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Studies on *Anopheles stephensi* Var*. mysorensis*- choice of water and effect of pH on oviposition

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ABSTRACT

Two experiments were designed to study the preference of different types of water for oviposition by *Anopheles stephensi* Var *mysorensis* in the laboratory. Nine types of water (pH range 6.0 to 7.0) viz; Pond water, Well water, Tube well water, Municipal tap water, Distilled water containing 1.0 per cent glucose, Distilled water containing 1.0 per cent glucose and 1.0 per cent yeast, Distilled water containing 1.0 per cent yeast, saline water and Drain water were provided to the blood fed mosquitoes. *Anopheles stephensi* Var *mysorensis* females did not prefer highly acidic (pH 2.0 to 5.0) or sufficiently alkaline medium (pH 9.0 and above) for oviposition. *Anopheles stephensi* Var *mysorensis* invariably preferred waters having pH value 6.0 and 7.0.

Key Words - *Anopheles stephensi* Var*. mysorensis,* Hatching, Pond water, Tube well water, Municipal water, Tap water, Distilled water, Well water, HCl, NaOH, pH, Buffer

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INTRODUCTION

Different stages of anopheline mosquitoes exhibit wide range of adaptation to the habitat. The characteristics of aquatic environment that make a medium favourable for life of mosquitoes are well known. Hydrogen ion concentration, or pH, is considered to be an important limiting factor and thus to be a promising overall indicator for determining the general ecological condition of aquatic environment. Early interest in pH in ecological work was due to the discovery by the physiologist that pH was very important in regulating respiration and enzyme systems within the body, very small differences being critical (Odum,1971). The chemical content of water has also been found to influence the breeding of anopheline. Of the many factors the pH, dissolved gases, inorganic salts and nitrates play a very important role. The pH of natural water varies considerably. Mosquito breeding has been noticed in water collections having varied pH. However, it is not clear that why certain breeding places are preferred by certain species. Some species for example *Anopheles sundaicus* is alkaliphilic (Sen, 1938), while some other like *Anopheles stephensi* and *Anopheles varuna* are acidophilic (Ramchandra Rao, 1981). Species breeding in water having pH between the two extremes are not uncommon. It appears that pH value has an indirect effect on larval breeding. The mosquito larvae also differ in their ability to absorb dissolved oxygen in water. In general, anopheline larvae requires more oxygen than others (Subba Rao, 1989).

Adult mosquitoes have wings. Male mosquitoes feed only on nectar, plant juice and other sources of liquid carbohydrates. Male mosquitoes usually emerge a few hours (up to a few days) before the females emerge. The males rest in vegetation surrounding the emergence site waiting for female to emerge. Female mosquitoes also feed periodically on nectar, plant sap and other sources of carbohydrates for energy. However, the females

must have a blood meal before they can produce egg.

Anopheline mosquitoes breed in clean water collections, therefore breeding is dramatically high in the rainy season as well as period beyond that because many artificial water collections are filled with rain water, which provide suitable breeding grounds. There are habitats favourable for anopheline breeding available everywhere in India. Breeding habitats include- pond, swamps, rice field, spring pools, dams, seepages, wells, etc. The breeding habits of anophelines have been studied by a large number of workers viz; Challam, 1924; Senior White and Adhikari,1939 and Muirhead Thomson, (1940a, 1940b, 1940c, 1941a, 1941b, 1941c, 1942a, 1942b, 1945 and Singh ,1999).

MATERIALS AND METHODS

blood fed *A. stephensi* Var. *mysorensis* were collected from in and around Hazaribag Town from various houses and cattle shed during evening and nights using test tube or an aspirator. Collected mosquitoes were brought to the laboratory and released in an especially designed breeding chamber. The breeding chamber consisted of a steel frame work (2.7 feet x 1.2 feet x 1.2 feet).

The four sides of the chamber were covered by a fine white nylon net which prevented the escape of mosquitoes, but allowed free entry of air and also permitted observations from outside. On one side of the net, a hole was made with which one-foot-long tube-like net was sewed. Through this tubular netting hand could be easily entered into the breeding chamber. This tubular netting was tightened at its base all the time except when there was a need to enter the hand for releasing mosquitoes.

Blood fed female mosquitoes collected from the field were released into the breading chamber containing 250 ml beakers almost filled with water. Such mosquitoes deposited eggs on the surface of water contained in the beakers. The eggs were laid singly.

