CATRINA (2024), 30 (1):51-63

Effect of the electric field generated from high voltage power lines on the biology and behavior of the fruit fly, *Drosophila melanogaster*

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ABSTRACT

The electric fields (EFs) generated by high-voltage power lines used to transmit electricity among cities and villages may have a considerable potential to significantly influence organisms in close proximity. This study aims to investigate alternations in *Drosophila melanogaster* biology and behavior following exposure to high EFs. Moreover, the levels of neurochemicals in flies exposed to EF were evaluated. A simulation system program was used in the laboratory to generate actual EF values in the vicinity of high-voltage power lines. The intensity of EF was adjusted to 12.0 kV/m and 5.7 kV/m, representing two different distances from a 220 kV power line. Flies were exposed to the simulated EFs for 6 days/8 hours a day. The results revealed that near-distance exposure to EFs negatively impacted development time, adult emergence, and hatchability rate. In behavior, EF exerted profound adverse effects on memory retention, climbing ability, male aggression, and adult food consumption. Additionally, near-distance exposure to EFs showed a significant increase in the levels of neurotransmitters, dopamine and serotonin. The results provide scientific evidence that anthropogenic EFs emitted from the transmission power lines in terrestrial habitats can be an environmental stressor potentially affecting the biology, behaviour, and neurochemicals of *Drosophila.* We assume a similar impact of these high EFs on vertebrates including humans living near high-voltage power lines. Therefore, the study recommends avoiding building under high-voltage lines, and people should reside far away from these lines when constructing any facility for humans or living organisms.
Keywords: Acetylcholine; Dopamine; *Drosophila;*Electric field; Power lines; Serotonin.

INTRODUCTION

Electric fields (EFs) are naturally occurring electrical charges between the earth’s surface and the outer atmosphere. EFs are generated by various sources, including a global electric circuit (Adlerman and Williams, 1996). By the 20th century, the ongoing need for electricity has increased. Thus, above-ground high-voltage transmission power lines are abundant in urban and rural areas of developed and developing countries, carrying large amounts of electric power. Power lines are anthropogenic sources of electric and magnetic fields called electromagnetic fields (EMFs) produced under and near power lines. Both fields are high around a power line and diminish rapidly with distance away from the source.

New high-voltage power lines are introduced into the environment as a result of the growing demand for electric power supplies. The introduction of such new power lines may have a considerable impact on all living organisms, including vertebrate and invertebrate animals, plants, and residents living nearby (Petri *et al.*, 2017, Schmiedchen *et al.*, 2018). EMFs produced by electric power lines rose clearly in the last five decades to become biologically active environmental pollutants potentially threatening public health (Levitt *et al.*, 2022, Thill *et al.*, 2023). Consequently, people who live or work near power lines have an increased risk of cardiovascular diseases, brain tumors, and childhood Leukemia (WHO, 2007).

Relative to the knowledge of the effects of EMFs on insects, less is known about the influences of EFs. The effects of EFs at different frequencies and intensities on adult insect behavior have been observed in both field and laboratory studies. Field studies on honey bees, *Apis mellifera* colonies underneath 765 kV power lines (EF ca. 7 kV/m) showed an increase in agitation at the entrance of the hive besides a decrease in foraging rates (Greenberg *et al.*, 1981). Laboratory studies have reported that EFs induce several altered behavioral responses in insects, including avoidance behavior in the cockroach, *Periplaneta americana* (Newland *et al.*, 2008), cigarette beetle, *Lasioderma serricorne* and the fruit fly, *Drosophila melanogaster* (Matsuda *et al.* 2011), disturbance in the flight behavior of the cabbage loopers, *Trichoplusia ni* (Perumpral *et al.*, 1978) and reduced walking activity of the ichneumon wasp, *Itoplectis conquisitor* (Maw, 1961) and cockroaches (Jackson *et al.*, 2011).

The effects of EFs on early life history traits and reproduction of insects are currently limited. Only, He *et al.* (2016) studied the effects of long-term exposure to EF over 30 consecutive generations on the development and reproduction of the wheat aphid, *Sitobion aveanae.* They found that exposure exerted adverse effects on nymph developmental duration, total longevity, and reproductive rate. Maw (1961) reported the effect of a weak static field on the oviposition rate of

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the ichneumon wasp, *I. conquisitor*.

The impacts of EFs on the physiology and molecular biology of insects are poorly understood. Early physiological studies reported the effects of EFs on metabolic activity, oxygen consumption, and food intake in bees, cockroaches, Indian stick insects, and wasps (Altmann 1959). A few studies analyzed the effects of EFs on insect neurochemicals. The biogenic amines serotonin, dopamine, and octopamine are neurotransmitters and play a major role in insect behavior and physiology. In *Drosophila,* they modulate circadian rhythms, learning and memory, mating behavior, locomotion activity, aggression, and many different mechanisms (Monastirioti, 1999, Wolfgang and Arnd, 2001, Banu *et al*., 2023). Newland *et al*. (2015) reported changes in three biogenic amines (serotonin, dopamine, and octopamine) levels in *Drosophila*'s brains after exposure to EFs.

Acetylcholine is a fast neurotransmitter that mediates communication between neurons in synapsis or between neurons and muscles to regulate locomotion, heartbeat, and other physiological functions in multicellular organisms (Picciotto *et al*., 2012, Cox *et al*., 2020, Showell *et al*., 2020, Giordani *et al.*, 2023). It plays a key role in the cognitive defects associated with aging in adult *Drosophila* (White *et al*., 2020). Most of the previous studies did not comprise modeling of high-voltage transmission lines. A simulation model power line where EF values were controlled was used in this study to determine whether exposure to EFs would affect the biology and behavior of *Drosophila*. Furthermore, the potential effect of EF exposure on *Drosophila*'s neurochemicals was assessed. *Drosophila* was used as a model organism to study these effects. *Drosophila* has a great history as a perfect model for research. It has simple phenotypes and huge genetics available (Hassaneen 2015) and 75% of human disease genes (Bier 2005) and 50% of protein sequences (Redlarski *et al.* 2015) are related to its genetic map. As a model, *Drosophila* was investigated to understand genes, chromosomes, and mutations (Ashburner and Bergman 2005, Yamaguchi 2018). Moreover, many different human diseases such as cancer (Mirzoyan *et al.* 2019), infectious diseases (Arch *et al*., 2022, Harnish *et al.* 2021), and neurological diseases (Suzuki *et al.* 2022) are under study. Therefore, in this study, a high-voltage transformer was employed in the laboratory to replicate the scenario where individuals reside in close proximity to high-voltage transmission lines. The outcomes obtained offer insights into the potential impacts of exposure to high-voltage transmission lines on organisms situated near such power lines.

