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POTENTIAL IMPACT OF GLOBAL WARMING ON POPULATION DYNAMICS OF DENGUE MOSQUITO, *Aedes albopictus* SKUSE (DIPTERA; CULICIDAE)

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Aedes mosquitoes, a vector of many fatal diseases including dengue fever and cause millions of deaths annually. Dengue fever has been reported from Pakistan during 1994 at a low pace. However, its first epidemic was recorded from Punjab during 2011 which was associated with 2010 heavy rainfall, floods and high *Aedes albopictus/aegypti* population. Thus, this study was carried out to examine the impact of changing climatic conditions on the population dynamics of *Aedes* mosquitoes. Collection of mosquitoes (larvae, pupae, and adults) was done from natural and artificial storage containers twice during different seasons, i.e., winter (October-February), summer (March-June) and monsoon season (July-September) from marked rural areas (fields, villages, towns) and urban areas using simple random technique. Years, months, seasons, relative humidity, and temperature were found to be significant in case of population dynamics of *Ae. albopictus*. The overall values of container index (CI), premises index (PI) and breteau index (BI) (19.9%, 8.1% and 8.9% respectively) were higher for 2011 than all other years. High population (61.7%) was recorded during rainy season due to high temperature (28-36°C) and high relative humidity (up to 75%) and very low populations (7.2%) were recorded from outside during winter due to low temperature (<10°C) and low relative humidity (<23%). *Aedes albopictus* was mostly collected from rural areas and in some city parks during rainy season. So, we can manage its population by decreasing the number of water bodies in our area especially during rainy season.
Keywords: Dengue fever, population dynamics, dengue mosquitoes, global warming, epidemiology.

INTRODUCTION

Mosquitoes are notorious due to their role as vectors of different diseases such as malaria, yellow fever, Zika virus and dengue (Yang *et al*., 2012; Mehmet *et al*., 2017). Among three medically important genera of mosquitoes, *Aedes* mosquitoes (vectors of dengue fever) are known as day time biters and widely distributed in tropical and subtropical regions of the world (Gratz, 2004). *Aedes albopictus* Skuse and *Ae. aegypti* (L.) are main vectors for dengue virus in humans (Knudsen, 1995). Dengue was a sporadic disease during the 19th century that caused epidemics after long time intervals. However, modern means of transportation increased this disease many folds during the previous half century and now it has become a major health concern in more than 100 countries of the world. Twenty two thousand deaths are reported annually mainly in children and young adults (Riaz, 2011). In Pakistan, although dengue fever (DF) was first recorded in Karachi in 1994 (Chan *et al*., 1995), by 2011, it appeared in its epidemic form in the province of Punjab with 100 confirmed cases daily (Anonymous, 2011).

Mosquitoes may adapt to any place near standing water bodies. The chemical properties of water (acidity or alkalinity; fresh, salty or brackish), the type or amount of vegetation present, and the distinctive egg laying habit of each species determines its offspring habitat. Thus, mosquitoes can be grouped by their preference for certain water body i.e. permanent, flood, and transient water body (Vanwambeke *et al*., 2007). Climatic dynamics such as global warming with unplanned urbanization and rapidly growing population has resulted in substandard housing and inadequate water and sewage problems. Due to global warming, temperature is increasing day by day and causing more rains to fall in one year and none in other years (Nasir *et al*., 2017). Due to heavy rains, floods occur irregularly. Moreover, inadequate and poor waste management systems have increased the mosquito population many times (Ahmad *et al*., 2017). Due to limited financial and human resources, most people have poor and inadequate public health facilities (Reiter, 2001).

Since dengue is a serious viral infection, currently neither a vaccine nor a treatment is available for public use. The only practical solution for this is in the hands of a vector biologist, who can better understand the vector and its dynamics, and provide a better management solution (Reisen *et al*., 2006). Along with the knowledge of prevailing climatic conditions, a person can help in the proper and timely implementation of environment friendly mosquito management approach (Gratz, 2004; Sharma *et al*., 2005). The capacity, location, and temperature of the water source can vary seasonally and have been reported as important ecological factors affecting the population dynamics of *Aedes* mosquitoes. *Ae. albopictus* is mostly found in fields or forests under the shade of trees and *Ae. aegypti* is found in or around human habitats. Thus, seasonal climatic fluctuations affect *Ae. albopictus* more than *Ae. Aegypti* (Focks and Barrera, 2006; Abbas *et al*., 2014). The amount of rainfall, temperature, and relative humidity are very important climatic factors that seriously affect the population of *Aedes* (Scott et al., 2000). Due to these factors, the relationship between global warming and the population dynamics of *Ae. albopictus* was investigated.

