



RESEARCH ARTICLE

## Aquatic Choice of Female Mosquitoes (*Aedes* and *Culex* spp.) for Egg-Laying Preferences Under Field Conditions

Adnan Usman<sup>1\*</sup>, Waseem Akram<sup>1</sup>, Fatima<sup>1</sup>, Tahir Bilal<sup>2</sup>, Fatima Khan<sup>3</sup>, Ahmad Ammar<sup>1</sup>, Waqas Ahmad<sup>1</sup>, Muhammad Zohaib Ashfaq<sup>4</sup>, Syed Muhammad Ali<sup>1</sup> and Muhammad Waseem<sup>5</sup>

<sup>1</sup>Department of Entomology, University of Agriculture Faisalabad, Pakistan; <sup>2</sup> Earth and Environmental Sciences, Macquarie University, North Ryde, Australia; <sup>3</sup>Life and health sciences, Ulster University, UK; <sup>4</sup>Department of Agronomy, University of Agriculture Faisalabad, Pakistan; <sup>5</sup>Plant Protection Entomology, Ghazi University, Dera Ghazi Khan, Pakistan.

\*Corresponding author: [adnanusm586@gmail.com](mailto:adnanusm586@gmail.com)

Article History: 26-035

Received: 20 Jan 2026

Revised: 27 Feb 2026

Accepted: 05 Mar 2026

### ABSTRACT

In this study, oviposition preferences of the *Aedes aegypti* and *Culex* spp. on four different water surfaces, trees hole debris, dry leaves, husk, and plain water were observed under natural field conditions. Plastic containers (5L) were used to test the preference of oviposition of the *A. aegypti* and *Culex* spp. on the above four water surfaces. Findings indicated that containers that had organic substrates and especially Tree Hole Debris and Dry Leaves, recorded higher oviposition levels than Plain Water. *A. aegypti* were found to be more attracted to darker and nutrient-rich substrates and *Culex* spp. was found to be more tolerant to treatments. Weekly oviposition were also affected by environmental changes like temperature, humidity, among others. On the whole, the results demonstrate the role of organic matter in the development of mosquitoes' breeding behavior, which can be useful in the development of specific control measures against the vectors.

**Key words:** *A. aegypti*, *Culex* spp, Mosquito ecology, oviposition preferences, Breeding substrate, Environmental factor.

### INTRODUCTION

Mosquitoes are insects of the order Diptera and family Culicidae and are associated with several arthropod-borne diseases that kill millions of people annually (Nebbak et al., 2022). Scientifically classified into 41 genera, there are nearly 3,500 species of mosquitoes (Mudi et al., 2021). Mosquito are important vectors due to their ability to transmit diseases in large numbers, recurrent of infection, and adapt to diverse of environment (Wilkerson et al., 2021). Diseases transmitted by mosquitoes include dengue, malaria, filariasis, Zika, and chikungunya (Huynh, et al., 2022). While adult mosquitoes are winged and capable of flight (Liu, et al., 2024) their larvae and pupae are aquatic and require water for development (Tyagi et al., 2025).

Female mosquitoes seek water for oviposition, often preferring dark water enriched with organic matter (Pautzke et al., 2024). Different species breed in various types of water, including fresh, brackish, or polluted

water, but not in saline water (Hoyochi et al., 2025). Laboratory trials have shown that many species deposit eggs with a preference for dark-colored containers (Hug et al., 2025). Around human dwellings, *Aedes* mosquitoes prefer clean water containers (Agus et al., 2023), while *Culex* species are more likely to breed in polluted water due to poor sanitation and drainage (Bodinga et al., 2025).

Phytotelmata, such as tree holes, bamboo internodes, pitcher plants, and leaf axils, are natural aquatic habitats rich in organic matter and serve as breeding sites for mosquitoes (de Almeida et al., 2025; Ceretti-Junior et al., 2025). These microhabitats support the complete development of larvae and pupae and provide suitable oviposition sites (Simons et al., 2023). *Aedes aegypti*, which lays eggs in both natural and artificial containers (Rubio et al., 2025), has led to the development of specific traps for monitoring and controlling this vector (Collins, 2025). *Culex* species prefer water that remains standing for more than two days, which supports larval growth (Meena, 2025).

**Cite This Article as:** Usman A, Akram W, Fatima, Bilal T, Khan F, Ammar A, Ahmad W, Ashfaq MZ, Ali SM and Waseem W, 2026. Aquatic Choice of Female Mosquitoes (*Aedes* and *Culex* spp.) for Egg-Laying Preferences Under Field Conditions. Trends in Animal and Plant Sciences 7: 99-106. <https://doi.org/10.62324/TAPS/2026.011>

Allochthonous plant material in these aquatic habitats serves as a food base for mosquito larvae and other detritivores organisms (McKie et al., 2023), influencing larval growth and development (Netshituni, 2025; Allan et al., 2021). Tree holes in tropical and temperate forests are among the most common (Kirsch et al., 2021) stable standing water bodies, making them important breeding grounds for mosquitoes (Leroy, 2021; Chowdhury et al., 2024). The choice of oviposition site is crucial for female mosquitoes, as it affects the survival of eggs (Phiri, 2024) and larvae in temporary aquatic habitats subject to drought, freezing, or nutrient limitations (Girard et al., 2021).

