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Seasonal Prevalence and Impact of Environmental Variables in Emergence of *Aedes* Mosquito in Multan, Pakistan

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Authors' Contribution
AF conceived and designed the study. FA performed research, provided reagents and materials; and drafted the manuscript. Both authors revised and approved the manuscript.

Key words
***Aedes*, Temperature, Rain fall, Relative humidity**

ABSTRACT

Mosquito borne infection is considered a most important public health problem all over the world including the dengue virus. The dominant occurrence of *Aedes* mosquitoes under the influence of environmental factors (temperature, relative humidity, and rainfall) of Multan city was assessed during 2014-2016. In this study, *Aedes* larvae were collected by using the entomological dipping method and identified in the laboratory. The results showed mean (±SD) temperature, rainfall, and relative humidity during 2014-2016 ranged 12.88-35.43°C, -0.33-87.43mm, and 36.17-72.17%, respectively. Likewise, maximum *Aedes* larvae were found during the month of November, with 348.67 *Aedes* larvae from indoor while 71.67 from outdoor. The emergence of *Aedes* larvae was found after rainy season while decline trend was observed at non-rainy, extreme temperatures and relative humidity conditions. This study could be used in further studies in order to evaluate *Aedes* mosquito emergence in relation with different environmental variables, and to establish integrated vector control strategies in the future.

INTRODUCTION

Dengue fever is a well-known viral infection, influenced by many environmental factors (Mahmud *et al*., 2019) that infect human and threatened 975 million peoples around the world specifically in tropically and sub-tropically areas of South-Eastern Asia, America and the Pacific region (Haider and Iqbal, 2016). Although, dengue virus belonged to family flaviridae which has 4 strains or serotypes that are serologically similar but different by genetically (Zanotto *et al*., 1996). Additionally, *Aedes aegypti* and *Aedes albopictus* are the mosquito vectors that are responsible for the transmission of the dengue virus in human causing symptoms such as high grade fever, painful muscles and joints, vomiting, nausea, skin rashes, severe headache, and pain behind the eyes (Manzoor *et al*., 2015; Habib *et al*., 2016). *Aedes* mosquito is primarily responsible for the spread of dengue in all regions of the world (Manzoor *et al*., 2015) while later is usually thought to be a weak with minor epidemics (Kim-Lien *et al*., 2015).

The *Aedes* population has a major effect on the human populations as its life cycle is closely related with the human activities (Madzlan *et al*., 2016) and can adopt different habitats such as *aegypti* occupied urban residences however *albopictus* invade rural, sub-urban and urban environmental conditions (Kim-Lien *et al*., 2015). *Aedes aegypti* established itself in the urban areas and lesser extent to semi-urban area but is not established in the rural area. On the other side, *Aedes albopictus* population had established itself in rural areas rather than urban areas. Moreover, for breeding, *Aedes aegypti* preferred indoor and *Aedes albopictus* preferred both indoor and outdoor (Herath *et al*., 2022).

Aedes is not only adopt the local domestic environments but can also adopt demographic growth and migrate through human (Carneiro *et al*., 2017). In tropical and sub-tropical lands of Asia, this viral infection is mostly spread by *Aedes aegypti* and *Aedes albopictus* and existed in the surroundings of human. On the other hand, *Aedes albopictus* is originate in sub-tropical zones than *Aedes aegypti* due to its tolerance to low temperature. Additionally, extreme invasion of dengue infestation is related with the adult vectors under the ideal setting of humidity and temperature in rainy seasons (Carneiro *et al*., 2017).

Moreover, *Aedes* mosquito required clean watery environment during initial three developmental stages i.e., egg, larvae and pupae both in natural and artificial conditions (Madzlan *et al*., 2016), depends upon water temperature and its environmental conditions (Gubler, 1997). Furthermore,

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mosquitos completed their three developmental stages (egg to adult mosquito) in 7-10 days. The female *Aedes* mosquito lays its eggs in potential water containers (CDC, 2019) where eggs are fastened with the walls of container and could survive up to 8 months. Eggs hatched within a few days to months-long duration when enclosed with water till emergence of larvae in water container and later converted into pupae in next 5 days. In next 2–3 days pupae develop into mature mosquito (CDC, 2019).

