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Assessment of population dynamics and breeding habitat diversity of *Culex quinquefasciatus*

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Key words: *Culex* mosquitoes, Breeding Preference Ratio (BPR), Container Index (CI), House Index (HI)

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Abstract

Mosquitoes are the most important group of insects well known for the public health importance. Mosquito vector control remains the cornerstone for the control of vector borne diseases. Larval control is the initial step in mosquito vector control, since they are killed at the breeding sites, prior to dispersing and infesting a community. Therefore, identification of breeding sites appears to be an easier means to check the mosquito population. Containers in and around human habitation are probably the most important factors facilitating the breeding of mosquito vectors. The present study was undertaken to determine the breeding preferences of *Culex* mosquitoes by conducting larval survey in 6 different localities of Ganjam district of Odisha state, India. Larval survey was carried out in outdoor as well as indoor containers and the Breeding Preference Ratio (BPR) was calculated. Result of current study showed a high BPR value (14.70%) for the indoor containers. In all most all study area, high rate of breeding preference was also observed for cement tanks in outdoor and plastic buckets in indoor studies. To support the study further, the Container Index (CI) and House Index (HI) percentages were also calculated. The CI% was found more in indoor and HI% was found more in outdoor studies respectively.

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Introduction

Despite of extensive research, public health problem related to mosquito vector borne diseases has reached its peak. Several mosquito vector borne diseases like Malaria, Dengue, Filariasis and Japanese encephalitis are major contributors to the communicable disease burden in the South-East Asia Region (Shivakumar *et al.*, 2010). Mosquitoes are found in all types of environments associated with lentic aquatic habitats for breeding such as sewage water, stagnant water, septic tanks etc (Gautam *et al.*, 2006) along with natural and artificial containers such as pools, gutters, coconut shells, tree holes, bamboo clumps, leaf axils, water tanks and so on (Mafiana, 1989; Aigbodion and Anyiwe, 2005).The abundance of the mosquito vectors transmitting diseases are closely associated with human dwellings (Rozilawati *et al.*, 2007).

Mosquito species requires water to complete their life cycle, both natural and artificial reservoirs favours mosquito breeding. Mosquitoes can thrive in a variety of habitats with fresh water, brackish water, or any water (clear, turbid or polluted) except in marine habitats (Rueda, 2008). It is also well known that the transmission of the disease depends on the density of adult mosquitoes which in turn directly dependant on the survival of the larval stages (Raghavendra *et al.*, 2010). Hence, larval control is the initial step in mosquito vector control, since they are killed at the breeding sites, prior to dispersing and infesting a community (Chen *et al.*, 2005). Therefore, identification and elimination of breeding sites appears to be an important and easier means to check the population of these mosquito vectors (Dame and Fasulo, 2003).

Containers in and around human habitation are probably the most important factors facilitating the breeding of mosquito vectors (Lee, 1991). Reproduction potential of a female mosquito can be influenced by nutrition deficiency in both adult and larval stages (Manorenjitha *et al.*, 2012). Thus laying eggs, larval development, emanation of the adult and other developmental processes in the larval habitats of mosquitoes, play a vital role in the determination of abundance and distribution of mosquitoes (Ali *et al.*, 2013). Lymphatic filariasis is one of the most common human diseases caused by the nematode worms, *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. Female *Culex* mosquitoes act as secondary host of these parasites and transmit the disease in human. Earlier studies reported that breeding of *Culex* mosquitoes specifically *Culex quinquefasciatus* in artificial and natural water bodies, such as catch-pits, septic tanks, stagnant drains, ground pools and ditches, which were invariably made or influenced by man (Kaul *et al.*, 1977). However, heavy breeding was found to be associated with high pollution conditions and water temperature in the range of 14 °C and 30°C.