The University of Agriculture Faisalabad hosts numerous trees with holes that collect rainwater during the rainy season, providing suitable mosquito breeding sites (Ishtiaq, 2021; DONATUS et al., 2022). Therefore, this study aims to determine the preferred breeding sites of *A. aegypti* and *Culex* spp., assess the seasonal impact of temperature on breeding, and identify the conditions that allow successful egg laying, development, and hatching.

## MATERIALS AND METHODS

### Study area

The study was conducted at three selected field locations characterized by semi-urban vegetation and natural mosquito breeding habitats.

### Experimental design

A completely randomized field experiment was conducted from October 2024 to March 2025. At each location, four substrate treatments were tested:

1. Tree-hole debris water
2. Leaf-infused water
3. Husk-infused water
4. Plain tap water (control)

For each treatment, three replicate plastic containers (5L capacity) were placed per location, giving 12 containers per site and 36 containers in total.

Each container was filled with 4L of water. Organic substrates were added at standardized amounts (100 g/L of dried tree debris, leaves, or husk). Containers were randomly arranged and exposed outdoors under natural conditions.

### Exposure and sampling

Containers were left undisturbed for 14 days prior to the first observation. Thereafter, larval counts were recorded biweekly (every two weeks) throughout the experimental period. After each sampling event, containers were cleaned, refilled, and re-prepared to maintain uniform experimental conditions.

### Environmental parameters

Ambient temperature and relative humidity were recorded at each biweekly sampling event using a calibrated digital hygrometer.

### Larval identification

Collected larvae were counted and morphologically identified to genus/species level using standard taxonomic keys.

### Statistical analysis

Larval abundance (mean  $\pm$  SE) was analysed using two-way ANOVA with substrate type and sampling date as fixed factors. Tukey's HSD test was applied for post-hoc comparisons at  $p \leq 0.05$ . Statistical assumptions of normality and homogeneity of variance were verified prior to analysis. All analyses were performed using SPSS.

## RESULTS

This study was conducted to investigate the oviposition preferences and seasonal population dynamics of *Aedes aegypti* and *Culex* mosquitoes in different breeding substrates under natural field conditions. The influence of organic materials and environmental parameters, particularly temperature and relative humidity (RH), on larval abundance was monitored over time. Variations in water quality and substrate composition were also evaluated to understand their effects on mosquito breeding patterns and population fluctuations.

The data demonstrated a strong association between *A. aegypti* larval density and climatic factors, especially temperature and RH. During the early observation period in October 2024, when the mean temperature remained stable at 26.1 °C and RH at 50%, *Aedes* populations were highest, reaching a peak of 80.33 larvae on October 31. With the seasonal transition into November, when the temperature decreased to 19.8 °C and RH slightly increased to 55%, the population declined moderately to 67.67 and 60.33 larvae by mid- and late November, respectively.

A sharp decrease in *A. aegypti* abundance was observed in December and January, as temperatures dropped further to 14.4°C and then to 12.5°C, and humidity levels increased to 60–66%. Larval counts fell drastically to 22.67 in mid-December and reached the lowest point of 11.67 on January 29, indicating that lower temperatures have a stronger negative impact on mosquito activity than higher humidity levels during winter. In contrast, as temperatures began to rise again in mid-February to 15.4°C and in March to 21.1°C, mosquito populations gradually recovered, increasing to 50.33 by March 30.

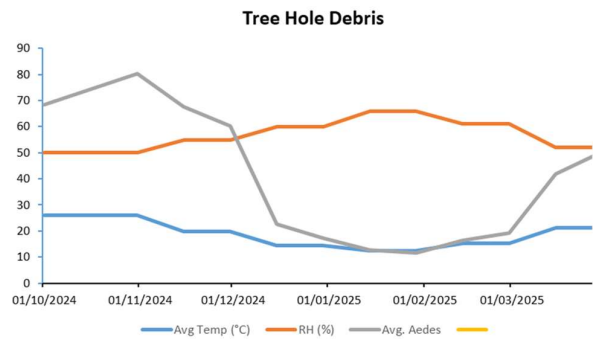
**Table 1:** Analysis of variance for *Aedes* sp. recorded in tree hole debris substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	8	4		
Treatment	12	26794.6	2232.88	1317.77	0
Error	24	40.7	1.69		
Total	38	26843.2			
Grand Mean	44.615				
CV	2.92				
Grand Mean	44.615				
CV	2.92				

**Table 1:** Mean comparison of *Aedes* sp. mosquito's species population recorded in tree hole debris substrate at different dates of observation

Date	Mean	Homogeneous Groups
10/31/2024	84.333	A
10/16/2024	78.333	B
10/1/2024	72.333	C
11/15/2024	71.667	C
11/30/2024	64.333	D
3/30/2025	53.667	E
3/15/2025	45.333	F
12/15/2024	24.667	G
2/28/2025	21	GH
12/30/2024	20	H
2/13/2025	17.667	HI
1/14/2025	14	IJ
1/29/2025	12.667	J

