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ORIGINAL RESEARCH ARTICLE

# Effect of physicochemical factors of breeding sites on larval density and detoxification enzymes activities of *Culex pipiens* (l.) (Diptera: Culicidae) in qalyubia governorate, Egypt

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**Abstract**

There is limited evidence on the effects of physicochemical factors of breeding sites on the density of *Culex pipiens* (L.) (Diptera: Culicidae) larvae in Qalyubia Governorate. Therefore, the aim of this study was to investigate *C. pipiens* larval density and the response of their detoxification enzymes to levels of various physicochemical environmental factors present in their breeding sites. Larvae were collected from three different zones (domestic, agriculture and petrochemical) from March-June 2020. Water samples were collected during larval collection and levels of 10 physicochemical parameters (temperature, pH, conductivity, turbidity, total dissolved solids, phosphates, sulphates, nitrates, oil and grease) were measured. Activities of the three major detoxification enzymes, P450, GST and α & β-esterases were determined on the sampled larvae. Data were assessed by One-way ANOVA followed by Tukey's post-hoc test and Pearson Correlation analysis. Results showed significant difference in the mean larval density of *C. pipiens* within the three study zones with the residential zone having the highest mean larval density. The activities of the detoxification enzymes were higher in the agricultural and petrochemical zones, which recorded higher levels of the physicochemical factors. There was a significant negative correlation between the density of *C. pipiens* larvae and temperature, pH, turbidity, phosphates, sulphates, nitrates, oil and grease levels, whereas conductivity and total dissolved solids had no significant correlation with larval density. The concentrations of oil and grease which were significantly higher in petrochemical zone were strongly positively correlated with P450 activities. While, phosphate, sulphate and nitrate which were significantly higher in agricultural zone were associated with GST and α & β-esterase activities. The correlations observed between the physicochemical factors and larval density as well as the inductive impact on detoxification enzymes can confirm the effect of these factors on mosquito breeding activity and these observations could have a significant effect on the environmental management and approach to mosquito control in Qalyubia Governorate.

**Keywords** *Culex pipiens* · Physicochemical factors · Larval density · Detoxification enzymes

## Introduction

The house mosquito *C. pipiens* is the most widespread mosquito species in rural and urban areas in Egypt and causes a human health risk. They transmit numerous serious pathogens of diseases, such as lymphatic filariasis, Rift Valley Fever and West Nile encephalitis (Tageldin et al. 2018). The amount of pollutants released in the natural environment by domestic and industrial human activities has increased during the past decades (Biney et al. 1994). Most of these pollutants accumulate in rivers and stagnant water bodies and the exposure of mosquito larvae to these compounds could reduce their level of susceptibility to pesticides which are used for control. Despite the intensive use of chemical insecticides to control *C. pipiens*. These measures are currently inadequate to halt diseases transmission. Researchers have been exploring several alternatives for controlling mosquitoes and one particular approach that appears to be gaining attention is an environmental management strategy that aims to reduce the adult population by targeting their aquatic immature stages (Imam and Deeni et al. 2015). The density of adult mosquitoes is determined by larval productivity of breeding sites and their proximity to human hosts where they can obtain a blood meal (Takken and Verhulst

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2013). Physicochemical characteristics of mosquito breeding sites are important factors in determining whether the larvae can survive and successfully complete their life cycle (Garba and Olayemi 2015). Mosquitoes breeding sites are sometimes contaminated with pollutants from several sources related to human activities such as, sewage and fertilizers from agricultural fields (Zayed et al. 2019). Water characteristics such as, pH, conductivity, turbidity, concentration of sulphate, phosphate, nitrate and total dissolved solids as well as climatic factors such as temperature have a significant effect on the occurrence and larval abundance of mosquitoes and any change in these factors in the larval habitats may create conditions unfavorable or favorable for mosquito biology (Bahgat 2013; Emidi et al. 2017; Nikookar et al. 2017b; Azrag and Mohammed 2018; Ukubuiwe et al. 2018; Musonda and Sichilima 2019). Insecticides, oil spillage and wastes derived from domestic and industrial discharges are the compounds responsible for the selection of mosquito resistance (Li et al. 2007). There are two major mechanisms are responsible for the mosquito tolerance: detoxification through increased enzyme activities and reduced sensitivity of target site due to DNA mutations. Three large detoxification Enzymes are involved in insecticide metabolism; the cytochrome P450s, glutathione S transferases (GSTs) and esterases (David et al. 2006; Fossog et al. 2012; Das and Dutta 2014; Viswan et al. 2018; Tomia et al. 2019). Ecological data, such as physicochemical parameters of breeding sites affecting mosquito density (Abai et al. 2016; Bashar et al. 2016; Dom et al. 2016; Mahgoub et al. 2017; Getachew et al. 2020) and understanding the cross resistance of the vector to insecticides may serve as the basis for designing and implementation of adequate mosquito control program that depends on the use of various larvicidal techniques and environmental management practices for reducing larval density and therefore reducing vector abundance. While several factors affecting the breeding of *C. pipiens* have been documented, there is limited evidence on the impacts of physicochemical factors on these mosquitoes in Qalyubia Governorate. Therefore, this study was conducted to evaluate the correlation between the physicochemical factors of larval habitat and larval density, and to demonstrate the potentiality of these factors for the emergence and development of resistance in *C. pipiens* in Qalyubia Governorate by increasing the activities of the detoxification enzymes.

