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Behavioural study in relation to oviposition of wild *Culex quinquefasciatus* in laboratory condition

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Abstract

Mosquito borne diseases are majorcontributors to the communicable disease burden in the South-East Asia region. In the present investigation, both immature and mature stages of various mosquito species were collected from different localities of Ganjam district, Odisha during 2016. Mosquitoes were identified based on the examination of the taxonomic keys and *Culex quinquefasciatus* mosquitoes were abundantly available in almost all selected study sites.In order to find out oviposition preference, gravid female *Cx. quinquefasciatus* were allowed to oviposit in ovitraps with different concentrations of NaCl, in different colouredovitraps, in water from different sources, in larva holding water and in different sized ovitraps. Results of laboratory oviposition bioassays showed that the number of egg rafts laid by gravid *Cx. quinquefasciatus* gradually decreased with increase in NaCl concentration. When gravid mosquitoes were allowed for oviposition in different colour edovitrap, black ovitrap was found to be the most preferred colour by this mosquito species. Water samples collected from different sources were used in the ovitraps to understand the oviposition preference of this mosquito species. Water collected from paddy field was found to be the most preferred water among all other water sample surveyed. Further, it was noticed that *Cx. quinquefasciatus* laid maximum number of egg rafts in ovitraps containing larva holding water than distilled water. In relation to the container size, female adult *Culex* mosquito deposited apparently more egg rafts in the largest ovitrap taken.

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Introduction

Vector-borne diseases account for approximately 20% of all infectious diseases, causing more than 1 million deaths annually among which mosquito vector borne diseases plays a major role (WHO, 2014). In recent years, vector-borne diseases have emerged as a serious public health problem in countries of the South-East Asia region, including India (Thankachan and Gopinath, 2017). Despite of intensive research and massive expenditure towards the control of mosquito vector borne diseases, no such strategies are effective till now. Adequate knowledge on mosquito biology is the foundation on which we can hope to control them and subsequently the mosquito vector borne diseases. The most important behaviours of mosquito vectors include feeding and oviposition. Mosquito species differ in the type of aquatic habitats they prefer for oviposition based on location, physicochemical condition of the water body and the presence of potential predators (Shilulu *et al.*, 2003; Piyaratnea *et al.*, 2005). Blaustein and Kotler (1993) stated that mosquitoes use chemical and biological cues to detect the presence of larval competitors and avoid ovipositing in such habitats. However, the specific cues that trigger oviposition behavior in mosquitoes are not clear.

Culex quinquefasciatus is the most widespread mosquito species found from forest to semi-desert (Rochlin *et al.*, 2009) and having the ability to adjust its seasonal cycle of reproductive activity to the environments ranging from temperate continental climates to humid tropics. This predominant species is endemic in all tropical areas residing mostly in houses (Kohn, 1990) and acts as major urban vectors of lymphatic filariasis (Tiwary *et al.*, 2007) along with the carrier of other disease causing pathogens like West Nile virus (WNV), Ross River virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Reticuloendotheliosis virus (Holder, 1999), Murray Valley encephalitis (Weinstein *et al.*, 1997), Reovirus type 3 and Chikungunya virus (Lee *et al.*, 1989; Holder, 1999). *Culex quinquefasciatus* is found all over India and accounts for 95% of the total cases of lymphatic filariasis (Mitchell, 1996). Successful implementation of this vector control strategies requires sound knowledge of ovipositional behavior and choice of breeding sites. *Cx. quinquefasciatus* is known to breed in diverse artificial and natural water bodies, such as catch-pits, septic tanks, stagnant drains, ground pools and ditches, which are invariably made or influenced by man.

The aim of the present study was to understand the oviposition behavior of *Culex quinquefasciatus* collected from the selected study sites of Ganjam district of Odisha.

Materials and methods

Collection and identification of field collected mosquitoes

Both immature and mature stages of various mosquito species were collected from selected study areas of Ganjam district, Odisha State of India, and were transported to the laboratory for mass rearing. Selected study area had a rich and diversified fauna of mosquitoes and many of these species were vectors of various diseases. After collection, mosquitoes were identified under a binocular stereo zoom microscope in the laboratory as per the identification keys. *Culex quinquefasciatus* was abundantly found in all most all the selected study areas. Therefore, all the experiments pertaining to the selection of oviposition medium and preference for oviposition, F_1 progeny of *Cx. quinquefasciatus* were used.

