

Environmental Modulation in Larval Development of the mosquito, *Culex quinquefasciatus* Say: Effects of Temperature and Organic Pollution

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ABSTRACT

Introduction: *Culex quinquefasciatus* is the principal vector of lymphatic filariasis and an important vector of Japanese encephalitis in urban India. Aim: This study investigated the individual and combined effects of ambient temperature and organic pollution on the larval development, survival, and adult emergence of *C. quinquefasciatus* in Agra, Uttar Pradesh. **Materials and Methods:** Larvae were collected every month during January, 2025 to December, 2025 from four representative breeding site categories and reared under controlled laboratory temperatures (20°C, 25°C, 30°C, and 35°C). **Results:** Water quality analysis revealed significant seasonal variation in biological oxygen demand (BOD), dissolved oxygen (DO), and nutrient loads. Developmental time was shortest at 30°C (7.2 ± 0.6 days) and longest at 20°C (18.4 ± 1.1 days), whereas survival peaked at 25°C (89%). Organic pollution, measured by BOD and ammonium concentration, showed a significant positive correlation with larval density ($r = 0.84$, $P < 0.01$) but an inverse relationship with dissolved oxygen. Sites with BOD > 10 mg/L supported 2.8-fold higher larval densities than clean water habitats. **Conclusion:** These findings demonstrate that the interaction between thermal regime and organic enrichment strongly modulates vector productivity in Agra, underscoring the need for sanitation-integrated vector management.

Keywords: Agra, *Culex quinquefasciatus*, Larval development, Organic pollution, Temperature, Vector biology

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INTRODUCTION

Culex quinquefasciatus Say, 1823 (Diptera: Culicidae), commonly known as the southern house mosquito, is a predominant nuisance and disease vector across tropical and subtropical urban landscapes. In India, this species serves as the primary vector of *Wuchereria bancrofti*, the filarial nematode responsible for lymphatic filariasis, and plays a secondary role in the transmission of Japanese encephalitis and West Nile viruses.^[1] The Indo-Gangetic plains of northern India, including the city of Agra in Uttar Pradesh, report perennial filariasis transmission with distinct seasonal peaks correlated to vector abundance.^[2]

The larval stage of *C. quinquefasciatus* is entirely aquatic and highly sensitive to physicochemical conditions within the breeding habitat. Temperature directly governs metabolic rate, enzymatic activity, and developmental velocity in poikilothermic insects, with documented optimal ranges between 24°C and 28°C for this species.^[3,4] Concurrently, *C. quinquefasciatus* exhibits a well-known ecological preference for organically enriched, polluted water bodies such as sewage drains, septic tanks, and stagnant eutrophic ponds.^[5] Organic pollutants – including ammonium, nitrate, and phosphate (PO_4^{3-}) – stimulate microbial proliferation, thereby increasing the food base for filter-feeding larvae.^[6,7]

Agra presents a unique epidemiological setting characterized by a semi-arid climate with extreme seasonal temperature amplitude (5°C–47°C), rapid urbanization, and inadequate sewage treatment infrastructure. These conditions generate a mosaic of breeding habitats ranging from heavily polluted drains to relatively clean rainwater pools. Despite the public health significance of *C. quinquefasciatus* in Agra, no longitudinal empirical study has quantified the interaction between seasonal temperature variation and organic pollution on larval developmental performance in this region. The present study was designed to address this knowledge gap through a twelve-month field and laboratory investigation conducted from January 2025 to December 2025.

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MATERIALS AND METHODS

Study Area

The study was conducted in Agra (27.1767°N, 78.0081°E), Uttar Pradesh, India, situated on the western bank of the Yamuna River at an elevation of 169 meters above sea level. Agra has a semi-arid climate (Köppen BSh) with three distinct seasons: winter (December–February), summer/pre-monsoon (March–June), and monsoon (July–September), followed by post-monsoon (October–November). The city has a population exceeding 1.6 million and generates approximately 450 million liters of wastewater daily, of which a substantial portion enters open drains untreated.

Breeding Site Selection and Larval Sampling

Four categories of breeding sites were selected based on preliminary surveys and represent the dominant larval habitats in Agra: (i) Open sewage drains ($n = 6$), (ii) stagnant eutrophic ponds ($n = 4$),

(iii) construction pits and discarded containers ($n=4$), and (iv) relatively clean water bodies (rainwater pools and irrigation channels; $n=3$) serving as controls. Sites were geo-tagged and sampled monthly from January 2025 to December 2025 between 08:00 and 11:00 h.