## Materials and methods

### Study area

This study was conducted in Qalyubia Governorate in the Southeast of the Nile Delta, north of Cairo, Egypt. Qalyubia is located 30°28′ N 31°11′E and it is 1001.09 km2 (0.1% total area of Egypt), comprised 195 villages and 11 cities. The earth's surface 17 m above sea level in the South and gradually decreased to be 10 m in the Northeast. According to the 2020 population, the Governorate had a total of 5,893,749 people. The climate is hot arid summer and mild rainy winter with annual rainfall 20–200 mm. The average annual temperature is 21.9 °C. It has various topographic strata including agricultural, semi-desert and desert areas. This has impact on diversity of mosquito breeding habitats. Six villages located in three districts, Tokh District (Mitt Kenana and Mushtuhur), Benha District (Marsafa and Shiblanga), El Khanka District (Arab El Elaykat and Abu Zaabal) were survived (Figs. 1 and 2). These villages with high population density (Mitt Kenana 180,000**,** Mushtuhur 48,857, Marsafa 43,867, Sheblanga 45,000, Arab El Elaykat 28,651 and Abu Zaabal 55,938 people**)**. The majority of villages have no sewage or garbage disposal systems, surrounded by agricultural lands and have many of water resources that are adjacent or descend through these villages leading to an increase in the mosquito density.

### Study design and sites

Mosquito breeding sites were categorized into three different study zones on the basis of human related activities (domestic activities, agriculture and petrochemical industries, respectively) taking place within and/or around the breeding sites. A total of 38 sampling points were inspected in the six mentioned villages. Three positive fixed breeding places in each village were visited and sampled (from 10—11:59 AM) once a week throughout the study period (March-June, 2020) and Water samples were collected from various breeding sites for the analysis of physicochemical parameters.