MATERIALS AND METHODS

Fly stocks and rearing conditions

All experiments were carried out on wild-type *Drosophila* Egyptian strain. The flies were maintained in vials (size 100 ml) containing approximately 25 ml standard cornmeal-yeast-agar diet consisting of sugar, cornmeal, and yeast; 63g of each and 12.5g agar dissolved in 1L distilled water. The flies were kept at a density of ~ 50 individuals of mixed sex. They were raised at 25 ± 2 ºC with 60%-80% RH under a 12:12 hrs light:dark photoperiod.

Electric field simulation and exposure

EF generation and simulation need two main steps: first, the evaluation of EF in the vicinity of high-voltage power lines at two distinct positions, and second, the simulation of this targeted EF in the laboratory. The EF distribution was evaluated in the vicinity of a 220kV above-ground high-voltage transmission power line using the finite element method, as shown in Figure (1A). The spacing of the lower high voltage line was about 15m from the ground level. EF was then plotted from the conductor surface up to the ground level, as depicted in Figure 1B. Two different separating distances from the high-voltage line were considered in the present study: 3 and 12m. EF was calculated at these separating distances. At 3 m, it was 12.0 kV/m; at 12 m, it was 5.7 kV/m.

A high-voltage module test system (AC module system, Type: WBS 5.8/100) was used to simulate the actual EF near the high-voltage power lines. The test system was designed to produce a continuously variable AC test voltage against the earth using a high-voltage testing transformer (Figure 2A) operated by the control panel shown in Figure (2B). Moreover, the exposure test cell where test *Drosophila* (in glass vials containing diet) were placed for exposure was created using two copper plates (50×25 cm) with a (15 cm) gap distance between them and settled on a wooden frame with an electrode to be connected to the transformer (Figure 2C). During experiment, the voltage was applied to the two parallel copper plates, creating an electrical potential difference between them. This indicates that no current is flowing between the plates, since the medium between the plates is air, which is an insulator. Thus, no heat was generated. The simulated EF in the laboratory was calculated using the equation: $V= Ed$ whereas voltage (V), electric field (E), and distance (d). To develop 12.0 kV/m, at a distance of 15 cm, a voltage of 1800 V (12.0 kV/m) was applied across the test cell. Meanwhile, to develop 5.7 kV/m, at the same distance (15 cm), a voltage of 850 V (5.7 kV/m) was applied across the test cell. All experiments in the laboratory were conducted under exposure to the two primary high electric fields: 5.7 kV/m and 12.0 kV/m. The control group did not undergo exposure to the electric fields (voltage = 0.0 kV/m).

In actual power line scenarios, the magnetic field effect from above-ground high-voltage lines is below 4 µT (Nichols *et al*., 2012; Tourab and Babouri, 2016), which is significantly lower than the limit set by the International Commission on Non-Ionizing Radiation Protection (Tong *et al*., 2016). Thus, it is believed that the magnetic field effect was negligible. Owing to these findings, we suppose that the expected influence on *Drosophila* would be due solely to EF exposure. In the high-voltage transformer used, there was no current flow; therefore, no magnetic field was generated.

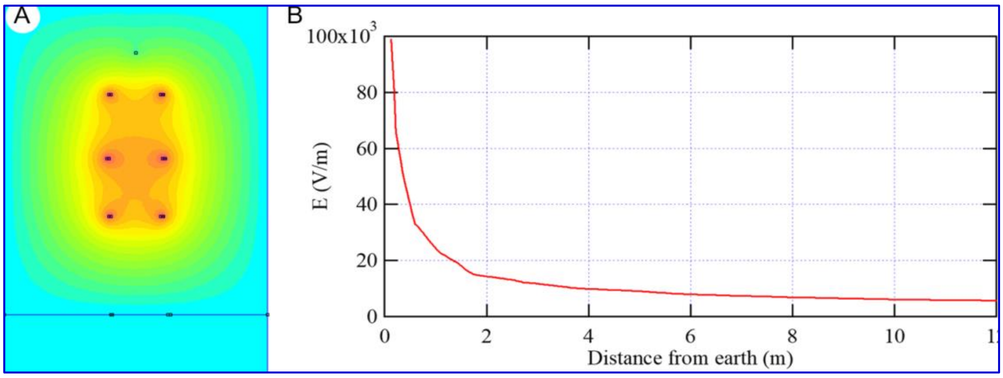

Figure (1): Electric field exposure system (A) and distribution (B) in the vicinity of 220 kV high voltage transmission line.

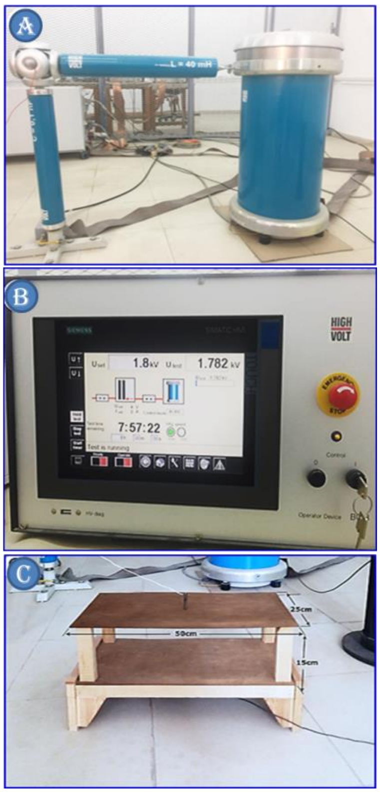

Figure (2): Instruments used for measurement the effect of EF on *Drosophila.* A, High voltage transformer generator; B, control panel for voltage setting operator device; and C, the exposure device test cell with dimensions.

Biological assays

Development time and adult emergence assay

To determine the effect of EF on the development time and adult emergence, 20 early 2nd instar larvae/ replicate were randomly collected and placed in vials containing a standard diet. This procedure was replicated 10 times. The larvae were exposed to 5.7 kV/m or 12.0 kV/m EF intensity for 6 days/ 8h a day. The control groups were at the same condition with no voltage (=0.0 kV/m). After exposure, the larvae were allowed to complete their development until adult emergence under laboratory conditions away from any electrical effect. The duration of the larval development was determined as the time larvae spent for development after exposure whereas; the emergence rate was calculated as the proportion of adults that emerged out of the exposed larvae.

