Combined effects of GSM 1800MHz smartphone radiation and antioxidant-rich diets on *Drosophila melanogaster* survival and reproduction

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Abstract. Electromagnetic fields (EMF) from devices like smartphones can negatively affect health through both thermal and non-thermal mechanisms. Thermal effects involve temperature changes that disrupt metabolism, while non-thermal effects can impair the nervous, hormonal, reproductive, and cardiovascular systems and potentially increase cancer risk. EMF radiation also promotes the formation of reactive oxygen species (ROS), causing oxidative stress and damage to RNA, DNA, and proteins. Antioxidants, such as vitamin C found in kiwi and dragon fruit, may counteract these effects. This study examines the impact of 4G smartphone EMF exposure (GSM 1800MHz) and diets enriched with kiwi or dragon fruit on the survival and reproductive morphometrics of fruit flies (Drosophila melanogaster). Fruit flies were exposed to EMF radiation for 2 hours per day at a distance of 3 cm for three days. The results showed that EMF exposure decreased development and survival rates in fruit flies raised on a standard banana medium. However, flies on nutrient-enriched mediums with kiwi or dragon fruit were more resilient to EMF effects. There were no significant differences in reproductive organ morphometry between diet groups. These results suggest that antioxidant-rich diets could potentially mitigate the adverse effects of EMF radiation.

1 Introduction

Electromagnetic waves, which carry both electric and magnetic charges, span a wide spectrum, including radio waves, microwaves, visible light, and X-rays, with diverse applications from communication to medical imaging [1, 2]. These waves can originate from natural sources like sunlight or from electronic devices such as smartphones, which are a significant source of electromagnetic radiation (EMF) in daily life [3]. As smartphone technology evolves, the level of EMF exposure increases, raising concerns about potential health effects. EMF exposure can have both thermal effects, such as increased tissue temperatures that disrupt metabolic functions, and non-thermal effects, including impacts on the nervous, hormonal, reproductive, and cardiovascular systems, as well as a heightened risk of cancer [4, 5].

EMF can also induce the formation of reactive oxygen species (ROS), leading to

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oxidative stress, which damages cellular components like RNA, DNA, and proteins, and contributes to diseases such as cancer and neurodegenerative disorders [6, 7]. The body's defense mechanisms against oxidative stress include antioxidants, which neutralize ROS and prevent cellular damage [8, 9]. Antioxidants like vitamins C and E, found in fruits such as kiwi (Actinidia deliciosa) and red-fleshed dragon fruit (Hylocereus polyrhizus), help protect cells by stabilizing free radicals and reducing oxidative stress [10, 11].

Drosophila melanogaster (fruit flies) serve as a model organism in studying the biological effects of EMF due to their rapid reproduction, easy maintenance, and shared genetic pathways with humans [12, 13]. Previous studies have shown that EMF exposure reduces survival rates and damages reproductive organs in fruit flies, but antioxidant-rich diets may mitigate these effects [14, 15].

Despite the known antioxidant properties of kiwi and dragon fruit, their potential to protect against EMF-induced damage remains unclear. This study investigates whether diets enriched with these fruits can improve survival rates and reduce reproductive organ damage in Drosophila melanogaster exposed to smartphone EMF radiation. By exploring these effects, this research aims to provide insights into the protective potential of these antioxidant-rich fruits against electromagnetic exposure.

2 Materials and Methods

2.1 Fruit fly trapping

Fruit flies for this study were collected using traps placed around the Faculty of Biology at UGM. Traps were constructed by cutting bananas into segments and placing them in containers. These traps were positioned in bright areas shaded from direct sunlight. They were left in place for several days to attract fruit flies, with monitoring conducted each morning. Once a sufficient number of fruit flies were trapped, they were captured and collected for culturing purposes.

