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Ovipositional preferences of *Anopheles stephensi* Liston, 1901 with respect to different pH, colour and water

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Abstract
Anopheles stephensi is the principle vector of malaria. The present study is based on the ovipositional site preferences of *An. stephensi* with respect to different pH, colour, and water types. For the study, we used different pH range such as 3, 5, 7, 9, and 11, different coloured bottom petridishes like, red, green, blue, yellow, white, and black were used. Different water samples were collected from various sites of Kota. We used different water like rainwater, fresh standing water, standing water with algae, sandy water, sewage water, distilled water, and tap water. The present study was carried out under laboratory conditions. Our findings showed that *An. stephensi* deposited more number of eggs in pH 7 (Tap water) suggesting that female mosquito prefer this pH range for oviposition whereas more number of eggs were observed in black bottom petridishes suggesting that black colour is comparatively more attractive than other colours. In the case of different water types, fresh standing water with algae showed a greater number of eggs laid than other water types.

Keywords: *Anopheles stephensi*, oviposition, pH, colour, water

Introduction
Mosquitoes are arthropods belonging to the Order Diptera, which are responsible for causing various infectious diseases to humans and animals such as Dengue, chikungunya, yellow fever, Japanese encephalitis, filariasis, zika virus etc. [1]. *Anopheles* genera are one of the medically significant genera, which consists approximately 481 species globally (http://mosquito-taxonomic-inventory.info/). Malaria is the most prominent infectious disease that have *Anopheles* species such as *Anopheles culicufacies* Giles, 1901, *Anopheles subpictus* Grassi, 1899, *Anopheles sundaicus (*Rodenwaldt 1925), *Anopheles stephensi* Liston, 1901, *Anopheles fluviatilis* James, 1902 etc. as vectors in India [2]. About 3.3 lakh cases of malaria and 73 deaths have been reported from India in 2019 (https://nvbdcp.gov.in/).
In India, *An. stephensi* is the crucial vector of human malaria in urban areas. It can transmit both *Plasmodium falciparum* and *Plasmodium vivax* parasites [3]. It is responsible for severe human suffering and mortality in children throughout the world. Management of such diseases becomes increasingly difficult due to high level of resistance of mosquitoes to pesticides [4]. Previous study shown that controlling measures of malaria involves either reduces in population of vector or reduces in human- vector contact [5]. Increasing behavioural resistance to mosquitoes for insecticides facilitates interest in behavioural manipulation for pest management [6].
The selection of oviposition site is a crucial behavioural phenomenon of gravid mosquitoes to the survival of their progeny. This selection is based on various physical and chemical factors [7]. Mosquitoes detect their host location and oviposition sites through volatile semio-chemicals [8].
In the present study, we investigated oviposition preference of *An. stephensi* for different pH, different colour and different types of water.

Method
Study area and mosquito collection
The present study is based on surveys in Kota city and extensive laboratory studies**.** Field trips were conducted in the different parts of Kota. Mosquito larvae were sampled using standard ladle and pipette. The larval habitat were located at an altitude from 250-300 m. Collected larvae were transferred into 1 lit. Plastic jars and transported to laboratory for further studies.

Mosquito rearing and identification
Larvae were provided Brewer's yeast (25mg/l) as food until pupation. Pupae were transferred to adult cage (30 cm x 30 cm x 30 cm) in fresh water containers. 5% sugar solution was provided as food to adult mosquitoes. Identification of mosquito was carried out using standard taxonomic keys [9, 10].

Data collection
The oviposition experiments were performed under laboratory condition *i.e.* 26±1 °C, 65±5% RH and 12:12 h light-dark photoperiod. 20 pairs of *An. stephensi* mosquitoes were used for analysis. Three sets of experiment (different pH, different colour and different water types) were designed for estimation of oviposition preference. Each experiment was repeated for five times and the mean readings were taken for interpretation. Here no. of eggs/female was used for analysis and it was calculated by division of total no. of eggs to total no. of females in the cage.

For pH, five ranges (3, 5, 7, 9 and 11) were used. Here tap water is considered as pH 7 solution. Different pH solutions were developed using pH tablets. Three petridishes of equal size and depth containing varying pH solution were kept in the cage. 20 pairs of mosquitoes were released in the cage. The male mosquitoes provided with 5% sugar solution as food and the females were given blood feed from back and belly shaved rabbit. The number of eggs laid in each of the petridish was counted daily for about 07 days.

