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Abstract

Although nitrate is one of the most common fertiliser-derived contaminants in mosquito habitats, its public-health implications for mosquito development and insecticide susceptibility remain poorly understood. This study examined how nitrate enrichment influences biological traits that directly affect vector proliferation and the effectiveness of insecticide-based control strategies. Larvae were reared from hatch across a gradient of nitrate concentrations (0–1024 mg/L) and monitored through emergence. Developmental outcomes (duration of immature stages, survivorship, larval growth rate), metabolic reserves (lipid, protein, glycogen, glucose), susceptibility to permethrin (0.75%), and detoxification enzyme activity (esterases, glutathione-S-transferase) were estimated following standard procedures. High nitrate concentrations significantly prolonged larval and pupal development, while survivorship remained high (>90%). Larval growth rate peaked at moderate enrichment (16 mg/L). All metabolic reserves increased with nitrate exposure, indicating improved physiological condition. Importantly, nitrate-reared mosquitoes exhibited substantially reduced susceptibility to permethrin and elevated detoxification enzyme activity. These findings highlight nitrate pollution as an overlooked environmental driver that can enhance mosquito robustness and reduce the efficacy of insecticide-based interventions. As nutrient runoff intensifies with agricultural expansion, integrating environmental nutrient management into vector-control planning may be essential for sustaining the impact of current dengue and arbovirus prevention strategies.

Keywords: *Aedes aegypti*, Fertilisers, Environmental pollution, Agrochemicals, environmental impact

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Declarations

Financial interests

The authors declare they have no financial interests. All authors certify that they have no affiliations with or involvement in any organisation or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Authors' Contributions

Conceptualisation: Ezekiel Bala, Azubuike Christian Ukubuiwe, Chinenye Catherine Ukubuiwe, Ifeanyi Famous Ossamulu, Zainab Shehu Abubakar, and Israel Kayode Olayemi; **Methodology:** Ezekiel Bala, Azubuike Christian Ukubuiwe, Israel Kayode Olayemi; **Formal analysis and investigation:** Ezekiel Bala, Azubuike Christian Ukubuiwe, Chinenye Catherine Ukubuiwe, Zainab Shehu Abubakar, Ifeanyi Famous Ossamulu; **Writing – original draft:** Ezekiel Bala, Chinenye Catherine Ukubuiwe, Zainab Shehu Abubakar; **Writing – review & editing:** Azubuike Christian Ukubuiwe, Ifeanyi Famous Ossamulu, Israel Kayode Olayemi; **Funding acquisition:** Israel Kayode Olayemi, Azubuike Christian Ukubuiwe, Ifeanyi Famous Ossamulu; **Resources:** Ezekiel Bala, Azubuike Christian Ukubuiwe, Chinenye Catherine Ukubuiwe, Zainab Shehu Abubakar, Ifeanyi Famous Ossamulu, Israel Kayode Olayemi; **Supervision:** Azubuike Christian Ukubuiwe, Israel Kayode Olayemi, Ifeanyi Famous Ossamulu; **Data curation:** Ezekiel Bala, Chinenye Catherine Ukubuiwe, Azubuike Christian Ukubuiwe, Ifeanyi Famous Ossamulu; **Investigation:** Chinenye Catherine Ukubuiwe, Azubuike Christian Ukubuiwe, Ifeanyi Famous Ossamulu; **Validation:** Azubuike Christian Ukubuiwe, Israel Kayode Olayemi

Introduction

Agriculture remains the backbone of many developing economies, providing food security, sustaining human and livestock populations, and contributing substantially to national income [1]. In Nigeria, the agricultural sector continues to play a central role in economic growth and remains a major contributor to the national gross domestic product [2]. Rapid population expansion has intensified the demand for food production, necessitating increased agricultural output to meet rising needs [3]. This demand has driven the widespread use of agrochemical inputs—including fertilisers, herbicides, insecticides, weedicides, and

pesticides—to enhance crop productivity [4]. While these inputs are effective in boosting yields, they also introduce substantial quantities of chemical residues into the environment, altering the physicochemical properties of agricultural ecosystems and affecting insect diversity [5], including mosquito populations [6].

Agricultural practices contribute to the creation of persistent mosquito breeding habitats, as irrigation systems, drainage channels, and fertiliser-enriched puddles provide stable aquatic environments that support mosquito breeding throughout the year [6, 7]. Several studies have linked agricultural inputs to fluctuations in the physico-chemical characteristics [8 – 10] and mosquito species abundance [11] in mosquito larval habitats. As these observations arise from the interplay of several environmental factors, it is often difficult to elucidate the contribution of individual agricultural input or their components on mosquito development. This limitation underscores the need for controlled laboratory studies that quantitatively evaluate how specific physico-chemical factors influence mosquito biology.

Existing laboratory-based investigations have demonstrated that agricultural inputs such as urea fertiliser [12] and rice-farming herbicides like 2,4-Dichlorophenoxyacetic acid [13] can significantly affect the development of *Culex pipiens pipiens*. Other studies have examined how water hardness [14, 15], temperature [16 – 18], and pH [19, 20] influence fitness indices in *Cx. quinquefasciatus* and *Anopheles* species. Despite these advances, there remains a paucity of laboratory-based research on how nitrate enrichment—a common consequence of fertiliser runoff—affects fitness indices in *Aedes aegypti*.

Aedes aegypti is a medically important mosquito species responsible for transmitting pathogens that cause yellow fever, dengue fever, Zika virus disease, and other arboviral infections. Nevertheless, little is known about how nitrate contamination in larval habitats influences their development, metabolic physiology, or susceptibility to insecticides. Understanding these interactions is essential, as environmental conditions that enhance mosquito robustness or reduce insecticide susceptibility may undermine current vector-control strategies.

This study investigates how nitrate enrichment modulates key biological traits in *Aedes aegypti* such as duration of immature development, growth rates, immature survivorship, metabolic reserve profiles (lipid, protein, and carbohydrate), susceptibility to permethrin insecticides, and detoxifying enzyme profiles. These traits are critical to the success of mosquitoes, and influence disease transmission dynamics.

We hypothesised that: increasing nitrate concentrations will prolong larval and pupal development but maintain high survivorship of *Ae. aegypti*; nitrate enrichment will elevate metabolic reserves; exposure to higher nitrate levels will reduce susceptibility to permethrin; nitrate enrichment will upregulate the activity of resistance-associated enzymes, particularly esterases and glutathione-S-transferases; and nitrate enrichment will positively correlate with multiple indices of biological fitness, potentially enhancing the resilience and vectorial capacity.

This study, thus, aims to provide mechanistic insights into how nitrate contamination affects mosquito development, physiology, and insecticide response—information that is critical for designing environmentally informed vector-control strategies.

Materials and Methods

Eggs Collection and Mosquito Rearing

Aedes aegypti eggs were collected using ovitraps deployed across diverse habitats within the Federal University of Technology, Minna, North-central Nigeria. Sampling was conducted between June and September 2023, coinciding with the peak of the rainy season, following the established protocols [21]. The collected eggs were preserved and subsequently transferred into plastic hatching trays containing distilled water. Hatching was stimulated by the addition of yeast and incubation at ambient room temperature for 24 hours, as described earlier [14]. The insectary was maintained under controlled conditions of a 12:12 h light: dark cycle, with a mean temperature of 28.00 ± 1.00 °C and relative humidity of $80.16 \pm 4.26\%$.

Hatched larvae were reared in well-labelled plastic trays ($30 \times 25 \times 5$ cm) at a density of one larva per 4 mL of water. Larvae were fed Coppens® fish feed, gently sprinkled on the water surface at a rate of 0.32 mg per 100 larvae every other day. Rearing water was replaced daily to prevent debris accumulation and scum formation. Pupae were separated daily into labelled bowls and allowed to emerge in adult-holding cages. Emerged adults were maintained on a 10% sucrose solution provided via sterile cotton wool [15].

Preparation of Nitrate Culture Media

Potassium nitrate (KNO_3 ; analytical grade) was used as the nitrate source for larval rearing experiments. Stock solutions were prepared by dissolving accurately weighed quantities of KNO_3 in distilled, deionised water to ensure purity and consistency. From these stocks, five working concentrations were formulated: 4, 16, 64, 256, and 1024 mg/L. These concentrations were selected to represent a gradient of nitrate enrichment, ranging from levels comparable to minimally impacted freshwater habitats to those characteristic of heavily nutrient-enriched environments.

All solutions were freshly prepared before use to minimise chemical degradation and microbial contamination. Each concentration was dispensed into labelled plastic rearing trays, which served as the culture media for larval development. Distilled, deionised water without nitrate supplementation was used as the control medium. The pH of the solutions was monitored to ensure consistency across treatments, and all trays were maintained under identical insectary conditions (12:12 h light: dark cycle, 28.00 ± 1.00 °C, $80.16 \pm 4.26\%$ relative humidity). These were to ensure that the observed differences in larval development, metabolic reserves, and insecticide resistance could be attributed to nitrate enrichment rather than extraneous environmental variation.

Duration of Immature Development

Larval duration of development was monitored systematically to quantify the duration of immature stages. Cohorts of larvae were observed twice daily, at 06:00 and 18:00 h, to record transitions between successive instars, pupation, and adult emergence, following established procedures [22]. Care was taken to minimise disturbance during observations to avoid stress-induced developmental delays. The total larval duration (TLD) was calculated as the cumulative time spent across the four instars (L1–L4). The total immature duration was defined as the sum of the duration of larval development (TLD) and pupal stages, representing the complete pre-adult developmental period. Adult emergence was recorded as the endpoint of immature development.

Larval Growth Rate

Growth rate was calculated as the ratio of adult dry weight at eclosion to larval development time, representing average daily biomass gain (Timmermann and Briegel, 1999). The growth rate of *Aedes aegypti* larvae was assessed as an integrative measure of biomass accumulation during development. Newly emerged adults were collected immediately upon eclosion to eliminate variation in body weight associated with post-emergence feeding or metabolic activity.

To obtain dry weight, adults were oven-dried at 60 °C for 48 hours until constant weight was achieved. Each individual adult mosquito was weighed using a calibrated analytical microbalance with a precision of ± 0.01 mg, ensuring accuracy and reproducibility of measurements. Larval development time was recorded as the number of days from hatching (L1) to adult emergence, based on twice-daily monitoring of instar transitions, pupation, and eclosion.

Determination of Larval Survivorship and Adult Longevity

Larval survivorship was assessed as a measure of cohort viability across successive developmental stages. Survivorship was expressed as the proportion of individuals successfully transitioning from one stage to the next, beginning with first instar larvae (L1) and continuing through pupation and adult emergence. Twice-daily monitoring allowed precise documentation of stage-specific transitions, and mortality was recorded whenever individuals failed to progress. This approach provided a quantitative measure of survival under varying nitrate treatments, enabling comparisons of environmental effects on developmental success.

Adult longevity was determined by monitoring newly emerged adults maintained under standardised insectary conditions. Following emergence, adults were transferred into labelled holding cages and provided with a 10% sucrose solution absorbed in sterile cotton wool as their sole nutritional source [22]. Longevity was recorded as the number of days each adult survived post-emergence, with daily checks conducted to remove and document deceased individuals. Care was taken to ensure uniform feeding conditions and minimise external stressors, thereby allowing longevity measurements to reflect intrinsic physiological differences attributable to larval rearing conditions.

Insecticide Susceptibility and Enzyme Activity

Adult susceptibility to permethrin insecticide was evaluated using World Health Organisation (WHO) standard insecticide-impregnated papers containing 0.75% permethrin, following established guidelines [23, 24]. Batches of non-blood-fed, 3–5-day-old adult *Aedes aegypti* mosquitoes were introduced into WHO test tubes lined with permethrin-treated papers. Each exposure assay was conducted for the recommended duration of 60 minutes, during which knockdown was monitored at regular intervals. Control groups were simultaneously exposed to untreated papers to account for background mortality.

Following exposure, mosquitoes were transferred into clean holding tubes lined with untreated papers and provided with a 10% sucrose solution absorbed in sterile cotton wool. Recovery was monitored for 24 hours under standard insectary conditions (12:12 h light: dark cycle, 28.00 ± 1.00 °C, $80.16 \pm 4.26\%$ relative humidity). Mortality was recorded at the end of the recovery period, with individuals failing to stand or fly considered dead.