Female fecundity and egg-hatchability assay

To track the effect of EFs on *Drosophila's* reproductive capacity, five virgin females/replicate aged 2-4 days were collected from rearing vials and divided into two red-colored hard agars medium vials designated as control and exposed. The females were exposed to 5.7 or 12 kV/m EFs for 6 days/8 hrs per day. The control was kept without any electric exposure (Voltage = 0.0 kV/m). After that, 7 males (approximately 7 days old) were introduced into each female's vial and allowed to mate for 24 hrs. Then, they were transferred to a new vial with fresh red medium for 3 successive days for egg laying. Eggs were counted after the adults were removed from the vial to a new vial with the red media for egg-laying. For hatchability, eggs were allowed to hatch and develop until the larval stage. Counting was achieved when the larvae reached the 3rd instar, especially the wandering stage, only to avoid miscounting as some larvae may be hidden in the medium; the larvae were counted 3 times per vial. This procedure was performed 10 times/EF.

Behavioral assays

The behavioral parameters for aversive learning and memory retention, negative geotaxis, aggression, courtship, and feeding rate of *Drosophila* were studied after EF exposure.

Learning and memory behaviors

Drosophila's learning and memory behaviors were determined after exposure of male flies to 5.7 and 12.0 kV/m for 6 days/8 hrs a day using the idea of engaging the positive photo-tactic behavior with an aversive stimulation to a bitter taste (Ali *et al*., 2011). Only one choice a fly had to take, to enter the lighted chamber with 5M NaCl and be considered as failed, or to avoid the bitter taste and enter the dark chamber and be considered as passed. After five tries, a typical fly would avoid the glowing chamber. Their short-term memory was assessed following the learning process to determine whether the flies would recall to steer clear of the illuminated chamber and toward the darker one.

a. Learning

Ten *Drosophila* males were placed in vials and starved for 3 h to be ready for the aversive taste. After starvation, flies were examined first for their sensitivity to light to ensure they were positively photo-tactic. Each male was transferred to the T-maze in the darkness only under the red-light effect, trapped in the dark chamber, and was allowed to acclimate in the darkness for 30 seconds. Then, the light source was turned on in the lighted chamber and the fly was allowed to enter it. If the fly ran to the light chamber within 10 seconds, it was considered positively phototactic and was chosen for the test.

For the aversive phototaxis, a fly was trapped in the dark chamber for 30 seconds at the same time a filter paper with a 5M NaCl solution was added to the light chamber. After the acclimation time, the light was turned on and the fly was allowed to enter the chamber. One minute later, the fly was tapped back into the dark chamber; this procedure trial was repeated 9 times (learning training). After learning training, the other 5 trials were recorded to calculate the learning index. When the fly entered the light chamber in 10 seconds; this was recorded as a failure. The fly that avoided the lighted chamber was recorded as a pass.

b. Memory

To investigate the memory retention of *Drosophila* males, each trained fly was returned after training to food vials for 3 h. Then, the flies were placed again in the T-maze for the memory assay, and the other 5 trials were tested for short-term memory. The observation was recorded as the fly that entered the lighted chamber, failed, and that avoided it, passed.

Negative geotaxis (Climbing ability assay)

Negative geotaxis, scored as the climbing ability of male flies exposed to 5.7 and 12 kV/m for 6 days/ 8 h per day was carried out based on the protocol described by Nichols *et al*. (2012). In each experiment, ten males were transferred to a ring apparatus which consisted of five glass cylinders (replicates) enclosed inside a wooden frame. The flies were left to accommodate for a minute. After that, the apparatus was tapped down strongly three times on the bench surface to locate the flies at the bottom of the cylinders forcedly. The flies were allowed to climb the cylinder wall. Vertical positioning of the flies 'climbers' in the cylinder was recorded after five seconds of tapping. A screenshot photo was taken from the video recording for the apparatus to easily analyze and measure the climbing distance of flies in each cylinder. All photos were analyzed, and the negative geotaxis was calculated by measuring the average distance that flies climbed in centimeters (cm) by five seconds. Image J software program (Schneider *et al*., 2012) was used to determine the distance flies climbed five seconds post-tapping.

Selection of mate (Courtship assay)

The ability of males to court and the acceptance of females to the competition between males till a successful copulation were tested in this experiment. Virgin males and females *Drosophila* (up to 8 h post-emergence) were sexed and separated into two vials based on gender. After 2-4 days of emergence, the separated adults were exposed to 5.7 or 12 kV/m for 6 days/8 h a day. The control flies were exposed to 0.0 kV/m EF. After exposure, each female was placed without anesthetizing in a test chamber with two males for ten replicates/EF. Flies were observed till successful copulation. The courtship index was calculated according to Nichols *et al*. (2012) as the time spent in courting was divided by the total time until copulation.

Male aggression

To determine the effect of EF exposure on male *Drosophila*'s aggressive behavior, the protocol of Sahu *et al*. (2020) was adopted. Eight males (2-4 days old) were exposed to voltage levels of 5.7 and 12 kV/m for 6 days/8 h per day in 10 replicates/EF. After exposure, males were placed in empty vials without food for 2 h to encourage aggressive behavior then, flies were transferred to a transparent cubical test chamber (2 cm in size) containing a small drop of food (standard medium). The small size of the chamber and the small drop of food after starvation were both stimulants for male aggression. The control groups were exposed to 0 kV/m EF. Males were observed for five minutes and aggressive behaviors like chasing; kicking, boxing, and wing threats were counted and recorded.

Adult feeding rate

To clarify whether the feeding rate of adult *Drosophila* could be affected by EF, mixed adult populations of 10 males and 10 females (2-4 days old) were collected and exposed to either 5.7 or 12 kV/m EF intensities for 6 days/8 h a day. The control groups were exposed to 0.0 kV/m EF. To calculate the feeding rate of adults in the exposed or control flies, a capillary feeder (CAFE) was adopted as described by Diegelmann *et al*. (2017). In brief, adults were kept in vials and starved for 3 hrs. Then, they were transferred to test vials; each vial contained six capillaries with 10µL liquid food consisting of sugar, yellow cornmeal, and yeast. Six grams of each was dissolved in 100 mL distilled water and 2 mL of red dye was added. Adults were kept in the vials for 24 hrs in the previously mentioned laboratory conditions. To avoid the evaporation factor, three reference vials with the capillaries and without the flies were kept at the same conditions to calculate the evaporation rate. Subsequently, the capillaries were labeled with a marker pen at the start of the liquid food level (m beginning) and at the end of the 24 hrs experiment (m end).The distance between the two marks was measured using a ruler to calculate the food uptake as the following equation:

$$\text{Food uptake } (\mu\text{L}) = \frac{\text{measured distance (mm)}}{5.4 \text{ (mm)}}$$

Where, the capillary was 89 mm long and contained 10µL of food solution. A 5.4 mm decrease in the meniscus reflected the uptake of 1µL solution.

