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Spatial Distribution Patterns and Relative Abundance of *Culex* Mosquito Larvae in Alexandria Governorate, Egypt

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ABSTRACT

The present study aimed to study *Culex* mosquito vectors' different patterns in Alexandria Governorate, Egypt. Four regions (Abis, Al Mamurah, El Ibrahimeya and El-Agamy) are selected for this study. Different physicochemical parameters and breeding water (temperature, pH, total dissolved solids, and dissolved oxygen) were measured; the relationship between measured parameters and the relative abundance of mosquito larvae were examined. Data revealed that *Culex* mosquito species in Alexandria Governorate were represented by four species: *Cx. pipiens*, *Cx. antennatus*, *Cx. theileri* and *Cx. tritaeniorhynchus*. *Culex pipiens* and *Cx. antennatus* were the most abundant mosquito species in the study area. The highest relative abundance of *Cx. pipiens* larvae were recorded in El Ibrahimeya region (56.19%). A significant variation ($P<0.05$) was detected between investigated species in their abundance during the study period, except *Cx. tritaeniorhynchus* and *Cx. theileri*, where there was no significant variation ($P>0.05$) in their abundance, compared to each other. In addition, a significant variation ($P<0.05$) was recorded between Abis comparison to Al Mamurah, El Ibrahimeya, and El-Agamy. On the other hand, the temperature was the most reliable factor affecting the abundance of all species at Abis, Al Mamurah and El-Agamy, while the total dissolved solids (TDS) represented a reliable factor for the abundance of all species at El-Agamy. Furthermore, all species abundance at El Ibrahimeya is associated with dissolved oxygen (DO).

INTRODUCTION

Mosquitoes are the most irritating blood-sucking insects annoying human beings **(Bahadur *et al.*, 2020).** Mosquitoes are a biological vector of many diseases such as filaria, malaria, dengue and Rift Valley Fever **(Hassan *et al.*, 2014; Qamandar & Shafeeq, 2018; Tamilventhan & Jayaprakash, 2019; Hassanain *et al.*, 2019; El-Mehdawy *et al.*, 2021)**. In Egypt, the most common mosquito-borne diseases transmitted to human beings are: the West Nile virus **(Taylor *et al.*, 1956)**, malaria **(Barber & Rice, 1937; Farid, 1940)** and Dengue Fever **(Gaber *et al.*, 2022; Fang *et al.*, 2022)**. Moreover, mosquito negatively impacts livestock by decreasing weight gain and milk production in dairy cows through disease transmission **(Islam *et al.*, 2017)**.

In Egypt, some members of the family Culicidae are widely distributed all over Egypt. Both *Culex pipiens* and *Cx. antennatus* are the primary vector of filariasis, *Wuchereria bancrofti* and Rift Valley Fever virus during the outbreak in the Nile Delta **(Khalil *et al.*,**

1932; Meagan *et al.*, 1980; Darwish & Hoogastrall, 1981; Gad *et al.*, 1995; Hanafi *et al.*, 2011). To eliminate the distribution of the previously mentioned diseases, controlling mosquitoes is an effective strategy that we must follow in addition to good knowledge and understanding of the relevant biology and ecology of the target species are of paramount importance **(Seghal & Pillai, 1970; Gimnig *et al.*, 2001)**. Additionally, understanding the nature of different breeding habitats influencing larvae is the first step to control mosquito vectors' survival, production, development, abundance and distribution **(Jemal & Al-Thukair, 2016)**.

Thus, the present study was performed to investigate different patterns, including the relative abundance and spatial distribution of different mosquito species larvae in Alexandria Governorate, Egypt and address the physicochemical parameters of breeding water habitats.

MATERIALS AND METHODS

1. Study sites

The present study was carried out at Abis (31°11'47.2" N, 30°00'46.4" E, altitude -4m), Al Mamurah (31°17'10.4" N, 30°03'01.2" E, altitude -2m), El Ibrahimeya (31°12'40.5" N, 29°55'37.4" E, altitude 14m) and El-Agamy (31°04'19.2" N, 29°44'57.4" E, altitude -1m), in Alexandria Governorate, Egypt from January to December 2019.

Fig. 1. Locations of Alexandria Governorate's study areas; Abis, Al Mamurah, El Ibrahimeya and El-Agamy. Map source: Landsat-7 satellite imagery using ArcGIS10.8

2. Larval collection and identification

Larvae were collected from different breeding habitats (canals, pools, drainages, ponds, swamps and ditches). A rounded net dipper (20 cm in diameter) with a stainless-steel handle (150cm in length) was used. Monthly, three dips were randomly made in each habitat; the larvae were transferred to the laboratory of the Medical Entomology Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt. Larvae were morphologically identified using previous keys described by **Harbach (1985).**

Fig. 2. Different breeding habitats of mosquito larvae in Alexandria Governorate, Egypt

3. Physico-chemical parameters of breeding water

Water temperature and total dissolved solids were determined using Adwa (AD31) apparatus (Waterproof Conductivity-TDS-Temp Pocket Testers with the replaceable electrode). While, the potential hydrogen (pH) was determined using Adwa (AD11) apparatus (Waterproof pH-TEMP Pocket Tester with the replaceable electrode). Dissolved oxygen (DO) was estimated using the Winkler method **(APHA, 1926)** aligned with the modifications of **Labasque *et al.* (2004)**. Briefly, water samples were collected carefully in 300cm glass stoppered bottles and fixed by adding immediately 2ml of manganous sulfate reagent ($MnSO_4$), followed by 2ml of alkaline potassium iodide reagent (KI), just beneath the surface of the bottle. The bottle stoppered and was kept in the dark box until reaching the laboratory. In Lab, 2ml of concentrated H_2SO_4 was added, mixed, and allowed to stand for at least 5min. About 100ml of fixed sample was titrated against sodium thiosulphate (0.025 N), using starch as an indicator until the blue color disappeared. The DO concentrations were calculated according to the following equation:

Dissolved Oxygen (mgl^{-1}) = N × V × 8 × 1000 /Volume sample (ML) (Where, N, Normality of sodium thiosulphate & V: Volume of sodium thiosulphate).

