Larval habitat prevalence of genus *Culex* (Diptera: Culicidae) mosquitoes in Udaipur district Rajasthan.

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Abstract

Research was conducted from April 2021 to March 2023 to examine the mosquito fauna and ecology in Udaipur district, Rajasthan, India. Mosquito larvae were collected using the dipping method, and the parameters of their environment were documented based on hydro-ecological aspects. A total of 825 Culex larvae were collected from various larval breeding sites. Eight species of the genus *Culex: Quinquefasciatus, Culex pipiens, Culex edwardsi, Culex whitei, Culex vagans*, and *Culex pseudovihnui. Culex gelidus* and *Culex vishnui* were identified b ased on the enumeration of adults that emerged in the laboratory from collected larvae. In Localit y 1, 108 specimens were recognised from puddle water bodies, whereas in Locality II, 72 specim ens were identified from tyres.

Keywords: - Mosquito, fauna, *Culex* larvae, breeding site, environment.

INTRODUCTION

At this time, mosquito-borne diseases are major public health problems in many parts of the world (Dass and Mariappan, 2014; Nadeeka *et al.,* 2014). Today there are more than 4500 species of mosquitoes found around the world, belonging to 34 genera; most of these species belong to genus *Aedes*, *Anopheles* and *Culex* (Chandra *et al.,* 2013).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 (Tolle, 2009). 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 (WHO, 2019).

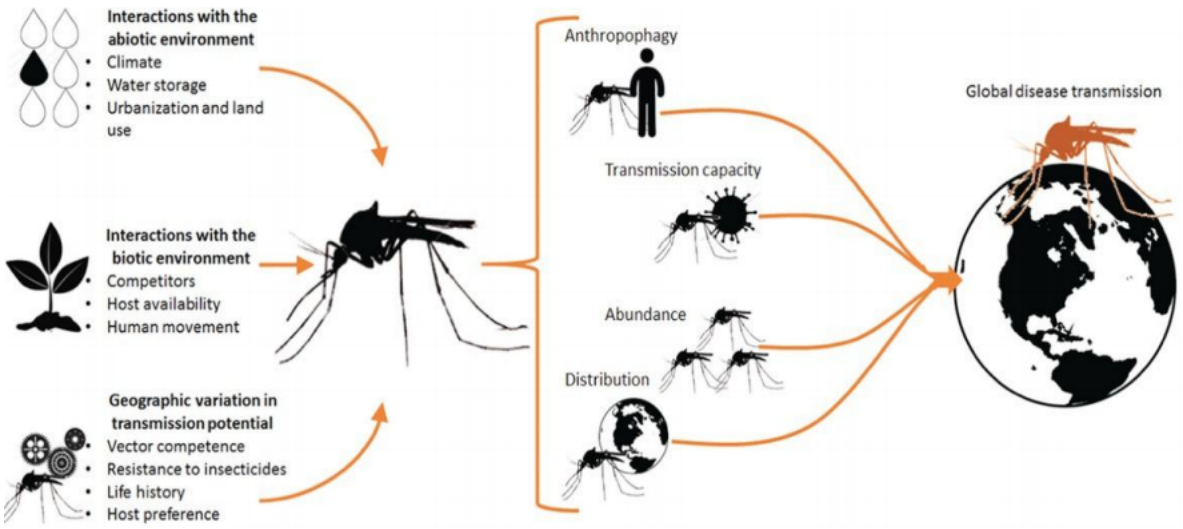

Figure 1.1: The complexity of interactions among mosquito vectors, arboviral pathogens, and transmission across environmental drivers (Shragai *et al.,* 2017).

Due to rapid increase in population and construction of buildings in an unplanned and unbalanced manner, the population density and species diversity of mosquitoes is increasing on large scale in urban environments. The lack of a functional sewage system has also led to pools of stagnant water and puddles. These water bodies are the most preferred breeding habitat for *Culex quinquefasciatus say,* the main vector of *Wuchereria bancrofti*. It also spreads Japanese encephalitis (Bhattacharya *et al*., 2016). Mosquito species vary in type of aquatic habitats, they give preference oviposition depending on the location, physicochemical condition of the water body and presence of the number of potential predators (Shililu *et al*., 2003).
Physicochemical factors that affect ovulation, survival and spatio-temporal distribution of important disease vector species contain dissolved salts, organic and inorganic substances, degree of eutrophication, turbidity, presence or absence of suspended mud, presence or absence of plants, temperature, light, shade and hydrogen ion concentration (Gimnig *et al*., 2001).

