



The effect of chia seed on the starvation in *Drosophila Melanogaster*

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Abstract

As a model organism, *Drosophila melanogaster* is commonly used by researchers to evaluate traits like starvation resistance. Severe hunger or insufficient food resources are two prevalent and ecologically relevant forms of environmental stress in animals. Consuming food helps with growth, reproduction, and food storage. The amount of food an animal consumes, how frequently it eats, and how well it digests and absorbs its food all affect its capacity to absorb nutrients through this system. One of the important external environmental factors influencing environmental stress is dietary issues. The purpose of this experiment is to determine how chia seeds affect hunger resistance by cultivating *D. melanogaster* in wheat cream agar media with 5g, 10g, and 15g of chia seed powder. The findings demonstrated that flies kept on cream of wheat agar media exhibited the least resistance to starvation, while flies given 10g Chia seed media had the highest resistance to starvation compared to those given 5g and 15g Chia seed, which had average resistance. This implies that an additional 10g of chia seeds offers greater nourishment and vitality to endure famine during the solo period. The present study also reveals the female flies were more starvation resistant than males on these three types of diets. Furthermore, unmated male and female flies were more resistant to famine than mated male and female flies on these four forms of food in both mated and virgin flies. According to this study, adding 10g of chia seeds to the diet provide enough energy and food storage to last longer starvation. Thus, the quality and quantity of nutrients present in different concentration of Chia seed may leads to more resistant to starvation.

Keywords Diet, starvation resistance, *Drosophila Melanogaster*, mated, virgins

Introduction

Animal's capacity to endure a shortage of food is known as starvation. Generally speaking, famine can happen quickly or gradually. Animal death may result from prolonged famine, for instance brought on by seasonal fluctuations and a lack of food supplies (McCue, 2010) [13]. Many animal species have difficulty finding food, and an animal's ability to forage is strongly related to its reproductive fitness (Chippindale *et al.*, 1996; Wayne *et al.*, 2006) [4].

Food scarcity is the most prevalent environmental issue that animals deal with, and an animal's diet and nutritional state affect its capacity to endure extended periods of famine. The nutritional basis of starvation resistance has not been fully examined, despite the fact that the ecology and evolution of starvation resistance have been extensively studied using fruit flies of the genus *Drosophila* (Lee and Jang, 2014) [10].

The rate and frequency of food ingestion, along with the effectiveness of the nutrients' digestion and absorption, determine how much food an animal gets through this process (Singh and Sisodia, 2012). The amount and quality of nutrients in the diet can alter regulatory feedback loops, which are known to closely govern this eating response in certain animals (Simpson *et al.*, 2012) [18]. It was once believed that an ageing animal's only concern was energy, but it is now known that animals balance their intake of various nutrients to optimise their fitness.

Numerous studies have demonstrated that sexual dimorphism occurs in insect features linked to hunger resistance. Because mated females consumed more food than males, they acquired more lipids (Lee *et al.*, 2013). Aggarwal (2014) examined the physiological impacts of sex and population-specific variations in *D. leontia* hunger resistance from our experiment we also found females have greater starvation resistance when compared to males.

In natural populations, the quantity of available food sources tends to decline. The physiology of hungry adaptation is relevant to the broader study of evolution and its potential impacts for human health. A comparative study of patterns of variation within and across populations of *D. melanogaster* species that experienced drought and famine was carried out by Hoffmann and Harshman (1999) [7, 6].

Since an animal's physiological capacity to withstand starvation is dependent on its nutritional condition and feeding history, the nutritional value of a feed is a determining element that determines an animal's resistance to starvation. *D. melanogaster* must eat, digest, and absorb accessible nutrients since it is a heterotroph. Consumed food is utilised for storage, development, and reproduction. The rate and frequency of food ingestion, as well as the effectiveness of the nutrients' digestion and absorption, all affect how much nutrition an animal gets during this process. (Anusree K.A *et al.*;2024) [2]

There is compelling evidence that enhanced hunger tolerance in adults is caused by a larger fat content. Some data suggests that this trait accounts for nearly all variance in hunger resistance. For instance, Chippindale *et al.* (1996) [4] assessed lipid levels and famine in a set of strains chosen for starving or alteration of life history traits. They discovered an almost perfect correlation between hunger and cholesterol levels after accounting for all of this. Lipid connections have also been documented from several starvation selection strains originating from different base populations, comparing allozyme genotypes and the age-dependent changes in lipid and starvation levels (Offmann and Harshman, 1999) [6].