The percentage mortality was calculated for each replicate, and Abbott's correction was applied when control mortality ranged between 5% and 20%. Assays with control mortality exceeding 20% were discarded and repeated. Susceptibility status was classified according to WHO criteria: mortality $\geq 98\%$ indicated susceptibility, mortality between 90–97% suggested possible resistance requiring confirmation, and mortality $< 90\%$ was considered indicative of resistance.

Determination of Detoxification Enzymes (Esterase and Glutathione S-Transferase) Activities

The activities of esterase and glutathione S-transferase (GST) were quantified to assess metabolic detoxification capacity in adult *Aedes aegypti*, following established procedures [25] with minor modifications. Non-blood-fed adults (3–5 days old) were immobilised by freezing and homogenised individually in 200 μL of ice-cold phosphate buffer (0.1 M, pH 7.5) using a glass tissue homogeniser. Homogenates were centrifuged at $10,000 \times g$ for 15 minutes at 4 °C, and the resulting supernatants were collected as crude enzyme extracts. Extracts were kept on ice and assayed immediately to minimise enzyme degradation.

Esterase activity was determined using α -naphthyl acetate (0.3 mM) as substrate. The reaction mixture contained 50 μL of enzyme extract and 450 μL of substrate solution in phosphate buffer. After incubation at room temperature (28 °C) for 15 minutes, the liberated α -naphthol was coupled with Fast Blue B salt (0.1% w/v), and absorbance was measured spectrophotometrically at 570 nm. Enzyme activity was expressed as $\mu\text{mol } \alpha\text{-naphthol}$ formed per minute per mg protein ($\mu\text{mol}/\text{min}/\text{mg}$ protein).

Glutathione S-transferase activity was assayed using reduced glutathione (GSH; 5 mM) and 1-chloro-2,4-dinitrobenzene (CDNB; 1 mM) as substrates. The reaction mixture contained 50 μL of enzyme extract and 450 μL of substrate solution and was incubated at 28 °C. The conjugation of CDNB with GSH was monitored by measuring the increase in absorbance at 340 nm over 5 minutes. Activity was expressed as nmol conjugated product formed per minute per mg protein ($\text{nmol}/\text{min}/\text{mg}$ protein).

Protein concentration in each enzyme extract was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard, and expressed in mg/mL [26]. Enzyme activities were normalised to protein content to ensure comparability across samples. All assays were performed in triplicate, and results were reported as mean $\pm$ standard error.

Biochemical Analysis of Nutritional Reserves

The nutritional reserves of *Aedes aegypti* were quantified to evaluate physiological condition under varying nitrate treatments. Ten individuals, each of fourth-instar larvae, pupae, and newly emerged adults from every treatment group, were analysed for lipid, glucose, glycogen, and protein content, following established protocols [17, 27].

Sample preparation: Each specimen was homogenised in 200 μL of 2% sodium sulfate solution using a glass tissue homogeniser. Homogenates were extracted with 400 μL of chloroform: methanol (1:1 v/v) and centrifuged at 3,000 rpm for 1 minute. The resulting fractions were separated: the organic phase was used for lipid analysis, the aqueous phase for sugar analysis, and the pellet for glycogen determination.

Lipid reserve quantification: The organic phase was evaporated at 100 °C to dryness, treated with 200 μL of concentrated sulfuric acid, and heated at 90 °C for 10 minutes. After cooling, samples were reacted with vanillin reagent (0.2% vanillin in 17% phosphoric acid).

Absorbance was measured at 625 nm using a spectrophotometer. Lipid content was expressed as micrograms of lipid per milligram of body weight ($\mu\text{g}/\text{mg}$).

Glucose and glycogen reserves quantification: The aqueous phase was evaporated to a final volume of 150 μL , treated with 200 μL of anthrone reagent (0.2% anthrone in concentrated sulfuric acid), heated at 90 °C for 10 minutes, cooled to room temperature, and the absorbance was read at 625 nm. Glycogen was quantified using the pellet fraction, which was resuspended in 200 μL of distilled water, treated with anthrone reagent, and processed similarly. Both glucose and glycogen contents were expressed as micrograms per milligram of body weight ($\mu\text{g}/\text{mg}$).

Protein reserve quantification: Protein concentration was determined using the Bradford assay [26] (Bradford, 1976). Homogenates were centrifuged at 400 rpm for 20 minutes at 4 °C, and 20 μL of supernatant was mixed with 200 μL of Bradford reagent. After incubation at room temperature for 10 minutes, absorbance was measured at 595 nm. Protein content was expressed as milligrams per millilitre (mg/mL), using bovine serum albumin (BSA) as the standard.

All biochemical reserve values were reported as mean $\pm$ standard deviation. Lipid, sugar, and glycogen contents were expressed relative to body weight ($\mu\text{g}/\text{mg}$), while protein concentration was expressed per unit volume (mg/mL). This standardised approach ensured comparability across developmental stages and treatments, thereby linking nitrate enrichment to physiological fitness in *Aedes aegypti*.

Data Analysis

All data were analysed using R (version 4.3.2) and SPSS (version 26.0). Before analysis, data were checked for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene’s test. Where assumptions of parametric tests were met, one-way analysis of variance (ANOVA) was used to compare means across nitrate concentrations. Significant differences were further explored using Tukey’s HSD post-hoc test. For non-parametric data, the Kruskal–Wallis test followed by Dunn’s multiple comparison test was applied.

Developmental parameters (larval duration, pupation time, survivorship, and growth rate) were analysed as continuous variables. Survivorship data were arcsine-transformed before analysis. Insecticide susceptibility was expressed as percentage mortality and analysed using probit regression to estimate lethal concentration (LC_{50}). Enzyme activity (esterase and glutathione-S-transferase) and metabolic reserves (lipid, protein, glycogen, and glucose) were compared across treatments using ANOVA. Correlations between nitrate concentration and biological fitness indices were evaluated using Pearson’s correlation coefficient. All results are presented as mean $\pm$ standard error (SE), and statistical significance was set at $p < 0.05$.

Results

Effects of Nitrate Enrichment on Developmental Indices of *Aedes aegypti*

Duration of Development:

Nitrate enrichment exerted a significant ($p < 0.05$) effect on the duration of post-embryonic development of *Ae. aegypti* (Figures 1A–1C). The total larval duration (TLD), pupal duration (PD), and total immature duration (TID) varied consistently across treatments, with the shortest developmental times observed at 16 mg/L and the longest at 1024 mg/L nitrate

concentration. At low nitrate (0–16 mg/L), development duration was relatively long; however, as nitrate increased (64–256 mg/L), duration of development shortened significantly ($p < 0.05$). At the highest level (1024 mg/L), duration of development increased again, suggesting possible stress or toxicity.

Analyses revealed that TLD ranged from 6.72 days (95% CI: 6.09–7.35) to 9.09 days (95% CI: 8.24–9.95) (Figure 1A). Pupal duration ranged from 1.55 days (95% CI: 1.36–1.74) to 2.08 days (95% CI: 1.79–2.36) (Figure 1B). Similarly, TID ranged from 8.28 days (95% CI: 7.79–8.76) to 11.18 days (95% CI: 10.24–12.11) (Figure 1C).

Larval Growth Rate (LGR): The LGR increases steadily with nitrate enrichment up to 256 mg/L, then at 1024 mg/L, it dropped sharply and was significantly ($p < 0.05$) different from the mid-range treatments. Quantitatively, the LGR was slowest at 1024 mg/L (Mean = 10.40×10^6 mg/day, 95% CI [9.24, 11.56]) and fastest at 16 mg/L (Mean = 19.99×10^6 mg/day, 95% CI [18.06, 21.92]) (Figure 1D).

Survivorship of Immature Stages: Nitrate enrichment did not exert a statistically significant ($p > 0.05$) effect on the survivorship of *Aedes aegypti* larvae or pupae (Figures 2A–2C). Across all treatments, survivorship of the immature stages remained consistently high, with mean values exceeding 90%. For the larval stage, average survivorship (ALS) was lowest at 64 mg/L (Mean = 98.99%, 95% CI: 97.13–100.87) and highest at 256 mg/L (Mean = 99.75%, 95% CI: 98.95–100.55) (Figure 2A). Pupal survivorship (PS) ranged from a minimum of 91.57% (95% CI: 74.83–108.31) at 4 mg/L to a maximum of 99.00% (95% CI: 95.82–102.18) at 1024 mg/L (Figure 2B). Across the entire immature period, average survivorship (AIS) was lowest at 4 mg/L (Mean = 97.71%, 95% CI: 93.52–101.91) and highest at 1024 mg/L (Mean = 99.40%, 95% CI: 98.18–100.62) (Figure 2C).

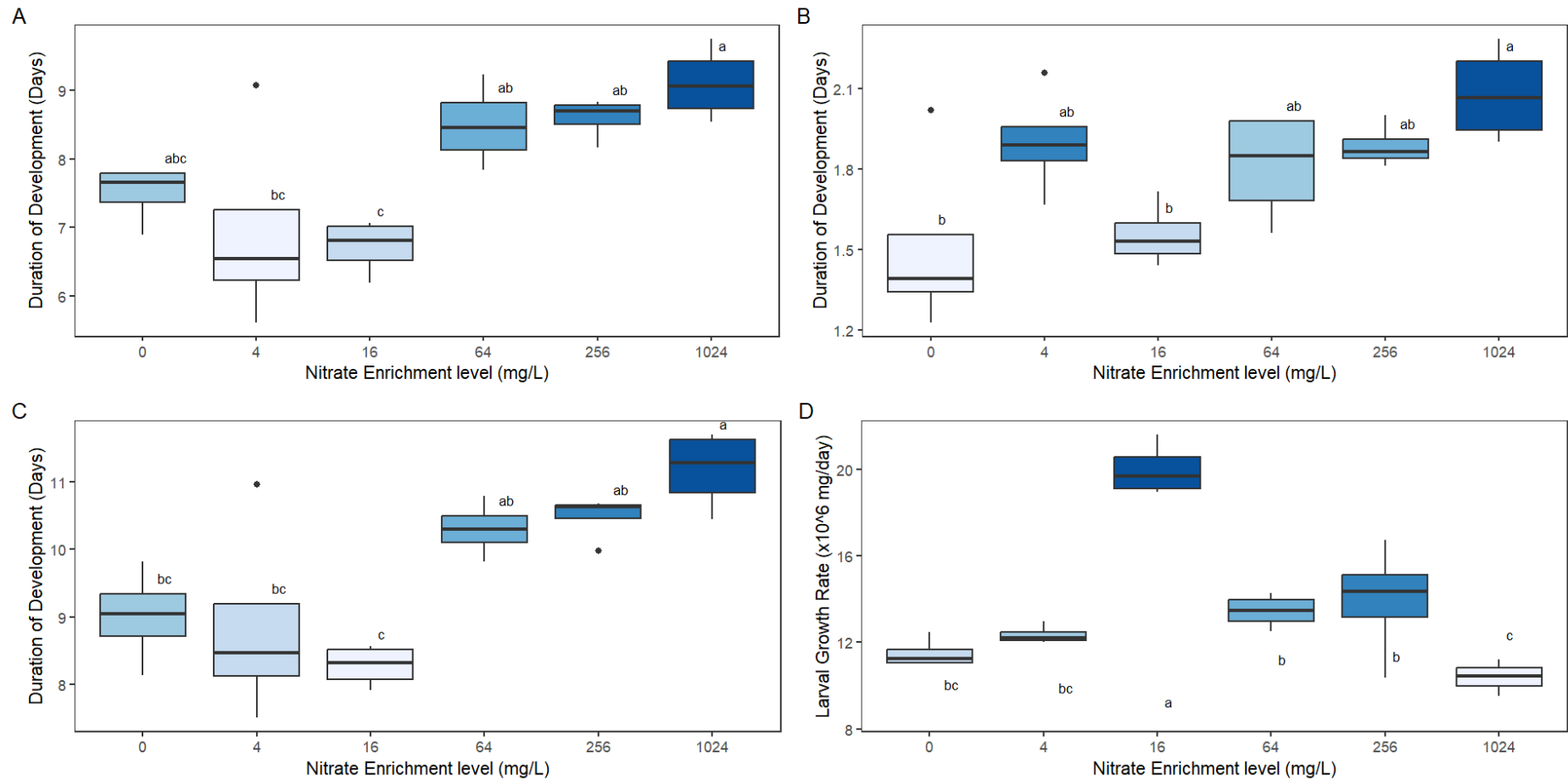


Figure 1: Non-linear effects of nitrate enrichment on *Aedes aegypti* Mosquito larval performance. (A) Total Larval (B) Pupal (C) Total Immature Duration of development (D) Larval Growth Rate. Moderate nitrate enrichment (64–256 mg/L) accelerated larval development and growth, while both low and excessive levels prolonged development and reduced growth.

