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The effect of larval exposure to acids and detergents on the life history of the major malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae)

Chia-Yu Chen[a,b*] and Shüné V. Oliver[a,b]

Abstract

BACKGROUND: *Anopheles arabiensis*, a highly adaptable member of the *Anopheles gambiae* complex, poses a challenge for control efforts due to its outdoor biting and resting behaviour. Consequently, indoor insecticide-based control methods are ineffective against *An. arabiensis*. Furthermore, *An. arabiensis* are adapting to breeding in polluted waters, and may be contributing to residual malaria and malaria in urban areas. There have been some advances in understanding the effect of rural pollutants on *Anopheles* mosquitoes, but the effect of urban pollutants is poorly understood. Thus, in this study, the effect of acidic pollutants [nitric acid (HNO_3) and hydrochloric acid (HCl)] and alkaline pollutants (phosphate-free and phosphate-containing detergent) on two laboratory-reared *An. arabiensis* strains – an insecticide susceptible strain (SENN) and an insecticide-resistant strain selected from SENN (SENN-DDT) – were determined.

RESULTS: The median lethal concentration (LC_{50}) and larval exposure on larval development, adult longevity and insecticide tolerance were evaluated. Nitric acid and phosphate-containing detergent were found to be more toxic than HCl and phosphate-free detergent in terms of LC_{50} values. Detergent exposure (both phosphate-containing and phosphate-free) increased adult longevity of both strains. Nitric acid reduced larval development time in both SENN and SENN-DDT, whereas HCl reduced larval development time in SENN only. By contrast, both phosphate-containing and phosphate-free detergents increased larval development time of both strains. Furthermore, HNO_3 and phosphate-containing detergent increased insecticide tolerance the most.

CONCLUSION: The two *An. arabiensis* strains responded to urban pollutants differently. Thus, this study provides insight into the adaptation of *An. arabiensis* to acidic and alkaline urban pollutants.
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Keywords: pest biology; pest control; larval development; longevity; insecticide resistance; environmental impact; vector control

1 INTRODUCTION

The incidence of malaria, the single most devastating vector-borne disease in the world, had been on a steady decline from 2000 to 2019. However, owing to interruptions caused by the COVID-19 pandemic, in 2020, malaria deaths increased by 12% to an estimated total of 627 000 globally compared to 2019. In 2021, of the 88 malaria-endemic countries in the world, more than half of all malaria deaths were from six countries in Africa.[1]

Challenges to malaria elimination efforts were evident even before the COVID-19 pandemic. This is because insecticide resistance, especially in *Anopheles arabiensis*, the major vector species, continues to be detected across Africa.[2–4] Between 2010 and 2020, 78 of 88 countries confirmed vector resistance to at least one insecticide.[1] Moreover, 29 countries confirmed the resistance of vectors to four insecticide classes – pyrethroids, organophosphates, carbamates and organochlorines – with 19 countries reporting resistance to all four classes of insecticides.[1,5]

Relative to its sibling species *Anopheles gambiae*, *An. arabiensis* is typically more zoophilic, exophagic and exophilic.[6,7] However, feeding and resting patterns have been found to vary widely depending on geographical location.[7] *An. arabiensis* can be found in dry savannah environments but also forested areas.[6] Furthermore, like most of the *An. gambiae* complex, *An. arabiensis* larvae generally inhabit small, temporary, sunlit, clear and shallow freshwater pools. However, larvae have also been reported in

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slow-flowing, partially shaded streams, natural and land-made pools, and even in brackish water.[6,8] As such, *An. arabiensis* is a highly adaptable vector species which contributes to the reason why it is responsible for the low-level outdoor malaria incidence known as residual malaria.[9]

The increased proportion of sub-Saharan Africans living in urban areas and the adaptability of *An. arabiensis* means that the outdoor life cycle of *An. arabiensis* is now influenced by the urban environment.[10] Thus, the polluted water bodies in which *An. arabiensis* breed in urban settings are especially important. Increased urbanization results in increased domestic pollutants which include cleaning agents such as hydrogen peroxide (H_2O_2) or detergents. Both of these agents have been demonstrated to increase pyrethroid tolerance in the *An. gambiae* complex.[11,12] A study in Cameroon that sequenced 941 mosquitoes of the *An. gambiae* complex has already shown that rural and urban mosquitoes of the same species are genetically different, with urban mosquitoes possessing more genetic markers associated with xenobiotic (synthetic compounds, usually used for domestic, agricultural and industrial purposes) resistance.[13] Furthermore, previous studies have shown that contaminants such as heavy metals, cigarette-associated pollutants, petroleum and domestic organic waste have larvicidal effects as well as affect the tolerance of the *An. gambiae* complex to chemical insecticides permethrin, deltamethrin and neonicotinoids.[14–16] Thus, in addition to the development of insecticide resistance being a major limiting step in malaria elimination in Africa, the environment in which mosquitoes inhabit is quickly becoming an important factor to consider with regard to vector control.

Additionally, mosquitoes do demonstrate a degree of tolerance towards acids. For example, there have been reports of mosquitoes surviving at a pH of 4.4.[17] A previous study demonstrated that *Culex pipiens* had a high tolerance to a range of acids, with acetic acid being tolerated the best and salicylic acid being poorly tolerated. The same study noted that the acids were ingested rather than penetrating cutaneously.[17] *Aedes aegypti* and *Ochlerotatus taeniorhynchus* are able to develop in water with a pH of 4 but require acclimatization to be able to develop at pH 3.[18] The capacity of mosquitoes to survive low pH conditions is significant owing to the acidification of water bodies as a result of industrialization and urbanization.[19] There have already been reports of mosquito larvae colonizing acidified water bodies in the absence of other taxa.[20] As such, the effect of such pollutants on an adaptable species such as *An. arabiensis* is worth considering.

The study aimed to evaluate the effect of acid [hydrochloric acid (HCl) and nitric acid (HNO_3)] and commercial liquid detergents on larval development, adult longevity and adult insecticide tolerance in both an insecticide-susceptible (SENN) and an insecticide-resistant (SENN-DDT) strain of *An. arabiensis*.

2 MATERIALS AND METHODS

2.1 Insects

Two laboratory strains of *An. arabiensis* were used in this study: SENN and SENN-DDT. The SENN strain is insecticide-susceptible and was colonized in 1980, from Gezira, Sudan.[21] From this, the insecticide-resistant strain SENN-DDT strain was selected through continuous exposure to the insecticide dichloro-diphenyl-trichloroethane (DDT) since 1995. In addition to DDT, SENN-DDT is also resistant to deltamethrin, permethrin, λ-cyhalothrin and malathion.[22] Resistance in SENN-DDT is mediated by elevated cytochrome P450, Glutathione-*S*-transferase and general esterase activity. SENN-DDT is also fixed for the L1014F mutation.[23] SENN and SENN-DDT were maintained in the Botha de Meillon insectary, Johannesburg, South Africa according to the procedures described by Hunt *et al.*[24] In brief, the strains were maintained at 25 °C (±2 °C) and 85% (±5%) humidity. The insectary was kept on a 12 :12 h, light:dark photoperiod with a 30-min dawn–dusk cycle. For each set of experiments, a separate set of treatments were prepared, and no adults were used from previous treatments.

