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(Research Article)

Mass gainer increases cold resistance in *Drosophila melanogaster*

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Abstract

Mass gainer is a powdered supplement that combines carbohydrates and protein which is usually used to increase body mass. A lot of people especially athletes and body builders consume mass gainers due to their beneficial effects on nutrition and health. In insects, food composition can have a strong effect on cold tolerance, in the present study *Drosophila melanogaster* was cultured in on wheat cream agar, 10g mass gainer, and 20g mass gainer. Flies obtained from these media were used to determine the effect of mass gainer on cold resistance. The results showed that flies raised in 20g of mass gainer media had greater cold resistance compared to flies that were given mass gainer of 10g which had an average cold resistance whereas flies that were reared on wheat cream agar media showed the least cold resistance. The present study also reveals that female flies were greater cold resistance than males on these three diets. In addition, mated female flies and unmated female flies, mated female flies had greater cold resistance on these three types of diets. However, in male we found mated male had greater cold resistance than unmated males. Thus, these studies suggests that mass gainer increases cold resistance in *D. melanogaster.*

Keywords: Diet; Cold resistance; *D. melanogaster*; Mated; Virgins**.**

1. Introduction

Starvation, drought and exposure to extreme cold are the most stressful environmental challenges for insects. In insects, cold tolerance is an important ecological characteristic closely related to species distribution that can be significantly influenced by their diet. Different types of cold stress can have varying impacts on physiology and fitness, for example locomotor abnormalities, reduced fertility and death (Littler *et al*, 2021).

In evolutionary biology, tolerance to harsh environmental conditions is considered a key issue. Severe conditions cause physiological strain, leading to directional selection for stress resistance. Temperature is an important abiotic element for ectothermic animals, especially insects, because it can cause possible cold stress (David *et al*, 1998). Cold tolerance is a major determinant of insect dispersal, because the physiological effects of temperature ultimately determine physical performance and reproductive success. *D. melanogaster* is an excellent model for linking temperature effects on biochemistry and physiology to ecological patterns and processes due to the wide interspecific and intraspecific variability in the genus's thermal tolerance (Macmillan *et al*, 2016).

In insects, temperature and cold tolerance can be significantly influenced by the makeup of their diet. Insect fitness and temperature interact to influence species distribution and population structure, adaptability to new environments, and climate change response (Littler *et al*, 2021). The amount and balance of macronutrients (proteins, lipids, and carbohydrate) and micronutrients (vitamins and minerals) in nutrition have an impact on an animal's fitness, Insect physiology, stress, fecundity, and cold resistance can all be strongly impacted by the makeup of the diet (Cotter *et al*, 2019).

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Compared to diets high in protein, previous research has demonstrated that diets high in carbohydrates tend to increase cold resistance. This could be because they provide substrates for the formation of cryoprotectants like sugars, polyols, and free amino acids. The consumption of meals high in carbohydrates leads to significant alterations in metabolic and lipidomic profiles, such as elevated blood sugar levels. This could potentially account for the observed correlation between diets high in carbohydrates and survival during cold weather (Andersen *et al*, 2010).

Carbohydrates are known to raise the fat level in flies. In *Drosophila*, the capacity to tolerate cold and starvation is positively correlated with body fat accumulation (Sisodia *et al*, 2015). In (2010), Andersen *et al* researchers discovered that *D. melanogaster* flies fed in carbohydrate rich media recovered from a cold coma more quickly than flies cultivated in protein-enriched media. Consequently, it is most likely because of their larger lipid stores that flies raised on carbohydrate-rich feed recover from cold comas more quickly.

Mass gainer is a powdered supplement that combines carbohydrates and protein which is usually used to increase body mass (Campbell *et al*, 2008). Mass gainer, often known as a "weight gainer," is a powder that is intended to replace meals with the goal of gaining muscle mass. In order to encourage an energy surplus and the synthesis of muscle protein, the majority of mass gainers are high in fat, protein, and carbohydrates. In order to speed up recuperation, mass gainers may also include additional muscle-building components like beta-hydroxymethylbutyrate (HMB) and creatine monohydrate.

