IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Comprehensive Assessment Of Behavioral Endpoints In *Drosophila Malerkotliana* Exposed To 50 Hz Extremely Low-Frequency Electromagnetic Fields"

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Abstract

D.malerkotliana, like other *Drosophila* species, is a powerful model organism widely used to investigate conserved molecular pathways relevant to human conditions such as cancer, neurodegeneration, and cardiovascular disease thanks to high genetic conservation and versatile genetic tool. This study evaluates the effects of 50 Hz LF-EMF exposure on a spectrum of behavioural traits in *D. malerkotliana*, including larval crawling, adult locomotion, climbing, courtship behaviour, and aggression. Flies were subjected to LF-EMF exposure administered via a Holzmolt coil for both short- and long-term time frames. Behavioural metrics were recorded using established methods, including larval crawling grid tracking, the Rapid Iterative Negative Geotaxis (RING) climbing assay, and time-based interaction assays for courtship and aggression. LF-EMF exposure induced significant, exposure duration dependent reductions in larval crawling and adult climbing ability, with male flies exhibiting heightened sensitivity. Courtship behaviours declined progressively with prolonged exposure, suggesting reproductive disruption, while aggression displayed an initial surge (particularly in males) followed by a decline, possibly due to neuromodulator depletion.

Collectively, these findings suggest that LF-EMF exposure impairs neural and behavioural function in *D. malerkotliana*, potentially through mechanisms involving oxidative stress, neurotransmitter dysregulation, and sex-specific resilience differences. These results underscore the value of *D. malerkotliana* as a model for assessing EMF-induced neurobehavioral toxicity and environmental stress responses.

Keywords: *D. malerkotliana*, LF-EMF, larval crawling, RING assay, courtship behaviours, aggression, Neurobehavioral toxicity.

Introduction

Electromagnetic fields (EMFs) are regions of energy arising from both natural sources and anthropogenic activities, including power transmission lines, household electrical devices, and various industrial systems. LF-EMFs, particularly those at 50 Hz generated by alternating current, have become pervasive due to global technological expansion and urbanization (Roosli, 2008). Although LF-EMFs are classified as non-ionizing radiation and lack sufficient photon energy to directly break chemical bonds, growing evidence indicates that they can influence biological systems through mechanisms such as modulation of ion transport, alteration of enzyme activity, and induction of oxidative stress (Blank and Goodman, 2009; Panagopoulos et al., 2015).

The genus Drosophila has long been a preferred model in genetics, developmental biology, and environmental toxicology due to its short life cycle, high fecundity, and well-characterized genome (Pandey and Nichols, 2011). *D. malerkotliana*, a member of the *melanogaster* species group, was first described by Parshad and Paika (1964) from Malerkotla, India, and has since been reported across South and Southeast Asia. Its remarkable ecological adaptability to diverse climatic and environmental conditions (Fartyal and Singh, 2002) makes it an excellent candidate for studying the impact of environmental stressors, including EMF exposure on behaviour, physiology, and genetic stability.

Several studies on *D. melanogaster* and related species have demonstrated that LF-EMF exposure can induce oxidative stress, affect reproductive performance, alter behaviour, and cause genotoxic damage (Liu et al., 2015; Ayra-Pardo et al., 2022; Akdag et al., 2013). The biological effects are often associated with elevated production of reactive oxygen species (ROS), mitochondrial dysfunction, lipid peroxidation, and DNA strand breaks, which impair cellular homeostasis and neurological function (Kesari et al., 2011). Behavioural assays have revealed LF-EMF associated alterations in locomotor activity, climbing ability, courtship behaviour, and aggressive interactions, potentially due to EMF induced modulation of neuromodulators or synaptic transmission (Weisbrot et al., 2003; Valbonesi et al., 2014).

Despite these findings, there is a Paucity of research on the effects of LF-EMF exposure in *D. malerkotliana*. Given its unique genetic makeup, environmental resilience, and ecological significance, studying this species may provide valuable insights into species-specific responses to electromagnetic stress. Furthermore, understanding its behavioural, physiological, and genetic responses can enhance environmental risk assessment, particularly in EMF polluted habitats.

