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Mosquito faunal diversity and species composition: A study based on seasonal prevalence in and around the rural settings of Kashmir, Jammu and Kashmir, India

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Abstract
Mosquitoes are the haematophagous insects of medical importance and colonize almost all types of lentic water habitats with transient, semi-permanent and permanent nature in order to breed and successfully continue their generation resulting in complex communities. The present study (June,2019-October,2019 and June,2020-October,2020) was carried out on mosquitoes seasonal diversity and species composition in Kupwara district, Jammu and Kashmir, India reveals the prevalence of seven mosquito species belonging to three genera notably *Culex* (59.37%), *Aedes* (26.78%) and *Anopheles* (13.83%). Out of the 896 specimens collected, three *Culex* species- *Culex* quinquefasciatus, *Culex vagans,* and *Culex mimeticus;* two *Aedes* species- *Aedes aegypti* and *Aedes albopictus;* two *Anopheles* species- *Anopheles gigas* and *Anopheles stephensi* were collected from selected breeding sites across five localities of the study area facing different directions. During the collection period, it was recorded that *Culex quinquefasciatus* species were abundantly found in almost all breeding points of the study area with species percentage composition of 37.38, Shannon-Weiner diversity index of 0.159 and Simpson dominance index of 0.139. Moreover the present data will serve as a baseline and suitable tool for designing and implementation of integrated larval management approaches in the future.

Keywords: *Culex, Aedes, Anopheles*, diversity, dominance, species composition

Introduction
Biodiversity (richness of all life forms on planet earth) is the result of 3.5 billion years of evolution. India highly contribute to the biological richness internationally as well as locally and is a country of mega diversity hotspot, ranked among top 10 or 15 biologically rich nations and representing 7% of world's total biodiversity [1]. Among fauna population, insects successfully evolved on earth in diverse environments with unique characteristics like presence of segmented bodies, one or two paired wings, paired antennae, three pairs of legs and comprise more than half (73%) of all known living creatures and are highly capable of sensing the levels of environmental changes [2, 3].

Insects like mosquitoes (Diptera: Culicidae) stands out by far, the most significant, successful and medically important group of holometabolous insects involved in pathogen transmission [4]. The information regarding mosquito diversity reveals the presence of 3541 species compiled into 112 genera and 2 subfamilies (anophelinae and culicinae) reported all over the globe except few islands and Antarctica and India accounts 404 species belonging to 50 genera [5]. Mosquitoes secure worldwide attention by means of their nuisance, notorious and undesirable potential agents of devastating and debilitating diseases [6, 7]. Mosquito-borne infections contribute to the public health burden causing millions of deaths and hundreds of millions of illnesses every year around the world. The heavy implications of mosquito borne diseases, notably malaria (transmitted by *Anopheles* mosquitoes); dengue, chikungunya, zika (caused by *Aedes* mosquitoes); lymphatic filariasis (transmitted by *Anopheles* and *Culex* mosquitoes) and japanese encephalitis (caused by *Culex* mosquitoes) on human populations, in turn, produces a serious impediment and adversities to economic development and productivity [8, 9].

Mosquitoes prefer diverse aquatic lentic environments in order to complete their life cycle [10, 11]. The mosquito developmental stage starts with an egg desiccation followed by immature stages (larvae and pupae) and finally culminates into an adult emergence. Their ability to withstand wide variety of factors due to the powerful mechanism of physiological regulation processes notably osmotic and ionic transport in the gut epithelium aid in internal homeostasis. In addition, the overlapping categories associated with each of the landscape modifications influenced by human activities like water retention systems, irrigated canals and deforestation creating heterogeneous environment is the over advantages available for mosquitoes to flourish [12, 13]. Mosquito diversity surveys remains as an important tool in order to prevail the status of medically important culicids. Earlier notable contributors of mosquito faunal studies in Kashmir are by James and Liston (1904, 1911) [14, 15], Adie (1913) [16], Gill (1920) [17]. Later their work was compiled by Christophers (1933) [18] and Barraud (1934) [19] in their monographs on anophelines and culicines respectively. Almost after four decades, Rao *et al*. (1973) [20], Bhat and Kulkarni (1983) [21], and Rao (1984) [22] carried out their extensive surveys on mosquitoes of Western Himalayas.