Mass rearing of mosquito vector

The mosquito colonies were maintained at 26 ± 1°C and at a relative humidity of ~75% under a photoperiod of 14:10 h L: D. Larvae were fed a mixture of dog biscuit powder and baker's yeast in the ratio of 2:1. Adults were kept in 30 × 30 × 30 cm plexiglas cages fitted with cotton surgical stocking tops and maintained on a 10% sucrose solution provided ad libitum.

Oviposition in response to NaCl concentration

Graded series of NaCl concentrations (of analytical grade) in dechlorinated tap water by volumetric dilution were tested for oviposition preference of *Cx.*

quinquefasciatus mosquitoes. The tested concentrations of NaCl were ranged from 0.5% to 2.0%. Five-six-day-old females were given a blood meal and transferred into cages (24″× 24″ × 24″) in batches of 100 females per cage. After 48hr of the blood meal, ovitraps were introduced into the cages containing variable concentrations of NaCl and dechlorinated water which served as control. No definite pattern of arrangement of ovitraps was followed in the cages. Egg rafts were collected after egg laying. The experiment was repeated 10 times and the data obtained were recorded for further statistical analysis.

Oviposition in response to colour of ovitrap

In order to find out if *Cx. quinquefasciatus* mosquitoes have a colour preference for oviposition, ovitraps were covered with different coloured velvet papers such as black, red, yellow, green, blue, and white dipped half in the water inside to provide moist surface for egg laying by the gravid females and kept arbitrarily inside the cages without any specific sequence of arrangement. Five-six-days-old gravid female *Cx. quinquefasciatus*, mosquitoes after a blood meal, in groups of 100 females per batch, were introduced into the cages for oviposition. The egg rafts were collected when the insectary was kept lighted for 12hr to facilitate the females to distinguish between different colours of the ovitrap. The experiment was repeated ten times and the data so collected were subjected to statistical analysis.

Oviposition in response to water collected from different sources

Experiments were conducted in cages (24”× 24”× 24”) to understand the oviposition behaviour in response to water from different sources under controlled environmental conditions. Five-six-days-old gravid female *Cx. quinquefasciatus* mosquitoes, after a blood meal, were grouped into 100 mosquitoes per cage separately. After 48hr of blood meal, circular ovitraps of 4″ diameter half-filled with water from different natural sources like, pond, well, paddy field, drain, river and tap water were introduced randomly into the cages. The numbers of egg rafts laid by female mosquitoes were counted. Ten replicate experiments were conducted and the data obtained were recorded for statistical analysis.

Oviposition in response to ovitrap with larva holding water

In this experiment, in order to find out if the gravid female *Cx. quinquefasciatus* have any preference for egg laying in water holding larvae or water without any larvae, one hundred blood-fed female *Cx. quinquefasciatus* mosquitoes per cage were kept in cages of dimensions of 24″ × 24″ × 24″. Ovitraps containing 10, 20 and 40 larvae separately and ovitrap with only dechlorinated tap water were arbitrarily introduced into the cages. The experiment was repeated ten times and the numbers of egg rafts laid were recorded. The data were subjected to statistical analysis.

Oviposition in response to ovitrap size

Blood-fed gravid female *Cx. quinquefasciatus*, 100 per batch, were transferred into cages. In order to find out the preference of the *Cx. quinquefasciatus* mosquitoes for egg laying if any, with respect to the size of ovitrap, ovitraps of different diameters ranging from 6.0 cm to 12.0 cm were kept inside the cages without any definite arrangement or sequence.

The number of egg rafts laid in each ovitrap was counted. The experiment was repeated 10 times and the data obtained were subjected to further statistical analysis.

Statistical analysis

Data was computed for means, standard error and further analysis of variance (ANOVA) using the statistical software package (SPSS) 17.0 version.

Results

In order to understand the oviposition behaviour of field collected *Cx. quinquefasciatus*, experiments were conducted in relation to varied NaCl concentration, choice of ovitrap colour, choice of oviposition with water collected from different

sources, larva holding water and size of ovitrap in the present investigation.