After the onset of the monsoon, dengue virus infestations occurred as a burning health issue due to higher arrival of *Aedes* mosquito in Pakistan (Manzoor *et al*., 2015). Due to greater suitable vector environment and habitats, dengue virus has occupied in many regions in Pakistan in last decade. This disease victimized mainly the adult population of human in Pakistan as well as all over the world (Naqvi *et al*., 2015). Present study was planned to evaluate the impact of different environmental variables such as rainfall, relative humidity, and temperature in the emergence of *Aedes* mosquito responsible for the infestation of dengue fever in district Multan, Punjab; Pakistan. Moreover, this study will be also helpful in future to control the dengue virus epidemics by earlier prediction by evaluating the impact of environmental variables that favored vector emergence in any region of the world.

Aim of the study was to evaluate the seasonal prevalence of *Aedes* mosquito and impact of environmental variables upon *Aedes* mosquito emergence in Multan, Pakistan during the year 2014-2016.

MATERIALS AND METHODS

Growth conditions

Previously it is demonstrated that the *Aedes* can adapt different habitats with human migration (Kim-Lien *et al*., 2015; Carneiro *et al*., 2017) as adapted different habitats of Multan. Multan is located at southern area of Punjab; Pakistan. Its climate is arid that usually experienced hot summer with higher temperature and cold winters with lowest temperature.

To determine the effect of arid climate of Multan in the emergence of *Aedes,* the vector larvae were collected from the natural environment of inside (Indoor) and outside (Outdoor) of the houses. During *Aedes* larvae sampling, temperature was recorded in Celsius degrees by using ordinary thermometer while relative humidity was measured in percentage by using wet and dry bulb thermometers and rainfall was measured in millimeters by using ordinary rain gauge.

Sample collection

Larvae were collected at different stages of its lifecycle from different natural water containers and stagnant water ponds via dipping method (Robert *et al*., 2002) by utilizing collection tools i.e., sampling scoop, transparent glass jar, magnifying glass, torch and strainer.

The wild urban populations of *Aedes* larvae were sampled randomly during the day times from two premises. In 1st premises (indoor sites), aqueous habitats of inside of houses which were considered for larvae sampling i.e., water containers used by human in washing and drinking, animal's drinking pots, room water coolers, main whole covers, used/spare tires, air condition water collection containers, leaking water taps, flower jars, house stagnant water, household rubbishes, roof top junks, refrigerator trays, garbage, and construction rubbish. Whereas, in 2nd premises (outdoor sites) aqueous habitats of outside of houses which were considered i.e., park shrubberies, tyres, street and road side trash, flower pots, leaked water taps, air coolers, open manholes/drains, water vessels used for puncture in the shops, sanitation wastes in buildings, park and tree holes, construction wastes, bird pots, air conditioner outlets, water tanks, fountains and stagnant water at roadside.

The *Aedes* larvae were collected during the year 2014-2016 with different month's interval at different temperature, average rainfall, and relative humidity (Table I). The collected *Aedes* larvae were taken to the laboratory and were identified by using standard taxonomic keys as prescribed previously (Darsie and Pradhan, 1990; Tyagi *et al*., 2015) with little modifications.

Table I. Mean values of metrological parameters and total *Aedes* larvae found in Multan Pakistan during the year 2014-2016.

Months	Climate			*Aedes* larvae (Vector)		
	Average temper-ature (ºC)	Average rain fall (mm)	Average relative humidity (%)	Indoor	Out-door	Total
January	12.88	1.63	72.17	8.33	0.00	8.33
February	16.55	10.60	64.67	7.33	1.33	8.67
March	21.10	47.17	63.17	41.67	10.67	52.33
April	27.98	14.17	44.00	83.00	12.67	95.67
May	33.35	27.00	36.17	59.67	9.33	69.00
June	35.43	16.83	42.83	12.33	2.00	14.33
July	33.62	87.43	60.50	52.00	6.67	58.67
August	32.62	60.80	62.67	134.67	32.00	166.67
September	31.47	5.27	59.67	188.00	37.00	225.00
October	27.68	11.23	56.00	257.67	53.00	310.67
November	20.62	0.07	61.00	348.67	71.67	420.33
December	15.32	-0.33	69.83	125.33	14.33	139.67

Statistical analysis

To analyze the emergence of *Aedes* larvae, variance analysis was performed. Temperature, average rainfall, and relative humidity were recorded daily in three repeats and average value was calculated for a month although error bars represent the average ± SD of recorded values.