Later on Jebanesan *et al*. (2012) [23] carried out work on tree hole mosquitoes in Baramulla and Budgam districts. Recent survey on mosquito fauna was carried out by Dar (2018) [24] in Jammu and Kashmir, India and recorded 26 species in Kashmir division. Most of the study carried out on mosquitoes in Kashmir were restricted towards southern, central and few northern parts. Little available published data is known about the diversity and mosquito species composition in northern rural settings of Kashmir division. Hence the sole aim of the present work is to predict the mosquito biodiversity in Kupwara district, Jammu and Kashmir, India. Therefore the current approach of study will yield valuable and significant information in order to update the prevalence of diverse mosquito fauna for the future reference.

Materials and Methods

1. Study area

The diligent study tours of aquatic habitats harboring mosquitoes were undertaken and split between 2019 (June to October) and 2020 (June to October) comprising summer and autumn months. The field survey areas were selected merely on the basis of varied landscape topography, availability of aquatic environment resources, agricultural inputs and rural settings. Land-use and land-cover changes, such as deforestation, agricultural expansion, infrastructure development and human population growth contribute to the proliferation of breeding sites of mosquitoes.

Kupwara district (latitude 34°31´15´´N longitude 74°15´37´´E; average elevation of 1589 m; area of 2379 km^2; average annual temperature of 14.1^0 C and precipitation of 869 mm/year) is located in the northern-most frontier, backward and rural settlement endowed with dense forests, hilly terrains and mountains on west, north and east boundaries; the southern parts of the district are plain. Most of the people are connected with agriculture practices. The district is bordered in the north by ladakh, bounded by Baramulla district in the south, its eastern side touch Bandipora district, northern and western areas borders with Pakistan.

2. Mosquito Larvae sampling

2.1. Outline of study sites

Considering the availability of different aquatic environments, longitudinal survey tours were conducted across eight potential mosquito breeding points of the five study localities. The oviposition habitats- [1. agricultural reservoirs (semi-permanent aquatic habitat) 2. farm ditches (comprising of vegetated and concrete temporary habitats); 3. sewage canals (stagnant human-made aquatic habitats); 4. Open drains (permanent slow-flowing water bodies); 5. ponds, 6. swamps (semi-natural aquatic environments with vegetation); 7. tree holes (natural containers); 8. puddles (semi-natural bodies without vegetation)] supporting immature mosquitoes. Meanwhile in different seasons, the alterations in the waters level were noticed and it was observed that temporary and semi-permanent breeding habitats hold water for a short period of time and changed accordingly on the basis of water availability. The aquatic habitats of permanent nature were stable and remained in their fixed locations.

2.2 Larval sampling

Five localities (Drugmulla-Lt.1 Handwara-Lt.2, Langate-Lt.3, Upper Qaziabad-Lt.4 and Lower Qaziabad-Lt.5) of the study area facing different directions were selected and larval collections were carried out every two weeks per month from transient, semi-permanent and permanent oviposition habitats. Standard dipper (350 ml capacity) as a survey tool used during sampling period was adjusted by fixing the length of its handle manually to certain levels for different varieties of mosquitoes harboring water habitats. Dipping (3 to 10 dips calculated as average number of larvae per dip sample *viz*; 3 dips from puddles and 10 dips along river beds; 5 dips from concrete and farm ditches; 8 dips from marshlands and swamps) was performed from each and every possible habitat between 9:00 hrs to 12:00 hrs and 15:00 hrs to 17:00 hrs by gently dragged along the edges of water surface of each habitat at an angle of 45° to avoid disturbance and for subsequent dips an interval of 2-3 minutes were maintained. Occasionally 5 ml graduated plastic pipettes proved helpful alternatives of standard dippers for small breeding sites and latter were sometimes pooled to get the required water sample volume for larval collection purposes. One to three sampling points were randomly selected according to the perimeter length of each aquatic habitat (three sampling points for perimeters more than 30 m; two sampling points for perimeters more than 10 m but less than 30 m and likewise one sampling point for perimeters equal to or less than 10 m).

2.3 Culture and Rearing

Proper labeled field samples of larvae and pupae were brought and maintained under controlled and ideal laboratory conditions (in separate plastic and enamel culture trays at ambient temperature of 27±2°C, natural photoperiod cycle of 14L:10D and relative humidity of 75-85% under hygienic environment). During the mean time, immature larvae culture was fed with the brewer's yeast and dog biscuits (artificial larval food in 3:1 ratio) on regular basis. Pupal emergence were noticed regularly and transferred to plastic jars kept in wooden framed cage (40×40×40) with front side covered with muslin cloth. Adults emerged from pupae were provided 10% sucrose soaked in cotton pad and placed in a petriplate at bottom edges of mosquito cage. Later on emerged adults were picked with the help of mouth aspirator, narcotized and pinned on corks in glass vials for further investigation [25, 26].