For different colour, six petridishes with different coloured bottom (red, green, blue, yellow, white and black) containing tap water were placed in the cage. 20 pairs of mosquitoes were released in the same cage. The males were provided with 5% sugar solution and the females were provided with a back and belly shaved rabbit for their blood feed. The number of eggs laid in each of the petridish was counted every day for about 07 days.

For different kind of water, eight water types (rain water, pond water, fresh standing water, standing water with algae, sand water, sewage water, distilled water and tap water were used for analysis. Eight petridishes of equal size and depth containing different water samples were kept in the cage. 20 pairs of mosquitoes were released in that cage. The males were provided with 5% sugar solution and females were provided with a back and belly shaved rabbit for their blood feed. The number of eggs laid in each of the water samples was counted for about 07 days.

Result

For pH, the observations revealed that tap water (pH 7) solution have highest preference (42 eggs/female), followed by pH 5, pH 3, pH 9 and pH 11 (Table 1) (Fig. 1) whereas for different colour, petridish with black coloured bottom (39 eggs/female) was found most prominent, followed by red, green, blue, yellow and white (Table 2) (Fig. 2). For different water types, fresh standing water with algae found most preferable (30 eggs/female), followed by fresh water, sand water, pond water, tap water, rain water, distilled water and sewage water (Table 3) (Fig. 3).

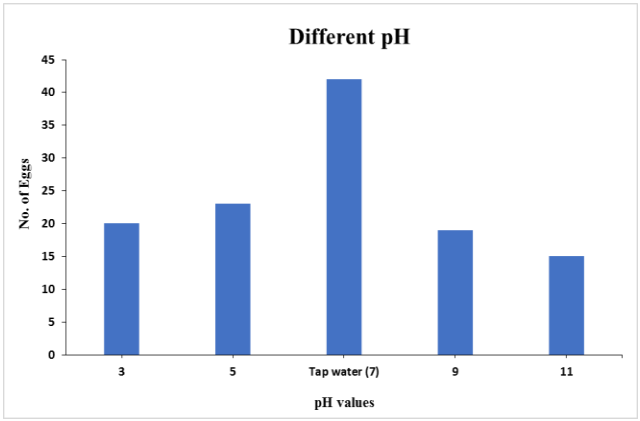

Fig 1: Observations on oviposition with respect to different pH values.

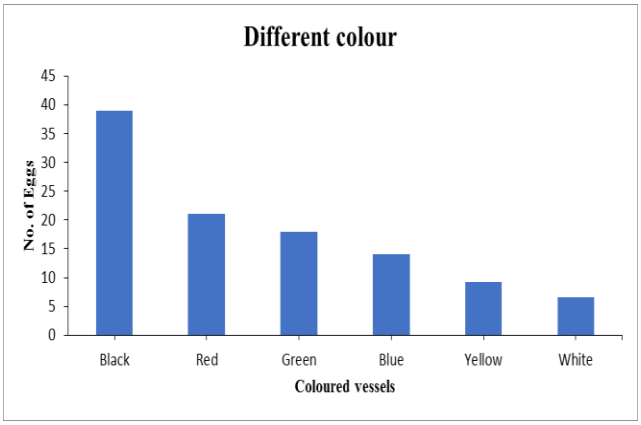

Fig 2: Observations on oviposition with respect to different coloured bottom of petridishes.