Correlation among total *Aedes* larvae and numerous metrological parameters were performed by using regression analysis in MS Excel. Asterisks over the values indicate a significance level among mean values of metrological parameters and total *Aedes* larvae was evaluated via regression analysis (*P < 0.05, **P < 0.01, ***P < 0.001).

RESULTS

Multan climate is hot and arid that favored *Aedes* mosquito breeding and its emergence in September, October and November. Consequently, the rainfall, relative humidity, and temperature were recorded in 2014-2016 (Table I). The maximum temperatures 35.43°C were recorded in June, while minimum values were recorded as 12.88°C in January during 2014 to 2016. Recorded data showed maximum rainfall 87.43 mm in July while maximum relative humidity was recorded (72.77%) in January during three consecutive years (2014-2016). Moreover, the mean prevalence of *Aedes* larvae were found highest in the month of November (348.67 from indoor and 71.67 from outdoor) while the lowest in January (8.33 from indoor and 0.00 from outdoor), showing that *Aedes* had the preference for indoor breeding, as described in Table I. Collectively, the maximum *Aedes* larvae (603 and 766) were collected in November, 2015 and October, 2016 when temperature, rainfall and relative humidity were found favorable for its breeding and development. Prevalence of *Aedes* (Dengue Vector) larvae in District Multan during years 2014-2016 is represented in Table II, indicating no significant difference between the prevalence of indoor and outdoor *Aedes* larvae. Table III shows the prevalence of *Aedes* (Dengue vector) larvae in District Multan during years 2014-2016.

Table II. Prevalence of *Aedes* (Dengue Vector) larvae in District Multan during years 2014-2016.

Year	*Aedes* (Indoor)	*Aedes* (Outdoor)
2014	0.0000 A	0.2500 A
2015	0.5833 A	0.1667 A
2016	0.0833 A	0.2500 A

Means bearing different letters are significantly different from each other.

Table III. Prevalence of *Aedes* (Dengue Vector) larvae in District Multan during years 2014-2016.

Month	*Aedes* (Indoor)	*Aedes* (Outdoor)
January	0.0000 A	0.0000 A
February	0.0000 A	0.0000 A
March	0.0000 A	0.0000 A
April	1.6667 A	0.0000 A
May	0.3333 A	0.0000 A
June	0.0000 A	0.0000 A
July	0.6667 A	0.0000 A
August	0.0000 A	0.3333 A
September	0.0000 A	1.3333 A
October	0.0000 A	0.3333 A
November	0.0000 A	0.6667 A
December	0.0000 A	0.0000 A

Means bearing different letters are significantly different from each other.

Regression analysis (r value) was performed to determine the co-efficiency among different metrological parameters and *Aedes* larvae in Multan (Table IV). Findings of regression analysis provided that total larvae showed significant (P<0.001) correlation with indoor and outdoor larvae, but insignificant (P>0.05) correlation with relative humidity, temperature and rain fall. However, temperature significantly affected the relative humidity (P<0.001) and rainfall (P<0.05).

Table IV. Relationship among different metrological parameters and *Aedes* larvae found in Multan Pakistan during 2014-2016.