Many experiments were conducted on starvation based on different products such as Mass gainer by Anusree *et al.*; (2024) [2], Ensure nutrition supplement by Aishwarya *et al.*; (2024), Jeeni millet traditional mix by Kiran *et al.*; (2023)

[11], Spirulina supplements by Shreeraksha *et al.* (2023) [17]. From the above research they found that in spite of the difference in products they used but all show great impact on starvation resistance on *D. melanogaster*. This may be due to the different quality and quantity of nutrients present in different compounds.

For mated and unmated flies, starvation may have distinct effects. Some research indicates that as compared to male flies, females exhibited more resistance. *et al.*, Aishwarya (2024). The reason female flies are larger than males are because they accumulate more lipids and glycogen, which are then digested to provide energy during starvation (Aishwarya *et al.*, 2024) [1]. More efficiently, the female flies use their energy (Kiran and Krishna, 2023) [11]. Some research indicates that unmated females exhibited a greater degree of resistance to starvation than mated females. Since mating requires a lot of energy, the mated females may have expended the majority of their energy during copulation. *et al.*, Aishwarya (2024) [1]. The major cells of the male accessory gland produce seminal fluid products, which are responsible for the cost of mating in females. Chapman *et al.* (1996) [3] found that increased exposure to these products increases the mortality rate of females and may lead them to die earlier than unmated females.

From the above literatures we found that there was no other experiment were conducted on the effect of Chia seed on the starvation in *D. melanogaster*. Since the Chia seeds are the plant's most consumed component, they are also the subject of the greatest research. Remarkably, the seeds' oil 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 for human needs. (Cristiane Freitas Rodrigues *et al.*, 2018) [5]

Materials Method

The Loyal World grocery in Mysore, Karnataka, is where the chia seeds were bought. The experimental media was made with this chia seed (True Elements brand). For usage as a treatment system, they are ground into a powder and stored.

Establishment of Stock

For the study, the experimental Oregon K strain of *Drosophila melanogaster* was obtained 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. The flies were housed in a laboratory environment with a temperature of $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 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.

Establishment of Experimental Stock

In order to prepare the flies from the wheat cream agar medium [control]-100g of jaggery, 100g of wheat cream rava powder, 10g of agar boiled in 1000ml of distilled water, and 7.5ml of propionic acid are added. Using the following approach, treated media containing chia seeds in varying concentrations (5g, 10g, and 15g) were prepared by follow. The 5g Chia seed by boiling 100g of jaggery, 100g of wheat cream rava, and 10g of agar in 1000ml of distilled water, along with 7.5ml of propionic acid and the 5g Chia seed powder. 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. And the 15g 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 15g of Chia seed powder. Flies obtained from this control and treated were maintained using the same laboratory circumstances as previously described, and the flies emerged from the wheat cream agar medium and other experimental concentration media Of Chia seed were used in the experiment.

Starvation Resistance

Five-day-old unmated (virgins) and mated flies were grown from wheat cream agar, and 5g of Chia seed medium, 10g, and 15g were used to investigate hunger resistance. A total of 15 flies each empty vials with five flies. (unmated male and female, mated male and female) were observed separately on wheat cream agar, 5g, 10g, and 15g of chia seed media. These vials were maintained at 22°C with continuous light, and each fly's resistance to starvation was noted every hour until it died. Three replicates were conducted for each of the following: 5g, 10g, and 15g of Chia seed medium, and wheat cream agar.

