International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

The Effect of Chia seed on The Heat resistance in *Drosophila melanogaster*

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ABSTRACT :

The quality and quantity of nutrients consumed play a critical role in determining an organism's stress resistance, life history traits, and reproductive success. To survive and reproduce under fluctuating environmental conditions, animals must maintain a balance between energy intake and expenditure. Nutritional availability and composition are central to an organism's capacity to adjust physiologically, behaviourally, or developmentally in response to environmental challenges such as temperature stress. In this study, we investigated the effects of chia seed supplementation at varying levels (control, 5g, 10g, 15g) on heat resistance in *Drosophila melanogaster*, focusing on differences related to sex and mating status.

Chia seeds are nutritionally dense, containing high levels of omega-3 fatty acids (especially alpha-linolenic acid), dietary fiber, protein, antioxidants, and essential minerals such as calcium, magnesium, and zinc. Despite their nutritional value, our results revealed that increased chia supplementation was associated with decreased heat resistance across all groups. Flies on the control diet (wheat cream agar) showed the highest thermal tolerance. Virgin males exhibited greater heat resistance than virgin females, particularly at lower chia concentrations. Mated individuals of both sexes showed a further decline in heat resistance, with females responding more sensitively to both diet and mating status. While males maintained relatively stable heat resistance regardless of mating, female tolerance was more variable—likely influenced by sex-specific physiological or hormonal factors interacting with diet. The observed reduction in heat resistance with increased chia intake may be due to metabolic shifts caused by chia's high lipid and fiber content, which can affect energy allocation, water retention, and cellular stress responses. These findings suggest that although chia seeds are rich in essential nutrients, their composition may compromise thermal stress adaptation in *D. melanogaster*, especially in mated females.

Keywords: *Drosophila melanogaster*, chia seeds, heat resistance, sex differences, mating status, thermal stress, nutrition

Introduction

D. melanogaster is frequently used as a model organism in research on the physiological and evolutionary responses to various stressors (Hoffmann *et al*., 2003, Sinclair *et al*., 2007, Kristensen *et al*., 2008).[11,28,14] However, research usually underestimates the importance of diet in its design, and little is known about how nutrition impacts life history traits and performance under heat stress (Prasad *et al*., 2003)[24].

As a result, there is a growing need to examine how diet contributes to diversity in features that are essential for fitness. Numerous factors, such as nutrition, temperature, cage density, and social environment, have been shown to affect fly lifetime. Stress resistance traits, including as tolerance to heat, cold, hunger, and desiccation, are significantly influenced by temperature and gender. Given that it affects practically every rate-dependent function, from biochemical kinetics to life history features, temperature is arguably the most significant extrinsic factor in regard to ectotherms (Cossins and Bowler, 1987; Hochachkaand and Somero, 2002)[4,12].

Temperatures between 11°C and 32°Care home to the well-researched and cosmopolitan species D. melanogaster. One of the most pervasive effects of climate change is rising temperatures, which also have a wide range of effects on life cycle features (Rincon *et al.*, 2014)[27]. All living organisms depend on food and other nutritional sources for development, survival, and maintaining good health. In response to an abundance of food, the body alters its metabolism to store resources for periods of famine or other tasks that need energy expenditure (Krittika and Yadav, 2024)[15]. Nutrition plays a major role in an insect's ability to withstand environmental stress (Andersen *et al*., 2010; Djawdan *et a*l., 1997; Sisodia and Singh, 2012)[1,5,29]

D. melanogaster grown on a diet high in protein shows better heat and desiccation tolerance but less recovery from cold coma than adults raised on a diet high in carbs (Andersen *et* al., 2010)[1]. Perhaps the solution lies in the presence of the aminoacids tyrosine and phenylalanine, which are building components for the production of melanin and which together give insects their ability to harden their cuticles and tolerate dehydration. Lipids also indirectly improve heat resistance by reducing air loss from evaporation at high temperatures and regulating the deposition of cuticular wax (Yosef *et al*., 2022)[39].

Age, marital status, and sex are some of the interacting variables that affect how nutrition affects temperature tolerance. Even yet, metabolic and physiological investigations are helping to clarify the physiological and biochemical foundation of the temperature response (Overgaard *et al*., 2007, Doucet *et a*l., 2009, Colinet *et al.,* 2012, Kostál *et al.*, 2012, Storey., 2012, Teets and Denlinger, 2013)[23,6,4,13,34,35]. The way that nutrition influences temperature tolerance depends on a number of interrelated factors, including age, marital status, and sex. The physiological and biochemical foundations of the temperature response are becoming clearer thanks to metabolic and physiological research.

