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Budding Researchers' Section

LABORATORY STUDIES ON OVIPOSITIONAL PREFERENCES OF MALARIA VECTOR *ANOPHELES SUBPICTUS* PREVALENT IN HOOGHLY DISTRICT, WEST BENGAL, INDIA

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ABSTRACT

Background: Mosquitoes serve as vector for several disease-causing pathogens in the world. The present study was conducted to investigate ovipositional preferences of the field-caught and blood-fed adult *Anopheles subpictus* mosquitoes in laboratory conditions.

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Methods: Mosquitoes were offered with seven different choices of water, viz., pond water, rice-field water, drain water, ditch water, tap water, distilled water and a mixture of distilled water and cow urine in 1:1 ratio; natural habitat water in different coloured (white, orange and green) and different sized cups for oviposition in caged condition.

Results: Number of eggs laid was highest in pond water (235.33 ± 6.74), followed by tap water (114.66 ± 3.17) and lowest number of eggs were laid in distilled water (12.66 ± 1.45) with intermediate values in other types of water. Among different coloured oviposition substrates provided, the highest amount of oviposition was recorded in white substrate (211.66 ± 4.33) and the lowest oviposition occurred in orange coloured substrate (20.66 ± 4.05). Mosquitoes laid higher number of eggs (221 ± 5.5) in large square shaped oviposition substrate when offered along with smaller ones. One way ANOVA showed that there was a significant difference ($p < 0.05$) in respect to the number of eggs laid in different oviposition substrate.

Conclusion: The present study demonstrates that *An. subpictus* preferred to lay eggs in white, square shaped and large containers than the coloured, small and round containers, which indicated that mosquitoes might use some behavioural and chemical cues during oviposition. A better understanding of this phenomenon would help in developing effective management strategies of this important vector in the peninsular India.

Keywords: *Anopheles subpictus,* oviposition, breeding habitat, eggs, management strategy

INTRODUCTION

Mosquitoes are small dipteran insects, serving as vectors of numerous pathogens like *Plasmodium* spp., *Wuchereria bancrofti*, dengue viruses, West Nile virus, etc., which are agents of deadly and/or debilitating diseases. Among these vectors several species of the genus *Anopheles* such as *Anopheles culicifacies, An. stephensi, An. fluviatilis, An. subpictus, An. sundaicus*, etc. transmit deadly malaria in India.[1] *Anopheles subpictus* Grassi (1899) acts as a potent malaria vector in several rural areas of Hooghly district, West Bengal.[2] Till today the most effective way to prevent malaria is vector control.[3] Mosquitoes of several genera prefer to lay eggs in specific types of aquatic environment, which ensures the survival and fitness of their progeny.[4,5] Availability of the preferable oviposition site leads to greater degree of oviposition by adult mosquitoes, which increases the vector prevalence. In nature some aquatic habitats are found to have greater vector populations while some remain un-colonized, which suggest that some habitats are more attractive to gravid females than others. There are several physical and chemical cues, including olfactory cues, cues from other immature larvae, colour and turbidity of oviposition substrate,[6] microbial activity of breeding habitats, soil nature,[7] which altogether influence the mosquito ovipositional activity. Proper identification of these cues may help to better understand the species-specific habitat preferences for ovipositional activity. Complete knowledge about several factors, which influence the daily oviposition pattern of gravid female mosquitoes, may help to successfully control the vector mosquitoes in the field condition.[7,8] Interruption of mosquito oviposition sites leads to reduced vector populations and gives a positive impact on mosquito control programme.[9] The behavioural ecology of oviposition of malaria vector still remains unknown and a proper elucidation of the principal components associated with oviposition behaviour of malaria vectors is still lacking.[10]

The present study aims to determine the ovipositional preferences of the field-caught adult *An. subpictus* in laboratory condition, which would provide a new insight for control of mosquito oviposition or to create an efficient ovitrap for vector-control programme in malaria endemic regions.

MATERIAL AND METHODS

Collection of adult mosquitoes:

Wild adult blood-fed female *An. subpictus* mosquitoes were hand-collected using test tubes from human dwellings and cattle-sheds in rural areas of Hooghly district. The adult mosquitoes were then brought alive to the Parasitology &

Microbiology Research Laboratory of University of Burdwan for further investigation.