MATERIALS AND METHODS

The key ecological factors and breeding habitats (water bodies) determine the risk areas of *Ae. albopictus*. This research was planned to study the seasonal distribution of *Ae. albopictus* for a period of seven years, from 2010 to 2017 in various districts (Faisalabad, Lahore, Gujranwala, Sargodha, Rawalpindi, Islamabad, Murree, Multan, D.G. Khan, Bahawalpur and Rahim Yar Khan) of the province Punjab, Pakistan by collecting its immature stages (larvae and pupae with dippers) and adults with aspirator (Fig. 1). Collection was done from each district (five rural and five urban localities) during every season.

***Habitat characteristics*:** As this species breeds in all aquatic media preferably in clean water, so all types of aquatic habitats including the waste water near houses, water channels, seepage areas, animal ponds, livestock and poultry farms (Nasir *et al*., 2017), cemented tanks, bamboo sticks (Bashar *et al*., 2005), tires, tree holes (Akram and Lee, 2004), broken bottles, pots, etc. were sampled as a whole (depending on the amount of water) or with the standard plastic dipper (depending upon the size of water body) for the collection the immature stages (larvae and pupae) of *Ae. albopictus*. Collection was done twice during different seasons, i.e., winter (October-February), summer (March-June) and monsoon season (July-September) from marked rural areas (fields, villages, towns) and urban areas of Punjab, Pakistan using simple random technique. For the evaluation of larval indices (premises index, container index and breteau index), hundred houses were also covered during every visit. The adults were collected with aspirators.

***Seasonal Abundance*:** Seasonal abundance of *Ae. albopictus* was studied by collecting it from different seasons; i.e. summer (March to June), rainy or monsoon (July to September), and winter (October to February) (Mohiuddin, 2007). Global positioning system (GPS) was used to mark the location of collection sites. The coordinates (longitude and latitude) were recorded, for every sampling site during the study.

Figure 1. Location of selected districts of the Punjab for the collection of *Aedes* mosquitoes.

***Biotic and abiotic factors*:** Abiotic factors including physical condition of water (clear, turbid, and foul), pH, temperature, relative humidity (RH), location (urban or rural), shady or exposed area, and distance from residential areas along with the type of vegetation and fauna (biotic factor) were recorded from each site (Mohiuddin, 2007).

***Handling of samples*:** Immature stages were kept in 300 ml plastic cups along with water from the source to keep them alive. The cups with mosquito larvae were tied with muslin cloth for aeration and transferred to Vector Biology Laboratory, Government College University, Faisalabad, Pakistan for rearing and identification. The collected samples of mosquitoes were identified under the microscope with the help of available taxonomic keys (Darsi and Pradhan, 1990). The population of mosquitoes (adults and immature) were counted to record the abundance and seasonal prevalence from all collection sites.

***Equipment*:** The temperature, relative humidity and pH were measured with a multi-meter (Elmetro Poland, Model # CPC 401). Coordinates were recorded with Magellan GPS (Explorist, 660) and the specimens were identified using the microscope (Nikon Binocular Microscope Eclipse, Model # E-100).

***Analysis of data*:** Larval indices (premises index, container index and breteau index) were calculated for the year 2010-17 from the data of collected larvae. The data about biotic and abiotic factors were subjected to software Statistica, for chi-square test and logistic regression model to determine the association between different variables and their influence on the population density of *Ae. albopictus*. P-value was calculated to identify which factor was critical and dominant that influenced the population of *Ae. albopictus.* A stepwise forward approach for logestic regression was applied (Vanwambeke *et al*., 2007).

RESULTS

Ae. albopictus was collected during the seasons of 2010-17 from different districts of the province Punjab, Pakistan i.e. generally from central and upper Punjab (Faisalabad, Lahore, Sargodha, Rawalpindi, and Murree) but few from Southern Punjab (Multan, Bahawalpore, and Rahim Yar Khan). The largest population of *Ae. albopictus* was recorded from Rawalpindi (forest area) during rainy season, while the smallest was recorded from Faisalabad district during the winter season. This species was mostly sampled from latitude (max. 33° 45.405, min. 29° 57.859) and longitude (max. 074° 45.405, min. 072° 57.859) with elevation (max. 1,220 m, min. 145 m).