2.2 Preparation of fruit fly culture media

The culture media used in this study included standard medium and nutrient-enriched medium with dragon fruit or kiwi. The standard medium was prepared by mixing banana, agar, palm sugar, yeast, distilled water, and cassava tape. For the nutrient-enriched medium, 15 grams of dragon fruit or kiwi was added to the previously prepared standard medium. Each type of medium was prepared in 5 bottles for replication. Bottles containing the banana standard medium were labeled BCON (control) and BEMF (EMF treatment). The kiwi-enriched medium was labeled KCON (control) and KEMF (EMF treatment), while the dragon fruit-enriched medium was labeled DCON (control) and DEMF (EMF treatment).

2.3 Rearing fruit fly adults

Adult fruit flies were reared on a control medium for this study. Upon observation of pupation, they were transferred to a fresh control medium to prevent mixing with virgin adults. This rearing process continued for approximately 14 days until the emergence of F1 generation adults. The rearing was conducted continuously to maintain a stock culture for ongoing experiments.

2.4 Isolation of virgin male and female fruit flies

Virgin male and female fruit flies were isolated by collecting imago from the parental culture aged no more than 24 hours. Imago were sexed using a stereo microscope following the method of [16] and then transferred based on their sex into a control medium. The collection of virgin imago was done simultaneously for each treatment group to obtain individuals of the same age. The following day, two pairs of virgin imago were placed into each treatment.

2.5 Preparation of experimental sets and smartphone EMF irradiation

Experimental sets were constructed using aluminum frames covered with anti-UV plastic to form cube-shaped covers with one side open. These covers were used to maintain environmental stability around the fruit fly cultures during rearing and treatments and to prevent environmental radiation other than from the smartphone (Fig. 1). The average temperature inside the experimental covers was 29.4°C, and humidity levels averaged 73.2%.

Fig. 1. Experimental sets for fruit flies culture

Fruit fly cultures in the EMF treatments (BEMF, KEMF, and DEMF) were arranged in a circular layout inside the experimental covers. EMF irradiation treatments were conducted using a 4G LTE (B3) smartphone operating at a frequency of 1800MHz. The smartphone was placed at the center of the culture bottle arrangement, maintaining a distance of 3 cm from each bottle. Conversely, control fruit fly cultures (BCON, KCON, and DCON) were placed outside the experimental covers. EMF irradiation treatments were conducted from 09:00 to 11:00 AM for three days. After the third day, the irradiation was stopped, and the growth of fruit flies was observed. The number of larval, pupal, and adult individuals was counted and recorded each afternoon until day 14.

2.6 Isolation of fruit fly reproductive organs

Reproductive organs of fruit flies from each treatment were isolated following the method of [16]. The isolation process began with collecting male and female fruit flies from each bottle in every treatment. Fruit flies were placed into tubes. Anesthesia of the fruit flies was performed by placing the tubes in a container filled with ice. The number of male and female individuals from each bottle was counted and recorded. Fruit flies were then treated with 70% alcohol, transferred onto glass slides, and positioned with their abdomen facing upwards to facilitate the surgical process. Wings of the fruit flies were gently lifted and secured by inserting a needle through the sixth and seventh abdominal segments. The needle was gently pressed against the terminal abdomen and slowly pulled towards the posterior direction. Testes and ovaries were isolated from other organs and surgical debris. Both organs were observed using a stereo microscope connected to OptiLab Viewer 2.2. Morphometric measurements were conducted for each treatment using Digimizer 6.4.3.

2.7 Data analysis

The survival rate of F1 generation fruit flies from each treatment was analyzed as follows [17] with modifications.

$$Survival\ Rate = \frac{Number\ of\ live\ adults}{Number\ of\ live\ individuals + Number\ of\ dead\ individuals} \times 100\% \qquad (1)$$

For statistical analysis, Two-way ANOVA was employed to evaluate the effects of EMF exposure and diet on survival rates and reproductive morphometrics. Tukey HSD post-hoc tests were used to identify significant differences between treatment groups. It is important to note that normality and homogeneity of variance were not explicitly tested in this study, and no data transformations were applied prior to analysis. All statistical analyses were conducted using RStudio 2022.12.0, with a significance level set at 5%.