Alpha 0.05  
Standard Error for Comparison 1.0628  
Critical Q Value for Comparison 5.176  
Critical Value for Comparison 3.8904

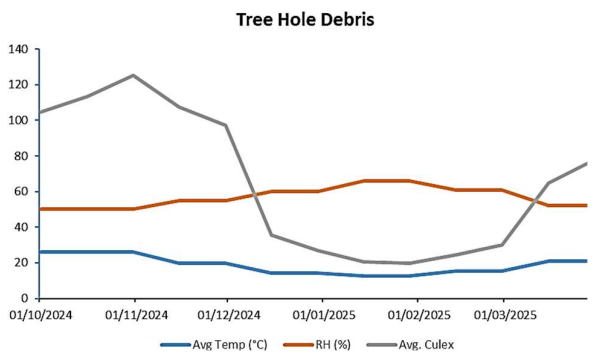


**Fig. 1:** Relationship between *aedes* population and effect of temperature and humidity in tree hole debris water at different time intervals.

**Table 2:** Analysis of variance for *Culex* sp. recorded in tree hole debris substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	16.7	8.33		
Treatment	12	65142.9	5428.58	804.23	0
Error	24	162	6.75		
Total	38	65321.6			

Grand Mean 69.564 CV 3.73



**Fig. 2:** Relationship between *Culex* SPP population and effect of temperature and humidity in tree hole debris water at different time intervals.

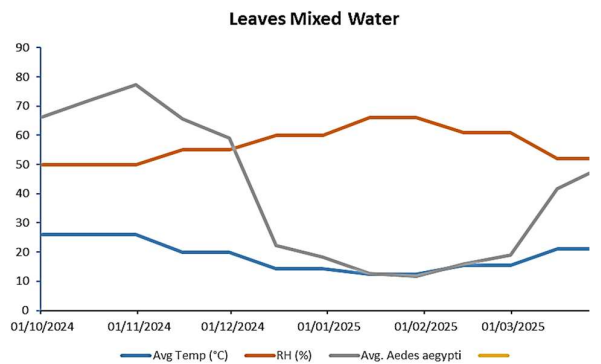
The population dynamics of *Culex* spp. of mosquitoes show in Figure 2 a clear seasonal trend in response to fluctuations in temperature and relative humidity. During the early observation period in October 2024, when the average temperature remained at 26.1°C with 50% RH, *Culex* spp were at their highest. The population peaked on October 31 at 125.33 larvae, highlighting this period as the optimal breeding window for *Culex* Sp under favorable climatic conditions. With a temperature drop to 19.8°C and a slight rise in RH to 55% during November, a moderate decline in population was observed, falling to 97.33 larvae by November 30.

As winter approached and temperatures decreased further to 14.4°C in December and 12.5°C in January, accompanied by increased RH levels of 60–66%, *Culex* abundance fell drastically. The larval population declined to 35.67 in mid-December, 20.67 by mid-January, and reached its lowest at 19.67 on January 29, confirming that cold temperatures significantly suppress *Culex* breeding activity, even when humidity is high. However, with the return of warmer temperatures in March (21.1°C and 52% RH), the population rebounded sharply to 65 on March 15 and 78 by March 30, indicating a strong positive correlation between rising temperature and *Culex* oviposition.

**Table 0:** Analysis of variance for *Aedes* sp. recorded in leaves in water substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	7.6	3.79		
Treatment	12	22678.4	1889.86	977.3	0
Error	24	46.4	1.93		
Total	38	22732.4			

Grand Mean 40.872 CV 3.40



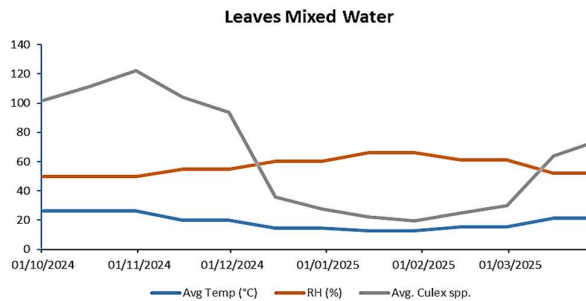
**Fig. 3:** Relationship between *A. aegypti* population and effect of temperature and humidity in leaves in water at different time intervals.

The population trend of *A. aegypti* in the leaves mixed water substrate closely followed seasonal changes in temperature and relative humidity Shown in Figure 3. During October 2024, when the average temperature was stable at 26.1°C and RH at 50%, *Aedes* populations were highest, peaking at 77.33 larvae on October 31, as shown in Figure 3. This suggests that *A. aegypti* thrives in warm, moderately humid environments when organic matter like leaf debris is

present, enhancing water suitability for oviposition. As the temperature declined to 19.8°C in November and humidity slightly increased to 55%, the mosquito population declined moderately to 65.67 (mid-November) and 59 (end of November), indicating a gradual sensitivity to falling temperatures. With the onset of winter in December and January, when temperatures dropped further to 14.4°C and 12.5°C and RH rose to 60–66%, the population decreased sharply to as low as 11.67 larvae on January 29, highlighting a strong inhibitory effect of cold temperatures on *Aedes* development and survival. While RH increased during this period, it did not counterbalance the adverse effects of low temperature. As the temperature began to rise again in February and March (15.4°C to 21.1°C), *Aedes* populations showed a notable rebound, increasing to 49.33 larvae by March 30, confirming the species' temperature-dependent oviposition behavior.

