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Diversity assessment of mosquito across habitats in the slum areas of Bankura, West Bengal, India

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Abstract
Objective: Spatial and temporal changes in climate will affect the biology and ecology of vector mosquitoes and consequently the risk of disease transmission. Periodic analysis of the changes in climatic conditions with respect to the changes in frequency, diversity and distribution of vectors on a regional basis is a prerequisite for ensuring public health and sanitation.
Methods: Sampling of adult mosquitoes was carried out from heterogeneous habitats of slums of Bankura to determine the species composition, relative abundance and habitat characteristics. Adult mosquitoes, collected from heterogeneous locations during morning and evening times were also subjected to species level identification following standard manuals. 20 mm^3 bloods were collected by finger prick and clinical examination of filariala disease of 2025 people (1250 male and 775 female) was done randomly. Symptoms of bancroftian filariasis from both sexes were recorded through physical examination.
Results: The mosquito species diversity *(H)* in summer and rainy was calculated to be 1.57 and in winter 1.63, while total number of individuals in rainy season was significantly higher than summer and winter. Endemicity rate (ER) was 18.37%. Causative parasite was identified as *Wuchereria bancrofti* and *Culex quinquefasciatus* was recorded as vector.
Conclusions: The findings of this study suggest serious threat of mosquito-borne diseases to public health, in Bankura, West Bengal, India promoted by anthropogenic alterations of the ecosystem.

Keywords: Mosquito, diversity, Endemicity rate, filarial transmission

1. Introduction

Rapid urbanization, excessive deforestation distribution range of both mosquitoes and mosquito borne diseases are proliferating in large number everywhere [1]. Day by day resistance among mosquitoes to insecticides has developed. Several attempts have been made to control them. But despite these, remarkably adapted insects continue to coexist with man, feeding on him and his domesticated animals [2].

A total of 3539 species of mosquitoes belonging to 112 genera are found on this earth Harbache, 2013 [3]. Mosquitoes co-exist with human [4]. They are well known group of insects, which transmit many dreadful diseases causing serious health problems to human beings. The females biting habit during their search for blood meal shortly before oviposition increases their propensity to transmit various diseases associated with high morbidity and mortality. Such diseases vectored by mosquitoes include: malaria, filariasis and yellow fever, which affect hundreds of millions of people every year, causing immense suffering and hindering development.

The warmer climates in tropical areas allow these insects to be active all year round since their ideal conditions for proliferation in an environment which is hot and humid with moderate rainfall a typical tropical condition. In hot climate they are able to be more active and the rainfall gives them aquatic sites for larval and pupal stages [5]. They exhibit spatial and temporal distribution on the basis of species, climatic conditions and environment [6]. They breed in natural or manmade temporary, semi-permanent as well as permanent water bodies with a variety of oviposition sites such as ground water sites (pools, rivers and lakes) and container sites including bottles, cups, and tree holes [7]. Larval distribution is greatly influenced by several factors such as elevation of water, water movement, water condition (polluted, fresh etc.), water temperature, vegetation, types of water source and many others [7]. Oviposition, development of larva, adult emergence and many other processes take place in mosquito larval habitats, plays an important role in determining adult distribution and abundance [8]. Many studies pertaining to the species diversity of mosquitoes have been carried out in different parts of India and other countries [9-11]. Because of their medical importance, it is essential to make an inventory of the diversity of mosquitoes in different places periodically, which forms the baseline to study bionomics of vector species as well as correlations with the abiotic factors of the environment and to make strategy for the control over mosquito born diseases [12].

In light of above, the present study has been attempted to assess the diversity, distribution and abundance of mosquito vectors from slums of Bankura, West Bengal, India with respect to two years from March 2007 to February 2009. Special attention was given to the filarial vector, *Culex quinquefaciatus* as the study area is noted to have repeated outbreaks of filariasis in recent years. The ultimate objective of the study was to provide information to help Bankura Municipal Council in instituting the appropriate control measures for populations at risk of mosquito-borne diseases.