4. Statistical analysis

Data have been analyzed using various softwares. Excel 365 was used for data entry and data representation purposes. Data were cleaned before running any statistical analyses. Data were first investigated using IBM SPSS V.25. The frequency of the key quantitative variables was determined as well as the descriptive statistics for the key quantitative variables. Inferential statistics were used to answer the major questions of the study. All variable parametric assumptions were tested. Data were presented as mean and SD, and *P*-value was considered significant at< 0.05. Data were tested for satisfying assumptions of parametric tests, continuous variables Shapiro- Wilk, and Kolmogorov-Smirnov test results for normality were listed. However, results showed that variables which followed a non-normal distribution pattern were transformed to meet the requirements for parametric analysis. Spearman correlations were used for testing correlation relationship between parameters; in addition, when needed, pairwise regressions were listed to estimate the relation between factor and response using Minitab V 21.2. Number of present data visualization becomes available using R-studio V.4.1.3.

RESULTS

1. Species composition and abundance of the mosquitoes' larvae

Culex mosquito species in Alexandria Governorate, Egypt was represented by *Culex pipiens, Cx. antennatus, Cx. theileri* and *Cx. Tritaeniorhynchus.* The obtained results showed that, *Cx. pipiens* and *Cx. antennatus* were the most collected species from the Abis region, with 46.03 and 28.35% (2242 and 1381) of the total mosquito larvae collected during all months. Moreover, the abundance of mosquito larvae recorded the highest level in summer (June, July and August) with 12.61, 17.41 and 22.23% of the total mosquito larvae collected, compared to winter (December, January and February) with 2.36, 0.55 and 1.07% of the total mosquitoes' larvae collected, respectively (Fig. 3).

Regarding Al Mamurah region, the highest relative abundance of mosquito species (52.52%) was recorded for *Cx. pipiens* 52.52% (1510) followed by *Cx. antennatus* 29.98% (862), *Cx. tritaeniorhynchus*11.72% (337) and *Cx. theileri* 5.78% (166), respectively. In addition, the highest relative abundance of mosquito larvae collected at El Ibrahimeya region (56.19%) was recorded for *Cx. Pipiens*, followed by *Cx. antennatus* (29.46%), respectively. Meanwhile, the relative abundance of *Culex pipiens* at El-Agamy region was 49.91% (1682), followed by *Cx. antennatus* (27.06%) (912), *Cx. Tritaeniorhynchus* (14.57%) (491) and *Cx. theileri* (8.46%) (285), respectively (Fig. 3). All species followed the bell shape trend, with a high peak in August.

As shown in Fig. (4), a significant variation (P<0.05) was recorded between the investigated species and their calculated abundance throughout the study period, except for *Cx. tritaeniorhynchus* and *Cx. theileri*, where there was no significant variation (P>0.05) in their recorded abundance, compared to each other (Fig. 4a). As shown in Fig. (4b), there was a significant variation (P< 0.05) between Abis, compared to Al Mamurah, El Ibrahimeya and El-Agamy. At the same time, there was no significant variation (P> 0.05) between Al Mamurah, compared to El Ibrahimeya and El-Agamy. Additionally, no significant variation (P>0.05) was detected between stations of El Ibrahimeya and El-Agamy with respect to their mosquitoes abundance.