Several indices have been used to monitor the *Culex* populations for vector borne disease transmission. Those related to the immature populations are the House index (HI), i.e. the percentage of house infested with larvae or pupae and the Container index (CI), i.e. the percentage of water holding containers infested with larvae or pupae. When using the HI, the definition of a house should be one unit of accommodation and the surrounding premises, irrespective of the number of people residing therein (Tun-Lin *et al.*, 1995). These indices are measures of positive containers for *Culex* larvae but are not intended to measure the actual number of larvae present at a particular location. These indices have guided successful eradication programs in some countries until late 90's when *Culex* populations start re-infesting in most part of the globe. Selected insecticide resistance and changes in urban and demographic structure have made eradication an infeasible target now-a-days (Braga and Valle, 2007), forcing a shift in entomological surveillance goals from eradication to reduction of mosquito populations, for which priority areas for intervention shall be identified.In this study, efforts have been made to understand the significance of entomological survey of *Culex* mosquitoes specifically *Culex quinquefasciatus* using the BPR, CI and HI in some selected towns and villages of Ganjam district,

Odisha, India and to incorporate the findings in the vector borne disease control protocol.

Materials and Methods

Selection of study site and sampling

In the present study, the outdoor survey was made from fixed localities across the three selected towns such as Aska (19° 36' 22.0536" N; 84° 40' 20.9604" E), Buguda (19° 48' 33.7716" N; 84° 47' 30.8796" E), Bhanjanagar (19° 56' 10.8924" N; 84° 34' 43.4784" E) and three rural areas like Bhetanai (19° 41' 2.6268" N; 84° 41' 22.5456" E), Ballipadar (19° 44' 11.526" N; 84° 42' 23.1696" E) and Baunsalundi (19° 55' 28.812" N; 84° 34' 15.3048" E) of Ganjam district of Odisha state to understand the types of breeding sites, breeding preference of certain wild mosquito species.

Mosquito collection and identification

Various immature stages of mosquitoes were collected from various breeding sites from the selected study area at monthly intervals using standard sampling methods during the year 2016. After collection, larvae were reared and 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.

Entomological indices

Entomological indices, namely, BPR (Breeding preference ratio), CI (Container index) and HI (House index) were computed from the recorded data collected during this work. To calculate these indices, the following formulae were used.

$$\text{House index (HI)} = \frac{\text{Number of houses positive with Culex larvae or pupa}}{\text{Total number of houses searched}} \times 100$$

$$\text{Container index (CI)} = \frac{\text{Number of wet containers found positive with Culex larvae}}{\text{Total number of wet containers searched}}$$

Culex larval surveillance

All potential breeding habitats were identified in all 6 localities through a preliminary survey conducted for a period of one month prior to the research study and certain fixed and temporary breeding places were identified for the larval survey. Larval collections were made randomly from indoor sites (earthen pot, cement tank, plastic drum, flower pot, and plastic bucket) and outdoor sites (earthen pot, cement tank, old vehicles, discarded tyre, plant axil, coconut shell, bamboo clump, plastic container, metal drum and plant pot). The details of number and type of habitats surveyed and mosquito species recovered were recorded. The immature stages were collected with the help of glass dropper and transferred to the laboratory in plastic containers. Larger water containers were sampled as per the protocol (Eshita and Kurihara, 1978; Wongkoon *et al.*, 2005), in brief, by dipping a fish net in the water starting at the top and continuing to the bottom in swirling motion, that were sample all edges of the containers.

Results

Assessment of CI and HI of selected study area

During the study, total 21,600 number of containers were screened both in outdoor and indoor, out of which total 2,292 containers were found positive for *Culex* mosquito larva (1,233 out of 14,400 and 1,059 out of 7,200 in outdoor and indoor containers respectively). In outdoor studies, out of six selected study area, CI was maximum in Buguda (9.41) followed by Baunsalundi, Aska, Bhetanai, Bhanjanagar, and minimum in Ballipadar (7.4). In case of HI, Buguda (50) was also found maximum followed by Ballipadar, Aska, Baunsalundi, Bhetanai and minimum in Bhanjanagar (32.91). Similarly in indoor study for CI, Bhanjanagar (19.58) was found maximum with the decreasing order of Bhetanai, Aska, Baunsalundi, Ballipadar, and Buguda (7.08) and with that of HI, Baunsalundi (46.25), Aska, Bhetanai, Bhanjanagar, Buguda and Ballipadar (27.91) (Fig 1 and 2).

