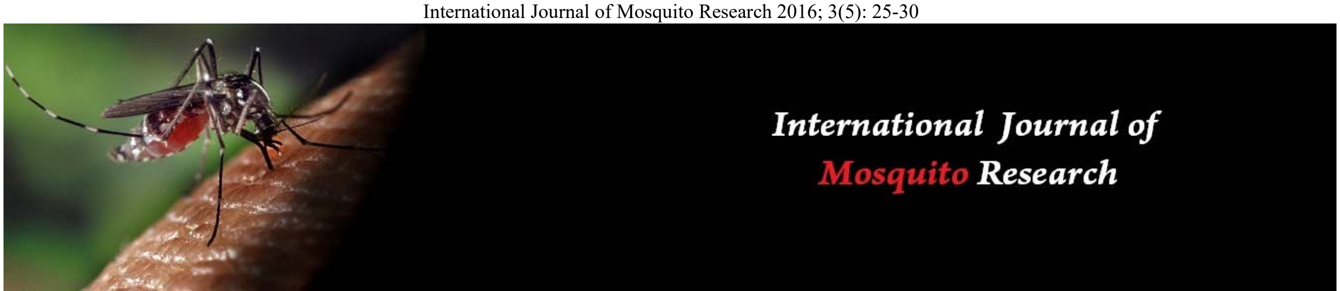

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2016; 3(5): 25-30

Received: 05-07-2016
Accepted: 06-08-2016

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Behavioral resilience of *Culex quinquefasciatus* Say 1823 at Nagpur district of Maharashtra

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Abstract
In Maharashtra state of India, 0.46% population is categorized as active microfilaria carriers perpetuated by the mosquito *Culex quinquefasciatus* Say 1823. The studies on breeding behavior of this vector species at Nagpur district revealed that it prefers to breed in organically polluted water bodies with alkaline tendency. In laboratory evaluation, although the preference of breeding was observed between the pH of 6.5 to 8.5, but the range between pH 7.5 and 8 was the most preferred values. The vector enters the house for feeding at dusk (6:00 pm to 8:00 pm) and prefers dark undisturbed areas for resting like cupboards, behind doors, toilet, bathrooms, dark articles, roof of cattle sheds adjacent to dwelling, dry grass kept for cattle feeding, wood beams of roof and rooms used for grain storage. Biting occurs from 8:00 pm onwards. During summer, cool places like water tanks, shades near water puddle and cracks of trees were found to be preferred site to rest during the day time.

Keywords: *Culex quinquefasciatus,* behavior, breeding, feeding, resting, pH, alkaline

1. Introduction
Lymphatic filariasis commonly known as elephantiasis is considered as a major public health problem due to its morbidity and social stigma caused by its symptoms. It is the second most common vector-borne parasitic disease after malaria and is found in 81 tropical and subtropical countries [1]. Over 120 million people are affected by filariasis; 1.3 billion live at the risk of infection and one third of them live in India [2]. Transmission of the parasite *Wuchereria bancrofti* by *Cx. quinquefasciatus*, accounts for 95% of the total lymphatic filariasis cases in India [3]. The vector of lymphatic filariasis, *Culex quinquefasciatus* Say 1823 is a markedly domestic, strong winged species found all over India, in and around human dwellings.
Rapid urbanization and industrialization without adequate drainage facilities are responsible for its increased dispersal [4]. Although humans are the preferred source, *Cx. quinquefasciatus* mosquitoes can also take blood meals from birds and livestock. In India highest microfilaria carriers are found in West Bengal (1.01%) and lowest in Chhattisgarh (0.14%), whereas for Maharashtra state the value is 0.46% [5]. Microfilariae of *Wuchereria bancrofti* ingested by *Cx. quinquefasciatus* pass through three developmental stages (L1, L2 and L3) and L3 are able to infect humans when the infectious mosquitoes return to feed again [6]. For mosquito borne diseases, vector control is an essential component of disease-eradication programme and is aimed at reducing the vector population below a minimum threshold level so that the transmission of the disease is interrupted [7]. This study makes an attempt to find out the preference of breeding sources, preference of pH value of breeding sources in field and in laboratory conditions, resting and feeding behavior of the *Cx. quinquefasciatus*, in Nagpur district of Maharashtra. This study can be useful for planning control methods for this vector species in this region.