Parameters	Indoor larvae	Outdoor larvae	Total larvae	Relative humidity	Temperature	Rain fall
Indoor larvae	1					
Outdoor larvae	0.916***	1				
Total larvae	0.998***	0.940***	1			
Relative humidity	0.014^{NS}	0.017^{NS}	0.015^{NS}	1		
Temperature	0.063^{NS}	0.074^{NS}	0.065^{NS}	0.690***	1	
Rain fall	0.200^{NS}	0.149^{NS}	0.194^{NS}	0.064^{NS}	0.369*	1

***P<0.001, *P<0.05, ^{NS}P>0.05.

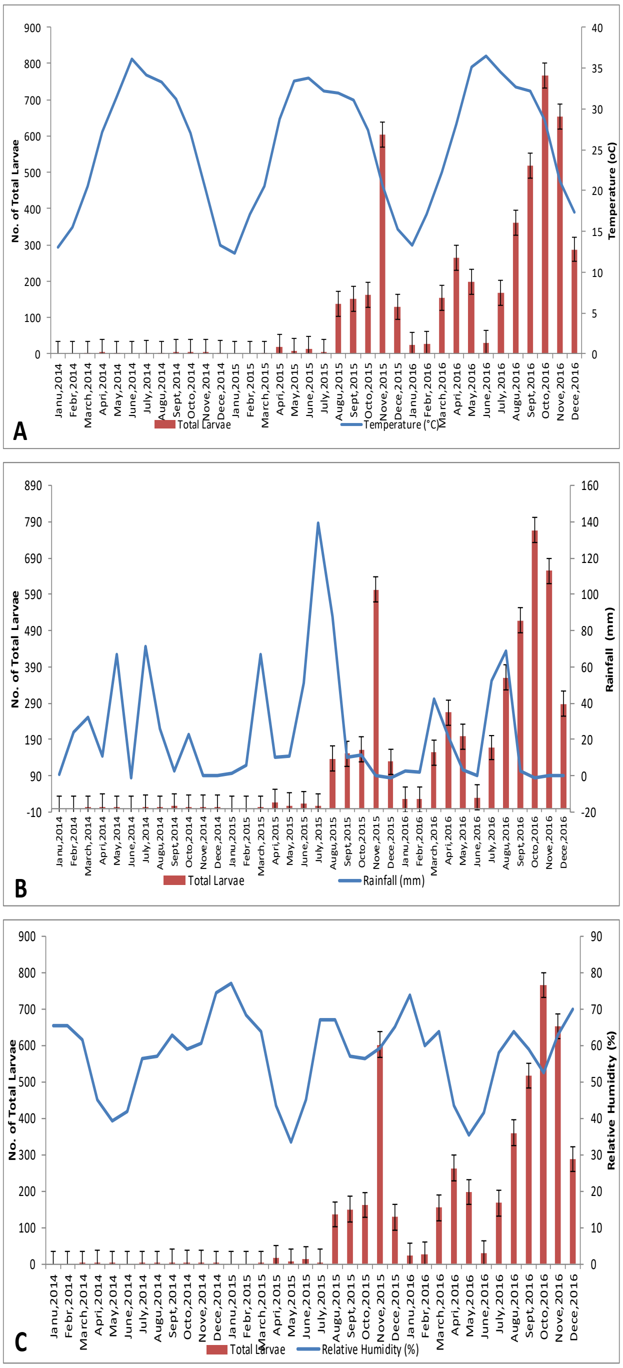

Fig. 1. Effect of temperature (A), rainfall (B) and relative humidity (C) on the total number of *Aedes* larvae found during the year 2014-2016 from District Multan.

Additionally, the dependent of temperature on the emergence of total number of *Aedes* larvae during the studied years (2014-2016) is shown in Figure 1A. Extremely high and low temperature suppressed the breeding and growth of the *Aedes* larvae. At extremely low temperature (0-18ºC) from January to February and high temperature (32-36ºC) in May to July, no or less *Aedes* larvae were observed. However, during September to November when temperature ranged from 20-30ºC, maximum larvae were found.

Furthermore, the role of rainfall in the emergence of *Aedes* larvae was also examined by measuring average rainfall and collecting total number of larvae (outdoor and indoor) after different month's interval in 2014 and 2016. Rainfall established water holdings and provide environment for female *Aedes* mosquito to lay its ova and then developed into larvae. Higher rainfall in July, 2015 and August 2016 gave ultimately larvae emergence during August to November with maximum larvae population in October, 2015 and November, 2016 (Fig. 1B). Hence, the results showed positive relationship between rainfall and larvae incidences.