Result

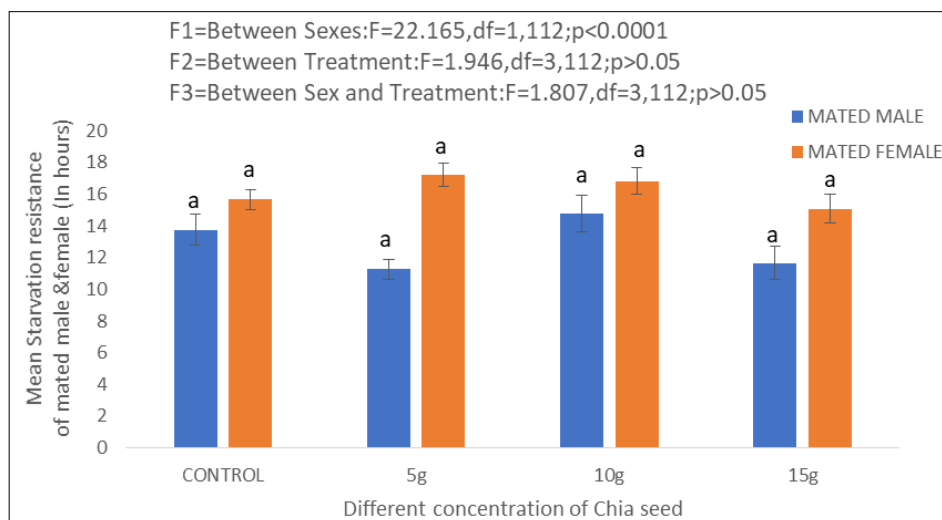


Fig 1: Effect of the Chia seed on the starvation resistance in the mated male and female of *Drosophila melanogaster*

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

Figure 1. Shows the mean and standard error of the starvation resistance of mated male and female flies reared on wheat cream agar, 5g of Chia seed, and 10g and 15g of Chia seed media. Data showed that the 10g of Chia seed had higher hunger resistance than the wheat cream agar and the

concentrations of 5g and 15g of Chia seed. According to the results, the mated female was more resistant to hunger than the mated males in various concentrations.

When the two-way ANOVA and Tukey's post hoc test were applied to the aforementioned data, the results indicated insignificant difference sexes, between treatment and there is a significant variation between sexes and treatment.

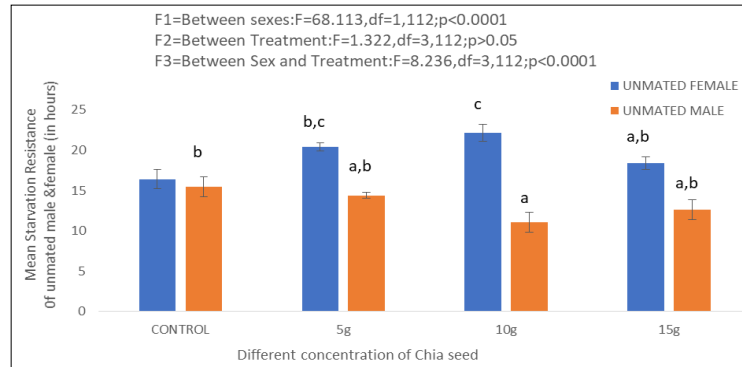


Fig 2: Effect of the Chia seed on the starvation resistance on the unmated male and female *Drosophila melanogaster*

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

Figure 2. demonstrates that unmated male and female flies raised on a concentration of 10g of Chia seed had greater mean and standard error values for hunger resistance than those raised on wheat cream agar or 5g,10 g of Chia seed.

According to the findings, unmated females were more resistant to hunger than unmated males at all concentrations. Significant differences in concentration were found when the data was analysed using a two-way ANOVA and Tukey's post hoc test. There was significant difference between the sexes, between treatment and between the sexes and concentrations.

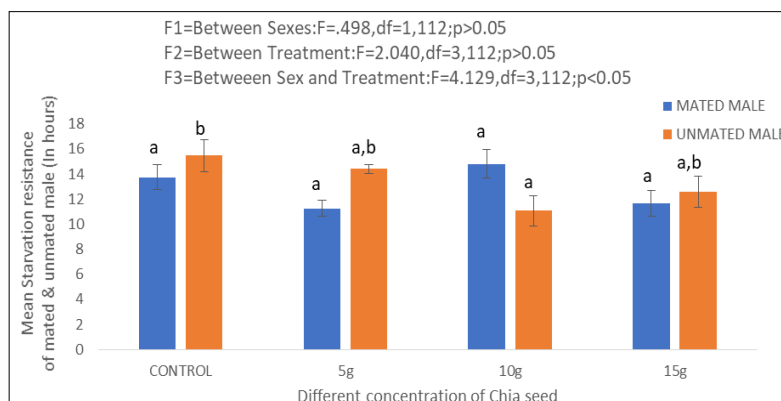


Fig 3: Effect of the Chia seed on the starvation resistance of the mated male and unmated male in *Drosophila melan*

The different letters on the bar graph indicate the significant variation between the different concentration by Tukeys post host at 0.05 level.