Mosquito identification and rearing:

The adult mosquitoes were identified morphologically following Nagpal & Sharma.[11] Mosquitoes other than *An. subpictus*, male *An. subpictus* mosquitoes and unfed mosquitoes were discarded. Adult gravid females of *An. subpictus* were then released in mosquito rearing cages (30cm × 30cm × 30cm). The cages were maintained at 28±2 ºC temperature, 80±5% relative humidity and 12:12 h (light:dark) photoperiod in an environmental chamber.

Ovipositional bioassay:

Ovipositional activity of field collected gravid *An. subpictus* was tested on different types of water, different coloured oviposition substrates and in different shaped oviposition substrate in laboratory condition in separate cages. Three replications were made for each experiment.

Cage 1: In different types of water

In this cage ten adult field-collected gravid *An. subpictus* mosquitoes were released and seven oviposition substrates of 100 ml capacity, each containing seven different types of water, *viz*., pond water, rice-field water, drain water, ditch water, tap water, distilled water, a mixture of distilled water and cow urine (1:1 ratio) were taken and were placed randomly inside the cage for mosquito oviposition. Pond water, rice-field water, drain water and ditch water were collected from the field. Distilled water was kept as control. Cow urine used in this study was freshly collected from the cattle shades in rural areas.

Cage 2: In different coloured oviposition substrates

In this cage, ten adult field-collected gravid *An. subpictus* mosquitoes were released and three different coloured oviposition substrates of 100 ml capacity, *viz*., white, green and orange-coloured plastic cups containing natural anopheline breeding habitat water (pond water) were placed randomly inside the cage for mosquito oviposition.

Cage 3: In different shaped oviposition substrates

Ten adult field-collected gravid *An. subpictus* mosquitoes were released in this cage and three different shaped oviposition substrates, *viz*., round substrate, small square shaped substrate (7cm × 7cm) and large square shaped substrate (14cm × 14cm) containing natural anopheline breeding habitat water (pond water) were placed randomly inside the cage for mosquito oviposition.

Record of data:

Number of eggs laid in each of the oviposition substrates was recorded during three consecutive days with the help of a dissecting binocular.

Statistical analysis:

Data were subjected to analysis by one way ANOVA using SPSS 16.0 to see whether there is any significant difference in the number of eggs laid among different oviposition substrates.

RESULTS AND DISCUSSION

Total number of eggs laid (mean ± S.E) in five different types of water in the cage 1 is given in Table 1. In this case it was found that the mosquitoes laid highest number of eggs in cups containing pond water (235.33 ± 6.74), whereas lowest number of eggs were laid in cups having distilled water (12.66 ± 1.45). One way ANOVA showed that different types of water samples have significant influence on the number of eggs laid by gravid female *An. subpictus* [$F(6, 14)=431.708$, $p< 0.05$]. A study in Africa showed that cow urine acted as an attractant for *Anopheles gambiae* oviposition in laboratory condition.[9] In the present study, however, we have recorded fewer numbers of eggs (25.33 ± 2.9) in distilled water + cow urine (1:1 ratio). Number of eggs laid in ditch water (89 ± 3.78) was comparatively higher than the number of eggs laid in drain water (52.33 ± 4.33). Moreover, mosquitoes laid more eggs in tap water (114.66 ± 3.17) than in the distilled water (12.66 ± 1.45). On the other hand, number of eggs laid in rice-field water (184 ± 3.46) was higher than tap water (114.66 ± 3.17) but lower than the number of eggs laid (235.33 ± 6.74) in pond water. The differences in the number of eggs laid between distilled water and distilled water + cow urine were not statistically significant ($p = 0.332$). Total number of eggs laid (mean ± S.E) in three different coloured oviposition substrates in cage 2 is given in Table 2. Gravid females of *An. subpictus* laid much higher number of eggs (211.66 ± 4.33) in white coloured oviposition substrate and lowest number of eggs (20.66 ± 4.05) in orange coloured oviposition substrate. Higher numbers of eggs were recorded in green substrate (81.66 ± 4.6) than orange one. There was a significant difference in the number of eggs laid in different coloured oviposition substrates [$F(2, 6) = 503.841$, $p < 0.05$]. Previous study showed that colour of oviposition substrate affected the mosquito oviposition.[6]