To calculate the food consumption/fly, the mean of the three capillaries of the reference vials was excluded according to the following equation:

$$\text{Food consumption/fly} = \frac{\text{Food uptake } (\mu\text{L}) - \text{Evaporation loss } ((\mu\text{L})}{\text{Total number of flies in the vial}}$$

Determination of acetylcholine, dopamine, and serotonin levels

The levels of acetylcholine, dopamine, and serotonin were measured in the whole body of the adult male

Drosophila after exposure to EF 12.0 kV/m for 6 days/8 hrs a day as a confirmatory experiment. 100 Adults per replicate were collected, freezed, and homogenized in acetonitrile:methanol (75:25) and centrifuged at 4°C for 30 min at 4500 rpm. This was replicated three times. After the centrifugation, the supernatant was collected. Acetylcholine, dopamine, and serotonin measurements were determined using the high-performance liquid chromatography (HPLC) system. The performance consisted of a pump (Thermo Ulti-mate 3000), a column (SVEA-RP-C18gold, sized; 5µm 250×4.6mm-NANOLOGICA-Sweden), and an associated DELL-compatible computer supported with Cromelion7 interpretation program. A diode array detector DAD-3000 was used. A volume of 20 µL was injected into the column in an isocratic mode as mobile phase was 0.05 M Potassium dihydrogen phosphate buffer (KH_2PO_4): Acetonitrile (90:10 v/v). Samples and standard solutions, as well as the mobile phase were degassed and filtered through a 0.45 µm membrane filter (Millipore) and sonicated 15 minutes before use. The flow rate was held at 1.0 ml/min. The column was kept at 30°C. The identification of the compounds was done by comparing their retention time and UV absorption spectrum at 325 nm with those of the standards. All standards were purchased from Sigma-Aldrich.

Statistical analysis

All response variables were checked for normality by the Anderson-Darling test and homogeneity of variances by Bartlett's test. Count data were transformed into LOG, while proportions were transformed using ARC.SIN (SQRT). Normally distributed variables were presented as mean ±standard deviation (M±SD), while non-normally-distributed variables were presented as Min-Max values with a median line. To compare the two high voltages used, 5.7 and 12.0 kV/m, we ran two control groups at 0.0 kV/m. However, we found it more convenient to combine the data of the two controls in one group for better presentation and easy comparison since there was no significant difference between them. One-way ANOVA and Kruskal-Wallis accompanied by Tukey multiple-comparison or Dunn Multiple Range test were used to compare the groups tested. An unpaired t-test was used to compare two-group data, based on normality. Statistical analysis was performed using GraphPad Prism 9.0.2 for Windows, GraphPad Software, San Diego, California USA (www.graphpad.com) or SAS 9.1 (SAS Institute, Cary NC).

RESULTS

Biological studies

Development time and emergence rate

The Welch's ANOVA showed that the development time of *Drosophila* was significantly affected by exposure ($F_{2,19.17}$ = 12.09, p =0.0004). Dunnett's Multiple Range tests revealed that the observed difference was only at the 12 kV/m exposure ($p \leq 0.0001$), where the exposed larvae needed more time (14.57 ±0.33 day) for development compared with the control (13.35 ±0.81 day) (Figure 3A). However, the development of larvae (14.22 ±1.17 day) exposed to 5.7 kV/m did not show a significant difference when compared with the control insects. Furthermore, one-way ANOVA of adult emergence showed significant differences among means due to EF exposure ($F_{2,37}$ = 10.03, p =0.0003). The Tukey multiple comparison tests clarified that the adult emergence rate (0.74 ±0.1) exposed as larvae to 12.0 kV/m was lower (p =0.0007) than the controls (0.92 ±0.06). Upon comparing both exposed groups, the multiple comparisons revealed that the emergence rate of *Drosophila* exposed to 12.0 kV/m was significantly lower (p =0.001) compared to those exposed to 5.7 kV/m (0.91 ±0.13). No significant difference was observed when comparing the controls with those exposed to 5.7 kV/m (Figure 3B).

Female fecundity and egg-hatchability

Female *Drosophila's* fecundity was not affected by EF high-voltage exposure for 6 days/8 hrs a day as reported by one-way ANOVA results ($F_{1,37}$ = 2.219, p =0.123). The female fecundity was 13.7 ±2.85, 11.5 ±2.84, and 13.5 ±2.91 eggs/female/day in the controls, insects exposed to 5.7 kV/m and 12.0 kV/m, respecttively (Figure 4A). In pairwise comparisons, there was no significant difference between those groups.

The egg hatchability was affected by EFs. The results of one-way ANOVA revealed that the exposure induced a highly significant difference among means ($F_{1,36}$ = 9.851, p =0.00004) (Figure 4B). The Tukey multiple tests indicated that the flies exposed to 5.7 kV/m had less (p =0.0007) hatchability rate (0.63 ±0.14) than those in the control (0.83 ±0.14). Peculiarly, the exposure to 12.0 kV/m significantly (p =0.0015) increased the egg hatchability rate (0.84 ±0.07) again when compared with those exposed to 5.7 kV/m. However, there was no significant difference ($p \geq 0.05$) between the means of the exposed group at 12 kV/m and the control groups at 12.0 kV/m (Figure 4B).

Behavioral studies

Aversive learning and memory retention rate

Kruskal-Wallis test showed no significant effect of EF exposure (*H(3)*=3.2, p =0.2) on the aversive learning rate of males. Moreover, the pairwise comparison (Dunn multiple test) between the tested groups did not reveal any significant difference (Figure 5A). How-ever, the memory retention rate of the same tested male flies was significantly affected by the exposure (*H(3)*= 8.85, p =0.012) (Kruskal-Wallis test). The multiple comparisons (Dunn test) revealed males exposed to the high voltage of 12 kV/m showed more deficiency (p =0.01) in the memory retention rate (0.3 Median) than the control (0.8 Median). However, the flies exposed to 5.7 kV/m showed non-significant differences when compared with those in the control or exposed to 12.0 kV/m (Figure 5B).