Among different years, 2011 showed higher values of CI, PI and BI (19.9%, 8.1% and 8.9%) than all other years (Table 1). The highest number of water bodies and positive water bodies were also observed during 2011. Populations of *Ae. albopictus* showed great variation in their density during all seasons (P-value <0.001), i.e., maximum (61.7%) was found in rainy season followed by summer (31.1%) and minimum (7.2%) in winter. Water quality (P-value < 0.001), color of water (P=0.04) and Light (P<0.002) also had a significant impact on population density. Most of the populations were recorded from clear water (46.4%) followed by turbid (27.8%), turbid foul (21.0%), and (4.8%) from clear foul water. It was found that the maximum population was found in standing water (97.1%) followed in exposed shady medium (62.8%) and minimum in flowing water (2.9%) as shown in Table 2.

Ae. albopictus was collected from both natural and artificial containers. Maximum population was collected from small water containers (64.3%) followed by tires (33.3%), cans (1.2%), and tree holes (1%). None was collected from plant pots as shown in Fig. 2. The locality also showed a significant effect on population level (P=0.019). Most of the population was recorded from rural areas (69.2%) outside the residential areas than urban (30.8%). The flora (algae, grass, and spirogyra) and fauna (copepods, dragonflies, damselflies, frog tadpoles, and water bugs) were also recorded among the mosquito population.

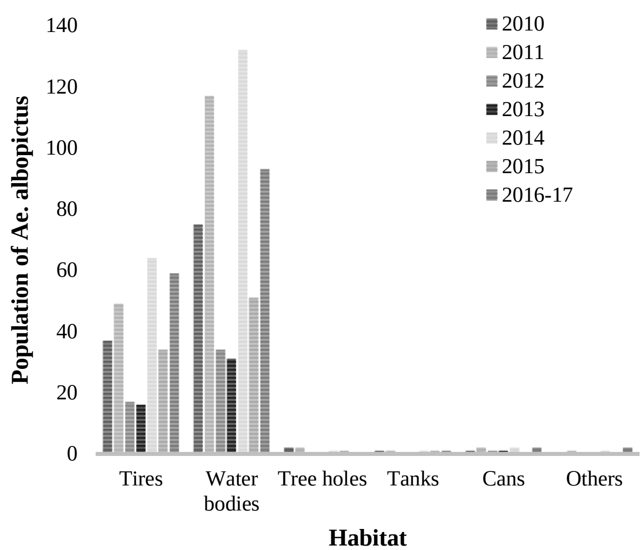

Figure 2. The relationship between habitat and population of *Aedes albopictus*.

The role of key factors affecting the presence of *Ae. albopictus* in different (urban and rural) localities of the Punjab was assessed using logistic regression analysis. Significant factors such as seasons, light, color of water, location, and distance from the residential areas were studied in this model. The seasons played a significant role in future

Table 1. *Aedes albopictus* larval indices in the Punjab, Pakistan during 2010-20117.

Years	Total no. of water bodies	No. of +ve water bodies	Container index (CI)	Premises index (PI)	Breteau index (BI)
2010	76	47	18.6 (14/73)	6.2 (4/64)	5.9 (4/69)
2011	83	58	19.9 (17/82)	8.1 (7/86)	8.9 (8/89)
2012	41	29	08.7 (05/53)	4.2 (2/47)	4.1 (2/49)
2013	37	23	07.4 (03/42)	2.7 (1/39)	3.0 (1/42)
2014	79	59	18.8 (15/79)	5.5 (3/48)	5.6 (3/53)
2015	44	33	16.4 (11/67)	3.5 (2/57)	3.7 (3/62)
2016-17	69	35	16.9 (13/77)	4.6 (3/65)	4.4 (3/67)