In the first design of experiment for determining the choice of the water type for oviposition following types of water were kept in the oviposition chamber: - 1. Pond water, 2. Well water 3. Tube well water 4. Municipal tap water 5. Distilled water containing 1% glucose 6. Distilled water containing 1% glucose and 0.5 % yeast. 7. Distilled water containing 1% yeast. 8. Drain water 9. Saline water

In another set of experiment, blood fed females were also supplied with water having different pH values ranging from pH 2.0 - 12.0, to see if there is any preference of female to deposit eggs in a particular pH. The eggs were usually laid by the females in between 7.00 pm to 11.00 pm. Mosquitoes were provided water with pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.5, 10.0 and 12.0. For obtaining desired pH buffer tablets (E. Merck India Limited) of different pH and HCl and NaOH were used.

RESULTS

The average numbers of eggs laid in different types of water were different (Table 1 and Figure 1.1). The average number of eggs laid in Pond water, Well water, Municipal tap water, and distilled water containing 1% glucose were 125.66, 89.33, 107.0,103 and 95 respectively. The experiment demonstrated that the females preferred pond water the most and the Well water the least. Further, the order of preference for oviposition in different types of water was as follows:

Pond water > Tube well water > Municipal water > Tap water > Distilled water containing 1.0 per cent glucose > Well water.

In the second experiment ten blood fed *Anopheles stephensi* Var *mysorensis* females were released in the breeding cage containing distilled water in eleven 250 ml beakers. These beakers contained distilled water with different pH, ranging from 2.0 to 12.0. The purpose of the study was mainly to know the range of pH of water in which the mosquito under study lay eggs, and to know the optimum pH of water. The results of these experiments are presented in table-2.1, which also shows the number of eggs laid. The experiment was repeated thrice. This experiment was conducted at room temperature (26°C to 28°C).

Table-1: Oviposition choice of *Anopheles stephensi* for different types of water

Sl. No.	Types of water	pH	Number of eggs laid			
			Replicate			
			1	2	3	Mean ± S.E.
1.	Pond Water	7.0	128.42	126.3	123.0	125.9 ± 1.56
2.	Well Water	7.0	81.0	92.75	95.5	89.75 ± 4.44
3.	Tube Well Water	7.0	112.0	113.5	96.0	107.16 ± 4.23
4.	Municipal Tap Water	6.0	113.0	105.7	91.0	103.23 ± 6.47
5.	Distilled Water Containing 1% Glucose	7.0	88.43	99.3	98.0	95.0 ± 3.42
6.	Distilled Water Containing 1% Glucose and 1% yeast	7 .0	00	00	00	00
7.	Distilled Water Containing 1% Yeast	7.0	00	00	00	00
8.	Drain Water	7.0	00	00	00	00
9.	Saline water	7.0	00	00	00	00

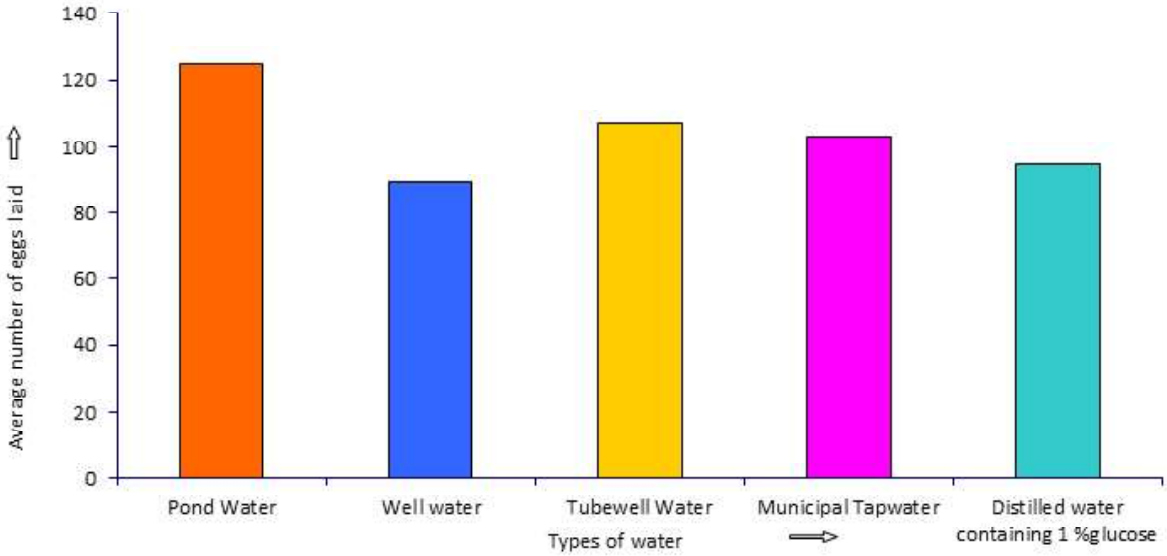

Figure 1- Oviposition choice of *Anopheles stephensi* for different types of water