Analysis of breeding preference ratio of selected study area

For the calculation of Breeding Preference Ratio (BPR), the number of containers examined (both in outdoor and indoor) was arbitrarily set to 240. Based on the percentage of positive cases found, the X%, Y% and BPR values were calculated.

Table 1. The breeding preference for different types of container/sites (indoors and outdoors) in *Culex quinquefasciatus* at Buguda, Odisha, India.

SL NO	Outdoor container	Examined	X%	+VE cases (%)	Y%	BPR (Y/X)
1	Earthen pot	240	10	13 (5.4%)	5.75	0.57
2	Cement tank	240	10	45 (18.7%)	19.91	1.99
3	Old vehicles	240	10	28 (11.6%)	12.38	1.23
4	Discarded tyre	240	10	24 (10%)	10.61	1.06
5	Plant axil	240	10	15 (6.2%)	6.63	0.66
6	Coconut shell	240	10	17 (7.08%)	7.52	0.75
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	56 (23.3%)	24.77	2.47
9	Metal drum	240	10	28 (11.6%)	12.38	1.23
10	Plant pot	240	10	0	0	0
	TOTAL	2400		226 (9.4%)		
	Indoor containers					
1	Earthen pot	240	20	02 (0.8%)	2.35	0.11
2	Cement tank	240	20	23 (9.5%)	27.05	1.35
3	Plastic drum	240	20	17 (20%)	20	1.0
4	Flower pot	240	20	0	0	0
5	Plastic bucket	240	20	43 (50.5%)	50.58	2.52
	Total	1200		85 (7.08%)		

The calculated BPR for outdoor containers was found maximum for cement tank in Ballipadar (2.359), Aska (2.546), Bhanjanagar (2.925) and Baunsalundi (2.522) whereas plastic containers in Buguda (2.477) and Bhetanai (2.463) but no larvae were found in the plant pot in all the study areas except in Bhanjanagar (0.106). Similarly, the BPR for indoor containers was maximum for cement tank in Ballipadar (1.954), Bhetanai (1.794), Bhanjanagar (1.659) and Baunsalundi (1.624) whereas plastic bucket in Buguda (2.52) and Aska (1.945) and minimum was for flower pot in Bhetanai (0.191) and Baunsalundi (0.253) butfor flower pot no larvae were found in other four study areas such as Buguda, Ballipadar, Aska and Bhanjanagar.

Table 2. The breeding preference for different types of container/sites (indoors and outdoors) in *Culex quinquefasciatus* at Ballipadar,

SL NO	Outdoor container	Examined	X%	+VE cases (%)	Y%	BPR (Y/X)
1	Earthen pot	240	10	18 (7.5%)	10.11	1.01
2	Cement tank	240	10	42 (17.5%)	23.59	2.35
3	Old vehicles	240	10	29 (12%)	16.29	1.62
4	Discarded tyre	240	10	20 (8.3%)	11.23	1.12
5	Plant axil	240	10	03 (1.25%)	1.68	0.16
6	Coconut shell	240	10	09 (3.7%)	5.05	0.50
7	Bamboo clump	240	10	02 (0.8%)	1.12	0.11
8	Plastic container	240	10	18 (7.5%)	10.11	1.01
9	Metal drum	240	10	37 (15.4%)	20.78	2.07
10	Plant pot	240	10	0	0	0
	Total	2400		178 (7.4%)		
	Indoor containers					
1	Earthen pot	240	20	07 (2.9%)	8.04	0.40
2	Cement tank	240	20	34 (14.1%)	39.08	1.95
3	Plastic drum	240	20	26 (10.8%)	29.88	1.49
4	Flower pot	240	20	01 (0.4%)	1.14	0.05
5	Plastic bucket	240	20	19 (7.9%)	21.83	1.09
	Total	1200		87 (7.2%)		

Odisha, India.