2. Materials and Methods
2.1 Study site
The present investigation was carried for three consecutive years (2011-2013) in and around Nagpur city of Maharashtra state, India. The climate is tropical with three seasons dry (February-June), monsoon (July-August) and post-monsoon (September-December). The average relative humidity is around 70% to 20% and average annual rainfall of 1205 mm. Thirteen sites at Nagpur city and surrounding villages were selected for mosquito collection{Mandavghorad, Shiva, Vihirgaon, Ghogli, Alesur, Gumthi (Fig. 1) and Ajani, Wadi, Ambazari, Wathoda, Babulkheda, Bajajnagar, Bardi (Fig. 2)}.

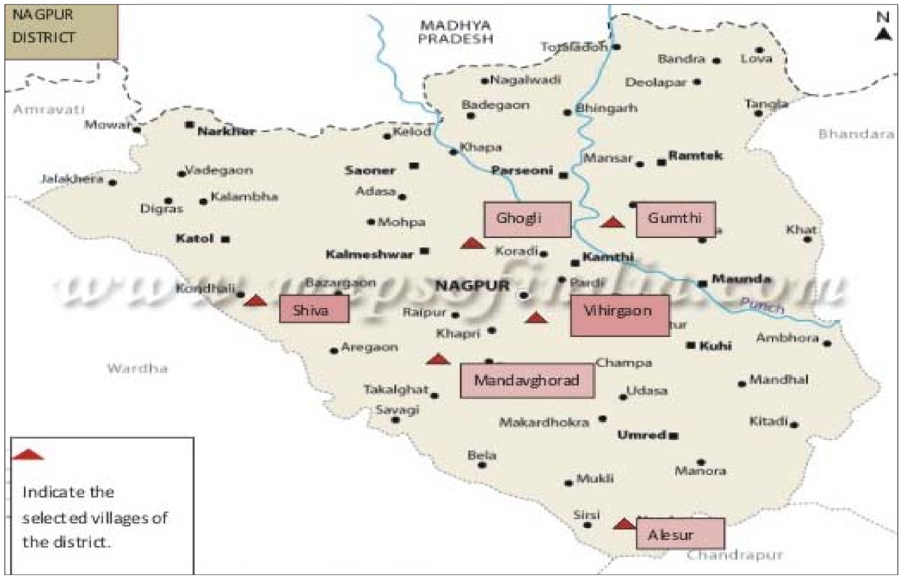

Fig 1: Mapping of selected sites for mosquito collection in Nagpur rural area

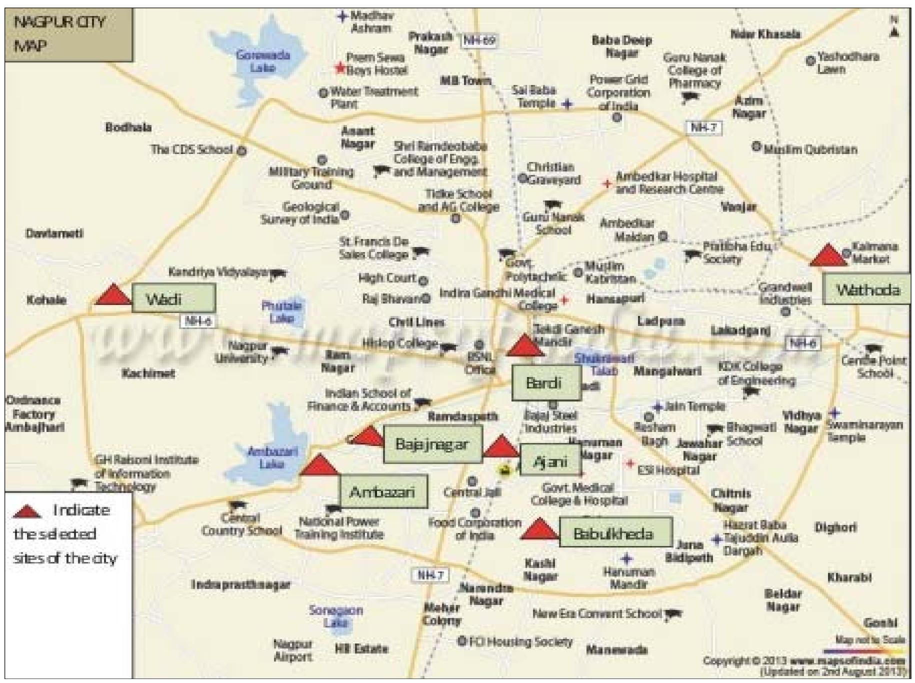

Fig 2: Mapping of selected sites for mosquito collection in Nagpur city area

2.2 Mosquito collection and identification

Mosquitoes were collected from randomly selected houses, cattle sheds and outdoor of selected thirteen sites according to standard entomological surveillance guidelines of WHO [8, 9]. All the adult mosquitoes were identified using standard identification keys of each genus and *Cx. quinquefasciatus* was separated for further evaluation [10-18]. The resting behaviour of *Cx. quinquefasciatus* was noted during surveillance. While entering a house for mosquito collection, the purpose of the investigation was explained to the head member. Permission to enter each of the household was sought and the right to refuse or withdraw at any time was respected.

2.3 Study of breeding preference in different pH

All open water bodies were taken as potential breeding sites and checked by dipper method [8]. The pH value of breeding sources preferred by *Cx. quinquefasciatus* in field was evaluated by pocket pH meter. To study the breeding preference of *Cx. quinquefasciatus* in different pH concentration in laboratory condition, 100ml of aqueous solutions ranging from 4.5 to 9 pH was prepared using sodium hydroxide and hydrochloric acid. Containers of these concentrations were kept in rearing cage and among field collected mosquitoes, ten gravid *Cx. quinquefasciatus* females were separated and introduced in the cage. The egg rafts were observed after 48 hours. Post ovipositor, the egg rafts were counted and preference of pH value noted [19-20].

3. Results

3.1 Breeding behaviour