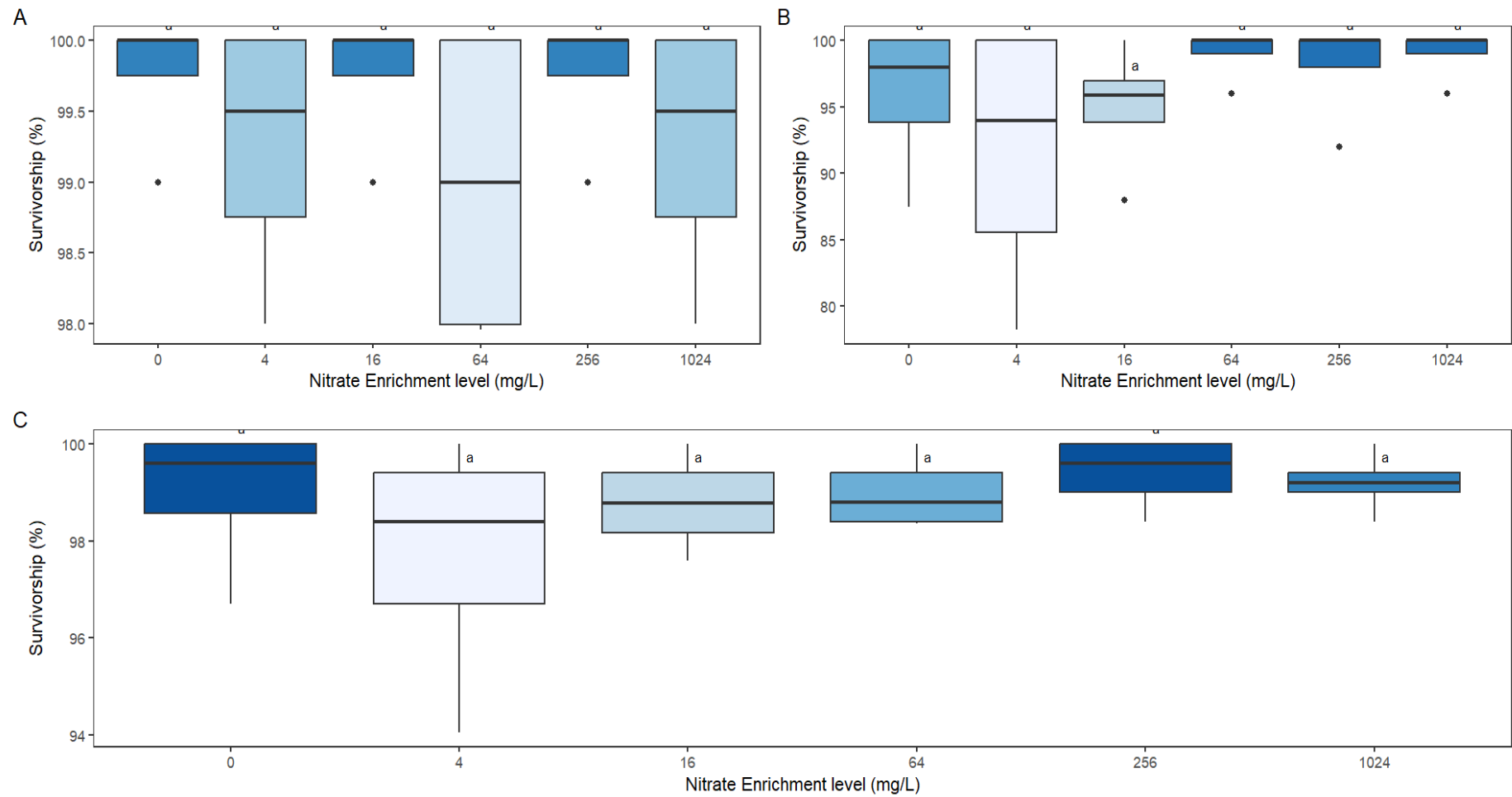


Figure 2: Effects of Nitrate Levels on (A) Average Larval (B) Pupal (C) Average Immature Survivorship of Adult *Aedes aegypti* Mosquito.

Effects of Nitrate Enrichment on Metabolic Reserve of Critical Life Stages of *Aedes aegypti*

Nitrate enrichment significantly ($p < 0.05$) affected the metabolic reserves of critical life stages of *Aedes aegypti*—the fourth larval instar, pupae, and adults (Figures 3–5). Analyses revealed a concentration-dependent increase across all reserve components, with reserve levels consistently and significantly ($p < 0.05$) lowest at lower nitrate concentrations (4 and 16 mg/L) and highest at elevated concentrations (256 and 1024 mg/L) (Figures 3 to 5).

Lipid reserves: This varied across life stages; being lowest in adults, ranging from 14.83 µg/mosquito (95% CI: 14.14–15.53) to 27.42 µg/mosquito (95% CI: 26.96–27.89) (Figure 5), and highest in pupae, ranging from 17.35 µg/mosquito (95% CI: 16.29–18.41) to 35.31 µg/mosquito (95% CI: 34.91–35.70) (Figure 4). Larval values fell between these extremes, ranging from 13.41 µg/mosquito (95% CI: 12.72–14.10) to 28.93 µg/mosquito (95% CI: 27.95–29.91) (Figure 3).

Protein reserves: Though with a different pattern, protein reserves were lowest in fourth-instar larvae, ranging from 16.42 µg/mosquito (95% CI: 15.35–17.49) to 39.94 µg/mosquito (95% CI: 39.29–40.59) (Figure 3). Adults exhibited the highest protein reserves, ranging from 25.22 µg/mosquito (95% CI: 24.43–26.01) to 43.80 µg/mosquito (95% CI: 42.89–44.70) (Figure 5). Pupae displayed intermediate but elevated values, ranging from 19.92 µg/mosquito (95% CI: 19.23–20.60) to 44.83 µg/mosquito (95% CI: 43.79–45.88) (Figure 4).

Glucose reserves were lowest in pupae, ranging from 6.05 µg/mosquito (95% CI: 5.14–6.95) to 18.64 µg/mosquito (95% CI: 17.64–19.64) (Figure 4). Adults had the highest glucose reserves, ranging from 7.78 µg/mosquito (95% CI: 7.49–8.07) to 18.86 µg/mosquito (95% CI: 18.25–19.46) (Figure 5). Larval values ranged from 8.09 µg/mosquito (95% CI: 7.25–8.94) to 15.77 µg/mosquito (95% CI: 14.92–16.62) (Figure 3).

Glycogen reserves were highest in fourth-instar larvae, ranging from 37.98 µg/mosquito (95% CI: 37.50–38.46) to 68.31 µg/mosquito (95% CI: 67.89–68.73) (Figure 3). Adults exhibited the lowest glycogen reserves, ranging from 29.26 µg/mosquito (95% CI: 28.29–30.22) to 67.85 µg/mosquito (95% CI: 66.88–68.81) (Figure 5). Pupae showed intermediate values, ranging from 41.31 µg/mosquito (95% CI: 40.63–41.98) to 76.41 µg/mosquito (95% CI: 75.76–77.07) (Figure 4).

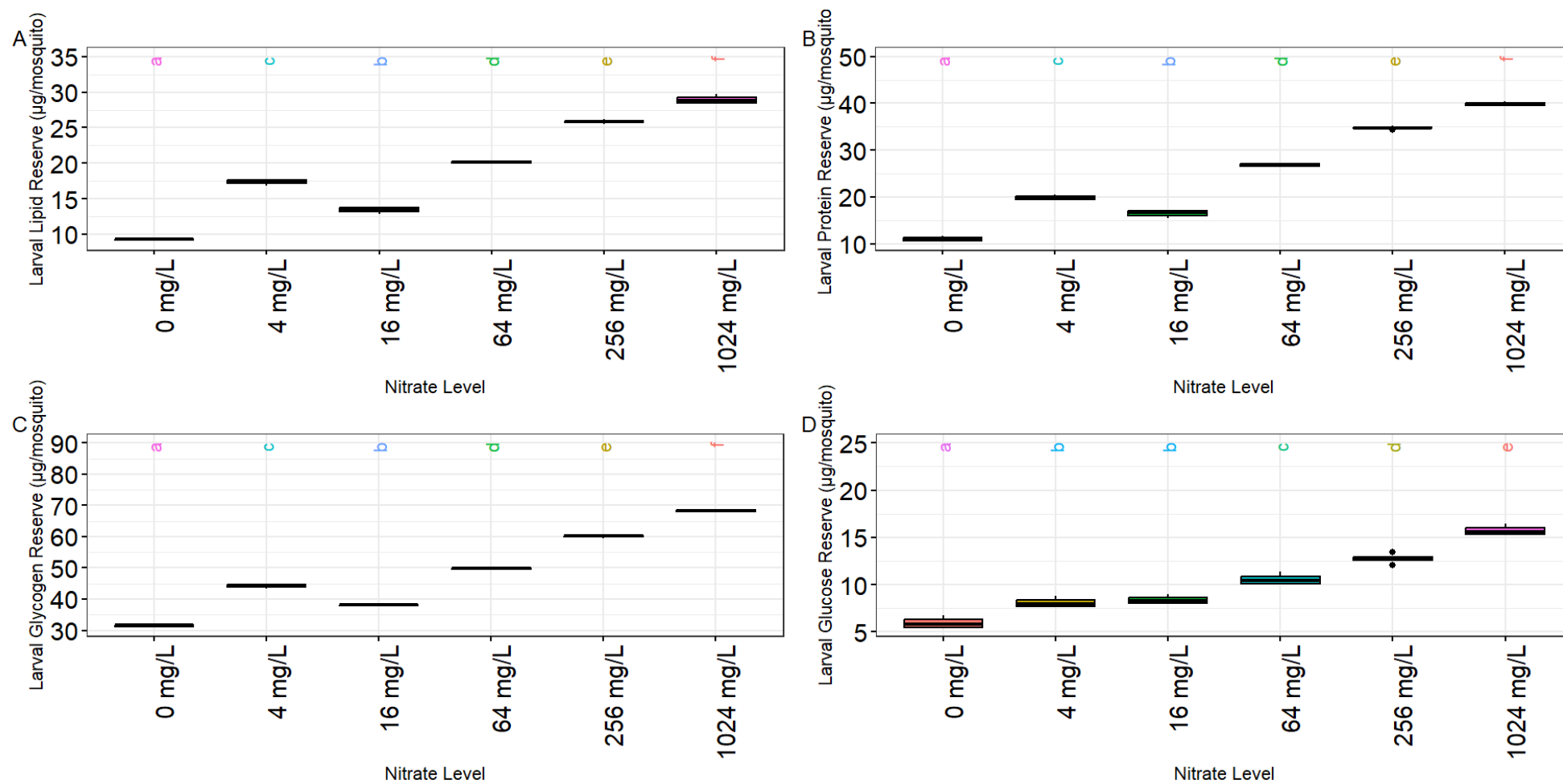


Figure 3: Influence of Nitrate Enrichment on Metabolic Reserves of Fourth Larval Instar of *Aedes aegypti* Mosquito (A) Lipid (B) Protein (C) Glycogen (D) Glucose Reserves