During screening the fixed 2,400 number of outdoor containers of Buguda, 226 (9.41%) containers were found positive for *Culex* mosquito and maximum positive cases were in plastic container (23.33%) followed by cement tank (18.75%), old vehicles (11.66%) and no larva were found in bamboo clump and during indoor study total fixed 1,200 containers screened of five types and 85 (7.08%) containers were found positive and maximum was in plastic bucket (50.58%) followed by the cement tank (9.58%) and with no larva in flower pot (Table-1).Out of 10 types of outdoor breeding sites surveyed, in Ballipadar the *Culex* larvae were recovered from almost all types except in plant pot. Total 2,400 number of containers were screened out of which 178 (7.41%) containers found positive and maximum was in cement tank (17.5%) followed by metal drum (15.41%) and minimum in plant axil (1.25%) and bamboo clump (0.8%). Regarding the study of indoor containers, total 1,200 containers of five types were screened out of which 87 containers were found to be positive in respect to maximum positive cases were cement tank (14.16%) followed by plastic drum (10.8%)with the minimum in flower pot (0.41%) (Table-2).

Out of 10 types of outdoor breeding sites surveyed, in Aska the *Culex* larvae were recovered from seven types of containers but no larvae were found in plant axil, bamboo clump and metal drum. Total 2,400 number of container screened and found 216 (9.0%) containers positive and maximum was in cement tank (22.91%) followed by plastic container (21.25%) and minimum in discarded tyre (5.83%) and in coconut shell (5.0%). Regarding the study of indoor containers, total 1,200 of five types containers were screened out of which 221 (18.41%) containers were found to be positive with respect to maximum cases in plastic bucket (35.83%) followed by plastic drum (32.08%) with the minimum in earthen pot (3.75%) and no larvae were found in flower pot (Table- 3).

Table 3. The breeding preference for different types of container/sites (indoors and outdoors) in *Culex quinquefasciatus* at Aska, Odisha, India.

SL NO	Outdoor container	Examined	X%	+VE cases (%)	Y%	BPR (Y/X)
1	Earthen pot	240	10	18 (7.5%)	8.33	0.83
2	Cement tank	240	10	55 (22.9%)	25.46	2.54
3	Old vehicles	240	10	31 (12.9%)	14.35	1.43
4	Discarded tyre	240	10	14 (5.8%)	6.48	0.64
5	Plant axil	240	10	0	0	0
6	Coconut shell	240	10	12 (5%)	5.55	0.55
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	51 (21.2%)	23.61	2.36
9	Metal drum	240	10	35 (14.5%)	16.20	1.62
10	Plant pot	240	10	0	0	0
	Total	2400		216 (9%)		
	Indoor containers					
1	Earthen pot	240	20	09 (3.7%)	4.07	0.20
2	Cement tank	240	20	49 (20.4%)	22.17	1.10
3	Plastic drum	240	20	77 (32.08%)	34.84	1.74
4	Flower pot	240	20	0	0	0
5	Plastic bucket	240	20	86 (35.8%)	38.91	1.94
	Total	1200		221 (18.4%)		

Out of 10 types of outdoor breeding sites surveyed, in Bhetanai, the *Culex* larvae were recovered from almost all types except in plant pot. Total 2,400 number of containers were screened and 207 (8.62%) containers found positive for *Culex* larvae and maximum was in plastic container (21.25%) followed by cement tank (17.5%) and minimum in bamboo clump (1.2%). Regarding the study of indoor containers, total 1,200 containers of five types were screened out of which 234 (19.5%) containers were found to be positive with respect to maximum cases in cement tank (35.0%) followed by plastic drum (26.25%) with the minimum in flower pot (3.83%) (Table-4).

Table 4. The breeding preference for different types of container/sites (indoors and outdoors) in *Culex quinquefasciatus* at Bhetanai, Odisha, India.