**Table 5:** Analysis of variance for *Culex* sp. recorded in leaves in water substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	15	7.49		
Treatment	12	54709.6	4559.14	708.86	0
Error	24	154.4	6.43		
Total	38	54879			
Grand Mean	63.974	CV 3.96			



**Fig. 4:** Relationship between *Culex* population and effect of temperature and humidity in leaves in water at different time intervals.

The oviposition pattern in Figure 5 of *Culex* mosquitoes in the leaves mixed water substrate revealed a strong climatic response, particularly to seasonal shifts in temperature. During October 2024, when average temperatures remained at 26.1°C and RH was 50%, *Culex* populations reached their peak levels, with the highest count recorded on October 31 (122.33 larvae). This period marked the most favorable breeding conditions, combining warm temperatures and nutrient-rich organic water from leaf matter. As temperatures declined to 19.8°C in November and RH rose to 55%, a moderate decrease in population was observed, with 104.33 and 93.67 larvae recorded on November 15 and 30, respectively.

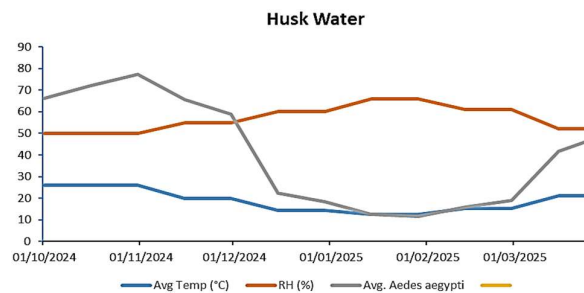
A sharp decline followed in mid-winter, with temperatures falling to 14.4°C in December and 12.5°C in January, and RH increasing to 60–66%. Correspondingly, *Culex* larval populations dropped significantly to 27.67

on December 30 and reached a seasonal low of 19.33 by January 29, indicating low-temperature inhibition of oviposition and larval survival, even in enriched substrates. As temperatures gradually recovered in February (15.4°C) and reached 21.1°C in March, *Culex* populations also rebounded, rising to 64 on March 15 and 74.67 on March 30, suggesting that *Culex* mosquitoes quickly respond to warming trends in late spring.

These findings confirm that *Culex* spp. favor warm conditions for breeding and show reduced activity below 20°C, regardless of relative humidity. The leaves mixed water substrate, rich in organic nutrients, provides ideal conditions during favorable weather, but temperature remains the primary limiting factor. This emphasizes the critical role of climate-aware vector surveillance and the importance of targeting organic water collections during peak months for effective *Culex* control.

**Table 6:** Analysis of variance for *Aedes* sp. recorded in husk substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	11.2	5.615		
Treatment	12	11724.8	977.064	604.85	0
Error	24	38.8	1.615		
Total	38	11774.8			
Grand Mean	42.077	CV 3.02			



**Fig. 6:** Relationship between *Aedes* population and effect of temperature and humidity in husk at different time intervals.

The seasonal trend shown in Figure 7 of *A. aegypti* in the husk substrate demonstrates a clear climatic dependency, particularly on temperature fluctuations. During October 2024, under favorable conditions of 26.1°C average temperature and 50% relative humidity, *Aedes* larval populations were at their highest, peaking at 77.33 on October 31. This reflects an optimal breeding environment supported by warm temperatures and semi-organic substrate like husk. As temperatures declined to 19.8°C in November and RH slightly increased to 55%, a gradual decrease was observed, with larval counts falling to 59 by the end of November, indicating moderate sensitivity to cooling conditions.

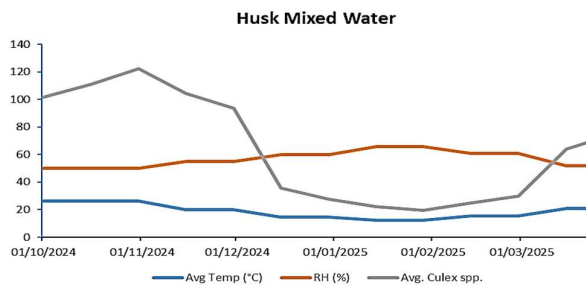
In December and January, when the average temperature further dropped to 14.4°C–12.5°C and RH rose to 60–66%, a sharp decline in population was evident, reaching a low of 11.67 larvae by January 29. Despite the nutrient-retaining nature of husk, the lower temperatures

likely inhibited adult activity and delayed larval development. However, as temperatures rose again to 15.4°C in February and reached 21.1°C in March, the population gradually rebounded, climbing to 49.33 larvae by the end of March, showing the species' capacity for rapid recovery in response to warming weather.

**Table 7:** of variance for *Culex* sp. recorded in husk substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	32	16		
Treatment	12	28204.3	2350.36	414.77	0
Error	24	136	5.67		
Total	38	28372.3			

Grand Mean 65.692 CV 3.62



**Fig. 8:** Relationship between *Culex* population and effect of temperature and humidity in husk at different time intervals.