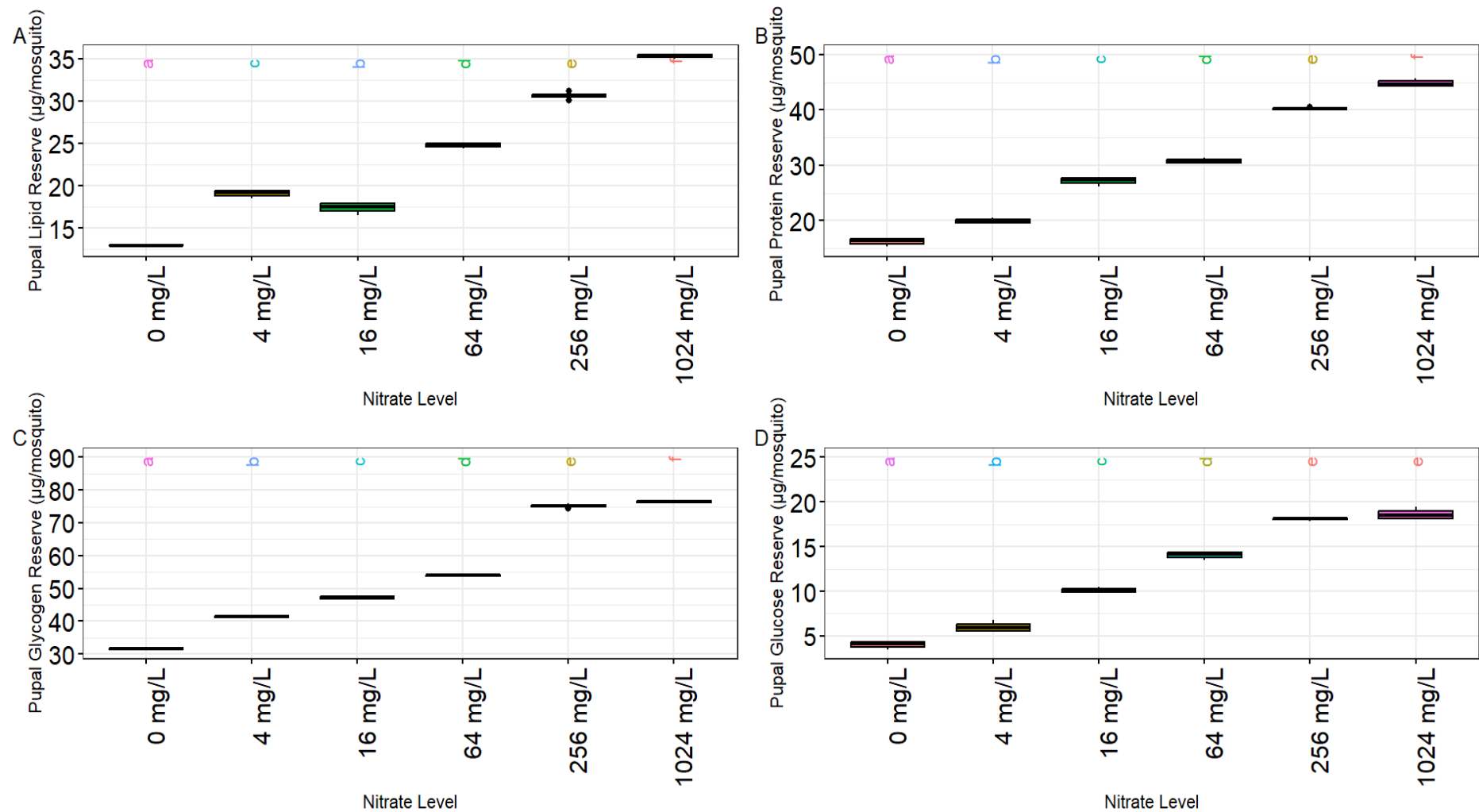


Figure 4: Influence of Nitrate Enrichment on Metabolic Reserves of Pupae (A) Lipid (B) Protein (C) Glycogen (D) Glucose Reserves of *Aedes aegypti* Mosquito

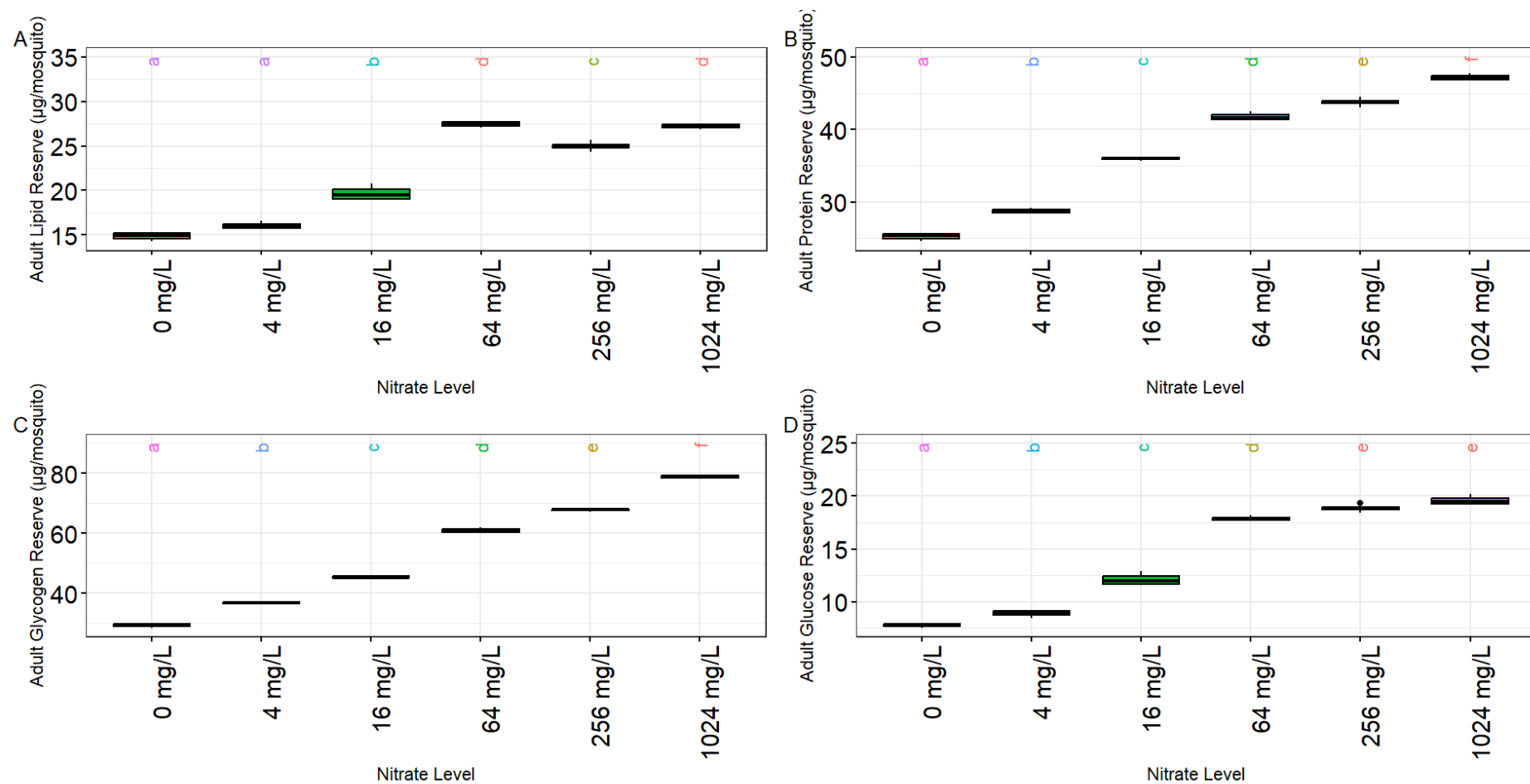


Figure 5: Effects of Nitrate Enrichment on Metabolic Reserves of Adult *Aedes aegypti* Mosquito (A) Lipid (B) Protein (C) Glycogen (D) Glucose Reserves

Effects of Nitrate Enrichment on Susceptibility of *Aedes aegypti* to Permethrin Insecticide

Exposure of immature life stages to nitrate enrichment significantly ($p < 0.05$) influenced the susceptibility of emergent adults to permethrin insecticides. Mortality increased with both exposure duration and nitrate concentration, but the pattern was concentration-dependent and non-linear. Adults reared under low enrichment (4 mg/L) were most susceptible, with mortality reaching ~70 % within 40 minutes (Mean = 69.8%, 95% CI: 66.2–73.4), a level significantly higher than all other treatments. Moderate enrichment (16 mg/L) produced intermediate susceptibility, with mortality peaking at ~55 % after 45 minutes (95% CI: 51.1–58.9). At higher concentrations (64–256 mg/L), mortality plateaued between 40–50% after 60 minutes, with overlapping confidence intervals (95% CI ranges: 38.7–52.1), while adults reared under extreme enrichment (1024 mg/L) were least susceptible, with mortality not exceeding ~35% even after 60 minutes (95% CI: 32.4–37.6). Control mosquitoes exhibited baseline mortality of ~40%, comparable to mid-range enrichment but significantly lower than the 4 mg/L group (Figure 6).

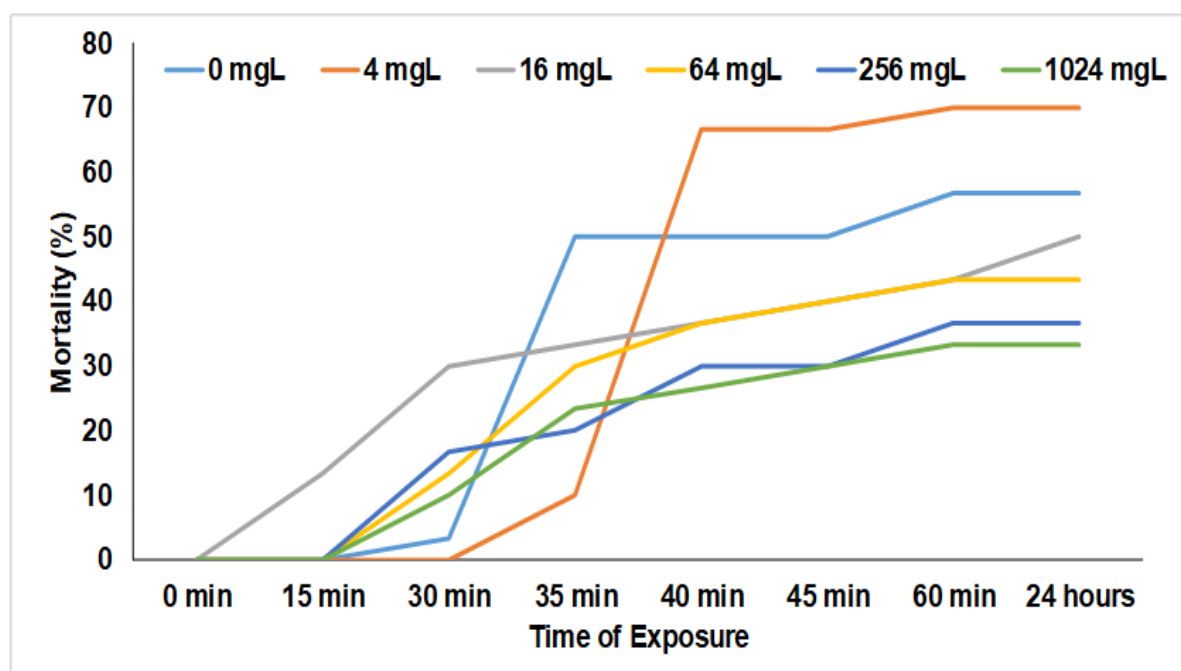


Figure 6: Effects of Nitrate Enrichment on Susceptibility of Adult *Aedes aegypti* Mosquito to Permethrin (0.75 %)

Effects of Nitrate Enrichment on Detoxifying Enzyme Activities in *Aedes aegypti*

Esterase activities in *Ae. aegypti* exposed to permethrin after larval exposure to nitrate enrichment were significantly (t -value = 6.014, $df = 23$, $p = 0.000$; Cohen's d : 1.2275) lower at enrichment levels between 4 and 64 mg/L both before (range = 140.11 ± 0.67 to 155.01 ± 0.22 nMoles 1-naphthol/min/mg protein) and after (range = 99.01 ± 0.67 to 122.07 ± 0.98 nMoles 1-naphthol/min/mg protein) exposure to permethrin 0.75% (Figures 7 and 8).

Glutathione-S-Transferase (GST) activities were also significantly (t -value = 5.822, $df = 23$, $p = 0.000$; Cohen's d : 1.1884) lower at nitrate levels between 4 and 64 mg/L both before (range = 0.24 ± 0.01 to 0.30 ± 0.01 nMoles CNG/min/mg protein) and after exposure (range = 0.16 ± 0.01 to 0.26 ± 0.01 nMoles CNG/min/mg protein) (Figure 9).