2.2 Calculation of lethal concentrations of detergents and acids

For the acid-treated samples, hydrochloric acid 32% (HCl) (Univar Solutions Inc., Downers Grove, IL, USA) and nitric acid 55% (HNO_3) (Univar Solutions Inc.) were used. HCl was chosen to represent common acids used in industry.[25,26] Although HNO_3 represents one of the acids found in acid rain, it also has a range of industrial uses.[26–29] Furthermore, HCl and HNO_3 have low pH and pKa values, classifying these acids as strong acids.

For the detergent-treated samples, a commercial liquid detergent, Sunlight Dishwashing Liquid (Unilever, UK) was used as a phosphate-containing detergent. The phosphate-free liquid detergent was represented by Acetron A (Associated Chemical Enterprises Pty Ltd, Johannesburg, RSA). These two classifications were chosen as they are common classifications for detergents.

Ranging bioassays were performed to determine lethal concentrations that would represent low, medium, and high toxicities against 4th-instar mosquito larvae. These representative lethal concentrations of the pollutants were calculated as per the protocol to calculate the concentrations of larvicides.[30] In brief, for the acid treatments, final concentrations of 0.125% (v/v), 0.25% (v/v), 0.5% (v/v), 1% (v/v) and 2% (v/v) were evaluated. For the detergent treatments, final concentrations of 0.05% (v/v), 0.1% (v/v), 0.2% (v/v) and 0.4% (v/v) were evaluated. For both experiments, controls did not contain acids and detergents, respectively. These volumes represented the amount used from the undiluted starting material. For each concentration, 20–25 4th-instar larvae were exposed. The experiments were replicated four times from larvae originating from separate egg batches (cohorts). Larval mortality was scored after 24 h as per the standard WHO protocol for evaluating larvicides in mosquitoes.[30] The lethal concentrations were determined by Probit analysis.[31]

2.3 Effect of acid and detergent exposure on larval development

The choices of concentrations used for the development assays were based on information from the representative lethal concentrations calculated above. The three concentrations represented low, medium and high toxicity. For both acid and detergent, these toxicities were 25, 50 and 100 µL/1000 mL, respectively.

In each of the treatments, 100 1st-instar larvae (<24 h old) of SENN and SENN-DDT were added to a container with dimensions 214 × 155 × 80 mm. A sample in untreated water served as a control. The treatments were fed the same amount of food which was 1 mg larva^{-1} once a day for the first 5 days (L1 to approximately L3), thereafter 1 mg larva^{-1} three times per day.[23] Larval development was measured as time to pupation. The experiment was replicated three times, with larvae from different egg batches constituting the three different cohorts.

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2.4 Effect of larval acid and detergent exposure on adult longevity

In order to determine the effect of acid and detergent exposure on adult longevity, larvae were prepared as per the development study, except that 200 larvae were prepared. For longevity, only one concentration was used (25 µL/1000 mL) as this was the only concentration that was successful for rearing both acids and detergents. This was to allow for the use of a uniform concentration between acid and detergent concentrations. SENN and SENN-DDT adults from each treatment were collected upon emergence and placed in a cage. Cages contained 50 males and 50 females and were allowed *ad libitum* access to 10% sucrose, but no access to blood. Cadavers were removed daily until all specimens were dead. The experiment was replicated three times.

2.5 Effect of larval acid and detergent exposure on adult insecticide tolerance

In order to determine the effect of acid and detergent exposure on adult insecticide tolerance, 200 SENN and SENN-DDT larvae were reared in 25 µL/1000 mL acid- or detergent-treated water. Adults were collected upon emergence, and not allowed access to blood. Thereafter, 3-day-old SENN and SENN-DDT adults were exposed to either deltamethrin or malathion in a CDC bottle bioassay. For SENN, the adults were exposed to 0.0001 mg mL^{-1} of insecticide and for SENN-DDT the exposure concentration was 0.001 mg mL^{-1}. The assays were performed as per Jeanrenaud *et al* and Samuel *et al*.[32,33] Samples exposed to acetone-coated bottles served as a solvent control and unexposed samples served as an environmental control. Four concentration replicates were used to calculate the median lethal times (LT_{50}), which were calculated using Probit analysis.[31]

2.6 Statistical analysis

Data were assessed for normality using the Shapiro–Wilk test.[34] Differences in means were compared using a one-way ANOVA followed by *post hoc* comparisons of the means using Tukey's honestly significant difference (HSD) test.[35] The time to event analysis (larval development and adult longevity) was analyzed using a Kaplan–Meier estimator,[36] where statistical significance was determined using a log-rank test.[37] All analyses were performed using a 95% confidence interval (CI).

3 RESULTS

3.1 Lethal concentrations of detergent and acid do not differ in insecticide-susceptible and resistant *an. Arabiensis*

There were no statistical differences in the tolerance of SENN and SENN-DDT larvae to HCl (two-sample Student's *t*-test: $P = 0.15$, $t = 1.78$, df = 4) and HNO_3 ($P = 0.06$, $t = 2.45$, df = 6), respectively. However, when comparing the LC_{50} values of SENN and SENN-DDT between HCl and HNO_3, there was a statistical difference in tolerance, with both SENN and SENN-DDT larvae having lower LC_{50} values for HNO_3 than HCl [one-way ANOVA: $P < 0.01$, $F_{(3;10)} = 13.66$] [Fig. 1(A)].

When comparing the LC_{50} values of the detergents, there was no difference in tolerance to phosphate-free detergent between the strains [$P = 0.60$, $F_{(1;\ 7)} = 0.30$]. This also was true for commercial phosphate-containing detergent [$P = 0.52$, $F_{(1;\ 5)} = 0.49$]. However, the LC_{50} values of phosphate-free detergent were significantly higher than those of phosphate-containing detergent for both strains [$P = 0.03$, $F_{(3;10)} = 4.29$] [Fig. 1(B)].

3.2 Acid exposure alters larval development time in *an. Arabiensis*

Neither SENN nor SENN-DDT larvae could develop in the 100 µL/1000 mL HCl concentration, and as such, only larvae treated with the 25 and 50 µL/1000 mL treatments could pupate (Fig. 2). In the SENN strain, the untreated controls had a significantly longer time-to-pupation than SENN treated with both HCl treatments (log-rank test: $P < 0.01$, $\chi^2 = 102.85$, df = 3). By contrast, untreated SENN-DDT controls had a significantly shorter time-to-pupation compared to SENN-DDT treated with HCl at both treatments.

