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Seasonal Prevalence and Phenomenal Biology as Tools for Dengue Mosquito *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) Management

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HIGHLIGHTS

- The effective approach to advance mosquito surveillance and monitoring program
- The impacts of diverse environmental conditions for better amalgamation of *Aedes aegypti* control
- Risk assessment studies and utilization of these tools for economically important vector control programs and disease prevention activities.

Abstract: Vector monitoring is a fundamental tool for mosquito control activities and assembles information on the relative population size, species composition and advantages in dengue disease prevention. Investigations have been initiated for the exploration of relative abundance and biology of the dengue vector *Aedes aegypti* (Linnaeus) mosquito to provide baseline information for effective vector control and dengue prevention strategies. The studies were conducted for two years (2018-2019) from January to December focusing on the developmental stages of mosquitoes. The results revealed that seasonal densities of *Ae. Aegypti* undergoes considerable variations throughout the year. The population dynamics of the vector exhibited well-defined increasing populations during the post-monsoon season. Consequently, observations found a rapid decline in its abundance during the months of December to February until it reached the next peak in March and April. Datasets exhibited that the vector population gradually decreased to a lower level from May to June and there was a population increase in July and August of the years. The developmental stages of *Ae. aegypti* (egg to adult) revealed that three days after the female mosquitoes had taken blood food of Balb/C mouse began to lay eggs. The egg stage lasted for 3 days and after hatching, the four larval instars; first, second, third and fourth earmarked about 1.3, 1.1, 1.2 and 2.4 days, respectively to develop into pupae. The pupal stage developed into adults in 2 days. The entire life cycle was completed within about 11 days including female longevity 28 and male longevity 21 days, respectively.

Keywords: Mosquito; *Aedes*; biology; dengue; population; vector monitoring.

INTRODUCTION

Mosquitoes (Diptera: Culicidae), are the most medically important insects and cause human diseases and billion dollars economic loss in the entire world. Mosquitoes blood-sucking creatures are among the highly recognized groups of insects. These are slender, long-legged and two-winged insects famous for the transmission of diseases to mankind and animal populations [1-4]. Mosquitoes are comprised of five genera, 2500 species and among these 900 species belong to the genus *Aedes. Aedes aegypti* mosquitoes are best-known vectors of yellow fever, malaria, encephalitis disease, chikungunya, zika fever, dengue fever, several filariasis and viral diseases [5-11].

The occurrence of dengue fever cases has been enhanced every year in the country through the bite of an infected person by *Ae. aegypti* causing the spread of infection, virus and dengue fever [12]. Dengue outbreaks happened in Pakistan and the maximum number of confirmed cases along with deaths due to fever had been reported during the year 2011 and these cases increased every year. During the last decade there was an estimation of 0.5 million confirmed dengue cases [1-3,6-7,13-18]. The menace of dengue plague and related diseases always exists with the presence of *Ae. aegypti.* The paramount tactic to reduce mosquito-borne diseases is through the control of mosquitoes by knowing about seasonal variations, biology, population abundance and endemic circumstances of the vector [14,17].

Studies and the survey on the biology of *Aedes* up till now remained poor in Pakistan. The deficient dengue-related information in the country provoked the present studies. One of the utmost important aspects of learning how to control mosquitoes, it is necessary to know how these creatures grow and develop in the ecosystem**.** As a result, the most viable and cost-effective method for dengue fever control lies in eliminating of mosquito vectors [15,18]. The ecological investigations have been initiated to provide contextual literature requirements for the ongoing dengue control research program. The present research programs commenced for the avoidance of the mosquito-syndromes within the vicinities of the country. Qualitative research investigations have been initiated to articulate the relative abundance and biology of mosquitoes causing diseases and to regulate the seasonal abundance and biology. These studies are of prominence to distinguish trends and possible environmental stimuli for the vector reproductive behavior and are essential for mosquito control tactics.

MATERIALS AND METHODS

The life table parameters and practical procedures of the *Ae. aegypti* were examined from the protocols of Aida and coauthors [19].