SL NO	Outdoor container	Examined	X%	+VE cases (%)	Y%	BPR (Y/X)
1	Earthen pot	240	10	19 (7.9%)	9.17	0.91
2	Cement tank	240	10	42 (17.5%)	20.28	2.02
3	Old vehicles	240	10	28 (11.6%)	13.52	1.35
4	Discarded tyre	240	10	24 (10%)	11.59	1.15
5	Plant axil	240	10	04 (1.6%)	1.93	0.19
6	Coconut shell	240	10	09 (3.7%)	4.34	0.43
7	Bamboo clump	240	10	03 (1.2%)	1.44	0.14
8	Plastic container	240	10	51 (21.2%)	24.63	2.46
9	Metal drum	240	10	27 (11.2%)	13.04	1.30
10	Plant pot	240	10	0	0	0
	Total	2400		207 (8.6%)		
	indoor containers					
1	Earthen pot	240	20	21 (8.7%)	8.97	0.44
2	Cement tank	240	20	84 (35%)	35.89	1.79
3	Plastic drum	240	20	63 (26.2%)	26.92	1.34
4	Flower pot	240	20	08 (3.8%)	3.41	0.19
5	Plastic bucket	240	20	58 (24.1%)	24.78	1.23
	Total	1200		234 (19.5%)		

Out of 10 types of outdoor breeding sites surveyed, in Bhanjanagar, the *Culex* larvae were recovered from almost all types except in bamboo clump. Total 2,400 number of containers were screened and found 188 (7.83%) containers were positive and maximum was in cement tank (22.91%) followed by plastic container (17.5%) and minimum in plant pot (0.83%), whereas no larvae were found in bamboo clump. Regarding the study of indoor containers, total 1,200 containers of five types were screened out of which 235 (19.58%) containers were found to be positive with respect to maximum cases in cement tanks (32.5%) followed by plastic drum (27.08%) with the minimum in flower pot (2.5%) (Table-5).

Table 5. The breeding preference for different types of container/sites (indoors and outdoors) in *Culex quinquefasciatus* at Bhanjanagar, Odisha, India.

SL NO	Outdoor container	Examined	X%	+VE cases (%)	Y%	BPR (Y/X)
1	Earthen pot	240	10	12 (5%)	6.38	0.63
2	Cement tank	240	10	55 (22.9%)	29.25	2.92
3	Old vehicles	240	10	32 (13.3%)	17.02	1.70
4	Discarded tyre	240	10	16 (6.6%)	8.51	0.85
5	Plant axil	240	10	03 (1.2%)	1.59	0.15
6	Coconut shell	240	10	08 (3.3%)	4.25	0.42
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	42 (17.5%)	22.34	2.23
9	Metal drum	240	10	18 (7.5%)	9.57	0.95
10	Plant pot	240	10	02 (0.8%)	1.06	0.10
	TOTAL	2400		188 (7.8%)		
	Indoor containers					
1	Earthen pot	240	20	32 (13.3%)	13.61	0.68
2	Cement tank	240	20	78 (32.5%)	33.19	1.65
3	Plastic drum	240	20	65 (27.08%)	27.65	1.38
4	Flower pot	240	20	06 (2.5%)	2.55	0.12
5	Plastic bucket	240	20	54 (22.5%)	22.97	1.14
	Total	1200		235 (19.5%)		

Out of 10 types of outdoor breeding sites surveyed, in Baunsalundi, the *Culex* larvae were recovered from almost all types except in bamboo clump and plant pot. Total 2,400 number of containers were screened and found 218 (9.08%) containers positive and maximum was in cement tank (22.91%) followed by plastic container (15.41%) and minimum in coconut shell (7.5%) and in plant axil (0.83%), whereas no larvae were found in bamboo clump. Regarding the study of indoor containers, total 1,200 containers of five types were screened out of which 197 (16.41%) containers were found to be positive with respect to maximum cases in cement tank (26.66%) followed by plastic drum(22.08%) with the minimum in earthen pot (10.0%) and flower pot (4.16%) (Table-6).

Table 6. The breeding preference for different types of container/sites (indoors and outdoors) in *Culex quinquefasciatus* at Baunsalundi, Odisha, India.