The 50 µL/1000 mL HCl treatment against SENN resulted in a significantly longer time to pupation than the 25 µL/1000 mL HCl treatment ($P < 0.01$, $\chi^2 = 9.53$, df = 1). Likewise, SENN-DDT larvae had a significantly longer time to pupation when treated with 50 µL/1000 mL HCl compared to the 25 µL/1000 mL HCl treatment ($P < 0.01$, $\chi^2 = 38.77$, df = 2) (Fig. 2).

HNO_3 treatments also resulted in a significant difference in the time-to-pupation of both SENN and SENN-DDT larvae. In the SENN strain, all HNO_3 treatments decreased time to pupation when compared to the untreated controls ($P < 0.01$, $\chi^2 = 162.88$, df = 3). There were, however, no significant differences in time to pupation between the HNO_3 treatments at the three different concentrations ($P = 0.47$, $\chi^2 = 1.80$, df = 2). Likewise, in the SENN-DDT strain, the untreated control had the longest time to pupation ($P < 0.01$, $\chi^2 = 138.96$, df = 3) compared to SENN-DDT treated with HNO_3. When comparing only the three HNO_3 treatments for SENN-DDT, the 50 µL/1000 mL HNO_3 treatment caused the longest time to pupation ($P < 0.01$, $\chi^2 = 17.94$, df = 2) whereas the 25 µL/100 mL HNO_3 treatment resulted in a longer time to pupation than the 100 µL/1000 mL treatment ($P = 0.04$, $\chi^2 = 4.33$, df = 1) (Fig. 2).

3.3 Detergent exposure alters larval development time in *An. arabiensis*

Overall, phosphate-free detergent resulted in a delay in larval development in the SENN strain when compared to the untreated controls (log-rank test: $P < 0.01$, $\chi^2 = 61.44$, df = 3) where the highest concentration of phosphate-free detergent treatments resulted in the longest time-to-pupation ($P < 0.01$, $\chi^2 = 24.44$, df = 2) (Fig. 3). However, there was no significant difference in the larval development time of SENN between the 25 µL/1000 mL and 50 µL/1000 mL phosphate-free detergent treatments ($P = 0.34$, $\chi^2 = 0.88$, df = 1) (Fig. 3). A similar pattern was observed for SENN-DDT, where all phosphate-free detergent treatments overall resulted in a longer time to pupation ($P < 0.01$, $\chi^2 = 43.10$, df = 3) (Fig. 3). As with SENN larvae, 100 µL/1000 mL phosphate-free detergent treatment resulted in the longest time-to-pupation in SENN-DDT ($P < 0.01$, $\chi^2 = 19.74$, df = 2) whereas no significant difference in the time-to-pupation between SENN-DDT larvae treated with the 25 µL/1000 mL and 50 µL/1000 mL treatments of phosphate-free detergent was observed ($P = 0.06$, $\chi^2 = 3.59$, df = 1) (Fig. 3). When comparing time-to-pupation between the SENN and SENN-DDT strains treated with phosphate-free detergent, there was no significant difference at the 25 µL/1000 mL concentration ($P = 0.17$, $\chi^2 = 1.84$, df = 1), 50 µL/1000 mL concentration ($P = 0.79$, $\chi^2 = 0.07$, df = 1) and the 100 µL/1000 mL concentration ($P = 0.52$, $\chi^2 = 0.40$, df = 1) (Fig. 3).

Larvae of both strains could only develop at the lowest concentration (25 µL/1000 mL), of the phosphate-containing detergent.

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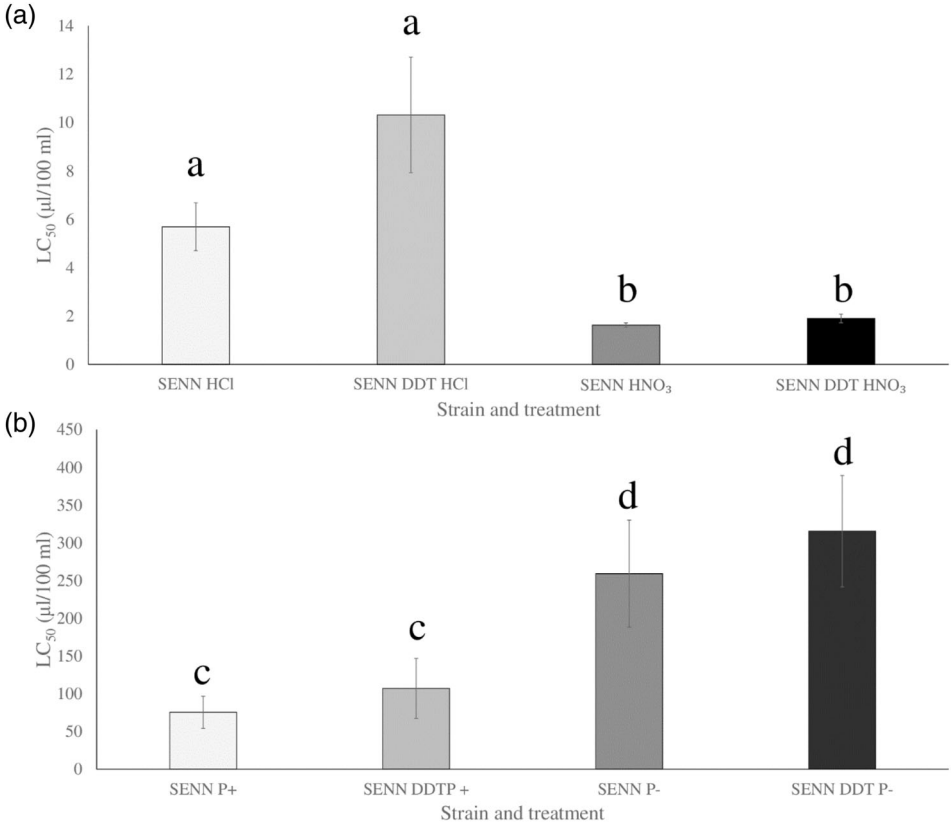

Figure 1. Median lethal concentration (LC_{50}) values for acid and detergent treatments against 4th-instar larvae of two *An. arabiensis* strains (SENN and SENN-DDT). (A) Mean LC_{50} values of 4th-instar larvae for acid treatments (HCl and HNO_3). (B) Mean LC_{50} values of 4th-instar larvae for phosphate-containing and -free detergent treatments. Significant differences are indicated by different letters.