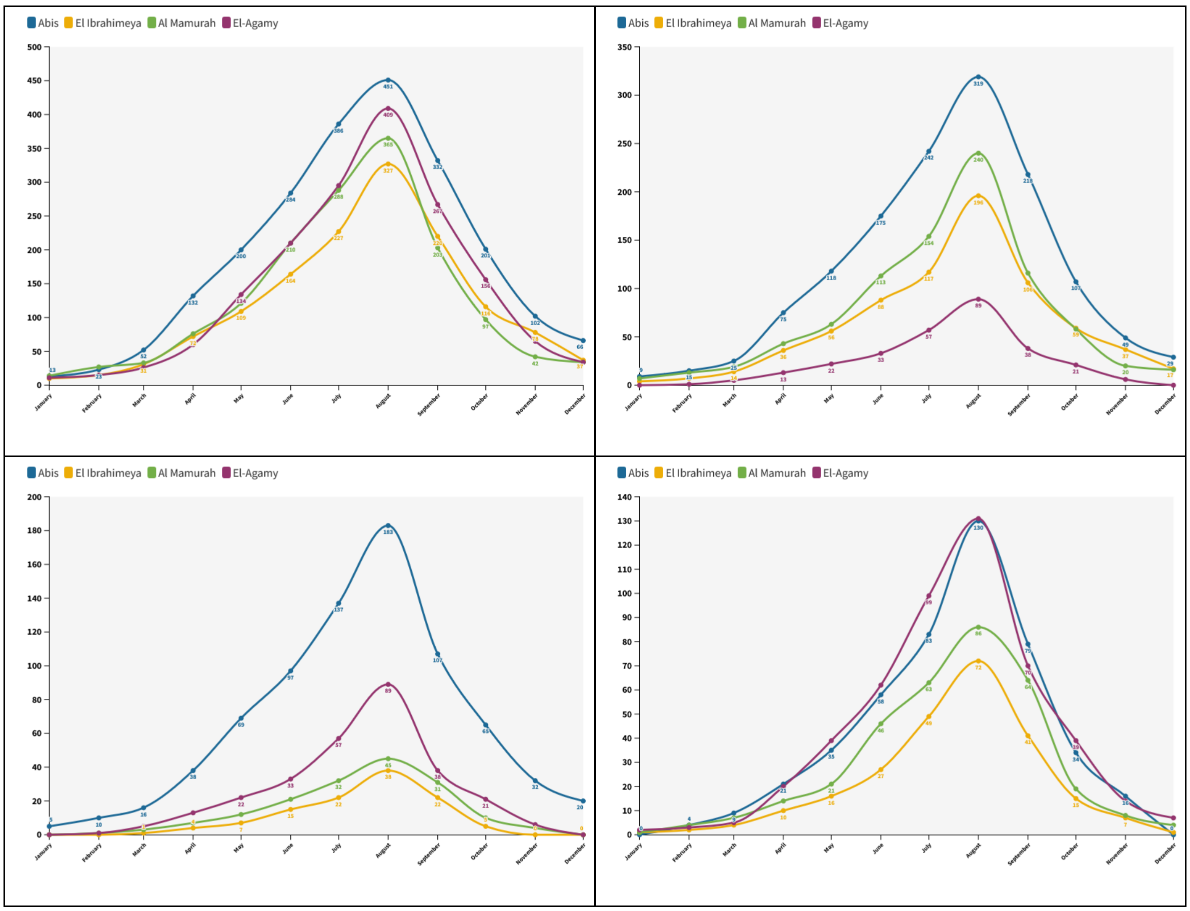

Fig. 3. The gradient line chart representing the average abundance of *Cx. pipiens*, *Cx. antennatus*, *Cx. theileri* and *Cx. tritaeniorhynchus* through the investigated months

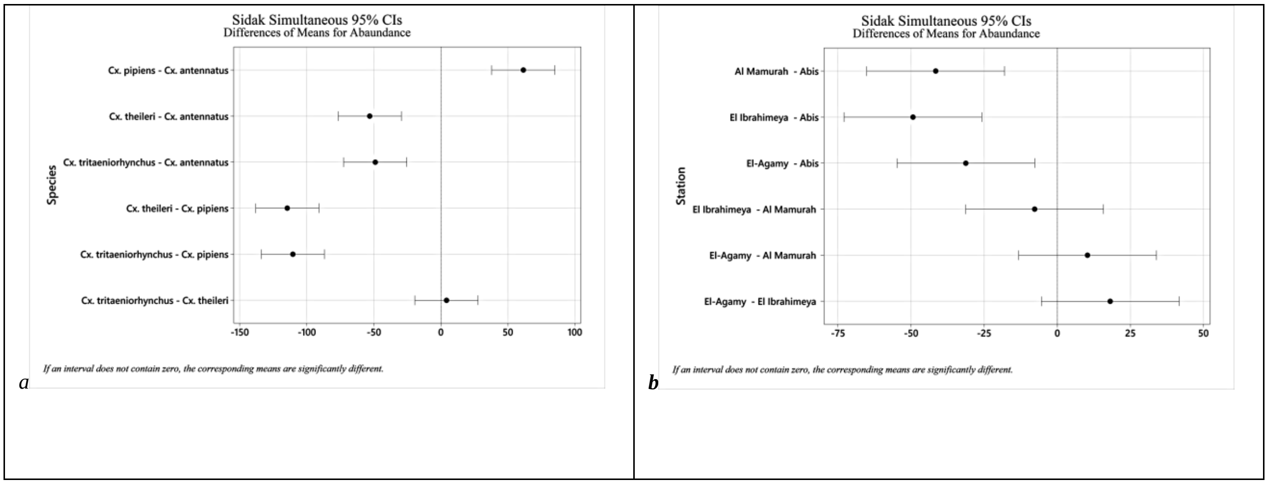

Fig. 4. Sidak simultaneous 95% CIs differences of the mean for (*a*) Species and (*b*) Stations

Fig. (5) displays the heatmap representing a comparison of mosquito abundance throughout the study period at different stations. As the color grade turns red, the abundance increases; at the same time, the color grade gets blue as the abundance of *Cx. pipiens, Cx. antennatus*, *Cx. theileri* and *Cx. tritaeniorhynchus* decreases.

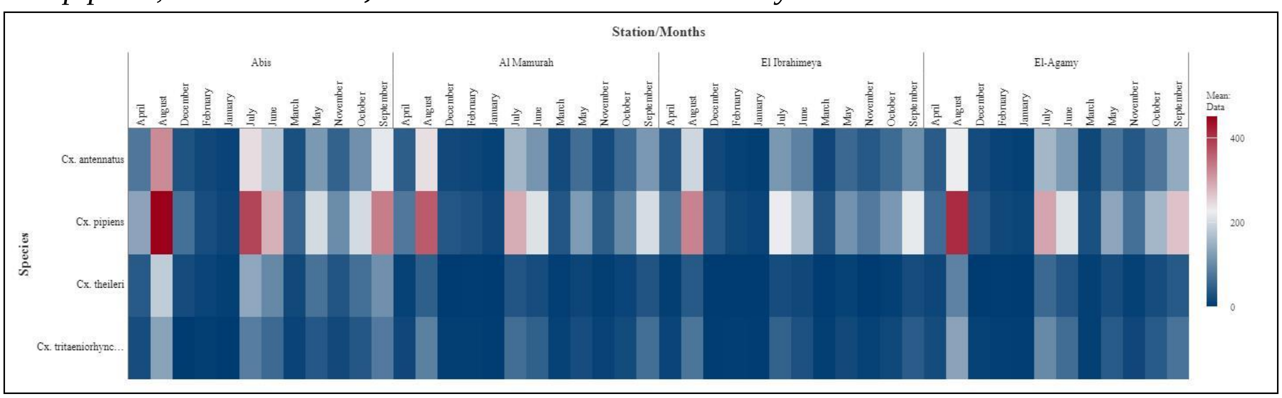

Fig. 5. Heatmap representing the abundance of species throughout the study period at a different stations

2. Physico-chemical parameters of breeding water

The lowest and the highest water temperature (9.6±0.2 and 31.2±0.4°C) were recorded in Abis region during Jan. and June, while the highest potential hydrogen (pH) value (8.8±0.4) was detected in May. In addition, the highest water dissolved oxygen (DO) content value (5.1±1.3 mg/l) was recorded in Jan. Regarding Al Mamurah region, the highest water temperature (28.7±0.1°C) was recorded in May, and the highest pH value (9.0±0.2) was recorded in May (Fig. 6). Additionally, the highest TDS values (985.3±436.4 and 903.7±594.3 ppm) were recorded in El Ibrahimeya and El-Agamy regions in May and July. Temperature tends to follow semi bell shape trend, while there was no significant variation (*P*>0.05) between the station at Al Mamurah, compared to El Ibrahimeya and Abis. For El Ibrahimeya and Abis, their recorded Temp. PH, TDS, and DO follow uneven trends throughout consecutive months. The pH value shows a significant variation (*P*< 0.05) between station El Ibrahimeya and stations of both Abis and El-Agamy. On the other hand, TDS was not significantly varied (*P*>0.05) between station El-Agamy, compared to Abis and Al Mamurah. At the same time, DO was significantly differentiated ($P < 0.05$) between all stations (Figs. 6, 7).