Choice of oviposition with varied NaCl concentration

Different concentrations of NaCl solutions from 0.5% to 2.0% were tested to understand the oviposition preference of *Cx. quinquefasciatus*. The present investigation reveals that *Cx. quinquefasciatus* females preferred the ovitraps containing distilled water without NaCl for oviposition and there was no egg rafts found in ovitrap containing 2.0% NaCl solution. Result indicates that there was a progressive decrease in the oviposition activity of female *Culex* mosquitoes in a dose dependant manner with the increase in the concentration of NaCl (Fig.1) which exhibits negative correlations between the number of egg rafts laid by the female and the concentration of NaCl in the oviposition medium.

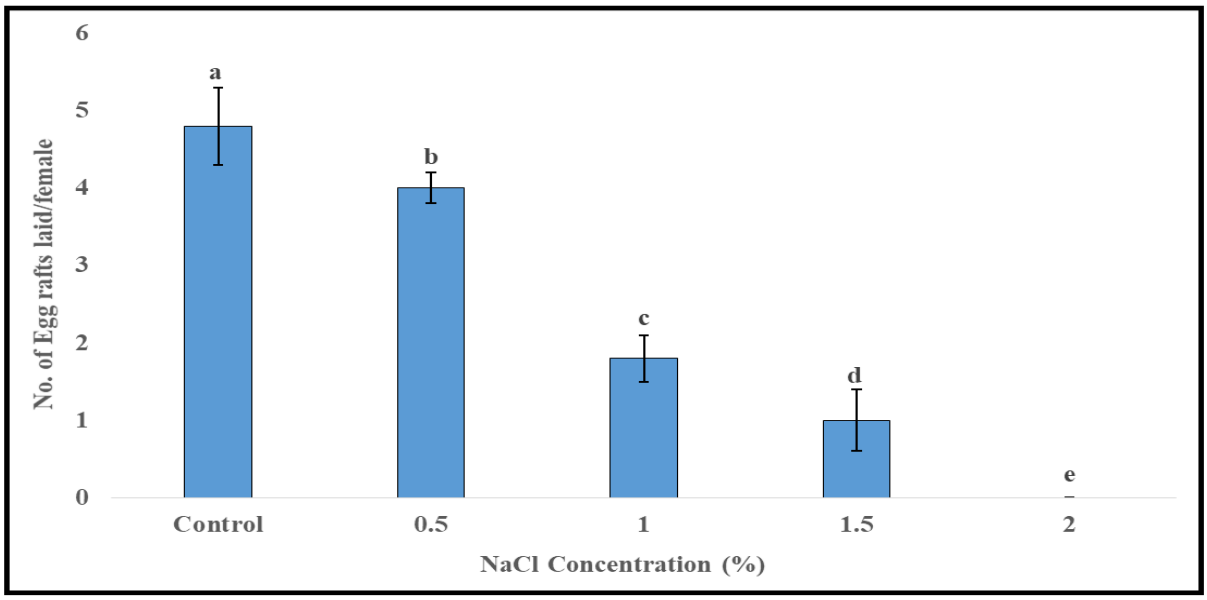

Fig. 1. Effect of NaCl concentration on oviposition of wild *Culex quinquefasciatus*. Mean ± SE followed by same letter in a column are not significantly different at P≤0.05 level (ANOVA followed by LSD posttest), n=10.

Choice of colour ovitrap for oviposition

Oviposition behavior of female *Culex* mosquito in relation to the colour of the ovitrap was ascertained and significant influence of colour on the oviposition was noticed. The data in the current study reflects that *Culex* mosquito preferred black colour ovitrap and blue colour was the least preferred one, as presented in Fig. 2.

The egg rafts laid by the female *Cx. quinquefasciatus* in different colour edovitraps with the decreasing order preference were white, green, yellow, red and blue.

Choice of water from different sources for oviposition

Experiments were undertaken to understand the oviposition behavior of *Cx. quinquefasciatus* in different ovitraps with water samples collected from different sources like tap, well, pond, river, paddy field and drain.