However, the effect of chia seeds on heat resistance has not been investigated yet, which led to the initiation of the current study.

Chia seeds are also rich in dietary fibre (18–30%), ashes (4–5%), proteins (15–25%), lipids (30–33%), and carbs (26–41%). Even though the oil contains a lot of polyunsaturated fatty acids (PUFAs), they also contain a lot of antioxidants, such as caffeic acid, myricetin, quercetin, and kaempferol, which serve to stabilise the oil. It has been demonstrated that these natural antioxidants offer protection against the damaging effects of reactive oxygen and nitrogen species, which are crucial in diseases associated with obesity.(Rodrigus C F *et al* 2018)[27]

Materials and Methods

Chia Seed Source and Preparation

Chia seeds utilized in this experiment were procured from the Loyal World supermarket in Mysore, Karnataka, under the True Elements brand. The seeds were finely ground into powder form and stored appropriately for use in preparing experimental diets.

Establishment of Stock

The experimental model organism, *Drosophila melanogaster* (Oregon K strain), was obtained from the *Drosophila* Stock Centre, Department of Studies in Zoology, University of Mysore. Flies were maintained in culture bottles containing a wheat cream agar medium prepared by boiling 100g of jaggery, 100g of rava powder, and 10g of agar in 1000ml of distilled water, with 7.5ml of propionic acid added as a mold inhibitor. Cultures were sustained under standardized laboratory conditions: 22°C ± 1°C temperature, 70% relative humidity, and a 12-hour light/dark cycle.

These flies were then used to establish experimental groups by incorporating varying concentrations of chia seed powder into the standard medium.

Preparation of Experimental Media

This cultured flies were used to establish the experimental flies with different diet media.[Wheat Cream Agar Medium (Control): The control medium, known as wheat cream agar, was formulated by mixing 100grams of jaggery,100grams of rava powder,and 10grams of agar into 1000ml of boiling distilled water, followed by addition of 7.5ml of propionic acid..Wheat Cream Agar with 5g Chia powder: The wheat cream agar medium was prepared by dissolving 100g of jaggery, 100g of rava powder, and 10g of agar in 1000ml of boiling distilled water. After thorough mixing, 7.5ml of propionic acid was added to the hot solution.The medium was then allowed to cool ,after which 5g of chia powder was added. The mixture was stirred well to ensure even distribution of chia powder..Wheat Cream Agar with 10g Chia Powder: To prepare the medium , 100g of jaggery, 100g of rava powder, and 10g of agar were dissolved in 1000ml of boiling distilled water. After complete dissolution,7.5ml of propionic acid was added while the mixture was still hot. The medium was then cooled and 10g of chia powder was added. the contents were mixed thoroughly to achieve uniform consistency..Wheat Cream Agar with 15g Chia powder: To prepare the medium , 100g of jaggery, 100g of rava powder, and 10g of agar were dissolved in 1000ml of boiling distilled water. After complete dissolution,7.5ml of propionic acid was added while the mixture was still hot. The medium was then cooled and 15g of chia powder was added . the contents were mixed thoroughly to achieve uniform consistency]

Each medium was freshly prepared and cooled before use to ensure consistency in nutrient content and texture. Flies from the stock population were transferred to these media and reared under the same laboratory conditions to examine their heat resistance in response to chia supplementation.

