their general activities, but also these behaviors promote different oviposition sites for different species of mosquitoes. Shape and size of opposition containers also have some role in the selection of oviposition site. Some of the mosquitoes lay their eggs on floating water; others lay out of water (Blosser and Burkett-Cadena, 2017). By disturbing the oviposition behavior of mosquitoes, their life cycle can be disturbed and hence the population can be controlled.in the present study, the preference of gravid female mosquitoes to lay eggs in different habitats and on different colors was studied. Mosquitoes usually prefer to oviposit in high precipitation compared to low precipitation (Banafshi et al., 2013). Oviposition usually reaches at its peak in the months June and September (Bareera et al., 2011).

This study was conducted to determine the oviposition color preference of certain mosquito species.

#### Materials and methods

The experiment was conducted at two places, in the laboratory and field conditions during 2016.

## Laboratory experiment

Immatures of mosquitoes were collected from standing water near different habitats, including forest area, animal sheds, houses, parks, graveyard and scrape yard. The collection was done with the help of dipper (Mehmood *et al.*, 2016). The immatures were brought to the laboratory. Laboratory temperature was maintained at  $25\pm 2C$  and  $75\pm 5\%$  relative humidity. The dimension of cages was 18"x18"x18".

These larvae were shifted to rearing jars in glass beakers containing water. These were fed with skimmed milk. On development into pupae, were shifted to separate beakers. After 3-5 days, these pupae emerged into adults.

Plastic ovitraps of different colors including black, red, yellow, green and blue were half filled with tap water having same colored paper strip in it, placed inside the rearing jars for oviposition. Adult mosquitoes were fed with sugar solution, chicken liver and chick's blood. After mating, gravid females laid eggs in different plastic ovitraps. These plastic ovitraps containing the eggs of mosquitoes were separated to differentiate each colored ovitrap's mosquito population.

Adult Population from each colored ovitrap were collected with an aspirator and killed with potassium cyanide containing killing jar. After killing, these were pinned, preserved and identified.

## Field experiment

Different habitats were selected for placement of colored plastic ovitraps containing water in them. These colored ovitraps were placed at those sites, where chances of disturbance were minimum. These ovitraps were half filled with tap water.

The habitats were visited twice a week to refill those ovitraps with water, as water evaporated. Eggs and larvae from those ovitraps were collected in jars, tagged and brought to laboratory for rearing. These jars were placed in separate rearing cages. On development into adults, these were collected, killed, preserved and identified.

#### Statiscal analysis

The data hence obtained was statistically

analysed using SPSS 16.0, while the graphs were made with the help of Microcal Origion 6.0.

#### **Results and discussion**

#### Laboratory Conditions

A total of 680 eggs were laid in the laboratory conditions in different colored ovitraps used in this experiment viz black, red, yellow, green and blue. All the species followed the same trend of oviposition viz black color was preferred the most, followed by red, yellow, green and blue was the least preferred color for oviposition.

The results obtained showed that black and red colors were significantly different (F [ $_{18.14}$ ] =0.00, P<0.05) and preferred the most. On an average 17.66 and 13.33 eggs were laid on the black color and red color respectively. Yellow color was non-significant compared with blue, the mean number of eggs laid on yellow color were 3.93 and 2.06 in case of blue color. Green color was significantly different, compared with yellow and blue (P=0.01, P=0.001), the mean number of eggs laid on green color ovitrap were 8.33. *Aedes albopictus* laid the most number of eggs (29 ± 2) on the black color ovitraps, followed by red (21.66 ± 1.52), green (8.66± 1.52), yellow (4 ± 1). There were no eggs found in blue color ovitraps (Fig. 1).



Fig. 1. Oviposition color preference in laboratory.

Anopheles annularis laid the highest number of eggs  $(20 \pm 1.73)$  on black color ovitrap, followed by red color  $(15.33\pm1.52)$ , green  $(7.33\pm1.52)$ , yellow  $(5\pm 1)$  and blue  $(3.66\pm1.52)$  (Fig. 1).