A popular and extensively used supplement among athletes, protein powders assist build and repair of skeletal muscle as well as improve overall health and aid in post-exercise recovery. Athletes are also advised to build more muscle mass and avoid breaking down proteins while engaging in extended activity (Memet *et al.*, 2014). Although protein supplements seem to have benefits, other scientists believe that taking too much of them could be harmful to one's health. According to a related study, consuming a lot of protein and using supplements could harm renal function (Baskan and Sezen, 2023).

These days, a lot of people especially athletes and body builders consume mass gainers due to their beneficial effects on nutrition and health. There is no published data on how mass gainer impacts an organism's ability to endure the cold or other environmental stresses, despite numerous studies demonstrating that its consumption can increase protein synthesis, increases in muscle protein net balance, and increase body weight, among other benefits, in various model organisms (Campbell *et al*, 2008). Therefore, this study has been undertaken to determine the influence of mass gainer on cold resistance in *D. melanogaster.*

2. Materials and Methods

The mass gainer was purchased through Flipkart App from A207, Lane No. 9, No. 4, Mahipalpur, Delhi, 110037, India. This mass gainer was used to prepare the experimental media.

2.1. Establishment of stock

Experimental Oregon K strain of *D. melanogaster* used in the study was collected from *Drosophila* stock center. Department of studies in Zoology, University of Mysore, Mysore and this stock was cultured in bottles containing wheat cream agar media [100g of jaggery ,100g of wheat cream rava,10g of agar was boiled in 1000 ml distilled water and 7.5 ml of propionic acid was added]. Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark and 12 hours light cycles and temperature 22°C ± 1°C.

The flies obtained as above were used to establish the experimental stock with different diet media [Wheat cream agar media: Wheat cream agar media was prepared from 100g of jaggery, 100g of wheat cream rava, 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to it.

20g of Mass gainer media: is prepared from100g of jaggery, 80 g of wheat cream rava, 20g of mass gainer powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.

10g of mass gainer media: is prepared from 100g of jaggery, 90g of wheat cream rava and 10g of mass gainer powder,10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it]. The flies emerged from the wheat cream agar media and other experimental treated media under the same laboratory conditions as mentioned above were used to study the cold resistance in *D. melanogaster*.]

2.2. Experimental procedure

Cold resistance: To study cold resistance five days old unmated (virgins) and mated flies obtained from wheat cream agar, 10g mass gainer and 20g mass gainer were used. Ten flies (unmated male / unmated female, mated male / mated female) were observed by transferring them to empty vials with each vial containing 5 flies. These vials were kept at -4° C under constant cool condition in the refrigerator and resistance to cold of each fly was observed in 1 hour interval until its death. A total of 2 replicates (each with 5 flies) were carried out for each of the wheat cream agar, 10g mass gainer and 20g mass gainer media. Separate experiment was carried out for mated and unmated flies.

3. Results

3.1. Effect of the mass gainer on the cold resistance in the mated male and female of *D. melanogaster*

The mean and standard error value of the cold resistance of mated male and female flies raised with wheat cream agar, 10g of mass gainer and 20g of mass gainer media are provided in the figure 1. According to data it was noticed that cold resistance was greater in the 20g of mass gainer compared to the wheat cream agar and 10g of mass gainer diet. The result was found that the mated female had the greater cold resistance than mated males in different diet.

The above data was subjected to the Two-way ANOVA followed by the Tukey's post hoctest showed the significant variation in cold resistance was found between the diets, between sexes and interaction between the diet and sex. However, significant variation was observed between the treatment and non significant variation was found between sexes and in the interaction between sex and treatment.