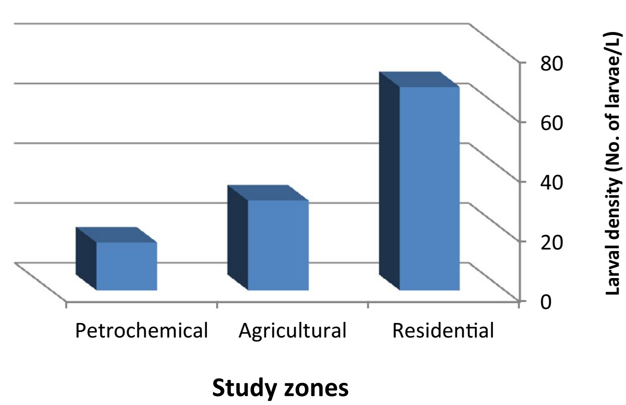


**Fig. 1** Larval density across three different *C. pipiens* breeding sites in Qalyubia Governorate, Egypt

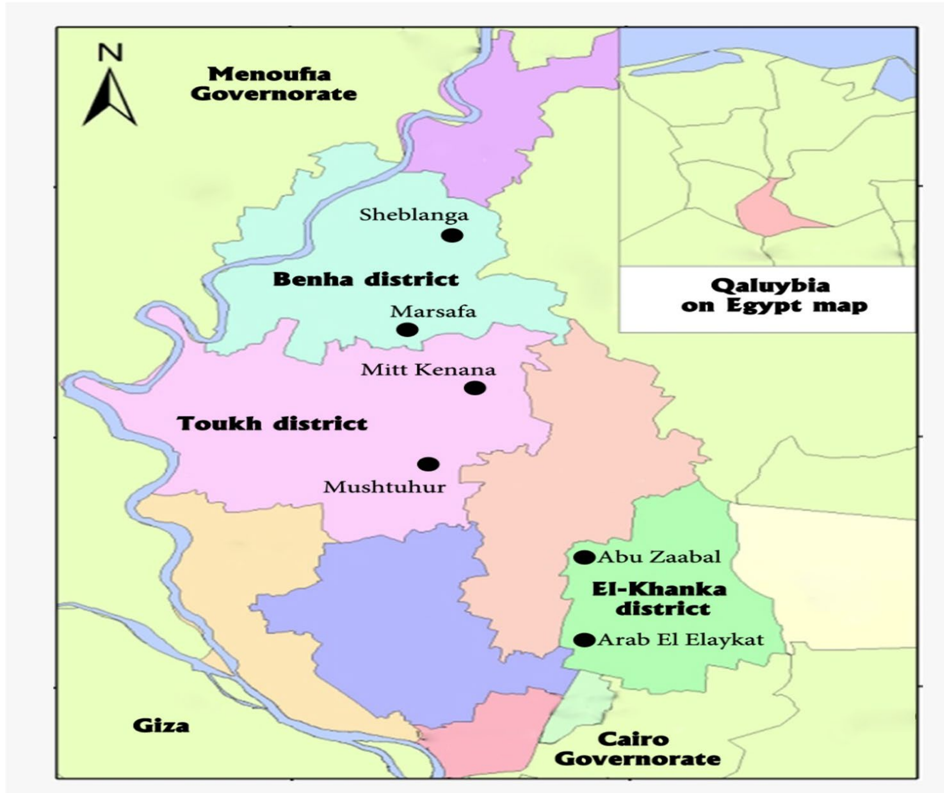


**Fig. 2** Qalyubia Governorate map showing the study area

## Larval habitat characterization

The breeding sites located in the residential zone include open basins (the water somewhat clear and invaded by algae), barrel (large container containing stored water used for domestic purposes), sewer cesspools, and water collection created after rains containing domestic wastes i.e. contaminated with organic products in decomposition (Fig. 3a–d). Breeding sites in the agricultural zone include canals and irrigation channels. Canals are the most commonly used structure for conveyance of water for irrigation. In this breeding site water flow is slow and contain debris of refused houses with grasses on some sides of canals. Irrigation channels are small channels used to irrigate the nearby fields and containing garbage (Fig. 3e, f). While the breeding sites located in the zone of discharged petrochemical


**Fig. 3** Different types of larval habitats in Qalyubia Governorate, Egypt: **a** Open basin, **b** Barrel, **c** Sewer cesspool, **d** Rainwater collection, **e** Canal, **f** irrigation channel, **g** puddle of stagnant water

products consist of small puddles of stagnant water and the water was found to be oily, muddy, dirty and obviously contaminated (Fig. 3g).

## Sampling and determination of larval density

Collection of larvae was conducted using a standard dipper (350 ml) and several dips (5–10) were made depending on the size of the breeding habitat. After each larval collection, the larvae were sorted first on the basis of species into Culicines and Anophelines. *Culex* sp. suspends there respiratory appendages at 450 to the water surfaces a feature which is particular to this species in water only, while Anopheles sp. position their body parallel to the water surfaces. The stage of the larval development (1st, 2nd, 3rd and 4th instars) was noted. All members of the Anopheline species were returned back to the breeding habitat after sorting and counting while the *Culex* larvae were retained in the same breeding water after counting and transferred to the laboratory in plastic containers for identification and biochemical assays. These larvae reared in the insectary and fed on fish food (Tetramin®) with grinded bread in the ratio of 3:1. The total larval densities were determined by manual counting and expressed as the number of larvae per liter of breeding water. Last third and fourth larval instars were identified to species level using keys of Harbach (1985).

