

PREVALENCE OF MALARIA DISEASE AND ITS VECTORS *ANOPHELES CULICIFACIES* AND *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE) IN MAIN MALARIA AFFECTED AREAS OF SINDH.

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ABSTRACT

Background: Malaria is a vector borne disease and one of the leading serious health issue in south Asia including Pakistan. Globally approximately 282 million cases and 610,000 deaths were reported during the year (2024). The main affected regions of the world are Africa and Asia, probably due to significant challenges poses to elimination efforts against its vectors, *Anopheles culicifacies* and *Anopheles stephensi*.

Aim: This study aimed to observe the frequency of malaria cases in endemic area of Sindh and seasonal diversity of its vectors which transmitted malaria parasite in different areas of studied localities.

Methods: Blood samples were collected through finger pick and then observed by thick and thin microscopy. While mosquito were collected by aspirator and insect neat at the time of dawn and dusk, larvae were collected by dipping method from different breeding habitats.

Results: During the study period twelve months (361247) blood samples were screened out of which (17127) were found positive for malaria parasites. Most frequent species *Plasmodium vivax* (11803) followed by *P. falciparum* (3969) and mixed infection (1355). Seasonal diversity of malaria vectors (121) adults were collected during dry season (pre-monsoon) and (239) were collected during wet season (post-monsoon).

Conclusion: Proper awareness should be important regarding to wearing of proper protective clothing to low socioeconomic people. Civilian bodies and Non-government should encourage local population to actively take part in clearing mosquito breeding places. As number of malaria patients are increasing day by day, it is recommended that malaria control program should also focus on research regarding development of novel synergistic and compound combination effective against drug resistant *Plasmodium* species and *Anopheles* mosquito.

Key words: *Anopheles* mosquito, Anti-malaria drugs Resistant, Control measurements, Health issue, Monsoon rain, *Plasmodium*.

INTRODUCTION

Malaria is an infectious disease of humans; the causative agent of malaria is a unicellular parasite *Plasmodium*, which belongs to phylum Protozoa, sub phylum Sporozoa and class Telosporea. *Plasmodium* is transmitted to human being through the bite of female infected *Anopheles* mosquitoes. The history of

malaria dates back to 2700 B C in China (Cox, 2002). Malaria is one of the major infectious disease which causing a serious health problem in many parts of the World especially in tropical and subtropical countries which include Africa, Latin America and Asia including Pakistan, (Layne, 2006). Among the all infectious diseases

malaria rank in third position, which cause mortality due to communicable disease, (Malaria case Management, 2010). Majority of the victims are children malaria kills a child in every a minute, because of it malaria is the top priority disease of tropical countries, it affects nearby five times as many people as AIDS, Leprosy, Measles and Tuberculosis combined, (WHO, 2024).

As malaria is a life threatening disease, (WHO, 2022), there are many species of *Plasmodium* which is responsible for malaria such as, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium Knowlesi*, (Mueller *et al.*, 2007; Collins, 2012). In endemic countries such as Afghanistan, Bangladesh, Bhutan, Srilanka, China, India, Indonesia, Iran, Malaysia, Nepal, Sudan, Tajikistan and Myanmar and Pakistan. Women are more affected from malaria attacks probably due to low immunity during pregnancy as a result the new born has low weight, infant mortality and still birth, particularly in *P. falciparum* infection but rarely with *P. vivax* (Rijken *et al.*, 2012). Malaria in Pakistan is typically unstable, (WHO, 2022), in Pakistan two species of *Plasmodium* is responsible for malaria disease *i.e.* *P. vivax* and *P. falciparum* (Yaseen and Ali, 2015); *P. falciparum* caused most dangerous form of malaria (cerebral malaria). (NMCP Pakistan, 2013).

Malaria infects red blood cells as a result deficiency of hemoglobin occurred beside the destruction of red blood cells it also give rise fever, anemia, chills, headache, shivering, muscle ache, tiredness, Nausea, vomiting, severance of disease lead to coma and sometimes death due to the disturbance of immune system of the infected person. There was about 282 million malaria cases were reported clinically Worldwide (WHO & UNICEF, 2024). Malaria not only causes mortality and morbidity but also disturbed the daily life, (Sachs and Malaney, 2002). Therefore, malaria & poverty is correlated, (Gallup and Sachs, 2001). Although now a day's malaria is easily curable and preventable disease but unfortunately the burden of disease is increasing because of some factors such as irrigation system, hydrological changes, anti-malarial drug and insecticide resistance in parasite and vectors and due to increase of

human population, improper urbanization and environmental pollution.