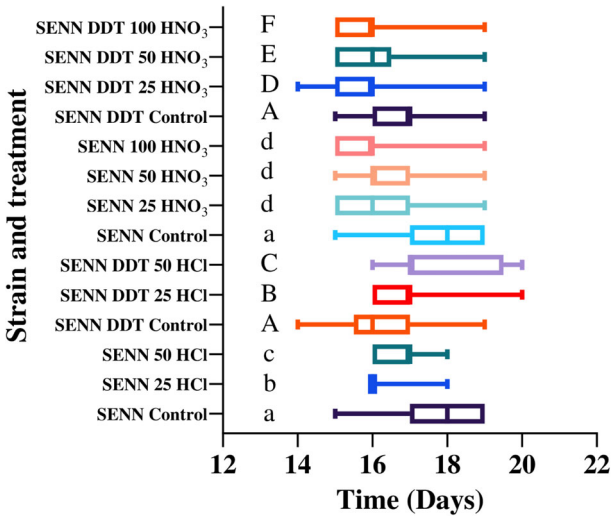

Figure 2. Effect of acid exposure on larval development time in two *An. arabiensis* strains (SENN and SENN-DDT). Different lowercase letters represent statistically significant differences in treatments against SENN larvae, whereas different uppercase letters indicate statistically significant differences in treatments against SENN-DDT larvae. Statistical significance was determined by a log rank test. Treatments were performed at 25, 50 and 100 µL/1000 mL for both acids.

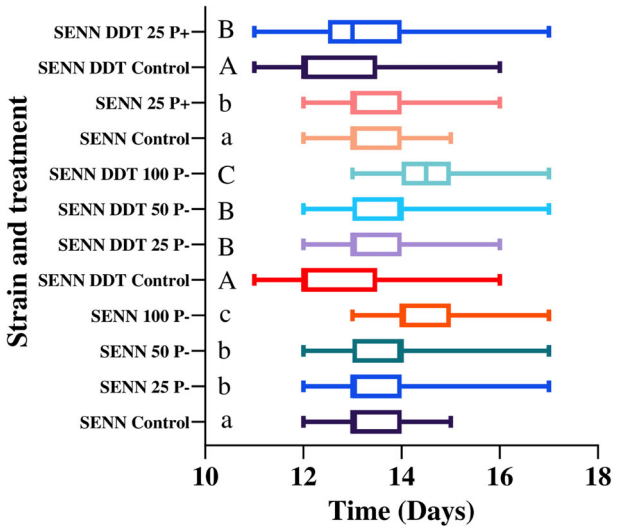

Figure 3. The effect of detergent exposure on larval development time in two *An. arabiensis* strains (SENN and SENN-DDT). Different lowercase letters represent statistically significant differences in treatments against SENN larvae, whereas different uppercase letters indicate statistically significant differences in treatments against SENN-DDT larvae. Statistical significance was determined by a log-rank test. For phosphate-free detergent (P−), treatments were performed at 25, 50 and 100 µL/1000 mL concentration. For phosphate-containing detergent (P+), treatment was only performed at 25 µL/1000 mL.

At 25 µL/1000 mL of the detergent-containing detergent treatment, slower larval development in the SENN strain ($P < 0.01$, $\chi^2 = 8.79$, df = 1) and SENN-DDT strain ($P < 0.01$, $\chi^2 = 16.2$, df = 1) were observed when comparing with the untreated controls. In a similar way to the phosphate-free detergent, there was no significant difference in the time-to-pupation between

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the two strains at the 25 µL/1000 mL concentration ($P = 0.42$, $\chi^2 = 0.62$, df = 1) (Fig. 3).

3.4 Larval acid exposure affects adult longevity in *An. arabiensis*

For all longevity studies, except for SENN male treatments [Fig. 4(B)], there were no significant difference in the longevity of the different concentrations of HCl and HNO_3. For the SENN strain, both acid treatments did not affect adult female longevity significantly when compared to that of the untreated SENN female controls (log-rank test: $P = 0.88$, $\chi^2 = 0.02$, df = 2) [Fig. 4(A)].

By contrast, for SENN males, HCl treatment resulted in significantly increased longevity ($P < 0.01$, $\chi^2 = 21.44$, df = 2) [Fig. 4(B)]. The trend for SENN-DDT females was similar, with acid treatments not significantly affecting adult longevity ($P = 0.09$, $\chi^2 = 4.71$, df = 2) [Fig. 4(C)]. As for SENN-DDT females, there were no significant changes in adult longevity in SENN-DDT males after acid treatments ($P = 0.13$, $\chi^2 = 4.03$, df = 2) [Fig. 4(D)].

3.5 Larval detergent exposure affects adult longevity in *An. arabiensis*

Detergent treatments resulted in more changes in adult longevity of *An. arabiensis* than acid treatments did. For SENN females, although adult longevity for phosphate-free and phosphate-containing detergent treatments did not differ significantly, both treatments resulted in significantly longer adult longevity than their untreated counterparts ($P < 0.01$, $\chi^2 = 57.41$, df = 2) [Fig. 5(A)]. For SENN males, phosphate-containing detergent resulted in significantly greater longevity than phosphate-free detergent, as well as untreated adult males ($P < 0.01$, $\chi^2 = 14.85$, df = 2) [Fig. 5(B)]. The SENN-DDT females displayed the same pattern as SENN females, where adult longevity after both phosphate-free and phosphate-containing detergent treatments did not differ significantly from each other but resulted in significantly longer longevity than their untreated counterparts [Fig. 5(C)]. Although both detergent treatments resulted in increased longevity compared to their untreated counterparts in SENN-DDT males, phosphate-free detergent resulted in the greatest increase in longevity which was significantly longer than the increase in adult longevity induced by phosphate-containing detergent ($P < 0.01$, $\chi^2 = 38.51$, df = 2) [Fig. 5(D)].

3.6 Larval detergent and acid exposure alter adult deltamethrin tolerance in *An. arabiensis*

Exposure to HCl did not have a significant effect on the LT_{50} of deltamethrin or malathion in SENN. For SENN females, HCl treatment did not result in a significant change in deltamethrin ($P = 0.11$, $t = 1.74$, df = 12) or malathion ($P = 0.57$, $t = -0.58$, df = 12) Tolerance. This was also true for SENN male deltamethrin tolerance ($P = 0.05$, $t = 2.51$, df = 6) as well as malathion tolerance ($P = 0.07$, $t = 2.25$, df = 6) [Fig. 6(A)]. However, this was not the case for SENN-DDT females where deltamethrin tolerance was significantly increased in females ($P = 0.04$, $t = 2.43$, df = 7) as was malathion tolerance ($P = 0.02$, $t = 3.01$, df = 7), after HCl