SL NO	Outdoor container	Examined	X%	+VE cases (%)	Y%	BPR (Y/X)
1	Earthen pot	240	10	20 (8.3%)	9.17	0.91
2	Cement tank	240	10	55 (22.9%)	25.22	2.52
3	Old vehicles	240	10	34 (14.1%)	15.59	1.55
4	Discarded tyre	240	10	22 (9.1%)	10.09	1.0
5	Plant axil	240	10	02 (0.8%)	0.91	0.09
6	Coconut shell	240	10	18 (7.5%)	8.25	0.82
7	Bamboo clump	240	10	0	0	0
8	Plastic container	240	10	37 (15.4%)	16.97	1.69
9	Metal drum	240	10	30 (12.5%)	13.76	1.37
10	Plant pot	240	10	0	0	0
	Total	2400		218 (9.08%)		
	Indoor containers					
1	Earthen pot	240	20	24 (10%)	12.18	0.60
2	Cement tank	240	20	64 (26.6%)	32.48	1.62
3	Plastic drum	240	20	53 (22.08%)	26.90	1.34
4	Flower pot	240	20	10 (4.1%)	5.07	0.25
5	Plastic bucket	240	20	46 (19.1%)	23.35	1.16
	TOTAL	1200		197 (16.4%)		

Discussion

Entomological surveillance is an appropriate tool for the prevention and control of various vector borne diseases until suitable controlling measures such as vaccines and drugs are made available. It was ascertained by the World Health Organization (WHO) that preventing or reducing the transmission of various vector borne diseases entirely depends upon the control of the vector or interrupting human-vector contact (Seng and Jute, 1994; WHO, 1998). Based on the above approaches, a non-chemical control was suggested rather than a chemical control, because there is an increasing resistance among mosquitoes. Above all, the reduction of *Culex* population through surveillance work yield good results and is mainly achieved by emptying the water-filled containers which are more favorable oviposition sites for the most of the mosquito vector species.

Monitoring of adult *Culex* mosquito population can be challenging in densely populated urban and rural areas where there are diverse potential feeding resting sites and the densities of mosquitoes can be low. Mosquito species may have shifted their niche with changing weather patterns and ecology in order to attain a wide dissemination in the environment.

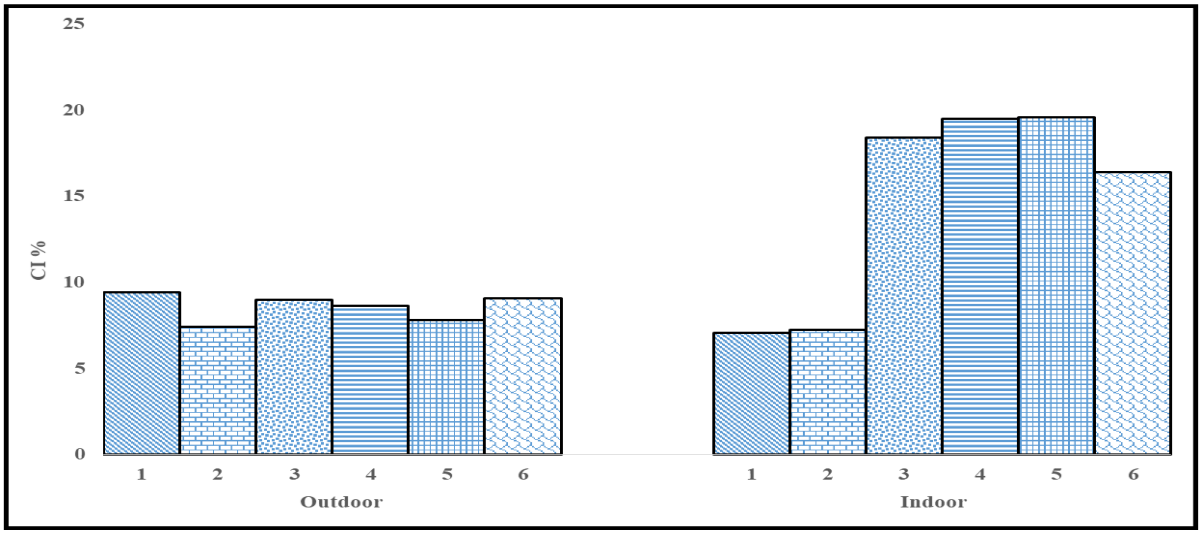

Fig. 1. The CI % of different localities under study.1- Buguda; 2-Ballipadar; 3-Aska; 4-Bhetanai; 5-Bhanjanagar; 6-Baunsalundi.