We report for the first time the molecular identification of *Culex quinquefasciatus* and *Culex pipiens* species while the molecular identification of *Culex tritaenirhynchus* and *Culex sitiens* was reported for the first time in the entire Saudi Arabia and Jazan region. This study used PCR for the first time to identify *Culex* mosquito species in the Jazan region. PCR is a complementary and integrated taxonomy-based identification tool for mosquito species. This integrated has the potential to promote and enhance programs, as well as define the genetic diversity of species in the region (Noureldin *et al*., 2021).The quality of breeding water is an important determinant of whether female mosquitoes will lay their eggs, and whether the resulting immature mosquitoes will successfully complete their development to adult mosquitoes (Piyaratnea *et al*., 2005). The proportional abundance of *Culex torrentium* may potentially be underestimated. This is particularly notable as Carbon dioxide containing traps have been described as attracting and

catching a wider range of different types of mosquitoes than other commonly used traps (Pezzin *et al*., 2016).

Materials and Methods

GEOGRAPHY OF STUDY AREA

Udaipur is in the state of Rajasthan in India. Udaipur is located at 24’58°N; 73’68°E. It has an average elevation of 598 meters (1961 feet) and total area 64 Km². Udaipur is bordered by Rajsamand district to the north, Chittorgarh and Pratapgarh district to the east. Banswara district to the South-east, Dungarpur to the South and Gujrat to the South-East. Udaipur is divided into 12 tehsils such as Mavli, Gogunda, Kotra, Jhadol, Girwa, Vallabhnagar, Lasadia, Salumber, Sarada, Rishbhdev, Kherwara and Semiri. Udaipur district is predominated hilly, the Western region of the district is irrigated by the Sabarmati River, which originates in the Aravalli range in Udaipur district and flows into Gujarat. The Northern area of the district is drained by tributaries of the Banas River including the Ahar River which flows through Udaipur city. In the Southern and Central region of the district the tributaries of Mahi River enter the Som and Gomti. Our study was divided into two localities in Udaipur district. This study area was selected based on their socio-ecological characteristics. The region has rivers and flowing standing water which is the main breeding habitat for *Culex* mosquitoes.

LOCALITY- I

This area includes hilly areas. The area mainly consists of peri-urban areas such as Gogunda, Jhadol, Kotra and Girwa areas. The area is surrounded by hilly terrain with seasonal rivers and waterfalls. The villages are located on hill peaks and are characterized by the presence of perennial streams and springs which are prime habitats for breeding mosquitoes.

LOCALITY- II

The area covers plains with seasonal rivers, streams and mainly includes urban areas such as Mavli, Udaipur city and some parts of Slumber regions.

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).

Larval Surveys

Female *Culex* mosquitoes prefer to lay their eggs in places where dissolved organic matter is abundant such as sewage waste and septic tanks. However, the immature stages of this mosquito can often be seen in artificial containers containing organically enriched or polluted water. The *Culex* mosquito, the Southern house mosquito has been very well studied in recent time due to its

major role in the transmission of diseases to humans. Larvae were collected from different types of water bodies like puddles, sewage, water tanks, riverbeds, tyre, cement tanks, tree holes and rock hole with the help of pipettes, droppers and dippers. The larvae were transported to the laboratory in small plastic wide mouth bottles marked with date and location.

Identification of *Culex* Mosquitoes

When at rest, the body exhibits hunchback (*i.e.* the thorax makes an angle with the abdomen). *Culex* stay parallel to the surface. It profusely breeds in dirty water contaminated with sewage. This identification features were used and visualized using stereoscopic microscope in the laboratory. The collected mosquitoes were brought to the laboratory and identified with standard (Reuben *et al.,* 1994; Rattanarithikul *et al.,* 2005) keys.