The results of the present study indicated that the most preferred ovitrap was the water of paddy field and followed by the decreasing order of pond, tap water, drain and river (Fig. 3).

There was not statistically different in egg laid by the female in drain and river water. It was further noticed that, *Culex* mosquito did not prefer the water collected from well for oviposition.

Choice of larva holding water for oviposition

Entomological investigations were made in the current study to understand the influence of larval presence in the water of the ovitrap for oviposition by

the female adult *Culex* mosquito. Five-six days oldmatured gravid female *Cx. quinquefasciatus* were allowed for oviposition in the ovitraps containing distilled water and water with 10, 20 and 40 larvae of same species separately. It was observed that there was a remarkable statistically significant difference in egg laying activity of *Cx. quinquefasciatus*.

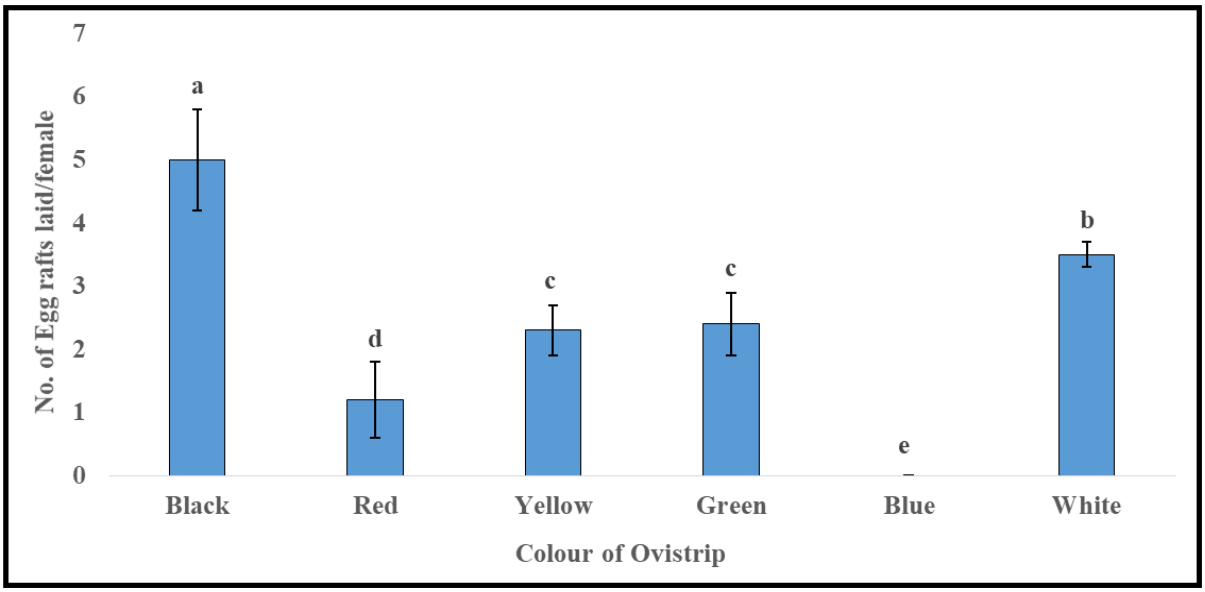

Fig.2. Oviposition preference of wild *Culex quinquefasciatus*for different colour edovitraps. Mean ± SE followed by same letter in a column are not significantly different at P≤0.05 level (ANOVA followed by LSD posttest), n=10.

The female mosquitoes laid maximum number of egg rafts in the larva holding water than only distilled water (Fig. 4).

Choice of ovitrap size for oviposition

Ovipitraps with different surface area (diameter in cm) varies from 6.0 to 12.0 cm were taken to study the preference of the oviposition of *Cx. quinquefasciatus* with respect to different size of the ovitraps. All the ovitraps are filled with dechlorinated tap water to provide oviposition surface at the time of experiment.

It was observed that the maximum numbers of egg rafts were laid in the largest ovitrap taken and the number gradually decreased with the decrease in the surface area of the ovitraps that exhibits positive correlation between the size of the ovitraps and number of egg rafts laid (Fig. 5).

Discussion

Selection of an appropriate oviposition site has a great influence on maternal reproductive success in species with aquatic larval and pupae stages (Millar *et al.*, 1994).