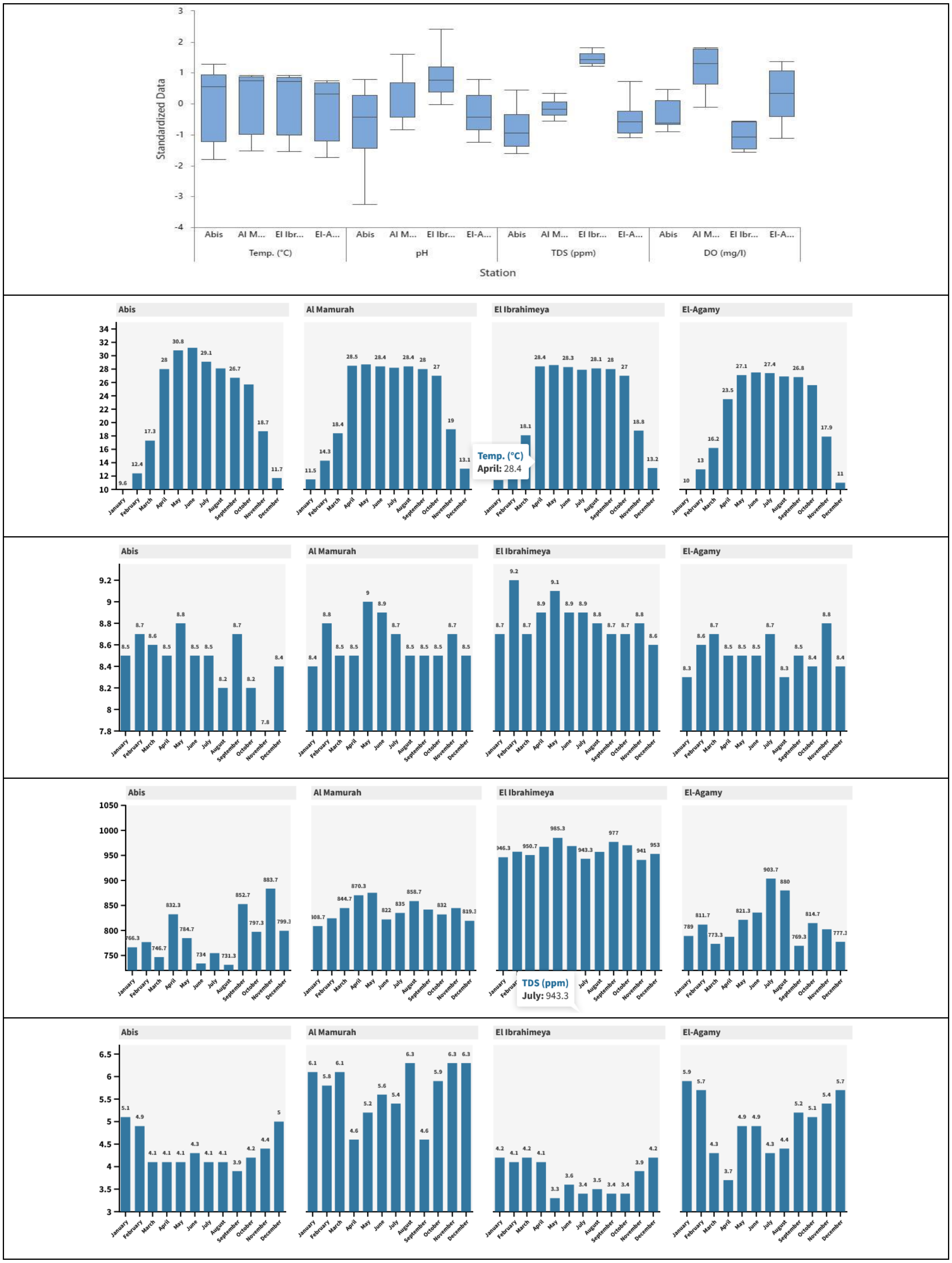

Fig. 6. (***a***) Box plot and (***b,c,d, &e***) Gradient column chart of analyzed physical parameters throughout the study period

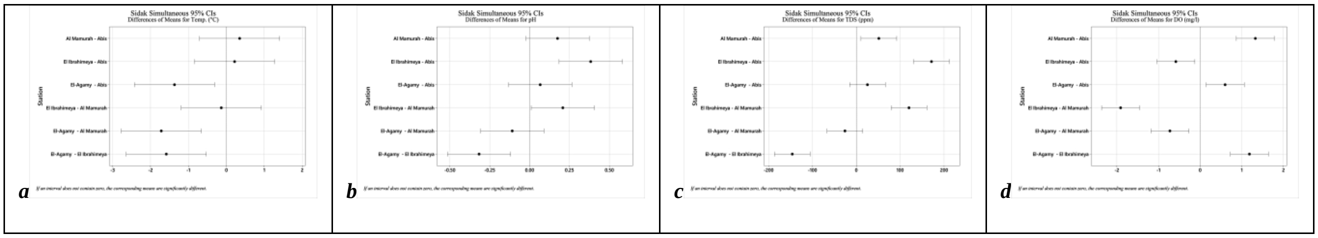

Fig. 7. Sidak simultaneous 95% CIs differences of the mean for ***(a)*** Temp, ***(b)*** PH, ***(c)*** TDS and ***(d)*** DO

To study the environmental-abundance relationship, pairwise regression was listed, as shown in (Fig. 8*a*- *d*); Temp is considered the most reliable factor that *Cx. pipiens* abundance depends on at Abis, Al Mamurah and El-Agamy, and TDS was also a reliable factor at El-Agamy. Besides, *Cx. pipiens* abundance at El Ibrahimeya relied on DO. The model summary for *Cx. pipiens* at four stations were presented with R^2= 59.32, 47.51, 54.4, and 67.84%, respectively.