The breeding sources in the study area for the present investigation were divided into three groups - domestic, peri-domestic and natural.

3.2 Domestic

The major breeding sources for *Aedes, Anopheles* group of mosquitoes are household tanks, overhead cement tanks, overhead plastic tanks, buckets, flower pots, coolers, fountains, drums, mud pots, well, terrace, the water spread cement concrete (used for 'curing'), puddle of water in and around the construction site. While *Cx. quinquefasciatus* and *Armigeres* group of species are found to breed in septic tanks, open gutter line and outlet channel of septic tanks.

3.3 Peridomestic

Coconut shells around the coconut water shop and around the hospital wards, battery boxes, tires, broken bucket, open boxes, cups and containers with water kept for birds, tire-track, pits, burrow, ditch, disposable cups used for tea and water, artificial hole, concrete hole were found to be the breeding sources of *Anopheles* and *Aedes* species while open and closed gutter lines form the breeding sources for *Cx. quinquefasciatus* and *Armigeres* mosquitoes.

3.4 Natural

The river banks are found to be with mixed dwelling of *Anopheles*, *Culex* and *Armigeres* species. Tree holes and rock pools were preferred by *Aedes* species.

3.5 pH preference

After evaluating 88 breeding sources of *Cx. quinquefasciatus* for the pH value preference. It was found that pH value varies between 6.5 and 8. Around 58.28% breeding sources had the pH value of 8. While lowest contributor among pH value with positive breeding sources is of 6.5 (42.85%). Breeding of *Cx. quinquefasciatus* was not found in water bodies of pH value of 6, 8.5 and 9 (Table 1; Fig. 3 and 4).

In controlled conditions, it was found that preference of breeding was observed in beaker contain water of pH values between 6.5 to 8.5, but pH 7.5 and 8 was the most preferred values for this species. Oviposition was not observed in pH value of 4.5 to 6 and 9 (Table 2; Fig. 5).

Table 1: Breeding preference report of *Cx. quinquefasciatus* in different pH.

Sr. No.	pH	No. of breeding sources	Positive breeding sources	Percentage of positive breeding sources.
1	6	7	0	0
2	6.5	21	9	42.85
3	7	18	8	44.44
4	7.5	15	7	46.66
5	8	17	10	58.82
6	8.5	7	0	0
7	9	3	0	0

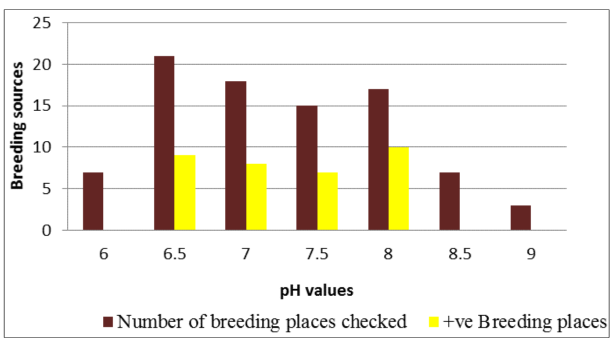

Fig 3: Graphical representation of relationship between breeding sources of *Cx. quinquefasciatus* and pH value.