3 Results and discussion

3.1 Shift in Growth Rate of EMF-Irradiated Fruit Flies

The growth rate of fruit flies, characterized by rapid development over approximately 14 days, involves distinct stages from egg to larva, pupa, and imago. This study meticulously tracked these stages through daily counts, using direct observation throughout the experimental period. Controlled environmental conditions were crucial, with an average temperature of 29.4°C and humidity levels averaging 73.2%. These conditions optimized fruit fly development and ensured consistency in experimental outcomes. They also provided insights into how environmental stressors, such as electromagnetic fields (EMF), might influence developmental trajectories and physiological responses.

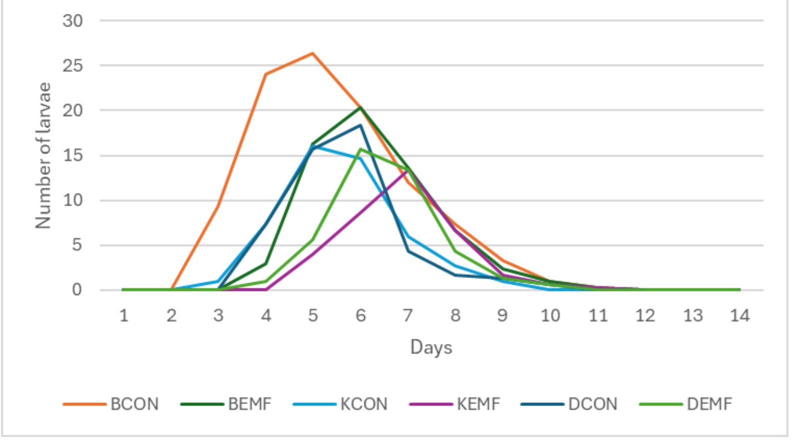

Fig. 2. Larval growth rate of F1 generation fruit flies

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The growth of fruit fly larvae generally follows a growth curve similar across all treatments. The highest number of individuals was observed in the BCON. Significant increases in individuals occurred between days 3 and 5. However, larvae in the EMF treatments showed delayed growth, emerging as fruit fly larvae only on day 4. This indicates that EMF exposure had a negative impact on the parental generation during the initial phase, potentially inducing stress and affecting their ability to mate and lay eggs. Previous studies have also shown that low-frequency electromagnetic radiation can influence the circadian rhythm and behavioral abnormalities of *Drosophila melanogaster* through the involvement of the light-sensitive photoreceptor, cryptochrome CRY [18,19]. The addition of nutrients, whether from kiwi or dragon fruit, also led to a significant decrease in the number of individuals, indicating that the parental generation might have experienced some initial dietary shock. However, this nutritional adjustment did not result in a significant decline in the number of larvae by the end of the phase, suggesting that while there was an initial adaptation period, the larvae ultimately maintained their numbers throughout the study.