Mosquito collection Arena

The present research was conducted for two years from January to December 2018-2019, in and the vicinity of Faisalabad, Punjab, Pakistan. The study sites are the most significant area including urban and rural peripheries of the Faisalabad city from where mosquito samples and baseline information on relative abundance and biology of dengue vector *Ae. aegypti* was collected. The areas selected for study were with crops in site for usual sampling, variable with the cyclic crop rotations, cutting of crops and location has variable four different weather patterns; a cool dry winter, a hot dry spring, the summer and rainy season.

Mosquito collection

The entomological observations were made from January to December and conducted on weekly basis. The collection study was conducted dependent on the convenience of adult mosquitoes and their reproductive stages. For mosquito surveillance, immature and adult mosquito populations were measured by the collections and counts of mature and immature populations through adult, egg, larva and pupa inspections from seven different randomly selected sites. The four sites were selected from urban and three from rural areas of Faisalabad city. The area of each site was about 200 acres. There were no insecticide sprays applied in the experimental area during the study period. The preventive measures to avoid mosquito breeding sites include the removal of any water from things like containers, canvases, or buckets. Collections were done from everything that can sustain water with the cover or in a dry place, including work equipment, excess materials, or trailers, and keep bins covered. Thrown out any rubbish lying around, like unused or empty vessels, tyres, additional materials and keep worksites clean [16].

The datasets were collected in replicates. The samples were also collected from different types of sewerage, irrigation, pond, ditch water and the vegetations around the fields. The unaffected fragments of the area were assessed every time through the choice of sampling fields. The marked ovitraps were installed to fascinate the female mosquitoes for eggs laying in the region of 200 m apart. The presence of *Aedes* species was confirmed by the laid eggs inside the ovitrap on the filter paper. The ovitraps at the ground level were placed close to vegetation for more mosquito shelter places. Further, the traps were retained in place to avoid direct sunlight and easy for mosquito detection. The eggs progenies were collected after every week and replacing water through regular monitoring. The filter papers with eggs were shifted to the laboratory in rearing trays. The aspirator was used to search adult mosquitoes, frequently resting in areas with relatively static air, dark places, temporary objects, or semi-permanent articles and furniture. The survey was accomplished through larval and pupal samples. These samples were collected weekly from vegetative plantations and all types of water vessels through an aspirator for their further identification in the laboratory. A fine sieve was used to pour the samples from small containers, whereas dipping the net in the water from the larger containers starting from top to bottom of the container in a churning motion through all edges of the container. The collected samples were shifted to a plastic container (500 mL) and the upper part covered with a cloth and taken to the laboratory. The collected specimens were identified up to genus level and only those belonging to the genus Aedes were identified up to species level.

Biology of *Aedes aegypti* (Linnaeus)

All stages of *Aedes* (eggs, larvae, pupae and adults) were reared separately in the controlled environmental conditions of (27 ± 2) °C temperature and (74 ± 4) % humidity in rearing rooms. Adults were reared in perplex chambers (36 L, 36 W, 36 H) with the provision of 10% sucrose solution, while latterly the females were nourished on a live Balb/C mouse to have a blood meal to develop their eggs. After hatching of eggs a small amount of water (100-300 mL) and an appropriate food viz: *Cladophora* and *Spirogyra* along with plant and animal origin debris were provided to the larvae. Durations of egg hatching, larval instars, pupal period, number of eggs laid per female, adult male and female longevities were recorded. The adults and immature mosquitoes collected were taken to the laboratory for verification and sorted according to sex and species. The adult mosquitoes were separated based on sex from the rearing chambers through an aspirator. These mosquitoes were killed through socked cotton with chloroform in an airtight bottle. Taxonomic and identification studies were performed up to species level with the help of taxonomic keys under the microscope [13, 20]. The evidence of the sample collection time was acknowledged with immense care and precision for decisive analysis for the conclusion allurement.

Environmental data

Meteorological datasets comprising daily mean temperature, rainfall and humidity were recorded at the Observatory of Plant Physiology Section, located in Ayub Agricultural Research Institute, Faisalabad during mosquito collection periods. Climatic datasets were linked with the ongoing program for mosquito monitoring to note possible influences on levels of the vector population.