2.4 Identification

Morphological differences among genera and species of family Culicidae can be determined on the basis of respiratory siphon, proboscis, head, thorax, abdominal segments, wing scales and hind legs. Few genera of mosquitoes were identified macroscopically during field collection surveys and were recognized based on their body surface positions (larval forms floating horizontally to the surface of the water, likely to be *Anopheles*; slender respiratory siphon at certain angle to the water surface, likely to be a *Culex*; respiratory tube of short stature with a certain angle to the surface of water, likely to be *Aedes*). Adults were counted and observed under Stereo zoom binocular microscope following systematic taxonomic keys [5, 18-19].

Results

In the present study, a total of 896 mosquito specimens recorded from the selected breeding habitats of five localities were identified morphologically into three genera (*Anopheles*, *Culex* and *Aedes*) and seven species (Table 1). The collected mosquito species were *Culex quinquefasciatus* (37.38%), *Culex vagans* (13.83%), *Culex mimeticus* (8.14%), *Aedes aegypti* (15.95%), *Aedes albopictus* (10.82%), *Anopheles gigas* (8.59%) *and Anopheles stephensi* (5.24%). *Culex quinquefasciatus* species were prevalent in most of the aquatic breeding points and is considered as most dominant species followed by *Aedes aegypti. Anopheles stephensi* were least recorded and account 5.24 % of the mosquito population. The aquatic sites ponds, farm ditches, agricultural reservoirs, sewage canals and open drains were dominantly accommodated by most of the larval mosquitoes species. Moreover mosquitoes were analyzed seasonally (summer and autumn) and monthly (2019 and 2020) as shown in Table 2. The current study reveals that the summer season provides an ideal environment for mosquitoes to flourish. It has been observed that the respective months of Augest and September support a maximum prevalence of mosquitoes population across all localities of the study area. The overall population, species composition, diversity index indices, proportion of the individual species and status of sampled mosquitoes species is mentioned in Table 3. Moreover the Shannon-Weiner diversity index reveals the highest diversity for *Cx.quinquefasciatus* (0.159) followed by *Ae.aegypti* (0.126) and least for *Anopheles stephensi* (0.066). Simpson diversity dominance index was also recorded highest for *Cx.quinquefasciatus* (0.139) followed by *Ae.aegypti* (0.025) and least for *An.stephensi* (0.002). Breeding habitats of the localities 4 and 5 depicts the highest proportion of individuals of particular species as shown in Table 4. The percentage composition of the specimens collected discloses that the *Culex* genera exhibit the more than half the population (59.37%) followed by *Aedes* (26.78%) and *Anopheles* (13.83%) as shown in figure 1. The habitat wise abundance of larval immatures in a particular habitat has been described in the table 5 and clearly indicates that the *Culex quinquefasciatus* as most abundant species.

Table 1: Mosquitoes species collected across five localities during June, 2019 to October, 2019 and June, 2020 to October, 2020.

Mosquito Genus	Mosquito species	Lt. 1			Lt. 2			Lt. 3			Lt. 4			Lt. 5			Total
		N	S	A	N	S	A	N	S	A	N	S	A	N	S	A	
Culex	*quinquefasciatus*	59	35	24	74	42	32	67	42	25	72	39	33	63	35	28	335
	vagans	27	17	10	21	11	10	20	12	8	23	15	8	33	16	17	124
	mimeticus	14	8	6	12	7	5	19	11	8	14	7	7	14	8	6	73
Aedes	*aegypti*	27	18	9	24	14	10	25	14	11	33	21	12	34	21	13	143
	albopictus	16	9	7	15	9	6	19	12	7	21	12	9	26	16	10	97
Anopheles	*gigas*	14	8	6	14	8	6	15	9	6	18	11	7	16	11	5	77
	stephensi	10	6	4	9	5	4	10	7	3	7	5	2	11	7	4	47
	Total	167			169			175			188			197			896

N= Total number of mosquito specimens, Lt= Locality, Lt.1= Drugmulla, Lt.2= Handwara, Lt.3= Langate, Lt.4= Upper Qaziabad, Lt.5= Lower Qaziabad S= Summer and A= Autumn

Table 2: Seasonal diversity of mosquito species from the five different localities of the study area (June, 2019- October, 2019 and June, 2020- October, 2020).