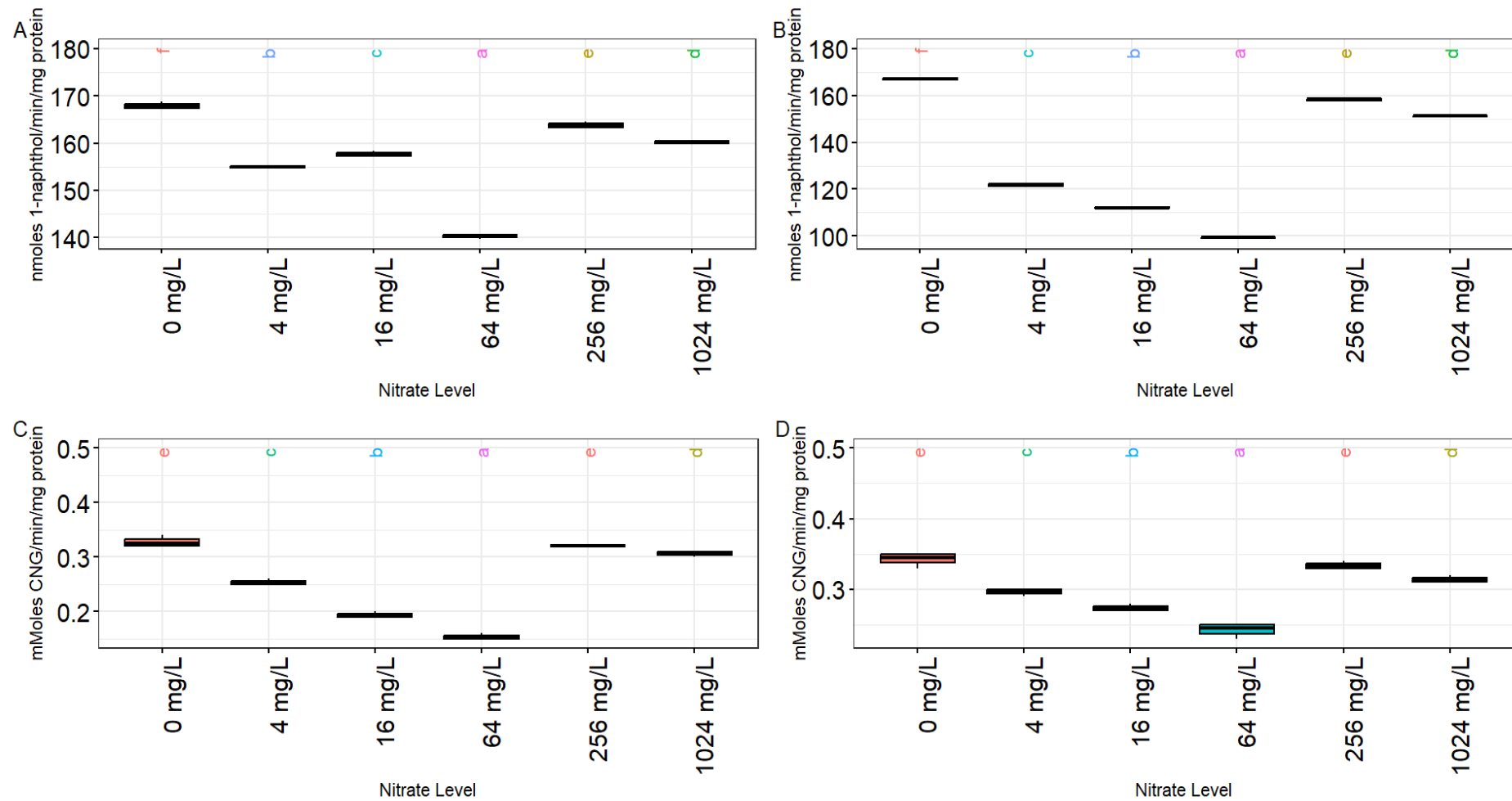


Figure 7: Insecticide Detoxifying Enzyme Levels in Adult *Aedes aegypti* Mosquitoes to Graded Nitrate Enrichment. (A) Esterase level before exposure (B) Esterase level after exposure (C) GST before exposure (D) GST level after exposure to permethrin

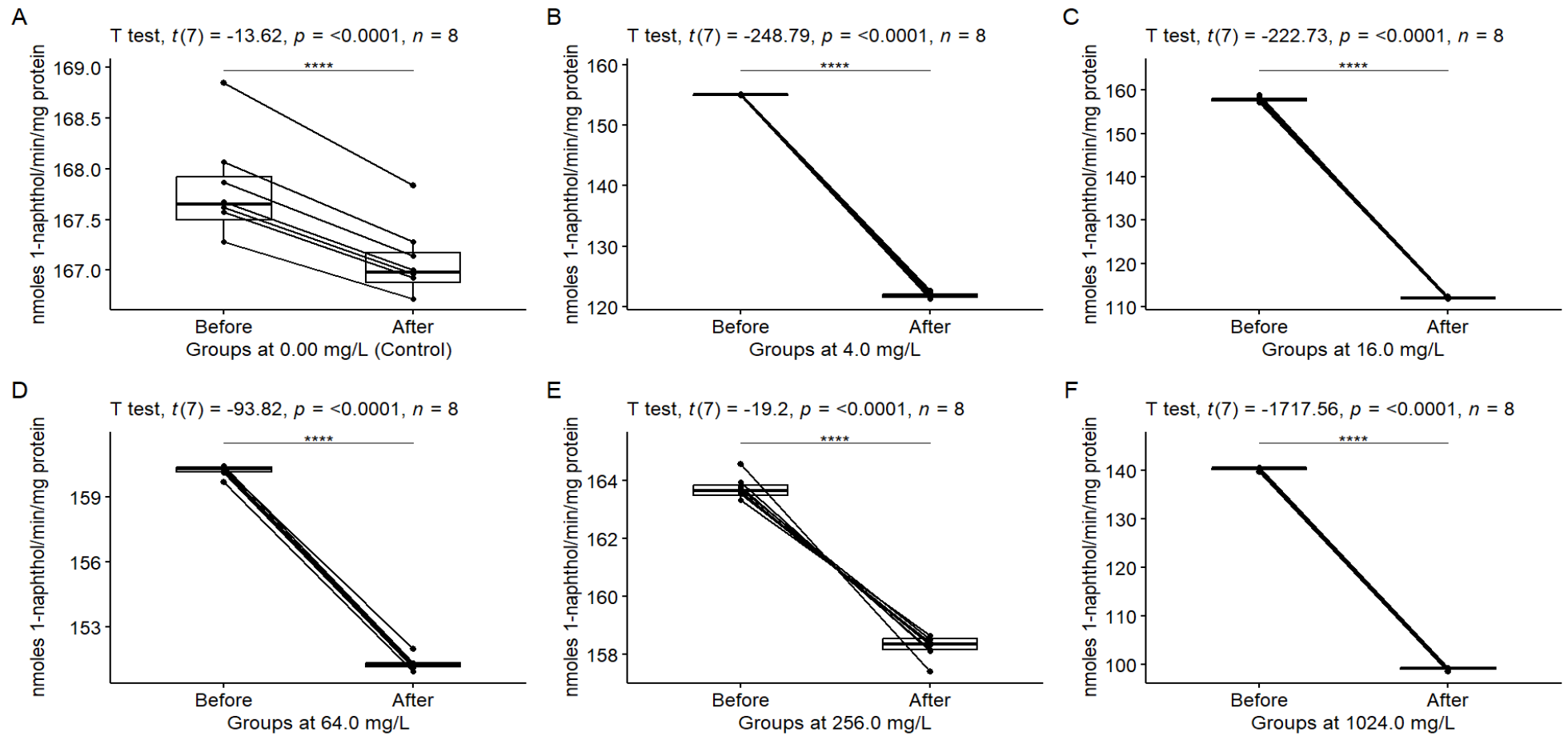


Figure 8: Influence of Graded Nitrate Enrichment on Esterase Activity Enzyme Levels before and after exposure to Permethrin in Adult *Aedes aegypti* Mosquito

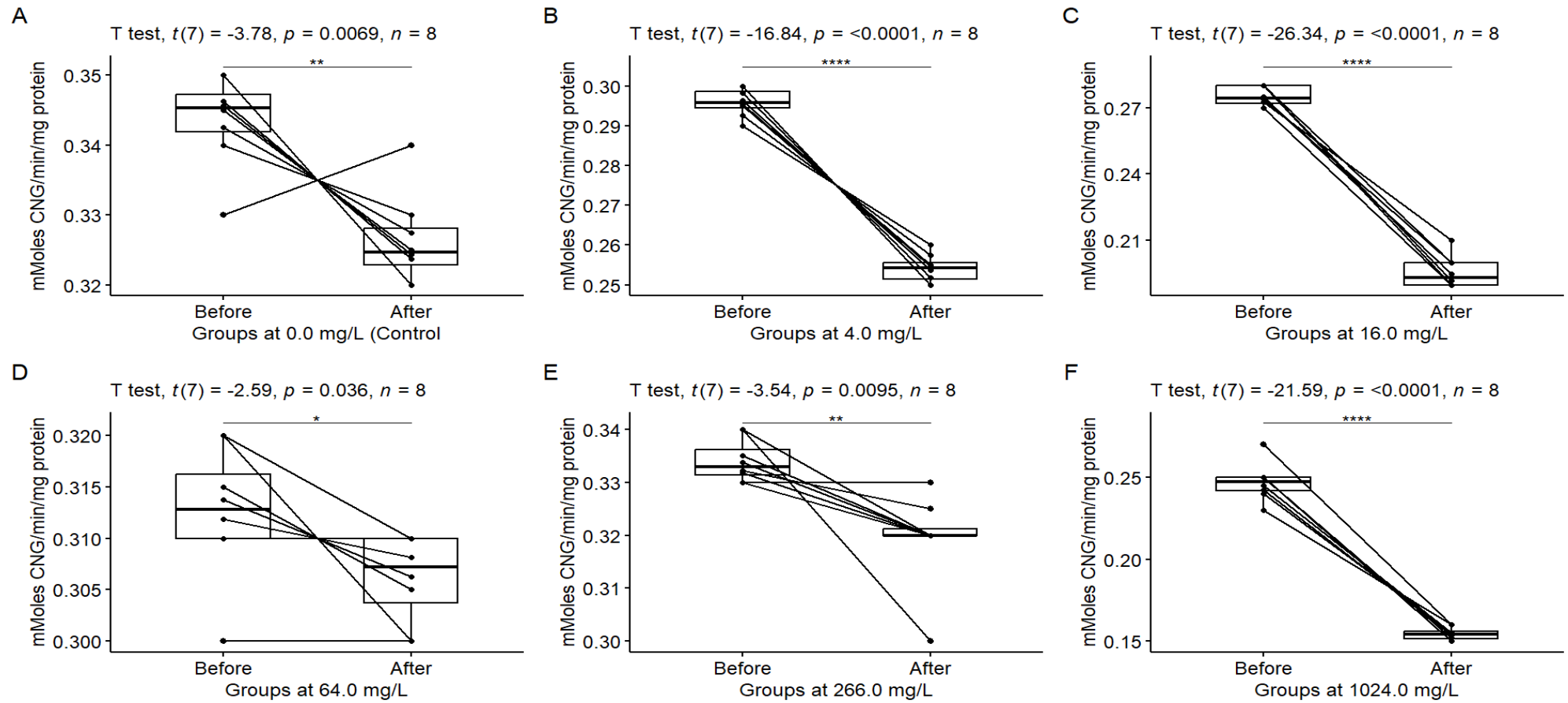


Figure 9: Influence of Graded Nitrate Enrichment on Glutathione-S-Transferase Activity Enzyme Levels before and after exposure to Permethrin in Adult *Aedes aegypti* Mosquito

Relationship between Nitrate Enrichment and Entomological Variables in *Aedes aegypti*

The correlations between duration of immature development (TLD, TID), GR and immature survivorship (ALS, PS and AIS) are shown in Figure 8. TLD correlated significantly with TID ($p < 0.05$; 0.98), PS ($p < 0.05$; 0.57), and AIS ($p < 0.05$; 0.43). Further, TID correlated strongly with PS ($p < 0.05$; 0.57), and AIS exhibited a significant positive relationship with PS ($p < 0.05$; 0.89) and ALS ($p < 0.05$; 0.56). The larval GR exhibited weak negative correlations with duration of development (TLD, -0.30 and TID, -0.33) and weak positive correlations with survivorship (ALS; 0.18, PS; 0.06, and AIS; 0.14) (Figure 8).