Statistical Analysis

All statistical analyses were done with Statistix software version 8.1 (P= 0.05). The numbers of total *Aedes* captured in traps from collection sites were calculated and numbers of insects per trap per week used for subsequent analysis. The overall variances in seasonal abundance from both indoor and outdoor active stages of *Aedes* were equated by the least significant difference (LSD) test. Durations of life parameters were compared by one-way analyses of variance (ANOVA) with hierarchical mean numbers [21].

RESULTS

The data indicated that the adults reproductive population of *Aedes aegypti* was detected throughout the year and also verified the presence of their eggs, larvae and pupae. Temperature, rainfall with peak relative abundances of vector happening during the post-monsoon period and to a lesser degree of relative humidity are the key factors for the population dynamics of most breeding mosquitoes (Tables 1 and Figure 1).

The eggs laid by females of *Ae. aegypti* generally peaked at the beginning of July till November (30.00-60.00 eggs per trap per week) and tapered off in December to February (12.42-18.42 eggs). The laid eggs displayed a relative increase in abundance during March (57.00 eggs), but at lessening levels from April to June (48.00 - 21.00 eggs). The larval and pupal frequencies were generally higher in October followed by September and November (48.00, 42.00 and 36.00 per site per week), respectively for transformation to adults (Figure 1). Seasonal prevalence reached its next higher peak in March and April (l30.00- 27.00) approached by July and August (21.00- 24.42). The immature stages of *Ae. aegypti* declined to their minimum abundance in May and June (18.00 and 15.00), respectively.

The largest flow of adult mosquitoes occurred in early to the end of October (15.00 per site per week) during the observation year, presumably due to the monsoon rainfall trend that stimulated the eclosion of overwintering eggs for succeeding generations. The population reached its higher peak abundance (13.28) in September followed by November (12.00) representing the main activity periods of the vector. The numbers of adults collected were much least from December to February (2.57- 5.14) than the relative abundance of mosquito species found for March to August (9.85- 7.28) as presented in (Figure 1).

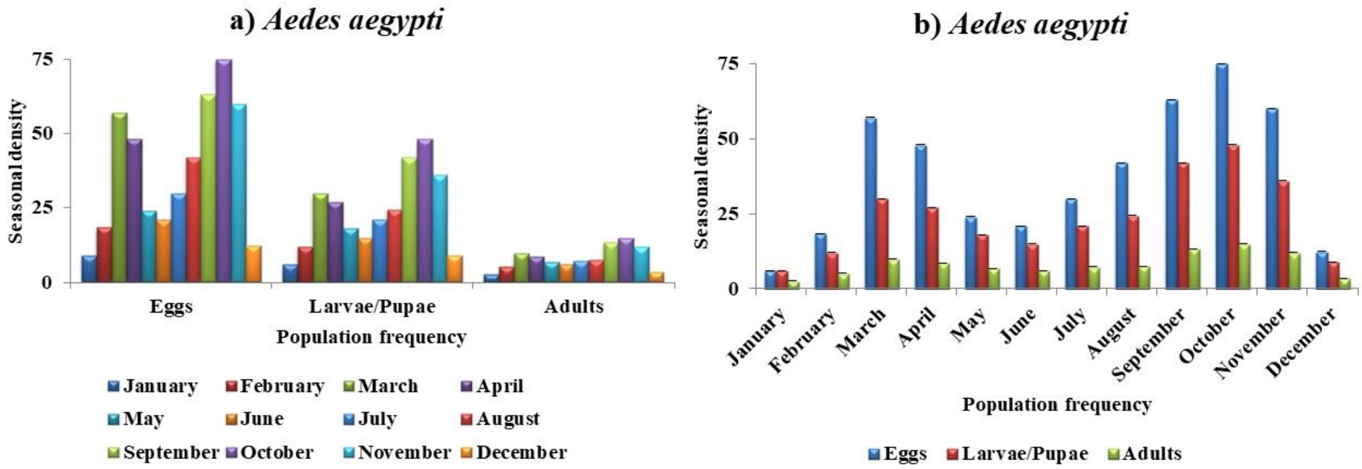

Figure 1. Population dynamics and seasonal abundance of primary dengue vector mosquito *Aedes aegypti*. a) Mosquito developmental stages b) Effects of environmental conditions on monthly population frequency.