2. Materials and Methods

2.1. Study sites

Bankura district is bounded by latitude 22˚38́ N and longitude 86˚36́ E to 87˚47́ E.

The climate in the area is subtropical, with summer (March-June), monsoon (July-Oct) and winter (Nov-Feb). The temperature generally ranges from 19°C to 35°C and humidity from 62% to 93%. The area lies in sub-humid zone having average annual rainfalls of 0 mm to 25.56 mm. Early morning mist is common in winter season.

Most of the human habitations are hutments, without or with very small windows and ventilations, single storied and some of which (very few) are of reinforced cement concrete structures.

2.2. Mosquito collection and Identification

Mosquitoes were collected from randomly selected human habitations, cattle sheds and outdoor of each sites during different seasons from March 2007 to February 2009. The catching of adult mosquitoes was carried out according to standard entomological surveillance guidelines [13-15]. While entering in the house purpose of the investigation was explained to the head of each of the households selected. Permission to enter each of the household was sought and the right to refuse or withdraw at any time was respected. Collected adults forms of mosquitoes were brought to the laboratory. All the adult mosquitoes were identified using standard identification keys of each genus [16-23].

2.3. Blood sampling and physical examination

A door to door random night blood survey was carried out from each of 4 slums between 1900 & 2300 h IST (Indian Standard Time). 20 mm^3 bloods were collected from all the individuals available at the time of survey by finger prick [24]. Then the slides were examined for detection of microfilariae (mff).

Symptoms of bancroftian filariasis from both sexes were recorded through physical examination. Age, sex and clinical history of each subject were also noted. The total surveyed population was divided into different age groups, as ≤ 10 years, 11-20 years, 21-30 years, 31-40 years and so on - as followed by many earlier epidemiologists [25, 26]. Endemicity Rate (ER) was calculated. It includes number of persons microfilaremic and diseased.

2.4. Meteorological data

Monthly average of maximum and minimum temperature and humidity, as well as rainfall data of study period obtained from Meteorological Department to analyze the mosquito population dynamic with the abiotic factors.

2.5. Data analysis

Seasonal analysis was performed on adult mosquitoes collected in the field. Seasonal variation was analyzed in terms of relative abundance and distribution using the following formulae (modified as the term density, "D" is changed to relative abundance) [27, 28].

Relative abundance (D) =I/L x 100

Where `I' is number of specimens of each mosquito species and 'L' is the total number of specimens.

The mosquito species were classified in following relative abundance classes: Satellite species, relative abundance <1%; subdominant species, relative abundance <5% and dominant species, relative abundance >5% [29].

Distribution (C) = n/N 100

Where' n' is number of sites where species was found, and 'N' is total number of sites. The following classes were used to represent distribution status of different species.C1 (sporadic) 0-20%; C2 (infrequent) 20.1-40%; C3 (moderate) 40.1-60%; C4 (frequent), 60.1-80%; and C5 (constant) 80.1- 100% [30].

Margalef richness index (M), Simpson's index (D), Simpson's Index of Diversity (1-D), Simpson's Reciprocal Index (1/D) [31], Shannon's diversity index (H) [32] and Pielou's evenness index (J), biodiversity indices were calculated.

2.6. Ethical Clearance

Ethical clearance for the study was obtained from the institutional Ethics Committee (IEC) of Vidyasagar University, Midnapore, West Bengal, India, IEC No. IEC/5-11/C-5/15.

