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The impact of electromagnetic radio waves on some biological aspects of *Culex (Culex) pipiens* Mosquitoes (Diptera: Culicidae)

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Experiments were conducted to investigate the impact of Radio-Frequency (RF) exposure on some biological aspects of immature stages of *Culex (Culex) pipiens* mosquito. Immature stages of *Cx. (Cx.) pipiens*mosquito were collected from Giza, Egypt and maintained at the Dept. of Entomology, Fac. of Science, Cairo University. 100 first in star larvae were exposed to a single dose of discontinuous RF using GSM multiband mobile phone for 4 hours simulating phone conversation conditions. Immature duration, percentage mortality, adult emergence, and sex ratio were calculated and statistically analyzed. RF-exposed insects exhibited significant reduction in developmental duration (19 and 10.55 days for control and RE-exposed, respectively), significant increase in percentage mortality (23.8, 11.2% and 38.6, 48.8% for control and RE-exposed larval and pupal mortality, respectively) and mail-biased sex ratio (1♀: 1.34♂ and 1♀: 3.46♂ for control and RE-exposed, respectively). These results provided an evidence for the negative impact of RF-radiation on the components of ecosystem. Additional research on the molecular effects of RF-exposure on living organisms is encourage

Keywords: Radio-Frequency exposure, *Culex (Culex) pipiens*, mosquito, Diptera, Insects

INTRODUCTION

Human beings are generally uncovered from the effect of both mechanical and electromagnetic waves. Unlike mechanical waves (MW), electromagnetic (EM) waves propagate in vacuum. Electromagnetic spectrum includes a wide range of wavelengths from radio waves to gamma-rays (Reed, 2018, Balmori, 2021). Mobile phone cell is an artificial EM source (WHO, 2002, Perrin and Souques, 2012, Wargo et al. 2012). A considerable increase in the number of mobile phone devices was determined worldwide. In 2021, more than 8.6 billion subscriptions of mobile phone were reported. It was less-than one billion subscriptions in 2000 (ITU, 2021). Consequently, the infrastructures like telecom companies' towers and telecom relay stations were installed. Several infrastructures were installed closer to densely populated urban residential areas (*e.g.* schools, nurseries, public playgrounds, commercial buildings, hospitals, university campuses, and amphitheaters) (Feynman, 2013). The increasing number of smart phones' users, in addition to the revolution of smart mobile technology that supports games, has led to the emergence of many studies confirming the impact of mobile phone use on the behavior of children and adolescents (*e.g.*Hardell, 2017, Kopecký et al. 2021).Thus, many risk-assessment studies investigated the effect of mobile phone radiation on living organisms (Adey, 1975a, Bawin et al. 1975, Dutta et al. 1984, Adey, 1988, Goodman et al. 1995, Velizarov et al. 1999, Xenos and Margas, 2003,Cucurachi et al. 2013).

As a considerable component of biomass and ecosystem, insects are very important for the ecosystem sustainability and human life continuation (Samways, 1993, Miller and Spoolman, 2012). The impact of electromagnetic field (EMF) on insect was investigated by many researchers: development dynamics of *Musca domestica* (Stanojević et al. 2005); reproductive capacity of *Drosophila melanogaster* (Panagopoulos et al. 2004, 2007); ant food sites cues (Cammaertset al. 2012); the generation time of *D. melanogaster* N strain (Fauzi et al. 2015); the generation time, sex ratio and filial number of *D. melanogasterebony* strain (Fauzi and Corebima, 2015); the development and performance of *Callosobruchuschinensis* (Coleoptera) (Maharjan et al. 2019a), oviposition preference and development of *Marucavitrata* (Lepidoptera) (Maharjan et al. 2019b), life-table parameters of perilla seed bugs (*Nysius* sp.) (Heteroptera: Lygaeidae) (Maharjan et al. 2020); and survival rate and reproductive organs morphology of *D. melanogaster* (Sudaryadiet al. 2020). Such potential effects may result in dramatic decline of insect populations

(Balmori, 2009, 2014, 2015, 2021, Hallmann, 2017, Thill, 2020). Cellular phone radiations were also incriminated in the changes of behavior and colony collapse disorder of honey bee in USA (Kimmel et al. 2007, Mixsonet al. 2009, Sharma and Kumar, 2010, Kumar et al. 2011, Favre, 2011, Sainudeen, 2011, Mal and Kumar, 2014, Taye et al. 2017, Kumar, 2018, Favre and Johansson, 2020, Di Noi et al. 2021).

The present study aimed to investigate the impact of Radio Frequency (RF) radiation emitted by cellular mobile phone on larval *Culex (Cx.) pipiens* mosquito..