The seasonal meteorological variability that occurred periodically during the years (2018- 2019) had a significant influence on the maximal and minimal abundance of *Ae. aegypti*. The inconsistency in population dynamics of *Ae. aegypti* was mainly due to seasonal variabilities in temperature, relative humidity and rainfall (Table 1). In this instance, the weather severity within the study season for the mean minimum (24.3- 27.9 ^{o}C) and maximum (39.7- 41.6 ^{o}C) temperatures reached a peak in the months of May to September and May to June, respectively. There was a rapid decline in the temperature from December to February, which indicated a possible link between vector densities and weather prevalence. Percentage humidity in the calendar time observed, the peak lowest (47.0- 28.0 %) during the months of August to November and the highest (96.0- 85.0%) from July to August. The rainfall pattern was enormously diverse and means ranged from 21.0- 147.9 mm in July to October and the next peak (26.0 mm) occurred in April. The variation in the population prevalence of the *Ae. aegypti* was accredited due to the seasonal variability of environment.

Table 1. Mean meteorological observations of the study area for two years (2018-2019).

Month	Temperature (^{o}C)		Relative Humidity (%)		Rainfall (mm)
	Minimum	Maximum	Lowest	Highest	
January	4.1	19.5	27.0	94.0	3.2
February	5.6	21.0	16.0	92.0	6.3
March	12.9	28.1	14.0	90.0	1.8
April	19.7	34.6	18	73	26.0
May	24.3	39.7	18.0	51.0	0.1
June	27.4	41.6	22.0	74.0	11.4
July	27.9	38.6	22.0	96.0	63.0
August	27.0	36.7	47.0	85.0	24.0
September	24.2	34.3	39.0	96.0	147.9
October	16.9	31.8	32.0	90.0	21.0
November	11.3	27.3	28.0	94.0	Traces
December	6.6	20.6	29.0	100.0	16.4

Biology of *Aedes aegypti* (Linnaeus)

There was an aquatic phase encompassing larval and pupal stages and a terrestrial phase comprising eggs and adults throughout the whole life cycle of *Ae. aegypti*. Three days after the female dengue mosquito *Ae. aegypti* had taken blood food of Balb/C mouse, began to lay eggs. After egg-laying, the egg stage lasted for 3 days to develop into larvae. The larvae passed through four instars; first, second, third and fourth instar has taken 1.3, 1.1, 1.2 and 2.4 days, respectively, to develop into pupae. Two days were taken by the pupae stage to become adults. The entire life cycle was completed within 11 days. Each female laid an average of 81.28 eggs per clutch often at a single time. The longevity of the adult female was 28 days and the male had a life span of 21 days (Table: 2).

Table 2. The mean duration of the lifespan developmental parameters of *Aedes aegypti*

Life Parameter	Duration (day)/ Number
Egg hatching	3.00 d
First instar larvae	1.30 e
Second instar larvae	1.10 e
Third instar larvae	1.20 e
Fourth instar larvae	2.40 de
Pupae	2.00 de
Adult Male longevity	21.00 c
Adult Female longevity	28.00 b
Number of eggs laid per female	81.28 a
Standard Error	0.663
LSD Value	1.334

The average values represented inside a column followed by the identical letter are not statically different using the LSD test (P= 0.05).

Adults

The adults of mosquitoes causing dengue fever were slender with legs long- shaped, medium-size about 7 millimeters in length. Whereas, during resting position body of the adult looked in two axes and further proboscis and wings generally uniform with shorter maxillary palps. Male mosquitoes hold short mouthparts for feeding on plant nectar in contrast to females having a long, sharp and thin proboscis adapted to feed on blood after penetration of the host skin. The proboscis of both sexes was dark and the clypeus had two clusters of white scales. Adult males were smaller than females and can be differentiated by small palps angled with white or silver scales. The male mosquitoes were with dense hairs named plumose and females had pilose antennae. The abdomen finished to a tippy point, which showed the distinction of all *Aedes* species. The dorsal thoracic portion in adults of *Ae. aegypti* had white scales that looked like the violin in shape. Two straight lines bounded by curved lyre-shaped outlines on both sides and white basal bands retained on the hind tarsal section of the legs, which appeared similar to the bands. The white scales lied on the abdomen and the color was dark brown to black.