This is because several factors of the water, both physical and chemical, influence hatching success and larval survival (Resetarits Jr. and Wilbur, 1989).

Therefore, mosquitoes have a strong selection for discrimination of potential oviposition sites based on offspring viability (Petranka and Fakhourry, 1991).

Impact of NaCl on oviposition behavior

In the present investigation, the authors were inclined to study the oviposition response of female *Cx. quinquefasciatus* mosquitoes to solution containing different concentrations of NaCl.

It was observed that maximum number of egg rafts were laid by *Cx. quinquefasciatus* females in distilled

water and gradually decreased with an increase in the concentration of NaCl which is in agreement with the findings of Anne Hudson (1956) on *Culex molestus*. However, Wallis (1954, 1955) reported that 0.5% NaCl solution was preferred over distilled water for oviposition by *Aedes* mosquitoes. It may be concluded that *Cx. quinquefasciatus* mosquitoes have preference for normal water than the saline water for the oviposition medium for oviposition.

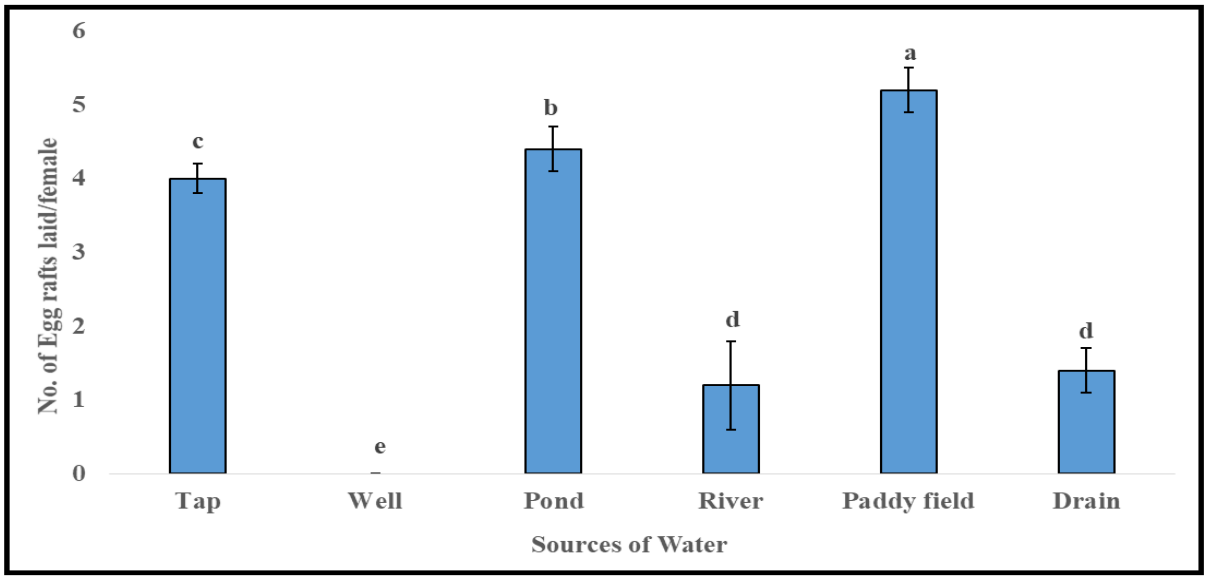

Fig. 3. Oviposition preference in water from different sources by wild *Culex quinquefasciatus*. Mean ± SE followed by same letter in a column are not significantly different at P≤0.05 level (ANOVA followed by LSD posttest), n=10.

Effect of container colour on oviposition behavior

Container colour was important in influencing the oviposition choices of some female mosquitoes breeding in artificial containers. In order to find out if ovipositing *Cx. quinquefasciatus* have any choice for the colour of the medium for oviposition, it was decided to use six different colour edovitraps to study their oviposition behaviour.