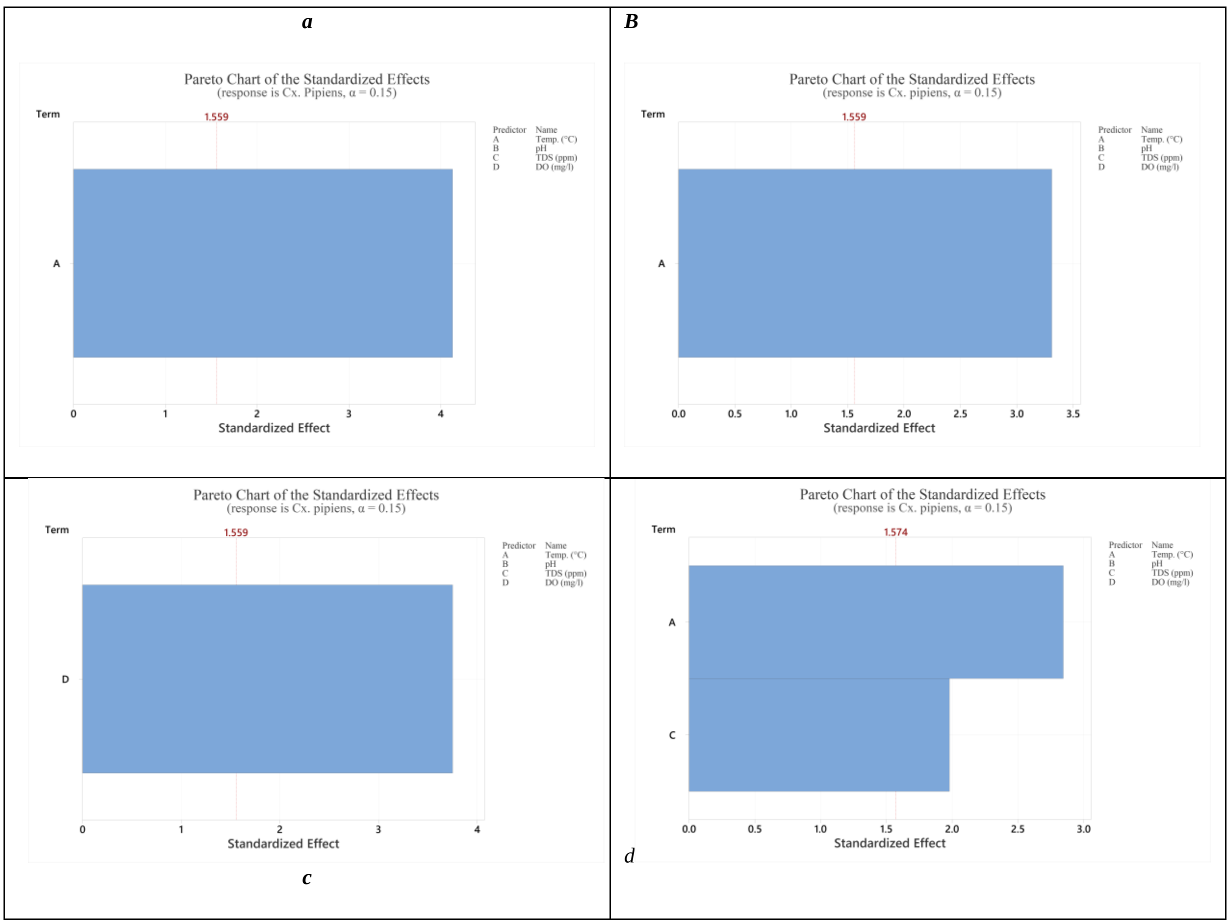

Fig. 8. Pareto chart of standardized effects for *Cx. pipiens* at ***(a)*** Abis, ***(b)*** Al Mamurah, ***(c)*** El Ibrahimeya, and ***(d)*** El-Agamy

Temp was considered the most reliable factor that *Cx. antennatus* abundance depends on in Abis, Al Mamurah and El-Agamy, while TDS was also a reliable factor at El-Agamy. *Cx. antennatus* abundance at El Ibrahimeya and Al Mamurah relied on DO. The model summary for *Cx. antennatus* at the four stations was presented with R^2= 51.86, 51.74, 47.31, and 67.67%, respectively (Fig. 9).