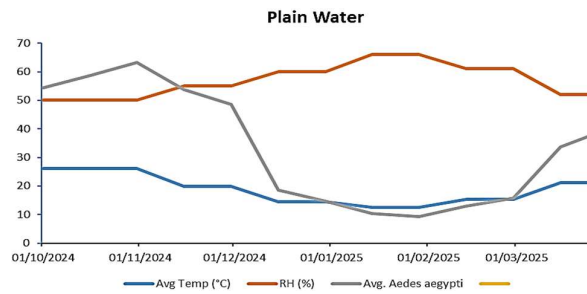
The breeding pattern shown in Figure 9 of *Culex* mosquitoes in the husk substrate show in Figure 6 a clear dependence on seasonal climatic changes, particularly temperature trends. In October 2024, when the temperature averaged 26.1°C with 50% relative humidity, *Culex* populations were at their highest, reaching 122.33 larvae on October 31, indicating that *Culex* mosquitoes prefer warm, semi-organic aquatic habitats like husk water for oviposition. The population remained robust during November, with a slight decrease to 93.67 larvae by the end of the month as the temperature declined to 19.8°C and RH rose slightly to 55%, suggesting moderate tolerance to early seasonal cooling.

A steep decline in mosquito numbers was observed during December and January, when temperatures dropped to 14.4°C–12.5°C and RH increased to 60–66%. Larval counts fell sharply to 19.33 on January 29, indicating that *Culex* activity is significantly suppressed by colder temperatures, even when humidity remains high. However, with the return of milder weather in February (15.4°C) and a further increase to 21.1°C in March, the population began to recover, reaching 74.67 larvae by the end of March, reflecting the species' sensitivity to temperature rise and ability to rebound in late spring.

**Table 8:** Analysis of variance for *Aedes* sp. recorded in plain water substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	2.5	1.23		
Treatment	12	152009	1267.49	1053.62	0
Error	24	28.9	1.2		
Total	38	15241.2			

Grand Mean 33.385 CV 3.29



**Fig. 10:** Relationship between *Aedes* population and effect of temperature and humidity in plain water at different time intervals.

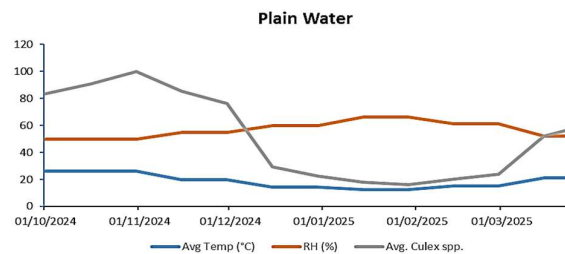
The oviposition trend of *A. aegypti* in plain water showed in Figure 7 a distinct seasonal pattern influenced primarily by temperature variations. During October 2024, when the average temperature was 26.1°C and relative humidity remained at 50%, *Aedes* populations peaked at 63.33 larvae on October 31, indicating that *Aedes* can breed effectively even in clean water when temperatures are favorable. As temperatures dropped to 19.8°C in November and RH rose slightly to 55%, larval counts decreased modestly to 48.67, suggesting initial climatic stress. The absence of organic matter in plain water likely made it less supportive compared to substrates like tree hole debris or leaf-infused water.

A dramatic population decline occurred in December and January, when temperatures fell to 14.4°C and later 12.5°C, with RH reaching 60–66%. During this period, *Aedes* populations declined to 9.33 larvae by January 29, the lowest in the study, confirming *A. aegypti* strong thermal sensitivity and reduced ability to breed in colder conditions, especially in nutrient-poor water. As temperatures started to rise again in February and March, a noticeable recovery occurred, with populations increasing to 40 larvae by March 30, indicating a positive response to warming temperatures despite relatively lower RH (52%).

**Table 9:** Analysis of variance for *Culex* sp. recorded in plain water substrate at different dates of observation

Source	DF	SS	MS	F	P
Replicate	2	8.8	4.41		
Treatment	12	36566.4	3047.2	727.6	0
Error	24	100.5	4.19		
Total	38	36675.7			

Grand Mean 52.179 CV 3.92



**Fig. 11:** Relationship between *Culex* population and effect of temperature and humidity in plain water at different time intervals.

The population dynamics of *Culex spp.* in plain water showed in Figure 8 a clear climatic response, similar to other substrates, but with slightly lower peak values due to the absence of organic matter. In October 2024, when average temperatures were 26.1°C and RH was 50%, *Culex* populations reached their highest levels, peaking at 100 larvae on October 31. This indicates that *Culex* mosquitoes can utilize clean water sources for oviposition effectively under favorable thermal conditions, though slightly less efficiently than in organically enriched substrates like leaf water or husk.

With the onset of cooler weather in November (19.8°C and 55% RH), a moderate decline was observed, with the population dropping to 76.33 by November 30. A much sharper decline occurred during December and January, when temperatures fell to 14.4–12.5°C and humidity increased to 60–66%, with the population reducing drastically to 16.33 larvae by January 29. This decline highlights *Culex* sensitivity to low temperatures, especially in nutrient-poor habitats like plain water, which offer fewer resources for larval development.

