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A Promising Approach to Control Aquatic Developmental Stages of *Culex Cx. univittatus* Theobald, 1901 Using 4G Mobile (HSDPA 2100) Radiation

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ABSTRACT
The International Telecommunication Union reported more than 7 billion cellphone subscriptions worldwide. Therefore, the net result is the production of a number of biological effects on the whole biomolecules, cells and organisms. Such effects induced changes in the intracellular ion concentrations, the rate of composition of different biomolecules, cell reproduction rates and animal reproductive capacity. Biologically, The emitted electromagnetic field (EMF) impacted the exposed insect populations. Both of the larval instars, and the pupal lifespan were significantly depressed due to exposure to mobile radiation. Additionally, the failure of adult emergence increased significantly. Moreover, the sex ratio of Cx. (Cx.) univitattus was significantly affected by exposure to mobile radiation. The duration of the first larval instar declined from 1.36 ± 0.13 days, compared to 0.90 ± 0.34 (P< 0.01) day for both the control and the exposed larvae, respectively. Meanwhile, the durations of the second larval instar decreased from 2.53 ± 0.27 to 1.2 ± 0.09 days for both the control and the exposed larvae (P< 0.05), respectively. Moreover, the duration of the third larval instar was declined from 3.78 ± 0.40, compared to 1.09 ± 0.05 days for both the control larvae and the exposed larvae group (P< 0.05), respectively. Additionally, the duration of the fourth larval instar decreased (5.27 ± 0.9 days) for the exposed larvae group compared to the control (8.33 ± 2.18 days; P < 0.001). The total larval duration was 16.00 ± 2.98 days compared to 8.46 ± 1.38 days for both the control and the exposed larvae (P< 0.001), respectively. Meanwhile, the pupal lifespan was significantly affected by the exposure to mobile radiation (2.2 ± 0.1 days, and 2.5 ± 0.9 days; P< 0.05) for the control and the exposed pupae, respectively. The adult emergency was significantly affected by the exposure to mobile radiation (4:3, and 16:1; P< 0.001) for the control and the exposed groups, respectively. The same pattern was true for the males and females emergency, which was significantly affected (1:1.8) for males, and (1:2.7) females of both the control group and the exposed groups, respectively.

INTRODUCTION

Culex Culex univittatus Theobald, 1901 (Diptera: Culicidae) is known as ornithophilic mosquito, which is capable of transmitting various viral diseases such as SINV (Sindbis virus) and USUV (Japanese encephalitis virus serocomplex). The former was first isolated from specimens collected in Cairo in 1952 **(Taylor *et al.* 1955)**, while the latter was originally isolated in South Africa in 1959 **(Williams *et al.*, 1964)**. In South Africa, *Cx. (Cx.) univittatus* is well known to be as an efficient vector of WNV (West Nile virus) to birds **(Jupp, 1974)** and Rift Valley fever (RVFV) **(Eisa *et al.*, 1977)**.

Recent telecommunications industry produced a magnificent prevalence within the number of wireless devices. Mobile services have led to a proliferation of infrastructure support in the form of cell towers that provide a link to and from a mobile phone. With no regulations on the status of cell towers, they are placed closer to schools, nurseries, public playgrounds, commercial buildings, hospitals, university campuses, and amphitheaters of densely populated urban residential areas **(Feynman *et al*. 2013)**. In 2015, the International Telecommunication Union reported more than 7 billion cellphone subscriptions worldwide. This led to changes in the intracellular ion concentrations, the rate of composition of different biomolecules, cell reproduction rates and animal reproductive capacity **(Lin-Liu & Adey, 1982; Dutta *et al*., 1984; Adey, 1988; Goodman *et al*., 1995; Kwee & Raskmark, 1998; Penafiel *et al*., 1997; Velizarov *et al*., 1999; Xenos & Margas, 2003)**.