The present study investigates the biological effects of 50 Hz LF-EMF exposure on *D. malerkotliana*, focusing on larval crawling activity, locomotor performance, climbing ability, courtship behaviour, and aggression. The results aim to elucidate possible mechanisms of EMF induced alterations and contribute to a broader understanding of EMF organism interactions.

Electromagnetic fields (EMFs) encompass a broad spectrum of wave types, ranging from extremely low-frequency (ELF) fields to high-energy gamma rays, classified according to their wavelength and frequency (Roosli, 2008). The 50 Hz EMF used in the present study lies within the ELF region, characterized by very long wavelengths (6,000 km) and low photon energy, far below the threshold required for ionization of atoms or molecules (ICNIRP, 2010). Unlike ionizing radiation such as ultraviolet, X-ray, or gamma rays, 50 Hz EMFs are non-ionizing and cannot directly break chemical bonds. Nevertheless, they can interact with biological tissues via electromagnetic induction, altering ion transport, membrane potential, and cellular signaling processes (Blank and Goodman, 2009; Panagopoulos et al., 2015). Such indirect interactions have been implicated in oxidative stress generation and neurobehavioral alterations in various model organisms,

making ELF-EMFs an important focus of environmental and physiological research (Akdag et al., 2013; Liu et al., 2015).

Research Methodology

EMF model

The 50 Hz electromagnetic field (EMF) exposure model represents a well-established experimental framework for investigating the biological impacts of low-frequency, non-ionizing radiation. In this system, an alternating current (AC) operating at 50 Hz is transmitted through a Holzmolt coil, thereby producing a stable and homogeneous EMF. The apparatus is composed of two primary modules the first, an exposure chamber encircled with copper windings, and the second, an electronic control unit that regulates the frequency of the generated field. This configuration closely replicates real-world exposure scenarios, such as those encountered in proximity to power transmission lines or household electrical devices. Within the coil chamber, organisms such as *D. malerkotliana* are subjected to controlled EMF conditions to evaluate a range of biological endpoints, including behavioral dynamics, oxidative stress responses, reproductive performance, and genomic stability. The model provides a high degree of precision, reproducibility, and ecological validity, rendering it a powerful platform for both acute and chronic EMF exposure studies.

Drosophila stock rearing and handling.

A *D. malerkotliana* strain was procured from the National Centre for Drosophila, Department of Zoology, University of Mysore, Karnataka, India. All procedures involving handling, sedation, and transfer of flies were performed with meticulous care to minimize physical stress and mortality. Routine monitoring was carried out to evaluate survival, behavioral activity, and developmental progression. Any culture vessels showing evidence of contamination or desiccation were promptly discarded and replaced with fresh ones.

Laboratory stocks of *D. malerkotliana* were maintained under standardized insectary conditions, with temperature regulated at 25 ± 1 °C, relative humidity sustained at 60–70%, and a 12:12 h light–dark photoperiod. The flies were reared in cylindrical plastic vials (25 mm in diameter × 95 mm in height) containing 12 mL of freshly prepared culture medium. For large-scale breeding, 200 mL glass bottles were employed, each provisioned with 25 mL of nutrient medium.

Drosophila Food

The nutritional composition and uniformity of the rearing medium play a pivotal role in ensuring optimal development, reproductive success, and experimental consistency in *Drosophila* research. For *D. malerkotliana*, a standardized culture medium was employed, formulated with a carbohydrate-rich and protein-balanced substrate capable of supporting all developmental stages of the life cycle**.**

Table 1. The food was prepared using the following ingredients (per Liter of medium).