MATERIALS AND METHODS

Mosquito rearing

Larvae of *Cx. (Cx.) pigpens* were originally collected on October to September, 2020 from a breeding site in Giza, Egypt. This colony was maintained in the in sectary of the Department of Entomology, Faculty of Science, Cairo University under controlled conditions (12Light: 12Dark photoperiod, 27± 1 °C temperature, and 70-80% RH). Larvae were fed with activated yeast. Adult mosquitoes were fed on the blood of tied domestic pigeon (*Columba liviadomestica*) placed on the top side of the mosquito cages twice a week as previously described (Galalet al. 2017).

Raio-Frequency irradiation and experimental setup

One hundred newly hatched first in star larvae of *Cx. (Cx.) Pigpens* were exposed to a single dose of discontinuous radio frequency (RF) signal produced by a 4G GSM multiband mobile phone (about 900/ 1900 MHz and power approximately 0.03 m W/cm2) for 4 hours under controlled conditions. To simulate a phone conversation, one mobile device called and the second answered. The two devices were in a continuous conversation during the RF-exposure period. Each device was hanged up in front of and touching a glass jar (10-cm diameter x 20-cm height). Each jar contains 50 newly hatched first instar larvae. Control group was subjected to all conditions,but free from RF-exposure. Following the end point of the irradiation, the 100 larvae were divided into 10 cups (10 larvae/ cup) to facilitate observation recordings. Immature duration, percentage mortality, adult emergence, and sex ratio were observed and calculated. Four readings per day were taken during the first 24 hours post-irradiation, followed by one reading per day until the end of the experimental period. All experiments were repeated three times.

Statistical analyses

Statistical analyses were done using SPSS Ver. 25.0 software (SPSS Inc., Chicago, IL). Comparisons between means were done by using *t-test* at the significance level *P*< *05*. Additionally, correlation analyses of duration-stage and mortality-stage were done for both control and RF-exposed insects. Sex ratio data were subjected to *Chi*^{2} test.

RESULTS

The effect of RF-exposure on the developmental period of immature stages of *Cx. (Cx.) pipiens*

The effect of RF-exposure on the developmental period of immature stages of *Cx. (Cx.) pipiens* was illustrated in Figure (1). Durations of the RF-exposed larvae were reduced to ≈half the value when compared to controls (RF-unexposed). Statistical analyses revealed that durations of the RF-exposed second larval, third larval, fourth larval, total larval, and total immature (larval+ pupal) were significantly reduced ($P< 0.05$) when compared to control. Duration differences between RF-exposed and controls were insignificant ($P> 0.05$) in the cases of the first larval and pupal durations (Figure 1).

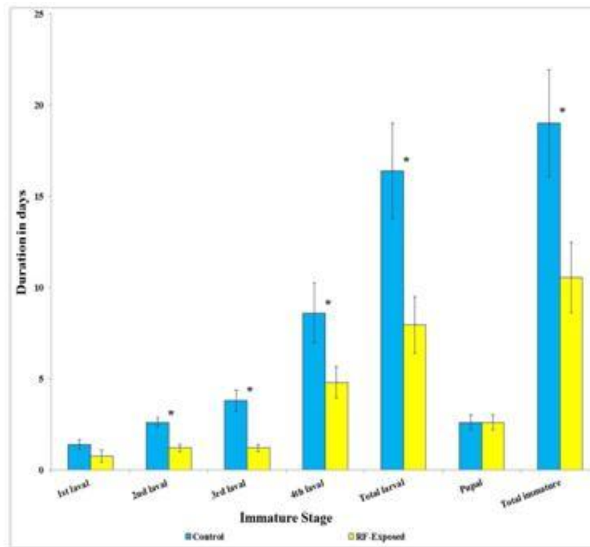

Figure 1: Effect of RF-exposure on the immature duration of *Cx. (Cx.) pipiens*. Asterisks refer to significant difference between RF-exposed insects and control at $P< 0.05$using *t*-test.

Correlation analysis indicated that there were positive correlations between duration and immature stage in both RE-exposed and control insects (Figure 2). The correlation was weak positive ($R^2= 0.49$) in control, and strong positive ($R^2= 0.62$) in RF-exposed insects (Figure 2).