The starvation resistance means and standard error values of mated male and unmated male flies reared on wheat cream agar, 5g Chia seed media, 10g Chia seed, and 15g Chia seed media are shown in Figure 3. According to the findings, it was discovered that in various concentrations, unmated males showed higher tolerance to hunger than mated males.

The data was examined using a two-way ANOVA and Tukey's post hoc test. The results showed that there were significant differences between diets, differences between sexes, and an interaction between diets and sexes.

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

Figure 4 displays the mean and standard error values for the starvation resistance of mated and unmated female flies raised on 5g, 10g, and 15g of Chia seed media, as well as wheat cream agar. When compared to flies raised on the 5g, 15g, and wheat cream agar media, the results indicated that the flies raised on the 10g Chia seed media exhibited the highest level of starvation resistance. Additionally, it was observed that unmated females showed greater hunger resistance than mated females on a range of media.

The Two-way ANOVA and Tukey post hoc test applied to the above-mentioned data revealed significant difference between the concentration and as well as an interaction between the concentration and sexes and difference between the sexes.

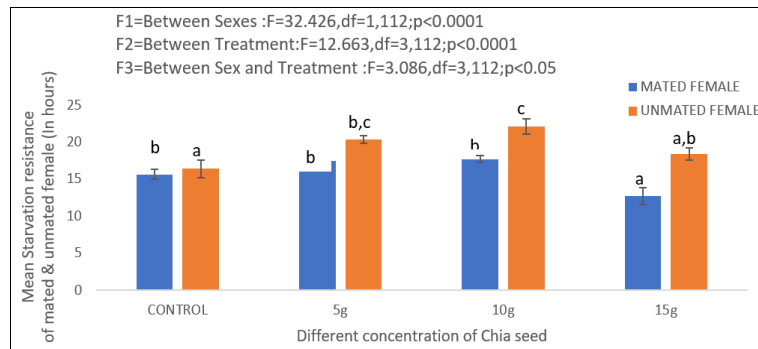


Fig 4: Effect of the Chia seed on the starvation resistance of the mated female and unmated female in *Drosophila melanogaster*

Discussion

Numerous facets of fly development are influenced by diet, including sex, health, reproduction, and even the organism's resistance to famine and capacity for survival. The results of this study showed that the *D. melanogaster* showed more resistance to famine when fed 10g of chia seeds as opposed to 5g, 15g, and wheat cream medium due to the different quality and quantity of nutrients such as rich in omega 3 fatty acid, proteins and fats may leads to greater resistant to starvation.

Many other experiments were undertaken on *D. melanogaster* on the starvation resistant by different products. In their investigation of the impact of Spirulina supplements on *D. melanogaster's* resistance to starvation, Shreeraksha *et al.* (2023) [17] also demonstrated that flies treated with Spirulina exhibited noticeably higher levels of resistance to starvation. This is because, in times of severe food scarcity, *Drosophila* flies mobilise their stored energy stores that were built up during periods of high nutrition availability because Spirulina has a higher protein concentration than the control media.

Kiran and Krishna (2023) [11] conducted similar research, examining the impact of the traditional Jeeni millet mix on *D. melanogaster's* tolerance to famine. The findings demonstrated that flies fed a combination of wheat cream and jeeni millet were more resistant to hunger. A popular and nutrient-dense grain that is strong in both protein and carbohydrates is millet. Resistance to famine varies according to the known amount, quantity, and quality of nutrition in diet. Therefore, these *Drosophila* experiments imply that starvation resistance is influenced by the quality of nutrition.