As the temperature gradually increased to 15.4°C in February and then 21.1°C in March, *Culex* populations began to recover, reaching 60.67 by March 30. This late-season resurgence emphasizes *Culex* adaptability and rapid response to warming, even in simple water collections. However, the overall larval densities in plain water remained consistently lower than in organic substrates, affirming the role of water quality and nutrient content in mosquito breeding success.

This study revealed strong seasonal dynamics and substrate-specific preferences in the oviposition behaviour of *A. aegypti* and *Culex* mosquitoes. Using ANOVA, significant variation in larval populations was recorded across all dates in every substrate, with extremely high F-values and  $P=0.000$ , reflecting genuine temporal differences and minimal experimental error.

## DISCUSSION

The present study investigated the oviposition preferences and seasonal population dynamics of *Aedes aegypti* and *Culex spp.* in four different aquatic substrates—tree hole debris, leaves mixed in water, husk-infused water, and plain water—under natural field conditions at the University of Agriculture, Faisalabad, from October 2024 to March 2025. Mosquito larvae were recorded every two weeks (biweekly), i.e., on specific sampling dates as defined in the Methods section), and their fluctuations were analyzed in relation to substrate type and environmental factors such as temperature and relative humidity.

A clear seasonal pattern was observed, with peak mosquito populations occurring during October 2024, coinciding with higher ambient temperatures (26.1°C) and moderate relative humidity (~50%). During this period, *A. aegypti* and *Culex spp.* reached maximum densities of 80.33 and 125.33 larvae, respectively, particularly in tree hole debris (as recorded in Table

2&3). A steep decline in larval populations was noted in January and February, with average temperatures dropping to 12.5°C and RH increasing to 66%. These trends strongly suggest that temperature is a primary driver of mosquito oviposition and larval development, whereas high humidity alone cannot compensate for thermal limitations during the colder months. This is consistent with the general understanding of mosquito biology; wherein higher metabolic and reproductive rates are sustained under warmer conditions.

The significant variation in larval densities among substrates revealed that oviposition site selection is not random but influenced by both physical and chemical properties of the breeding medium. Tree hole debris and leaf-infused water consistently supported the highest *A. aegypti* populations, indicating a strong preference for organic-rich substrates. These findings closely align with those reported by Chemjor, (2023), who demonstrated that *A. aegypti* showed a marked preference for ovipositing in organic media such as sewage water and guava leaf extract. In their study, plant-based infusions, especially those aged to enhance microbial activity, significantly increased oviposition rates. Similarly, in the current field study, the decomposing organic matter in tree hole and leaf-mixed water likely released volatile compounds and microbial metabolites that acted as oviposition attractants for *A. aegypti*.

Further support comes from Santos *et al.* (2010), who investigated the influence of odor types fetid versus non-fetid on *A. aegypti* oviposition using various plant-based infusions. They found that both infusion concentration and odor characteristics significantly impacted oviposition, with non-fetid *Anacardium occidentale* infusions attracting the highest egg counts. This supports our observation that mosquito larvae were more abundant in naturally balanced organic substrates that may have emitted attractive but non-repelling odors. While our study did not chemically analyze these substrates, the results suggest that a moderate level of decomposition may optimize oviposition attractiveness, aligning with findings that overly fetid or overly clean water can be less favorable to gravid females.

Similarly, Mulatier *et al.* (2022) found that hay-based and rice straw infusions significantly stimulated oviposition in *Aedes albopictus* (*A. albopictus*), another major dengue vector. Their high oviposition activity index (OAI = 0.62) reflects strong attractiveness, further validating that organic plant matter, whether in natural or artificial forms, can serve as effective oviposition stimulants. Although their study focused on *Aedes albopictus*, the behavioral overlap with *A. aegypti* in terms of substrate preference is noteworthy and supports the broader ecological understanding that members of the *Aedes* genus are highly responsive to decomposing vegetation and microbial byproducts in water.

This concentration-dependent response suggests that while organic cues are attractive, excessive

amounts may lead to repellent effects or indicate habitat overcrowding or poor quality. In our study, the naturally occurring balance of organic matter in tree hole and leaf-infused water likely provided optimal conditions—neither too weak to be unnoticed nor too strong to be avoided. These parallels indicate that organic infusions and substrates need to be carefully balanced to maximize attractiveness in ovitraps and natural habitats alike.

Regarding *Culex* spp., the results presented a somewhat different pattern. While *A. aegypti* showed strong substrate selectivity, *Culex* spp. exhibited greater flexibility, successfully ovipositing in both organic-rich and plain water environments. This aligns with findings from the New South Wales wetland study, which reported that *Culex annulirostris* preferred habitats with specific aquatic macrophytes like *Salvinia molesta*, whereas *Culex quinquefasciatus* showed no particular preference. In our field conditions, *Culex* populations remained consistently high across husk and plain water substrates, reinforcing their broader ecological tolerance and opportunistic breeding behavior. These differences in oviposition strategy between genera suggest that *Culex* spp. may thrive in a wider range of environmental conditions, including urban water bodies, drainage channels, and artificial containers.