The correlations between indices of development (TID, GR and AIS) and metabolic reserve components (lipid, glucose, glycogen and protein) of the fourth larval instars are shown in Figure 9. Under the influence of nitrate enrichment, TID negatively correlated with all the larval metabolic reserve components (range = - 0.08 to - 0.19). Similarly, GR correlated negatively with the reserve components (range = - 0.14 to - 0.28), while AIS exhibited weak positive correlations (range = 0.08 to 0.13) with the reserve components (Figure 9).

The correlations between larval survivorship (AIS and ALS) and metabolic reserve components (lipid, glucose, glycogen and protein) of the fourth larval instars are shown in Figure 10. Analyses revealed that AIS correlated positively and significantly with ALS ($p < 0.05$; 0.563), but weakly with the reserve components (range = 0.17 to 0.25). Similarly, AIS exhibited a weak positive correlation with the reserve components (range = 0.08 to 0.13) (Figure 10). However, all larval metabolic reserve components correlated positively and significantly with themselves (range = 0.96 to 0.99).

The correlations between susceptibility of *Aedes aegypti* to permethrin insecticide and activities of detoxifying enzymes (Esterase and Glutathione-S-transferase, GST) before and after exposure to permethrin insecticide are shown in Figure 11. Analyses revealed that susceptibility to permethrin exhibited very weak positive correlations with the level of esterase before (0.25) and after (0.06) exposure to permethrin, and the level of GST before (0.23) and after (0.15) exposure to permethrin (Figure 11). However, all enzyme group activities correlated positively and significantly with themselves (range = 0.88 to 0.97) (Figure 11).

The correlations between metabolic reserve components of the adult life stage and activities of detoxifying enzymes before and after exposure to permethrin insecticide are shown in Figure 12. Analyses revealed that irrespective of nitrate enrichment condition, all metabolic reserve components correlated strongly and positively with themselves (range = 0.80 to 0.99) (Figure 12). The detoxifying enzyme levels of the species, however, exhibited weak positive correlations (range = 0.02 to 0.37), except lipid reserve, which had a weak negative correlation with esterase before exposure (- 0.13) (Figure 12).

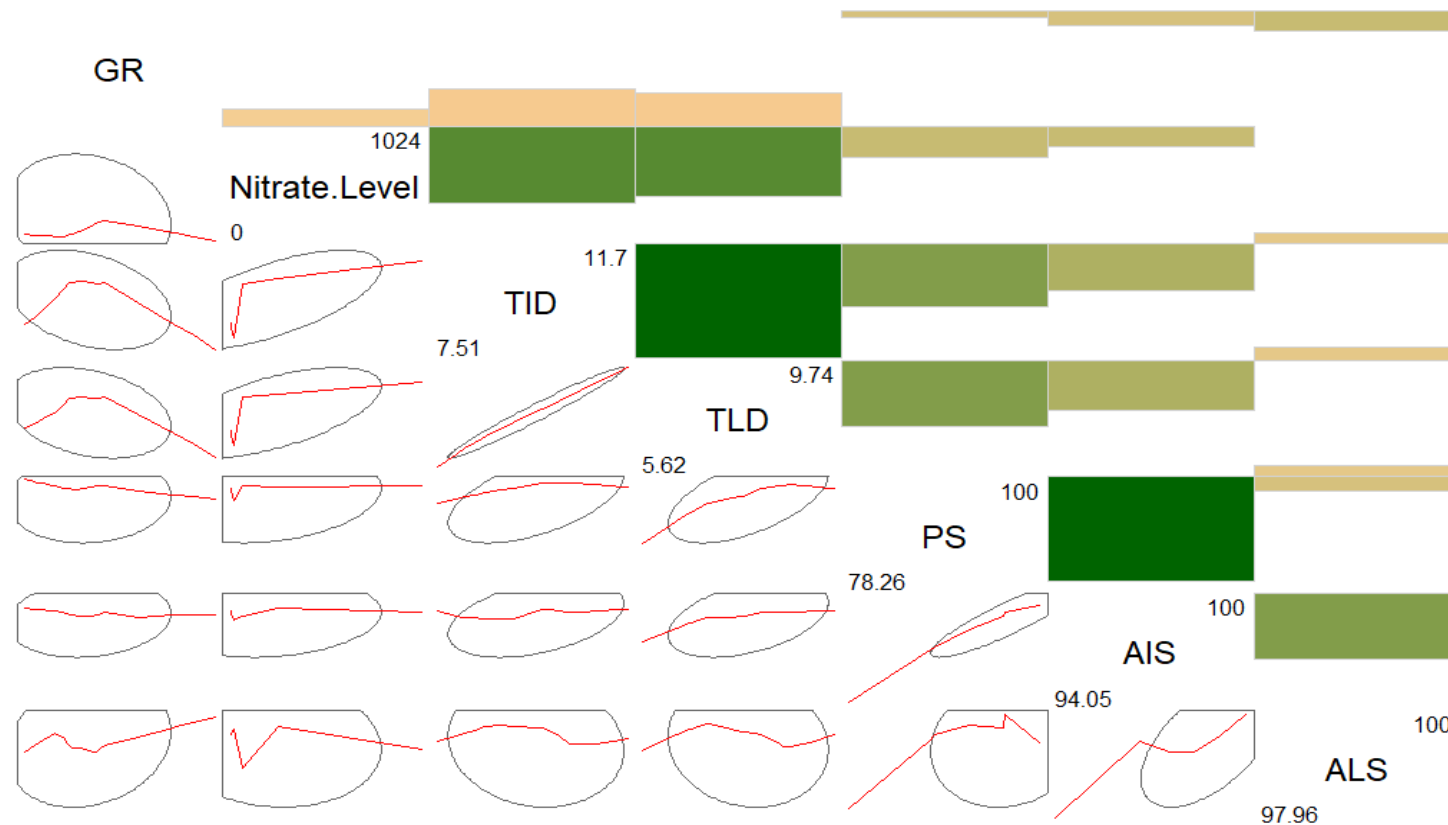


Figure 10: Correlation between Indices of Development in *Aedes aegypti*. GR – Growth Rate, Nitrate.Level – Nitrate enrichment level, TID – Total Immature Development, TLD – Total Larval Development, PS – Pupal stage survivorship, AIS – Average Immature Survivorship, ALS- Average Larval Survivorship.

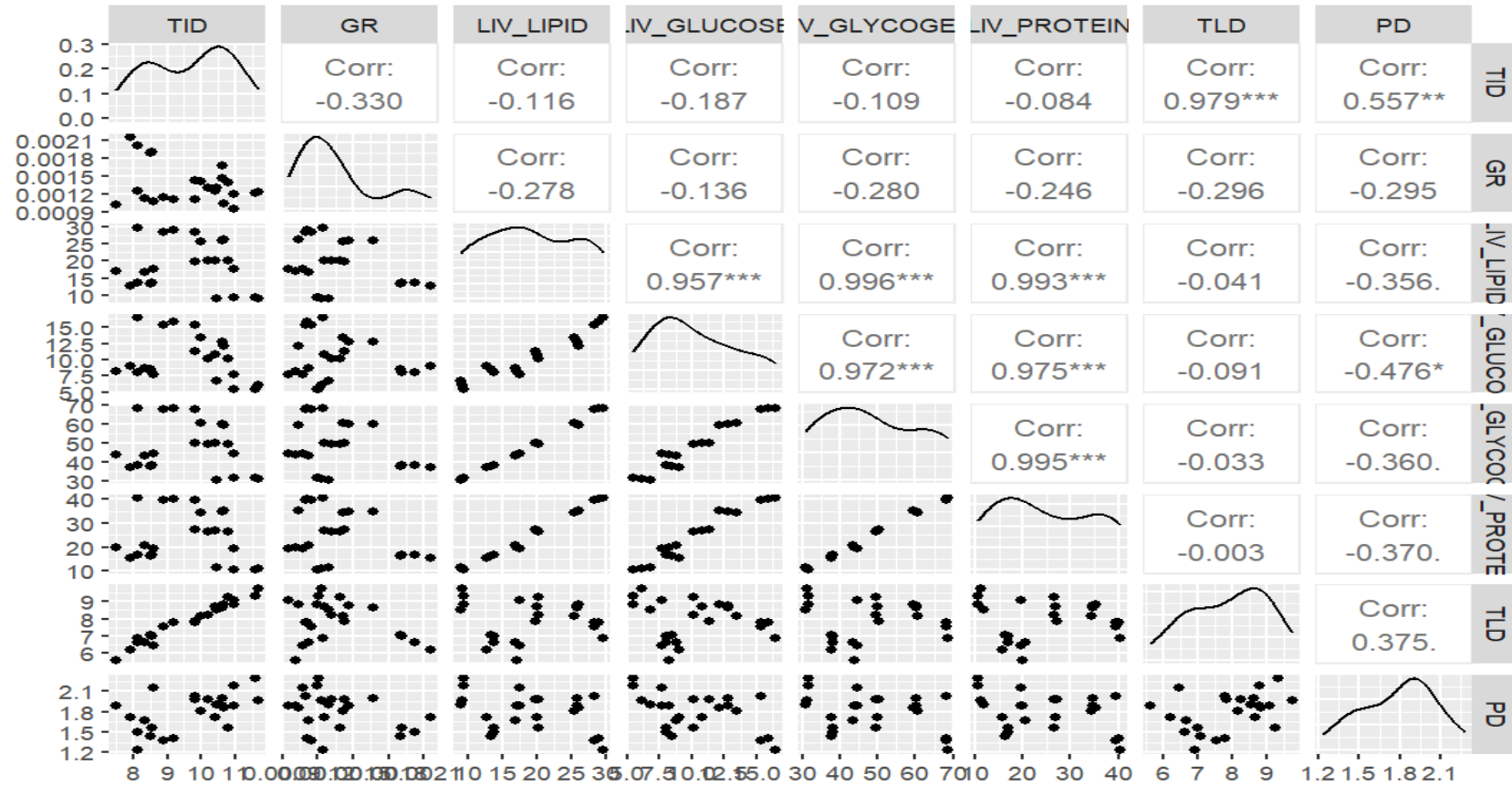


Figure 11: Correlation between Developmental Indices and Metabolic Reserve of Fourth Larval in *Aedes aegypti*. TID – Total Immature Development, GR – Larval Growth Rate, TLD – Total Larval Development, PD – Pupal stage Duration of Development, LIV_Lipid – Lipid reserve of fourth larval instar.