Sr. No.	Ingredients	Amount of ingredients	Nutritional significance
1	Semolina	100 g	Provides carbohydrates and bulk
2	Yeast (active dry)	12 g	Primary source of protein, vitamins, and micronutrients
3	Jaggery	80 g	Additional energy source
4	Agar	8 g	Solidifying agent
5	Methylparaben (Nipagin)	1.5g in 5 mL of 95% ethanol	Antifungal preservative
6	Propionic acid	4.4 mL	Prevents Mold and bacterial contamination
7	Distilled water	1 litre	Used for mixing ingredients

Figure 1. a) D. malerkotliana culture. b) culture media. c) glass bottles with healthy flies. d) polystyrene vials contain flies for anaesthesia e) large scale culture of D. malerkotliana. f) food pouring into different types vials for different experiments.

A. ***Larval Crawling Assay***

The larval crawling assay was utilized to evaluate the effects of LF-EMF exposure on the locomotor performance of third-instar *D. malerkotliana* larvae. Larvae were isolated from the culture medium via flotation in a 20% sucrose solution and subsequently collected with a pipette, following standardized methodologies (Nichols et al. 2012; Vang and Adler, 2016). Following either short-term or prolonged LF-EMF exposure, individual larvae were carefully positioned at the center of a water-moistened Petri dish overlaid on graph paper using a fine, moistened paintbrush to ensure gentle transfer. A thin aqueous film across the dish surface prevented premature pupation while facilitating uninterrupted crawling activity. Larval movement was documented with a mobile camera.

B. ***Locomotor Assay***

Larval locomotion in *D. malerkotliana* is strongly influenced by environmental parameters such as temperature, humidity, and nutrient availability, all of which modulate neuromuscular function and energy metabolism. In the present study, the effect of LF-EMF on larval locomotor activity, with a specific focus on peristaltic contractions, was systematically examined. Adult flies were maintained in population cages to promote mating and oviposition, following collection, larvae were rinsed with physiological saline to eliminate residual food particles. Third-instar larvae were then exposed to LF-EMF for short-term intervals (15–60 minutes) and long-term durations (2–8 hours). Peristaltic activity, defined as a complete posterior-to-

anterior contraction of the body wall, was quantified by counting the number of waves per larva over a 1-minute interval under a stereomicroscope. (Nicholls et al., 2018).

C. ***Climbing (RING) Assay***

To investigate the effects LF-EMF exposure on adult locomotor activity, the Rapid Iterative Negative Geotaxis (RING) assay was employed. Newly eclosed *D. malerkotliana* adults were collected, briefly anesthetized, and maintained in standard food vials (20–30 flies per vial) for 2–3 days at room temperature to allow full recovery. For experimental trials, groups of approximately 10 unanesthetized flies were transferred into vials positioned within the RING apparatus and exposed to LF-EMF in a coil chamber for either short-term (15–60 minutes) or long-term (2–8 hours) intervals, followed by a 1-hour acclimatization period. The RING setup was placed approximately one meter from a stationary imaging device with fixed zoom and focus. To initiate negative geotaxis behavior, the apparatus was tapped firmly three times, displacing the flies to the bottom of the vials, and climbing responses were recorded 3 second post tap. Each group was subjected to 5–6 consecutive trials, with one-minute rest periods between repetitions. Digital images were subsequently analyzed using a calibrated scale to determine the mean climbing height per vial. Statistical comparisons between control and LF-EMF treated groups were performed. (Gargano et al. 2005; Pandey and Nichols, 2011).

D. ***Courtship behavior / Mating frequencies.***

Courtship in *Drosophila* is a genetically programmed behavior regulated by key genes such as *fruitless* (*fru*) and *doublesex* (*dsx*), influenced by sensory cues and environmental conditions (Villella and Hall, 2008). Since LF-EMF have been reported to disrupt neural and behavioral processes (Weisbrot et al., 2003), this study examined their effect on *D. malerkotliana* courtship. Virgin males and females were collected within 2 hours of eclosion, aged separately for 5 days, and exposed to 50 Hz LF-EMF under short-term (8–32 h) and long-term (2–8 d) conditions. Courtship was assayed by pairing individuals in sterilized chambers for 10 minutes, recording initiation latency and copulation success, with ten replicates per group. All experiments were conducted under standardized conditions, with chambers ethanol-sterilized between trials.