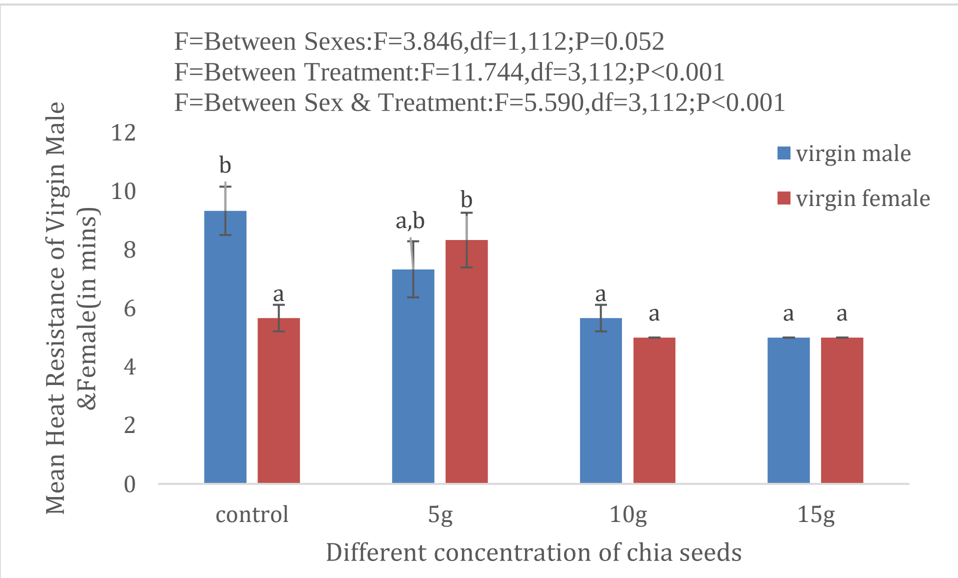
Experimental Procedure

Heat Resistance Test

To assess heat resistance, five-day-old flies—both virgins (unmated) and mated individuals—were collected from groups reared on wheat cream agar and on media supplemented with 5g, 10g, and 15g of chia seed powder. For each treatment, 15 flies were tested. The flies were gently transferred into clean, empty vials, with each vial holding five flies of the same group. The vials were then placed in a water bath set to 37°C, a temperature chosen to apply heat stress without causing immediate death. Every 5 minutes, the vials were taken out briefly to check on the flies. Observations continued until all the flies in each group had died. This process was repeated separately for virgin and mated flies, ensuring consistent conditions across all treatments.

Results

Figure 1 :Effect of the chia seed on the heat resistance in the unmated male and female of *D.melanogaster*



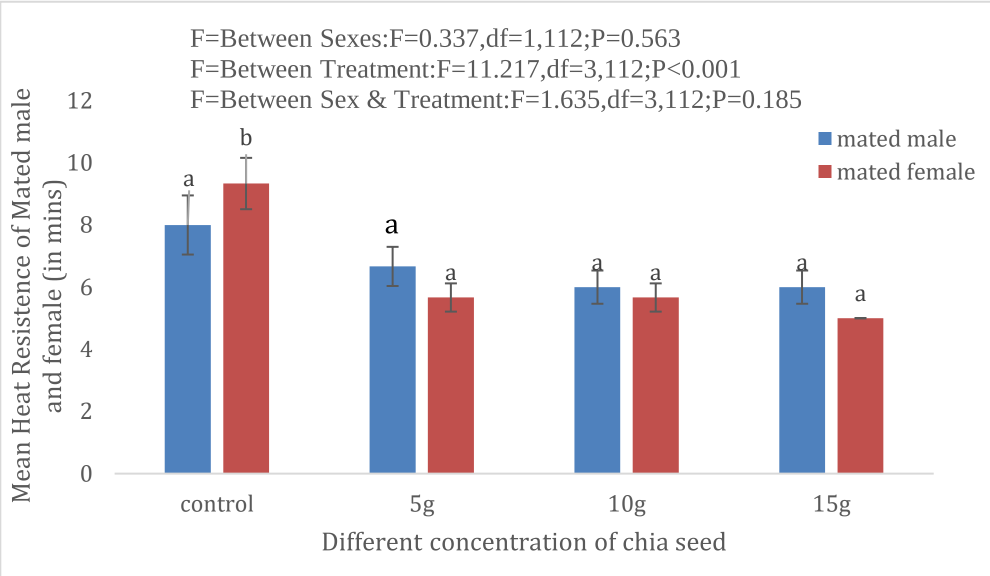
The different letters on the bar graph indicate the significant variation between the diffoperent diet by Tukey's post hoc test at 0.05 level.

Figure 1 illustrates the mean and standard error values of heat resistance in unmated male and unmated female *D. melanogaster* raised on wheat cream agar and diets supplemented with 5g, 10g, and 15g of chia seed powder. From the graph, it is evident that the flies raised on the control (wheat cream agar) and 5g chia seed diet showed higher levels of heat resistance compared to those raised on 10g and 15g chia seed diets. Among the groups, the virgin males in the control group exhibited the highest heat resistance, while the lowest was observed in both sexes at the 15g chia level.