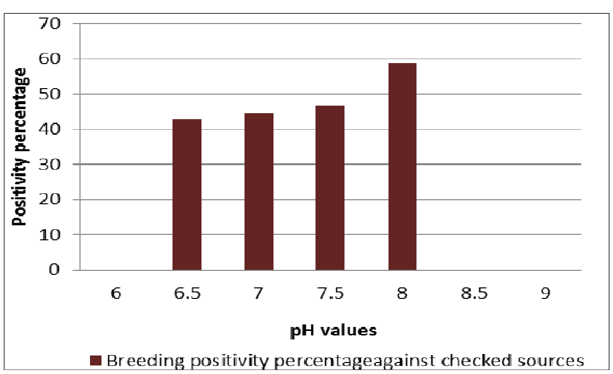

Fig 4: Graphical representation of breeding positivity percentage against checked breeding sources in different pH concentrations.

Table 2: Number of female *Cx. quinquefasciatus* ovipositing in water of different pH.

pH values	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9
No. of female deposited eggs	0	0	0	0	1	1	3	3	2	0

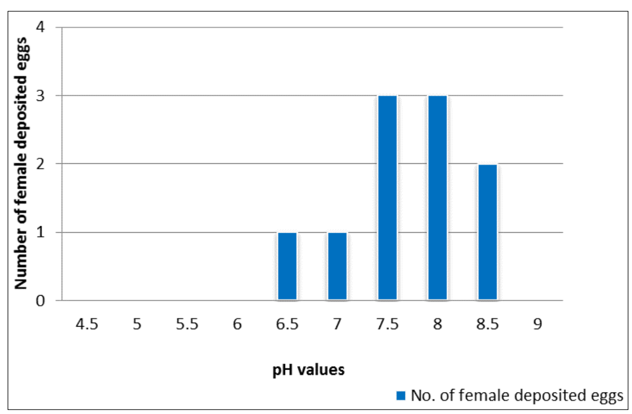

Fig 5: Number of female *Cx. quinquefasciatus* ovipositing in water of different pH

3.5 Resting and biting behaviour

During the present investigation it was observed that *Cx. quinquefasciatus* species enter the house for feeding at dusk (6:00pm to 8:00pm) and prefers the dark undisturbed areas like walls in shade, hanging clothes, cupboards, behind doors, toilet, bathrooms and dark articles for resting. In village, this species was found to rest on the roof of cattle sheds adjacent to dwelling, dry grass kept for cattle feeding, wood beams of roof and rooms used for grain storage. During summer seasons, cool places like water tanks, shades near water puddle were found to be a preferred site to rest during the day time. Almost 95% of the female collected were found full fed, semi-gravid or gravid during the early morning collection. The dusk collection species were found to be very active but unfed (Table 3).

Table 3: Gravidity rate of *Cx. quinquefasciatus* in different season at dawn and dusk.

Year	Season	Time of coll.	Total Coll.	Female	Unfed	Full-fed	Gravidity			
							Semi-gravid	Gravid	Total	Rate In %
2011	Summer	Dawn	41	35	4	7	8	16	24	59.52
		Dusk	12	7	6	0	0	1	1	
		Total	53	42	10	7	8	17	25	
	Rainy	Dawn	101	72	0	11	21	40	61	75.3
		Dusk	11	9	9	0	0	0	0	
		Total	112	81	9	11	21	40	61	
	Winter	Dawn	82	55	0	12	19	24	43	70.96
		Dusk	8	7	6	0	1	0	1	
		Total	90	62	6	12	20	24	44	
2012	Summer	Dawn	54	40	7	9	11	13	24	54.54
		Dusk	6	4	4	0	0	0	0	
		Total	60	44	11	9	11	13	24	
	Rainy	Dawn	138	99	3	10	27	59	86	78.57
		Dusk	16	13	11	0	1	1	2	
		Total	154	112	14	10	28	60	88	
	Winter	Dawn	128	85	10	9	29	37	66	68.68
		Dusk	19	14	12	0	2	0	2	
		Total	147	99	22	9	31	37	68	
2013	Summer	Dawn	55	43	3	3	16	21	27	77.08
		Dusk	6	5	5	0	0	0	0	
		Total	61	48	8	3	16	21	37	
	Rainy	Dawn	151	113	22	17	28	46	74	62.29
		Dusk	12	9	7	0	1	1	2	
		Total	163	122	29	17	29	47	76	
	Winter	Dawn	118	76	1	13	30	33	63	74.41
		Dusk	11	10	8	0	1	0	1	
		Total	129	86	9	13	31	33	64	

