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The effect of chia seed on cold resistance in *Drosophila Melanogaster*

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Abstract
An established model organism for genetic studies is *Drosophila melanogaster*. In recent decades, fruit flies have gained more recognition as a new and useful model in experimental food and nutrition research. Due of its many parallels to mammalian species, *D. melanogaster* can be employed in research involving dietary interventions. In insects, cold resistance can be significantly impacted by the makeup of their diet.

By growing *D. melanogaster* in wheat cream agar media with 5g, 10g, and 15g of chia seed powder, the experiment aims to ascertain how chia seeds impact cold resistance. The results showed that, in comparison to flies given 10g and 15g of Chia seed, which had average tolerance to cold, flies kept on wheat cream agar media showed the least amount of cold resistance, while flies given 5g of Chia seed media showed the most resilience. The current study shows that on all four diets, female flies had higher levels of cold resistance than males. Furthermore, mated female flies exhibited higher levels of cold tolerance on these four types of diets compared to unmated female flies. These findings imply that chia seeds help *D. melanogaster* become more resistant to cold. The Chia seed is rich with omega 3 fatty acid, antioxidants, fats, proteins and complex carbohydrates which may provide the energy to withstand the cold resistance in *D. melanogaster*.

Keywords: Cold resistance, Diet, *D. melanogaster,* mated, virgins

Introduction

For insects, the three most severe environmental conditions are starvation, thirst, and exposure to extremely low temperatures. The ability of insects to withstand cold is a crucial ecological trait that is strongly tied to species distribution and can be greatly impacted by their food. For instance, locomotor abnormalities, decreased fertility, and death are just a few of the ways that different forms of cold stress can affect physiology and fitness (Littler *et al*, 2021) [13].

Tolerance to severe environmental conditions is regarded as a crucial topic in evolutionary biology. Physiological strain brought on by extreme circumstances results in directional selection for stress resistance. Since it may result in cold stress, temperature is a crucial abiotic factor for ectothermic species, particularly insects (David *et al*, 1998) [9]. Since physical performance and reproductive success are ultimately determined by the physiological effects of temperature, cold tolerance is a key factor in insect dispersal. Because of the great interspecific and intraspecific variety in the genus's thermal tolerance, *D. melanogaster* is a great model for connecting temperature impacts on biochemistry and physiology to ecological patterns and processes (Macmillan *et al*, 2016) [16]. In insects, the composition of their diet can have a major impact on their resistance to cold and temperature. Temperature and insect fitness interact to affect species distribution and population structure, climate change response, and environmental adaptability (Littler *et al*., 2021) [13].

An animal's fitness is influenced by the quantity and balance of macronutrients (proteins, fats, and carbohydrates) and micronutrients (vitamins and minerals) in its diet. Insect physiology, stress, fecundity, and cold resistance can all be significantly impacted by the diet's composition (Cotter *et al*, 2019). Previous studies have shown that diets heavy in carbs tend to promote cold resistance more than diets high in protein. This might be as a result of the substrates they offer for the synthesis of free amino acids, sugars, and polyols, which are cryoprotectants. Consuming carbohydrate-rich meals causes major changes in lipidomic and metabolic profiles, including higher blood sugar levels. This may explain the reported association between carbohydrate-rich diets and cold-weather survival (Andersen *et al*, 2010) [2]. It is well known that carbohydrates cause flies to become more obese. In *Drosophila,* body fat increase is positively connected with the ability to withstand cold and famine (Sisodia *et al*, 2015) [17]. Andersen *et al.* (2010) [2] found that *D. melanogaster* flies raised in media high in carbohydrates recovered from a cold coma faster than flies raised in media rich in protein. Therefore, flies fed on carbohydrate-rich feed recover from cold comas faster, perhaps due to their greater lipid stores.

Many experiments were conducted on cold resistance based on different products such as Mass gainer by Anusree *et al.;* (2024) [1], Ensure nutrition supplement by Tanmayi kishore *et al;*(2024) [20]. From the above research they found that in spite of the difference in products they used but all show great impact on cold resistance on *D. melanogaster*. This may due to the different quality and quantity of nutrients present in different compounds. Chia seeds are made up of proteins (15–25%), fats (30–33%), carbohydrates (26–41%), high dietary fibres (18–30%), ashes (4–5%), and vitamins. They also contain a lot of antioxidants, including myricetin, quercetin, kaempferol, and caffeic acid, which make the oil stable even though it contains a lot of polyunsaturated fatty acids (PUFA). These natural antioxidants have been shown to have the potential to protect against the harmful effects of reactive oxygen and nitrogen species, which are important in diseases linked to obesity. (Cristiane Freitas Rodrigues *et al*.,2018) [8].

Materials and Methods

The chia seeds were purchased from the Loyal World supermarket in Mysore, Karnataka. This chia seed (True Elements brand) was used to make the experimental media. They are ground into a powder and kept for usage as a treatment system.