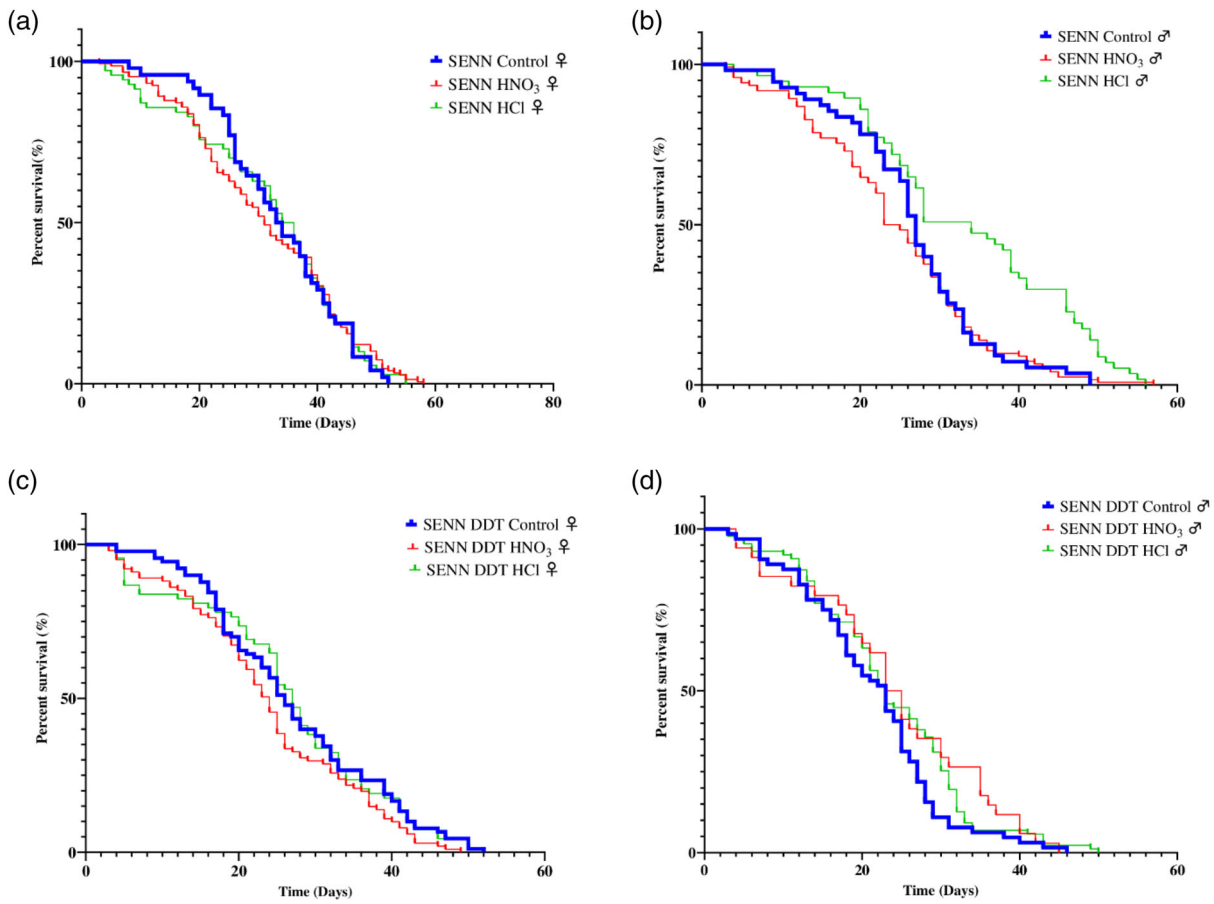

Figure 4. Effect of larval exposure to acids (HCl and HNO_3) on adult *An. arabiensis* longevity. (A) Longevity of SENN females (♀). (B) Longevity of SENN males (♂). (C) Longevity of SENN-DDT females (♀). (D) Longevity of SENN-DDT males (♂).

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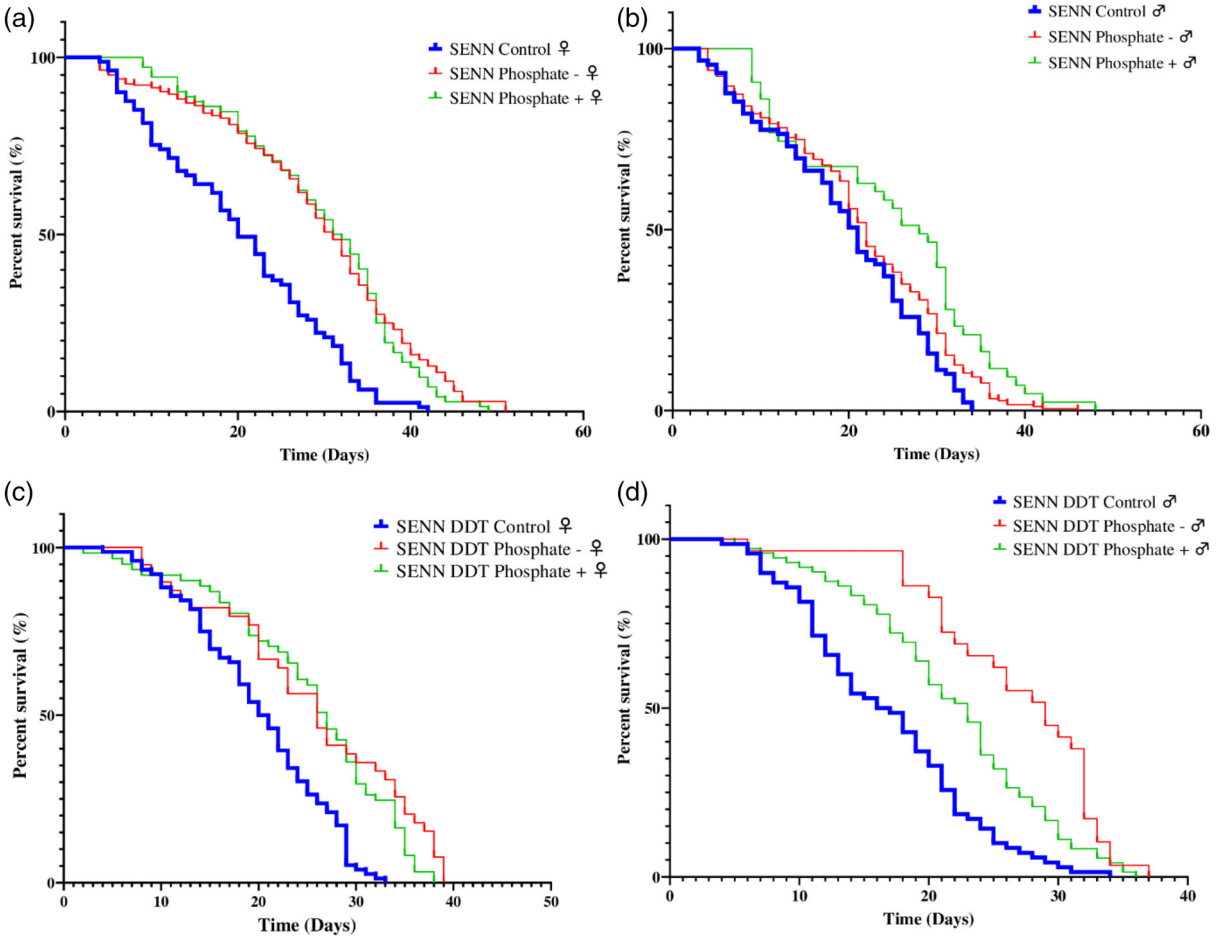

Figure 5. Effect of larval exposure to phosphate-free and phosphate-containing detergents on adult *An. arabiensis* longevity. (A) Longevity of SENN females (♀). (B) Longevity of SENN males (♂). (C) Longevity of SENN-DDT females (♀). (D) Longevity of SENN-DDT males (♂).

treatment. This pattern was not repeated in males, where although malathion tolerance was significantly increased ($P = 0.03$, $t = 2.91$, df = 6) after HCl treatment, deltamethrin tolerance was not affected by HCl treatment ($P = 0.14$, $t = 1.73$, df = 6) [Fig. 6(B)].