Additionally, such probable effects may be relevant for insects, essentially for those which live passionate about learning and memory. The radiation emitted from mobile phones,—could have contributed to the dramatic decline in insect populations **(Balmori, 2006, 2009, 2014, 2015, 2021; Hallmann, 2017; Thill, 2020)**.

Pervasive media reports asserted that cellular phones were responsible for the collapse and disorder of the honey bee colonies **(Good Morning America, 2007; Kimmel *et al.*, 2007; Mixson *et al.*, 2007; Sharma & Kumar, 2010; Kumar *et al.*, 2011; Favre, 2011; Sainudeen, 2011 ; Mal & Kumar, 2014; Taye *et al*., 2017; Kumar, 2018; Favre & Johansson, 2020)**.

The biological impacts of the electromagnetic field (EMF) on insects were detected, including the effect on the reproductive capacity of *Drosophila melanogaster* **(Panagopoulos *et al.*, 2004, 2007)**; the pupae of the house fly *Musca domestica* **(Stanojević *et al.*, 2005)**; ant food sites cues **(Cammaerts *et al.*, 2012)**; the developmental periods, the adult longevity, the adult weight, and the fecundity of subsequent generations of *Callosobruchus chinensis, Marucavitrata, Nysius plebeius* and *Nysius hidakai* **(Maharjan *et al.*, 2019a, 2019b, 2020)** in addition to the impact on the survival of fruit flies and their reproductive organs' morphology **(Sudaryadi *et al.*, 2020)**.

The main goal of the existing experimental study aimed to record the expected insecticidal effects of mobile phone radiation on the developmental stages of *Cx (Cx) univittatus* (Diptera: Culicidae).

MATERIALS AND METHODS

Field collection, identification and mass rearing

In October 2020, a laboratory colony of *Cx. (Cx.) univitattus* Theobald, 1901 was initiated by collecting larvae from a breeding site in East Gara, Sakaka, Aljouf, Saudi Arabia. Random samples were killed in boiling water, dehydrated in alcohol and preserved in alcohol (70%). The preserved larvae were mounted in euparal until microscopic examination. The larvae were identified according to **Harbach (1988)**. For mass rearing, larvae were maintained under controlled laboratory conditions at 24- 25°C and 75-85% relative humidity with a light: twilight: dark cycle of 12L:1twilight:10D:1dawn. To feed the adult female mosquitoes, a domestic pigeon (*Columba livia domestica*) was used as a blood meal source, which was provided to the mosquito cages twice a week, following the method of **Galal and Seufi (2017)**.

Radiation impact experiments

Fifty newly hatched mosquito larvae (n=50) were subjected to a single (acute) discontinuous EMF signals produced by using a 4G mobile phone (HSDPA 2100). Two mobile phones were hanged between bottles containing the larvae, and all were surrounded by screen covered to prevent environmental bias. EMF radiation ranged from 900/ 1900 MHz, and power approximately 0.03 mW/cm. Larvae were exposed to that EMF for four hours, followed by observations without radiation treatment. Larval duration, pupal duration, percentage of the adult emergence (%), and the sex ratio were calculated for both the controlled, and the exposed larvae. The experiment was repeated thrice.

Statistical analyses

Data were compiled, and calculated using descriptive statistics (the means, standard errors and ranges). Statistical analyses were carried out using SPSS ver. 19 program (SPSS Inc., Chicago, IL). Unpaired, two- tailed Student's *T- test* were carried out to compare between group means and determine the significance at ($P<0.05$).

RESULTS AND DISCUSSION

The duration of the 1st larval instar was 1.36 ± 0.13 days and 0.90 ± 0.34 day for both the control and the exposed larvae ($P< 0.01$), respectively. Meanwhile, the duration of the 2nd larval instar recorded 2.53 ± 0.27 days for the control against 1.2 ± 0.09 day for the exposed larvae ($P< 0.05$). While, the duration of the 3rd larval instar reached 3.78 ± 0.40 days for the control compared to 1.09 ±0.05 day for the exposed larvae ($P<0.05$). For the duration of the 4th larval instar, it was 8.33 ± 2.18 days compared to 5.27± 0.9 days for both the control and the exposed larvae ($P< 0.001$), respectively. Additionally, the total larval duration (16.00 ± 2.98 days) of the control significantly exceeded the whole lifespan of the treated larvae (8.46±1.38 days, *P*<0.001) (Table 1).