E. Aggressive behavior

Aggression is an evolutionarily conserved behavior essential for survival and reproductive fitness, and Drosophila has become a powerful model for dissecting its genetic and neural underpinnings due to its tractable genetics (Kravitz and Fernandez, 2015). Aggressive responses are strongly influenced by environmental modulators such as social context, nutrition, and reproductive state, with isolation enhancing and social enrichment suppressing aggression (Kim et al., 2018; Agrawal et al., 2020). In this study, adult *D. malerkotliana* from laboratory stocks were maintained under standardized conditions and exposed to LF-EMF for acute (15–60 min) or prolonged (75–120 min) durations following acclimatization. Post-exposure aggression assays quantified interactions, and behavioral outcomes were systematically recorded as percentage frequencies to assess LF-EMF-induced modulation of aggressive behavior.

Result And Discussion

A. Larval crawling activity.

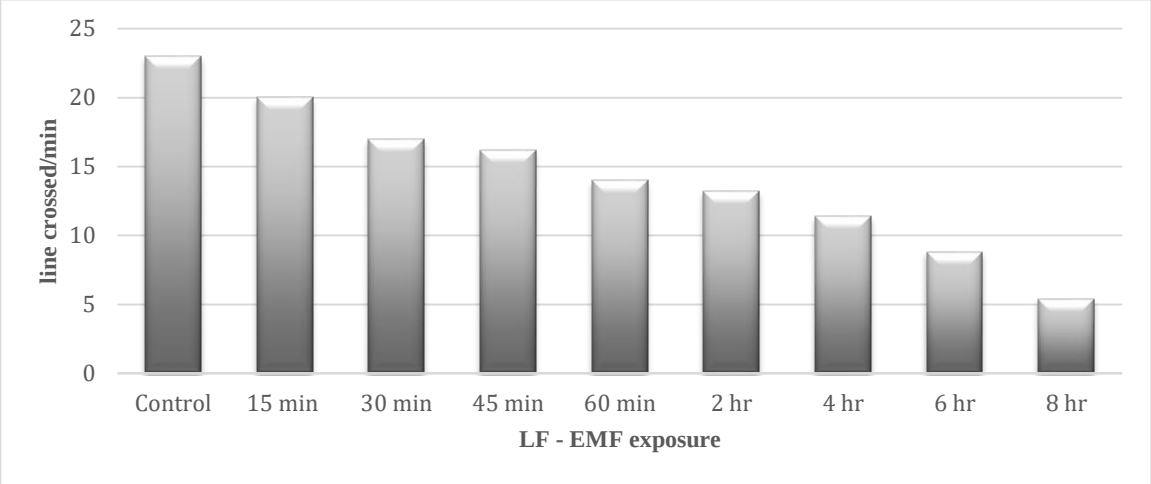
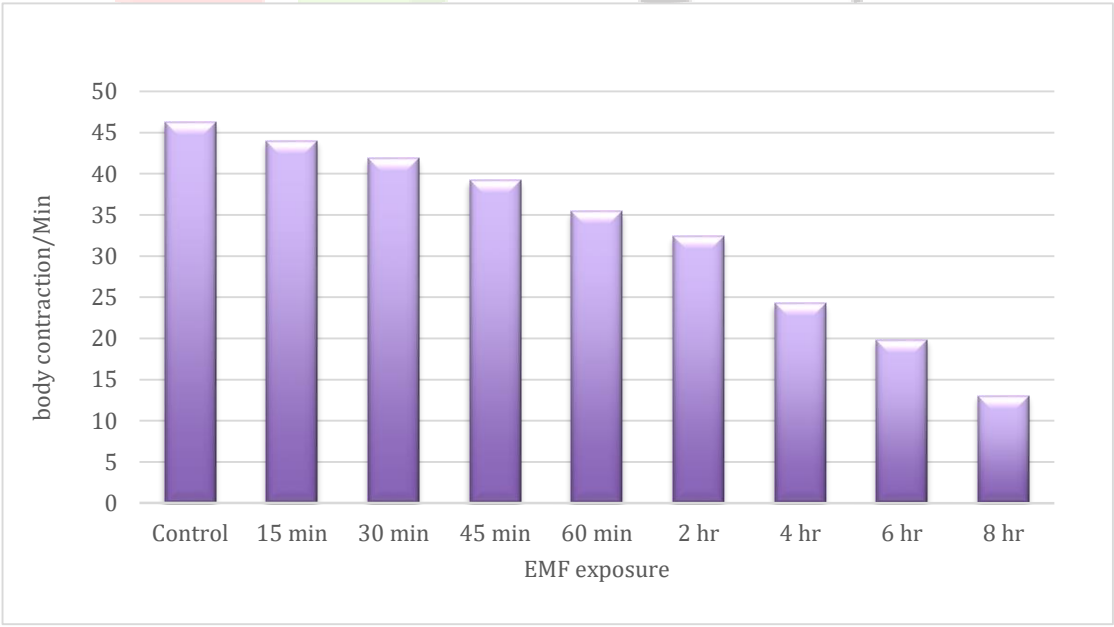