## Water analysis

Ten physicochemical parameters of larval habitats were determined. Conductivity (EC) in micro Siemens per cm (µS/cm), temperature (°C), acidity (pH), and Total Dissolved Solids (TDS) (mg/l) were measured at the time of collection using combo TDS/pH/EC/ temperature meter (HANNA instruments, Rhode Island, United States). Turbidity in Nephelometric Turbidity Unit (NTU) was determined using a portable handheld turbidimeter PCE-TUM 20 (PCE instruments, Germany). The chemical factors including, nitrate (NO3-), phosphate (PO32-) and sulfate (SO42-) expressed in mg/l were measured by a Unicam UV2-300™ spectrophotometer (Unicam Ltd., Cambridge, UK) according to standard methods (Rice et al. 2012). Levels of oil and grease (mg/l) were determined by the liquid–liquid extraction method described by Maiti (2012).

## Biochemical assays

- **Preparation of mosquito homogenate**
  Using a chilled glass Teflon homogenizer, 50 mg of mosquito samples were mechanically homogenized in 1 ml distilled water. The homogenate was centrifuged in a cooling centrifuge for 15 min at 8000 r.p.m. and supernatant was kept at -18 0C till used.

- **Cytochrome P450 (P450) assay**
  The activity of P450 was measured by adding 200 µl of the working solution (15 ml of 0.25 M sodium acetate buffer; pH 5.0 and 5 ml methanol solution of 0.002 mg/ml of 3,31,5,51-tetramethyl benzidine) to a mixture contained 20 µl of mosquito homogenate mixed with 80 µl of potassium phosphate buffer in a microliter plate well and 25 µl of 3% hydrogen peroxide was added to the well finally. At room temperature the mixture was incubated for two hours. The absorbance was read at 650 nm using a micro-plate reader. Varying concentrations of standard cytochrome c (calibration standards) were treated similarly. P450 activity was estimated by comparing the absorbance values with the standard calibration curve of absorbance for the known concentrations of cytochrome c. The P450 activity reported as equivalents units of cytochrome P450/mg protein (Imam and Deeni 2015).

- **Glutathione S-transferase (GST) assay**
  GST activity was evaluated by mixing 0.01 ml mosquito homogenate with a mixture (working solution) contained 0.15 ml of 10 mM reduced glutathione (GSH), 0.05 ml of 50 mM 1-chloro-2,4dinitrobenzene (CDNB) were added to 2.79 ml of 40 mM buffer saline; pH 6.8. The mixture was incubated at $20^{0}$C for three minutes. The increase in absorbance was recorded at 340 nm for 5 min. The GST activity (µmol CDNB conjugated/min/mg protein) was then calculated (Habig et al. 1974).

- **Esterase assays**
  α and β-esterase activities were measured according to Mulyaningsih et al. (2017). Fifty (50) µl mosquito homogenate was mixed with 50 µl of α-naphthyl acetate working solution (0.5 ml α-naphthyl acetate in acetone (6 g/l) mixed with 50 ml phosphate buffer solution (PBS) of 0.02 M and pH = 7.0) and β -naphthyl acetate working solution (0.5 ml β-naphthyl acetate in acetone (6 g/l) mixed with 50 ml phosphate buffer solution of 0.02 M and pH = 7.0) in separate microtitre plate wells and incubated for 60 s for α and β-esterases assay respectively. Then, 50 µl coupling reagent (150 mg of Fast Blue B salt in 15 ml $H_2O$ and 35 ml aqueous sodium dodecyl sulfate) was added to each well and incubated for 10 min. When the developed red color turned to blue, 50 µl of 10% acetic acid was added immediately into each well to stop the reaction. In each study zone, 10 determinations were examined and replicated three times. The intensity of the final color was measured at λ 450 nm by a microplate reader. The results were reported as µmol of the product formed/min/mg protein.

- **Data analysis**
  One-way ANOVA followed by Tukey's post-hoc test for multiple mean comparisons were used for the inves-

tigation of significance in mean distribution of the physicochemical environmental factors, larval density and detoxification enzymes across the three study zones. The relation of the physicochemical factors to the larval density and detoxification enzyme activities was assessed by Pearson Correlation analysis using SPSS software, version 25.0 (IBM Crop. Released 2013) and the values were considered significantly different if $p < 0.05$ for all tests.