Establishment of stock
For the study, the experimental Oregon K strain of *D. melanogaster* was obtained using *Drosophila*. From the *Drosophila* stock centre, Department of Studies in Zoology, University of Mysore, Mysore, this stock was grown in bottles using wheat cream agar medium (100g of jaggery, 100g of wheat cream rava, and 10g of agar were boiled in 1000ml of distilled water and 7.5ml of propionic acid was added). The flies were housed in a laboratory environment with a temperature of 22°C ± 1°C, 70% humidity, and cycles of 12 hours of darkness and 12 hours of light. The flies that were obtained as previously mentioned were used to create the experimental stock with different doses of chia seed and wheat cream *agar* media.
The flies of control and treated media were obtained as follows; [Wheat cream agar medium(control)-100g of jaggery, 100g of wheat cream rava powder, 10g of agar were boiled in 1000ml of distilled water, and 7.5ml of propionic acid was added. The 5g Chia seed media- was prepared by adding 100g of jaggery, 100g of wheat cream rava, 5g of chia seed powder, and 10g of agar are boiled in 1000ml of distilled water with 7.5ml of propionic acid added to media. The 10g of Chia seed media- was made by boiling 10g of agar in 1000ml of distilled water, adding 7.5ml of propionic acid, and combining 100g of jaggery, 100g of rava and 10g of Chia seed powder. The 15g of Chia seed media- was prepared by boiling 10g of agar in 1000ml of distilled water, adding 7.5ml of propionic acid, and combining 100g of jaggery, 100g of rava and 15g of Chia seed powder]. The flies emerged from the above wheat cream agar medium and other experimentally treated media were maintained under the same laboratory conditions as previously mentioned above.

Experimental Procedure
Five days old mated and unmated (virgins) flies were cultured from wheat cream agar media, 5g, 10g, and 15g Chia Seed media in order to study cold resistance. Fifteen flies (mated male/mated female and unmated male/unmated female) were observed by moving them to empty vials, in three replicas each of which held five flies. These vials were stored in a refrigerator at a constant, frigid temperature of -4° C, and each fly's resistance to the cold was measured every hour until it died. Three duplicates, each containing five flies, were conducted for the 5g, 10g, and 15g Chia Seed media, as well as the wheat cream agar. Separate experiments were conducted for mated and unmated flies.

Result

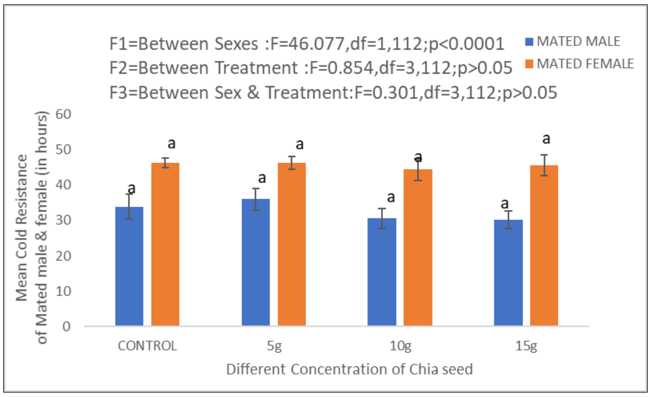

Fig 1: Effect of Chia Seed on the Cold Resistance in the Mated male and female of *D. melanogaster*

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

The mean and standard error value of the cold resistance of mated male and female flies who were raised on wheat cream agar, 5g, 10g, and 15g of Chia Seed media are provided in figure1. Based on data, it was observed that the 5g Chia seed diet had higher cold resistance than the 10g, 15g, and wheat cream agar diets. Based on the data, it was observed that the mated female had a higher level of cold resistance than the mated males in different diets.
The above data was subjected to the Two-way ANOVA followed by the Tukey's post hoc test showed the insignificant variation in cold resistance was found between the diets and between sexes and interaction between diet and sexes.

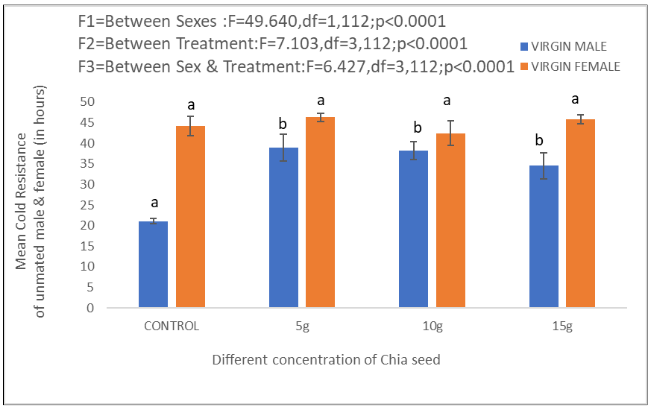

Fig 2: Effect of the Chia seed on the cold resistance on the unmated male and female of *D. melanogaster*.