Table 1. Effect of mobile radiation on the larval and pupal duration of *Cx.* (*Cx*) *univitattus*

	Duration of larval instar/day					Pupal duration /day
	1st	2nd	3rd	4th	Total larval duration	
Control	(2- 1) 1.36 ± 0.13	(3- 2) 2.53 ± 0.27	(2-5) 3.78 ± 0.40	(12- 3) 8.33 ± 2.18	(22- 8) 16.00 ± 2.98	(2- 4) 2.2 ± 0.1
Exposed	(2- 0.4) 0.90 ± .34 **	(2- 1) 1.2 ± 0.09*	(2- 1) 1.09 ±0.05*	(8- 3) 5.27± 0.9***	(14- 5.4) 8.46±1.38***	(2- 4) 2.5 ± 0.9*

The symbol (*) refers to a statistically significant difference at *P*< 0.05; n= 50.

The pupal duration was significantly affected (*P*<0.05) by the exposure to mobile radiation since the pupal duration of the control was 2.2 ± 0.1 days, compared to 2.5 ± 0.9 days for the exposed pupae (Table1). In this context, **Dimitrijević *et al.* (2014)** concluded that, ELF magnetic field shortens the developmental time of *Drosophila subobscura*, and **Galal and Seufi (2022)** reported a significant reduction in the developmental time of immature stages of RE-exposed *Culex pipiens* when compared to the control.

The percentage of mortality (%) for all the larval instars, and the pupal stage were significantly (*P*= 0.00) affected by the exposure of mobile radiation. The mortality percentages of the first larval instar were recorded as 1.36 ± 0.13%, and 3.9 ± 2.61% for both control and exposed larvae (*P<0.05*), respectively. Whereas, the mortality of the second larval instar was 6.65 ± 1.50 % in case of the control, compared to 7.34 ± 2.31% for the exposed larvae (*P< 0.05*). On the other hand, the recorded mortality of the third larval instar was lower (9.63 ±2.70 %) than that recorded for the exposed group (10.35 ±1.31%; *P<0.05*). The mortality percentage of the 4th larval instar was lower (10.82 ± 2.18%) when compared to the exposed larvae (12.26 ± 3.25%) (*P*<0.05). To sum up, the total larval mortality increased from 30.85 ± 8.70% for the control to 33.85 ± 9.48% for the exposed larvae (*P*<0.05), respectively (Table 2).

Table 2. Effect of mobile radiation on the percentage mortality (%) of the larval and the pupal stages of *Cx.* (*Cx.*) *univitattus*

	% mortality					Pupal % mortality (Failed to emerge)
	1st	2nd	3rd	4th	Total % mortality	
Control	(1.80- 5.30) 1.36 ± 0.13	(5.68- 9.35) 6.65 ± 1.50	(5.65- 14.43) 9.63 ±2.70	(8.53- 18.16) 10.82 ± 2.18	(21.66- 47.24) 30.85 ± 8.70	(10.58- 15.95) 13.79 ±0.93
Exposed	(2.30- 6.22) 3.9 ± 2.61*	(5.85- 10.42) 7.34 ± 2.31*	(7.11- 15.33) 10.35 ±1.31*	(8.65- 18.35) 12.26 ± 3.25*	(23.91- 50.32) 33.85 ± 9.48*	(40.43- 60.21) 57.14 ± 0.9***

The symbol (*) refers to a statistically significant difference at $P< 0.05$; n= 50.

The pupal mortality was significantly affected by radiation exposure (control: 13.79 ±0.93 %, and exposed: 57.14 ±0.9 %, $p<0.001$).