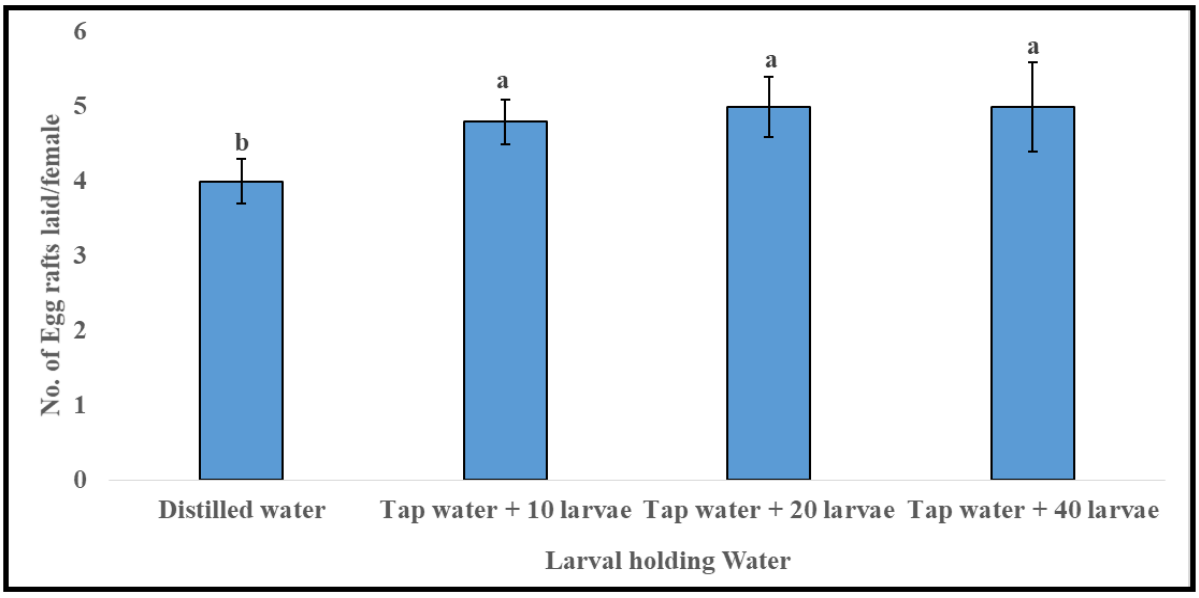

Fig. 4. Oviposition preference in Ovitraps with larva holding water by wild *Culex quinquefasciatus*. Mean ± SE followed by same letter in a column are not significantly different at P≤0.05 level (ANOVA followed by LSD posttest), n=10.

It was noticed that the maximum number of egg rafts were laid on black ovitrap which is similar to the findings of Panigrahi *et al.*, (2014) on *Aedes* mosquitoes. Similar observations were also made in various mosquito species by Williams,1962; Wilton, 1968; Mc Daniel *et al.*, 1976; Yap and Foo, 1984; Beehler *et al.*,1992; Jones and Schreiber, 1994; Yanoviak 2001; Colton *et al.*, 2003. However, Badamasi *et al.*, (2008) reported that red being the most attractive colour, followed by brown, black, blue, purple, pink, yellow and white by certain mosquito species. Further, it has been reported that both black and red containers acted as oviposition stimulant and attractant for *Toxorhynchites amboinensis* females (Collins and Blackwell, 2000). Earlier reports of Hilburn *et al.*, (1983) and Jones and Schreiber, (1994) suggested that mosquito species prefers other colour in the absence of black coloured container for oviposition.

