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Influence of Low Static Magnetic Field (SMF) on Immature Development and Survival of The Mosquito, *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

In this study, experiments were performed to investigate the effect of the low static magnetic field (SMF) on hatchability, development, and survival of *Culex pipiens* mosquito immature under laboratory conditions. Egg-rafts of *Cx. pipiens* were exposed to SMF at different intensities of 5 mT, 25 mT, and 50 mT for 20 minutes at a temperature of 28±1 ºC. Hatching rate, hatching delay time, and larval development significantly affected by SMF. The numbers of hatched larvae decreased with increasing SMF at 50 mT (133.33 ± 6.0) as compared to the control (302.23 ± 10.9). Also, the hatching rate significantly lengthened ($P < 0.05$) with high intensity of SMF. A total of 88.3% and 44.33% eggs hatched at 5 mT and 50 mT, respectively. For *Cx. pipiens* immatures, the duration time shortened and survival (%) reduced after exposure to high dose (50 mT) than to low dose (5 mT). We concluded that SMF affects egg hatchability and immature development which could be applied as a strategy in mosquito control.

INTRODUCTION

There is a considerably great interest in the effects associated with the magnetic field or due to the application of this field on living organisms (Levengood and Shinkle 1960; Azizoğlu *et al.*, 2011; He *et al.*, 2012). A lot of animal species are directly affected by or have the benefit of the magnetic field in orientation, finding homes, building their nests, and more (Walker and Bitterman 1985; Kirschvink *et al.*, 2010). The precise mechanism underlying the effects of the Static Magnetic Field (SMF) on living organisms is still vague although there are several hypotheses have been proposed to explain the mechanism by which SMF interacts with biological systems (Ghodbane *et al.*, 2013; Ibrahim 2015; Zhang *et al.*, 2017; Ji *et al.*, 2009).

SMF has been shown to influence the distribution, oviposition, development, and fecundity of many different species of insects (Walker and Bitterman 1985; Martin *et al.*, 1989; Jackson and McGonigle 2005). The application of a dose of 4.5 mT of SMF over a 48 hours period decreased the hatchability of Drosophila's eggs (Ramirez *et al.*, 1983). Also, a continuous exposure dose of 60 mT induced weaker viability of two Drosophila species (Savić *et al.*, 2011). Moderate SMF caused deformation in the biological cell membrane ion channels (Rosen 2003). Negative geotaxis in Drosophila melanogaster has been disrupted by SMF (Fedele *et al.*, 2014). In a study on the effect of SMF on honeybees (Martin *et al.*, 1989), it was found that a dose of 375 mT reduced honeybee's flying activity.

Moreover, it has been a recorded delay in the hatching time of mosquito eggs exposed to SMF at 9.4T and 14.1T (Pan and Liu 2004). In a study on the influence of exposing Tenebrio species to 50 mT SMF (Todorović *et al.,* 2013), it was found that SMF affected their motor behavior, although it did not alter their pupa to adult development.

As evident, many researchers have focused on the study of the phenomenon of SMF on the biological aspects of insects, but there are few studies on the effect of SMF on mosquitoes (Strickman *et al.,* 2000; Benelli *et al.,* 2016). Mosquitoes represent one of the major arthropod vectors of human disease (e.g., malaria, lymphatic filariasis, and arboviruses) worldwide (Ghosh *et al.,* 2012; Benelli *et al.,* 2016).

Mosquito is one of the insect's species that faces continuous daily exposure to SMF which could have a serious impact on their life cycle, development, and other activities. Therefore, in this paper, we monitored the effect of low SMF on the immature *Cx. pipiens* mosquito, aiming to evaluate some of the biological parameters such as hatchability and development.

MATERIALS AND METHODS

Mosquito Culture:

Culex pipiens L. larvae were obtained from the Medical and Molecular Entomology Section, Entomology Department, Faculty of Science, Benha University. They were maintained at 27 ± 2°C with a photoperiod of 14:10 h (light/dark) in the insectary. Larvae were fed on fish food (Tetramin®) with ground bread in the ratio of 3:1. Pupae were transferred from the enamel pans to a cups containing de-chlorinated tap water and caged in screen cages (35 × 35 × 40 cm dimensions), (Fig. 1a) where the adults emerged. The adult colony was provided with a 10% sucrose solution and was periodically blood-fed on pigeons. After three days, engorged female mosquitoes started to oviposit egg-rafts on small cups that contained tap water (Baz 2013).