Mosquitoes belong to order Diptera, sub order Nematocera and family culicidae. They are among the most important group of flying insects which affect the health of human being and domestic animals throughout World. This family contains over 3,500 species and is divided into three subfamilie, Anophelinae, Culicinae and Toxorhynchitinae, (Harbach, 2007). The sub family Anophelinae consists of three genera: *Anopheles*, *Bironella* and *Chagasia*. The mosquitoes of genera *Anopheles* only have medical importance, (Service, 1996). The genus *Anopheles* is of particular concern to human health because *Anopheles* species are major vector of malarial parasites. Over all about 70 species of *Anopheles* mosquitoes act as a malaria vectors from which 40 are major vectors of malaria, (Service, 1993).

In Pakistan about 24 *Anopheles* species are reported, (Felix *et al.*, 2002), out of these 24 species, only two species are reported as a vector of malarial parasites, one is *Anopheles culicifacies* and other is *Anopheles stephensi*, (Jahan and Sarwar, 2014). According to Rehman, 2026), the main malaria causing vectors in Sindh are *Anopheles culicifacies* and *Anopheles stephensi*. The *Anopheles culicifacies* is mostly found in rural areas, (Ganesh *et al.*, 2003), while *Anopheles stephensi* found in the urban and semi urban areas, (Rehman and Mutalib, 1967). *Anopheles* mosquitoes are found throughout the World except Antarctica, mostly distributed in tropical and subtropical areas of the World because of their favorable weather conditions, warm temperature, rain fall, high humidity and frequent availability of breeding places. Adult Mosquitoes are found in an area where favorable resting places, hosts and breeding places are present, (Avramov *et al.*, 2024). The ecological habitats of aquatic larvae varies greatly from species to species, such as permanent water surfaces to temporary puddles, *Anopheles* mosquitoes breed in a variety of natural water bodies and their breeding increases specially after rainy season when water is collected in buckets, bottles, tenders, tins, tyres and coconut shells etc, (Hamza, *et al.*, 2016).

Objective of the Study

Malaria disease is increasing rapidly day by day and the field surveys are essential for the planning, operation and evaluation of any vector control program. The main theme of this work is to survey different areas of Sindh, mainly those where the possibility of the prevalence of malaria is more. To identify the species of *Anopheles* mosquitoes, present in Sindh, to understand the ecology of *Anopheles* and causes of occurrences of *Anopheles* mosquitoes, in order to eradicate the breeding places of mosquitoes which will ultimately help us in the control of malaria disease.

MATERIALS AND METHODS

Study sites

The survey in six districts of Sindh, where malaria is prevalent was conducted, which

includes Larkana, Dadu, Jamshoro, Hyderabad, Tando Mohammad Khan and Tando Allahyar, (Fig. 01), three villages of each district were selected for the study. The survey and field collection was continued for 01 year *i.e.* from January to December 2025 seasonally. Covering six districts of Sindh, Larkana includes Goth Ghana, Rasulabad & Gulab Bhutto, Dadu includes Pir Ghazi, Goth Dad Muhammad & Tando Rahim Khan, Jamshoro includes Sindh University colony, Mehran University colony & WAPDA colony Hyderabad includes Qasimabad, Phulali & Husri, Tando Mohd Khan includes Ghaja mori, Sairi & Tando Saendad and Tando Allahyar includes Jhando mari, Khaisana mori & Buqara sharif.



Figure 01: Map of Sindh Province showing studied districts

Presently we have done taxonomy and ecology of *Anopheles* mosquitoes. The specimens of mosquitoes were randomly collected from studied areas. We have conducted weekly surveys for the collection of larvae and adult mosquitoes, the first visit for the survey and collection of *Anopheles* mosquitoes was carried out during dry period *i.e.* From January to June 2025, (pre monsoon) and the second visit for the survey and collection of mosquitoes was

made during wet period *i.e.* From July to December 2025, (monsoonal rain & post monsoonal season).