## Results

Mosquito larvae were found in a variety of water collections (7 types). A total of 28 breeding sites were found to be positive for mosquito larvae. The breeding sites included two open basins and a sewer cesspool in Mitt Kenana village, a sewer cesspool, rain pool and a barrel in Marsafa village, canal and two irrigation channels in Mushtuhur village, canal and two irrigation channels in Sheblanga village and three stagnant water puddles in each of Arab El Elaykat and Abu Zaabal villages. A total of 5974 larvae collected from 18 breeding sites in the six villages. Last third and fourth larval instars were identified and the most common species was *C. pipiens* in all breeding sites, where the relative abundance was 91.6% (5472 larvae). In the seven types of breeding sites, a total of 1632, 744, 540, 348, 872, 568, and 768 larvae were collected from open basins, rain pool, sewer cesspools, barrel, canals, irrigation channels and stagnant water puddles, respectively.

Results of one way ANOVA indicated that, there was a significant difference ($p=0.01$) in the mean larval density of *C. pipiens* within the three study zones with the residential zone having the highest mean larval density 68/L (figure, 1). The samples collected from breeding sites across the agricultural zone with a mean larval density of 30/L were second to those in residential zone which is about 55% less than those recorded for residential zone. The mean larval density in the petrochemical zone was 16/L and about 4 and twofold lower than those recorded for the residential and agricultural zones, respectively. This means that, the petrochemical zone had the lowest larval density.

## Physicochemical parameters across the three study zones

Breeding sites were chosen in order to cover the different habitats (residential, cultivated, petrochemical) and almost all districts in each city. Results of water chemistry analysis showed that, temperature and pH were not statistically significant across the three study zones, while, the differences in mean distribution of conductivity and turbidity were significant. Turbidity in the petrochemical zone was 136.6 NTU, which was 8.4 and 4.2-fold in comparison to residential & agricultural zones (16.2 and 32.5 NTU), respectively. Similarly, the differences in mean concentration in the total dissolved solids, phosphates, sulphates, nitrates, oil, grease and carbon content were significant ($p < 0.05$) across the three study zones. The concentrations of the chemical parameters; TDS, $PO_3$, $SO_4$ and $NO_3$ measured were high in the breeding sites across the agricultural zone (56, 6.8, 5 and 7.3 mg/L, respectively) which were 3.1, 4.85, 3.12 and 3.65-fold increase than the residential zone and 9.3, 5.7, 3.3 and 5.6 times higher than petrochemical zone. While, the levels of oil and grease were very high in the petrochemical zone (9.1 and 8.9 mg/L) compared to the other two zones which recorded zero (Table 1).

## Distribution of the detoxification enzymes within the three study zones

Results of biochemical analysis revealed that, the differences in mean distribution of *C. pipiens* larval cytochrome P450 activities was statistically significant ($P=0.01$) across the three study zones with highest mean activity recorded in the petrochemical zone (0.176 EU P450/mg protein). P450 activities of the residential and agricultural zones were 4.4 and 1.6 -fold lower than that of the petrochemical zone. In contrast, *C. pipiens* larvae from the agricultural zone recorded the highest mean GST (0.360 µmol/min/mg protein), and α & β-esterase activities (3.39 & 2.12 µmol of α & β-naphthol /min/mg protein, respectively) compared to the other two zones. The differences in mean activities of these two detoxification enzymes between agricultural & residential zone and agricultural & petrochemical zone were significant ($p < 0.05$) while differences between residential & petrochemical zone was not significant (Table 2).

## Correlation between larval density and physicochemical factors of breeding sites

Correlation coefficient between the larval density and each of the physicochemical parameters of *C. pipiens* breeding sites showed that, the larval density was negatively associated with temperature, pH and turbidity. While conductivity was the physical environmental factor that appeared not to affect the larval density. Furthermore, the chemical environmental parameters (phosphates, sulphates and nitrates) induced a moderate negative association with *C. pipiens* larval density while oil and grease produced significant negative correlations ($P=0.001$) with *C. pipiens* larval density. This suggested that, the density of *C. pipiens* larvae decreases as the level of each of these chemical factor increase. As was observed with conductivity, TDS had no significant correlation with larval density (Table 3).