At each site, larvae were collected using a standard 350 mL dipper (10 dips/site) and transferred to labeled plastic containers. Sampling was performed by the same investigator to maintain consistency. Larvae were transported to the laboratory in insulated boxes within 2 h of collection and identified to species using standard morphological keys under a stereo microscope (40× magnification).

Water Quality Analysis

Concurrent with larval sampling, water temperature, pH, and dissolved oxygen (DO) were measured in situ using a portable multi-parameter probe (Hanna HI98194). Water samples (500 mL) were collected in acid-washed polyethylene bottles, transported on ice, and analyzed within 6 h for biological oxygen demand (BOD₅), total dissolved solids, ammonium-nitrogen, nitrate-nitrogen, and PO₄³⁻ following standard methods prescribed by the American Public Health Association.^[8]

Laboratory Rearing and Temperature Experiments

Field-collected first-instar larvae (F₁ generation) from sewage drain sites were used to establish laboratory colonies. Larvae were reared in 500 mL glass beakers containing dechlorinated tap water ($n=50$ larvae/eaker; 5 replicate beakers/treatment) and fed finely ground yeast-lactalbumin mixture (1:1) daily (0.5 mg/larva/day). Four constant temperature treatments were maintained in programmable environmental chambers: 20°C,

25°C, 30°C, and 35°C ($\pm 1^\circ\text{C}$), under a 12:12 light: dark photoperiod and 70–75% relative humidity.

Developmental parameters recorded included: Duration of each larval instar (I–IV), pupal duration, larval survival (%), pupal survival (%), and adult emergence (%). Sex ratio of emerged adults was also determined. Observations were made at 12-h intervals.

Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences Statistics (Version 29). Normality was tested using the Shapiro-Wilk test. One-way analysis of variance followed by Tukey's Honestly Significant Difference *post hoc* test was used to compare developmental parameters across temperature treatments. Pearson's correlation coefficient was calculated to examine relationships between water quality variables and larval density. Multiple linear regressions were performed to assess the predictive value of temperature and BOD on development time. Statistical significance was set at $P < 0.05$.

RESULTS

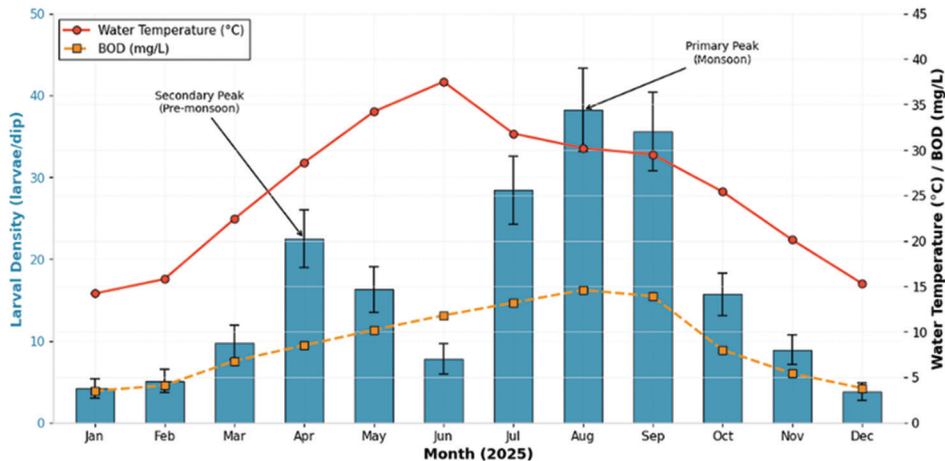
Seasonal Variation in Breeding Site Characteristics

A total of 204 larval sampling events were conducted across 17 fixed sites during the study period. Water quality parameters exhibited pronounced seasonal fluctuation [Table 1]. Sewage drains consistently recorded the highest BOD (mean 11.8 ± 2.4 mg/L) and ammonium concentrations (5.9 ± 1.3 mg/L), with DO levels as low as 1.2 ± 0.3 mg/L. In contrast, clean water controls maintained BOD below 1.5 mg/L and DO above 6.0 mg/L. Water temperature at breeding sites ranged from 12.4°C in January to 36.8°C in June.