Result

Collection sites covering both urban and rural habitats were selected. The surveys were conducted for two years from April, 2021 to March, 2022 and April, 2022 to March, 2023 to catch larval samples. These sites were further categorized into seven different water bodies such as rock hole, tree hole, cement tank, tyre, riverbed, sewage water and puddle. A total 161 adult mosquitoes of *Culex* were identified after emergence of larval stage during the collection of larvae. The genus *Culex* was represented by 8 species *Culex quinquefasciatus*, *Culex edwardsi, Culex whitei, Culex vagans, Culex pseudovishnui, Culex gelidus, Culex pipiens* and *Culex vishnui*. Out of the maximum 51 specimens were catched from puddle water bodies followed by 35 specimens from riverbeds, 29 specimens from cement tanks, 14 specimens from rock holes, 12 specimens from sewage, 11 specimens from tyres and 9 specimens from tree holes in the rainy, winter and summer seasons.

Culex quinquefasciatus was the dominant, 21 specimens reported from puddle water bodies with maximum 21 specimens, riverbeds 14 specimens and 11 specimens. *Culex pseudovishnui* was the second dominant reported species. Collection sites covering both urban and rural habitats were selected. The surveys were conducted for two years from April, 2021 to March, 2022 and April 2022 to March 2023 to catch larval samples. These sites were further categorized into seven different water bodies such as rock hole, tree hole, cement tank, tyre, riverbed, sewage water and puddle.

A total 194 adult mosquitoes of *Culex* were identified after emergence of larval stage during the collection of larvae. The genus *Culex* was represented by 8 species *Culex quinquefasciatus, Culex edwardsi, Culex whitei, Culex vagans, Culex pseudovishnui, Culex gelidus, Culex pipiens* and *Culex vishnui*. Out of the maximum 57 specimens were catched from puddle water bodies followed by 40 specimens from riverbeds, 18 specimens from cement tanks, 23 specimens from rock holes, 18 specimens from sewage, 14 specimens from tyres and 24 specimens from tree holes in the rainy, winter and summer seasons.

Data of Larval Survey (Locality-I) April, 2021 to March, 2022 and April, 2022 to March, 2023 (Table 1.1 and 1.2).

Table 1.1: Calculate of adult emerged in the laboratory from collected larva, Locality-I (2021-2022)

Seasons	Mosquitoes	Rock hole	Tree hole	Cement tank	Tyre	River bed	Sewage water	Puddle	Total
Rainy	*Culex quinquefasciatus*	2	0	4	1	5	3	7	22
	Culex edwardsi	0	0	0	0	2	0	3	5
	Culex whitei	0	2	0	2	0	0	0	4
	Culex vagans	0	0	2	0	0	0	0	2
	Culex pseudovishnui	3	0	0	0	4	3	5	15
	Culex gelidus	0	2	0	0	0	0	2	4
	Culex pipiens	1	0	0	1	1	2	0	5
	Culex vishnui	0	0	3	0	0	0	0	3
	Total	6	4	9	4	12	8	17	60
Winter	*Culex quinquefasciatus*	0	2	3	1	4	2	8	20
	Culex edwardsi	0	0	1	0	2	0	0	3
	Culex whitei	2	0	1	0	0	0	3	6
	Culex vagans	0	0	3	0	0	0	0	3
	Culex pseudovishnui	2	0	0	2	3	0	6	13
	Culex gelidus	1	0	0	0	0	0	2	3
	Culex pipiens	0	0	1	0	2	0	1	4
	Culex vishnui	1	0	3	0	0	0	0	4
	Total	6	2	12	3	11	2	20	56
Summer	*Culex quinquefasciatus*	1	0	4	2	5	1	6	19
	Culex edwardsi	0	0	0	0	0	0	1	1
	Culex whitei	0	0	1	0	0	0	2	3
	Culex vagans	0	1	0	0	3	0	0	4
	Culex pseudovishnui	0	2	0	0	0	1	2	5
	Culex gelidus	1	0	2	0	0	0	0	3
	Culex pipiens	0	0	0	0	2	0	2	4
	Culex vishnui	0	0	1	2	2	0	1	6
	Total	2	3	8	4	12	2	14	45
Grand total		**14**	**9**	**29**	**11**	**35**	**12**	**51**	**161**