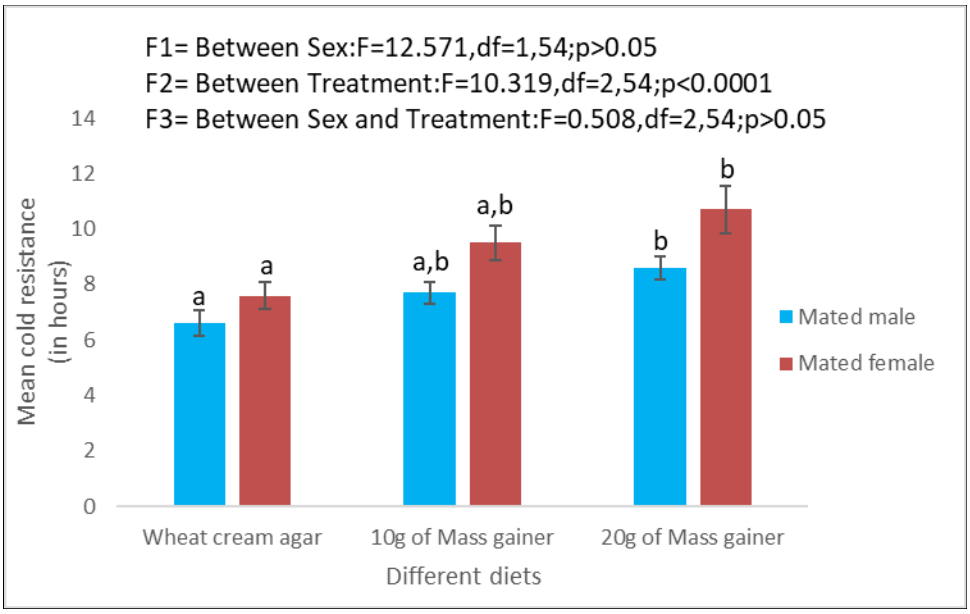

Figure 1 Effect of the mass gainer on the cold resistance in the mated male and female of *D. melanogaster.*

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.

3.2. Effect of the mass gainer on the cold resistance in unmated male and female of *D. melanogaster*

Data in figure 2 shows that the mean and standard error values for cold resistance were higher in unmated male and female flies raised on a diet with 20g of mass gainer compared to those raised on wheat cream agar or 10g of mass gainer. The results indicated that unmated females exhibited greater cold resistance than mated males across different diets.

The data was analyzed 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. However in cold resistance significant variation was observed between treatment and also non significant variation was noticed between sexes and in the interaction between sex and treatment in these three diets.

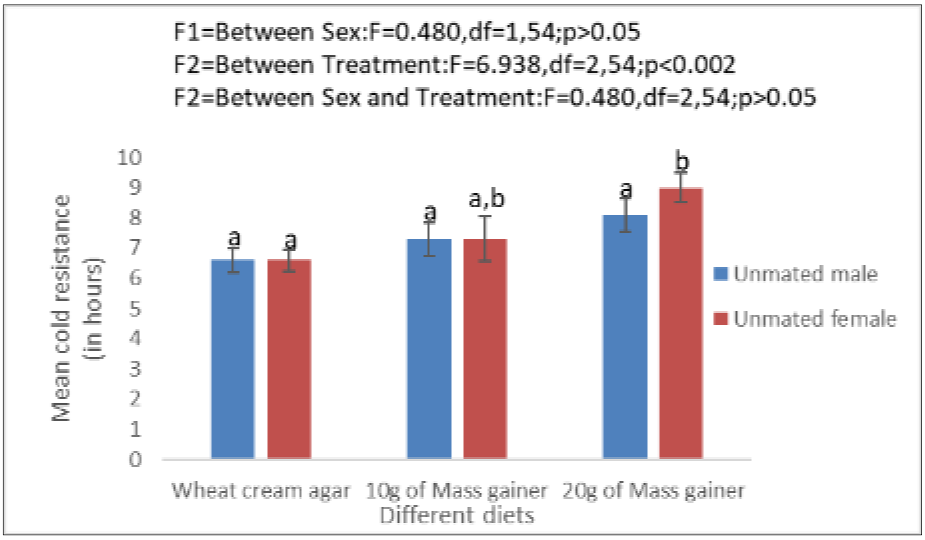

Figure 2 Effect of the mass gainer on the cold resistance on the unmated male and female of *D. melanogaster*

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.