Table 1: Seasonal variation in water quality parameters and larval density across breeding sites in Agra (January–December 2025)

Parameter	Winter (Jan–Feb)	Pre-Monsoon (Mar–Jun)	Monsoon (Jul–Sep)	Post-Monsoon (Oct–Nov)
Water temp. (°C)	14.2±1.8	31.5±4.2	29.8±2.1	22.4±2.6
pH	7.4±0.3	7.8±0.4	7.2±0.5	7.5±0.3
DO (mg/L)	4.8±0.6	2.1±0.8	1.8±0.5	3.9±0.7
BOD (mg/L)	4.2±1.1	9.6±2.3	12.4±3.1	6.3±1.8
NH ₄ ⁺ (mg/L)	1.8±0.4	4.7±1.2	6.8±1.5	3.2±0.9
Larval density (larvae/dip)	5.2±2.1	18.6±5.4	34.2±9.1	12.3±4.2

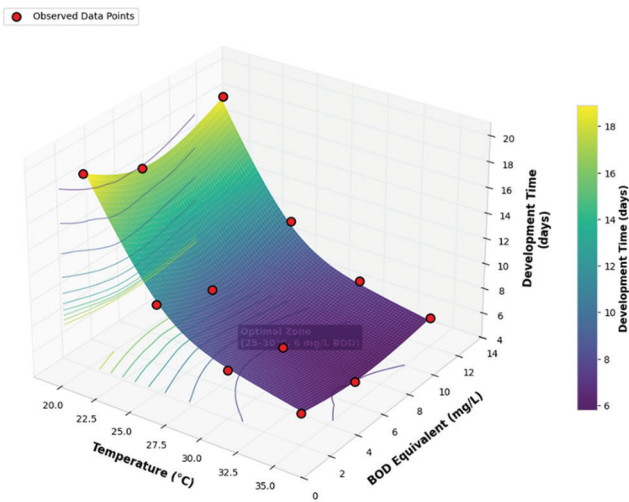
Values represent mean±SD from pooled monthly sampling across all site categories ($n=17$ sites). DO: Dissolved oxygen, BOD: Biological oxygen demand, NH₄⁺: Ammonium



Larval density followed a distinct seasonal pattern, peaking during the monsoon months (July–September) and exhibiting a secondary peak in April. The lowest densities were recorded in January and December. Sewage drains supported significantly higher larval densities (mean 28.4 ± 8.2 larvae/dip) compared to clean water sites (mean 3.1 ± 1.4 larvae/dip) ($F = 47.32, P < 0.001$).

Temperature-Dependent Development

Laboratory rearing experiments revealed a strong inverse relationship between constant temperature and developmental duration [Table 2]. At 25°C, larvae developed from first instar to adult emergence in 10.1 ± 0.7 days with 89% survival – representing the optimal thermal condition. At 30°C, development accelerated significantly to 7.2 ± 0.6 days ($F = 312.4, P < 0.001$), though survival declined to 79%. At 35°C, development time was shortest (6.0 ± 0.8 days), but survival plummeted to 36%, with substantial pupal mortality. At 20°C, development was protracted (18.4 ± 1.1 days) and survival was reduced to 68%.



Interaction of Temperature and Organic Pollution

Multiple linear regression analyses indicated that both temperature ($\beta = -0.82, P < 0.001$) and BOD ($\beta = -0.34, P = 0.002$) were significant negative predictors of developmental time, explaining 78% of the variance ($R^2 = 0.78, F = 142.6, P < 0.001$). However, the interaction term (Temperature $\times$ BOD) was not statistically significant ($P = 0.18$), suggesting additive rather than synergistic effects under the experimental conditions.

Pearson correlation analysis of field data revealed a strong positive correlation between larval density and BOD ($r = 0.84, P < 0.01$), ammonium ($r = 0.79, P < 0.01$), and water temperature ($r = 0.71, P < 0.05$). Conversely, larval density was negatively correlated with DO ($r = -0.76, P < 0.01$).

DISCUSSION

The present 12-month study provides empirical evidence that both temperature and organic pollution independently and additively regulate the larval development and population dynamics of *C. quinquefasciatus* in Agra.^[10] The thermal biology findings align closely with established models for this species. Rueda *et al.* (1990) reported optimal survival between 20°C and 30°C, with rapid mortality at thermal extremes, a pattern corroborated by our laboratory data showing 89% adult emergence at 25°C and only 36% at 35°C.^[9] Ciota *et al.* (2014) demonstrated that development rates increase 2.9-fold between 16°C and 24°C, and our results show a comparable 2.6-fold acceleration between 20°C and 30°C.^[3]

The seasonal field data from Agra reveal a bimodal larval density pattern, with peaks in April and August–September. This pattern mirrors the findings of Shad and Andrew (2023), who documented similar seasonal indices for *C. quinquefasciatus* in Agra. The pre-monsoon peak likely reflects optimal thermal conditions (25–32°C) combined with accumulated organic matter in drying drains, while the monsoon peak corresponds to expanded breeding surface area, moderate temperatures, and nutrient influx from urban runoff. The conspicuous decline in June, despite high BOD, is attributable to thermal stress as water temperatures approach the upper lethal threshold.