In terms of sex differences, virgin males generally showed greater heat resistance than virgin females, particularly in the control group. However, at higher chia concentrations (10g and 15g), the difference between sexes became minimal, with both showing reduced resistance.

The data were analyzed using Two-way ANOVA followed by Tukey's post hoc test. The analysis revealed a highly significant effect of diet on heat resistance, indicating that the different levels of chia seed supplementation had a measurable impact on thermal tolerance. Additionally, a significant interaction was observed between sex and diet , suggesting that males and females responded differently to the dietary treatments. However, the main effect of sex alone was not statistically significant, although it approached the threshold for significance. These findings indicate that diet plays a more prominent role in influencing heat resistance than sex, though the response to diet may still vary slightly between male and female flies.

Figure 2 :Effect of the chia seed on the heat resistance in the mated male and female of *D.melanogaster*

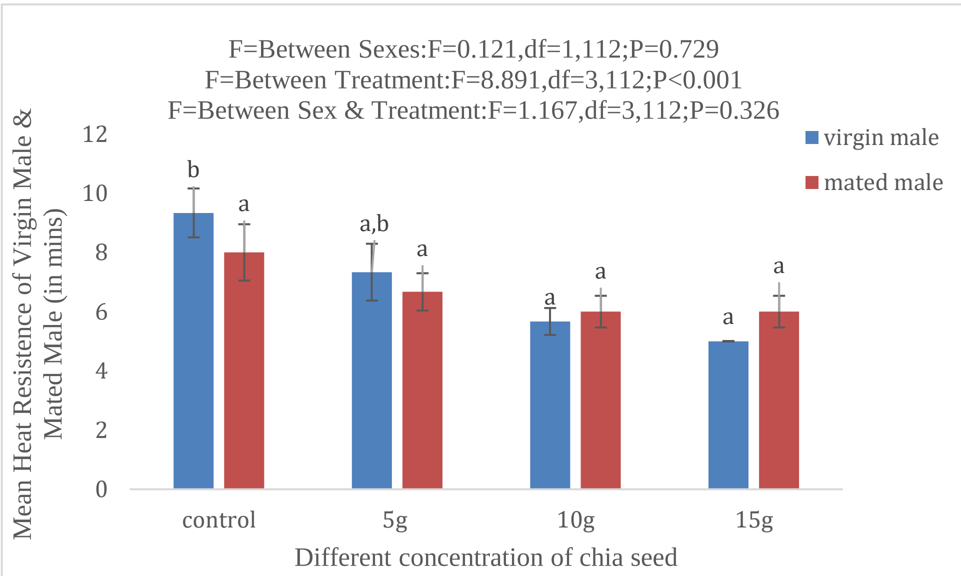


The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Figure 2 presents the average heat resistance (with standard error) of mated male and mated female *Drosophila melanogaster* raised on wheat cream agar, as well as diets supplemented with 5g, 10g, and 15g of chia seed. The results show that flies on the control diet (wheat cream agar) generally exhibited higher heat resistance compared to those fed with chia-supplemented diets. Among all groups, mated females on the control diet showed the highest heat resistance.

The statistical analysis using Two-way ANOVA followed by Tukey's post hoc test revealed a significant effect of diet on heat resistance ,indicating that different chia concentrations had a clear influence on thermal tolerance. However, no significant difference was found between males and females nor was there a significant interaction between sex and diet . This suggests that while chia seed supplementation reduced heat resistance overall, the effect was consistent across both sexes.

Figure 3:Effect of the chia seed on the heat resistance in the unmated male and mated male of *D.melanogaster*