Eggs

Female mosquitoes laid eggs above the water not floating though can be spread out over hours or days on a singly basis rather than in masses on the humid sides of vessels. Most often, females laid long, smooth, ovoid-shaped about one millimeter long eggs. The color of the eggs was white to shiny black and soft in appearance and laterally become hard within a few minutes. Female mosquitoes spread egg clutches over two or more sites rather than on a single site.

Larvae

The larval variation of *Ae. aegypti* from other water species happened by the appearance without legs, the occurrence of a distinct head with brushes on the mouth and antennae. Thorax was bulbous broader than the head and abdomen. Larval breathings of oxygen were through a posteriorly sited siphon (stout air tube) with one pair of hair tufts near the end of the abdomen. While the rest of the body was vertically detained above the water surface. Larval differentiation from other *Aedes* species was due to the identification of an unaided eye with a siphon tube. The comb scales and comb teeth had well-established lateral denticles, while pectin teeth with less defined denticles on the siphon. Larvae rested at an angle during feeding, present around the surface of the water and if bothered they swim to the bottom water. The whole larval period finished in four instars. There was a transverse appearance of dark bands on the thorax during the course of ecdysis.

First instar larva

The first instar larva was found twofold and 1 mm in length size on hatching. This first instar encompassed the sharp, stout sclerotized, enormously pointed, twisted and flattened conical setting in an oval region with the flexible membrane recognized by the survival of an egg surfer on the dorsal side of the head.

Second instar larva

During this phase, larvae were of identical size as the previous full-grown substantial larval phase, while the head was distended immensely after ecdysis. Throughout this phase, the siphon had a swollen shape and the tracheal trunk was bulbous and wrinkly with taenidia in the terminal portion. Similar to the previous larval phase the head became dark, while the rest of the body endured 3 mm length development with a cylindrical shape.

Third instar larva

The head capsule was bulbous shaped, short pointed with bulky head size and variability as compared to two other stages. Conspicuously, combed spine tails on the eighth abdominal segment having owed assembly. During this larval phase, the overall length extended up to 4 mm.

Fourth instar larva

This larval phase revealed the pupal trumpets for respiration, which were rudimental and stouter owing to the evolution of the buds on the thorax. The teeth with solid-built denticles on the horizontal side were the most imperative structure during the development of this instar. The fourth larval stage during this phase was prolonged up to 6 mm.

Pupae

After the fourth instar, the pupal ecdysis was derived in *Ae. aegypti* and become plumpy and pretentious. The larva stayed at rest at the water surface and stopped feeding. The pupa was observed as comma shape with pigment changes as white color on emergence. The expansion of the abdomen resulted in splitting open the pupal case and the head emerged by ingesting air during this stage.

DISCUSSION

The life cycle of the *Ae. aegypti* is often very complex and there is great variability in the length of the life cycle from some hours to a few days. The current finding represented the average life span of 28 days for the adult female and a male had 21 days, whereas, the entire life cycle could be completed within about 11 days. Comparable to current findings, earlier studies observed 6 to 8 days larval and 1 to 2 days pupal periods of *Ae. aegypti* with a total of 9 to 10 days for a complete life cycle. The longevity of male adults was less than 10 to 29 days as compared to 12 to 56 days in females [22, 23]. The differently reported increase of life cycle duration by earlier researchers is due to the differences in the scale of the studies, for example, the mosquito larvae in 5 to 10 days go through four larval stages [24]. Such inconsistent duration is temperature-dependent [25] or larval nourishments [24] and lasts for 1 to 2 days shorter pupal stage [22]. Both female and male *Aedes* live up to an average of 20 to 30 days as demonstrated by other studies [9-11]. These differences are due to various factors, including the environmental aspects, microhabitats, food and vector‘s behavior for adaptation to different environments. Moreover, the development rate of mosquito species was dependent and influenced by the water quality (temperature, pH and ion concentration) [9, 26].