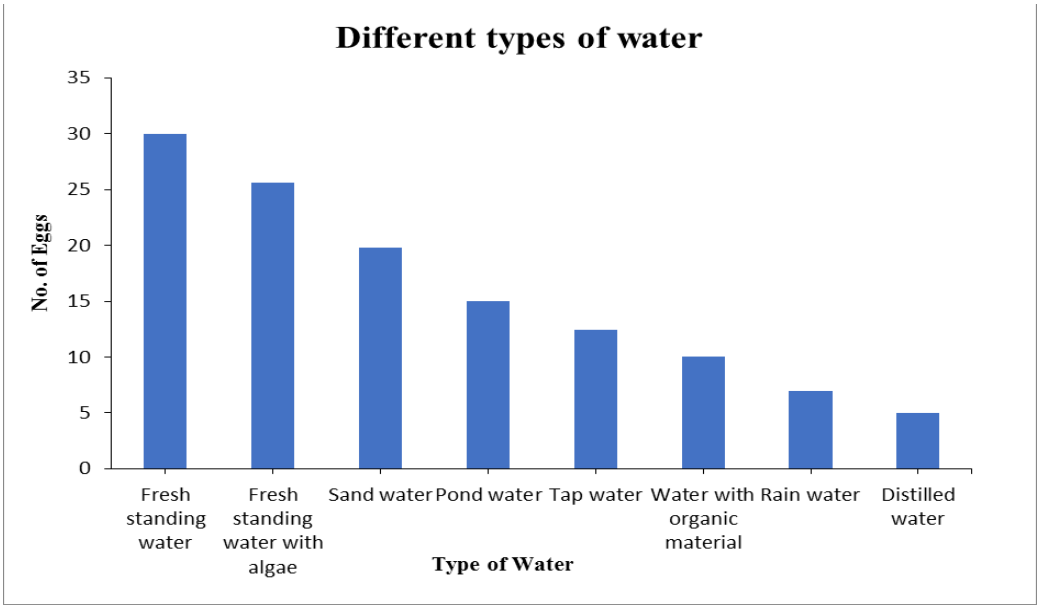

Fig 3: Observations on oviposition with respect to different kind of water

Table 1: Observations on oviposition with respect to different pH values. All values represent in mean form

pH	No. of eggs/female
3.0	20.0
5.0	23.0
Tap water (7.0)	42.0
9.0	19.0
11.0	15.0

Table 2: Observations on oviposition with respect to different coloured bottom of petridishes. All values represent in mean form

Coloured vessels	No. of eggs/female
Black	39.0
Red	21.0
Green	18.0
Blue	14.0
Yellow	9.2
White	6.6

Table 3: Observations on oviposition with respect to different kind of water. All values represent in mean form

Type of water	No. of eggs/female
Fresh standing water with algae	30.0
Fresh water	25.6
Sand water	19.8
Pond water	15.0
Tap water	12.4
Rain water	10.0
Distilled water	7.0
Sewage water	5.0

Discussion

Mosquito usually lay their eggs shortly after they have matured but oviposition may be delayed or prevented by cold as reported by Nichoison (1921); Mayne (1926), absence of water; Woke (1955) or by failure to mate as noticed by Macfie (1915), Tate and Vincent (1936).

Kennedy (1942) investigated the attraction of gravid females of Anopheles, Aedes and Culex from a distance in the laboratory by displaying different surfaces. It was found that the surfaces which were dark in contrast to their surroundings appeared more attractive and in nature a water surface which is shaped appears darker than its surroundings when seen at night from near the ground and hence appears more attractive for egg laying.

The experiments of Russell and Rao (1942) showed that the presence of rich plankton and organic amorphous matter in the water of borrow pits deflected Anopheles culicifacies from egg laying, so also the presence of blue green algae. Therefore, Anopheles culicifacies may show some preference or avoidance in egg laying. Buxton and Hopkins (1927); Manefield (1951) found that the presence of organic matter, whether sterile or putrid, rendered water highly attractive for oviposition. Aedes aegypti prefer such water to fresh water, the strength of the preference reflecting the extent of contamination normally found in the natural larval habitats.

Beckel (1955) revealed that many species laid their eggs on a moist surface just above the edge of a body of water and the colour and texture of the surface affected the number of eggs laid. All species tested in the laboratory preferred a dark surface to a light one and a rough surface to a smooth one. Bhatia and Wattal (1958) studied the viability of the eggs of anophelines. It was found that the viability periods of the eggs of Anopheles culicifacies, Anopheles subpictus, Anopheles annularis and *Anopheles stephensi* ranged from 0to 96 hrs. But the hatching ability decreased with the time of submergence. The eggs float on the water surface and unlike larve and pupae cannot withstand long submergence.

Bar-zeev (1960) showed that the tarsi of *Aedes aegypti* were sensitive to contact with water. Observations of mosquito behavior suggest that the females use their tarsal sense organs in selecting an oviposition site. These organs are sensitive to a number of inorganic ions, and females of Aedes aegypti select certain concentrations of sodium chloride from others for oviposition [23, 24].

Conclusions

The present study provides the information that gravid females of *An. stephensi* can use neutral pH, black colour and fresh water with algae reservoir when orienting towards potential oviposition sites. The findings would also be helpful to manufacture an efficient oviposition trap thereby we can estimate mosquito population size and targeting residual malaria transmission.

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