Magnetic Field Source:

Magnetic field apparatus located in Physics Department, Faculty of Science, Benha University was used as a source of SMF (Fig. 1b), and the strength of the SMF was kept stable on the specimens during the experiments. SMF device (Electromagnetic, Nuis Technologies Put, Ltd) is composed of two cylindrical magnets surrounded by a copper coil which connected to an AC-DC converter (CD power supply ST- 4181, output 10 Amperes). Egg rafts were placed in the mid-point of the two magnets and exposed for twenty minutes at room temperature (28±2 ºC). The intensity of SMF (B) was measured by Gauss meter or alternatively, it can be calculated from Ampere's formula

$$B = \frac{\mu_o I}{2\pi r}$$

Where μo is the free space permeability, I is the electric current in Amperes, r is the cylindrical tube radius in meters. The sensor was placed in the mid-point of the sample container. The insect samples were contained in a cylindrical tube of diameter ≈ 4 cm and immediately exposed to the SMF.

Laboratory Assay:

1. Mosquito Oviposition:

After female mosquitoes were blood-fed, they were caged as shown in Fig. 1c under experimental condition waiting for oviposition. A total of 24 similar egg-rafts were collected and divided into four groups of six rafts each for the control and the three SMF doses. Then, each group was divided again into three subgroups of two rafts each for experimental replication.

2. Effect of Magnetic Field on Mosquito Hatchability.

To study the effect of SMF on mosquito hatchability, two egg-rafts were separately exposed to SMF of each of 5 mT (milli-Tesla), 25 mT, and 50 mT. After exposure, egg-rafts were transferred to plastic trays (10 × 13 × 7 cm), (Fig. 1d). Two other egg-rafts were transferred to plastic trays without SMF exposure as control. Egg rafts were under the same experimental conditions for the remainder of the investigation. The number of hatched eggs (number of larvae) of the control and SMF exposed egg rafts were counted daily until no further egg hatch was observed. Three replicates were performed for each group.

Fig. 1: Experimental aspects of the effect of the magnetic field on mosquito immature stages. Mosquito rearing (a), SMF exposing facility (b), blood-feeding (c), and hatchability (d).

3. Effect of Magnetic Field on Survival and Development of Mosquito Immatures:

A newly hatched 50 larvae from each of the control and SMF exposed egg rafts were transferred into small cups in cages to study their development and survival. The larvae were daily observed until pupation and adult emergence. The survival rate, developmental periods, and pupation rates were determined for each group. The experiment was replicated three times and compared to the control groups.

Statistical Analysis:

Means and Standard Errors were calculated for the examined attributes and compared by ANOVA at a significance level of 0.05 using SPSS (ver. 22, for windows, SPSS Inc., Chicago, IL, USA) statistical software (Steel *et al.*, 1997). Multiple comparisons were carried out applying LSD.

RESULTS AND DISCUSSION

There were a lot of studies that focused on the effect of magnetic field on vertebrates, however, relatively fewer studies have been carried out on insects (Starick *et al.*, 2005), and with the majority of these studies were done using gamma rays, high-energy electrons, and X-rays on sterile mosquito (Lindquist 1955; Knipling 1979; Mansour 2010). Magnetic field; ultraviolet light (UV) radiation and microwave were used against different insects (Rosen 2003; Faruki *et al.*, 2007; Azizoğlu *et al.*, 2011). Our data showed that SMF has an impact on the biological aspects of mosquito immature.

The effect of SMF on the hatchability of *Cx. pipiens* eggs are represented in **Table**

1. It was noticed that the hatching periods and rates for the exposed and control eggs varied significantly where the time of hatching has taken 27.36 hrs and 51.84 hrs in the control and 50 mT respectively. As well as, the hatching rate was 88.30% of eggs hatched at 5 mT, while only 44.33% of eggs hatched at 50 mT.