3. Results

A total of 7996 (3934 during March 2007-Februray 2008 & 4062 during March 2008 to February 2009) were collected from four different slums of Bankura, West Bengal, India (Table 1). The collected mosquitoes belonged to five different genera (*Culex, Anopheles, Armigeres, Mansonia* and *Aedes*) and eight different specimens. The population of *Armigeres subalbatus* (35%) was highest followed by *Culex quinquefasciatus* (34%) (Fig. 1). Maximum density of mosquito population occurred during the month of July, August, September and October, i.e. Rainy season (Table 2). A significant variation in mosquito density and species richness was observed in the different study sites (Table 3). Diversity indices (Margalef richness index (M), Simpson's index (D), Simpson's Index of Diversity (1-D), Simpson's Reciprocal Index (1/D), Shannon's diversity index (H) and Pielou's evenness index (J), for three different seasons were calculated (Table 4). Relative abundance and distribution status of the total mosquito species collected in the in three different seasons were calculated (Table 5 A, B, C). 2025 people (1250 male and 775 female) were brought into the study and overall ER was assessed as 18.37% respectively (Table 6). The scatterplots, histogram and correlation matrix representing the relationship of the environmental variables with the relative abundance of *Cx. quinquefasciatus* observed during the two year (2007 – 2009) study period from Bankura were plotted in Fig. 2.

Table 1: Combination of different species (and percent) of indoor-resting mosquitoes during March 2007 to February 2009 at Bankura, West Bengal, India

Sl No.	Species	Mar. 2007 to Feb. 2008	Mar. 2008 to Feb. 2009	Total/Overall
1	*Culex quinquefasciatus*	1375 (34.95)	1335 (32.87)	2710 (33.90)
2	*Culex vishnui* group	255 (6.48)	218 (5.37)	473 (5.92)
3	*Anopheles annularis*	184 (4.68)	296 (7.29)	480 (6.00)
4	*Anopheles barbirostris*	199 (5.06)	212 (5.21)	411 (5.14)
5	*Anopheles subpictus*	327 (8.31)	425 (10.46)	752 (9.40)
6	*Armigeres subalbatus*	1423 (36.17)	1411 (34.74)	2834 (35.44)

7	*Mansonia annulifera*	67 (1.70)	57 (1.40)	124 (1.56)
8	*Aedes albopictus*	104 (2.64)	108 (2.66)	212 (2.65)
Total		3934	4062	7996

Table 2: Season wise number (and percent) of different species of indoor-resting mosquitoes

Mosquito Species	Summer			Rainy			Winter		
	March 2007 to February 2008	March 2008 to February 2009	Total/ Overall	March 2007 to February 2008	March 2008 to February 2009	Total/ Overall	March 2007 to February 2008	March 2008 to February 2009	Total/ Overall
Culex quinquefasciatus	521 (37.56)	489 (36.28)	1010 (36.93)	568 (36.34)	594 (34.55)	1162 (35.41)	286 (29.07)	252 (25.33)	538 (27.19)
Culex vishnui group	88 (6.34)	82 (6.08)	170 (6.22)	81 (5.18)	74 (4.30)	155 (4.72)	86 (8.74)	62 (6.23)	148 (7.48)
Anopheles annularis	57 (4.11)	64 (4.75)	121 (4.42)	86 (5.50)	101 (5.88)	187 (5.70)	41 (4.17)	131 (13.17)	172 (8.69)
Anopheles barbirostris	58 (4.18)	56 (4.15)	114 (4.17)	79 (5.05)	77 (4.48)	156 (4.75)	62 (6.30)	79 (7.94)	141 (7.12)
Anopheles subpictus	134 (9.66)	124 (9.20)	258 (9.43)	108 (6.91)	229 (13.32)	337 (10.27)	85 (8.64)	72 (7.24)	157 (7.93)
Armigeres subalbatus	463 (33.38)	463 (34.35)	926 (33.86)	570 (36.47)	573 (33.33)	1143 (34.83)	390 (39.63)	375 (37.69)	765 (38.66)
Mansonia annulifera	27 (1.95)	16 (1.19)	43 (1.57)	26 (1.66)	32 (1.86)	58 (1.77)	14 (1.42)	9 (0.90)	23 (1.16)
Aedes albopictus	39 (2.81)	54 (4.01)	93 (3.40)	45 (2.88)	39 (2.27)	84 (2.56)	20 (2.03)	15 (1.51)	35 (1.77)
Total	1387	1348	2735	1563	1719	3282	984	995	1979