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 shows the mean and standard error value of the cold resistance of unmated male and unmated female flies who were raised on wheat cream agar, 5g, 10g, and 15g of Chia Seed media. Based on data, it was observed that the 5g Chia seed diet had higher cold resistance than the 10g, 15g, and wheat cream agar diets. Based on the data, it was observed that the unmated female had a higher level of cold resistance than the unmated males in different diets.
The data was analysed using a Two-way ANOVA followed by Tukey's post hoc test, revealing significant variation in cold resistance was found between the diets, between sexes, and the interaction between diet and sex. And also, in cold resistance significant variation was observed between treatment and significant variation was noticed between sexes and in the interaction between sex and treatment in these four diets.

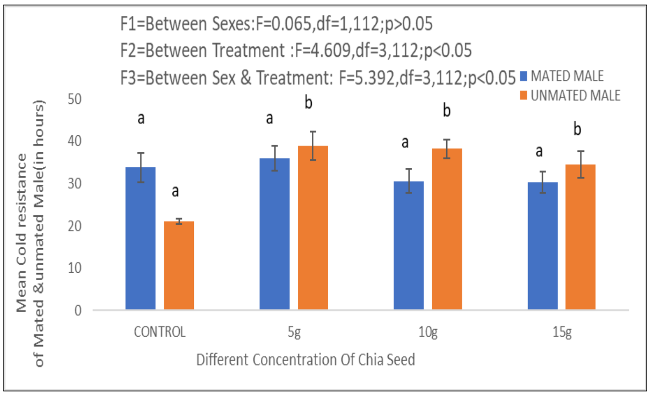

Fig 3: Effect of the Chia seed on the cold resistance of the mated and unmated male in *D. melanogaster*.

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 and standard error value of the cold resistance of mated male and unmated male who were raised on wheat cream agar, 5g, 10g, and 15g of Chia Seed media. Based on data, it was observed that the 5g Chia seed diet had higher cold resistance than the 10g, 15g, and wheat cream agar diets. Based on the data, it was observed that the unmated male had a higher level of cold resistance than the mated males in different diets.

The data was analysed using a Two-way ANOVA followed by Tukey's post hoc test, revealing significant variation in cold resistance was found between the diets, between sexes, and the interaction between diet and sex.

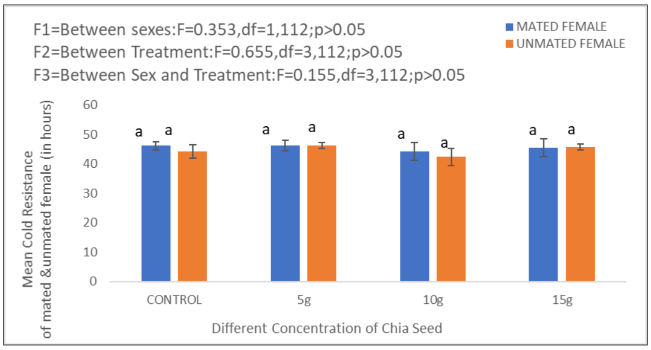

Fig 4: Effect of the Chia seed on the cold resistance of the mated and unmated female in *D. melanogaster.*

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

Figure 4 shows the mean and standard error value of the cold resistance of mated and unmated female who were raised on wheat cream agar, 5g, 10g, and 15g of Chia Seed media. Based on data, it was observed that the 5g Chia seed diet had higher cold resistance than the 10g, 15g, and wheat cream agar diets. Based on the data, it was observed that the mated female had a higher level of cold resistance than the unmated females in different diets.

The data was analysed using a Two-way ANOVA followed by Tukey's post hoc test, revealing insignificant variation in cold resistance was found between the diets, between sexes, and the interaction between diet and sex.

Discussion

The Effect of Chia seed on the cold Resistance in *D. melanogaster.*

Food is an essential element that all living things require for growth, development, physiology, resistance to famine, and survival. Reproduction and stress tolerance are two life history processes that are significantly impacted by the amount and quality of nutrients an organism consumes (Sisodia and Singh, 1988) [16]. Therefore, the focus of the current study was on how Chia seeds affected *D. melanogaster's* resistance to cold. It was discovered that flies treated with chia seeds had a significantly better level of cold resistance than control flies. This suggests that including chia seeds into meals could enhance *D. melanogaster's* resistance to cold. Increased cold tolerance necessitates physiological alterations that may compromise other fitness-related characteristics.