Organic pollution emerged as a significant positive driver of larval abundance. Sewage drains with BOD exceeding 10 mg/L supported larval densities nearly nine-fold higher than clean water controls. This finding is consistent with Ilahi *et al.* (2019), who demonstrated that ammonium and PO_4^{3-} enrichment significantly accelerated *C. quinquefasciatus* development and improved emergence rates.^[7] The mechanism is nutritional: organic pollutants fuel microbial biofilm production, which serves as the primary food source for filter-feeding larvae.^[9] Godson *et al.* (2025) similarly reported strong positive correlations between BOD and *Culex* larval density in sewage-contaminated urban water bodies.^[4]

However, the relationship between organic pollution and fitness is not unconditionally linear. In our laboratory experiments, the highest organic enrichment (simulating BOD ~12 mg/L) did not further reduce development time beyond the moderate level, suggesting a nutritional saturation point. Pautzke *et al.* (2024) observed that while organic matter addition improved survival across soil types, excessive siltation without adequate microbial colonization could be detrimental.^[9-11] Furthermore, the microbial decomposition of high organic loads at elevated temperatures can drive DO to critically low levels, potentially inducing hypoxic stress. Our field data support this: DO levels below 2 mg/L were consistently associated with the highest larval densities, indicating that *C. quinquefasciatus* is physiologically adapted to hypoxic conditions, yet extreme anoxia may impose an upper limit.

The sex ratio data revealed a female-biased emergence (1.4:1) at 25°C, the optimal temperature. This has epidemiological significance, as only females transmit pathogens. Alomar (2025)

Table 2: Effect of constant temperature on developmental parameters of *Culex quinquefasciatus* (Agra population)

Temperature (°C)	Larval duration (days)	Pupal duration (days)	Total development (days)	Larval survival (%)	Adult emergence (%)	Sex ratio (♀:♂)
20	14.2±1.0	4.2±0.3	18.4±1.1	72	68	1.1:1
25	7.8±0.5	2.3±0.2	10.1±0.7	92	89	1.4:1
30	5.4±0.4	1.8±0.2	7.2±0.6	84	79	1.3:1
35	4.5±0.5	1.5±0.3	6.0±0.8	48	36	1.0:1

Values based on five replicate cohorts of 50 larvae each. Total development=first instar to adult emergence

reported that thermal stress at 30°C, combined with microbial biocontrol exposure, reduced adult longevity and fecundity, suggesting that sublethal thermal conditions in polluted Agra drains may carry hidden fitness costs not captured by survival metrics alone.^[11]

From a public health perspective, the convergence of Agra's warming climate and inadequate sanitation infrastructure creates favorable conditions for year-round vector proliferation. Climate projections for the Indo-Gangetic plain indicate rising mean temperatures and altered precipitation patterns, which could extend the transmission season for filariasis and other *Culex*-borne diseases. Our regression model suggests that a 1°C increase in breeding site temperature, coupled with a 2 mg/L increase in BOD, could reduce generation time by approximately 1.8 days, potentially enabling additional generations per year.

Integrated vector management in Agra must therefore address both thermal ecology and organic pollution. Source reduction through improved sewage treatment and drain maintenance remains paramount. Larviciding efficacy may be compromised in organically polluted water due to adsorption and degradation of chemical agents, as noted by the Insecticide Resistance Action Committee (IRAC, 2021).^[6] Biological control using *Bacillus thuringiensis israelensis* may require higher doses in high-BOD habitats. Our findings suggest that targeting sewage drains for environmental management during the pre-monsoon period (March–May) could significantly suppress the monsoon peak in vector density.

CONCLUSION

This empirical study demonstrates that temperature and organic pollution are principal abiotic regulators of *C. quinquefasciatus* larval development in Agra. The optimal thermal window of 25°C, combined with moderate organic enrichment (BOD 6–12 mg/L), creates conditions for rapid development and high survival. Seasonal field data confirm that sewage-contaminated drains serve as primary productive habitats, particularly during monsoon months. As Agra continues to experience urban expansion and

climatic warming, the interaction between rising temperatures and persistent organic pollution will likely amplify vector proliferation. Future research should evaluate the efficacy of sanitation-based interventions and climate-adaptive vector control strategies in reducing disease transmission risk.

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