Similar to the present findings are those of **Ramirez *et al.* (1983)** who confirmed a higher mortality % of eggs, larvae, and pupae of *D. melanogaster* flies, placed for 48h under both pulsed (100 Hz, 1.76 mT). In this respect, **Agrawal *et al.* (2021)** postulated that, the third larval instar of *D. melanogaster* exposed to chronic and acute electromagnetic radiation showed a significant decline in the number of flies ($p =0 .007$) compared to the control.

It was noticed that, adult emergency and sex ratio were significantly affected by exposure of mobile radiation ($P<0.001$) as 4:3 and 1:16 (E: F) for adult emergency, and 1:1.8 and 1:2.7 (♀: ♂) for both the control and the exposed independently (Table 3).

Table 3. Effect of mobile radiation on adult emergency and sex ratio of *Cx.* (*Cx.*) *univitattus*

	Adult emergency: Emergency failure (E:F)	sex ratio (♀: ♂)
Control	(1.0: 0.5 - 5.0: 4.0) 4.0:3.0	(1.0: 1.35- 1: 1.9) 1.0 :1.8
Exposed	(1.0: 8.7- 1: 20) 1.0 : 16.0***	(1.0: 4.2- 1: 8.3) 1.0 :2.7*

Similar findings are those recorded in the study of **Agrawal *et al.* (2021)**. The authors reported that under chronic electromagnetic radiation, a low number of *D. melanogaster* flies eclosed was detected compared to the control. Additionally, adult flies of *D. melanogaster* exposed to chronic electromagnetic radiation emerged one day later, compared to the control **(Agrawal *et al.*, 2021)**. However, in contrast to the obtained results, **Dimitrijević *et al.* (2014)** stated that ELF magnetic field does not affect the sex ratio of *D. subobscura.*

Results proved that radiation increased the failure to reach the adult stage and the failure to switch to the extreme phase. The exposure to mobile radiation affected the survival rate significantly during the experimental period. This result coincides with that of **Aday (1975)**. The author postulated that the radiation of 900 MHz is explosively bioactive, causing conspicuous revise in the physiological function of living organisms. GSM 900 MHz radiation was reported to inhibit ants' association between food spots and encountered cues **(Adey, 1988)**. Electromagnetic field (EMF) could incite natural goods on biomolecules and cells **(Cammaerts *et al.*, 2012)**. **Panagopoulos *et al.* (2004)** reported a decrease of reproductive capacity of the insect *Drosophila melanogaster* by 50%– 60%, as an effect of GSM 900 MHz mobile phone radiation. Moreover, **Panagopoulos *et al.* (2007)** compared between the natural conditioning of the two systems, GSM (900 MHz) and DCS (1800 MHz). The authors concluded that both types of radiation dropped the reproductive capacity of fruit canvases significantly. Mobile phone is a device that emits the strongest EMF radiation which can suppress the survival of fruit canvases and change the morphology of their reproductive organs **(Sudaryadi *et al.*, 2020)**. **Stanojević *et al.* (2005)** reported that the pupae of the house fly *Musca domestica,* exposed to an EMF (50 Hz) showed braked down transformation. EMF radiation can be a problem for insects resulting from exposure **(Balmori 2006, 2009, 2014, 2015)**. The EMF affected the experimental period, adult life, adult weight and the fecundity of posterior generations of *Callosobruchus chinensis* (Coleoptera), *Maruca vitrata* (Lepidoptera), *Nysius plebeius* and *Nysius hidakai* (Hemiptera). The same conclusion was postulated by **Maharjan *et al.* (2019a, 2019b, 2020)**.

CONCLUSION

To conclude, all larval instars and pupal life span were significantly delayed by exposure to mobile radiation. In addition, the failure of adult emergency increased significantly as the sex ratio of *Cx.* (*Cx.*) *univitattus* was significantly affected by exposure to mobile radiation, and this failure increased specifically in females.

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