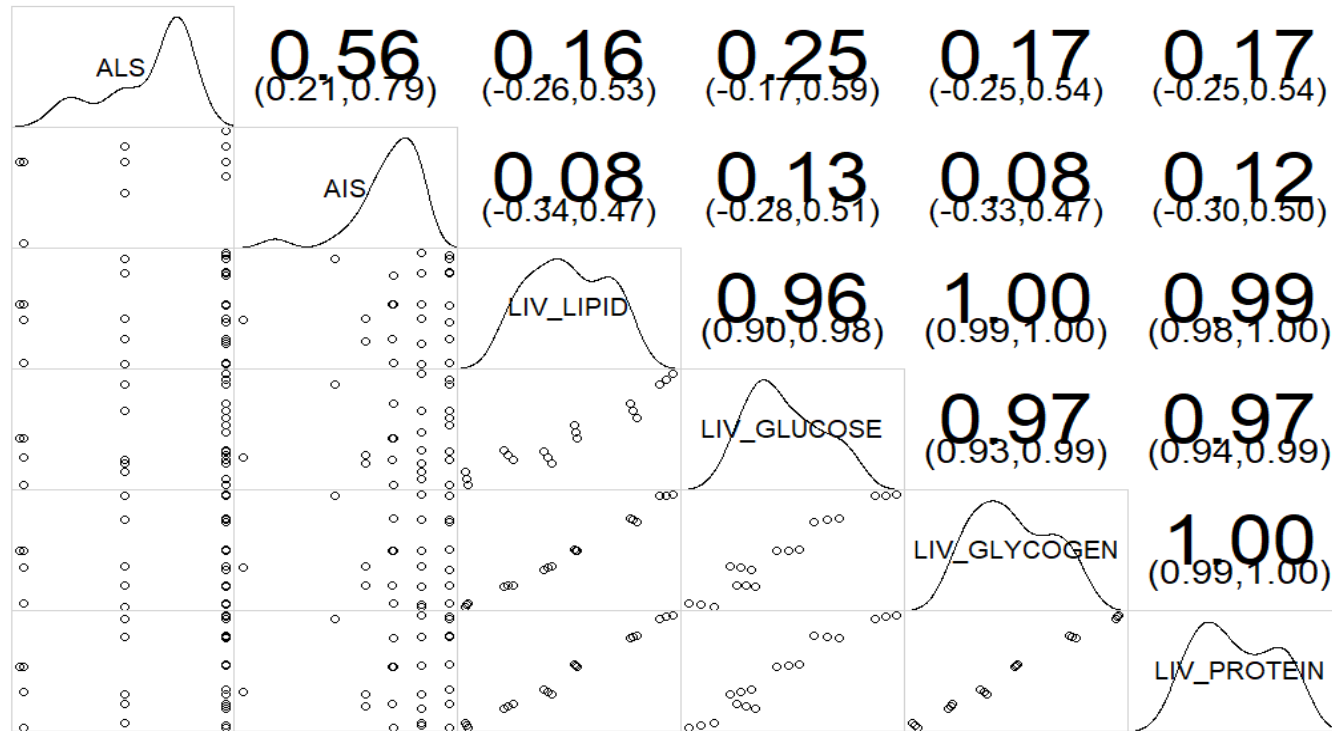


Figure 12: Correlation between Larval Survivorship and Larval Metabolic Reserve in *Aedes aegypti*. ALS - Average Larval Survivorship, AIS – Average Immature Survivorship, LIV_Lipid – Lipid reserve of fourth larval Instar

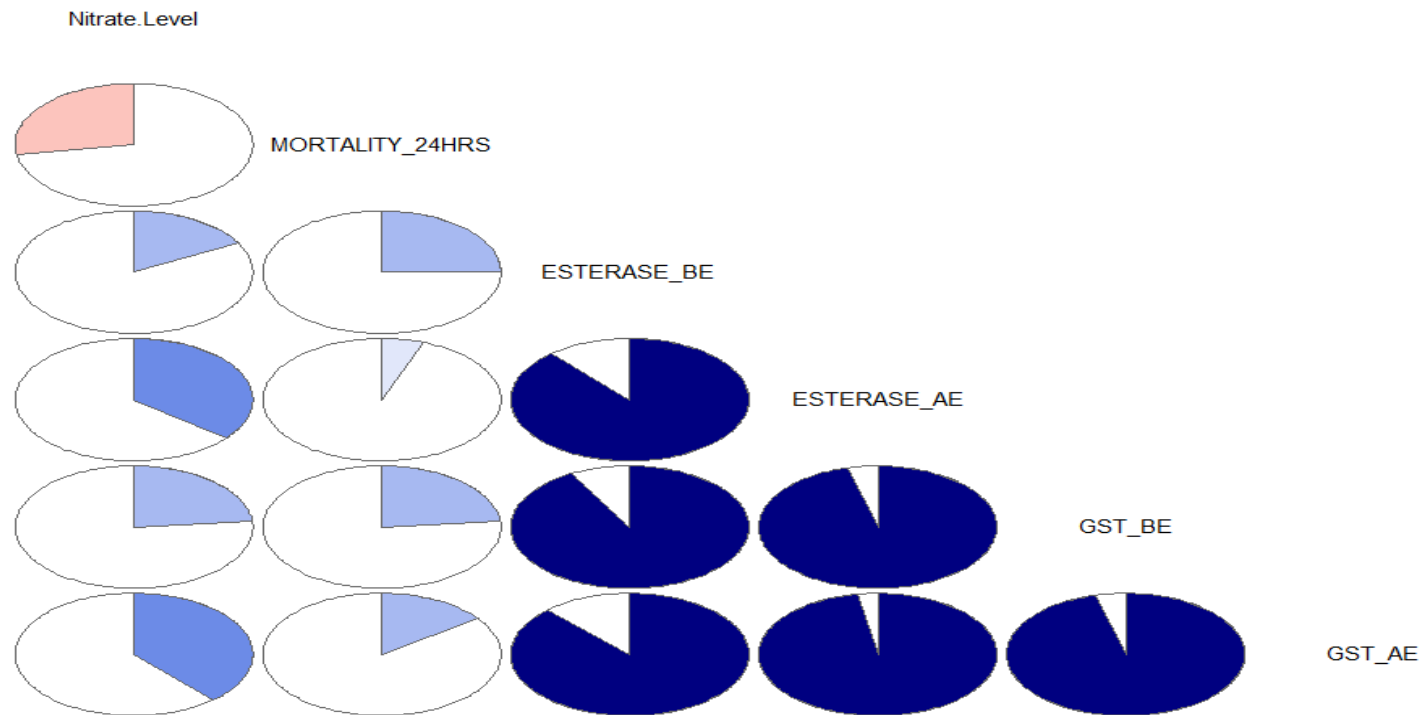


Figure 13: Correlation between Susceptibility to Permethrin and Detoxifying Enzyme in *Aedes aegypti*. ESTERASE_BE – Esterase Level before exposure to permethrin, GST_AE – Glutathione S- Transferase Level after exposure to permethrin, Nitrate.Level – Nitrate Enrichment Level.

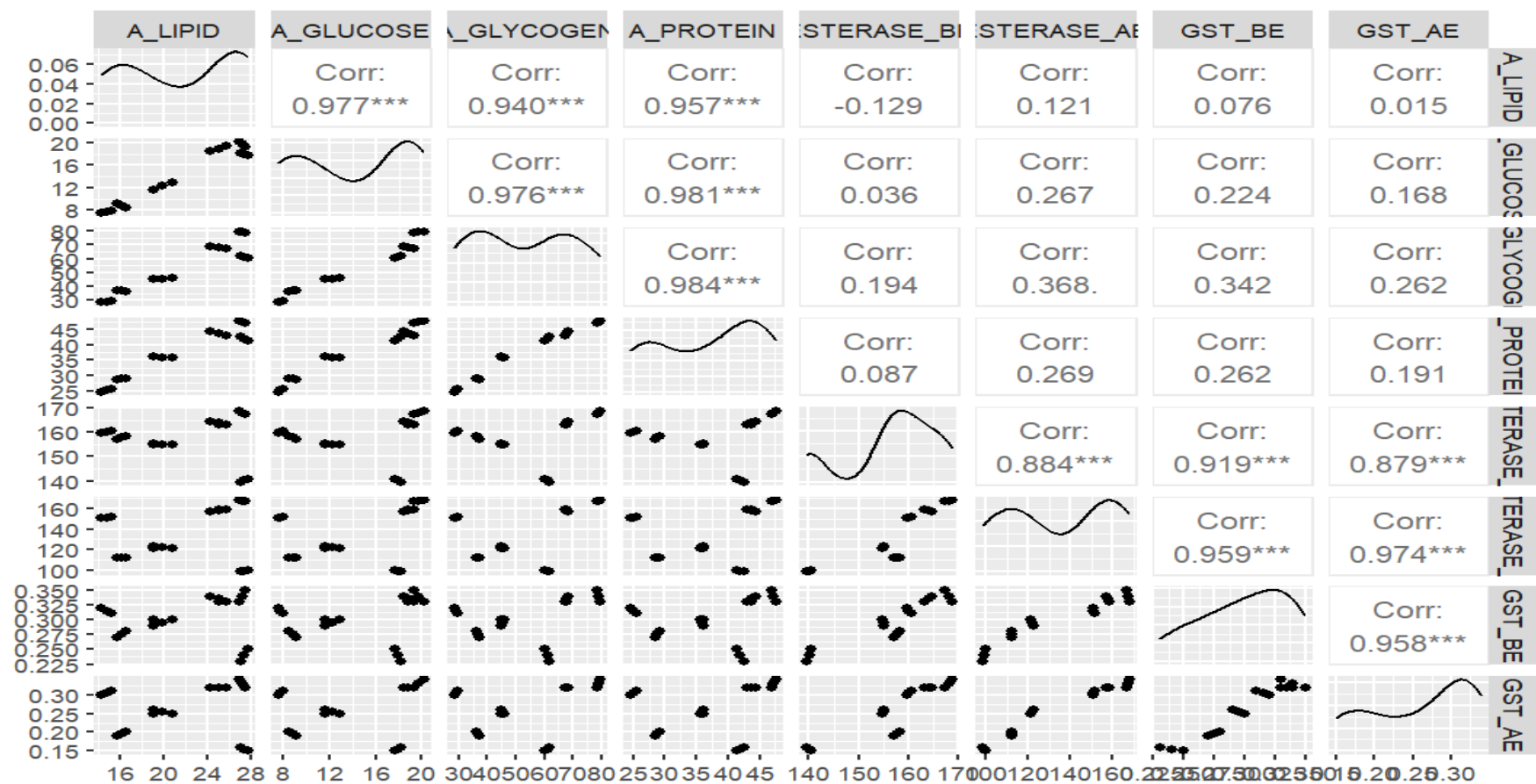


Figure 14: Correlation between Metabolic reserve and detoxifying Enzyme activities in *Aedes aegypti*. A_LIPID – Adult Lipid reserve, ESTERASE_BE – Esterase Level before exposure to permethrin, GST_AE – Glutathione S- Transferase Level after exposure to permethrin

Discussion

This study provides important insights into the influence of nitrate enrichment on the developmental biology of *Aedes aegypti*. Our findings revealed that nitrate enrichment modulated the duration of post-embryonic development and larval growth rate in this mosquito, while survivorship of immature stages remained consistently high across treatments. These represent possible ecological resilience of the mosquito in nutrient-enriched habitats [28] and highlight the potential public health risks associated with nitrate-induced environmental pollution.

Larval and pupal stages developed fastest at moderate nitrate enrichment (16 mg/L), whereas prolonged development was observed under high enrichment (1024 mg/L). This dose-dependent pattern suggests that nitrate availability directly influences developmental physiology. We postulate that moderate enrichment could have stimulated microbial productivity, thereby increasing food availability for larvae and accelerating growth [29]. In contrast, excessive enrichment may have disrupted microbial community balance or generated toxic by-products which could impose physiological stress [30] that slows progression. Such shifts in developmental timing can alter population dynamics by influencing emergence rates and overall mosquito abundance [8, 31].

Larval growth rate mirrored the developmental duration trends, peaking at 16 mg/L and declining progressively at higher nitrate concentrations. This trend suggest that moderate nitrate enrichment could provide optimal conditions for biomass accumulation, while excessive enrichment reduced growth efficiency [32]. Growth rate is a critical determinant of mosquito fitness, as faster-growing larvae reach pupation earlier and contribute to rapid adult emergence. Declines in growth rate under high enrichment could suggest that extreme nutrient loading may impose metabolic costs [15], potentially reducing competitive ability.

Survivorship of the immature life stages remained consistently high across nitrate levels, indicating that enrichment may not suppress mosquito populations but could enhance their physiological quality. The high immature survivorship even at elevated nitrate levels may suggest that eutrophic habitats may still be productive breeding sites, sustaining mosquito populations despite environmental stressors. Such adaptability have been reported to contribute to species' success in colonising urban and peri-urban environments, where nutrient pollution is prevalent [33]. These plasticity of immature stages to nitrate enrichment highlights the adaptability of *Ae. aegypti* mosquito to anthropogenically altered habitats. Nutrient enrichment, commonly resulting from agricultural runoff, sewage discharge, and urban pollution, enhances microbial productivity and organic matter availability, thereby supporting larval feeding and survival [30]. Further, these polluted habitats can serve as hotspots for vector proliferation, intensifying transmission of dengue, Zika, chikungunya, and yellow fever.