**Table 1** Physicochemical parameters of water samples collected from three different larval habitats of *C. pipiens* in Qalyubia Governorate

| Study Zones | Mean ± SE | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Conductivity (µS/cm) | Temperature (°C) | Acidity (PH) | Turbidity (NTU) | TDS (mg/l) | Phosphate (mg/l) | Sulphate (mg/l) | Nitrate (mg/l) | Oil (mg/l) | Grease (mg/l) |
| **Residential Zone** | 380±33.35 | 27. 1±0.95 | 7.13±0.35 | 16.2±2.35 | 18±1.4 | 1.4±0.31 | 1.6±0.09 | 2.0±0.12 | 0.01±0.003 | 0.00±0.00 |
| **Agricultural Zone** | 550±24.30 | 28.30±1.04 | 7.3±0.42 | 32.5±5.59 | 56±3.1 | 6.8±1.11 | 5.0±0.33 | 7.3±0.85 | 0.00±0.000 | 0.00±0.00 |
| **Petrochemical Zone** | 510±43.86 | 28.74±1.58 | 7.7±0.33 | 136.6±10.72 | 6±0.12 | 1.2±0.08 | 1.5±0.04 | 1.3±0.01 | 9.10±2.100 | 8.9±0.88 |
| **P value** | 0.050 | 0.109 | 0.402 | 0.001 | 0.05 | 0.002 | 0.003 | 0.001 | 0.000 | 0.000 |

*SE* standard error, p value significant if $p \leq 0.05$

## Correlation between detoxification enzyme activities and physicochemical factors of breeding sites

Correlation coefficient between detoxification enzyme activities and each physicochemical factor showed that, the activity of larval cytochrome P450 was statistically positively correlated ($P = 0.01$) with temperature, pH and turbidity, while conductivity showed no significant correlation with larval cytochrome P450. A significant correlation was detected between some chemical parameters and larval cytochrome P450 activity. Oil and grease were positively associated with the cytochrome P450 activity. This means that, P450 activity increased as the level of oil and grease increased. While nitrates, phosphates, sulphates and TDS showed no significant negative correlations with cytochrome P450 activity. Concerned with larval GST and α & β-esterase activities, pH was statistically correlated with the activity ($P < 0.05$), while conductivity, temperature and turbidity were not associated. In contrast to cytochrome P450 the chemical environmental factors; phosphates, sulphates, nitrates and TDS showed highly significant ($P = 0.001$) positive correlations with GST activity and significant ($P < 0.05$) positive correlations with α & β-esterase activities, while oil and grease showed non-significant negative correlations (Table 4).

## Discussion

Our study demonstrated that, the density of *C. pipiens* larvae were significantly influenced by several physicochemical environmental parameters that are associated with mosquito breeding sites. These environmental parameters were functions of the related human activities going on around these breeding sites. The levels of phosphates, sulphates, nitrates and TDS as well as conductivity were significantly higher in the breeding sites within the agricultural zone. This might be due to the use of phosphate and nitrate-base fertilizers as well as pesticides in the cultivated areas that are located around these breeding sites (Imam and Deeni 2015). While the levels of oil and grease were highest in the breeding sites that are located in the petrochemical zone. In this zone there is a widespread discharge of petrochemical products due to auto repair shops as well as fuel vending. These human activities are the major sources of the discharged lubricants and spent fuel into the surrounding water bodies and the mosquito breeding sites. In this study the density of *C. pipiens* larvae was negatively correlated with temperature, pH, turbidity, phosphates, sulphates, nitrates, oil and grease levels. This means that, increase in the levels of these factors lead to decrease in *C. pipiens* larval density. Physicochemical parameters are nutritional sources of water bodies. However, an excessive rise in these factors

**Table 2** Distribution of the detoxification enzymes across the three study zones

| Study zones | Enzyme activity (Mean ± SE) | | | |
|---|---|---|---|---|
| | **P450** EU/mg protein | **GST** μmol/min/mg protein | **α-esterase** μmol α-naphthol / min/mg protein | **β-esterase** μmol β-naphthol /min/mg protein |
| **Residential Zone** | 0.040±0.01a | 0.02±0.001a | 0.088±0.01a | 0.125±0.03a |
| **Agricultural Zone** | 0.110±0.03b | 0.360±0.02b | 3.39±0.26b | 2.12±0.18b |
| **Petrochemical Zone** | 0.176±0.07c | 0.04±0.009ac | 0.175±0.04ac | 0.178±0.08ac |
| **P value** | 0.01 | 0.06 | 0.061 | 0.056 |

Values within columns not followed by the same letter are significantly different ($p \leq 0.05$); Tukey's HSD test.