Relationship between number of *Aedes* larvae and relative humidity was also plotted as Figure 1C, which showed peak of *Aedes* larvae incidences at 50 to 65%. Relative humidity in the atmosphere due to higher rainfall in July, 2015 and August 2016 gave favorable environment for maximum emergence of larvae during August to November 2015 and October 2016. Collectively, these results showed the positive roles of moderate temperature, rainfall and relative humidity in the emergence of larvae suggested and confirmed the important roles of these environmental factors in the hatching and breeding of *Aedes* larvae which in turn caused dengue epidemic in Multan in late 2015.

DISCUSSION

Dengue is usually considered to be an urban disease and its infestations are increasing because it is caused by the species of the *Aedes* mosquito (*Aedes aegypti* and *Aedes albopictus*). The incidence of dengue vectors to new geographical areas, its climate, concentration of population, household water and trash storage to recycle the waste materials can serve as risk factors for dengue virus infections (Madzlan *et al*., 2016).

In this study, *Aedes* larvae were found higher from inside of the houses than outside of the houses of Multan during 2014-2016. These findings are in general agreement with that of reported by Herath *et al*. (2022), who have documented that *Aedes* was found moderately in the urban area and to a lesser extent in semiurban but not in the rural area and *Aedes* mostly preferred indoor breeding.

The higher number of *Aedes* larvae were collected in November, 2015 and October, 2016. The same findings were reflected by Sarwar and Rasool (2022) that revealed the highest breeding of *Aedes albopictus* from Faisalabad district during 2019-2020 in the months of September-November and previously by Asrar *et al*. (2017) whom found *Aedes* prevalence in Multan city, during February-March and later in July, September and maximum in November.

Although temperature has great impact on mosquitoes growth because mosquitoes are poikilothermic organisms on which external environment directly influence their body temperature (Hawley, 1988) but the effect of meteorological variables on vector populations could be different amongst geographical locations with diverse climate conditions (Tuladhar *et al*., 2019). In the current study, decline in *Aedes* population during extreme temperatures of cold weather (January to March and December) and hot weather (May to July) was observed in Multan. The peak population of the *Aedes* larvae during 2014-2016 was collected from Multan when temperature was calculated between 20-32 °C during September to November, and was in agreement with the results reported previously by Delatte *et al*. (2009) who found optimum immature stages development at 29 °C and Mohammed and Chadee (2011) whom proposed significant mosquito's eggs hatching success at 24-35 °C.

As it is reported previously that in the South-Eastern Asia, female vector preferred to lay its eggs in domestic containers and precipitations plays vital role in its distribution and abundance (Aziz *et al*., 2012) indicated that water is one of the important factors involved in the growth and breeding of larvae. In this study, the highest rainfall recorded in July, 2015 (138.9 mm) then in August, 2016 (69 mm), increased the emergence of *Aedes* larvae population from August to November during 2015 and 2016 suggested that the rainfall has positive correlation with the breeding of larvae. These results are in accordance with the earlier findings that higher rainfall plays vital role to enhance the *Aedes* population (Vanzie, 2008; Aziz *et al*., 2012). Additionally, the maximum population of *Aedes* larvae at 50-65% relative humidity in September to November during 2015 and 2016 demonstrated that relative humidity in the atmosphere due to higher rainfall had positive association with the emergence of larvae. The same findings were reported by Costa *et al*. (2010) that the gradual reductions in egg fertility at 60% relative humidity with increase in temperature, although such effect was not found at 80% relative humidity at 25 ºC and 30 ºC witch also confirmed and authenticated this study findings.

CONCLUSION

The environmental variables played major role in seasonal emergence of *Aedes* mosquito. Favorable environmental variables (watery environment or rain fall, temperature between 20-33 °C and suitable relative humidity between 50-65%) enhanced the growth of the *Aedes* population in Multan during this study especially in November 2015. Designed study and its findings will help in forecasting dengue epidemic especially in dengue endemic regions and will be helpful to control the spread of *Aedes* populations and dengue fever infestations under the impact of environmental variables by earlier adopting integrated vector management strategies.

DECLARATIONS

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Statement of conflict of interest

All authors declared that there is no conflict of interest.

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