Table 3: Site wise abundance of mosquito species

Name of mosquito species	Distribution patterns of mosquito at slums				Percentage Distribution
	Rampur	Kenduadihi	Lokepur	Pratapbagan	
Culex quinquefasciatus	*	*	*	*	100%
Culex vishnui group	*	*	*	*	100%
Anopheles annularis	*	*	*	*	100%
Anopheles barbirostris	*	*	*	*	100%
Anopheles subpictus	*	*	*	*	100%
Armigeres subalbatus	*	*	*	*	100%
Mansonia annulifera	*	*	*	-	75%
Aedes albopictus	*	*	*	*	100%

*=Present, - = Absent

Table 4: Biodiversity Indices

Index	Summer	Rainy	Winter
Total No. of Species (S)	8	8	8
Total No. of Individuals (N)	2735	3282	1979
Natural Log of Species (ln S)	2.08	2.08	2.08
Natural Log of Individuals (ln N)	7.92	8.09	7.59
Margalef's Index (M)	0.88	0.86	0.92
Simpson's Index (D)	0.268	0.265	0.247
Simpson's Index of Diversity (1-D)	0.732	0.735	0.752
Simpson's Reciprocal Index (1/D)	3.73	3.77	4.03
Shannon Index (H)	1.57	1.57	1.63
Pielou's Index (J)	0.754	0.754	0.784

Table 5: Relative abundance and distribution status of mosquito species in different seasons A. Summer season

Name of mosquito species	Relative Abundance	Distribution	Relative Abundance Status	Distribution Status
Culex quinquefasciatus	36.92	100	Dominant	Constant
Culex vishnui group	6.21	100	Dominant	Constant
Anopheles annularis	4.42	100	Subdominant	Constant
Anopheles barbirostris	4.16	100	Subdominant	Constant
Anopheles subpictus	9.43	100	Dominant	Constant

Armigeres subalbatus	33.85	100	Dominant	Constant
Mansonia annulifera	1.57	75	Subdominant	Frequent
Aedes albopictus	3.40	100	Subdominant	Constant

B. Rainy season

Name of mosquito species	Relative Abundance	Distribution	Relative Abundance Status	Distribution Status
Culex quinquefasciatus	35.40	100	Dominant	Constant
Culex vishnui group	4.72	100	Subdominant	Constant
Anopheles annularis	5.69	100	Dominant	Constant
Anopheles barbirostris	4.75	100	Subdominant	Constant
Anopheles subpictus	10.26	100	Dominant	Constant
Armigeres subalbatus	34.82	100	Dominant	Constant
Mansonia annulifera	1.76	75	Subdominant	Frequent
Aedes albopictus	2.55	100	Subdominant	Constant

C. Winter season

Name of mosquito species	Relative Abundance	Distribution	Relative Abundance Status	Distribution Status
Culex quinquefasciatus	27.18	100	Dominant	Constant
Culex vishnui group	7.47	100	Dominant	Constant
Anopheles annularis	8.69	100	Dominant	Constant
Anopheles barbirostris	7.12	100	Dominant	Constant
Anopheles subpictus	7.93	100	Dominant	Constant
Armigeres subalbatus	38.65	100	Dominant	Constant
Mansonia annulifera	1.16	75	Subdominant	Frequent
Aedes albopictus	1.76	100	Subdominant	Constant

Table 6: Filarial endemicity rate according to sex and age group among the population