Negative geotaxis

Kruskal-Wallis test indicated that EF exposure influenced (*H(3)*=11.8, p =0.0003) the climbing ability of males (Figure 6A). Dunn Multiple tests indicated that flies exposed to 12 kV/m showed significantly (p =0.018, p =0.0003) reduced climbing ability (4.87cm/5

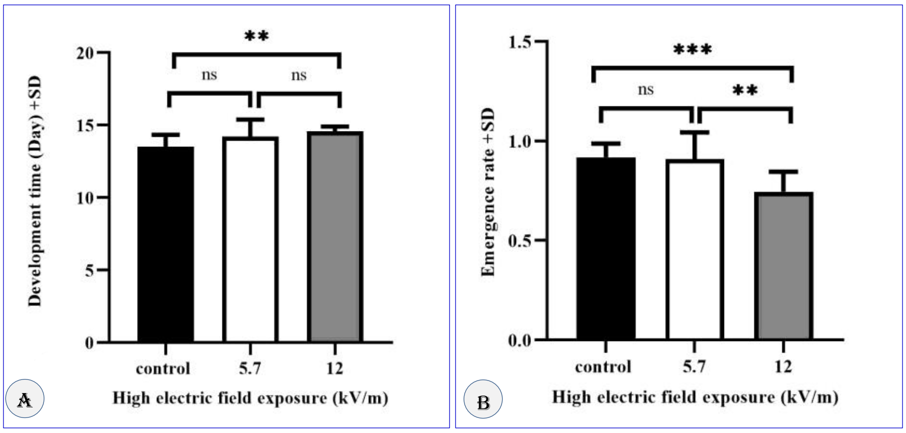

Figure (3): Larval development time (A) and adult emergence rate (B) of *Drosophila melanogaster* after exposure to electric field at 5.7 and 12 kV/m. One-way ANOVA results indicated that there is a significant difference among means at $p < 0.05$; ns, refers to a non-significant difference between means at $p \geq 0.05$, while (**) and (***) refer to a significant difference between means at $p < 0.01$ and $p < 0.001$, respectively (Dunnett's T3 multiple or Tukey test). n=10.

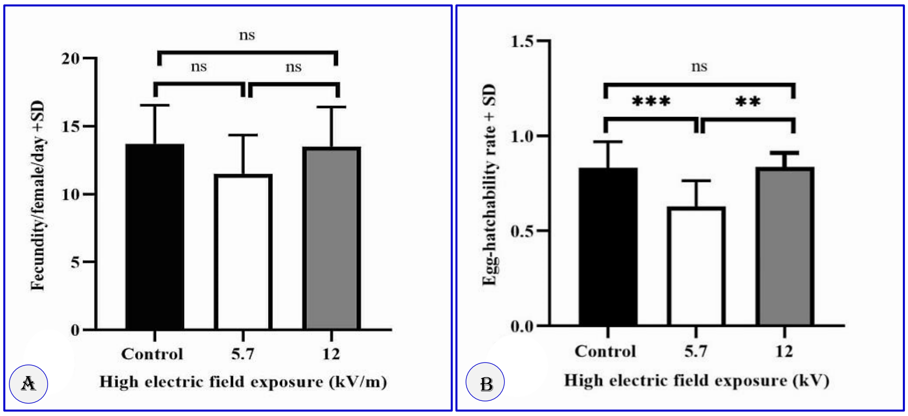

Figure (4): Female fecundity (A) and egg-hatchability (B) of *Drosophila melanogaster* after exposure to an electric field at 5.7 or 12.0 kV/m. One-way ANOVA revealed non-significant differences among means at $p \geq 0.05$ in the female fecundity, whereas revealed a significant difference among means due to EF at $p < 0.05$ in egg-hatchability. (ns) refers to a non-significant difference between means at $p \geq 0.05$. while (** & ***) refer to significant differences among means at $p \leq 0.01$ & ≤ 0.001, respectively (Tukey multiple-test), n=10.

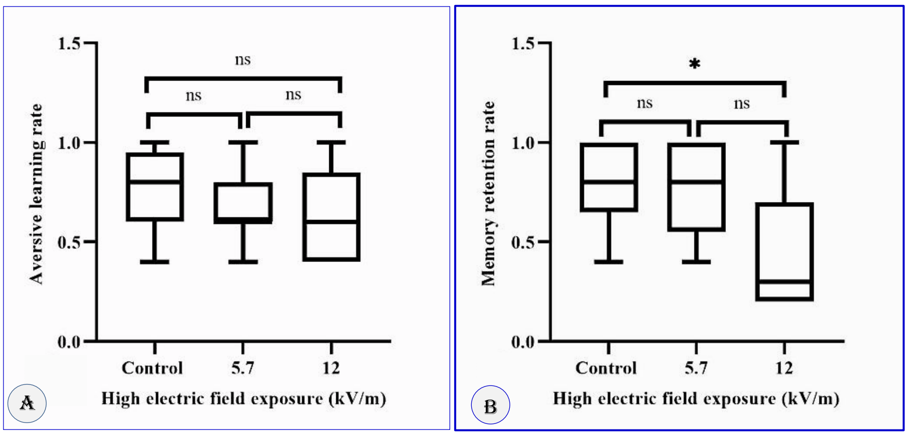

Figure (5): Aversive learning rate (A) and memory retention rate (B) of *Drosophila melanogaster* males after exposure to the electric field at 5.7 or 12.0 kV/m. Kruskal-Wallis test revealed non-significant differences among means at $p \geq 0.05$ in (a). (ns) refers to a non-significant difference between means at $p \geq 0.05$, while (*) refers to a significant difference between means at $p \leq 0.05$ (Drunken Multiple Range test). Values are presented as Min – Max with a Median line, n=10.

sec., Median) than those in the control (7.22 cm/5 sec., Median) and those exposed to 5.7 kV/m (7.49 cm/5 sec., Median), respectively.

Selection of mate (Courtship)

There were no significant differences in the courtship index of flies due to the EF exposure (one-way. ANOVA, $F_{1,37}$ =2.93, p =0.066). The changes between the means (0.77 ±0.08, 0.83 ±0.11, and 0.73 ±0.08) of the control, exposed to 5.7 and 12 kV/m groups did not show any significant differences (Figure 6B).

Male aggression

The evaluated parameters of male *Drosophila*'s aggressive behavior were kicking, wing threats, and chasing. EF exposure resulted in significant changes in aggressive male activity ($F_{1,37}$ =5.47, p =0.008) (Figure 7A). This was evident by the observation that only males exposed to 12.0 kV/m had significantly (p = 0.014) reduced aggressive (31.9 ±8.07) behavior than unexposed males (41.3 ±8.27). Meanwhile, the aggression of males (33.9 ±8.13) exposed to 5.7 kV/m did not differ from the control groups.