Table 2- Effect of pH on egg laying of *Anopheles stephensi*

Parameters	Replicates	pH										
		2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
Number of eggs laid when all choices were available to mosquitoes	01	00	00	00	00	116	180	119	00	00	00	00
	02	00	00	00	00	105	127	98	00	00	00	00
	03	00	00	00	00	99	139	117	00	00	00	00
Average	-	-	-	-	-	**106.66**	**148.66**	**111.33**	-	-	-	-
Number of eggs laid when only one choice was available to mosquitoes	01	00	00	48	68	123	138	97	83	89	00	00
	02	00	00	90	103	119	126	90	87	33	00	00
	03	00	00	78	96	114	117	106	93	94	00	00
Average	-	-	-	**72**	**89**	**118.66**	**127**	**97.66**	**87.66**	**72**	-	-

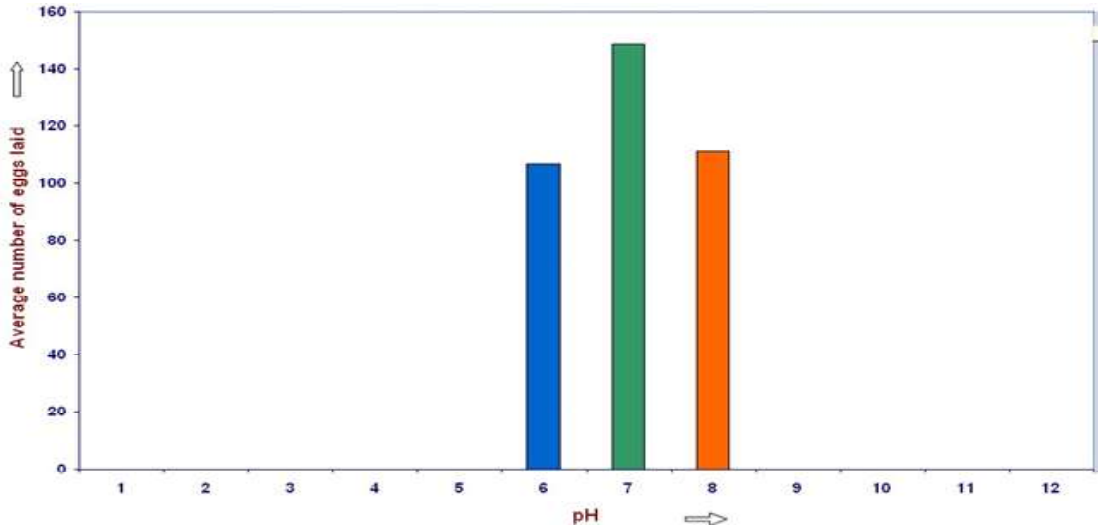

Figure 2- Effect of pH on egg laying of *Anopheles stephensi* Number of eggs laid when all choice were available

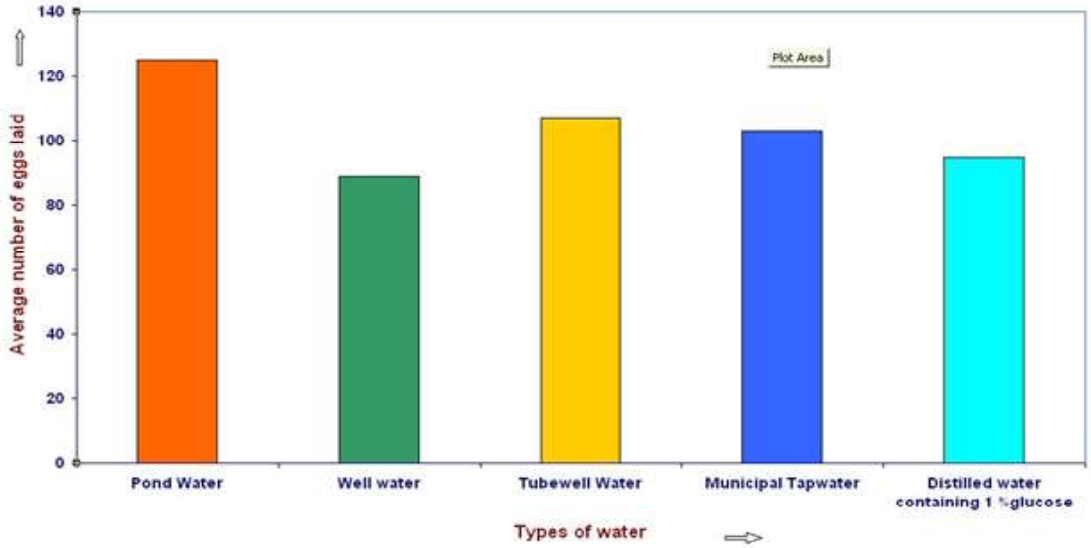

Figure 3- Oviposition choice of *Anopheles stephensi* Number for different types of water