Swan et al. (2018) explored oviposition behavior in *A. aegypti* and *Ae. albopictus*, focusing on the role of container surface texture. They found that lined, rugose surfaces attracted significantly more eggs compared to smooth, unlined containers. Although our study did not directly assess surface texture, the preference of *A. aegypti* for substrates like tree hole debris typically lined with decaying organic matter may similarly provide tactile and moisture-retaining properties conducive to egg laying. The study by Swan et al. emphasizes the multi-modal nature of oviposition site selection, where both chemical cues and physical structures play roles. Our field-based results complement these findings by confirming that naturally occurring textured and organic-rich substrates are consistently favored in real environmental settings.

Collectively, these studies underscore the complexity of mosquito oviposition behavior and its sensitivity to both biotic and abiotic cues. The findings of the current research confirm that organic-rich aquatic environments, moderate temperature, and microhabitat feature significantly influence mosquito breeding success. *A. aegypti* showed selective oviposition behavior, concentrating in substrates that likely offered favorable cues for egg and larval development, while *Culex* spp. adopted a more generalist strategy, thriving even in less favorable water conditions.

These insights have important implications for vector control. Timely interventions during peak breeding months (October–November), along with targeted removal or treatment of organic breeding substrates, could significantly reduce larval

development and subsequent adult mosquito emergence. Additionally, understanding the microhabitat preferences of different mosquito genera can inform the design of more effective ovitraps and larval source management programs, particularly in urban and peri-urban areas where container breeding is common.

## Conclusions

This research indicates that both *A. aegypti* and *Culex* spp. prefer oviposition sites that have high organic material and especially the ones with dry leaves and tree hole debris. Besides the fact that breeding sites are made more attractive, natural organic matter may also enhance the breeding environment in terms of larval survival. Plain water was the least preferred in both species. The above findings are significant and should be considered in controlling mosquitoes through the management or eradication of organically-enriched water-holding containers in both residential and peri-urban areas. It is possible to support more precise and permanent methods of managing vectors, and this is because of understanding species-specific oviposition behavior.

**Author Contributions:** A.U. conceived and designed the study. W.A. and F. conducted field sampling and data collection. T.B. and F.K. assisted in experimental setup and laboratory processing. A.A. and M.M. performed data analysis and interpretation. M.Z.A. and S.M.A. contributed to statistical analysis and manuscript drafting. M.W. critically reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

**Acknowledgment:** The authors gratefully acknowledge the support and facilities provided by the Department of Entomology, University of Agriculture, Faisalabad, Pakistan, for conducting this research.