Table 1.2: Calculate of adult emerged in the laboratory from collected larva, Locality-I (2022- 2023)

Seasons	Mosquitoes	Rock hole	Tree hole	Cement tank	Tyre	River bed	Sewage water	Puddle	Total
Rainy	*Culex quinquefasciatus*	2	3	0	0	6	3	9	23
	Culex edwardsi	1	0	0	2	0	0	1	4
	Culex whitei	2	0	1	0	3	0	0	6
	Culex vagans	1	1	0	0	2	0	1	5
	Culex pseudovishnui	2	1	2	0	4	1	8	18
	Culex gelidus	2	3	0	0	0	0	2	7
	Culex pipiens	0	2	0	0	0	2	1	5
	Culex vishnui	3	0	0	3	0	0	0	6
	Total	13	10	3	5	15	6	22	74
Winter	*Culex quinquefasciatus*	0	0	0	0	0	0	0	0
	Culex edwardsi	1	0	2	0	7	2	6	18
	Culex whitei	1	2	0	2	0	2	0	7
	Culex vagans	0	0	3	0	0	2	1	6
	Culex pseudovishnui	0	3	0	2	4	1	7	17
	Culex gelidus	2	2	0	0	0	2	0	6
	Culex pipiens	0	0	2	0	1	0	3	6
	Culex vishnui	0	0	0	1	2	0	2	5
	Total	4	7	7	5	14	9	19	65
Summer	*Culex quinquefasciatus*	0	2	0	0	5	0	5	12
	Culex edwardsi	0	2	0	1	0	1	0	4
	Culex whitei	3	0	2	0	0	0	1	6
	Culex vagans	0	0	3	0	2	0	0	5
	Culex pseudovishnui	1	3	0	0	4	0	4	11
	Culex gelidus	1	0	0	0	0	2	0	3
	Culex pipiens	2	0	3	0	0	0	3	8
	Culex vishnui	0	0	0	3	0	0	3	6
	Total	6	7	8	4	11	3	16	55
	Grand total	**23**	**24**	**18**	**14**	**40**	**18**	**57**	**194**

In locality II, the collection of immature mosquitoes of *Culex* were done for two consecutive years from April, 2021 to March, 2023 in the following water bodies namely, rock hole, tree hole, cement tanks, tyre, riverbed, sewage water and puddle. A total of 227 specimens of *Culex* were identified after larval emergence during larval collection in 8 species namely, *Culex quinquefasciatus, Culex edwardsi, Culex whitei, Culex vagans, Culex pseudovishnui, Culex pipiens, Culex gelidus* and *Culex vishnui*.

All of the total 227 identified mosquitoes were collected from different water bodies such as 40 samples from riverbed, 36 samples from cement tanks, 35samples from rock holes, 32 samples from tyres, 30 samples from sewage water, 30 samples from puddles and 24 samples from tree holes. *Culex quinquefasciatus* was the most dominant species in 73 samples or 31.73% and was found mainly in the riverbed with a maximum of 17 samples and also found in rock holes (12 samples). *Culex pseudovishnui* was the second most dominant species at 52 specimens or22.60% and found mainly in rock hole with a maximum of 9 specimens.

During second year out of grand total 243 identified mosquitoes were reported from different water bodies like 47 samples from riverbeds, 40 samples from tyres, 36 samples from sewage water, 33 samples from puddles, 31 samples from tree holes, 30 samples from cement tanks and 26 samples from rock holes. The survey was conducted from April, 2022 to March, 2023, the number of *Culex quinquefasciatus* and *Culex pseudovishnui* larva were recorded to be similar in different water bodies, which shows that the prevalence of these mosquitoes were higher in both years. If we compare the three seasons, it was found that the highest number of 95 specimens were recorded in the summer season, followed by 83 specimens recorded in the rainy season and the lowest number of 65 specimens recorded in the winter season. This proves that the prevalence of *Culex* mosquito the best season for considered to be the summer season between April, 2022 to March, 2023.
Data of Larval survey (Locality-II) of Year April, 2021 to March, 2022 and April, 2022 to March, 2023 is given in following Table (1.3 and 1.4).