It's interesting to note that the oil in the Chia seeds contains triglycerides, 25–40% fat acids, 60% ω-3 alpha linolenic acid, and 20% ω-6 linoleic acid—all of which are thought to be necessary fatty acids that humans require. Chia seeds also contain substantial levels of dietary fibre (18–30%), ashes (4–5%), proteins (15–25%), fats (30–33%), and carbs (26–41%). Additionally, they are rich in antioxidants such as myricetin, quercetin, kaempferol, and caffeic acid, which help to stabilise the oil despite its high polyunsaturated fatty acid (PUFA) content. (Cristiane Freitas Rodrigues *et al*.,2018). Thus, contents in the Chia seed had supported the Cold resistance in *D. melanogaster*.

Caloric restriction boosts the flies' resistance to cold by limiting their access to food, particularly protein (yeast), which can make a twofold difference between females that were previously fed ad labium yeast and those that were not. (McCue, 2010; Laparie *et al*., 2012; Ribeiro *et al*., 2010; Burger *et al*., 2017; Cown and Nicolson, 2004). Additionally, our study supports previous research in *Drosophila* that found that, in comparison to diets high in protein, diets high in carbs tend to increase cold tolerance to insect illnesses (Andersen *et al*., 2010 [2]; Burger *et al*., 2007 [3]; Colinet *et al*., 2013) [6]. Sai Shresta and Krishna (2023) [18] conducted similar research, examining how prebiotic supplements improve *D. melanogaster*'s resilience to heat and cold. The findings demonstrated that *D. melanogaster*'s resilience to heat and cold is improved by dietary prebiotic supplements. They showed that resistance to heat and cold was significantly influenced by the quantity and calibre of nutrients ingested.

The sexes showed comparable changes in gene expression in response to cold, indicating that males and females use essentially the same mechanisms to adapt their physiology. According to a number of researches, dietary composition may also have an impact on cold resistance in both sexes. The amount of cold stress trade-offs is expected to depend on a variety of characteristics, such as age, condition, and life cycle. Surprisingly little research has been done on sex, a potentially significant component. In all three diets used in this study, the females are more resilient to the males. may vary across the sexes as a result of variations in regulatory architecture or relative variations in the costs of reproduction or survival (Grath and Parsch, 2016; Mank, 2017).

The current study additionally examined the male and female flies' tolerance to cold in 5g, 10g, and 15g chia seed media and wheat cream agar media. According to the findings, female flies were noticeably more resistant to cold than male flies. This can be explained by the fact that females had a comparatively higher fat content, which would have helped them survive amid famine and the cold. However, as there would have been less weight to carry around, greater thermogenesis would have been the more efficient reaction to cold if food supplies had been normally plentiful (Hoyenga, and Hoyenga, 1982) [11].

Additionally, this study examined the differences in cold resistance between mated and unmated males and females, finding that mated females had higher cold resistance than unmated females (Carvalho *et al*., 2006; Lee *et al*., 2013) [4, 12]. According to a study, mating can change a female *D. melanogaster*'s resilience to cold. It has been demonstrated that mating can cause a female *D. melanogaster* to significantly expand her midgut, increasing her post-ingestive nutrition usage and enabling mated females to meet their increased energy requirements for egg laying. This is most likely due to the fact that mated females consumed more food and accumulated more lipids than males (Service, 1989; Goenaga *et al*., 2012) [10, 15]. Resistance to cold temperatures in *Drosophila* may be influenced by alterations in phospholipid composition, triacylglycerol buildup, and proline accumulation (Chen and Walker, 1994; Misener *et al*., 2001) [5]. An Ensure® supplement in the diet improves *D. melanogaster*'s resilience to cold, claim Tanmayi Kishore *et al*., (2024) [20]. While many extrinsic and intrinsic factors, such as age, social interactions, environmental temperature, and diet, can influence an organism's ability to withstand stress, genetic variation can also have an impact. However, in order to investigate the cold tolerance of *D. melanogaster*, we fed flies of the same age and temperature with varying diets. Therefore, the variance in *D. melanogaster*'s cold resistance was caused by variations in the quantity, quality, and amount of nutrients that were present in the diet.5g of Chia seed provide more energy, triglycerides, anti-oxidants, proteins and carbohydrate; and helps for longer food storage to withstand the cold resistance than the 10g,15g chia seed media and wheat cream agar. Chia seeds therefore improve cold resistance in *D. melanogaster*; in every diet examined, female flies were more resistant to cold than male flies. Additionally, the mated females had greater resistance to the cold compared to unmated male and female.

Conclusion

The current study shows that on all four diets, female flies had higher levels of cold resistance than males. Furthermore, mated female flies exhibited higher levels of cold tolerance on these four types of diets compared to unmated female flies. These findings imply that chia seeds help *D. melanogaster* become more resistant to cold. The Chia seed is rich with omega 3 fatty acid, antioxidants, fats, proteins and complex carbohydrates which may provide the energy to withstand the cold resistance in *D. melanogaster*.

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