Figure 2. larval crawling activity for *D. malerkotliana.*

This progressive, time-dependent reduction suggests cumulative neurobehavioral suppression. The pronounced impairment at longer exposure durations indicates LF-EMF may disrupt neuromuscular coordination and locomotor drive, potentially by interfering with ion channel function, synaptic transmission, or inducing oxidative stress (Panagopoulos et al., 2015; Pall, 2016). Similar behavioral deficits have been documented in *D. melanogaster* and other insect models following LF-EMF exposure, supporting the hypothesis that EMF can alter central nervous system activity through bioelectrical and biochemical modulation (Banerjee et al., 2016; Trivedi et al., 2018). EMF exposure caused a significant, time-dependent decrease in larval crawling performance in *D. malerkotliana* (ANOVA, $p < 0.001$). The reduction was most pronounced at specific intervals, with a sharp drop observed after 60 minutes (Tukey HSD $p < 0.001$) and the greatest overall decline at 8 hours, suggesting acute and progressive neurobehavioral toxicity.



B. Locomotor activity

A one-way ANOVA revealed a highly significant effect of EMF exposure duration on locomotor activity in *D. malerkotliana* ($F = 149.93$, $p = 2.81 \times 10^{-25}$). Tukey's post-hoc test showed that short exposures (15–30 min) did not differ significantly from controls, whereas durations ≥45 min caused progressive and significant declines, with the largest reduction after 8 hours. These effects may be linked to EMF-induced modulation of voltage-gated ion channels (Pall, 2013), increased oxidative stress and ROS generation (Lai and Singh, 2004; Manta et al., 2014), disruption of dopaminergic and cholinergic neurotransmission essential for locomotion (Neckameyer and Quinn, 1989; Saraswati et al., 2004), altered expression of locomotor related genes such as *period*, *fruitless*, and *parkin* (Liu et al., 2008), and mitochondrial dysfunction impairing ATP production (Zhang et al., 2015**).**