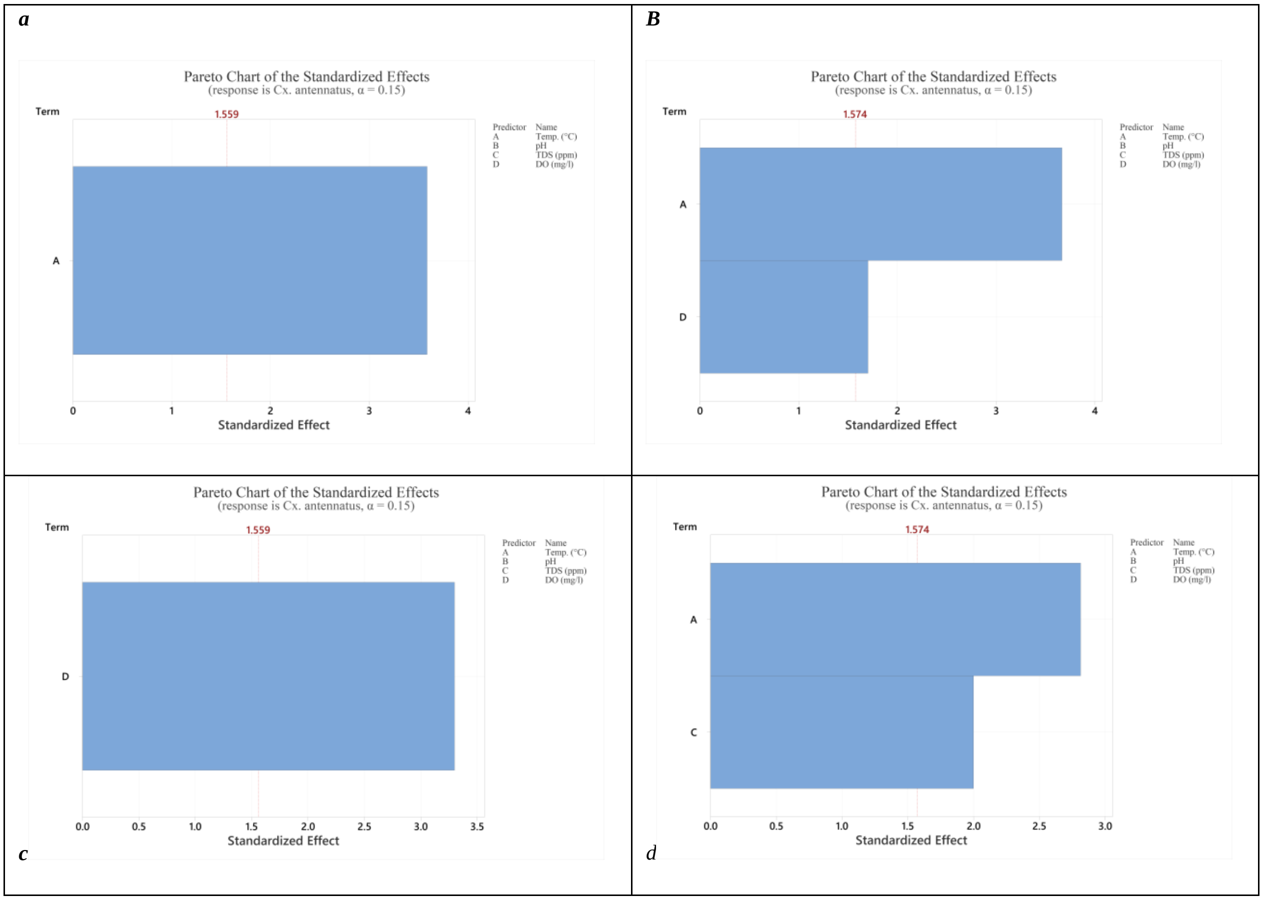

Fig. 9. Pareto chart of standardized effects for *Cx. antennatus* at ***(a)*** Abis, ***(b)*** Al Mamurah, ***(c)*** El Ibrahimeya, and ***(d)*** El-Agamy.

Temperature was considered the most reliable factor that *Cx. theileri* abundance depends on in Abis, Al Mamurah and El-Agamy, while TDS was also a reliable factor at El-Agamy. *Cx. theileri* abundance at El Ibrahimeya relied on DO. The model summary for *Cx. theileri* at four stations were presented with R^2= 50.53, 46.34, 37.97, and 67.22%, respectively (Fig. 10).