Table 1: Mean duration (per days) of immature stages produced from egg-rafts exposed to SMF

SMF Dose	No. of eggs	No. of larvae	Dead larvae %	Hatching rate %	Hatching delay time (hrs)
Control	315.33± 12.13^{a}	302.33± 10.90^{a}	4.10± 0.87^{d}	95.90± 0.87^{a}	27.36± 1.36^{d}
5 mT	302.00± 11.60^{a}	266.67± 5.21^{b}	11.70± 1.72^{c}	88.30± 1.72^{b}	34.8± 1.87^{c}
25 mT	300.67± 10.09^{a}	203.67± 4.26^{c}	32.20± 0.86^{b}	67.80± 0.86^{c}	41.0± 2.06^{b}
50 mT	300.67± 6.96^{a}	133.33± 6.01^{d}	55.67± 1.52^{a}	44.33± 1.52^{d}	51.84± 1.56^{a}

a, b, c, d: Means, within the same column have the same small letters and means within the same row have the same capital letters are not significantly different (P>0.05, LSD)

From Table 1, the SMF intensity significantly affected the mortality of larvae where it was increased with increasing of the applied SMF intensity. The mortality rate was significantly higher than that of the control (approximately 55.67 and 4.1% at 50 mT respectively). Although we believe that the obtained hatching delay time could be altered by changing experimental temperature because it is inversely proportional to temperature (Pan and Liu 2004), SMF also increased the hatching time at the used temperature.

The number of hatched larvae and consequently, the percentage of dead larva were influenced by SMF intensity. The higher the SMF intensity, the more the embryos which couldn't hatch out normally or they have died inside their eggs. We propose that this could be due to the hardening of the egg membrane or weakening of the embryos which couldn't come out of their eggs due to the effect of SMF.

This finding is in agreement with the finding that low doses of SMF would destroy the cellular membrane of E-coli bacteria (Ji *et al.,* 2009). On the other hand, the development of the hatched larva was observed to record how many larvae can reach the adult stage which is e able to produce new colonies. This means that SMF may consequently affect mosquito final numbers that reach the adult stage which will be exposed to the environment and causing offspring weakening and obstacle disease prevalence. Therefore, the duration time and survival (%) of *Cx. pipiens* immature stages under different SMF are shown in T**able (2)** and represented in Figure 2. The development time was observed to be elongated after exposure especially at 50 mT than at 5 mT, where the duration of larvae to adult emergence reached 18.70 and 11.28 days, respectively. The Percentage of larvae that reached the adult stage was reduced at 50 mT as compared to control (Fig 2).

Finally, data obtained show that there is a correlation between SMF intensity and the larva-adult number (increasing mortality) and periodicity (longer emergence time).

Table 2: Effects of the static magnetic field (Mean±SE) on egg development of *Cx. pipiens* mosquitoes*

SMF Dose	Larvae instar				Pupa	Larva-adult
	1st	2nd	3rd	4th		
Control	1.53± 0.09 ^{dE}	2.47± 0.06 ^{cC}	3.03± 0.06 ^{cB}	3.33± 0.10 ^{cA}	2.15± 0.12 ^{dD}	10.36±0.45
5	1.96± 0.07 ^{cD}	2.69± 0.20 ^{bC}	3.13± 0.07 ^{cB}	3.50± 0.08 ^{bA}	3.14± 0.13 ^{cB}	11.28±0.53
25	2.21± 0.11 ^{bE}	2.78± 0.17 ^{bD}	3.86± 0.20 ^{bC}	4.66± 0.06 ^{bA}	4.10± 0.26 ^{bB}	13.52±0.70
50	2.68± 0.12 ^{aE}	4.37± 0.12 ^{aD}	5.32± 0.17 ^{aB}	6.34± 0.18 ^{aA}	4.67± 0.12 ^{aC}	18.70±0.71

* in each column, means with different letters are not significantly different) LSD, P>0.05)