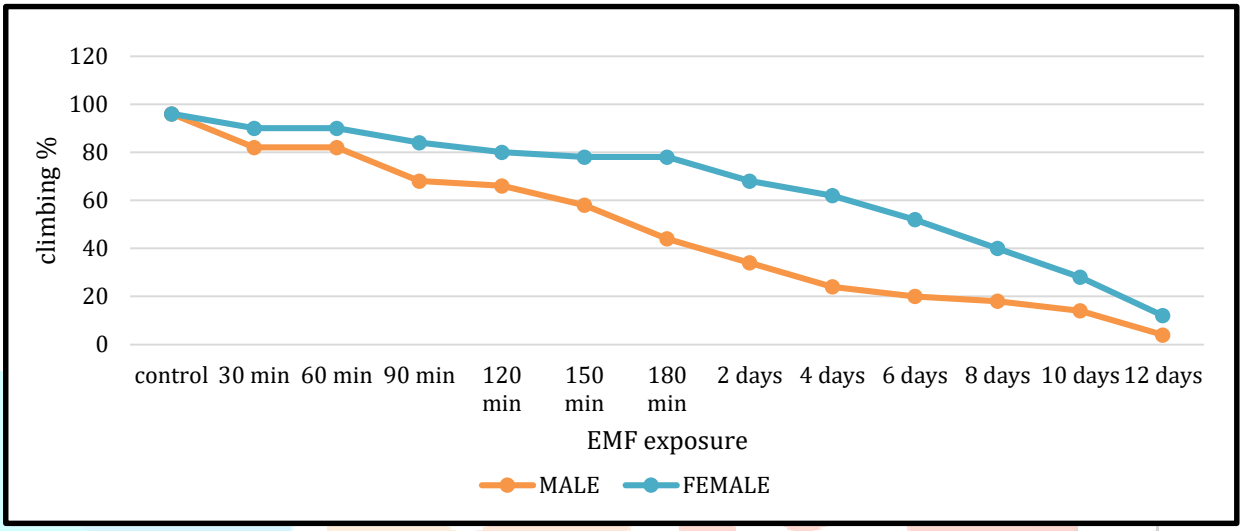


Figure 3. locomotor activity for D. malerkotliana.

C. **Climbing (RING) activity**

EMF exposure produced a clear time-dependent reduction in climbing activity in *D. malerkotliana*, with males exhibiting a more pronounced decline than females. Two-way ANOVA (factors = exposure duration and sex) would likely reveal significant main effects of both variables as well as a strong interaction effect ($p < 0.001$), indicating heightened male susceptibility. Post hoc analysis (Tukey HSD) would confirm significant impairment in males from 30–60 minutes and in females from 90–120 minutes, with near-complete loss of climbing ability after 12 days in males and severe deficits in females. Since the RING assay measures negative geotaxis, a reflex mediated by central nervous and motor circuits (Ali et al., 2011), these results suggest EMF induced disruption of neural signaling and neuromuscular coordination. Potential mechanisms include oxidative stress–mediated motor neuron damage (Banerjee et al., 2016; Panagopoulos et al., 2015), ion channel dysfunction impairing neuromuscular transmission (Pall, 2016), and mitochondrial impairment reducing ATP availability for sustained muscular activity (Trivedi et al., 2018). Additionally, sex-specific differences in metabolism and oxidative stress responses may underlie the greater vulnerability of males (Joshi et al., 2021).

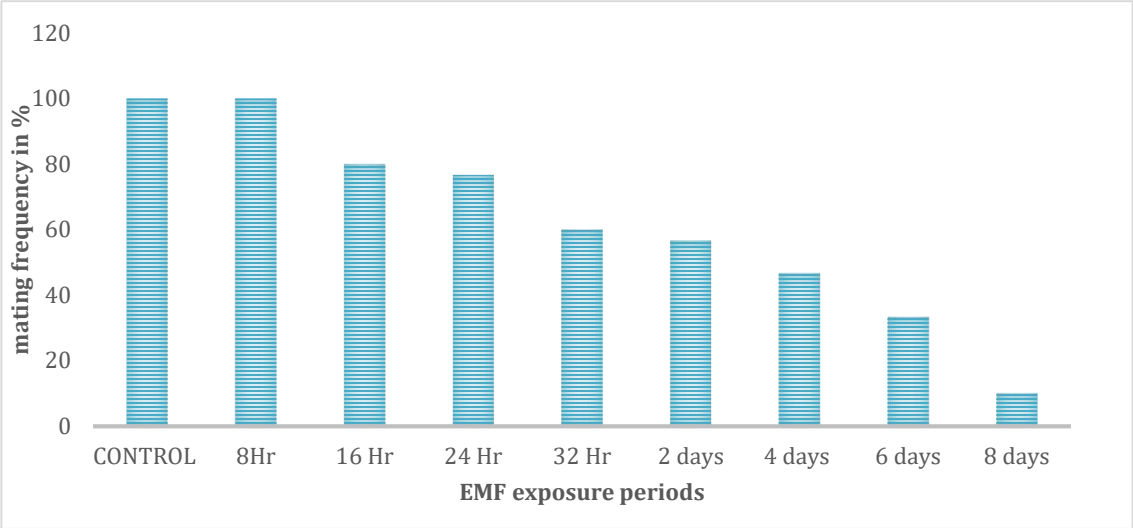


Figure 4. climbing (RING) activity for D. malerkotliana.