Table 2. Key parameters for the presence of *Aedes albopictus.*

	Percent (%) population during different years							% age	p-value
	2010	2011	2012	2013	2014	2015	2016-17		
Seasons									<0.001
Winter	7	15	3	5	14	5	11	7.2	
Summer	34	52	18	16	56	25	57	31.1	
Rainy	74	102	31	27	131	57	89	61.7	
Habitat									<0.001
Tyres	37	49	17	16	64	34	59	33.3	
Water bodies	75	117	34	31	132	51	93	64.3	
Others (Tree holes, cans, tanks and any other)	3	3	1	1	5	2	5	2.4	
Water quality									<0.001
Clear	52	82	25	22	93	38	73	46.4	
Turbid	37	47	14	13	57	29	43	27.8	
Turbid foul	21	41	11	10	42	16	33	21.0	
Clear foul	5	9	2	3	9	4	8	4.8	
Watercondition									0.100
Standing	112	164	51	47	195	85	151	97.1	
Flowing	3	5	1	1	6	2	6	2.9	
Light									<0.002
Exposed	38	48	16	15	61	27	48	30.5	
Exposed shady	69	111	33	30	126	53	99	62.8	
Shady	8	10	3	3	14	7	10	6.2	
pH									0.370
≤ 7.00	0	0	0	0	0	0	0	0	
7.01-8.00	27	50	13	12	51	16	48	26.2	
8.01-9.00	71	101	32	29	121	53	93	60.3	
>9.01	17	18	7	7	29	18	16	13.5	
Area size (m)									<0.001
≤ 1.00	79	107	34	32	134	60	104	66.3	
1.01-10.00	13	27	7	6	27	9	18	12.9	
10.01-100.00	10	23	6	6	23	11	19	11.9	
> 100	13	12	5	4	17	7	16	8.9	
Location									0.019
Urban	36	51	16	14	60	25	53	30.8	
Rural	79	118	36	34	141	62	104	69.2	
Temperature(°C)									<0.001
≤ 10	2	5	1	1	5	2	3	2.3	
11-20	9	19	5	4	16	3	17	8.8	
21-30	19	31	9	8	36	16	30	18.0	
31-40	85	114	37	35	144	67	107	71.6	
>41	0	0	0	0	0	0	0	0	
Relative humidity (%)									<0.001
≤ 30	4	5	2	2	8	5	6	3.9	
31-40	27	51	14	13	57	25	47	28.2	
41-50	47	60	19	18	74	30	65	37.8	
>51	37	53	17	15	62	27	39	30.1	
Distance from houses (m)									0.160
≤ 25	61	85	27	25	106	49	75	51.6	
26-100	32	55	16	14	61	24	53	30.7	
>100	22	29	9	9	34	14	29	17.7	

population dynamics such as rainy (OR: 0.342, P=0.003) were more predictors than summer (OR: 0.058, P = 0.005) (Table 3). There were more breeding sites due to more rain fall and floods in 2010, 2011 and 2014. These mosquitoes were mostly collected from forest (Faisalabad, Lahore and Murree) and rural areas while smaller populations were collected from small containers such as buckets, cans, and used tires near or around residential areas as shown in Figure 2.

Table 3. Logistic regression as Predictor forthe presence of *Aedes albopictus*.

Variables	Odds Ratio (OR)	95% confidence interval of OR		P-value
		Lower	Upper	
Seasons:				
Summer	0.058	0.007	0.439	0.005
Rainy	0.342	0.199	0.800	0.003
Light:				
Exposed	0.599	0.201	1.962	0.397
Exposed Shady	0.295	0.102	0.899	0.32
Colorless water	1.099	0.976	1.354	0.069
Location:				
Urban	0.498	0.261	1.102	0.048
Distance from houses:				
≤ 25	12.097	2.098	15.907	0.002
26-100	19.097	3.978	78.098	0.010
>100				

Abiotic factors also had a significant impact on the population of *Ae. albopictus.* More rainfall was observed during 2011 and 2014 resulting in more mosquito populations. Due to floods and rains, more breeding sites were observed during the rainy seasons, particularly in 2010, 2011 and 2014. As this species is dependent on natural water habitats, smaller populations were observed in 2010, 2012-13 and 2015 because of higher temperatures and lower rain fall as shown in Figure 3.

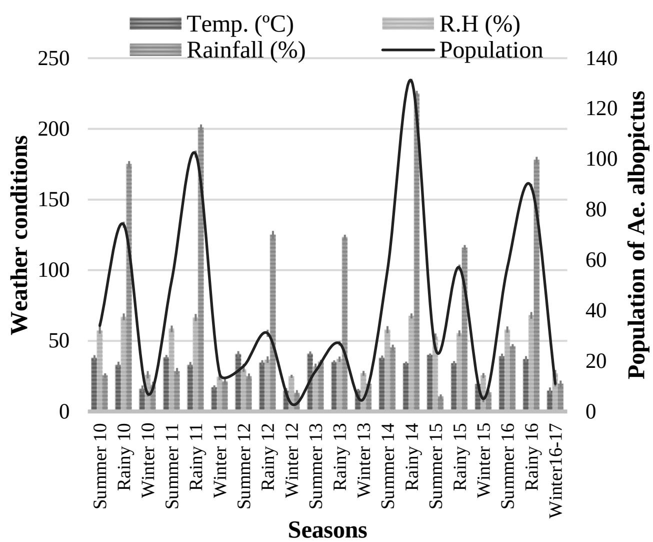

Figure 3. The relationship between abiotic factors during different seasons from 2010-17 and population of *Aedes albopictus*.