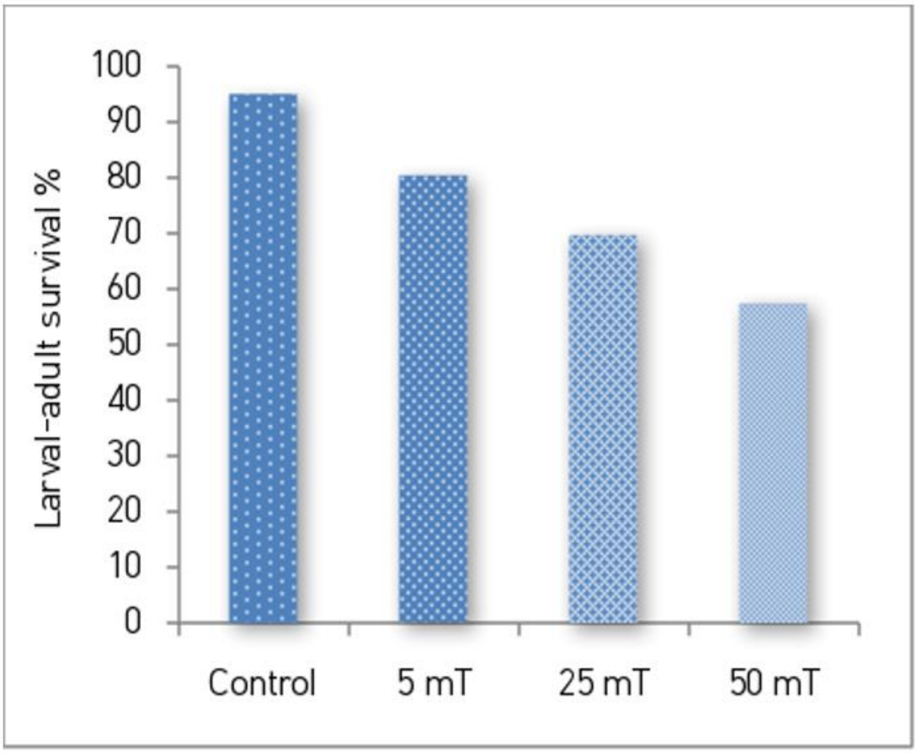

Fig. 2: The percentage of larvae survived to adult of *Cx. pipiens* as a function of SMF dose compared to the control.

Conclusions

From the obtained results and discussion, it can be concluded that although the applied SMF is low, it still affects the mortality, oviposition, development, and survival of *Cx. pipiens* immature stages which could be applied as a tool for mosquito control. Therefore, we conclude the following:

1. Mosquito's life period and development can be interrupted and affected by SMF they are facing in real life, outside laboratories.
2. Oviposition and hatching rate was affected by SMF and the effect was shown to increase with increasing SMF intensity.
3. The hatching delay time and development show that the number of mosquito complete life cycles can be reduced by the external impact of SMF.

4. Finally, SMF alters an insect's life periodicity positively or negatively, depending upon the way you are looking at it.

The study was conducted on the effect of SMF on eggs, in upcoming studies, we suggest a study of the effect of low SMF on adult mosquitoes that investigate the same parameters to test how adults are affected.

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ARABIC SUMMARY

تأثير المجال المغناطيسي الساكن المنخفض على تطور وبقاء يرقات بعوض كيولكس بيبنز

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يتأثر العديد من أنواع الكائنات الحية بما فيها الحشرات بشكل مباشر بالمجال المغناطيسي المنتشر بكثافة في انحاء العالم والذي يؤثر على اتجاة طيران الحشرات، هجرتها أو العثور على أعشاشها. وفي هذه الدراسة تم إجراء تجارب تأثير جرعات منخفضة من المجال المغناطيسي الساكن (SMF) على فقس كتل البيض، نمو وبقاء يرقات البعوض تحت الظروف المعملية. حيث تم تعريض كتل البيض من نوع بعوض الكيولكس بيبنز لجرعات منخفضة من SMF (5 ملى تسلا، 25 ملى تسلا، و50 ملى تسلا) لمدة 20 دقيقة عند درجة حرارة 28 ± 1 درجة مئوية، حيث لوحظ تأثر كلا من معدل الفقس، تأخر فقس البيض وتطور اليرقات بشكل كبير نتيجة تعرضها لتلك الجرعات من المجال المغناطيسي الساكن . (SMF)كما لوحظ انخفض عدد اليرقات (133.33 ± 6.0) الناتجة من الفقس عند زيادة جرعات المجال المغناطيسى الساكن SMF عند 50 ملى تسلا مقارنة بالمجموعات غير المعرضة (302.23 ± 10.9) على التوالى. أيضا، كما وجد ان معدل الفقس يتأثر بشكل كبير (P <0.05) عند الجرعات العالية من مجالSMF ، لذلك نجد ان معدل فقس البيض انخفض الى 44.33 ٪ عند 50 ملى تسلا و88.30 ٪ عند 5 ملى تسلا. تم اطالة المدة الزمنية لتطور يرقات البعوض كما اختزل عدد يرقات البعوض التى وصلت الى الطور اليافع بعد التعرض الى 50 ملى تسلا مقارنة بالجرعة المنخفضة (5 ملى تسلا).

استنتج من هذه الدراسة، أنه على الرغم من تعرض اناث البعوض الى جرعات منخفضة من المجال المغناطيسي الساكن (SMF) الا انه قد أثر على الخواص البيولوجية لمعدل زمن وفقس البيض وتطور اليرقات مما يتيح لنا امكانية تطبيقه ضمن استراتيجية مكافحة البعوض.