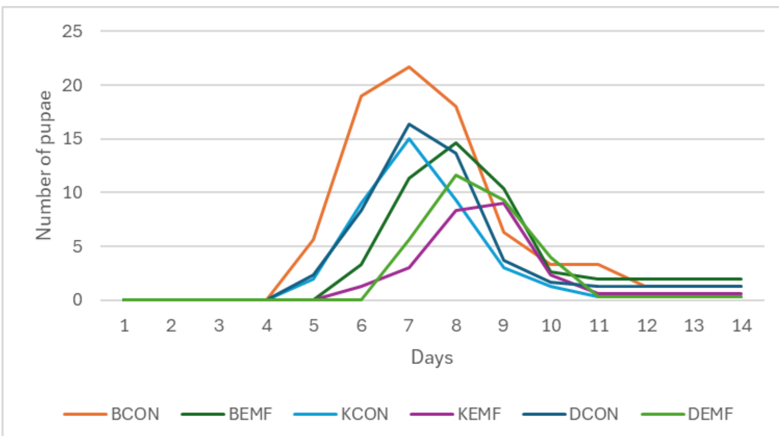

Fig. 3. Pupal growth rate of F1 generation fruit flies

Alongside larval growth, the highest number of pupae was also observed in the BCON treatment. The growth curve during the pupal phase followed a similar trend, with an increase in pupal numbers occurring from day 5 to day 8. During this period, there was a decline in the total number of individuals as some larvae did not survive to reach the pupal stage. Pupae in the control also appeared earlier compared to the EMF treatments, consistent with the delayed larval growth observed due to EMF exposure.

The pupal stage is crucial in the fruit fly life cycle, marked by a complex metamorphosis where internal organs undergo significant development to prepare for adulthood. Initially, fruit fly pupae are pale yellowish-white, gradually darkening as they undergo metamorphosis into adults. Throughout the observation period, no discernible effect of the EMF irradiation conducted during the first 3 days was observed on the fruit fly metamorphosis process.

In cultures with added nutrients, the number of pupae was lower compared to the banana control medium. However, the decrease in pupal numbers was more stabilized throughout the pupal phase in nutrient-enriched mediums compared to the control and EMF treatments for each type of diet. This indicates that the addition of nutrients helps maintain a more resilient pupal stage against EMF exposure. Despite the initial impact, the enhanced nutritional content appears to mitigate some of the adverse effects of EMF, leading to a more stable pupal development.

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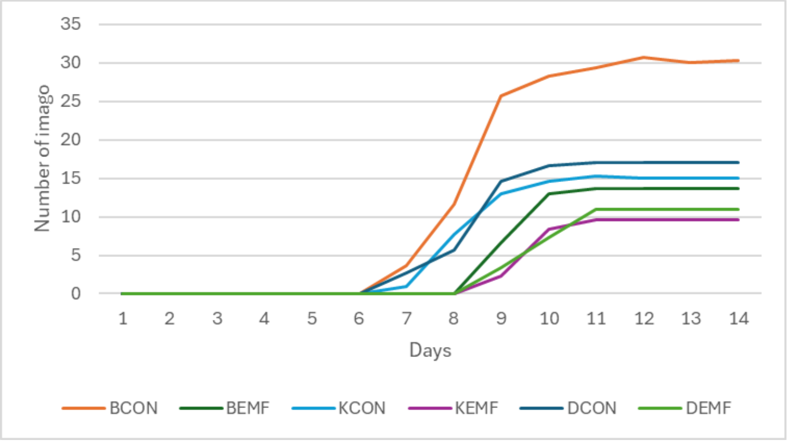

Fig. 4. Imago growth rate of F1 generation fruit flies

In the imago stage, the development of fruit flies shows varying dynamics depending on the treatment received during the preceding phases. The BCON treatment, which was not exposed to smartphone electromagnetic fields (EMF), consistently exhibited the highest number of imago individuals compared to other treatments. In BCON, DCON, and KCON, the emergence of imago individuals significantly increased from day 8 to day 10, indicating that an EMF-free environment allows optimal development from egg to imago stages. The growth curve of imago in these treatments peaked around days 11-12 and remained stable until the last day.

Conversely, in BEMF, DEMF, and KEMF treatments, the emergence of imago individuals was slower and lower compared to BCON. Significant increases in imago numbers in both EMF treatments occurred only from day 10 to day 11. This delay is attributed to the negative impact of early EMF exposure, which likely disrupted mating and egg-laying processes. As a result, the life cycle of fruit flies in these treatments experienced delayed timing and produced fewer imago individuals.

Overall, the data indicate that EMF exposure adversely affects fruit fly development. Although individuals from EMF treatments still completed their life cycles, it took longer, and fewer individuals successfully reached the imago stage. This suggests that EMF can act as an environmental stressor for fruit flies, potentially causing thermal stress that accelerates mortality and may disrupt circadian rhythms and locomotor activity, as indicated by previous studies [20]. But it should be underlined that the EMF source and intensity used in the study might not fully represent real-world exposure scenarios, as the intensity can vary depending on the type of source and distance from the radiation source, potentially affecting the generalizability of the results.