Mosquitoes were captured by:

Bait collection: - mosquitoes were collected while feeding on men and animal.

Resting places collection: - Adult collected from indoor resting places such as homes (bed rooms, wash rooms, store rooms and grain

stores) and outdoor resting places, such as, animal shelter and animal dung. Females were mostly collected from indoor resting places while males were collected from outdoor (around the bushes and tall grasses), at the time of dusk when mosquitoes form swarming.

Collection of adult mosquitoes

Adult mosquitoes were captured by using suction tube (Aspirator) and insect collecting net by following (Waseem *et al.*, 2009). Aspirator or sucking glass tube was used for indoor collection and insect hand net was used for outdoor collection.

After collection mosquitoes were kept in paper cups and brought to laboratory for identification, where these were killed by keeping them in the fridge for an hour, and then these mosquitoes were kept in petri dish with forceps and identified individually under the binocular microscope and magnifying glass; the identification was based on external morphology particularly mouth parts and scales on wings, with the help of type material and literature particularly the key given by (Das *et al.*, 1990). Photographs of mosquitoes were taken under the microscope and the diagram of Head, Thorax and Abdomen of *Anopheles* mosquitoes were drawn on graph paper using ocular graph and traced on tracing paper and then material was mounted and preserved properly and carefully in specific wooden insect boxes, labeled as per standard procedures. Naphthalene balls were used to protect mosquitoes from predators (ants) and fungus.

Collection of larvae (immature stage)

We have collected larvae from different breeding habitats of mosquitoes which includes temporary as well as permanent breeding sites. Larvae were collected by dipping method with the help of dipper, following (Ponalawat *et al.*, 2005) and brought to laboratory. *Anopheles* Larvae were identified with the help of morphological key given by (Das *et al.*, 1990).

Collection of malaria cases data

We have performed monthly visits to different hospital of studied districts for collection of month wise malaria cases data.

Collection of meteorological data

The effect of different meteorological parameters like rain, temperature and humidity on *Anopheles* mosquito population was observed month wise. The meteorological data was taken from metrological station Hyderabad monthly.

RESULTS

This study was conducted during two seasons (a) dry season pre monsoon, (January to June 2025) and (b) wet season monsoonal rain and post monsoonal season, (July to December 2025) to observe the prevalence of malaria vector mosquitoes *Anopheles culicifacies* and *Anopheles stephensi* in studied districts.

Entomological survey in (Larkana, Dadu, Jamshoro, Hyderabad, Tando Mohd Khan and Tando Allahyar) during dry season.

As malaria is a seasonal disease so the environmental factors are directly co related to the life of *Anopheles* mosquitoes. (Table 1) During dry season the maximum prevalence of *Anopheles* mosquitoes was from March to April. A total 121 adults of *Anopheles* mosquitoes were collected from different resting sites and while feeding on human bait and animal bait and 49 *Anopheles* larvae were collected from different breeding sites of studied localities. (Table 2) Maximum occurrence of *Anopheles* was found in district Hyderabad particularly its localities (Husri & Phulali areas) and minimum occurrence of *Anopheles* was found in two districts Jamshoro and Tando Allahyar, while they were not found from urban areas of any of the studied districts during dry season. Out of 121 adults mosquitoes 89 were female and 32 were males.

Table 1. Showing the month wise density of adult *Anopheles culicifacies* and *Anopheles stephensi* (females & males) during dry season (January – June 2025).

Month	<i>Anopheles culicifacies</i>		<i>Anopheles stephensi</i>		Total
	♀	♂	♀	♂	
January	08	0	0	0	08
February	0	0	0	0	00
March	13	05	08	02	28
April	22	08	14	06	50
May	15	06	09	05	35
June	0	0	0	0	00
Total	58	19	31	13	121

Table 2. Showing Prevalence of adult *Anopheles culicifacies* and *Anopheles stephensi* (females & males) in studied districts during dry season (January – June 2025).