In the present investigation, it has been cleared that the *Culex* mosquitoes preferred mostly cement tanks followed by plastic buckets over other types of indoor containers for oviposition in all the 6 localities under study. Whereas, in the case of outdoor container survey, *Culex* mosquitoes shows high preference for cement tanks which was same as in indoor study followed by plastic containers in most of the localities. Based on the calculated BPR values, it may be concluded that the *Culex* mosquitoes preferred outdoor site for oviposition. This could possibly due to the reason that, *Culex* population specially *Culex quinquefasciatus* produces and responds to oviposition pheromones with other population of this species, confirming that oviposition site selection is influenced by the pheromone emanating from apical droplets on the eggs (Mboera *et al.*, 2000).

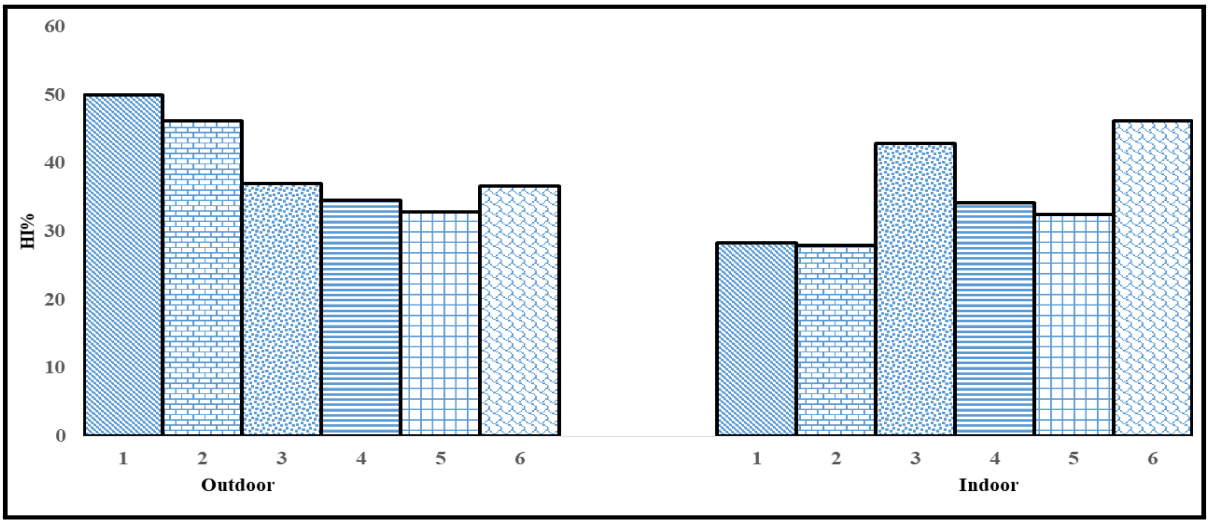

Fig. 2. The HI% of different localities under study.1- Buguda; 2-Ballipadar; 3-Aska; 4-Bhetanai; 5-Bhanjanagar; 6-Baunsalundi.

During this study, there may minimal variation in temperature and humidity that have a contribution towards lack of relationship between the abundance of *Culex* population and climatic variables. Mosquitoes were collected in monthly intervals and it may be possible that rapid changes in weather conditions and availability of breeding sites within the month may have influenced our results.

Conclusion

Mosquito vectors have been found breeding in a great variety of aquatic habitats. Therefore, aquatic habitats are crucial for mosquito population dynamics where many vital life processes such as oviposition, larval development and emergence of adult take place. Results of the present study indicated the breeding preference of *Culex quinquefasciatus* in both indoor and outdoor breeding sites. Understanding the characteristics of various aquatic habitats and breeding preference by the mosquitoes can be useful in improving larval source management operations for the control of mosquito vectors. Further, health-education and community awareness are equally important for the elimination of such aquatic habitats of mosquito vectors.

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Conflict of Interest

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

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