A comparison of reproductive activity with local weather conditions including monthly temperature, relative humidity and rainfall showed a clear link to the breeding of *Ae. aegypti*. The results revealed the seasonal densities of *Ae. aegypti* had undergone considerable variations during the studied years. The current decrees presenting the population fluctuation of vector displayed nearly a distinct rising tendency throughout the post-monsoon rainfall season (September to November). Afterward, interpretation observed a fast decline in its density during the months of December to February until it reached a subsequent climax in March and April, and steadily reduced to a lesser level from May to June. Observations demonstrated that the numbers of vectors attained the next increase in July and August of the study years. For here in the reported study, an increase in rainfall from July to October and the next peak in April encouraged enormous natural flooding and emerged to have been liable for the sharp upsurge in vector abundance. Actually, with the occurrence of flooding, *Aedes* species positioned for egg-laying, which resisted dryness [5]. During spring when the study areas were naturally flooded, the post-overwintering females originated resulting population growth and increased with the increase in air temperatures [27]. Additionally, the rate of larval development and population growth increased with the increase in mean temperature throughout the summer season [28]. During the summer period natural breeding sites desiccated up and due to slight rainfall from May to June may clarify the splendid fall of *Aedes* catch values in July and preservation at a low level throughout July. The population dynamics of irritant mosquitoes depend on many climatic factors such as temperature rainfall and anthropogenic factors like water management in the landscape, and mosquito control activities [29]. The population dynamics of mosquitoes were gripped by temperature, evaporation, rainfall, and photoperiod. Hence, the availability of weather forecasts up to several days can allow the prediction of mosquito abundance [30-31]. Their population dynamics were strongly related to seasonal temperature. The highest density of *Aedes* sp., was found in the month of April and the lowest found in the months of December and January [32]. An obvious awareness of these dynamics is required to envisage changes in risk assessment factors and subsidizing disease transmission. Consequently, the population density of *Aedes* acts more rapidly in response to succeeding periods of artificial or natural flooding and dryness.

The information on biology, abundance and seasonal occurrence of *Ae. aegypti* is still incomplete though its occurrence and impact in Pakistan as a vector of dengue fever have also been acquainted. However, the mosquito has perceived a recent renowned role in plague outbreaks of dengue fever. Notably, earlier information on the distribution of *Ae. aegypti* showing all the localities of species, density and seasonal prevalence in the subcontinent and South-East Asia was provided [13,33-34]. In the literature concerning the mosquitoes of Pakistan, a specification gradient has arranged 134 existing mosquito species [35]. The central part of Punjab was with the ventured status of *Ae. Aegypti.* Lahore, Ferozepur and Multan areas were found with quantitative mosquito populations. A total of twelve *Aedes* species were detected during these findings including *Ae. aegypti* [36-37] with a relative abundance of 2 other specimens reaching a total of 14 mosquito species [38]. Evaluations between collections supported only one other second common *Aedes* species of mosquito (*Aedes albopictus* Skuse). Further, molecular studies based on DNA analysis of mosquitoes in Pakistan had established a barcode reference library for the mosquitoes of this region [39]. The levels of genetic diversity varied among species and DNA barcoding supported an accurate tracking of mosquito populations [33]. Further, the present research was of restricted retro and not the only effort to statistical recitation abundance to concurrent quantification of biotic and abiotic variable shreds of evidence on population dynamics over a vast locality.

The prevalence of the *Ae. aegypti* status in Pakistan can generally be of the view that the infection of the global species occurred perhaps over the mid 19th century with increased trade activities and travel. A supportive view is available about the occurrence of dengue outbreaks due to these species near to Karachi, which is a coastal city though later on identified topical times in some of the interior regions. The dispersal of these mosquitoes is a consequence of its drive and one should have an understanding of this incredible concern. The present study might be cooperative in mosquito management strategies for effective dengue control to decrease repercussions in the health area.

CONCLUSION

Vector monitoring is a vital approach for mosquito control and deliberates information on the relative population size. The findings support population biology and weather effects monitoring on the growth stages of mosquitoes. The findings provide baseline information and revealed the seasonal abundance of *Ae. aegypti* that endured considerable variations throughout the study years resulting an impact on the population densities besides the growth stage of mosquitoes. These descriptions are convenient for vecttors borne diseases prevention activities locally and globally.

