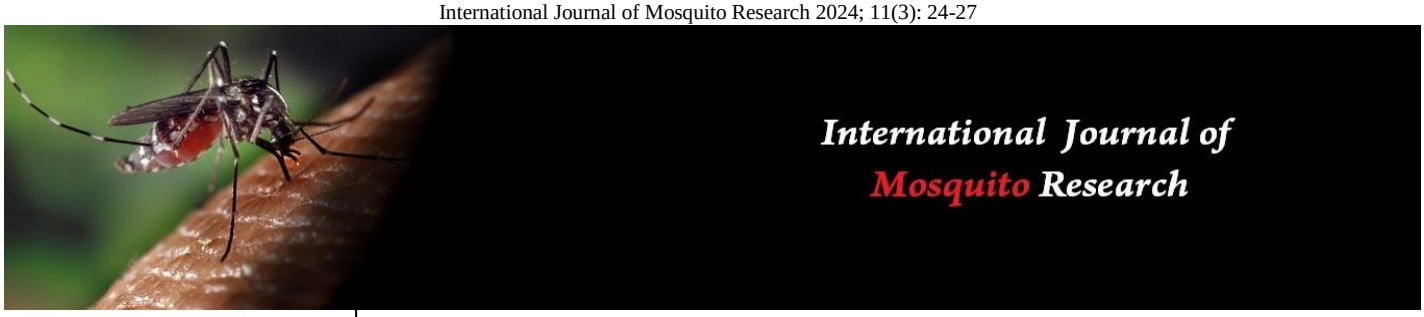

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Behavioral resting preference of *Culex* mosquitoes in indoor and outdoor sites in Udaipur district of southern Rajasthan

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Abstract
An investigation was conducted to find out the relative season wise abundance of *Culex* mosquitoes in indoor and outdoor sites using aspirator (hand catch method) in urban and rural areas of Udaipur district of Rajasthan. A total of 1115 mosquitoes were collected, out of which 1021 mosquitoes were found in indoor sites and 94 in outdoor sites. In Indoor site, 59.84% *Culex* mosquitoes were found in human dwellings and 40.15% in cattle sheds. While outdoor resting sites included puddle and river bed which had the highest number of mosquitoes. Comparing the three seasons in the resting study, it was found that mosquito preference was highest in the rainy season, followed by summer season and lowest in the winter season.

Keywords: Mosquitoes, *Culex*, cattle shed, human dwellings, resting sites

Introduction
Mosquitoes, blood-sucking dipteran insects are well-known vectors of transmission. There are many deadly diseases around the world and all of them are mosquito-borne diseases. It remains a major problem in almost all tropical and subtropical countries [1]. Mosquito species are estimated to spread diseases to more than 700 million people annually in Africa, Mexico, South America, Central America and most Asian countries with over one million deaths each year globally [2].

Globally, in 2013, mosquito-borne diseases included malaria, which caused to total of 5, 84, 000 deaths and 198 million cases more than 50-100 million new cases per year [3]. Which cases human suffering, loss of life and significantly reduced economic growth. Various mosquito species, including *Culex*, *Aedes* and *Anopheles* spp., are known to carry arboviruses [4].

The distribution of mosquito species is influenced by physical environmental factors to breeding and resting, in addition to various factors that can be altered by human activities and modify disease transmission dynamics [5]. Jos, a city in Nigeria, like most African urban centres, is slowly expanding with a growing population. This expanding could lead to increased contact between humans and wild monkeys living in the risk of spread of zoonoses as well as arboviruses clearly increases [6].

Various factors are considered to influence sampling efficiency from artificial resting sites, while a systematic evaluation of the various factors is missing. Thus, we studied three different factors that may affect sampling efficiency: resting site establishment environment (here: Tree type). This study was conducted in rural areas where mosquito breeding sites (such as drinking through ditches etc.) are abundant. One deciduous and one coniferous tree sampling site was selected to establish resting sites. Environmental conditions, for example, microclimate or lighting [7].

Although the exact relationship of these factors is mostly unknown, various studies highlight their relevance for mosquitoes resting site selection [8, 9].