3.3. Effect of the mass gainer on the cold resistance in the mated male and unmated male of *D. melanogaster*

Figure 3 provides the mean and standard error values for the cold resistance of mated male and unmated male flies raised in wheat cream agar, 10g of mass gainer, and 20g of mass gainer media. The data indicates that cold resistance was highest in flies raised in the 20g mass gainer diet compared to those in wheat cream agar and the 10g mass gainer media. Additionally, it was found that mated males exhibited greater cold resistance than unmated males in different diets.

By using Two-way ANOVA followed by Tukey's post hoc test, the data was analyzed, which revealed significant variations in cold resistance was found between sexes and diets. However in cold resistance non significant variation was observed in the interaction between condition and treatment.

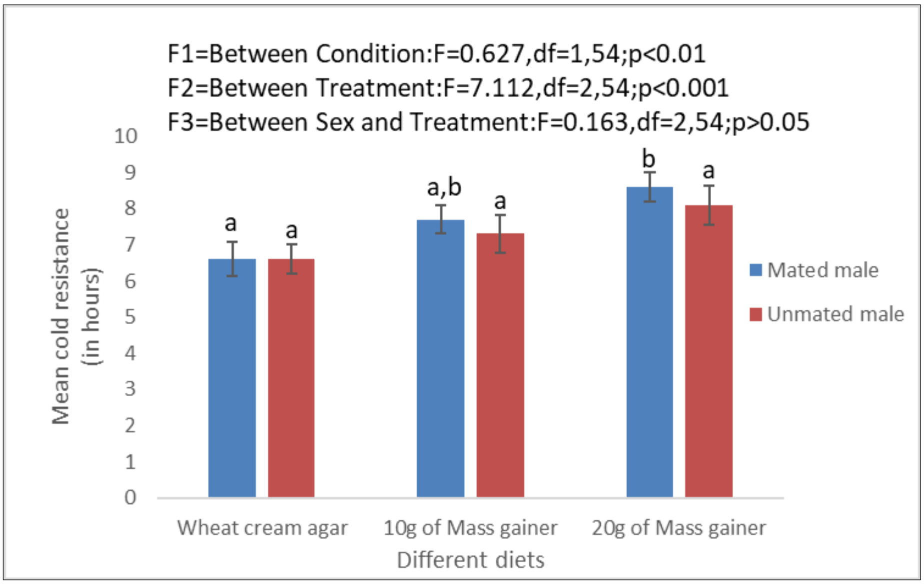

Figure 3 Effect of the mass gainer on the cold resistance of the mated and unmated male in *D. melanogaster.*

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.

3.4. Effect of the mass gainer on the cold resistance of the mated and unmated female in *D. melanogaster*

The cold resistance of mated and unmated female flies reared on wheat cream agar, 10g of mass gainer, and 20g of mass gainer media is shown in Figure 4 along with the mean and standard error values. According to the results, flies raised on the 20g mass gainer media showed the highest level of cold resistance when compared to those raised on the 10g mass gainer media and wheat cream agar media. Furthermore, it was noted that, irrespective of media, mated females demonstrated a higher level of cold resistance than unmated females.

The Two-way ANOVA and Tukey's post hoc test applied to the above mentioned data revealed significant variation in cold resistance between the sexes and between diets. However non significant variation was noticed with interaction between the diet and sex.