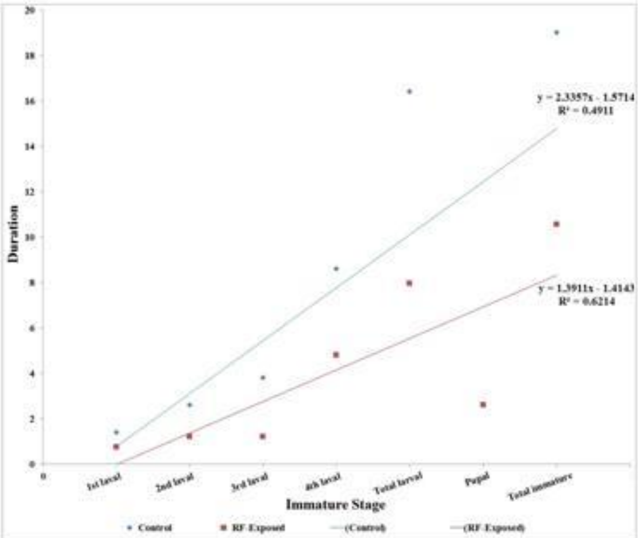

Figure 2.Duration-stagecorrelation of RF-exposed and control *Cx. (Cx.) pipiens*.R^2= *0.49* for control (weak correlation), and R^2= *0.62* for RF-exposed (strong correlation).

The effect of RF-irradiation on the percentage mortality of immature stages of *Cx. (Cx.) pipiens*

The effect of RF-exposure on the percentage mortality of immature stages of *Cx. (Cx.) pipiens* were illustrated in Figure (3). Percentage mortality of the RF-exposed larvae were increased to ≈double the value when compared to controls. Statistical analyses revealed that mortalities of the RF-exposed first larval, second larval, fourth larval, total larval, and pupal stages were significantly increased ($P < 0.05$) when compared to control mortality. Mortality difference between RF-exposed and control was insignificant ($P > 0.05$) in the case of the third larval stage (Figure 3).

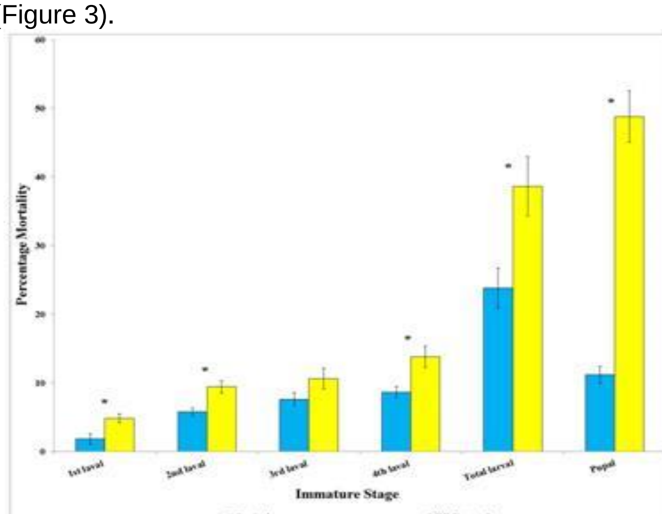

Figure3: Effect of mobile radiation on the immature mortality of *Cx. (Cx.) pipiens*. Asterisks refer to significant difference between RF-exposed insects and control at *P< 0.05* using *t*-test.

Correlation analysis indicated that there were positive correlations between percentage mortality and immature stage in both RF-exposed and control insects (Figure 4). The correlation was strong positive (R^2= *0.84*) in control, and moderate positive (R^2= *0.53*) in RF-exposed insects (Figure 4). Additionally, RF-exposure led to increased number of adults' failure to emerge from pupae.

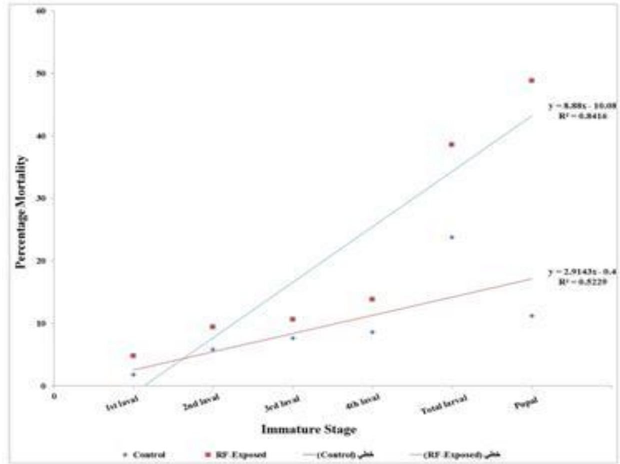

Figure 4: Mortality-stage correlation of RF-exposed and control *Cx. (Cx.) pipiens*. R^2= *0.84* for control (strong correlation), and R^2= *0.52* for RF-exposed (moderate correlation).

The effect of RF-irradiation on the sex ratio of *Cx. (Cx.) pipiens*

The effect of RF-exposure on the sex ratio of *Cx. (Cx.) pipiens* were illustrated in Figure (5).