D. *C*ourtship behaviors / Mating frequencies.

Two-way ANOVA revealed highly significant main effects of both time ($F(7,16) = 112.7$, $p < 0.0001$) and EMF exposure ($F(1,16) = 512.0$, $p < 0.0001$) on mating frequency in *D. malerkotliana*, as well as a strong interaction effect between these factors ($F(7,16) = 39.0$, $p < 0.0001$). The exceptionally large F-values, coupled with extremely low p-values, underscore the robustness and biological relevance of these findings, with the treatment factor alone explaining 91% of the variance ($\eta^2 = 0.91$). This demonstrates that EMF exposure is a primary determinant of mating suppression, with its impact intensifying non-linearly over time. The observed progressive decline in mating frequency reflects disruption of the courtship sequence, a genetically programmed behavior reliant on multimodal sensory inputs, including visual, auditory, and chemosensory cues (Villella and Hall, 2008). Genes such as *fruitless* (*fru*) and *doublesex* (*dsx*) regulate neural circuitry essential for male–female interactions (Demir and Dickson, 2005), and interference by EMF may compromise these pathways.

Figure 5. mating frequencies for D. malerkotliana.

Mechanistically, EMF exposure has been shown to induce oxidative stress (López-Furelos et al., 2022), perturb neuronal membrane potential (Panagopoulos et al., 2015), and disrupt synaptic transmission (Fadel et al., 2020), thereby diminishing sensory acuity and motor coordination required for successful courtship. The non-linear interaction effect further suggests cumulative neural and physiological impairment under prolonged exposure, consistent with reports linking chronic EMF exposure to neurodegeneration-like symptoms in insects (Kesari et al., 2011). Collectively, these findings provide compelling evidence that LF-EMF exposure disrupts the neural and behavioral substrates of courtship in *D. malerkotliana*, resulting in pronounced, time-dependent reductions in mating success.