Age group (years)	No. of person Examined			No. of person positive for Microfilaria			No. of diseased persons			Total no. of persons microfilaremic & diseased			Endemicity Rate		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
≤10	204	147	351	0	0	0	4	0	4	4	0	4	1.96	0	1.14
11-20	345	175	520	15	2	17	31	4	35	43	5	48	12.46	2.86	9.23
21-30	265	190	455	28	3	31	89	8	97	112	10	122	42.26	5.26	26.81
31-40	200	126	326	15	12	27	67	24	91	82	36	118	41.00	28.57	36.20
41-50	117	75	192	11	7	18	35	2	37	46	9	55	39.32	12.00	28.65
51-60	76	36	112	4	3	7	14	0	14	18	3	21	23.68	8.33	18.75
61-70	30	21	51	2	0	2	2	0	2	4	0	4	13.33	0	7.84
≥71	13	5	18	0	0	0	0	0	0	0	0	0	0	0	0
Total/Overall	1250	775	2025	75	27	102	242	38	280	309	63	372	24.72	8.13	18.37

4 (3 male & 1 female), 6(5 male & 1 female) microfilaremic persons in age group of 11-20 and 21-30 were found to be positive for filarial disease also and they are counted once when Endemicity rate was calculated
Note: (Male-15(mf) + 31(Disease) = 43, Female- 2+4 = 5 instead of 46 & 6
Male-28(mf) + 89(Disease) = 112, Female- 3+8 = 10 instead of 117 & 11)