**Conflict of Interest:** All authors have no conflict of interest

**Funding:** No funding was received for this study.

**Data Availability:** All relevant data are included within the article and its supplementary materials.

**Ethics Statement:** Not required

**Generative AI Statements:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

**Publisher's Note:** All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or

claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

## REFERENCES

- Allan, J. D., Castillo, M. M., & Capps, K. A. (2021). Trophic relationships. In *Stream ecology: Structure and function of running waters* (pp. 247–284). Springer International Publishing.
- Bodinga, M. M., Malami, M., Magaji, M., Sifawa, M. S., & Bodinga, L. I. (2025). A comparative study of mosquito breeding sites within and around the college environment: A case study of Sultan Abdulrahman College of Health Technology, Gwadabawa, Sokoto State, Nigeria. *Kashf Journal of Multidisciplinary Research*, 2(9), 16–32.
- Ceretti-Junior, W., Medeiros-Sousa, A. R., de Paula, M. B., Evangelista, E., Barrio-Nuevo, K. M., Wilk-da-Silva, R., ... & Marrelli, M. T. (2025). Species composition and ecological aspects of immature mosquitoes (Diptera: Culicidae) in phytotelmata in Cantareira State Park, São Paulo, Brazil. *Insects*, 16(4), 376.
- Chemjor, R. (2023). *The effects of plant infusions on the collections of the gravid Aedes aegypti for arbovirus surveillance* (Doctoral dissertation, University of Nairobi).
- Chowdhury, R., Faria, S., Chowdhury, V., Islam, M. S., Akther, S., & Akter, S. (2024). Bamboo stumps that are artificially in use put pressure on dengue and chikungunya vector control in Dhaka City, Bangladesh. *Journal of Vector Borne Diseases*, 61(2), 227–235.
- Collins, E. L. (2025). *Utilising genomic approaches to explore genetic diversity and insecticide resistance in Aedes aegypti populations* (Doctoral dissertation, London School of Hygiene & Tropical Medicine).
- de Almeida, N. M., Dias, R. F., Alves, D. C. V., Machado, S. L., Silva, J. D. S., de Mello, C. F., & Alencar, J. (2025). Mosquito fauna (Diptera: Culicidae) in phytotelmata environments of the Guapiaçu Ecological Reserve, Cachoeiras de Macacu, Rio de Janeiro, Brazil. *Frontiers in Ecology and Evolution*, 13, 1525202.
- DONATUS, O. O., Auta, I. K., Ibrahim, B., Yayock, H. C., & Johnson, O. (2022). Breeding site characteristics and mosquito abundance in selected locations within Kaduna metropolis. *FUDMA Journal of Sciences*, 6(6), 70–75.
- Girard, M., Martin, E., Vallon, L., Raquin, V., Bellet, C., Rozier, Y., ... & Minard, G. (2021). Microorganisms associated with mosquito oviposition sites: Implications for habitat selection and insect life histories. *Microorganisms*, 9(8), 1589.
- Hoyochi, I., Padonou, G. G., Tokponnon, T. F., Konkon, A. K., Zoungbédji, D. M., Salako, A. S., ... & Akogbétó, M. C. (2025). Influence of the physicochemical characteristics of mosquito breeding sites in domestic environments on the distribution of Anopheles, Aedes, and Culex mosquitoes in Benin. *Tropical Medicine and Health*, 53(1), 100.
- Hug, D. O., Hugo, E., & Verhulst, N. O. (2025). Avoid the light to avoid the heat? Thermal and light preferences of Aedes aegypti mosquito larvae. *Animal Behaviour*, 219, 123011.
- Ishtiaq, M., Babar, A., Ullah, U. N., & Ikram, R. M. (2021). Assessing the linkage of mosquito diversity with tree holes in Multan. *Agricultural Sciences Journal*, 3(1), 20–25.
- Leroy, B. M. L. (2021). *Effects of aerial insecticide treatments on forest arthropod communities: Limitations and opportunities of ecological impact assessment* (Doctoral dissertation, Technische Universität München).
- Meena, A. R. (2021). *Spatial distribution of dengue vectors*. Booksclinic Publishing.
- Mulatier, M., Boullis, A., & Vega-Rúa, A. (2022). Semiochemical oviposition cues to control Aedes aegypti gravid females: State of the art and proposed framework for their validation. *Parasites & Vectors*, 15(1), 228.
- Nebbak, A., Almeras, L., Parola, P., & Bitam, I. (2022). Mosquito vectors (Diptera: Culicidae) and mosquito-borne diseases in North Africa. *Insects*, 13(10), 962.
- Netshituni, T. V. (2023). *Effects of ash from native and alien plants on phytoplankton biomass and mosquito abundances: A mesocosm experiment*.
- Santos, E., Correia, J., Muniz, L., Meiado, M., & Albuquerque, C. (2010). Oviposition activity of Aedes aegypti L. (Diptera: Culicidae) in response to different organic infusions. *Neotropical Entomology*, 39, 299–302.
- Phiri, J. S. (2024). *Effects of field and laboratory larval inhabited water physical chemicals and background colour on oviposition site selection by gravid female anopheline mosquitoes in Lusaka province* (Doctoral dissertation, The University of Zambia).
- Mudi, S. D., Das, D., & Banerjee, S. (2024). Mosquito Diversity and Mosquito-Borne Mosquitoes: *Biology, Pathogenicity and Management*, 105
- Swan, T., Lounibos, L. P., & Nishimura, N. (2018). Comparative oviposition site selection in containers by Aedes aegypti and Aedes albopictus (Diptera: Culicidae) from Florida. *Journal of Medical Entomology*, 55(4), 795–800.
- Tyagi, B. K., Sarkar, M., Kandasamy, C., & Bhattacharya, S. (2025). Mosquitoes as vectors, pests, and allergens. In *Mosquitoes of India* (pp. 173–190). CRC Press.
- Wilkerson, R. C., Linton, Y. M., & Strickman, D. (2021). Mosquitoes of the world. Johns Hopkins University Press.
- Pautzke, K. C., Felsot, A. S., Reganold, J. P., & Owen, J. P. (2024). Effects of soil on the development, survival, and oviposition of Culex quinquefasciatus (Diptera: Culicidae) mosquitoes. *Parasites & Vectors*, 17(1), 154.
- Simons, J., Oxbrugh, A., Menéndez, R., & Ashton, P. (2023). Micro-habitat features determine oviposition site selection in High Brown and Dark Green Fritillaries. *Journal of Insect Conservation*, 27(5), 841–853.
- Kirsch, J. J., Sermon, J., Jonker, M., Asbeck, T., Gossner, M. M., Petermann, J. S., & Basile, M. (2021). The use of water-filled tree holes by vertebrates in temperate forests. *Wildlife Biology*, 2021(1), 1–4.
- Liu, Y., Liu, L., & Sun, M. (2024). Power requirements in hovering flight of mosquitoes. *Physics of Fluids*, 36(2).
- Rubio, A., Melgarejo-Colmenares, K., Kligler, M., Cardo, M. V., & Vezzani, D. (2025). Container emptying alone falls short: mechanical egg removal enhances Aedes aegypti management. *Pest Management Science*, 81, 3474–3481.
- McKie, B. G., Taylor, A., Nilsson, T., Frainer, A., & Goedkoop, W. (2023). Ecological effects of mosquito control with Bti: evidence for shifts in the trophic structure of soil-and ground-based food webs. *Aquatic Sciences*, 85(2), 47.
- Agus Nurjana, M., Srikandi, Y., Wijatmiko, T. J., Hidayah, N., Isnawati, R., Octaviani, O., ... & Ningsi, N. (2023). Water containers and the preferable conditions for laying eggs by Aedes mosquitoes in Maros Regency, South of Sulawesi, Indonesia. *Journal of Water and Health*, 21(11), 1741–1746.