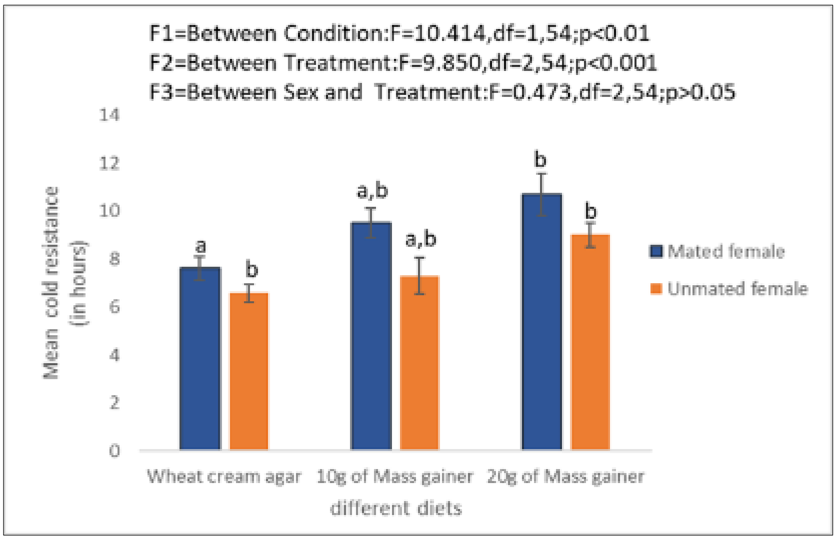

Figure 4 Effect of the mass gainer on the cold resistance of the mated and unmated female in *D. melanogaster*

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.

4. Discussion

Diet can have an impact on an insect's capacity to proliferate, tolerate stress, grow, and maintain immunity. Cold resistance also depends on changes in nutritional composition, and the amount of diet induced variability varies among individuals based on their genetic background. (Littler *et al*, 2021). In this present study, in the *D. melanogaster* results (figure 1-4) revealed that cold resistance was greater in the 20g of mass gainer compared to 10g mass gainer media and wheat cream media.

Our Study also confirms the earlier studies in *Drosophila* of (Andersen *et al*., 2010; Burger *et al*., 2007; Colinet *et al*., 2013), a diet rich in carbohydrates tends to increase cold tolerance to insect diseases compared to a diet rich in protein. This trend is proposed to be mediated by carbohydrate-induced changes in metabolism and lipidomic profiles. Flies reared on carbohydrate-rich feed had increased cold shock survival compared to house-reared flies on carbohydrate-poor BM and CM1 diets. While a low-carbohydrate SF diet also improved survival from cold shock, CM2 and SF diets both had higher lipid concentrations. And previous studies had higher lipid concentrations suggests lipids are an important energy source during stress and that certain classes of lipids maintain membrane function in cold weather (Potts *et al*., 2020; Shreve *et al*., 2007; Zhu *et al*., 2017).

Colinet *et al*., (2013) while studying dietary sugars affect cold tolerance of *D. melanogaster* have also showed that increasing body sugars levels, via dietary enrichment, will promote the cold tolerance of *D. melanogaster* adults. Dietary supplementation of sugars resulted in these compounds being incorporated and stored as fat and body fat help keep the *Drosophila* warm.

Similar studies have also been carried out by Sai Shresta and Krishna (2023), they studied about prebiotics supplement increases heat and cold resistance in *D. melanogaster*. The result showed that prebiotic supplement in the diet enhances cold and heat resistance in *D. melanogaster*. They demonstrated that the amount and quality of nutrients consumed had a significant impact on the ability to withstand cold and heat. Additionally, Sisodia and Singh (2012) found that south Indian *D. ananassae* groups that eat fruits high in carbohydrates were less susceptible to a food crisis than north Indian tribes that eat fruits high in protein.

In the present study we have cultured the flies in same temperature and humidity and other conditions, further we have used same aged flies therefore observed variation in the cold resistance was not resulted due to the age, and environmental conditions in the flies.