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REFERENCES

1. Sarwar M. Mosquito-borne viral infections and diseases among persons and interfering with the vector activities. Intern. J. Vac. Vaccin. 2016a; 3: 00063.
2. Sarwar M. Mosquitoes (Diptera: Culicidae) as malaria transmitters and procedures for suppression to exposure and spread of vectors. Biomed. Heal. Inform. 2016b; 1:38-43.
3. Sarwar M. Typical flies: natural history, lifestyle and diversity of Diptera. p. 50. In: Life cycle and development of Diptera. In: Sarwar M, Editor. Intech Open Ltd., London, UK. 2020; 170 pp.
4. Walter Reed Biosystematics Unit. *Aedes aegypti* species page. Walter Reed Biosystematics Unit, 2021; available in: http://wrbu.si.edu/ vector species/mosquitoes/aegypti (accessed 24 April 2022).
5. Kettle DS. Medical and Veterinary Entomology. CAB International, Wallingford, U.K. 1995. 116-7.
6. Sarwar M. Proposing Solutions for the Control of dengue fever virus carrying mosquitoes (Diptera: Culicidae) *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). J. Pharmacol. Toxicol. Stud. 2014a; 2: 1-6.
7. Sarwar M. Elimination of dengue by control of Aedes vector mosquitoes (Diptera: Culicidae) utilizing copepods (Copepoda: Cyclopidae). Internat. J. Bioinf. Biomed. Engin. 2015a; 1: 53-8.
8. Service MW. Importance of ecology in *Aedes aegypti* control. Southe. Asi J Trop Med Pub Heal. 1992;23:681-90.
9. Walter Reed Biosystematics Unit. *Aedes aegypti* species page. Walter Reed Biosystematics Unit, 2021; available in: http://wrbu.si.edu/ vector species/mosquitoes/aegypti (accessed 24 April 2022).
10. World Health Organization. A global brief on vector-borne diseases. WHO, 2014; Geneva, Switzerland.
11. World Health Organization. Global vector control response 2017-2030. WHO, 2017; Geneva, Switzerland.
12. Khan HAA, Akram W, Shehzad K, Shaalan, EA. First report of field evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. Paras. Vect. 2011; 14: 1-11.
13. Barraud PJ. The fauna of British India, including Ceylon and Burma. Diptera. Family Culicidae. Tribe Megarhinini and Culicinae. 1934; 5.
14. Sarwar M. Proposals for the control of principal dengue fever virus Ttansmitter *Aedes aegypti* (Linnaeus) mosquito (Diptera: Culicidae). J. Ecol. Environ. Sci. 2014b ;2: 24-8.
15. Sarwar M. Dengue fever as a continuing threat in tropical and subtropical regions around the world and strategy for its control and prevention. J. Pharmacol. Toxicol. Stud. 2014c; 2: 1-6.
16. Sarwar M. Defeating malaria with preventive treatment of disease and deterrent measures against Anopheline vectors (Diptera: Culicidae). J. Pharmacol. Toxicol. Stud. 2014d; 2: 1-6.
17. Sarwar M. Reducing dengue fever through biological control of disease carrier Aedes mosquitoes (Diptera: Culicidae). Inter. J. Preven. Med. Res. 2015b;1: 161-6.
18. Sarwar M. Controlling dengue spreading Aedes mosquitoes (Diptera: Culicidae) using ecological services by frogs, toads and tadpoles (Anura) as predators. American J. Clin. Neurol. Neuros. 2015c; 1: 18-24.
19. Aida HN, Dieng H, Ahmad AH, Satho T, Nurita AT, Salmah MRC, Miake F, Norasmah B. The biology and demographic parameters of *Aedes albopictus* in northern peninsular Malaysia. Asian Pac. J. Trop. Biomed. 2011; 1, 6: 472-477.
20. Christophers SR. The Fauna of British India, including Ceylon and Burma. Diptera. Family Culicidae. Tribe Anophel. 1933. 4.
21. Steel RGD, Torrie JH, Dickey DA. Principles and procedures of statistics. A Bio-metrical approach, 1997; 3rd ed. McGraw Hill Book Co. Inc., New York, USA.
22. Lee DK. The strategies for effective mosquito control in Korea. Gr. Cul. Pub. Heal. 2000; 1: 72-8.
23. Vythilingam I, Chiang GL, Lee HL, Singh KI. Bionomics of important mosquito vectors in Malaysia. Southe. Asi. J. Trop. Med. Pub. Heal. 1992; 23: 587- 603.
24. Hawley WA. The Biology of *Aedes albopictus.* J. Amer. Mosq. Cont. Assoc. 1988; 4: 1-40.
25. Neto PL, Navarro-Silva MA. Development, longevity, gonotrophic cycle and oviposition of *Aedes albopictus* Skuse (Diptera: Culicidae) under cyclic temperatures. Neotrop. Entomol. 2004; 33: 29-33.
26. Rydzanicz K, Lonc E. Species composition and seasonal dynamics of mosquito larvae in the Wroclaw, Poland area. J. Vect. Ecol. 2003; 28: 255-66.
27. Ludwig A, Bicout, DJ, Chalvet-Monfray K, Sabatier P. Modelling the aggressiveness of the Culex modestus, possible vector of West Nile fever in Camargue, as a function of meteorological data. Environ. Risq. et San. 2005; 4: 109-113.
28. Jetten TH, Takken W. Anophelism without malaria in Europe. A review of the ecology and distribution of the genus Anopheles in Europe. Landbouwuniversiteit Wageningen (Wageningen Agricultural University), Wageningen, Netherlands. 1994; 94-5:1-69.
29. Ponçon N, Toty C, L'Ambert G, Le Goff G, Brengues C, Schaffner F, et al. Population dynamics of pest mosquitoes and potential malaria and West Nile virus vectors in relation to climatic factors and human activities in the Camargue, France. Med. Vet. Entomol. 2007; 21:350-7.
30. Hongfei G, De Gaetano A. Harrington LC. A climate based mosquito population model. Proc. of the World Congress on Engineering and Computer Science, October 24-26, 2007; San Francisco, USA.
31. Sarwar M, Roohi A. New advances in insect vector biology and virus epidemiology. p. 301-311. In: Applied Plant Virology: Advances, Detection, and Antiviral Strategies. In: Awasthi LP, Editor. Elsevier Inc., London. 2020; 682p.
32. Farkhanda M, Aniqa N, Sabiha F. Population dynamics of different mosquito species at Lahore College for Women University Campus, Lahore. J. Mosq. Res. 2013; 3: 82-8.