3.2 Decrease in Survival Rate of EMF-Irradiated Fruit Flies

Survival rate is a measure of an individual's ability to survive over a specific period. The survival rate of fruit flies was measured by comparing the number of living imago to the total number of individuals, both living and dead. In this study, the survival rate was expressed asthe percentage of imago that survived until the 14th day of observation.

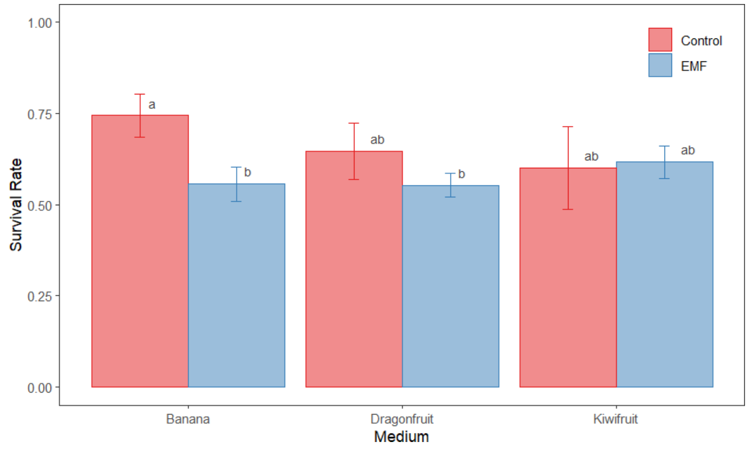

Fig. 5. Survival rate of F1 generation fruit flies

The results of the Two-way ANOVA analysis with Tukey HSD post-hoc test indicate that EMF irradiation on fruit fly cultures in banana medium significantly reduced survival rates ($p < 0.05$). Conversely, no significant difference in fruit fly survival between control and EMF-treated groups was observed in the kiwi dan dragon fruit medium. This suggests that nutrient-enriched medium with dragon fruit or kiwi possesses mechanisms that effectively maintain fruit fly vitality against EMF irradiation.

The difference in fruit fly survival response to EMF irradiation between standard and nutrient-enriched is closely related to the physiological characteristics and nutritional profiles of these fruits. Kiwi and dragon fruit is known for its richness in vitamin C, vitamin E, phenolics, flavonoids, and other phytochemicals with high antioxidant activity. Some antioxidants known to mitigate the negative effects of EMF include vitamin E, melatonin, and ferulic acid [21]. On the other hand, bananas have relatively lower levels of phenolic compounds and antioxidant capacity compared to these fruit, which may account for the significant difference in response to EMF irradiation [22].

3.3 Morphological and Morphometric Comparison of EMF-Irradiated Fruit Fly Reproductive Organs

Studies on the morphology and morphometry of fruit fly reproductive organs are highly relevant for understanding biological responses to environmental stressors. The reproductive organs of fruit flies not only reflect their reproductive processes but also serve as indicators of sensitivity to environmental changes.

Previous research has indicated that exposure to EMF radiation can affect the reproductive ability of male fruit flies and reduce the fecundity of females [23,24]. Additionally, studies have shown that EMF exposure in fruit flies can reduce the size of their reproductive organs, indicating that EMF irradiation not only affects reproductive function directly but also impacts the morphological and morphometric parameters of reproductive organs [25, 15]. In this study, the most representative morphology of reproductive organs was selected based on comprehensive observations from each treatment group. Representative morphology refers to having a morphology that is similar in shape and size to the average of all samples observed. Observations were focused solely on the main reproductive organs, namely the ovaries in female fruit flies and the testes in male fruit flies.