Adult feeding rate

The results of one-way ANOVA indicated significant changes ($F_{1,37}$ = 7.4, p =0.002) in the food consumption rates following EF exposure (Figure 7B). It was observed that the Tukey multiple tests clarified that the food consumption rate of adults exposed to 12.0 kV/m (1.29 ±0.23) was significantly (p = 0.025) lower than the control (1.56 ±0.27) and those exposed to 5.7 kV/m (1.71 ±0.21) (Figure 7B).

Acetylcholine, dopamine, and serotonin levels

Exposure to EF at an intensity of 12.0 kV/m for 6 days/8 h a day did not cause a statistically significant change in the acetylcholine level in the exposed male's bodies (t = 0.91, df = 4, p= 0.415) compared with the control groups; 137.0 ±8.35 µg/ml and 122.67 ±26 µg/ml, respectively (Figure 8). By contrast, dopamine and serotonin levels showed significant differences compared with the control groups.

Dopamine level measured in the exposed flies was significantly elevated (94.2 ±3.37 µg/ml) compared to the control group (37.87 ±1.5 µg/ml). Additionally, serotonin levels were higher in the exposed groups (1025 ±36.5 µg/ml) than in the control flies (830.9 ±±76.27 µg/ml). The unpaired t-test analysis was t = 26.44, df = 4, p ≤0.0001 and t = 3.98, df = 4, p =0.016 for both the dopamine and serotonin levels, respectively, equivalent to 5.7 kV/m, and another voltage, 1800 V, as a simulation for 12.0 kV/m.

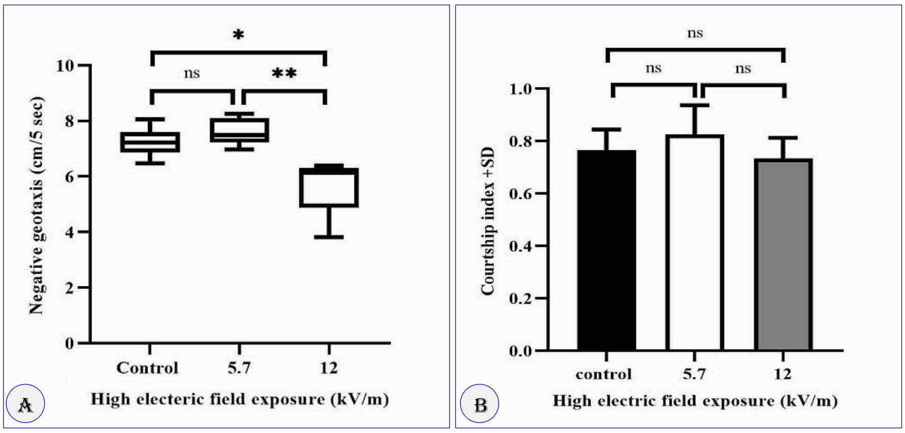

Figure (6): The negative geotaxis (A) and courtship index (B) of *Drosophila melanogaster* males after exposure to an electric field (5.7 or 12.0 kV/m). Kruskal-Wallis test indicated significant differences among groups in (A). One-way ANOVA indicated that there is a significant difference among means at $p \leq 0.05$; ns, refers to a non-significant difference between means at $p \geq 0.05$; while (* & **) refer to significant differences between means at $p \leq 0.05$ & ≤ 0.01, respectively (Dunn multiple Range tests), n =5 in (A) and 10 in (B).

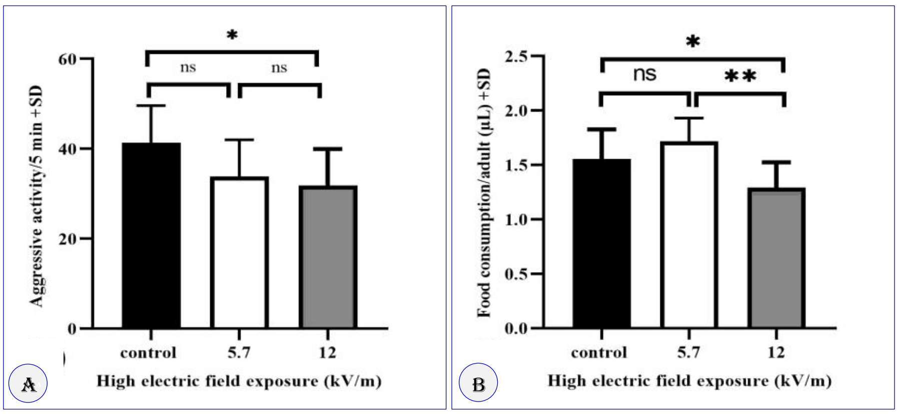

Figure (7): The aggressive activity of males (A) and food consumption of *Drosophila melanogaster* adults (B) after exposure to two high electric fields 5.7 or 12.0 kV/m. One-way ANOVA indicated that there were significant differences among means at $p \leq 0.05$; ns, refers to a non-significant difference between means at $p \geq 0.05$; while (* & **) refers to a significant difference between means at $p \leq 0.05$ and ≤ 0.01, respectively (Tukey multiple tests), n=10.

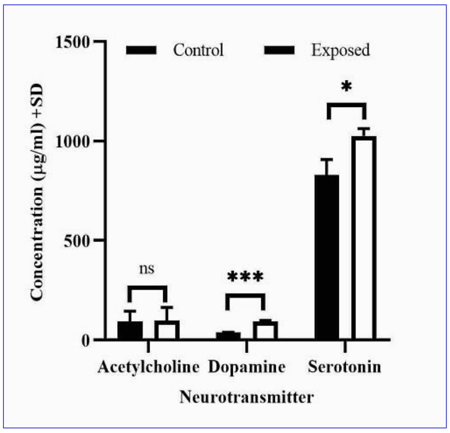

Figure (8): The acetylcholine, dopamine, and serotonin levels in *Drosophila melanogaster* males' bodies after exposure to an electric field (12.0 kV/m). An unpaired t-test was used to compare the control and exposed groups. ns, refers to a non-significant difference between means at $p \geq 0.05$; (* & ***) refer to significant differences between means at $p \leq 0.05$ and ≤ 0.0001, respectively. $n = 3$.