*Armigeres obturbans* preferred black color the most for oviposition and laid  $15.66\pm2.08$ eggs on black ovitrap, followed by red ( $12.33\pm1.52$ ), green ( $10\pm1$ ), yellow ( $2.33\pm1.52$ ) and blue ( $3.33\pm1.52$ ) (Fig. 1).

*Culex vagans* preferred black color highly for oviposition and laid  $16\pm2.64$  eggs on black ovitrap, followed by red (13.33±3.21), green (9 ± 1), Yellow (3.33±1.52) and blue (3.33±1.52) (Fig. 1).

*Lutzia raptor* laid the most number of eggs  $(7.66\pm1.52)$  on black color ovitrap, followed by green  $(6.66\pm2.08)$ , yellow  $(5\pm1)$ , red  $(4\pm1)$ . There were no eggs laid on blue color ovitraps (Fig. 1).

#### Field Conditions

A total of 1398 eggs were laid on different colors. The trend of oviposition in case of different species observed was same viz black color was preferred the most, followed by red, green, yellow and blue color was preferred the least except in case of *Lutzia raptor*, which showed a peculiar behavior in field conditions.

The results obtained suggest that black and red color ovitraps were significantly different ( $F_{[31.15]} = 0.00$ , P<0.05) compared with all other ovitraps and preferred the most. On an average 42.8 and 32.93 eggs were laid on the black color and red color respectively. Yellow color was non-significant compared with blue, the mean number of eggs laid on yellow color were 6.6 and 2.26 in case of blue color. Green color was significantly different, compared with yellow and blue (P=0.01, P=0.001), the mean number of eggs laid on green color ovitrap were 8.6. Aedes albopictus laid the most number of eggs (71.33 ± 3.21) on the black color ovitraps, followed by red  $(39 \pm 5.29)$ , green (12.33 $\pm$  3.21), yellow (7  $\pm$  2.64). There were no eggs found in blue color ovitraps (Fig. 2).

Anopheles annularis laid the highest number of eggs  $(39.66 \pm 4.61)$  on black color ovitrap, followed by red color  $(38.33\pm3.51)$ , green  $(10\pm0)$ , yellow  $(3\pm 2.64)$  and blue  $(5\pm2)$  (Fig. 2).



Fig. 2. Oviposition color preference in field.

Armigeres obturbans preferred black color the most for oviposition and laid  $55.66\pm3.05$ eggs on black ovitrap, followed by red ( $53.66\pm3.05$ ), yellow ( $9\pm2$ ), green ( $8.66\pm1.52$ ) and blue ( $2.66\pm3.05$ ) (Fig. 2).

*Culex vagans* preferred black color highly for oviposition and laid  $36\pm2.64$  eggs on black ovitrap, followed by red (27.33±2.51), green (9 ± 2), Yellow (7.66±2.30) and blue (3.66±3.21) (Fig.2).

*Lutzia raptor* laid the most number of eggs  $(11.33\pm3.21)$  on black color ovitrap, followed by red  $(6.33\pm2.51)$ , yellow  $(6.33\pm2.30)$ , green  $(3\pm1.73)$ . There were no eggs laid on blue color ovitraps (Fig. 2).

Different containers have different effects on the oviposition of mosquitoes regarding their color and major habitats. Black and red colors are considered to be the most attractive colors for oviposition of mosquitoes (Panigrahi *et al.*, 2014).

In our experiments we found out that black color was the most preferred color for oviposition, after that red was preferred, followed by green, yellow and blue. Blue color ovitrap was the least preferred colored for oviposition by mosquitoes. There was non-significant difference between red and black color, as well as yellow and green colors. Color adaptations are helpful in insect survivorship and species fitness (Farnesi *et al.*, 2017). William, (1962); Wilton, D.P. (1968); McDaniel *et al.*, (1976); Behler *et al.*, (1992); Jones and Schreiber (1994) found that black and red colors are not distinguished by mosquitoes that's why the number of eggs laid on these two colors were almost the same. Same is the case with yellow and green colors (Panigrahi *et al.*, 2014). It may be to survive the eggs and offsprings from the attack of predators, as the color of the eggs is usually black. The light colors are specially avoided by mosquitoes as is obvious from the results and previous findings by Gjullin (1947).

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## **Conflict of Interest**

Authors have no conflict of interest with any other scientists.

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