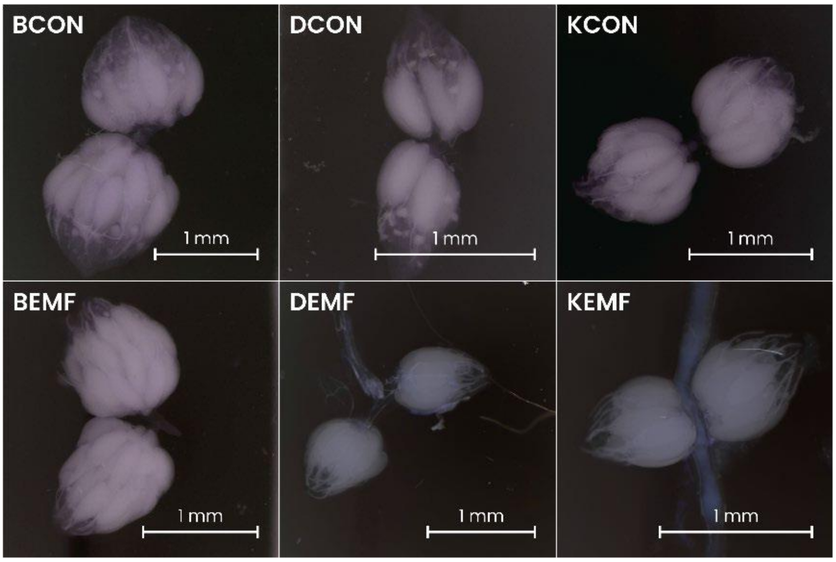

Fig. 6. Ovarian morphology of F1 generation fruit flies

Based on observations of the morphology of the ovaries in female fruit flies across each treatment, no significant differences or degeneration were found. However, ovaries in treatments with the banana diet, both BCON and BEMF, showed more mature development compared to those with the kiwi diet and dragon fruit diet, including KCON, KEMF, DCON, and DEMF. This was evidenced by the presence of mature ovaries in fruit flies with banana medium that had reached advanced developmental stages and were ready for fertilization. Fruit flies on the banana diet reached the imago stage faster than those on the kiwi diet and dragon fruit diet. This acceleration facilitated earlier maturation of ovaries, indicating that the banana diet supports more optimal development of reproductive organs compared to the kiwi diet and dragon fruit diet.

In contrast, the effects of EMF exposure on ovarian morphology were less pronounced. The lack of significant degeneration suggests that EMF exposure, while affecting overall development, does not directly impact the reproductive organ morphology to the same extent as dietary factors. This differentiation underscores that the banana diet's influence on reproductive development is more pronounced, while the impact of EMF is more subtle.

Table 1. Ovarian morphometry of F1 generation fruit flies

Parameter (mm^2)	**Treatment**					
	BCON	**BEMF**	**DCON**	**DEMF**	**KCON**	**KEMF**
Right ovary area*	$0,95 \pm 0,18^a$	$0,73 \pm 0,12^{ab}$	$0,66 \pm 0,24^{ab}$	$0,57 \pm 0,11^{ab}$	$0,53 \pm 0,18^{ab}$	$0,36 \pm 0,05^b$
Left ovaryarea*	$0,85 \pm 0,12^a$	$0,73 \pm 0,18^a$	$0,66 \pm 0,36^a$	$0,64 \pm 0,13^a$	$0,43 \pm 0,04^a$	$0,39 \pm 0,04^a$

Note: *) Different letters in each row indicate a significant difference at the 0.05 significance level (p < 0.05).

The reproductive morphometry of female fruit flies focused on the area of both the right and left ovaries. The results indicate a significant influence of diet type on ovarian size in fruit flies. Ovarian size was larger in the BCON and BEMF treatments compared to KCON, KEMF, DCON, and DEMF. This suggests that the banana diet, as provided in the BCON and BEMF treatments, is more optimal in supporting ovarian development. Nutritious diets that align well with the physiological needs of fruit flies can enhance ovarian size, directly impacting female reproductive potential.