Materials and Methods

Sample Collection

Survey was planned for two years April, 2021 to March, 2022 and April, 2022 to March, 2023. Sample collections were carried out three times in each season at each selected sites. The selection of survey site was based on the prevalence of *Culex* obtained from Chief Medical Officer, Udaipur. Three seasons were selected for the entire study, namely Rainy (July to October), winter (November to February) and summer (March to June).

Adult mosquito's collection (Outdoor and Indoor)

During surveys of resting habitats, both cattle sheds and human dwellings, mosquitoes were collected with oral aspirators and light torches. At outdoor localities, mosquitoes were collected from garden belts, villages, slum areas, around home and ground cavities. Mosquitoes were caught from main entry points, basement, living room, under the stairs etc. Mosquito collection was carried out from cattle shed and human dwellings using hand catch method between morning to evening. The collected mosquitoes are transferred to plastic containers with cuts on their sides. The containers are covered with cloth and tied with rubber bands and the mosquitoes are taken to the laboratory for identification.

Resting collection (indoor)

Culex mosquitoes are mostly inactive during the day and rest mostly in dark places such as corners of rooms and in shelters. Indoor adult mosquitoes were collected from cattle sheds and human dwellings between 7:00 am to 9:00 am in all seasons during the entire period of the survey. Adult mosquitoes prefer a resting places rather than most of the time. Most species rest entirely outdoors in natural resting sites and only a few species prefer artificial shelters. Only a few indoor resting mosquito species are known to be carriers of malaria, filariasis and arboviruses. Mosquitoes were collected and taken to the laboratory for identification. Different species were correctly identified by identification keys and their number were recorded.

Resting collection (Outdoor)

Outdoor resting mosquitoes were collected from urban, semi-urban, rural areas and crowded streets. Mosquitoes were collected with the help of oral aspirator in the evening for about 2 hours from the puddle, sewage, water tanks, tyre, cement tank, tree hole and rock hole.

Identification of *Culex* mosquitoes

The collected mosquitoes were brought to the laboratory and identified with standard (Reuben *et al.*, 1994; Rattanrithikul *et al.*, 2005) [10, 11] keys.

Results and discussion

Resting behaviour of *Culex* mosquitoes

Seasonal sampling was recorded for two years that is during the sampling locality-I and locality-II 1115 adult *Culex* mosquitoes were found in indoor resting while 94 mosquitoes were found in outdoor resting. Six habitats of *Culex* mosquitoes were recorded in outdoor places, namely puddles, sewage water, river bed, tyre and tree hole.

In indoor and outdoor habitat, *Culex quinquefasciatus* was recorded at 42.81 percent, followed by *Culex pseudovishnui* also recorded at 34.20 percent and after that the *Culex gelidus* was recorded at 16.42 percent and finally the *Culex vagans* was recorded at 12.38 percent. From April, 2021 to March, 2022, 57.26% *Culex* mosquitoes were recorded in human dwellings while, 42.72% *Culex* mosquitoes were recorded in cattle sheds. Similarly, in locality-I, 56.56% *Culex* mosquito were recorded in human dwellings while in cattle sheds 43.46% while in locality-II also 57.91% *Culex* mosquitoes were recorded in human dwelling and 42.08% *Culex* mosquitoes were recorded in the cattle sheds. From April, 2022 to March, 2023, 61.96% *Culex* mosquitoes were recorded in human dwelling while, 38.03% was recorded in cattle sheds. Similarly, in locality-I, 61.76% was recorded in human dwelling while in cattle sheds 38.23%. While in locality-II also 62.15% was recorded in human dwelling and 37.84% was recorded in cattle sheds.

Outdoor surveys from April, 2021 to March, 2022 observed the spread of *Culex* mosquitoes across six habitats, with a total of 27 *Culex* mosquitoes recorded in locality-I, while 28 *Culex* mosquitoes were recorded in locality-II. *Culex* mosquitoes were recorded to have the highest prevalence in similar number of habitat preferences in puddles and cement tanks and 5 in sewage water, followed by 4 in tyres and least two in tree holes.

Similarly, when an outdoor survey was conducted from April, 2022 to March, 2023, a total of 38 *Culex* mosquitoes were recorded of which 20 were found in locality-I and 18 in locality-II similarly, when comparing habitats, most *Culex* mosquitoes were found in puddle and river bed followed by cement tanks.