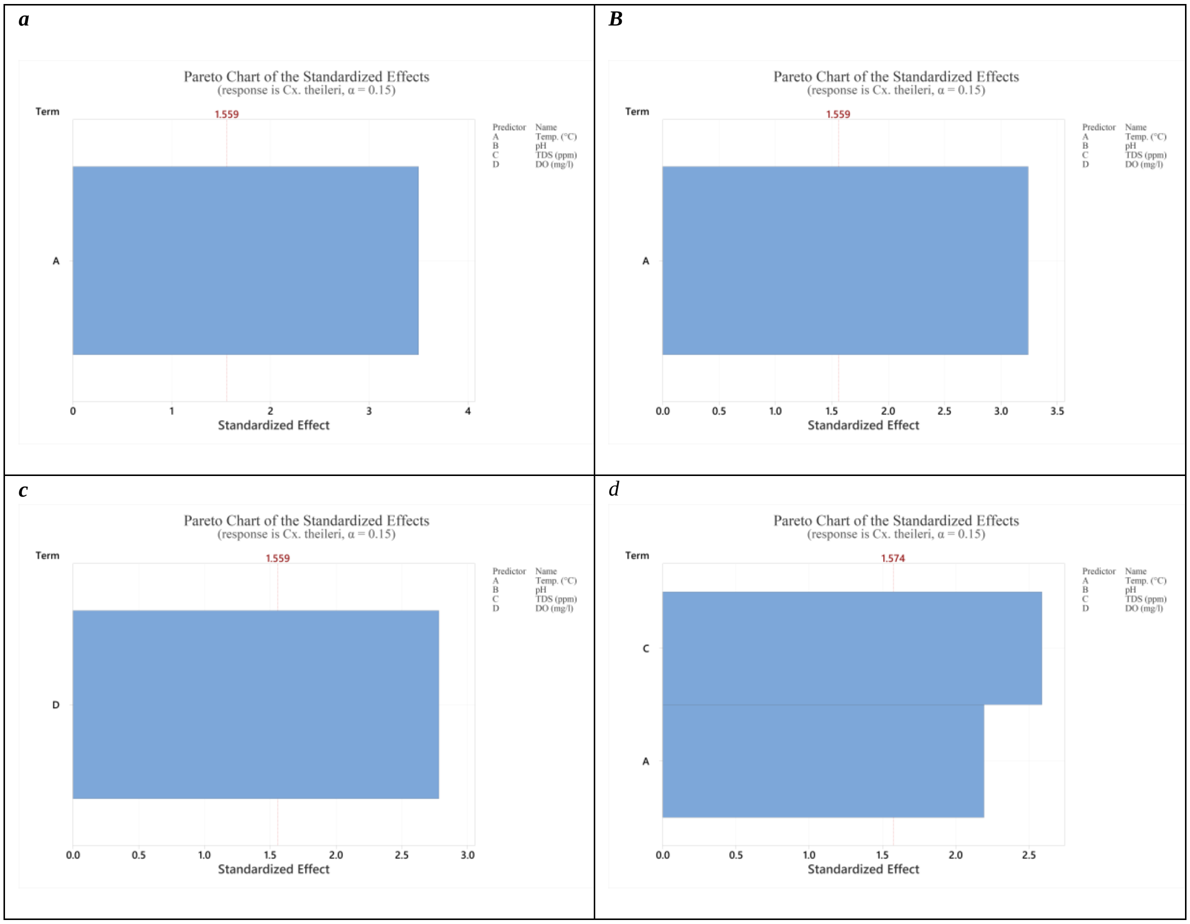

Fig. 10. Pareto chart of standardized effects for *Cx. theileri* at ***(a)*** Abis, ***(b)*** Al Mamurah, ***(c)*** El Ibrahimeya, and ***(d)*** El-Agamy.

Temperature was considered the most reliable factor that *Cx. tritaeniorhynchus* abundance depends on in Abis, Al Mamurah and El-Agamy, and TDS was a reliable factor at El-Agamy. *Cx. tritaeniorhynchus* abundance at El Ibrahimeya relied on DO. The model summary for *Cx. tritaeniorhynchus* at the four stations was presented with R^2= 43.81, 43.92, 42.71 and 71.95%, respectively (Fig. 11).

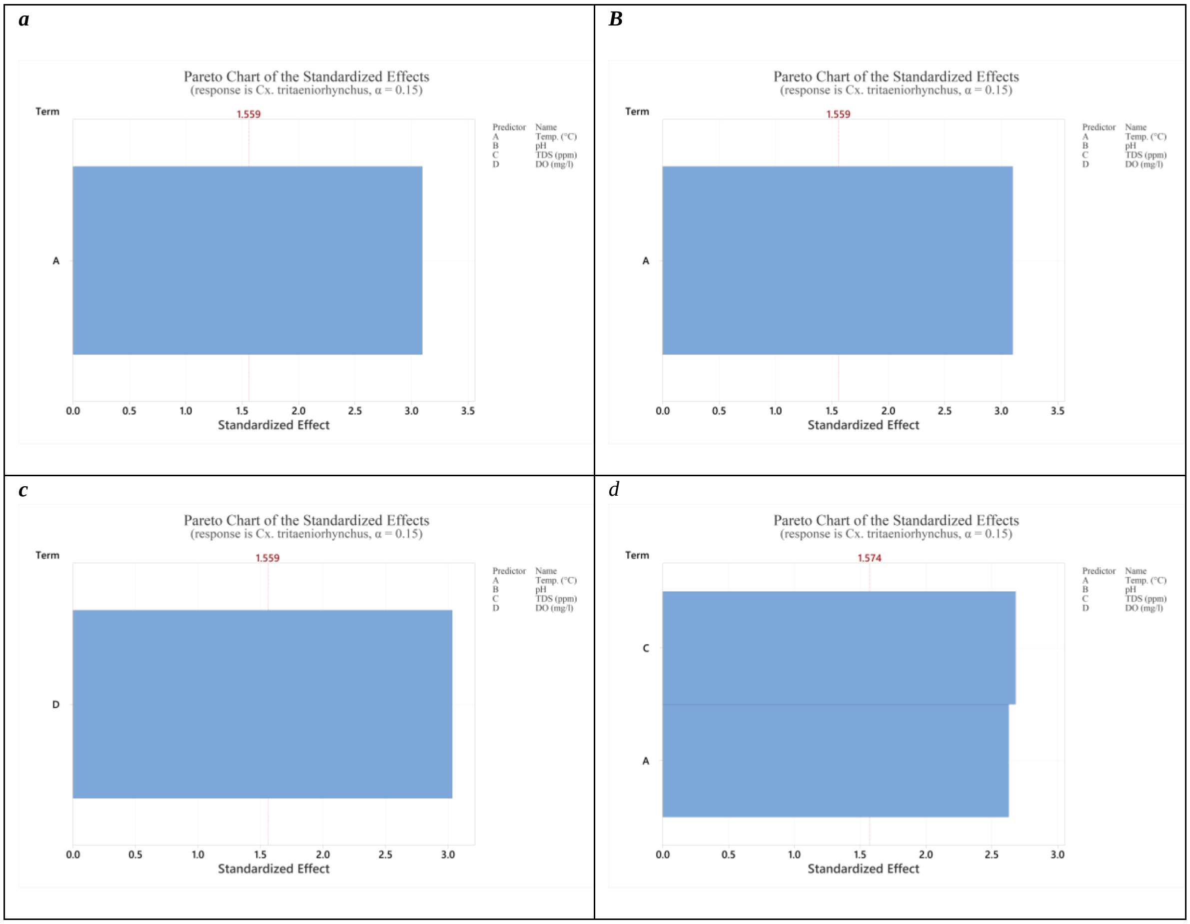

Fig. 11. Pareto chart of standardized effects for *Cx. tritaeniorhynchus* at ***(a)*** Abis, ***(b)*** Al Mamurah, ***(c)*** El Ibrahimeya, and ***(d)*** El-Agamy.

In addition, the correlogram revealed the most correlated physical parameters throughout the investigated stations, showing the highest negatively correlated parameters where DO and temperature recorded values of -0.85 at Abis. The temperature was positively correlated with TDS (0.63); at the same time, it correlated negatively with D.O. (-0.59) at Al Mamurah. Moreover, the temperature was positively correlated with TDS (0.59), while it recorded the highest negatively correlated value with DO (-0.81). Furthermore, TDS was negatively correlated with DO (-0.53) at El Ibrahimeya. On the other hand, the temperature was positively correlated with TDS (0.53), while it negatively correlated with the value of DO (-0.60) at El-Agamy (Fig. 12*a*- *d*).