These findings underscore that ovarian morphology and morphometry can vary depending on the nutritional content available in the diet, reflecting phenotypic plasticity— the ability of a single genotype to produce different phenotypes in different environments. Research has shown that diet composition variations can lead to significant changes in ovarian size and the number of developing oocytes [26]. Research also noted that diets high in protein significantly increase oocyte production compared to low-protein diets. In the context of this study, the banana diet appears to provide nutrients more supportive of ovarian development compared to the kiwi diet and dragon fruit diet.

Overall, while there were no significant morphological differences or degeneration observed due to EMF radiation, it is evident that the banana diet supports faster maturation of ovaries, as evidenced by their larger size compared to the other diet.

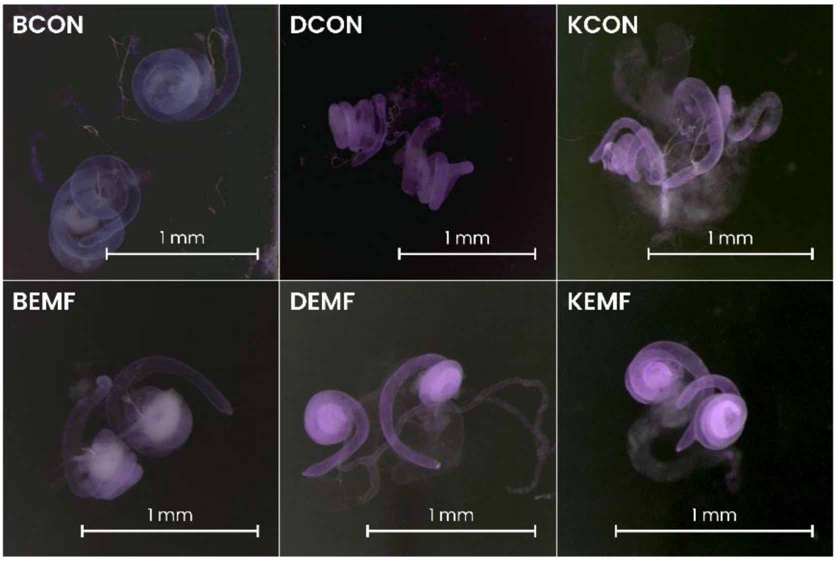

Fig. 7. Ovarian morphology of F1 generation fruit flies

The morphology of male fruit fly testes observed across all treatments did not exhibit significant differences. The testes in each treatment displayed normal conditions without signs of degeneration or meaningful morphological changes. The testis structure consisted of organized and coiled seminiferous tubules, clearly distinguishable and exhibiting typical morphology.

However, while there were no overt differences in testis morphology across the different treatments, it is important to note that the impact of diet and EMF exposure on reproductive organ development may not always be immediately apparent in testicular morphology alone. The absence of significant changes in testis structure suggests that while EMF exposure may not cause visible alterations in testis morphology, the dietary influences could still affect overall reproductive health in more subtle ways.

Table 2. Testes morphometry of F1 generation fruit flies

Parameter (mm)	Treatment					
	BCON	**BEMF**	**DCON**	**DEMF**	**KCON**	**KEMF**
Right testis length*	3,10 ± 0,35[a]	2,49 ± 0,19[a]	3,12 ± 0,51[a]	2,67 ± 0,42[a]	2,38 ± 0,55[a]	2,48 ± 0,55[a]
Left testis length*	3,10 ± 0,57[a]	2,46 ± 0,15[a]	3,24 ± 0,33[a]	3,00 ± 0,63[a]	2,31 ± 0,40[a]	2,48 ± 0,47[a]

Note: *) Different letters in each row indicate a significant difference at the 0.05 significance level ($p < 0.05$).

The reproductive morphometry of male fruit flies was analyzed by measuring the length of the right and left testes. The analysis revealed that there were no significant differences in testes size among all treatments. The measurements indicated that the length of the testes was generally uniform across all treatments. This suggests that neither the type of diet nor exposure to smartphone electromagnetic fields (EMF) had a significant impact on the development of male fruit fly testes.