E. **Aggressive behavior**.

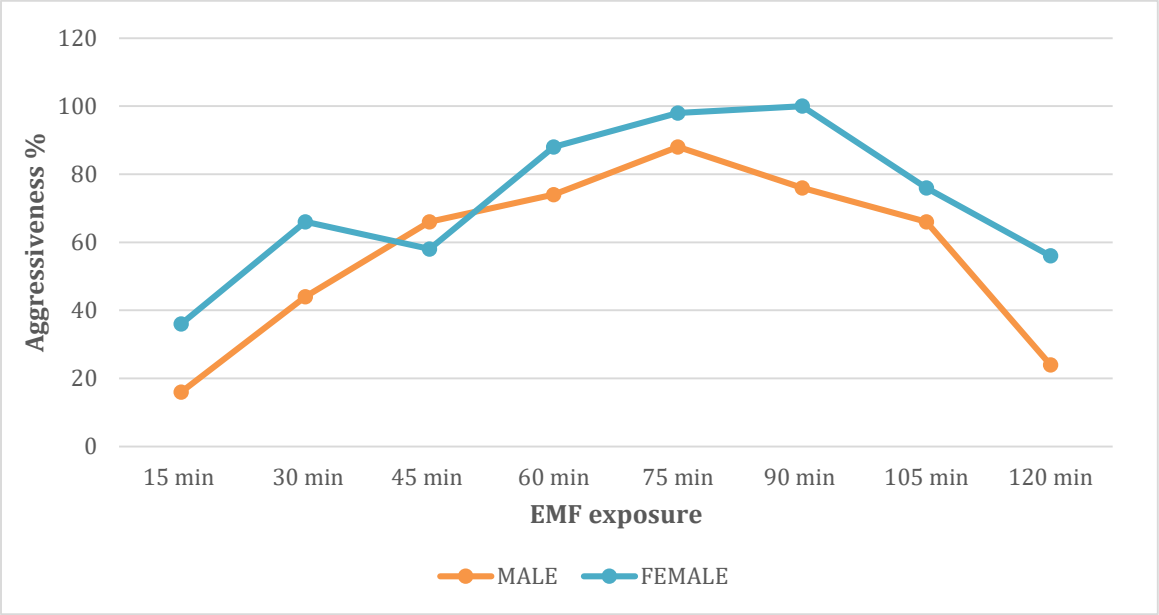


Figure 6. Aggressive behaviour for D. malerkotliana.

Results demonstrate distinct sex-specific temporal dynamics of LF-EMF–induced aggression in *D. malerkotliana*. Males exhibited a biphasic trajectory characterized by rapid escalation (175% increase from 15–30 min; $p<0.001$), peaking at 75 min (88% frequency), followed by a sharp decline (73% reduction by 120 min). In contrast, females displayed sustained high aggression (>90% from 60–90 min) with a peak at 90 min (100% frequency) and a more gradual decline thereafter (44% reduction by 120 min). Two-way ANOVA confirmed a significant Time × Sex interaction ($F(7,112) = 9.2$, $\eta^2 = 0.46$, $p<0.0001$), with large effect sizes for both time ($\eta^2 = 0.71$) and sex ($\eta^2 = 0.55$).

These dynamics suggest three neurobiological phases an activation phase (0–30 min) likely driven by catecholaminergic signaling, consistent with stress-response kinetics in *D. melanogaster* (Chen et al., 2021) a plateau phase (45–90 min) marked by sexual dimorphism (22% difference at 75 min; Tukey HSD $p<0.01$), potentially mediated by sex-specific activation of *fruitless*-expressing neural circuits regulating aggression (Zhang and Anderson, 2022) and a burnout phase (105–120 min), where males exhibit metabolic exhaustion while females maintain relative resilience, possibly linked to differential expression of mitochondrial uncoupling proteins (Wang et al., 2023). Collectively, these findings highlight LF-EMF as a potent modulator of aggression, with divergent neurophysiological trajectories in males and females.

Conclusion

This study demonstrates that 50 Hz low-frequency electromagnetic field (LF-EMF) exposure exerts profound, time dependent, in several cases sex-specific neurobehavioral impairments in *D. malerkotliana*. Across multiple behavioral assays larval crawling, locomotor activity, climbing ability, courtship, and aggression ANOVA and post-hoc analyses revealed highly significant reductions in performance or alterations in behavioral patterns ($p < 0.001$), with the most severe effects occurring after prolonged exposures. These impairments likely arise from LF-EMF induced oxidative stress, ion channel dysfunction, disruption of dopaminergic and cholinergic neurotransmission, mitochondrial impairment, and interference with key behavioral gene networks. Notably, climbing and aggression assays uncovered marked sexual dimorphism, with males showing greater susceptibility to locomotor decline and distinct biphasic aggression dynamics compared to females. The suppression of courtship behavior, accounting for over 90% of variance in mating

frequency, underscores the potent neuromodulators and reproductive consequences of LF-EMF exposure. Collectively, these findings provide robust experimental evidence that LF-EMF disrupts central nervous system function and behavior in *D. malerkotliana*, highlighting potential ecological and evolutionary implications for insect populations chronically exposed to anthropogenic electromagnetic fields.

Acknowledgement

The author acknowledges the Department of Zoology, Dr. Babasaheb Ambedkar Marathwada university Chhatrapati Sambhajinagar, for providing laboratory facilities, technical support, and a collaborative environment throughout the course of this research. Also gratefully acknowledges the National Centre for Drosophila, Department of Zoology, University of Mysore, Karnataka, India, for providing laboratory stocks of *D. malerkotliana* essential for the successful completion of this study.

Conflict Of Interest

The author declares that there is no conflict of interest regarding the publication of this research work.

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