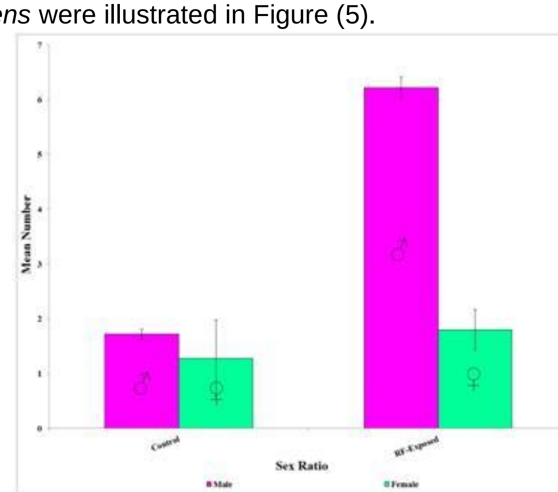

Figure 5: Effect of RF-exposure on the sex ratio of RF-exposed and control*Cx. (Cx.) pipiens*.

Sex ratio of the RF-exposed insects was male-biased (1♀: 3.46♂) when compared to controls (1♀: 1.34♂). Increase in the number of males emerged from RF-exposed insects was statistically significant (P= *0.00*) when compared to controls (*t-test*). However the difference in the number of females was insignificant (P= *0.25*) when compared to controls (*t-test*). Chi^2 statistical analysis of the sex ratio revealed that the difference in sex ratios of both RF-exposed and control insects was insignificant ($X^2= 18.0$ and $P= 0.08$).

DISCUSSION

The huge number of mobile phone devices, telecom towers, and relay stations emit a lot of EMF radiations within the ecosystem. The present study aimed to investigate the effect of RF irradiation on the immature stages of *Cx. (Cx.) pipiens*. Significant reduction was recorded in the developmental time of RE-exposed immature insects when compared to controls. This reduction was strongly correlated to RF-exposure. Contrarily, 50 Hz EMF-exposed *M. domestica* pupae showed significant retardation of metamorphosis (Stanojević et al. 2005). Additionally, significant elongated generation time of the first generation of two different strains of *D. melanogaster* was reported due to EMF-exposure when compared to control (Fauzi and Corebima, 2015, Fauzi et al. 2015). Furthermore, significant elongated developmental time was demonstrated for RF-treated *M. vitrata* when compared to control (Maharjan et al. 2019b). It is well-known that the shorter generation duration, the greater the insect population (Schowalte, 2006).The contradiction of the above-mentioned results and our results might correspond to the dose of irradiation, the irradiated stage and/ or the insect species. Furthermore, the effect of EMF on the generation time might correlate to the cellular and/ or hormonal processes of gonad development (Panagopoulos et al. 2004), or due to the concentration of ecdysteroid hormone (Gilbert et al. 2002, Atli and Unlu, 2007).

Another factor-influencing population is the percentage mortality. Significant increase was recorded in the percentage mortality of RE-exposed immature insects when compared to controls. This increase was moderately correlated to RF-exposure. As the effects of RF-exposure are non-thermal, the percentage mortality of RE-exposed insects might correlate to some proteins induced by the RF-exposure (Seufi et al. 2007, Wargo et al. 2012). These proteins might affect cellular protein functions (Weisbort et al. 2003). Consequently, RF-exposure could increase the failure of adults to emerge from pupae.

The last tested variable was sex ratio. We found that sex ratio of RF-exposed insects was significantly male-biased. Whereas, the *Chi*^{2} test clarified that the difference in sex ratios of both control and RF-exposed insects was insignificant. Similarly, Mirabolghasemi and Azarnnia (2002) and Fauzi and Corebima (2015) reported insignificant effect of EMF exposure on sex ratio of the first generation *D. melanogaster* when compared to control. Generally, sex ratio of most species tends to be 1♀: 1♂ (Fisher's principle), however in some cases, sex ratio bias in favor of male or female may happen in favor of the entire population fitness (Hamilton, 1967, Edwards, 1998).

CONCLUSION

The present study demonstrated that the RF-exposed insects exhibited reduced developmental duration, increased percentage mortality and mail-biased sex ratio.Therefore, our findings provided additional evidence to the adverse impacts of RF-radiation on the components of ecosystem. Additional research on the molecular effects of RF-exposure on living organisms is encouraged.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

GFH designed and performed practical work, data analysis and also wrote the manuscript. SAM participate in practical work, data analysis, writing the MS draft andreviewed the final manuscript. All authors read and approved the final version.

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