DISCUSSION

This study aimed to evaluate the effect of EF generated from the power lines on *Drosophila* as a model organism. Two high voltages were chosen for exposure to be simulated in the laboratory: 850 V, equivalent to 5.7 kV/m, and another voltage, 1800 V, as a simulation for 12.0 kV/m.

The development time of *Drosophila* was found to be affected by EF exposure. Exposure of *Drosophila* larvae to the highest EF 12.0 kV/m increased the developmental time compared to control groups. Meanwhile, no increase in development was observed by exposure to 5.7 kV/m. This result agrees with other researchers who documented clear developmental deviations. For instance, He *et al.* (2016) reported that the long-term exposure of the wheat aphid, *S. avenae* to static EFs at intensities of 200, 400, or 600 kV/m prolonged the developmental duration. Otherwise, the emergence rate of *Drosophila* herein was affected when exposed to different EFs; the stronger the EF, the greater the effect.

The reproductive success of insects includes two measures: fecundity and egg-hatching. The present results showed that female *Drosophila*'s fecundity exposed to 5.7 or 12 kV/m was not affected. In contrast, Levengood and Shinkle (1960) reported that the progeny yields of cultures of *Drosophila* in an EF were higher than that in control groups. Similarly, Panagopoulos (2015) noticed an increase in *Drosophila's* reproductive capacity by 30% following exposure to different EF intensities of 100, 200, 300, and 400 kV/m. However, Edwards (1961) observed that the geometrid moth, *Nepytia phantasmaria* females laid fewer eggs when exposed to an experimental EF magnitude of 18.0 kV/m. The contrasting results of these studies on reproductive behavior may have been due to the large range of applied EF strengths, exposure duration, stage, and kind of insect species studied. In the current study, exposure to the high EF of 5.7 kV/m significantly decreased the egg-hatchability of *Drosophila*. This may reflect that the EF could not affect the gonads but may impair the hatching ability. In this instance, EF exposure may impact some traits and enhance others (Pall, 2013).

The results indicated that the exposure of male *Drosophila* to EF at 12 kV/m (8 hrs daily for 6 days) caused a significant reduction in memory retention rate. In contrast, there was no change in male aversive learning. Little is known about insect aversive learning, and how it is affected by EFs and EMFs. Shepherd *et al*. (2019) reported that short-term exposure to 50 Hz extremely low-frequency EMFs, reduced the aversive learning performance of the honeybee, *A. mellifera*, by over 20%. This means that aversive learning depends on exposure duration, type, and strength of exposure as well as differences among insect species. Neural control plays another vital role in aversive learning behavior and memory retrieval (Schwaerzel *et al*., 2003, Schroll *et al*., 2006, Masek *et al*., 2015). This could mean that EF as an environmental stressor affected the neural pathway that controls the memory retention rate and not the aversive learning. Reduction in memory retention could be detrimental to *Drosophila's* survival.

The results of this study demonstrated that negative geotaxis in *Drosophila* males was disrupted by EF exposure at 12.0 kV/m. These findings support previous observations. For example, Watson (1984) showed that *Drosophila*'s walking ability was correlated with EF strength. Moreover, studies on *A. mellifera* exposed to EF of 150 kV/m led to vibrations of wings and antennae. By increasing the field strength up to 300 kV/m, bees appeared to have difficulty in walking (Bindokas *et al*., 1989). The result strongly suggests that naturally existing EF could adversely impact the geotaxis behavior of animals on Earth.

The courtship index depends mainly on the courting time spent by males. Its calculation in the normal wild-type *Drosophila* is usually in the range of 0.6-0.8 (Nichols *et al*., 2012). Nevertheless, there was no statistically significant change in the courtship index of the pairs exposed to 5.7 or 12 kV/m compared with those in the control. This behavior was found to be controlled by sensation (Greenspan and Ferveur, 2000, Villella and Hall, 2008, Nichols *et al*., 2012) and motor (Becnel *et al*., 2011). It became clear that EF did not affect this behavior.

Aggressive behavior between conspecifics is common for gaining access to desirable resources such as territory, food, and mates. Hence, aggression is critical for determining individual success. Both male and female *Drosophila* display aggressive behavior toward individuals of the same sex. In males, wing threats and boxing participate either in the establishment or in the maintenance of dominance (Simon and Heberlein, 2020, Legros *et al*., 2021). Fights can escalate to include boxing, holding, and tussling (Fernandez *et al*., 2022). The results showed that EF treatments resulted in a significantly reduced aggressiveness of male *Drosophila* upon exposure to 12.0

kV/m. This may be because the high voltage reduces locomotion and movements. This result could be inferred by the results of the negative geotaxis and the aggression. The EF of the higher strength (12.0 kV/m) impacted the male *Drosophila*'s motor control.

The exposure of *Drosophila* adults to EF at 12.0 kV/m reduced food consumption. The higher the EF intensity, the stronger the observed effect. Decreased food intake was found in bees during exposure to extremely low-frequency EMFs (Shepherd *et al.*, 2018). *Drosophila* adults were challenged to climb to the capillary and feed upside-down. In this assay, the motor and muscular control must facilitate that feeding way. It is well-known that muscles control the stand of the fly for a long time. So, if there is any motor control deficiency, feeding reduction could happen (Banu *et al.*, 2023). Therefore, EF of the higher strength (12.0 kV/m) impacted the ability of flies to stand on the capillary and compete to feed for the 24-hrs time allowed. Furthermore, this study showed changes in *Drosophila*'s behavior only under the influence of 12.0 kV/m EF. Therefore, it was important to compare these changes with the activities of neurochemicals that control behavior in *Drosophila*. Acetylcholine is known as a neurotransmitter that modulates physiological activities (Giordani *et al*., 2023). Exposure to 12.0 kV/m voltage did not affect the acetylcholine level in *Drosophila*. One possibility is that the strength of the voltage was not strong enough to exert change in the acetylcholine level in *Drosophila*.

The results demonstrated that flies exposed to 12.0 kV/m EF for 6 days/8 hrs a day, exhibited increased dopamine and serotonin levels compared with the control flies. However, Newland *et al*. (2015) reported a significant decrease in dopamine levels in the heads of flies exposed to an electric field (EF) of 70 kV/m for 4, 24, and 72 hours, while serotonin levels showed only a slight decrease. The discrepancy between our results and those of Newland and colleagues may be attributed to variations in experimental conditions, including differences in EF strength levels, exposure durations, and the specific insect tissue analyzed. In *Drosophila*, dopamine is known to play multiple vital roles in regulating aversive learning and memory, motor functions, aggression, courtship, and sexual behavioral patterns, as well as appetite or motivation to feed (Neckameyer, 1998, Monastirioti, 1999, Stevenson *et al*., 2000, Kume *et al*., 2005, Pizzo *et al*., 2013, Waddell, 2013, Yamamoto and Seto, 2014, Shepherd *et al*., 2019).