	Summer			Autumn		Summer			Autumn	
Mosquito Species	**June, 2019**	**July, 2019**	**Aug, 2019**	**Sep, 2019**	**Oct, 2019**	**June, 2020**	**July, 2020**	**Aug, 2020**	**Sep, 2020**	**Oct, 2020**
Cx. quinquefasciatus	15	35	51	47	23	17	30	45	44	28
Cx. vagans	7	14	18	17	9	5	14	13	16	11
Cx. mimeticus	6	7	12	12	6	1	4	11	8	6
Ae. aegypti	9	16	21	16	12	5	13	24	15	12
Ae. albopictus	6	10	14	11	7	4	10	14	12	9
An. gigas	2	10	13	9	6	1	9	12	9	6
An. stephensi	0	7	9	6	2	0	5	9	6	3
Total	282			183		246			185	

Table 3: Estimation of Diversity indices in the selected study area during the study period of selected months of 2019 and 2020.

Diversity indices	*Cx quinquefasciatus*	*Cx. vagans*	*Cx. mimeticus*	*Ae aegypti.*	*Ae albopictus.*	*An. gigas*	*An. stephensi*
Total number of Individuals (N)	335	124	73	143	97	77	47
Individual proportion of the species (Pi)	0.373	0.138	0.081	0.159	0.108	0.085	0.052
Species percentage composition` (%)	37.38	13.83	8.14	15.95	10.82	8.59	5.24
Shannon-Weiner diversity Index (H´)	0.159	0.118	0.088	0.126	0.104	0.090	0.066
Simpsons dominance Index (D)	0.139	0.019	0.006	0.025	0.011	0.007	0.002
Status	D	S	SD	D	SD	S	D

D= Dominant species, SD= Sub-dominant species and S= Satellite species

Table 4: Relative frequency (%) of mosquitoes species during the study period of selected months of 2019 and 2020.

Study area	Lt.1	Lt. 2	Lt. 3	Lt. 4	Lt.5
Cx. quinquefasciatus	35.32	43.78	38.28	38.50	31.97
Cx.vagans	16.16	12.42	11.42	12.29	16.75
Cx.mimeticus	8.38	7.10	10.85	7.48	7.10
Ae.aegypti	16.16	14.20	14.28	17.64	17.25
Ae.albopictus	9.58	8.87	10.85	11.22	13.19
An.gigas	8.38	8.28	8.57	9.62	8.12
An.stephensi	5.98	5.32	5.71	3.74	5.58

Lt= Locality, Lt.1= Drugmulla, Lt.2= Handwara, Lt.3= Langate, Lt.4= Upper Qaziabad, Lt.5= Lower Qaziabad

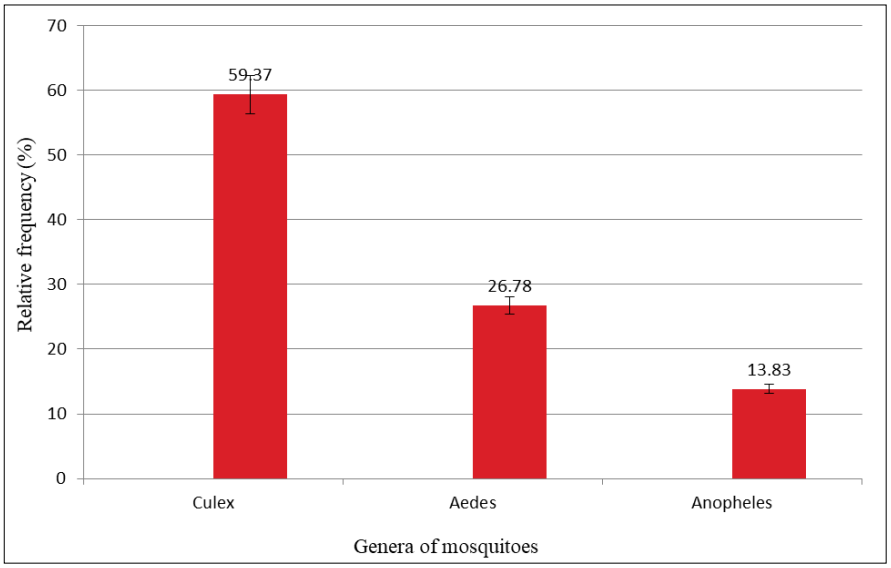

Fig 1: Percentage distribution of mosquitoes genera collected from the study area during 2019 and 2020.