The LT_{50} of deltamethrin was significantly increased after HNO_3 treatment of SENN females ($P = 0.02$, $t = 2.96$, df = 7) and SENN males ($P < 0.01$, $t = 5.34$, df = 7) [Fig. 6(C)]. By contrast, treatment of SENN with HNO_3 acid did not significantly alter the tolerance of SENN males and females to malathion [one-way ANOVA: $P = 0.18$, $F_{(3;\ 21)} = 1.80$] [Fig. 6(C)]. A similar pattern was observed for deltamethrin tolerance in the insecticide-resistant female and male SENN-DDT strains where female and male SENN-DDT had a significantly increased LT_{50} for deltamethrin after HNO_3 treatment: female, $P < 0.01$, $t = -7.05$, df = 5; male, $P = 0.02$, $t = -3.53$, df = 5[Fig. 6(D)]. However, unlike the SENN strain, HNO_3 treatment altered malathion tolerance in SENN-DDT because the LT_{50} values for both females ($P < 0.01$, $t = -3.72$, df = 6) and males ($P < 0.01$, $t = -3.66$, df = 7) increased significantly [Fig. 6(D)].

Phosphate-containing detergent significantly increased deltamethrin LT_{50} in SENN females ($P < 0.01$, $t = 5.58$, df = 6) as well as SENN males ($P = 0.01$, $t = 3.65$, df = 6) [Fig. 7(A)]. For malathion treatment, however, although SENN females had a significantly increased malathion LT_{50} ($P < 0.01$, $t = 5.82$, df = 11) after phosphate-containing detergent treatment, SENN males ($P = 0.91$, $t = 0.12$, df = 5) did not [Fig. 7(A)]. For SENN-DDT the phosphate-containing detergent treatment increased deltamethrin LT_{50} significantly in SENN-DDT females ($P < 0.01$, $t = 3.74$, df = 6) as well as males ($P = 0.04$, $t = 2.44$, df = 7). By contrast, phosphate-containing detergent did not increase the LT_{50} of malathion in SENN-DDT females ($P = 0.63$, $t = 0.51$, df = 7) or SENN-DDT males ($P = 0.44$, $t = 0.85$, df = 5) [Fig. 7(B)].

Phosphate-free detergent increased the LT_{50} of deltamethrin in SENN females ($P = 0.01$, $t = 3.48$, df = 5) as well as SENN males ($P < 0.01$, $t = 8.62$, df = 5). By contrast, treatment with this detergent did not increase malathion tolerance in SENN females ($P = 0.61$, $t = 0.23$, df = 12) or males ($P = 0.61$, $t = 0.53$, df = 6) [Fig. 7(C)]. In SENN-DDT, the phosphate-free detergent treatment significantly increased the LT_{50} of deltamethrin in females ($P = 0.01$, $t = 3.51$, df = 6) but not in males ($P = 0.11$, $t = 1.88$, df = 6). Like SENN, phosphate-free detergent treatment did not significantly increase malathion treatment in SENN-DDT females ($P = 0.20$, $t = 1.42$, df = 6) or males ($P = 0.09$, $t = 2.00$, df = 6) [Fig. 7(D)].

4 DISCUSSION

With the rapid urbanization of the African continent comes a host of challenges. This is particularly true for public health concerns. In

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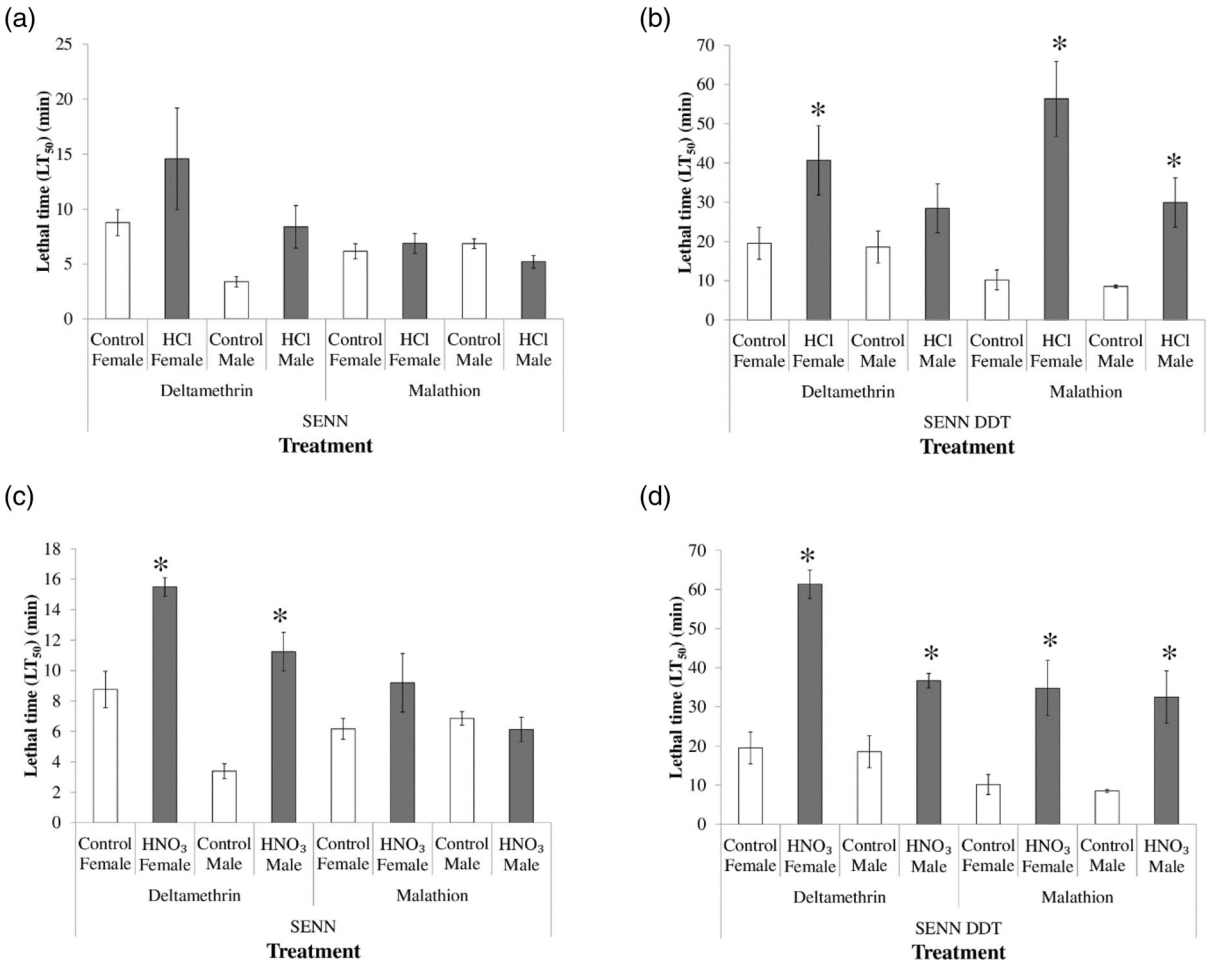