The testes of fruit flies in the control treatment (BCON) exhibited sizes within the normal range and did not differ significantly from other treatments. Additionally, although there was a slight indication of shorter testis length in flies exposed to EMF, this difference was not significant enough to conclude that EMF directly influences testis development. This indicates that male fruit flies may have adaptation mechanisms or better tolerance to EMF.

Other studies have noted that high levels of reactive oxygen species (ROS) reduce the number of germline stem cells (GSCs), while low ROS levels due to antioxidant activity lead to excessive growth of GSC-like cells [27]. GSCs are the precursor cells for testis development during the embryonic phase. The absence of significant differences between control and EMF treatments in each medium indicates that both banana, kiwi, and dragon fruit can maintain redox homeostasis, allowing normal testis development to proceed.

3.4 Changes in the Sex Ratio of Fruit Flies with Different Diets

Changes in the male-to-female sex ratio within a fruit fly population can reflect adaptive responses to specific environmental factors, such as variations in food availability. When the food source changes, the fruit fly population may adjust the proportion of males and females to enhance reproductive success and survival chances.

Table 3. Sex ratio of F1 generation fruit flies

Parameter	Treatment					
	BCON	**BEMF**	**DCON**	**DEMF**	**KCON**	**KEMF**
Femaleratio*	0,62 ± 0,05[a]	0,63 ± 0,06[a]	0,48 ± 0,05[a]	0,51 ± 0,18[a]	0,51 ± 0,17[a]	0,42 ± 0,09[a]
Maleratio*	0,38 ± 0,05[a]	0,37 ± 0,06[a]	0,52 ± 0,05[a]	0,49 ± 0,18[a]	0,49 ± 0,17[a]	0,58 ± 0,09[a]

Note: *) Different letters in each row indicate a significant difference at the 0.05 significance level ($p < 0.05$).

In treatments with a banana diet (BCON and BEMF), it was found that the fruit fly generations produced had a higher female ratio compared to males. The banana diet appears to facilitate better development and survival of female fruit flies.

Meanwhile, in treatments with the kiwi diet and dragon fruit diet, male-to-female ratios in the F1 generation tended to approach 1:1. This indicates that both diets did not significantly influence the sex ratio of fruit flies. Kiwi and dragon fruit provide a more stable environment regarding their effect on the sex ratio, possibly due to their nutritional content being sufficient to support the development of both sexes without specific bias.

Although there was variation in sex ratios between banana and other diet treatments, these differences were not statistically significant. This means that despite certain trends, the variability in the data was not large enough to show consistent and meaningful differences. Previous research also noted that the sex ratio of fruit flies tends to remain stable under various conditions, such as high temperatures, darkness, or exposure to EMF [28].

4 Conclusion

The GSM 1800MHz smartphone EMF radiation significantly impacts fruit fly development, affecting mating and egg-laying processes and reducing survival rates. However, no significant differences were observed in the morphology and morphometry of fruit fly reproductive organs due to EMF exposure. Notably, the kiwi and dragon fruit diets provided a more positive impact compared to EMF radiation, particularly with regard to the development of the ovaries. Supplementing with kiwi and dragon fruit helps maintain fruit fly survival rates under EMF radiation, comparable to those observed in control conditions. Both diets also preserve the morphology and morphometry of reproductive organs under EMF exposure similarly to control conditions. Nevertheless, the size of the ovaries on kiwi and dragon fruit mediums is smaller than that on the banana medium, likely due to differences in growth and development rates.

The findings highlight the protective role of antioxidant-rich diets, such as those including kiwi and dragon fruit, against the detrimental effects of EMF exposure. These diets appear to counteract the adverse impacts of EMF on fruit fly survival and reproductive health. Therefore, incorporating antioxidant-rich foods can be an effective strategy for mitigating the effects of environmental stressors like EMF radiation on biological organisms.

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