Table 1. Number of eggs laid by *An. subpictus* in different types of water

Types of water	Number of eggs laid (Mean ± S.E)
Natural breeding habitat water (Pond water)	235.33 ± 6.74
Rice-field water	184 ± 3.46
Drain water	52.33 ± 4.33
Ditch water	89 ± 3.78
Tap water	114.66 ± 3.17
Distilled water	12.66 ± 1.45
Distilled water + Cow urine	25.33 ± 2.9

Table 2. Number of eggs laid by *An. subpictus* in different coloured oviposition substrates

Colour of oviposition substrates	Number of eggs laid (Mean ± S.E)
White	211.66 ± 4.33
Orange	20.66 ± 4.05
Green	81.66 ± 4.6

Table 3. Number of eggs laid by *An. subpictus* in different shaped oviposition substrates

Shape of oviposition substrates	Number of eggs laid (Mean ± S.E)
Round	24.33 ± 3.4
Small square	112 ± 4.35
Large square	221 ± 5.5

The result of the present study also provided an indication that there might be some visual cues which could affect mosquito oviposition. The adult *An. subpictus* could differentiate among different colours and were more attracted to white substrate than the coloured ones (orange and green). Total number of eggs laid (mean ± S.E) in different sized oviposition substrate is given in Table 3. Square shaped oviposition substrates were found to be more preferred for oviposition by *An. subpictus* than round oviposition substrates and among the square shaped substrate more number of eggs (221 ± 5.5) were laid in large square shaped substrate than small square ones (112 ± 4.35). Differences in the number of eggs laid in different shaped oviposition substrates were statistically significant [$F(2, 6) = 473.958$, $p < 0.05$]. These data suggested that availability of surface area was an important factor of *An. subpictus* oviposition. As the large square shaped oviposition substrates have a relatively more surface area than small square and round ones, so significantly more amount of eggs were laid in large square shaped oviposition substrate only.

The tendency of mosquito oviposition is related to location and availability of their suitable breeding grounds. In an African laboratory study of mosquito oviposition, both the wild caught and greenhouse reared *An. gambiae* mosquitoes laid more number of eggs in anopheline larval water than culicinae larval water or distilled water in a choice bioassay which was statistically significant.[10] In the present study, gravid female *An. subpictus* mosquitoes also laid significantly high number of eggs in the oviposition substrate containing their natural breeding habitat water (pond water) than tap water, distilled water and other types water like drain water, ditch water etc. In another study, gravid *An. gambiae* mosquitoes also showed a marked preference towards water taken from a site which was naturally inhabited by anopheline larvae.[6] Several studies have suggested that breeding habitats mixed with cow urine can act as attractant sites of oviposition by gravid female mosquitoes.[12,9] Components of cow urine have also been found to attract other arthropods like *Hybomitra* house flies.[13] The present study depicted that cow urine did not significantly influence oviposition by gravid *An. subpictus* mosquitoes.

There are several biotic and abiotic factors which have significant influences on mosquito oviposition. Different types of water differentially affect gravid mosquito oviposition pattern not only due to differences in physico-chemical parameters of water[14,15,16,17] but also for the microbial activity.[18,19,20] In addition, mosquitoes also use visual cues to select their suitable breeding grounds that may increase the survivability and fitness of their progeny.[6]

CONCLUSION

The present study shows that the field collected blood-fed female *An. subpictus* mosquitoes prefer to lay eggs in their natural breeding habitat water than other types of water when provided in oviposition cups in laboratory condition. They also use visual cues and preferred white and large square substrates to lay their eggs than coloured and small oviposition substrates. These experiments elucidate that specific breeding habitat water containing a good number of substances exhibit a significant level of attractiveness for the ovipostion of *An. subpictus*. The synergistic activity of the attractants could provide a feasible and real-world tool to be exploited in the management of this vector mosquito. More detailed work however needs to be done to elucidate the effect of these factors on mosquito oviposition.

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REFERENCES

1. Panicker KN, Geetha BM, Bheema Rao US, Viswam K, Suryanarayanmurthy U. *Anopheles subpictus*, vector of malaria in coastal villages of South-East India. Curr Sci. 1981; 50: 694-5.