4. Discussion

Integrated Vector Management requires correct understanding of the bionomic of vector species. For mosquitoes type availability and abundance of breeding habitats regulate their population and diversity [21, 22]. *Cx. vishnui* and *Cx. tritaeniorhynchus* mainly breed in paddy fields [23], *Ar. subalbatus* prefers to breed in water containing sewage [24]. *Ae. aegypti, Ae. albopictus* and *Ae. vittatus* predominantly preferred to breed in artificial containers, *An. subpictus* and *Cx. gelidus* prefers stagnant pools while *Cx. pseudovishnui* and *Cx. quinquefasciatus* predominantly breed in water with heavy organic content [25]. In Nagpur city and surrounding areas, cisterns, pits, latrines, sewage polluted ditches, septic tanks, storm drains, gutter lines with heavy organic content were predominantly found to be the breeding sites for *Cx. quinquefasciatus,* as found by other workers at various states of India [25, 26] and costal region of Nigeria [27], Sweden [28] but in Goa and Tamil Nadu breeding of *Cx. quinquefasciatus* was reported from domestic water containers [29, 30]. In the present study, such domestic containers were found to be positive breeding sources for species of *Anopheles* and *Aedes* along with other species of *Culex.*

Many species of mosquito preferred breeding site with an almost neutral pH of 6.8 – 7.2 [31]. Mosquitoes belonging to *Aedini* group complete their larval development in breeding habitat within a pH range of 4 – 11 [32]. pH value of less than 7.3 is associated with high culicine larva [33], but the pH value of 7.6 and above is ideal for the proliferation of *Cx. quinquefasciatus* [26]. The pH value of breeding sites of different species of mosquitoes generally varied between 7.77 and 10.70 [20]. The present study reports that *Cx. quinquefasciatus* in and around Nagpur district of Maharashtra prefers to breed in pH between 7.5 and 8; in highly polluted undisturbed water bodies indicating that this species possesses alkaline tendency.

The feeding and resting behaviour of the mosquito species is an important component for the planning of vector control operation [34]. Malaria transmission by *An. sundaicus* in southern Java could not be controlled by indoor residual spraying of dichloro-diphenyl-trichloroethane (DDT), because of increased exophillic behavior of this species [35]. “Bite and run” behavior of *An. gambiae* was observed in the Tanga region of Tanzania [36]. Complete change in the behavior of *An. minimus*, from endophilic to exophagic and exophilic was reported in the Zhougsha area of Hainan Island [37]. In Suriname and Colombia *An. darlingi* developed a shorter indoor resting period owing to insecticide pressure [38]. Previous studies reported endophilic nature of *An. gambiae* and *An. funestus* in Africa, *An. culicifacies* in India, and *An. minimus* in East and Southeast Asia [39]. During this study, it was found that *Cx. quinquefasciatus* has a tendency to feed and rest indoor and outdoor *i.e.* it posses both exophillic and endophillic and exo and endophagic nature while other studies reported only endophillic and endophagic nature of this species [40, 41]. The dual nature of feeding and resting of *Cx. quinquefasciatus* as conformed by this study should be taken into consideration when planning strategies to control in this region of the country. Although peak biting time of *Cx. quinquefasciatus* is between 18:00 to 20.00 hours as observed in the coastal region of Nigeria [27]. In this study, the dawn collection found 90% of *Cx. quinquefasciatus* females in fed status, semi-gravid or gravid stage while in the late dusk collection only 10% were observed in fed condition. Similar findings were also reported by other workers [42, 45]. Clearly indicating that the biting occur mostly from midnight to early morning.

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