In the present study, we also studied the cold resistance of the male and female flies in 20g of mass gainer, 10g of mass gainer and wheat cream agar media. The results (Figure1- 2) revealed that female flies were significantly had greater cold resistance than male flies. We can explain this as follows, female had a relatively higher fat content, the fat would have facilitated survival both in the cold and during starvation. On the other hand, if food supplies had been typically abundant, then increased thermogenesis would have been the more effective response to cold since this would not have involved an increase in weight to be carried around (Hoyenga, and Hoyenga, 1982). Consumption of a carbohydrate rich diet triggers considerable changes in metabolic and lipidomic profiles, example increasing body sugar levels which could explain the apparent relationship between carbohydrate rich diets and the ability to survive in the cold temperature (Colinet *et al*., 2013; Enriquez and Colinet, 2019).

Further, in this study we also studied the variation in the cold resistance in the mated and unmated male and females (Figure 4) revealed that cold resistance was greater in mated females compared to unmated females, according to (Carvalho *et al*., 2006; Lee *et al*., 2013). Study revealed that mating can alter cold resistance in *D. melanogaster* females.

The most likely reason for this is that mated females consumed more food and built up more lipids than male, it has also been shown that mating can cause a female *D. melanogaster* midgut to significantly expand, thus increasing their post-ingestive nutrition usage, allowing mated females to fulfill their increased energy requirements for egg laying *(Service, 1989; Goenaga et al., 2012).* In *Drosophila*, changes in phospholipid composition, triacylglycerol accumulation and proline accumulation can play a role in resistance to cold temperatures (Chen and Walker, 1994; Misener *et al*., 2001).

5. Conclusion

In the present study, the cold resistance of mated and unmated males was also examined (figure 3), it was found that mated males considerably outperformed unmated males in both the 10g of mass gainer and 20g of mass gainer.

20g of mass gainer provide more energy, carbohydrate, and helps for longer food storage to withstand the cold resistance than the 10g of mass gainer and wheat cream agar. Thus in *D. melanogaster* mass gainer increases cold resistance, in all the diet studied female had greater cold resistance than male flies. Furthermore, the mated males and females had greater resistance to the cold compared to unmated male and female.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Baskan,A and Sezen, B,"Knowledge levels, opinions and attitudes of individuals going to the gym about supplements. evista Gestão e Secretariado (GeSec) São Paulo," Revista De Gestão E Secretariado., vol.14 (10), pp. 17845-17857, Oct. 2023.

[2] Campbell, B., Kalman, D., Greenwood, M and Antonia, J, "Muscle Mass and Weight Gain Nutritional Supplements, Nutritional Supplements in Sports and Exercise," Nutritional Supplements in Sports and Exercise, pp.189-223, Jan. 2008.

[3] Memet, D., Weliakim, A and Wolach, B, "Proteins and amino acid supplementation in sports: Are they truly necessary, " The Israel Medical Association journa]., vol. 7(5), pp. 321-329. June. 2005.

[4] Sisodia, S and Singh, B. N, "Experimental Evidence for Nutrition Regulated Stress Resistance in *Drosophila ananassae*, " Journal pone., vol. 7(10), pp. e46131. Oct. 2012.

[5] Littler, A.S., Garcia, M. J and Teeth, M. N, "Laboratory diet influences cold tolerance in a genotype-dependent manner in *Drosophila melanogaster*, " Comp Biochem Physiol A Mol Integr Physiol., vol. 257, pp. 10948. Apr. 2021.

[6] MacMillan, N. J. M., Dennis, A. B, Udaka, H., Marshall, K. E., Merritt, T. J. S and Sinclair, B. J,"Cold acclimation wholly reorganizes the *Drosophila melanogaster* transcriptome and metabolome," Scientif report., vol. 6, pp. 28999. Jun. 2016.