33. Bibi M, Zahoor MK, Zahoor MA, Ashraf HM, Majeed HN, Nasir S, et al. Genetic analysis of mosquitoes from rural and urban areas of Sialkot, Pakistan. Int. J. Agric. Biol. 2015; 17: 809-814.
34. Rao TR. Distribution, Density and Seasonal prevalence of *Aedes aegypti* in the Indian subcontinent and South-East Asia. Bull. Wld. Hlth. Org. 1967;36:547-51.
35. Aslam KM. The Mosquitoes of Pakistan: I. A Checklist. Mosq. Syst. Newsletter. 1979; 3: 147-159.
36. Reisen WK, Hayes CG, Azra K, Niaz S, Mahmood F, Parven T. West Nile virus in Pakistan. II. Entomological studies at Changa Manga National Forest, Punjab Province. Transac. Roy. Soci. Trop. Med. Hyg. 1982; 76:437-48.
37. Reisen WK. A quantitative mosquito survey of 7 villages in Punjab province, Pakistan with notes on bionomics, sampling methodology and the effects of insecticides. Southeast Asi. J. Trop. Med. Pub. Heal. 1978; 9: 587-601.
38. Reisen WK, Milby MM. Population dynamics of some Pakistan mosquitoes: Changes in adult relative abundance over time and space. Ann. Trop. Med. Parasitol. 1986; 80: 53-68.
39. Ashfaq M, Hebert PDN, Mirza JH, Khan AM, Zafar Y, Mirza MS. Analyzing mosquito (Diptera: Culicidae) diversity in Pakistan by DNA barcoding. PLoS One. 2014; 9: e97268.