2. Chatterjee S, Chandra G. Role of *Anopheles subpictus* as a primary vector of malaria in an area in India. Jpn J Trop Med Hyg. 2000; 28(3): 177-81.
3. Lindh JM, Okal MN, Herrera-Varela M, *et al*. Discovery of an oviposition attractant for gravid malaria vectors of the *Anopheles gambiae* species complex. Malar J. 2015; 14(1):119. https://doi.org/10.1186/s12936-015-0636-0.
4. Bentley MD, McDaniel TN, Lee H, Stiehl B, Yatagai M. Studies of *Aedes triseriatus* oviposition attractants producted by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). J Med Entomol. 1976; 13: 112-5.
5. Bentley MD, Day JF. Chemical ecology and behavioral aspects of mosquito oviposition. Ann Rev Entomol. 1989; 34: 401-21.
6. McCrae AW. Oviposition by African malaria vector mosquitoes: II. Effects of site tone, water type and conspecific immatures on target selection by freshwater *Anopheles gambiae* Giles, sensu lato. 1984. Ann. Trop. Med. Pub. Hlth. 1984; 78(3): 307-18.
7. Sumba LA, Guda TO, Deng AL, Hassanali A, Beier JC, Knols BG. Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. Int J Trop. 2004; 24(3):260-5.
8. Wondwosen B, Birgersson G, Seyoum E, *et al*. Rice volatiles lure gravid malaria mosquitoes, *Anopheles arabiensis*. Sci Rep. 2016; 6: 37930.
9. Kweka EJ, Owino EA, Mwang'onde BJ, Mahande AM, Nyindo M, Mosha F. The role of cow urine in the oviposition site preference of culicine and *Anopheles* mosquitoes. Parasites Vectors. 2011; 4: 184.
10. Sumba LA, Okoth K, Deng AL, *et al*. Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae) on different types of aqueous substrates. J Circadian Rhythms 2004; 2(1): 6.
11. Nagpal B, Sharma VP. Indian Anophelines. Science Publishers, New York. 1995.
12. Mahande AM, Mwang'onde BJ, Msangi S, *et al*. Is aging raw cattle urine efficient for sampling *Anopheles arabiensis* Patton? BMC Infect Dis. 2010; 10:172.
13. Mihok S, Mulye H. Responses of tabanids to Nzi traps baited with octenol, cow urine and phenols in Canada. Med Vet Entomol. 2010; 24(3): 266-72.
14. Chakraborty A, Chatterjee S. Studies on the fitness components and comparative oviposition preferences of *Aedes albopictus* in various larval microhabitats of Burdwan, West Bengal. Int J Mosq Res. 2015; 2(3): 50-60.
15. Ahamadi SM, Vatandoost H, Zare M. Characterization of larval habitats for anopheline mosquitoes in a malarious area under elimination program in the southeast of Iran. Asian Pac J Trop Biomed. 2014; 4 (Suppl. 1): S73-S80.
16. Chatterjee S, Chakraborty A, Sinha SK. Spatial distribution & physicochemical characterization of the breeding habitats of *Aedes aegypti* in & around Kolkata, West Bengal, India. IJMR. 2015; 142 (Suppl. 1): S79- S86.

17. Alam MS, Al-Amin HM, Elahi R, *et al*. Abundance and Dynamics of *Anopheles* (Diptera: Culicidae) Larvae in a Malaria Endemic Area of Bangladesh. J. Med. Entomol. 2017; 55(2): 382-91.

18. Pavlovich SG, Rockett C. Color, Bacteria, and Mosquito Eggs as Ovipositional Attractants for *Aedes Aegypti* and *Aedes Albopictus* (Diptera: Culicidae). The Great Lakes Entomologist. 2000; 33(2): 141-53.

19. Huang J, Miller JR, Chen SC, Vulule JM, Walker ED. *Anopheles gambiae* (Diptera: Culicidae) oviposition in response to agarose media and cultured bacterial volatiles. J Med Entomol. 2006; 43(3): 498-504.

20. Nilsson LK, Sharma A, Bhatnagar RK, Bertilsson S, Terenius O. Presence of *Aedes* and *Anopheles* mosquito larvae is correlated to bacteria found in domestic water-storage containers. FEMS Microbiol Ecol. 2018; 94(6). doi: 10.1093/femsec/fiy058. PMID: 29617987.

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