[7] Cottera, S. C., Reaveyb , C. E., Tummalab, Y., Randallb, J. L., Holdbrookb, R., Ponton, P., Simpsonc , S. J .,Smithe, J. A and Wilson, K, "Diet modulates the relationship between immune gene expression and functional immune responses, " Insect Biochem Mol Biol., vol. 109, pp 128-141. Apr. 2019.

[8] Andersen, L. H.,Kristensen, T. N., Loeschcke, V., Toft, S and Mayntz, D, "Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult Drosophila melanogaster, " J Insect Physiol., vol. 56 (4), pp. 336–340. Nov. 2009.

[9] Sisodia, S.,Verma, P and Singh, B. N, "Effect of diet quality and associated metabolic changes in adult stress response and life-history traits in *Drosophila ananassae*,"Biology Environmental Science., vol. 109(9), pp. 1687-1696. Nov. 2015.

[10] Colinet, H., Larvor, V., Bical, R and Renault, D, "Dietary sugars affect cold tolerance of *Drosophila melanogaster*, "Metabolomics., vol. 9, pp. 608–622. August. 2013.

[11] Shresta, S. C. M and Krishna, M. S, "Prebiotics supplement increases heat and cold resistance in *Drosophila melanogaster*, "International Journal of Advanced Research in Biological Sciences., vol 10(8), pp. 155-165. August. 2023.

[12] Hoyenga, K. B and Hoyenga, K. T, "Gender And Energy Balance: Sex Differences In Adaptations For Feast And Famine, " Physiology and Behavior., Vol. 28, pp. 545- 563. March 1982

[13] Lee, K. P., Kim, J.S and Min, K. J, "Sexual dimorphism in nutrient intake and life span is mediated by mating in *Drosophila melanogaster*," Animal Behaviour., vol. 86 (5), pp. 987-992. Nov. 2013

[14] Carvalho, D. C. L. D., Cardoso, S. C and Cliquet, A, "Improvement of metabolic and cardiorespiratory responses through treadmill gait training with neuromuscular electrical stimulation in quadriplegic subjects, " Artif organs., 30(1), pp. 56-63. Jan. 2006

[15] Burger, J.M.S., Hwangbo, D.S., Corby-Harris, V., Promislow, D.E.L., "The functional costs and benefits of dietary restriction in *Drosophila*," Aging Cell., vol. 6, pp. 63–71. Feb. 2007.

[16] Chen, E.H., Hou, Q.L., Wei, D.D., Jiang, H.B and Wang, J.J, "Phenotypic plasticity, trade-offs and gene expression changes accompanying dietary restriction and switches in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), "Sci. Rep., vol. 7, pp. 1–12. Mei. 2017.

[17] Chen, C. P and Walker, V. K, "Cold-shock and chilling tolerance in *Drosophila*. Journal of Insect Physiology, "Journal of Insect Physiology., vo. 40 (8), pp. 661–669. August. 1994.

[18] Enriquez, T and Colinet, H, "Cold acclimation triggers lipidomic and metabolic adjustments in the spotted wing *Drosophila suzukii* (Matsumara), " Am J Physiol Regul Integr Comp Physiol., vol. 316(6), pp. 751-763. Apr. 2019.

[19] Service, P.M, "The effect of mating status on lifespan, egg laying, and starvation resistance in *Drosophila melanogaster* in relation to selection on longevity, "Journal of Insect Physiology., vol. 35 (5), pp. 447-452. August. 1989.

[20] Goenaga, J., Mensch, J., Fanara, J.J and Hasson, E, "The effect of mating on starvation resistance in natural populations of *Drosophila melanogaster*, " Evol. Ecol., vol. 26, pp. 813–823. Nov. 2012.

[21] David, J.R., Gibert, P., Pla, E., Pétavy, G., Karan, D and Moreteau, B, "Cold stress tolerance in Drosophila: analysis of chill coma recovery in *D. melanogaster*, "Journal of Thermal Biology., vol. 23, pp. 291–299. Jan. 1998.