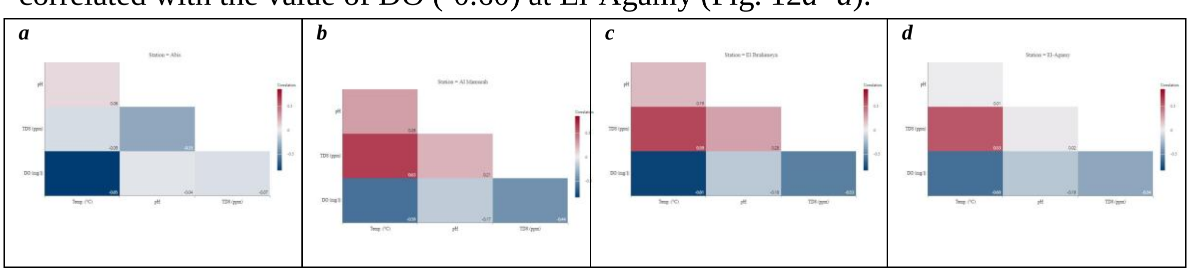

Fig. 12. Correlogram of stations' physical parameters representing their correlation relationship at ***(a)*** Abis, ***(b)*** Al Mamurah, ***(c)*** El Ibrahimeya and ***(d)*** El-Agamy.

DISCUSSION

The purpose of this study was to update our understanding of the most common *Culex* mosquito vectors distributed in Alexandria (Abis, Al Mamurah, El Ibrahimeya and El-Agamy) Governorate, Egypt. Four mosquito species were collected from different areas of Alexandria Governorate during 2019; namely, *Culex pipiens, Cx. antennatus, Cx. theileri* and *Cx. tritaeniorhynchus*. All species seemed to be native to the Egyptian fauna, as **Abdel-Hamid *et al.* (2011a, b)** reported the presence of *Cx. pipiens, Cx. antennatus* and *Cx. theileri* in El Gharbia and El Menoufia Governorates, Egypt. Similarly, **Abdel-Hamid *et al.* (2013)** and **Selim *et al.* (2019),** in Eldakahlia Governorate and Wadi El-Rayan protected area reported the occurrence of *Culex pipiens* and *Cx. antennatus.* Relative abundance of mosquito larvae collected from different breeding environments in Alexandria Governorate was recorded at 35.77% in the Abis region, 21.11% in Al Mamurah region, 18.37% in El Ibrahimeya region and 24.75% in El-Agamy region. These results agree with those of **Mostafa *et al.* (2019)** in the Red Sea Governorate.

In the present study, the temperature registered in the four-breeding regions in Alexandria Governorate affected the number of mosquitoes collected; for Abis region, about 1083 mosquito larvae (7.95% of the total mosquito larvae collected) were recorded in August 2019 at 28.1±0.2°C, and the lowest number was 27 mosquito larvae (0.20% of the total mosquitoes larvae) in January at 9.6±0.2°C. For Al Mamurah region, the abundance of mosquito larvae recorded the highest level in the summer season with 537, 736 and 414 of the total mosquito larvae collected, as compared with winter season 54, 22 and 45 of the total mosquito larva collected, respectively, where the temperature ranged between 11.5±0.4 & 28.7±0.1°C. Additionally, in El Ibrahimeya region, the abundance of mosquito larvae recorded about 633 mosquito larvae (4.65% of total mosquito larvae collected) in August 2019 at 28.1±0.2°C, compared to January 2019, about 15 mosquito larvae (0.11% of total mosquito larvae collected) at 11.4±0.5°C. For El-Agamy region, the abundance of mosquito larvae recorded the highest level in the summer season with 607, 853 and 515 of the total mosquito larvae collected, compared to winter season with 19, 28 and 51 of total mosquito larva collected, respectively, where the temperature ranged between 10±0.5 & 27.5±0.1°C. These results contradicted with those of **Kenawy *et al*. (2013)** who noticed a temperature range of 17-30°C for *Cx. pipiens*, and *Cu. Longiareolata* in Cairo Governorate, and **Elhawary *et al*. (2020)** who noticed a temperature range of 21-32°C for *Cx. pipiens*, and *Cu*. *Longiareolata* at different breeding sites in Egypt. Considering the pH of the studied breeding sites in the four regions, it ranged from 7.8±0.3 to 9.2±0.3, indicating that the water is neutral or slightly alkaline. These results coincide with those of **Pelizza *et al.* (2007)**, **Oyewole *et al.* (2009)** and **Elhawary *et al.* (2020)** who observed that, mosquito larvae prefer neutral or slightly alkaline water. Concerning the total dissolved solids (TDS), the highest values recorded were 883.7, 875.3, 985.3 and 903.7ppm, respectively, in Abis, Al Mamurah, El Ibrahimeya, and El-Agamy and varied significantly among mosquito breeding habitats. Anthropogenic activities and salt inputs in drainage water may be responsible for the high salinity at El Ibrahimeya breeding location **(Williams, 2001)**. The dissolved oxygen (DO) levels were high at the four breeding sites, with a range of 3.3±0.3 - 6.3±0.6mg/ L. These results agree with those of **Tadesse *et al.* (2011)** in Ethiopia. The high DO levels at the four sites may have a positive relationship to larval abundance significance.

CONCLUSION

From the present study, we can conclude that, *Culex pipiens*, *Cx. antennatus*, *Cx. theileri* and *Cx. tritaeniorhynchus* represent the patterns of *Culex* mosquito species in Alexandria Governorate, Egypt. *Culex pipiens* collected throughout the study period was the most prevalent species of mosquito in the studied area, which showed the risk of spreading diseases transmitted by this species. In addition, the temperature was considered the most reliable factor for the abundance of all species at Abis, Al Mamurah, and El-Agamy; TDS was another reliable factor for the abundance of all species at El-Agamy. On the other hand, all species abundance at El Ibrahimeya depend on DO.

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