Data of resting collection of Year April, 2021 to March, 2022 and April, 2022 to March, 2023 is shown in following Table (1-4)

Table 1: Indoor Resting collection of *Culex* mosquitoes in two localities (April, 2021- March, 2022)

Localities	Mosquitoes collected	Indoor Resting					
		Cattle sheds		Human dwellings		Total	
		Collected	%	Collected	%	Collected	%
Locality-I	*Culex quinquefasciatus*	42	44.21	53	55.78	95	100
	Culex pseudovishnui	23	42.59	31	57.40	54	100
	Culex gelidus	18	43.90	23	74.19	41	100
	Culex vagans	13	41.93	18	58.06	31	100
	Total	96	43.43	125	56.56	221	100
Locality-II	*Culex quinquefasciatus*	43	39.09	67	60.90	110	100
	Culex pseudovishnui	27	42.18	37	57.81	64	100
	Culex gelidus	18	47.36	20	52.63	38	100
	Culex vagans	13	46.42	15	53.57	28	100
	Total	101	42.08	139	57.91	240	100
Locality-I+II	*Culex quinquefasciatus*	85	41.46	120	58.53	205	100

	Culex pseudovishnui	50	42.37	68	57.62	118	100
	Culex gelidus	36	48.64	43	58.10	74	100
	Culex vagans	26	44.06	33	55.93	59	100
	Grand Total	197	42.73	264	57.26	461	100

Table 2: Indoor Resting collection of *Culex* mosquitoes in two localities (April, 2022- March, 2023)

Localities	Mosquitoes collected	Indoor Resting					
		Cattle sheds		Human dwellings		Total	
		Collected	%	Collected	%	Collected	%
Locality-I	*Culex quinquefasciatus*	43	37.06	73	62.93	116	100
	Culex pseudovishnui	28	39.43	43	60.56	71	100
	Culex gelidus	19	39.58	29	60.41	48	100
	Culex vagans	14	37.83	23	62.16	37	100
	Total	104	38.23	168	61.76	272	100
Locality-II	*Culex quinquefasciatus*	47	42.34	64	57.65	111	100
	Culex pseudovishnui	26	32.91	53	67.08	79	100
	Culex gelidus	24	40.00	36	60.00	60	100
	Culex vagans	12	31.57	26	68.42	38	100
	Total	109	37.84	179	62.15	288	100
Locality-I+II	*Culex quinquefasciatus*	90	39.64	137	60.35	227	100
	Culex pseudovishnui	54	36.00	96	64.00	150	100
	Culex gelidus	43	39.81	65	60.18	108	100
	Culex vagans	26	34.66	49	65.33	75	100
	Grand Total	213	38.03	347	61.96	560	100

Table 3: Outdoor resting collection of *Culex* mosquitoes in two localities (April, 2021- March, 2022)

Localities	Seasons	Mosquitoes collected	Puddle	Sewage water	River bed	Tyre	Cement tank	Tree hole	Total
Locality-I	Rainy	*Culex quinquefasciatus*	1	2	1	1	2	0	7
		Culex pseudovishnui	2	0	3	0	0	0	5
		Culex vagans	0	0	0	1	0	0	1
	Winter	*Culex quinquefasciatus*	2	0	0	0	0	1	3
		Culex pseudovishnui	1	0	0	0	0	0	1
	Summer	*Culex quinquefasciatus*	2	1	0	1	1	0	5
		Culex pseudovishnui	1	0	2	0	0	0	3
		Culex vagans	0	0	1	0	0	1	2
		Total	9	3	7	3	3	2	27
Locality-II	Rainy	*Culex quinquefasciatus*	2	0	2	0	1	0	5
		Culex pseudovishnui	1	1	3	1	0	0	6
		Culex whitei	1	0	1	0	0	0	2
	Winter	*Culex quinquefasciatus*	1	0	0	0	0	0	1
		Culex pseudovishnui	0	0	2	0	1	0	3
	Summer	*Culex quinquefasciatus*	3	0	3	0	0	0	6
		Culex pseudovishnui	2	1	0	0	0	0	3
		Culex whitei	0	0	1	0	1	0	2
		Total	10	2	12	1	3	0	28
		Grand total	19	5	19	4	6	2	55