Name of district	Name of locality surveyed	<i>An. culicifacies</i>		<i>An. stephensi</i>		Total female and male collected
		♀	♂	♀	♂	
Larkana	village Goth Ghana, Rasulabad and Gulab Bhutto)	07	0	11	05	23
Dadu	Pir Ghazi, Goth Dad Muhammad and Tando Rahim Khan	05	0	06	03	14
Jamshoro	Sindh university (Departments, hostiles & colony), Mahran University (Departments, hostiles & colony), and WAPDA colony	09	04	0	0	13
Hyderabad	Qasimabad, Husri and Phulali areas	15	07	06	02	30
Tando Mohd Khan	Ghaja mori, Sairi and Tando Saendad,	12	05	08	03	28
Tando Allahyar	Jhando mari, Khaisana mori and Buqara Sharif	10	03	0	0	13
Total		58	19	31	13	121

Table 03. Showing the population of adult *Anopheles culicifacies* and *Anopheles stephensi* females and males in studied areas during dry season (January-June 2025).

Name of Species	Female	Male	Total
<i>Anopheles culicifacies</i>	58	19	77
<i>Anopheles stephensi</i>	31	13	44
Total	89	32	121

Entomological survey in Larkana, Dadu, Jamshoro, Hyderabad, Tando Mohd Khan and Tando Allahyar) during wet season.

During monsoonal rains from July to September (Table 4 & 5) and during irrigation of rice cultivation season from July to October there was a high number of breeding places which left the positive effect on the population of both species, *Anopheles culicifacies* and *Anopheles stephensi* and we recorded a great number of *Anopheles culicifacies* and *Anopheles stephensi* from all studied areas. About 391

samples (152 larvae & 239 adults) were collected from different places of studied localities. Out of adults specimens 170 were females and 69 were males. It was observed that the mosquito population started to increase in late July and reached at its peak point in September and then started to decline in November. It was also observed that the high population of mosquitoes related to the availability of breeding habitats.

Table 4. Showing the month wise density of adult *Anopheles culicifacies* and *Anopheles stephensi* (females & males) during wet season (July –December 2025).

Month	<i>Anopheles culicifacies</i>		<i>Anopheles stephensi</i>		Total
	♀	♂	♀	♂	
July	15	06	10	3	34
August	19	09	14	5	47
September	36	16	18	6	76
October	28	13	9	3	53
November	15	06	6	2	29
December	0	0	0	0	0
Total	113	50	57	19	239

Table 05. Showing Prevalence of adult *Anopheles culicifacies* and *Anopheles stephensi* (females & males) in studied districts during wet season (July – December 2025).

Name of district	Name of locality surveyed	<i>An. culicifacies</i>		<i>An. Stephensi</i>		Total female and male collected
		♀	♂	♀	♂	
Larkana	village Goth Ghana, Rasulabad and Gulab Bhutto)	11	6	14	5	36
Dadu	Pir Ghazi, Goth Dad Muhammad and Tando Rahim Khan	10	4	12	5	31
Jamshoro	Sindh university (Departments, hostiles & colony), Mahran University (Departments, hostiles & colony),	19	8	5	0	32

	and WAPDA colony					
Hyderabad	Qasimabad, Husri and Phulali areas	32	14	9	3	58
Tando Mohd Khan	Ghaja mori, Sairi and Tando Saendad,	23	11	9	4	47
Tando Allahyar	Jhando mari, Khaisana mori and Buqara Sharif	18	7	8	2	35
Total		113	50	57	19	239

Table 06: Showing the population of adult *Anopheles culicifacies* and *Anopheles stephensi* females and males in studied areas during wet season (July-December 2025).

Name of Species	Female	Male	Total
<i>Anopheles culicifacies</i>	113	50	163
<i>Anopheles stephensi</i>	57	19	76
Total	170	69	239

During both (dry and wet) seasons majority of the larvae (63%) were collected from agriculture field like irrigation canals, rice fields and mango gardens, remaining 37% of larvae were collected from non-agricultural breeding places such as open stagnant water pools, puddles of standing water outside the houses, underground and over roof water tanks, water buckets, drainage containers of air conditioners and ornamental ponds, among these breeding places about 20% of larvae were collected from open stagnant water pools and puddles of standing water outside the houses which seems the most favorite breeding places of larvae, followed by underground and over roof water tanks and water buckets that is about 10% and minimum no. of larvae 7 % were collected from drainage containers of air conditioners and ornamental ponds. The adult mosquitoes were collected from indoor resting places as well as from outdoor resting places, but the majority of mosquitoes 60% were collected from outdoor

resting sites. It was observed that the both species *Anopheles culicifacies* and *Anopheles stephensi* were found in all the studied localities during the studied period. It was also observed that *Anopheles culicifacies* was the dominant vector species followed by *Anopheles stephensi* in all studied districts of Sindh.