Table 5: Presence/Absence of larval mosquito species in breeding habitats.

Mosquito Species	Breeding habitats								
	SC	AR	OD	FD		PD	SP	TH	PL
				Vg	Cn				
Cx. quinquefasciatus	*	*	*	*	*	*	*	-	-
Cx. vagans	*	-	*	*	-	-	*	-	-
Cx. mimeticus	*	*	*	-	-	*	-	-	-
Ae. aegypti	-	-	-	-	*	*	*	*	-
Ae. albopictus	-	*	-	*	*	-	*	*	-
An. gigas	-	*	*	-	-	-	-	-	*
An. stephensi	*	-	-	-	-	*	-	*	*

SC- Sewage canal, AR- Agricultural reservoir, OD- Open drain, FD- Farm ditch, Vg- Vegetated, Cn-concrete PD- Pond, SP- Swamp, TH-Tree hole, PL- Puddle

Discussion

In India, *Anopheles, Aedes* and *Culex* genera of mosquitoes are commonly known as vectors and are capable of transmitting diseases through pathogen transmission. Mosquito larval life forms harboring aquatic breeding habitats are crucial determinants and subsequently determine the status of adult distribution and abundance [27]. Different aquatic habitats were diligently observed across five localities of the study area for the distribution, diversity and abundance of mosquito species at Kupwara Jammu and Kashmir, India. The present investigation reveals the diversification of seven mosquitoes species in the study area belonging to the three genera of culicids. Among *Culex* mosquitoes, *Culex quinquefasciatus* was abundantly found in the aquatic habitats and is considered as a major vector of Japanese encephalitis across the globe [28]. Previous epidemiological studies carried out by Nair, (1973) [29] on malaria causing mosquitoes in upper reaches of Kashmir valley investigated *Anopheles fluviatilis* as a major vector in Baramulla district villages. However in the present survey, two *Anopheles* species were recorded namely *Anopheles gigas* and *Anopheles stephensi* from semi-permanent and temporary habitats like puddles, tree holes, ponds, agricultural reservoirs etc. Results obtained from the present study showed that *Aedes aegypti, Aedes albopictus* and *Anopheles stephensi* mosquitoes mostly preferred tree holes (natural containers) as breeding habitats and were recorded dominant in the months of July and Augest. Similarly mosquito tree hole studies on tree holes of Kashmir carried out by Jebanesan *et al*., (2012) [23] collected six species of mosquitoes and found *Aedes* aegypti and *Aedes albopictus* as dominant tree hole harboring species during the seasonal months of July, Augest and September. Moreover a large number of mosquito individuals were procured from their breeding habitats in the seasonal months of July, Augest and September and mostly it is due to the favorable physical (precipitation, temperature and humidity) and climatic factors supporting their survival and growth. Accordingly the breeding habitats of permanent and semi-permanent nature supported large specimens of mosquitoes and is mainly due to the accumulation of water for longer period of time [30]. Temporary habitats because of their transient nature accommodate individuals of mosquitoes like tree holes, puddles, farm ditches etc., for shorter period of time only [31]. The results of the present study coincides with the previous work carried out by Devi and Jauhari (2004) [32] recorded 4 species of mosquitoes- *Culex vagans, Culex quinquefasciatus, Culex mimeticus* and *Anopheles gigas* between altitudinal range of 500 and 2000m Garhwal region, Uttrakhand, India. It has been predicted that in the present study, the *Anopheles* species were least recorded from the breeding localities and may be due to the fact of their sensitiveness to environmental variables.

Conclusion

In the present study, the investigation on the mosquito fauna population was conducted at five localities of the Kupwara district, Jammu and Kashmir, India. A total of 896 mosquitoes specimens belonging to three genera and five species were recorded from all kinds of selected breeding habitats with permanent, semi-permanent and temporary nature. *Culex quinquefasciatus* mosquitoes were documented as most dominant species exhibiting almost all breeding sites of all localities studied. It is hypothesized that broad spectrum of aquatic breeding environments harboring immature forms may be convenient for particular range of species and inconvenient for others and their identification might lead to amend the prevalent management practices for constructive deployment of control measures. Hence it is important to mention that mosquitoes population can only be reduced through implementation of effective control approaches of their breeding environments.

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