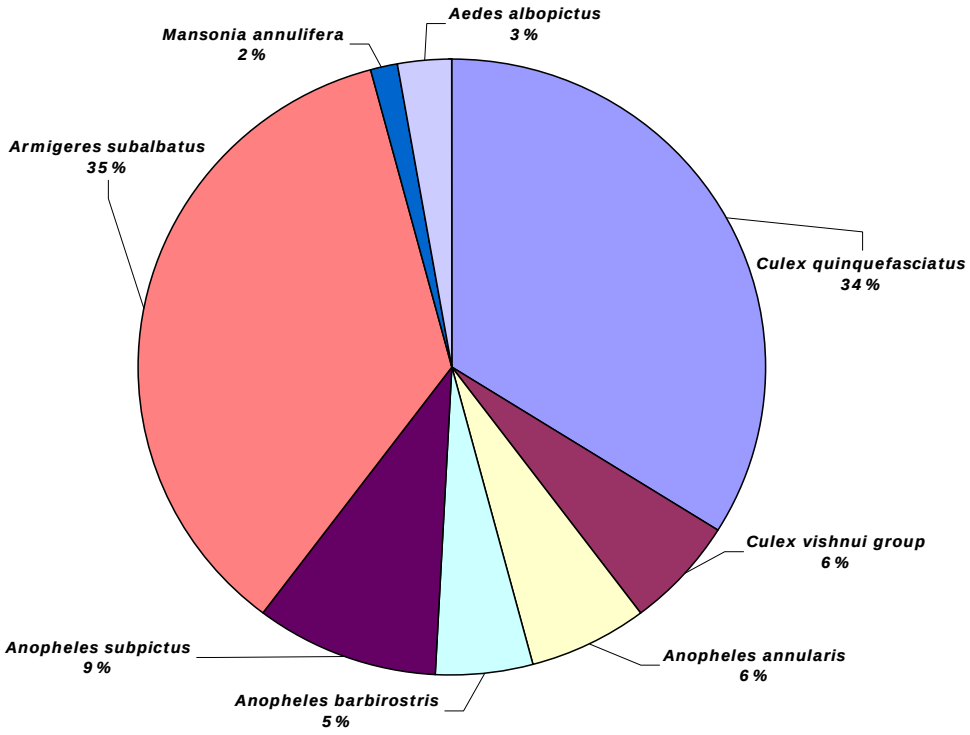

Fig 1: Vector species contribution percentage

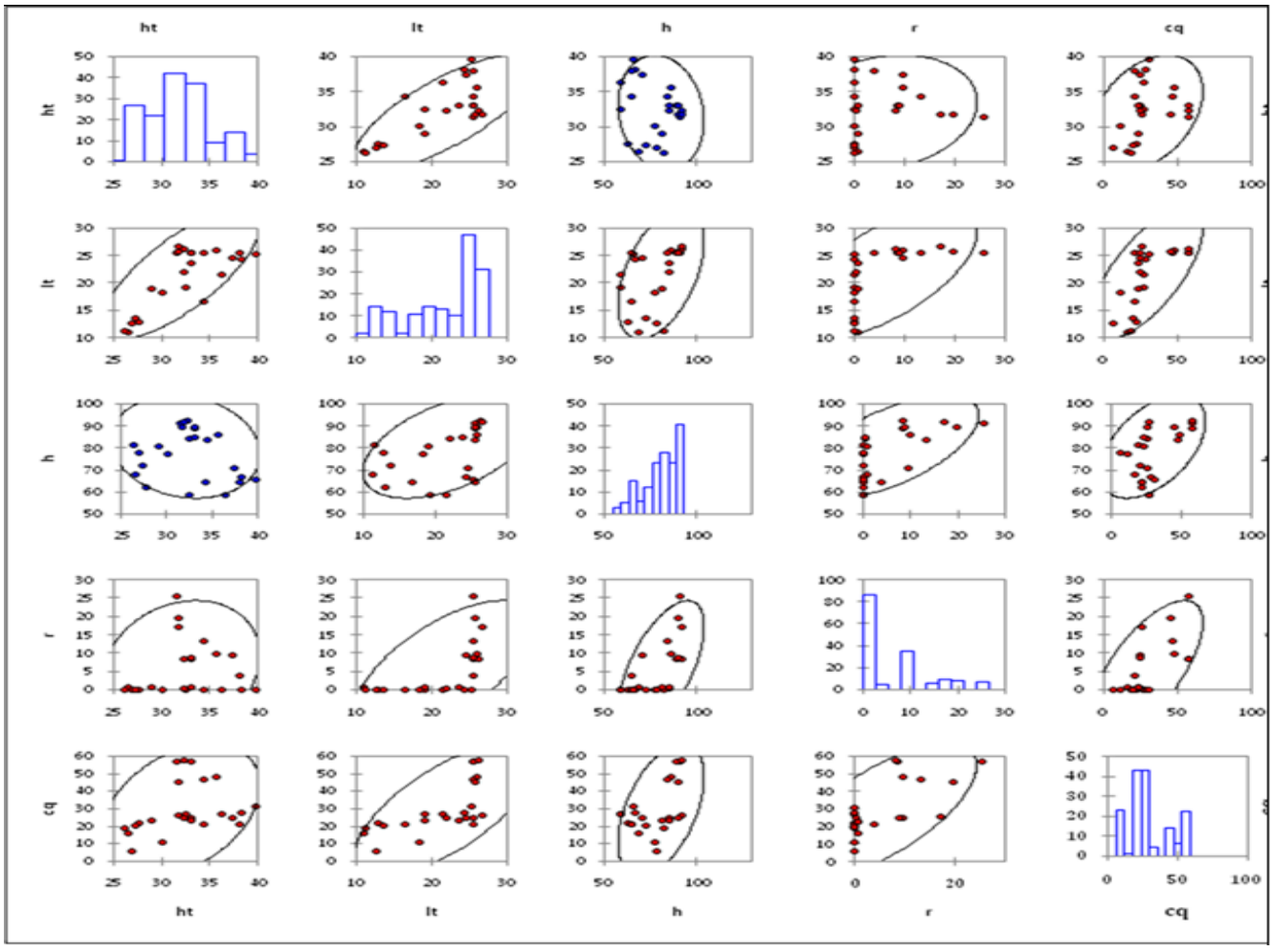

Fig 2: The scatterplots, histogram and correlation matrix representing the relationship of the environmental variables with the relative abundance of *Cx. quinquefasciatus* observed during the two year (2007 – 2009) study period from Bankura, West Bengal, India. Note the values in bold represents significance at $P < 0.001$ level.