can have adverse effects on some aquatic organisms and subsequently reduce food sources of mosquito larvae (Liu et al. 2012). While conductivity and TDS had no significant correlation with larval density. Similar findings were reported by Gopalakrishnan et al. (2013) who reported that, turbidity was negatively correlated with abundance of *C. quinquefasciatus* Say (Diptera: Culicidae), Imam and Deeni (2015) reported that, conductivity and TDS appeared to have no effect on *Anopheles gambiae* (Diptera: Culicidae) larval density, while the density of larvae was negatively correlated with temperature, pH, Kipyab et al. (2015) mentioned that, physicochemical factors in mosquito breeding sites have effect on survival and distribution, Dom et al. (2016) showed that, the percentage of dengue vectors larvae was higher in the lower readings of analyzed physicochemical parameters of breeding containers, Emidi et al. (2017) reported that, temperature and pH were associated with a decrease in larval density and neutral pH between 6.8 and 7.2 is a preferred breeding site by *Culex* mosquitoes, while conductivity and TDS appeared to have no influence, Nikookar et al. (2017b) who study the correlation between mosquito larval density and physicochemical factors of their breeding sites and observed negative correlations between larval density of *C. pipiens* and temperature, pH, turbidity and phosphate, Chumsri et al. (2018) observed that, the density of *Aedes* larvae was higher in clear water than in turbid water, Azrag and Mohammed (2018) found that, pH, conductivity, TDS, turbidity, oil and nitrate levels were higher in polluted water than control site and reported that, the density of *An. arabiensis* (Diptera: Culicidae) larvae increased as these factors decreased, Musonda and Sichilima (2019) observed that, total dissolved solids have insignificant relationship on the abundance of *Anopheles* mosquito in various breeding sites of Kapiri Mposhi districts, Zayed et al. (2019) stated that, the physicochemical factors; temperature, salinity and pH have a significant effect on the occurrence and the larval abundance among mosquito species and Getachew et al. (2020) reported that, The density of mosquito larvae was high in habitats closer to human habitation. In contrast with our research, *C. pipiens* showed a significant positive correlation with temperature, pH and nitrate (Kenawy et al. 2013), electrical conductivity (Nikookar et al. 2017b), this may indicate that, other factors are involved in the abundance of *C. pipiens*. The presence of *C. pipiens* larvae in habitats containing high levels of physicochemical factors suggest a gradual potential emergence of resistance of *C. pipiens* larvae to these factors, especially that, the lower larval densities were recorded in the agricultural and petrochemical zones in comparison with the residential zone. Water which is turbid from not nutritious particles for mosquito larvae could disfavor growth and development of larvae and can be considered a limiting factor Compatibility of *C. pipiens* to breeding sites in the vicinity of human habitats especially level of organic contamination could be important in its abundance (Nikookar et al. 2017a). One of the major mechanisms for the development of mosquitoes resistance is the detoxification enzymes mechanism. This involves induction in the activities of P450, GST, α and β-esterase which lead to rapid metabolism of the insecticides before it reaches its target sites (David et al. 2013). Therefore, exposure of *C. pipiens* to different chemical factors in its breeding sites, which could induce induction in activities of these enzymes could potentially produce acquired resistance to insecticides in mosquitoes emerging from such breeding sites. Biochemical assays revealed that, GST and α & β-esterase activities were significantly higher in *C. pipiens* larvae collected from breeding sites located in the agricultural zone while P450 activity was significantly higher in larvae collected from the petrochemical zone. Results also showed that, the concentrations of phosphate, sulphate and nitrate which were significantly higher in agricultural zone were associated with GST and α & β-esterase activities, while, oil and grease which were significantly higher in petrochemical zone were strongly positively correlated with P450 activities. This means that, oil and grease produced the most significant effect on larval density as well as P450 activities across the three zones. Similarly, Lima et al. (2011), Fossog et al. (2012) and Namountougou et al. (2012) have established a correlation between the induction of detoxification enzymes and the prior exposure to physicochemical environmental factors and this increase mosquito resistance to insecticides. Sridhar et al. (2014) reported that, resistance of mosquitoes is caused as a result of the pressure from the