Table 1.3: Calculate of adult emerged in the laboratory from collected larva, Locality-II (April, 2021- March, 2022)

Seasons	Mosquitoes	Rock hole	Tree hole	Cement tank	Tyre	River bed	Sewage water	Puddle	Total
Rainy	*Culex quinquefasciatus*	4	2	3	3	5	2	3	22
	Culex edwardsi	1	0	2	1	2	0	1	7
	Culex whitei	1	1	0	2	3	0	2	9
	Culex vagans	0	1	2	1	0	0	1	5
	Culex pseudovishnui	3	4	3	1	4	4	4	23
	Culex gelidus	2	0	1	0	0	1	0	4
	Culex pipiens	0	1	0	0	1	0	0	2
	Culex vishnui	1	2	2	0	0	1	0	6
	Total	12	11	13	8	15	8	11	78
Winter	*Culex quinquefasciatus*	3	2	3	3	5	3	3	22
	Culex edwardsi	0	1	1	2	1	0	1	6
	Culex whitei	0	1	1	1	0	1	2	6
	Culex vagans	2	0	0	0	1	2	0	5
	Culex pseudovishnui	3	1	2	4	3	3	0	16
	Culex gelidus	1	2	1	1	0	2	0	7
	Total	9	7	8	11	10	11	6	62
Summer	*Culex quinquefasciatus*	5	3	4	3	7	3	4	29
	Culex vagans	2	1	3	4	3	4	3	20
	Culex pseudovishnui	3	0	2	3	2	1	2	13
	Culex pipiens	1	0	4	2	0	2	2	11
	Culex vishnui	3	2	2	1	3	1	2	14
	Total	14	6	15	13	15	11	13	87
	Grand total	**35**	**24**	**36**	**32**	**40**	**30**	**30**	**227**

Table 1.4: Calculate of adult emerged in the laboratory from collected larva, Locality-II (April, 2022- March, 2023)

Seasons	Mosquitoes	Rock hole	Tree hole	Cement tank	Tyre	River bed	Sewage water	Puddle	Total
Rainy	*Culex quinquefasciatus*	3	3	4	3	4	3	5	25
	Culex edwardsi	0	1	1	0	2	2	0	6
	Culex whitei	0	0	0	2	3	0	1	6
	Culex vagans	0	0	0	1	2	0	2	5
	Culex pseudovishnui	3	1	0	3	4	5	3	19
	Culex gelidus	1	0	2	1	1	1	1	7
	Culex pipiens	0	1	1	2	2	2	2	10
	Culex vishnui	2	0	0	0	1	1	1	5
	Total	9	6	8	12	19	14	15	83
Winter	*Culex quinquefasciatus*	2	3	4	5	3	4	3	24
	Culex whitei	3	1	0	1	2	0	3	10
	Culex pseudovishnui	4	2	5	5	3	5	2	26
	Culex vishnui	1	0	1	3	0	0	0	5
	Total	10	6	10	14	8	9	8	65
Summer	*Culex quinquefasciatus*	2	3	4	5	6	3	4	27
	Culex vagans	1	4	3	3	3	4	3	21
	Culex pseudovishnui	3	7	2	5	7	5	2	31
	Culex pipiens	1	3	2	1	2	0	0	9
	Culex vishnui	0	2	1	0	2	1	1	7
	Total	7	19	12	14	20	13	10	95
	Grand total	**26**	**31**	**30**	**40**	**47**	**36**	**33**	**243**

CONCLUSION

This paper emphasises that *Culex* larvae can thrive in contaminated water. These water sources have little effect on their survival Eight species of the genus *Culex* were identified: *Culex quinquefasciatus, Culex pipiens, Culex edwardsi, Culex whitei, Culex vagans, Culex pseudovihn ui, Culex gelidus, and Culex vishnui.*

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