Figure 6. Effect of larval acid exposure on adult insecticide tolerance in two *An. arabiensis* strains (SENN and SENN-DDT). (A) LT_{50} values of SENN reared in HNO_3. (B) LT_{50} values of SENN-DDT reared in HNO_3. (C) LT_{50} values of SENN reared in HCl. (D) LT_{50} values of SENN-DDT reared in HCl. For SENN, the adults were exposed to 0.0001 mg/mL of insecticide and for SENN-DDT the exposure concentration was 0.001 mg/mL. * indicates a significant difference from the untreated control.

terms of urban vector-borne diseases, the challenges are usually associated with infections transmitted by *Culex*, *Aedes* and *Anopheles stephensi* mosquitoes,[38–40] which is in part a consequence of the cosmopolitan nature of these mosquitoes.[41] However, it may also be a consequence of the breeding habits of these mosquitoes and their capacity to breed in polluted water.[42]

By contrast, the distribution of malaria in rural areas is generally caused by members of the *An. gambiae* complex. This is a result of the preference of the *An. gambiae* complex to breed in clean, clear, sunlit bodies of water, which are more abundant in rural settings.[6] Although the other major vector, *An. funestus* has not been reported to breed in polluted water,[43] there have been reports of both *An. gambiae* and *An. arabiensis* breeding in polluted water.[10,11] In the case of the highly adaptable *An. arabiensis*, this is particularly concerning because there have already been reports of this species being associated with urban malaria.[44,45]

A range of studies have described the differential effects of pollutants in insecticide-resistant and susceptible *An. arabiensis*. In general, these studies found that toxic pollutants favoured the survival of the insecticide-resistant SENN-DDT strain,[46,47] whereas less toxic pollutants such as inorganic and organic fertilizers favoured the survival of the insecticide susceptible SENN strain.[32] By contrast, there has been very little work comparing the effects of pollutants across a range of pHs within a single study. Although there have been previous studies on the effect of H_2O_2 and detergent on mosquitoes,[11] and studies on the effect of larval acid exposure,[48] these have never been compared in the same study.

As with previous studies using other species, we found that *An. arabiensis* was reasonably tolerant to HCl but less so to HNO_3. By contrast, the detergent tolerance was not affected by the presence or absence of phosphate in the detergent. What is notable about the acute exposure studies is that there was no difference in the tolerance to either of these stressors between the strains. This is notable, as the SENN-DDT strain was previously demonstrated to be more tolerant to larval heavy metal and herbicide exposure.[46,47] The lack of strain differences in pollutant tolerance suggests that strain-specific xenobiotic detoxification enzymes may not play a large differential role in the tolerance of these pollutants as they did for heavy metals or herbicides. This is not surprising for the detergents, as one of the major challenges of larval survival in detergent-polluted water is the reduction of oxygen availability to the anophelines that obtain oxygen from the surface of the water.[49] Thus, the difference in tolerance is most likely to result more from the water chemistry rather than strain-dependent detoxification enzymes.

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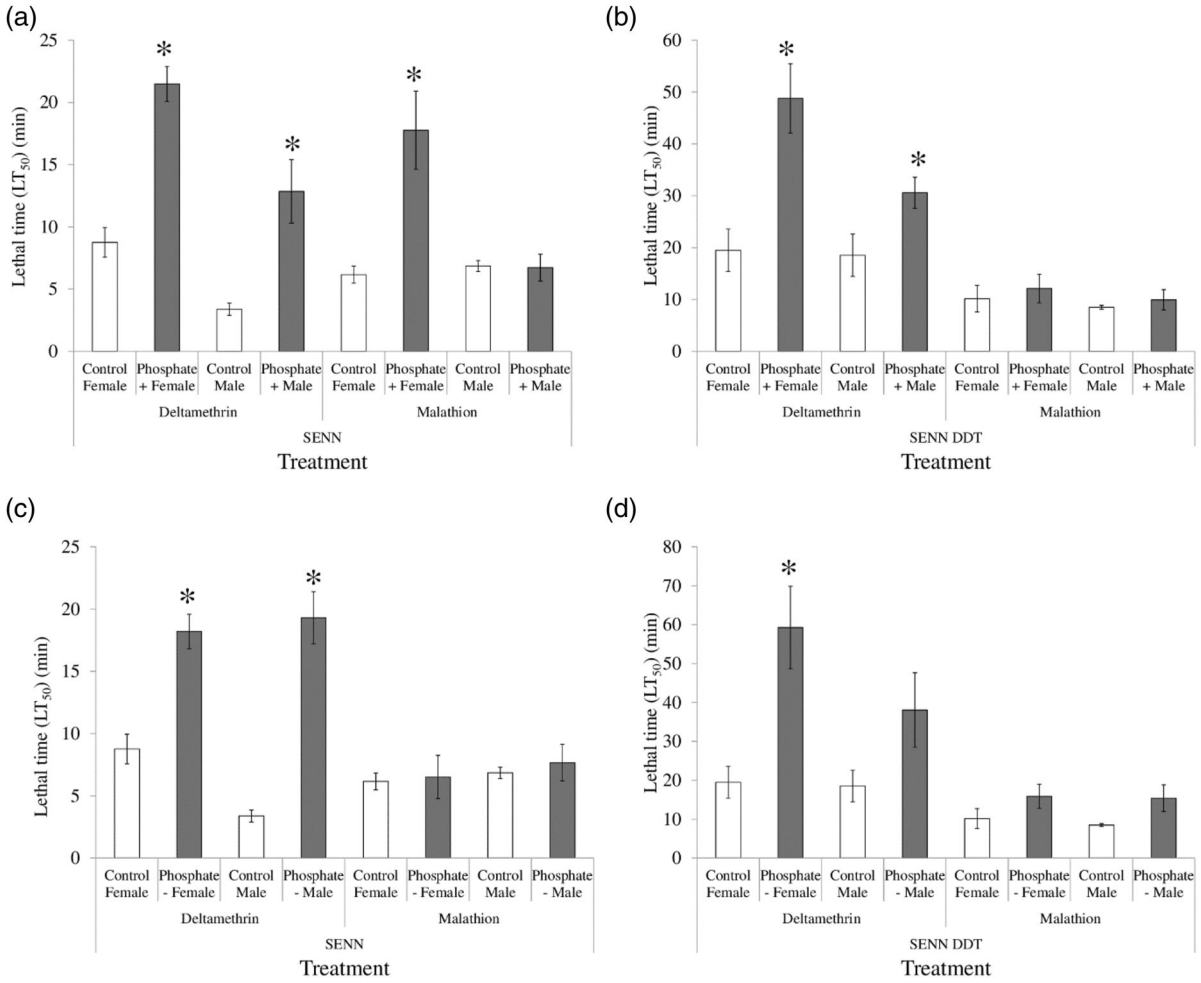