**Table 3** Correlation between larval density and physicochemical parameters of *C. pipiens* breeding sites

| | | Zone | Conductivity (µS/cm) | Temperature (ºC) | Acidity (PH) | Turbidity (NTU) | TDS (mg/l) | Phosphate (mg/l) | Sulphate (mg/l) | Nitrate (mg/l) | Oil (mg/l) | Grease (mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Density** | Pearson Correlation | -.966 | -.882 | -1.000 | -.883 | -.791 | -.028 | -.227 | -.233 | -.153 | -.707 | -.708 |
| | Sig. (2-tailed) | **.016** | .312 | **.001** | **.031** | **.041** | .982 | .154 | .150 | .102 | **.001** | **.001** |
| | Sum of Squares and Cross-products | -52.00 | -4220.00 | -45.680 | -13.900 | -2779.20 | -40.000 | -38.800 | -25.000 | -27.000 | -199.900 | -195.800 |
| | Covariance | -26.00 | -2110.00 | -22.840 | -6.950 | -1389.60 | -20.000 | -19.400 | -12.500 | -13.500 | -99.950 | -97.900 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

*N* number of zones, p value significant if ≤0.05

**Table 4** Correlation between detoxification enzymes activities and physicochemical parameters of *C. pipiens* breeding sites

| | | Zone | Conductivity (µS/cm) | Temperature (ºC) | Acidity (PH) | Turbidity (NTU) | TDS (mg/l) | Phosphate (mg/l) | Sulphate (mg/l) | Nitrate (mg/l) | Oil (mg/l) | Grease (mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P450** | Pearson Correlation | 1.000 | .743 | .970 | .970 | .915 | -.213 | -.015 | -.008 | -.090 | .857 | .857 |
| | Sig. (2-tailed) | **.011** | .467 | **.01** | **.01** | **.01** | .863 | .991 | .995 | .943 | **.034** | **.033** |
| **GST** | Pearson Correlation | .052 | .719 | .309 | -.176 | -.339 | .960 | .996 | .997 | .987 | -.455 | -.454 |
| | Sig. (2-tailed) | .967 | .489 | .800 | **.041** | .780 | **.001** | **.001** | **.001** | **.001** | .699 | .700 |
| **α-esterase** | Pearson Correlation | .023 | .699 | .281 | -.204 | -.367 | .968 | .999 | .999 | .992 | -.481 | -.480 |
| | Sig. (2-tailed) | .985 | .507 | .819 | **.037** | .761 | **.042** | **.035** | **.031** | **.043** | .681 | .681 |
| **β-esterase** | Pearson Correlation | .023 | .699 | .281 | -.204 | -.366 | .968 | .998 | .999 | .992 | -.481 | -.480 |
| | Sig. (2-tailed) | .985 | .507 | .819 | **.048** | .761 | **.041** | **.035** | **.031** | **.033** | .681 | .682 |

p value significant if ≤0.05

physicochemical environmental factors that has made the susceptible population become resistant, Imam and Deeni (2015) observed that, petrochemicals in breeding sites induced significant P450 activities in *An. gambiae*, Viswan et al. (2018) reported that, GST and monooxygenase levels were higher in field populations of *C. quinquefasciatus* than laboratory strain. The results of biochemical analysis conducted by Zayed et al. (2019) revealed elevation in GST activity of *C. pipiens* larvae and the observed high level of GST is supported the increased resistance in field populations of sewage water than fresh water bodies. Tomia et al. (2019) found that, the nonspecific esterase and monooxygenase enzyme activities of *Ae. aegypti* (Diptera: Culicidae) have increased in 20 urban villages in Ternate due to the excessive use of insecticides for long period.

## Conclusion

The present study has established significant correlations between various physicochemical factors of breeding sites and the density of *C. pipiens* larvae as well as the activities of the detoxification enzymes, P450, GST and α & β esterase. The characteristics and levels of these factors were related to the different human activities taking place around the mosquito breeding sites. Therefore, physicochemical characteristics of the breeding sites may determine the abundance of *C. pipiens* in this area. Findings in this study could be useful in comprehending mosquito ecology and should be taken into consideration when designing and implementing larval control programs especially using source reduction through the environmental modification and manipulation in addition to biological and chemical controls. This is the first study looking at the physicochemical characteristics associated with larval density and detoxification enzymes of *C. pipiens* in Qalyubia Governorate. Further studies are needed during different seasons to enable the use of this information in mosquito control programs.

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