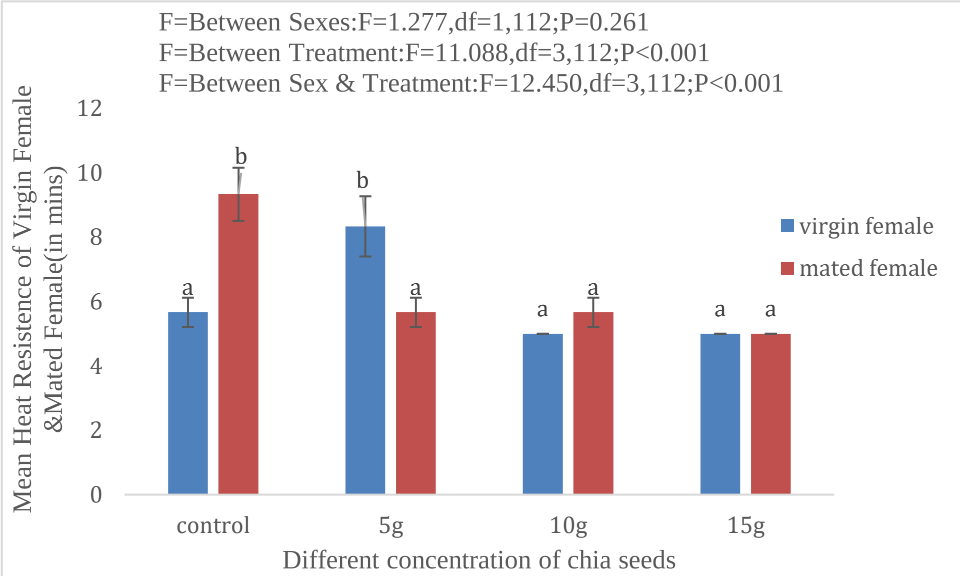


The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Figure 3:shows the mean heat resistance (± standard error) of virgin and mated male *Drosophila melanogaster* raised on a control diet (wheat cream agar) and on diets supplemented with 5g, 10g, and 15g of chia seed. The data indicate that flies raised on the control diet had the highest heat resistance overall, especially virgin males. However, heat resistance decreased with the inclusion of chia seed in the diet, particularly at the 10g and 15g levels.

Statistical analysis using Two-way ANOVA followed by Tukey's post hoc test revealed a significant effect of diet on heat resistance ,meaning the type of diet had measurable impact. In contrast, there was no significant difference between virgin and mated males, and the interaction between mating status and diet was also not significant. This suggests that while chia seed levels influenced heat resistance, the effect was similar regardless of whether the male flies were mated or unmated.

Figure 4:Effect of the chia seed on the heat resistance in the unmated female and mated female of *D.melanogaster*



The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Figure 4 presents the mean heat resistance (± standard error) of virgin and mated female *Drosophila melanogaster* raised on four diets: a control (wheat cream agar) and media supplemented with 5g, 10g, and 15g of chia seed. The control group exhibited the highest heat resistance overall, particularly among mated females. However, heat resistance decreased with increasing chia seed concentration in both virgin and mated females.

Statistical analysis using Two-way ANOVA followed by Tukey's post hoc test revealed a significant effect of diet on heat resistance and a significant interaction between diet and mating, suggesting that the effect of diet varied depending on whether the females were mated or virgin. However, the difference in heat resistance between virgin and mated females alone was not statistically significant .

Discussion

The Effect of Chia seed on the Heat Resistance in Drosophila melanogaster

Heat resistance in *Drosophila melanogaster* is widely used as a physiological indicator to assess the impact of diet, sex, and mating status on stress tolerance. Previous studies have shown that dietary composition, especially protein and carbohydrate content, plays a crucial role in shaping thermal resilience. For instance, flies raised on protein-rich or carbohydrate-balanced diets—such as yeast extract, spirulina, or mass gainer formulations—exhibited significantly improved resistance to heat and desiccation stress (Yosef *et a*l., 2022; Anderson et al., 2020)[39,1]. Similarly, spirulina-enriched diets also enhanced thermotolerance, likely due to their high protein content, suggesting that the type and quality of protein source matters greatly. These diets likely provided better metabolic support during thermal stress through enhanced energy reserves or more efficient synthesis of heat shock proteins (Hsps). Protein-enriched diets, such as those containing spirulina, have been associated with increased expression of Hsp70, a heat shock protein that helps maintain cellular function under thermal stress (Singh and Singh, 2008; Sorensen *et al*., 2003)[30,32].

However, our study using chia seed supplementation presents a contrasting pattern. While chia is considered a "superfood" rich in omega-3 fatty acids, fiber, and antioxidants, our findings indicate that higher levels of chia (10g and 15g) were associated with a decline in heat resistance across multiple experimental groups. Unlike spirulina or mass gainers that enhanced survival rates under stress, chia-fed flies—especially at higher concentrations—showed a notable reduction in thermotolerance, regardless of sex or mating status. This discrepancy may be due to chia's high fiber content or specific secondary metabolites, which might interfere with nutrient absorption or metabolism under stress conditions. Whereas other supplements offered direct macronutrient benefits, chia may have introduced physiological trade-offs, especially during the energetically demanding process of coping with heat stress. Thus, while dietary supplementation can modulate stress responses, the type and balance of nutrients involved play a critical role in determining whether those effects are beneficial or detrimental.