Malaria Cases Data

Malaria cases were high in the month of September and October (Table 07), total 17127 malaria cases were reported during the year 2025 in studied districts out of which 11803 were *Plasmodium vivax*, 3969 were *Plasmodium falciparum* and 1355 were mix infection (in which *Plasmodium vivax* and *Plasmodium falciparum* found together in the blood of the same patient). Malaria cases data were collected monthly from different hospitals of studied localities.

Showing the districts and Month wise malaria cases from the Month of January-December 2025

Districts	Months	Malaria Cases				Total
		Slides	P.v	P.F	Mix	
Larkana, Dadu, Jamshoro, Hyderabad, Tando Mohd Khan, Tando Allahyar	January	21847	226	248	43	517

Do	February	21787	229	108	31	368
Do	March	23331	291	101	26	418
Do	April	21830	365	86	38	489
Do	May	21891	553	80	14	647
Do	June	23486	804	67	22	893
Do	July	24665	954	53	146	1153
Do	August	23451	940	78	86	1104
Do	September	49375	2363	1006	308	3677
Do	October	52566	2361	992	306	3659
Do	November	51446	1762	1040	190	2992
Do	December	25572	955	110	145	1210
Total		361247	11803	3969	1355	17127

As malaria is a seasonal disease so the environmental factors (rainfall, humidity & temperature), directly affect the life of *Anopheles* mosquitoes, but the effect of humidity on the mosquito population is less than the other two factors (temperature & rainfall), (Jamieson *et al* 2006).

During these two studied periods (dry & wet) the majority of *Anopheles* was collected from Hyderabad region the main factor might be the moderate temperature (20°C - 30°C), which left favorable effect on the survival of *Anopheles* mosquitoes.

During this study it was observed that the population of *Anopheles* mosquitoes had two peaks at first they started to increase from the mid of March to mid of May and its second activity started from the end of July and reached to its peak point in September and then gradually declined from November probably due to low temperature.

Malaria is prevalent in both rural and urban areas of Sindh and it had become the burning issue after the super flood of (2022), which had left poor shelters, poverty, and mass number of stagnant water, poor sanitation and environmental pollution, along with poor health facilities. About 17127 malaria cases were reported during the year (2025) in studied

districts, which had the main flood affected areas. According to (Himeidan *et al* 2003), there are many climatic factors which have important effect on *Anopheles* mosquitoes and the transmission of malarial parasites but the rainfall has significant effect to initiate the epidemic of malaria. Now a day the burden of malaria is rising annually and its economic worth is also increasing and causing an enormous losses. Recent data of malaria cases reported from Sindh province indicated that *Plasmodium vivax* has a higher prevalence in human, about 11803 cases were reported during the year 2025 in studied districts followed by *Plasmodium falciparum* 3969 cases and least number of cases 1355 was mixed infections.

Conclusion and Recommendations

This study has been mainly focused on diversity and ecology of malaria vector mosquitoes *i.e.* *Anopheles culicifacies* and *Anopheles stephensi* in main malaria affected areas of Sindh. This study reported that these two vectors of malaria are widely spread throughout the Sindh. My study have also have shown that these two vectors *Anopheles culicifacies* and *Anopheles stephensi* were present in all studied districts.

Climatic conditions played an important role in the occurrence of mosquitoes. The health education and community awareness is very important to reduce malaria incidence especially during wet season, also to introduce small local fishes in irrigation canals like *Tilapia mosambicus* or *Cyprinus carpio* and *Poecelia reticulata* as these fishes have been found to be very effective against larvae of mosquitoes in Karachi.

Author's Contributions

N. Jehajo, designed and supervised the experiment, analyzed the data, wrote the initial and final draft. Author approved the final draft.

Conflict of Interest

The author declares no conflict of interest.

Generative AI Statement

The author declare no generative AI was used in the creation of this manuscript.

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