Figure 7. Effect of larval detergent exposure on adult insecticide tolerance in two *An. arabiensis* strains (SENN and SENN-DDT). (A) LT_{50} values of SENN reared in phosphate-containing detergent. (B) LT_{50} values of SENN-DDT reared in phosphate-containing detergent. (C) LT_{50} values of SENN reared in phosphate-free detergent. (D) LT_{50} values of SENN-DDT reared in phosphate-free detergent. For SENN, the adults were exposed to 0.0001 mg/mL of insecticide and for SENN-DDT the exposure concentration was 0.001 mg/mL. An asterisk (*) indicates a significant difference from the untreated control.

Despite the lack of difference in tolerance to acute exposure of the pollutants in the present study, there was a clear hormetic effect (favourable response to a low dose of usually toxic stressor) in the larval development after HCl treatment.[50] For SENN, hormesis was particularly evident after HCl exposure, where the highest HCl concentration (100 μL/1000 mL) was lethal for SENN, whereas the two lower HCl concentrations (25 and 50 μL/1000 mL) increased the development rate. Hormesis has been observed in similar studies before, where cigarette and metal pollution at certain concentrations caused hormetic effects in *An. arabiensis*.[16,47] Furthermore, the observation that hormesis was more evident in the SENN strain was similar to the exposure to cigarettes, where the SENN strain rather than the SENN-DDT strain developed faster in cigarette-polluted water.[16] In that case, however, there was evidence that this was a consequence of increased consumption of cigarette material in the SENN strain.[16] The reason for the hormetic effect of HCl in the present study is unclear.

For the detergent treatments, no hormetic effects for larval development were observed. Instead, detergent exposure increased larval development time of both SENN and SENN-DDT where the time-to-pupation increased. Again, this is possibly a result of the restriction of oxygen in the detergent-treated water. Moreover, what is notable about the detergent treatment is that it resulted in increased adult longevity. Longer larval development time has been associated with increased longevity before in *An. arabiensis* and *An. funestus*.[51,52] An increase in male longevity is of less epidemiological significance than the changes in female longevity because older males are not preferred for mating.[53] By contrast, older females are the malaria-transmitting population as a result of the intrinsic incubation period of the parasite. As such, small changes in longevity can have a significant effect on disease transmission.[54] Thus, the finding that both phosphate-containing and phosphate-free detergents significantly increased female adult longevity is directly relevant to malaria transmission and may affect vector control strategies.

In addition to differences in how SENN and SENN-DDT trade-off between altered development time and longevity, another key difference between the strains is the effect on insecticide tolerance. The SENN-DDT strain did not have a big advantage in terms of decreased development time, yet overall, they had a much bigger advantage in terms of insecticide tolerance after acid treatment. This was the same pattern as that observed in the case of larval organic fertilizer exposure where the change in insecticide was so marked, that it resulted in a change in resistance

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intensity.[32] Although the tolerance change was not as marked in this experiment, the SENN-DDT strain seems to preferentially increase detoxification enzyme activity, as evident by the larger LT_{50} values after acid treatments. Although enzyme activity was not measured in this experiment, previous studies on this strain demonstrated that deltamethrin and malathion resistance in this strain is mediated by increased enzyme activity rather than target site resistance.[23]

By contrast, HCl had no significant effect on insecticide tolerance in SENN. These findings have a two-fold implication. First, it implies the relative trade-offs and advantages obtained by the insecticide-resistant and susceptible strains. Although SENN had a clear hormetic advantage in terms of larval development, the advantage for SENN-DDT was in terms of insecticide tolerance. This suggests a preferential partitioning of resources to xenobiotic detoxification rather than growth in the SENN-DDT strain. The second implication is about the nature of the two acids themselves. HCl was less toxic than HNO_3 but induced a hormetic effect in the SENN strain, whereas HNO_3 was more toxic than HCl but resulted in increased deltamethrin tolerance in the SENN strain. For SENN-DDT, HNO_3 exposure increased both deltamethrin and malathion tolerance in both sexes. This suggests a potential for an optimal zone for hormetic advantages. If the hormetic concentration band is wider, it may result in greater advantages, as can be seen for insecticide tolerance.

Overall, the detergent treatments increased deltamethrin tolerance more than malathion tolerance. Furthermore, phosphate-containing detergent was the most effective at increasing insecticide tolerance in both SENN and SENN-DDT strains overall. The phosphate-containing detergent also had the greatest chronic effect on larval development as they could not develop past a 25 µL/1000 mL concentration. As such, phosphate-containing detergent represents the greater detergent challenge, and this could possibly account for the increased tolerance relative to phosphate-free detergent. The changes in insecticide tolerance are yet another demonstration of the variable response to larval pollutant exposure in insecticide-resistant and susceptible *An. arabiensis*.

This study has therefore demonstrated that pollution associated with urban environments has the capacity to alter the life history of *An. arabiensis*. Crucially, the response of the insecticide-resistant and susceptible strains is markedly different. Although the effects of detergents are similar, the two acids differed markedly. This may be a result of the differences in activity. Previous studies suggested that acid exposures may be to the result of ingestion rather than cuticular exposure.[17] As such, the two acids could affect the gut differently in a range of manners. This could include slight shifts in gut microbiota in the larvae.[55]

By contrast, the effect of the detergents may largely be due to their capacity to restrict oxygen. It must be noted, however, that commercial detergent has been shown to be toxic to mosquito larvae, as well as being known to be insecticidal. It is also an oxidant and could induce oxidative stress. This is another potential source of variability between the strains; SENN-DDT has been shown to have a greater defence against oxidative stress.[56]

In conclusion, this study adds to the body of data demonstrating that larval pollutant exposure in the *An. gambiae* complex, *An. arabiensis* in particular, can alter insecticide resistance. This is particularly true for pyrethroids, as deltamethrin tolerance was generally more affected than malathion resistance. Furthermore, exposure to detergent and acid had a hormetic effect on larval development and adult longevity. This differed between insecticide-resistant and -susceptible *An. arabiensis*. Therefore, urban pollutants have the capacity to alter the life history of this species in a manner that varies in insecticide resistance phenotype.

AUTHOR CONTRIBUTIONS

CYC: produced initial drafts and contributed to final drafts of MS. SVO: performed experiments, analysed data, produced initial drafts and contributed to final drafts of MS.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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