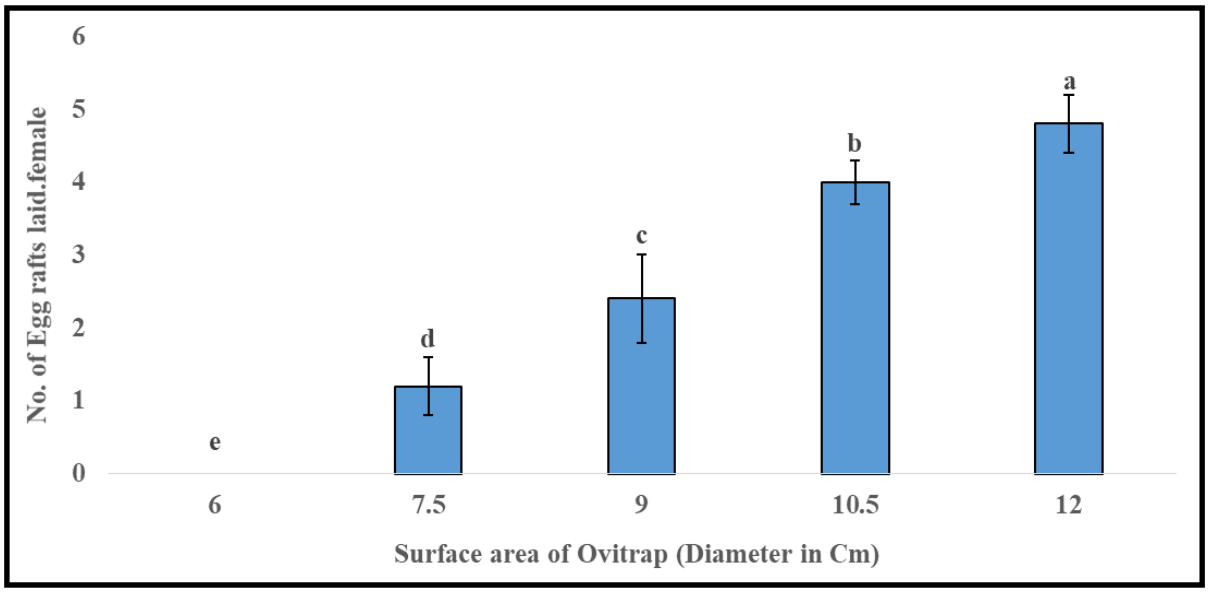

Fig. 5. Oviposition preference by wild *Culex quinquefasciatus*in response to size of the Ovitrap. Mean ± SE followed by same letter in a column are not significantly different at P≤0.05 level (ANOVA followed by LSD posttest), n=10.

Influence of water collected from different sources on oviposition behavior

In the present study, maximum number of egg rafts were found in the ovitrap contained water of paddy field followed by the water of pond, tap, drain and river but no egg rafts were laid in the well water which is in contrast to the findings of our previous report of Panigrahi *et al.*, (2014) where maximum number of eggs were laid by female *Aedes* mosquitoes in distilled water.

Influence of Larva holding water on oviposition behavior

Review of literature says that ovipositing females usually choose water with the presence of life (Bentley and Day, 1989; Lee, 1991) and normally mosquitoes avoid oviposition where interspecifc competitors are present (Blaustein and Kotler, 1993) but are attracted to sites where other mosquito larvae are present (Beehler and Mulla, 1995) because the presence of conspecific larvae may provide a reliable cue that the site offers suitable conditions for larval development (Stav *et al.*, 1999). In the present experiment too, the gravid females of *Cx. quinquefasciatus* preferred larval holding water over distilled water for oviposition which is also similar to the findings of Panigrahi *et al.*, (2014) on *Ae. albopictus* and *Ae. aegypti*. However, oviposition of female *Culex quinquefasciatus* was not statistically different in ovitrap having 10, 20 and 40 larvae of same species in the current study might be due to the repellent effect

of overcrowded larvae on ovipositing females (Suh *et al*., 2016).

Effect of container size on oviposition behavior

In the current study, it was found that *Cx. quinquefasciatus* females prefer larger surface area of ovitrap than the smaller ones for oviposition. The number of egg rafts laid exhibiting a direct linear relationship between the surface area of the ovitrap for oviposition which was similar to the observations made by Derraik and Slaney, (2005) and Panigrahi *et al*., (2014). It is believed that the larger containers may provide large, protected, humid resting surfaces for females preparing for oviposition (Reiskind and Zarrabi, 2012). Further, in larger containers, the chances of desiccation are less and are likely to contain more amou]nt of food and thus are likely to increase the chances of larval survival. Thus it may be concluded that *Cx. quinquefasciatus* mosquitoes prefer large sized containers holding water for oviposition compared to the ones with smaller dimensions.

Conclusion

Results of the current study related to oviposition behavior of *Culex* mosquito has immense scope to design and develop effective oviposition attractants for use in trapping mosquito species for the management of mosquito borne diseases.

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Conflict of Interest

There was no conflict of interest regarding the publication of this manuscript.

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