Table 4: Outdoor resting collection of *Culex* mosquitoes in two localities (April, 2022- March, 2023)

Localities	Seasons	Mosquitoes collected	Puddle	Sewage water	River bed	Tyre	Cement tank	Tree hole	Total
Locality-I	Rainy	*Culex quinquefasciatus*	2	0	3	0	0	0	5
		Culex pseudovishnui	1	0	0	0	0	0	1
		Culex vagans	0	0	1	0	0	0	1
	Winter	*Culex quinquefasciatus*	0	0	0	0	1	0	1
		Culex pseudovishnui	0	0	2	1	2	0	5
	Summer	*Culex quinquefasciatus*	2	0	0	0	0	0	2
		Culex pseudovishnui	2	0	0	0	0	2	4
		Culex whitei	0	0	0	0	1	0	1
		Total	7	0	6	1	4	2	20
Locality-II	Rainy	*Culex quinquefasciatus*	3	0	4	0	0	0	7
		Culex pseudovishnui	0	1	0	0	2	0	3
		Culex whitei	0	0	0	0	0	1	1
	Winter	*Culex quinquefasciatus*	0	0	2	1	0	0	3
		Culex pseudovishnui	0	1	0	0	0	0	1
	Summer	*Culex quinquefasciatus*	2	0	0	0	0	0	2
		Culex pseudovishnui	0	0	1	0	0	0	1

		Culex gelidus	1	0	0	0	0	0	1
		Total	6	2	7	1	2	1	19
		Grand total	13	2	13	2	6	3	39

With its clinical consequences, *Culex quinquefasciatus* is also responsible for nocturnal anxiety and allergic reactions due to its nuisance biting behavior throughout the night, both indoors and outdoor. During the day, they are mostly inactive and rest mostly in dark places such as corners of rooms and shelters. The nuisance caused by bites generally affects most people rather than the transmission of diseases. Sometimes mosquito bites become very troublesome and unbearable. Many new breeding habitats are created by human activities which become suitable for other mosquitoes to breed as well. For effective control of mosquitoes, it is important to know about their behavior [12, 13].

In indoor and outdoor habitat, *Culex quinquefasciatus* was recorded at 42.81 percent, followed by *Culex pseudovishnui* also recorded at 34.20 percent and after that the *Culex gelidus* was recorded at 16.42 percent and finally the *Culex vagans* was recorded at 12.38 percent.

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Although the specific relationship of these factors is generally obscure, different investigations feature their importance for mosquitoes resting site selection [8, 9].

Outdoor surveys from April, 2021 to March, 2022 observed the spread of *Culex* mosquitoes across six habitats, with a total of 27 *Culex* mosquitoes recorded in locality-I, while 28 *Culex* mosquitoes were recorded in locality-II. *Culex* mosquitoes were recorded to have the highest prevalence in similar number of habitat preferences in puddles and cement tanks and five in sewage water, followed by four in tyres and least two in tree holes. Similarly, when an outdoor survey was conducted from April, 2022 to March, 2023, a total of 38 *Culex* mosquitoes were recorded of which 20 were found in locality-I and 18 in locality-II similarly, when comparing habitats, most *Culex* mosquitoes were found in puddle and river bed followed by cement tanks.

Collections containing sites represent only a temporal snapshot of the resting mosquito population. Mosquitoes can enter and leave the resting site at any time and their resting behavior is influenced by various environmental conditions, for example, temperature or light [14, 15].

Conclusion

The seasonal study was reported for two years which is April, 2021 to March, 2022 and April, 2022 to March, 2023. During Locality-I and Locality-II, 1115 adult *Culex* mosquito samples were recorded, out of which 1021 mosquitoes were recorded at indoor resting sites, while 93 mosquitoes were recorded at outdoor resting sites. Six resting habitats of *Culex* mosquitoes were recorded at outdoor sites such as puddle, sewage water, river bed, tyres and tree holes. Comparing the three seasons in the resting study revealed that the preference of mosquitoes was highest in the rainy season, followed by summer season and lowest in winter season.

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