DISCUSSION

The responsibility for the choice of proper oviposition site rests upon the parent insects. Mosquitoes must do so in a place where the eggs are in water. They are also quite specific as to the choice of oviposition site locating it by means of a variety of different stimuli. When an insect is highly selective in the choice of a site for oviposition, it does not mean that it has a foreknowledge of the needs of the offspring. Oviposition in response to specific stimuli that are associated with an environmental stimulation in which the young can survive have, during the course of evolution, been selected for (Romorser, 1981).

Mosquitoes are very selective in respect to oviposition site as they have the capacity to discern between right and wrong kind of water with help their tarsal sensilla (Wallis, 1954). The investigation of Muirhead Thomson (1940, 1941) on the responses of egg laying female of *Anopheles minimus* have shown that several factors viz; temperature, purity, salinity composition, organic pollution, silt, water movement etc have an influence on the ovipositing female.

Experimental studies on the choice of different types of water indicated that *Anopheles stephensi* Var *mysorensis* females when given choice of different types of water were attracted to lay eggs in the selected types of water. The species did not prefer to lay eggs in drain water, distilled water containing 1.0 per cent glucose and 1.0 per cent baker's yeast, distilled water containing 1.0 per cent yeast, and saline water. Thus, the species preferred clean waters when given a choice of different waters. On the other hand, Roy (1931) had found *Anopheles stephensi* breeding in water contaminated by sewage. Similarly, Russell and Mohan (1939) were able to rear *Anopheles stephensi* larvae in many types of waters including contaminated waters. The difference between the findings of the present study and those cited above is perhaps due to availability of only contaminated water for breeding purpose in case of earlier observations. Our results demonstrate that when given a choice of both contaminated and clean waters *Anopheles stephensi* Var *mysorensis* preferred only clean water.

The pH of natural waters varies considerably. Most organisms have a well-defined range of pH tolerance range, death will occur due to respiratory or osmoregulatory failure (Kimmel, 1983). Low pH causes disturbance of balance of sodium and chloride ions in the blood of aquatic animals. At low pH, hydrogen ion may be taken into cell and sodium expelled (Morris *et al*., 1989). It is therefore, most aquatic organism survives best within a limited pH range. Even small range in pH is harmful to pH sensitive species viz; May flies (Sutcliffe and Hildrew, 1989). *Aedes aegypti* and the euryhaline mosquito *Ochelerotatus taeniorhynchus* have been found to complete larval developments in water ranging from pH 4.0 to 11.0, but the larvae always die in water with pH 8.0 or 12.0 (Clarke *et al*, 2004). Similarly, most fish can tolerate pH values outside this range can create problems for reproduction and survivals (Cooper and Wager, 1973). Acid water typically has fewer species and a lower abundance and biomass of macro invertebrates than near-neutral pH waters (Earle and Callaghan., 1998). Rao (1984) has found that *Anopheles stephensi* and *Anopheles varuna* prefer acidic medium. Sen (1938), while working on *Anopheles sundaicus* found a pH range of 7.5 to 8.5 suitable for breeding.

In a set of experiments conducted by us show that *Anopheles stephensi* Var *mysorensis* females have a choice to deposit eggs in waters having pH value ranging from 6.0 to 8.0. This species do not prefer highly acidic and sufficiently alkaline media. This happens when the gravid females were given a choice of waters with pH range 2.0 to 12.0. However, when such mosquitoes were provided only one choice of pH (ranging from 2.0 to 12.0), thus forcing them to deposit their eggs, these preferred a pH range of 4.0 to 10.0. The results thus suggest that under duress *Anopheles stephensi* Var. *mysorensis* may be forced to lay eggs in highly acidic and highly alkaline media. Although some anopheline appear to be acidophilic while other are alkaliphilic, *Anopheles stephensi* Var *mysorensis* under normal conditions preferred waters with pH range 6.0 to 8.0 but under forced conditions they may lay egg in both highly acidic and highly alkaline water.

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