DISCUSSION

Ae. albopictus was recorded in rural and urban areas of Punjab, Pakistan. This species breeds in natural as well as in manmade habitats. Formally it was considered that it breeds only in natural habitats (Akram and Lee, 1994) including tree holes, bamboo stumps, rock pools, etc but now it is well-documented world wide as a container breeder also. All the larval indices (premises index, container index and breteau index) were above the critical level (i.e. >1). Dengue cases reported from the Province Punjab showed different trend during different years. During 2010 and 2011, there was an epidemic of this disease with thousands of confirmed cases while there were few cases in 2012-13 and 2015 and again number of cases increased in 2014. The highest levels were recorded in the year 2011 and 2014. Our findings regarding the larval indices corroborate the dengue situation in the Punjab, Pakistan.

During this study, *Ae. albopictus* was recorded from 93 sites (out of 810) showing a great variation during all seasons. Mostly, it was observed during the rainy season than the summer and winter. Breeding sites containing small reservoir with clear water and exposed shady water pools were the favorable sites for *Ae. albopictus* than smaller breeding habitats around or inside the residential areas, old tires, and exposed shady water pools. Water temperature ranging from 31-40 ºC and relative humidity (41-50%) supported *Ae. albopictus* population density in rural areas than urban environment.

These results agree with Vanwambeke*et al*. (2007) who also observed a lot of breeding habitats of *Ae. albopictus* in the exposed shady conditions during the rainy season. This study showed that seasons affect the distribution patterns of *Ae. alboictus* and moreover, the winter season during December and January had great stress on the survival of adults and larvae but less on eggs as was also found by a researcher in Nagasaki (Mori and Wada, 1978). These results are statistically at par with the present findings. Population density was variable throughout the year depending on the larval breeding habitats. Rainfall played an important role in the activation of *Aedes* population density (Ndiaye *et al*., 2006). *Ae. albopictus* showed an enormous plasticity (Delatte *et al*., 2008) in different ecosystems with relatively differing environmental conditions such as temperature and relative humidity. Results from this study showed that *Ae. albopictus* was found in rainy as well as in dry summer season, whereas, these conditions mostly occur in areas where elevation is relatively higher and ecological conditions are not so harsh (Preechaporn *et al*., 2007). In Bangladesh, some workers collected mosquitoes from all sampling sites in the rural areas (Bashar *et al*., 2005). However, some workers from other regions also collected *Ae. albopictus* from auto mobile tires, cans, basements of apartments, and green houses (Mohiuddin, 2007). The consistently productive container types in the

Punjab area were primarily domestic water storage containers such as jars, buckets, and tanks followed by tires and smaller domestic containers. The containers that were mainly filled with rain water such as tires increased during the rainy season. Hence, the *Aedes* population did not reach low levels during the time when rainfall was scant. This was due to the production of mosquitoes in the artificial containers and it was also found that a few key types of containers play an important role in a large proportion of their pupal and adult production (Koenraadt *et al*., 2004; Barbazan *et al*., 2008). Protective and control measures such as lids, larvicides, removal of discarded and unused containers, or biological agents have reduced adult vector populations density. Container capacity, water temperature, source of water, and container location, all of which could vary seasonally (Koenraadt *et al*., 2004; Kay and Nam, 2005) have been reported to be important ecological factors affecting production of *Ae. aegypti* adults. It was also found that buckets, barrels, and used tires were the most productive containers during the dry and wet seasons and some variables such as habitat density, water source, seasonal variations, and neighborhood conditions during surveys played a significant role in container productivity (Lambdin*et al*., 2009). The typical rainy season in the Punjab, normally during July-August was associated with the peak population of *Aedes* species.

A better understanding of the environmental factors is an important part of adaptive vector control efforts (Scott and Morrison, 2008). In the tropical regions of the world, some workers are working at the effect of container removal on *Aedes* population (Arunchalam*et al*., 2010). Incorporating developmental site density into these efforts along with research on the ecology within larval development sites will lead to a more cohesive understanding of *Aedes* population dynamics and contribute to the design of increasingly effective vector intervention.

Conclusion**:** From this study, it can be concluded that the rains play a vital role in the population dynamics of *Aedes* mosquitoes. During moon soon season, when temperature and relative humidity is high, its population increases tremendously and during winter, when the temperature and relative humidity is low, then its population decreases dramatically. So, we should be vigilant during the rainy season and small water bodies should be filled with sand to avoid accumulation of water.

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