These findings have considerable public health implications. Faster larval development and higher growth rates of mosquitoes at moderate nitrate enrichment may lead to more rapid adult emergence, increasing the frequency of host-seeking adults and amplifying transmission of arboviruses such as dengue, Zika, and chikungunya [34, 35]. Conversely, the prolonged immature developmental stages under high enrichment may reduce adult emergence but could select for individuals with enhanced stress tolerance, potentially influencing longevity and insecticide resistance [36]. The consistently high survivorship of immature stages may ensure that adult populations remain robust.

These ecological and physiological responses could also be interpreted within the broader contexts of climate change and urbanisation. Rising global temperatures have been reported accelerate mosquito development and expand geographic ranges [33], while altered rainfall patterns have been reported to increase the availability of temporary breeding sites [37]. Urbanisation exacerbates nutrient pollution through improper waste management and fertiliser runoffs, creating eutrophic habitats that favour mosquito proliferation [10]. These factors synergise with nitrate enrichment to enhance vectorial capacity [38], complicating control efforts in rapidly growing tropical cities.

The study also demonstrated that nitrate enrichment could significantly enhance the metabolic reserves of *Ae. aegypti* mosquito across the critical life stages; as elevated nitrate concentrations (256–1024 mg/L) consistently increased lipid, protein, glucose, and glycogen reserves. This may suggest that nitrate pollution act as a nutrient supply, that could improve mosquito physiological quality. Stage-specific allocation patterns—lipid peaks in pupae, glycogen in larvae, and protein/glucose in adults—highlight possible adaptive strategies that could support rapid development, successful emergence, and reproductive potential [39]. Enhanced metabolic reserves have been reported to have direct implication for vectorial capacity. Adults with higher protein and glucose reserves are likely to exhibit increased longevity and reproductive output, both of which are critical determinants of arbovirus transmission [40]. Elevated lipid reserves in pupae and glycogen in larvae further ensure efficient emergence and sustained population turnover [41]. Similar energetic shifts have been linked to survival likelihood and transmission potential in *Anopheles gambiae* [42]. These results align with previous studies showing that agricultural runoff and fertilizer use enrich mosquito breeding habitats, altering mosquito breeding ecology, microbial communities, and physiological traits, thereby influencing population dynamics and, hence, vector abundance [30, 31, 43], acting as synergistic drivers of vector-borne disease transmission [28]. These results further reinforces the ecological risks associated with nutrient pollution in urban and peri-urban environments.

Therefore, the dual ecological–epidemiological pathway revealed in this study highlights the need to integrate pollution management into vector control strategies. Reducing nitrate runoff from agriculture and improving sanitation can limit the creation of nutrient-rich larval habitats, thereby reducing mosquito fitness gains. Monitoring water quality, particularly nitrogen levels, may also serve as a predictive tool for mosquito abundance and disease risk. Policy frameworks should incorporate agrochemical regulation and land-use management into integrated vector control programs, as anthropogenic nutrient inputs directly influence mosquito ecology and disease transmission dynamics [44]. Addressing nitrate pollution is therefore not only an environmental priority but also a public health necessity.

The present study also demonstrated that exposure to nitrate enrichment during immature stages of *Ae. aegypti* mosquito could significantly alter the susceptibility of emergent adults to permethrin, a widely used pyrethroid insecticide. The study revealed a non-linear, concentration-dependent pattern: adults reared under low enrichment (4 mg/L) were most susceptible, with mortality approaching 70% within 40 minutes, while those reared under extreme enrichment (1024 mg/L) were least susceptible, with mortality not exceeding 35% even after prolonged exposure. Intermediate concentrations produced moderate susceptibility, and control mosquitoes exhibited baseline mortality comparable to mid-range enrichment but significantly lower than the 4 mg/L group. These findings suggest a possible complex interplay between environmental pollution and insecticide resistance in the mosquito.

The study suggests that low nitrate enrichment, suggestive of nutrient limitation may impair detoxification pathways [45], thereby rendering adults more vulnerable to insecticides. Conversely, high enrichment could enhance metabolic reserves and detoxification capacity, thereby reducing susceptibility [46, 47]. The result of this study aligns with evidence from *Anopheles gambiae* and *Culex quinquefasciatus*, where agrochemical pollutants have been shown to upregulate detoxification enzymes such as cytochrome P450s and glutathione-S-transferases, thereby promoting resistance [48]. The high enrichment could also contribute to fortification of the adult integument through cuticular hydrocarbon enhancement [49], thereby contributing to insecticide resistance. The ecological risk is clear: polluted habitats not only sustain mosquito populations but also foster adaptive responses that undermine chemical control measures. Therefore, mosquitoes may evolve resistance more rapidly in environments with chronic nitrate pollution, thereby complicating long-term vector management.

Detoxifying enzymes such as esterases and GSTs are central to metabolic resistance in mosquitoes, enabling them to neutralize or sequester insecticides [52]. In the present study, the activities of these enzymes in *Ae. aegypti* mosquitoes were influenced significantly by nitrate enrichment. Both esterase and GST activities were markedly reduced at enrichment levels between 4 and 64 mg/L, before and after exposure to permethrin. These findings may highlight a critical link between environmental nutrient enrichment and the biochemical pathways underpinning insecticide susceptibility. The observed reduction in enzyme activity at low-to-moderate nitrate enrichment may suggest that nutrient limitation during larval development may impair the biochemical machinery required for detoxification. This impairment may likely explain the heightened susceptibility of adults reared under 4 mg/L enrichment to permethrin, as reduced esterase and GST activity diminishes their capacity to metabolise pyrethroids [53]. Conversely, at higher enrichment levels, metabolic reserves and enzyme activity are typically enhanced, supporting resistance phenotypes [48]. Thus, nitrate enrichment exerts a concentration-dependent effect on detoxification pathways, shaping insecticide susceptibility in emergent adults.

These results suggest that nutrient enrichment from agricultural runoff and sewage effluents may alter enzyme activity profiles, thereby influencing population-level resistance dynamics. Polluted habitats may therefore act as selective environments, fostering resistance at high enrichment levels while transiently increasing susceptibility at lower concentrations. This dual effect complicates ecological predictions, as resistance outcomes depend not only on insecticide exposure but also on background nutrient conditions. Similar interactions between agrochemical pollutants and mosquito physiology have been documented in *Culex* and *Anopheles* species, where fertilizers and pesticides synergistically drive resistance evolution [30].

The epidemiological consequences are profound. Reduced resistance enzyme activity at low nitrate enrichment level may temporarily enhance control efficacy, as mosquitoes from these regimens were more susceptible to pyrethroids. However, in nitrate-rich habitats, elevated enzyme activities and metabolic reserves can reduce susceptibility, undermining the effectiveness of pyrethroid-based interventions. Further, insecticide resistance driven by environmental enrichment exacerbates the risk of arbovirus transmission, as better-fit mosquitoes with enhanced detoxification capacity are more likely to survive interventions and sustain transmission cycles [51, 54].

Epidemiologically, the implications of these findings are equally concerning. Pyrethroids remain the cornerstone of vector control programs, particularly through insecticide-treated nets (ITNs) and indoor residual spraying (IRS). The observed reduced susceptibility of *Ae. aegypti* to permethrin at high nitrate enrichment levels could suggest that mosquitoes from polluted environments may survive these interventions, thereby sustaining transmission cycles of arboviral diseases such as dengue, Zika, chikungunya, and yellow fever. This finding resonates with broader evidence that environmental pollutants act as synergistic stressors, driving vector-borne disease transmission by enhancing mosquito resilience and reducing the efficacy of control tools [28]. The non-linear nature of the response further complicates epidemiological predictions, as susceptibility profiles may vary across habitats depending on local nitrate concentrations. This heterogeneity poses challenges for resistance monitoring and control planning, particularly in urban and peri-urban areas where agricultural runoff and sewage contamination are prevalent [50].

Addressing nitrate pollution is, therefore, not only an ecological priority but also a public health necessity. Monitoring nitrate levels in breeding habitats should, therefore, become part of insecticide resistance surveillance, enabling early detection of pollution-driven resistance hotspots. Further, deducing agrochemical runoff and improving wastewater management can mitigate environmental drivers of resistance, while adaptive strategies such as rotating insecticides and incorporating non-chemical interventions may help counteract pollution-mediated resistance evolution [51]. Policy frameworks must therefore recognize environmental management as a critical component of integrated vector management (IVM), in line with WHO recommendations for sustainable resistance management.

The correlation analyses revealed complex relationships between developmental indices, survivorship, metabolic reserves, and detoxifying enzyme activity in *Aedes aegypti* under nitrate enrichment. Developmental duration (TLD, TID) correlated positively with survivorship indices (PS, AIS), while larval growth rate (GR) showed weak negative correlations with development time and weak positive correlations with survivorship. Metabolic reserves of fourth instar larvae correlated negatively with development time and growth rate, but weakly positively with survivorship indices. Detoxifying enzyme activities (esterase and GST) exhibited only weak correlations with susceptibility to permethrin, though they correlated strongly with themselves. Similarly, adult metabolic reserves correlated strongly with themselves but only weakly with detoxifying enzyme activities, with lipid reserves showing a slight negative correlation with esterase before exposure.

These findings could indicate an intricate physiological trade-offs in mosquito development exposed to nutrient-enriched environment. The negative correlations between metabolic reserves and developmental duration may suggest that enriched nutrient conditions could accelerate development but reduce reserve accumulation efficiency. Weak correlations between detoxifying enzymes and insecticide susceptibility could indicate that resistance phenotypes are not solely determined by enzyme activity but are influenced by broader metabolic and ecological contexts. This aligns with evidence that multiple mechanisms—including target-site mutations and cuticular resistance—interact with metabolic detoxification to shape insecticide resistance in *Aedes aegypti* [52, 55]. The strong self-correlations among metabolic reserves and enzyme activities underscore the stability of these physiological systems, while the weak cross-correlations highlight the complexity of resistance evolution.

The positive correlations between survivorship indices and metabolic reserves may suggest that nutrient-rich habitats enhance population resilience, even if growth rates and developmental timing are variably affected. While weak correlations between reserves and detoxifying enzymes may imply that pollution-driven enrichment may not directly translate into resistance but could create conditions that favour adaptive responses over time.

From an epidemiological perspective, these correlations underscore the challenges of predicting vectorial capacity and resistance outcomes in polluted environments. Weak associations between detoxifying enzymes and insecticide susceptibility suggest that resistance monitoring must account for multiple physiological and ecological factors. Enhanced survivorship and metabolic reserves in nitrate-enriched habitats increase the likelihood of larger, better-fit adult populations capable of sustaining arbovirus transmission. The resilience of these populations, even under insecticide pressure, threatens the effectiveness of pyrethroid-based interventions such as insecticide-treated nets and indoor residual spraying. The epidemiological implication is clear: nutrient enrichment and pollution must be integrated into resistance management and vector surveillance frameworks to safeguard public health.

Limitations

Though, the study highlighted important physiological and ecological effects of nitrate enrichment on *Aedes aegypti*, several limitations are acknowledged to avoid overgeneralisation of the findings. First, the experiments were conducted under controlled laboratory conditions, which may not fully replicate the complexity of natural breeding habitats. Secondly, although a wide range of nitrate concentrations was tested, the ecological relevance of extreme levels (e.g., 1024 mg/L) may vary depending on local environmental contexts. Natural habitats may not consistently reach such concentrations. Thirdly, while enzyme activity was measured, the study did not directly assess gene expression or molecular mechanisms underlying detoxification pathways. Without these data, it is difficult to fully explain how nitrate enrichment modulates resistance phenotypes at the genetic or regulatory level. Lastly, the epidemiological implications discussed are inferred from physiological and biochemical changes rather than direct transmission studies. Although enhanced fitness and altered susceptibility suggest potential impacts on vectorial capacity, actual effects on arbovirus transmission dynamics were not measured.

Conclusion

The present study demonstrated that increasing nitrate level exerts measurable effects on the physiology and performance of *Aedes aegypti* mosquito, influencing developmental timing, survivorship, metabolic reserves, detoxifying enzyme activity, and susceptibility to pyrethroid insecticides. At low enrichment levels, impaired detoxification capacity increased vulnerability to insecticides, while higher enrichment enhanced metabolic reserves and enzyme activity, reducing susceptibility to permethrin. Further research is recommended to understand the relative contributions of genetic variation, and other environmental stressors to resistance evolution and vectorial capacity. Nonetheless, the evidence in this study supports the integration of pollution management into vector control strategies

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