In the present study, dopamine levels were significantly elevated in EF-exposed male flies, which could explain the observed reduction in memory retention rate. Zhang *et al*. (2008) confirmed that elevated dopamine levels induced memory impairment in *Drosophila*. Nevertheless, the role of dopamine in memory was debated; both increased and reduced dopamine levels negatively affect memory acquisition and retention in *Drosophila* (Kim *et al*., 2007, Yamamoto and Seto, 2014).

Otherwise, sexual behaviors in *Drosophila* are characterized by male movements, "courtship dance" and female acceptance. Dopamine is essential for normal female sexual receptivity and male courtship rituals (Neckameyer, 1998). Therefore, the increased dopamine levels may decrease successful mating (Andretic *et al*., 2005, Chang *et al*., 2006). Dopamine plays a modulatory role in how *Drosophila* locomotion is maintained (Pizzo *et al*., 2013) including the baseline locomotion and the touch response circuits (Simon *et al*., 2009).

In the present study, EF exposure increased the level of dopamine, which in turn induced climbing disability in flies that appeared as a decline in the negative geotaxis. Dopamine has been linked to aggression in *Drosophila* (Stevenson *et al*., 2000, Chen *et al*., 2002, Johnson *et al*., 2009, Alekseyenko *et al*., 2014). The results showed that exposure to EF at 12.0 kV/m dramatically reduced aggressive interactions in male flies. So, the observed increase in dopamine levels could explain the adverse effect on aggressive behavior which is important in mate competition, and most likely insect fitness.

Serotonin is involved in the regulation of feeding nutrient intake and digestion in many animals. It has been proven to control hunger and satiety and modulate specific aspects of feeding in different invertebrate phyla (Tierney, 2020). The results of many different approaches support the idea that serotonin suppresses feeding (Pooryasin and Fiala, 2015) and foraging honeybees (French *et al*., 2014). In agreement to these studies, our results support that the increase in serotonin levels in EF-exposed flies generally may decrease feeding or consumption in *Drosophila*.

Based on the previous results, exposure to EF of the high voltage affects *Drosophila* biogenic amine levels which control many different behavioral and biological traits. The negative effect was observed in development time, adult emergence, hatchability, memory, climbing ability, male aggression, and adult food consumption. A lesson could be learned to understand the similar effects on humans and many other organisms. The high-voltage transmission power lines could have an impact on the population's health. Future studies should focus on the biochemical and molecular aspects underlying these effects in *Drosophila* or other animal models.

CONCLUSION

Our study investigated the effect of the electric field generated by high voltage power lines on the biology and behavior of the fruit fly, *D. melanogaster*. The results revealed significant alterations in neurotransmitter levels, particularly dopamine and serotonin in flies exposed to the high EF strength. The observed increase in dopamine and serotonin levels suggests a potential impact of EF exposure on relevant activities and behaviors in the fruit flies. Furthermore, our findings along with prior research highlight the importance of considering experimental variables such as EF strength levels, exposure times, and tissue specificity when studying the biological responses of insects to electric fields. Overall, our study contributes valuable insights into the potential effects of electric fields on the physiology and behavior of *D. melanogaster*, emphasizing the need for further research to elucidate the underlying mechanisms and implications for insect populations in proximity to high voltage power lines.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

ETHICS APPROVAL

All protocols used in this study were approved by the Faculty of Science Ethics Committee, Tanta University, Egypt (Code: IACUC-SCI-TU-0135).

AUTHORS' CONTRIBUTIONS

WSM and DAM contributed to the study conception and design. Material preparation, data collection, and analysis were performed by DHE, DAM, and WSM. The draft of the manuscript was written by DHE. WSM, DAM, EAN, and AIS revised the manuscript. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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تأثير المجال الكهربائي المتولد من خطوط الكهرباء ذات الجهد العالي على بيولوجية وسلوك ذبابة الفاكهة *Drosophila melanogaster*

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الملخص العربي

قد يكون للمجال الكهربى المتولد من خطوط الجهد العالى المستخدمة لنقل الكهرباء بين المدن والقرى تأثير كبير على الكائنات الحية التى تعيش قريبة منها. لذلك تهدف هذه الدراسة إلى دراسة التغيرات في بيولوجيا ذبابة الدروسوفيلا وسلوكها بعد التعرض لمستويات عالية من المجال الكهربى. وعلاوة على ذلك، تم تقييم مستويات بعض النواقل العصبية في الذباب المعرض لـلمجال الكهربى. وقد تم استخدام نظام المحاكاة في المختبر لتوليد قيم المجال الكهربى الفعلية في محيط خطوط الكهرباء ذات الجهد العالي. وتم ضبط شدة هذه المجالات عند 12 كيلو فولت/م و5.7 كيلو فولت/م، وهو ما يمثل مسافتين مختلفتى البعد عن خطوط الكهرباء 220 كيلو فولت. تم تعريض الذباب للمجالات الكهربية لمدة 6 أيام/8 ساعات في اليوم. وأوضحت النتائج أن التعرض القريب للمجال الكهربى الصادر عن خطوط الجهد العالى أثرت سلبًا على مدة التحول فى الأطوار غير الناضجة ومعدل خروج الطور البالغ منها وكذلك معدل فقس البيض. أما بالنسبة للسلوك فقد كشفت النتائج أن تعرض الذباب للـمجال الكهريى كان له آثار سلبية شديدة على الذاكرة، وقدرة الذبابة على تناسق الحركة والتسلق، وعدوانية الذكور، ومعدل التغذى فى الذباب. وأظهر التعرض القريب كذلك زيادة ملحوظة في مستويات بعض النواقل العصبية مثل الدوبامين والسيروتونين. وبناء على ماسبق، توفر النتائج دليلاً علميًا على أن التعرض القريب للمجال الكهربى المتولد عن خطوط الجهد العالى فوق الأرض يمكن أن يؤثر على بيولوجيا ذبابة الفاكهة وسلوكها والمواد الكيميائية العصبية فيها. وتوصى الدراسة بعدم البناء تحت خطوط الضغط العالى و البعد قدر الإمكان عن هذه الخطوط عند إقامة إى منشأة للبشر أو الكائنات الحية.