The current study explored how varying concentrations of chia seed supplementation influence heat resistance in *D. melanogaster*, considering the factors of sex and mating status. Across all experimental groups, one consistent observation emerged: flies reared on the control diet (wheat cream agar) demonstrated the highest resistance to heat stress, while those raised on 10g and 15g chia seed-supplemented diets showed significantly reduced thermotolerance. This suggests that, contrary to expectations, chia seed supplementation at moderate to high levels may negatively affect the flies' physiological ability to cope with thermal stress.

In unmated flies (Figure 1), males displayed higher heat resistance than females, especially in the control group, aligning with earlier studies indicating that virgin males often show better baseline stress resistance (Kristensen *et al*., 2003). Female flies may have higher thermotolerance due to greater lipid and glycogen reserves, which can be metabolized for energy during stress (Maness and Hutchison, 1980). However, this sex-based difference diminished at higher chia concentrations, possibly due to a common dietary effect overshadowing sex-specific physiological traits. The significant interaction between sex and diet reinforces the idea that males and females respond differently to dietary interventions, even if the main effect of sex alone did not reach statistical significance.

For mated flies (Figure 2), both sexes exhibited a uniform decline in heat resistance when reared on chia-supplemented media. Mated females on the control diet recorded the highest resistance among all mated groups, but there was no significant sex-based difference in thermal tolerance , nor a significant interaction between sex and diet. This implies that mating, when combined with chia supplementation, may induce physiological stress or energetic trade-offs that reduce heat resistance equally in both sexes. This improved resistance in mated females under optimal conditions could be attributed to accessory gland proteins (Acps) transferred during mating, which trigger physiological changes like increased egg production and altered metabolism (Wolfner, 2002; Gillott, 2003)[38,9].

In male flies (Figure 3), virgin individuals maintained higher resistance than their mated counterparts, particularly on the control diet, although this difference was not statistically significant . The effect of diet, however, was strongly significant, confirming that chia supplementation reduced the flies' ability to withstand heat stress regardless of mating status. These findings suggest that mating may exert energetic costs in males that do not enhance, and may even impair, their capacity to respond to acute environmental stress. Interestingly, some studies suggest mated males may sometimes show higher resistance due to pheromonal signaling or altered physiology (Goenaga, 2011)[10], but such effects were not evident here.

Similarly, in females (Figure 4), both virgin and mated groups showed a gradual decline in heat resistance with increasing chia concentration. Mated females on the control diet again outperformed all others, indicating that while mating might boost stress resilience under optimal nutritional conditions, this benefit disappears or reverses with chia supplementation. A significant interaction between mating status and diet further highlights how the physiological effects of reproduction are influenced by dietary quality.

Overall, the results demonstrate that diet composition critically shapes heat resistance in *D. melanogaster,* and that the physiological responses to dietary changes are context-dependent, varying by sex and reproductive status., chia seed supplementation did not enhance thermal tolerance; instead, it appeared to reduce it, particularly at higher concentrations. These findings underscore the importance of evaluating novel or "functional" food components in a species- and context-specific manner, especially when considering their effects on stress resistance and life-history traits.

In conclusion, our findings challenge the assumption that nutrient-rich supplements like chia seeds universally enhance stress resilience. Instead, the data reveal that higher concentrations of chia seed in the larval diet of *D. melanogaster* are associated with a marked decrease in adult heat resistance, regardless of sex or mating status. While virgin males and mated females on the control diet exhibited the greatest thermal tolerance, this benefit diminished or reversed with increasing chia concentration. These results suggest that beyond a certain threshold, chia seed components may disrupt metabolic or physiological pathways critical for heat stress adaptation. Importantly, diet emerged as the most significant factor influencing thermotolerance, overshadowing the effects of sex and reproductive status.

Acknowledgement

The authors sincerely acknowledge the Chairman, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru, for his valuable support. We are also grateful to the *Drosophila* Stock Centre, a National Facility at the University of Mysore, for providing essential facilities for carrying out the major project work. Additionally, we extend our heartfelt thanks to Mr. Kiran for his assistance with the statistical analysis.

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