Variables	ht	lt	h	r	cq
ht	1	0.751	-0.143	0.189	0.374
lt	0.751	1	0.490	0.642	0.689
h	-0.143	0.490	1	0.618	0.497
r	0.189	0.642	0.618	1	0.675
cq	0.374	0.689	0.497	0.675	1

4. Discussions

Mosquitoes are well known group of insects, which transmit many dreadful diseases causing serious health problems to human beings. Climate change and variability are highly likely to influence the biology and ecology of mosquito vectors and consequently the risk of disease transmission. Mosquitoes exhibit spatial and temporal distribution on the basis of species, climatic conditions and environment [6].

Bankura municipal area is known to be endemic towards many mosquito borne diseases especially filariasis. Repeated outbreaks of the disease was reported in recent years signifies the ultimate necessity of diversity studies in the area. There is few published information regarding species composition of mosquitoes in Bankura municipal area of West Bengal. With the aim of contributing to this knowledge, occurrence of species and habitats used by mosquitoes were investigated. The mosquito communities sampled included several important vectors of infectious diseases such as filariasis and malaria. We showed that both the diversity of mosquito communities and the relative abundance of disease vectors varied by habitat, with the lowest diversity and highest abundance of certain vectors occurring in selected environments, whereas other vectors were most abundant in different habitats depending on their biology. In collections of three seasons *Culex quinquefaciatus*, *Anopheles subpictus* and *Armigeres subalbatus* were the most predominant species in terms of relative abundance and distribution. The filarial vector *Culex quinquefaciatus* was found to be the dominant and constant species in all the seasons in accordance with some earlier studies regarding mosquito fauna in different localities [33, 34].

A significant variation in mosquito density and species richness was observed in the different study sites may be due to the observed differences of breeding sources available, planned and unplanned area of the slums. The highest population of mosquito species observed in the rainy season, when the maximum and minimum humidity and temperature was 92%/84%; 32˚C/22˚C, respectively, and rainfall was 63 mm. While minimum density of mosquito species was observed in the winter, when the maximum and minimum humidity was 81%/62%; temperature was 29˚C/12˚C and rainfall recorded was 2 mm. Environmental parameters around these levels can be used as early warning for the outbreaks of mosquito population which is directly related to mosquito vector borne diseases. Age group wise distribution shows that, in the study area ER was generally higher among the peoples of younger to middle age [21-30 and 31-40], which is somewhat similar to some other areas [35,36]. Among the aetiologies, frequency of hydrocele and adeno-lymphangitis were higher than all other symptoms. A steady state of transmission was indicated by the fact that, different disease symptoms were distributed unevenly in different age groups and in both genders, in both the areas. Different aetiologies in both the sexes and almost in all the age groups were more or less higher in human population of slums under study. Presence of dense human population closely situated human habitations, favourable mosquito breeding place, gathering of too many outsiders every day etc. probably aided to this situation.

Due to the occurrence of high density of vector species in the study area, vector borne disease epidemics could easily be initiated by the arrival of parasite source or favorable climatic factors for the quiescent parasites to become active [37], and each of the species observed could easily adopt the parasites and may become potential vector. The epidemiological significance of the diverse mosquito species observed in the study area should therefore not be underestimated.

5. Conflict of interest statement

We declare that we have no conflict of interest.

6. Acknowledgments

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7. Author Contributions

Conceived and designed the experiments: GC, JK. Performed the experiments: BM, IB. Analyzed the data: BM, IB. Contributed reagents/materials/analysis tools: GC, JK. Wrote the paper: BM, IB. Carried out the field survey: BM. Carried out statistical analysis: BM and IB. Contributed equally in compiling the manuscript: GC, BM, IB and JK.

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