Figure 1-2 demonstrated that at varying concentrations of chia seeds, i.e., control and varied concentrations, both unmated and mated female flies exhibited much higher starvation resistance than those of mated and unmated male flies. The findings of Kiran and Krishna (2023) [11]; Sebastian and Krishna (2023); and Shreeraksha *et al.*; (2023) [17] are supported by our research. Additionally, their study revealed that female flies were more resistant to hunger than males. The following explanation for this result is that female flies are larger than male flies because they have accumulated more lipids and glycogen. These glycogen and stored lipids metabolised (broke down) to provide energy during famine. The female makes better use of energy (Kiran and Krishna, 2023) [11]. Hoffman and Harshman (1999) [6, 7] provided an explanation of body weight, stating that it is an indication that quantifies the total amount of chemicals carried by an organism that are stored as energy. As to Shreeraksha *et al.* (2023) [17], females may be more tolerant of resistance because they use the lipid and

glycogen contents as an energy source. However, the breakdown of body lipids as a source of stored energy causes the starving male to show decreased resilience to starvation.

It has been demonstrated that, even within *D. melanogaster*, the pattern of sexual dimorphism in starvation resistance varies significantly based on age, strain, mating status, and test situation (Service, 1989 [16]; Huey *et al.*, 2004 [9]; Vermeulen *et al.*, 2006; Matzkin, 2009) [12, 20]. Because males and females have different nutritional needs, many species display sex-specific characteristics of famine resistance. We employed the same strain, age, and media in our investigation, along with environmental conditions; the only change was sex. Thus, the individual's sex was the cause of the observed starving resistance.

We can use a few potential processes to explain the outcomes. For example, 1) females have a higher proportion of fat and/or protein than males, 2) a larger percentage of this content is available for energy metabolism (i.e., females going hungry have less fat and/or protein left over than males), and 3) females are better at using energy (Rion and Kawecki, 2007) [14].

There is a lot of evidence that sexual dimorphism occurs in traits related to hunger resistance in insects. According to Lee *et al.*; (2013), partnered females accumulated more lipids because they ate more food than males. Aggarwal (2014), who looked at the physiological effects of population-specific differences in *D. leontia* hunger resistance as well as the effects of sex.

While starving males solely used digested body lipids as an energy source, females under starvation stress stored more body lipids and glycogen and used both of these energy sources. Increased resilience to famine is associated with either better energy storage or a slower rate of depletion of these reserves (Hoffmann and Harshman, 1999) [7, 6]. Kiran and Krishna (2023) [11], Shreeraksha *et al.* (2023) [17], and Mamba and Krishna (2023) also demonstrated sexual dimorphism in *D. melanogaster* with regard to starvation resistance, and they discovered that females exhibited noticeably higher levels of famine resistance than males.

Starvation resistance has been shown to be significantly impacted by mating, particularly in *D. melanogaster*. The main reason for the increase in starvation resistance in female *D. melanogaster* is the post-mating increase in food consumption and the resulting rise in lipid storage. Mating is known to increase food intake, suppress sexual receptivity, stimulate egg production, and transfer male seminal fluid peptides to females (Rush *et al.*, 2007) [15]. According to more recent research, mating can lead a female *D. melanogaster's* midgut to enlarge considerably, increasing the amount of nutrients the animal uses after

eating. This enables mated females to meet their higher energy needs for egg laying (Service, 1989^[16]; Goenaga *et al.*, 2012).

In this study, we also examined how mated and unmated (virgin) flies differed in their ability to withstand famine. Because mated males lose energy during mating and transfer sperm and accessory gland proteins (ACPs), the results showed that unmated males were more resistant to starvation than mated males (figure 3). Unmated males, on the other hand, do not lose energy or sperm, which may result in increased resistance to starvation. Mating status had a distinct effect on male hunger tolerance (Kiran and Krishna, 2023)^[11].

Since we employed the same aged flies and cultivated them under the same temperature, humidity, and other conditions, the observed variance in the flies' resistance to starving was not caused by their age or environmental circumstances. The current study additionally examined the male and female flies' resilience to starving in 5g, 10g, and 15g of wheat cream agar and chia seed medium. The findings showed that, across the three concentrations, female flies had a considerably higher level of hunger resistance than male flies.

We therefore draw the conclusion from our experiment that *Drosophila* starvation resistance was significantly influenced by the rich content of omega 3 fatty acids, fats and proteins. Compared to 5g and 15g of Chia seed media and wheat cream agar, 10g of Chia seed gives you more energy and helps you store food for longer so you can tolerate fasting. Starvation resistance is increased when 10g of chia seeds are added to the meal.

Female flies exhibited higher hunger resistance than male flies across all diets examined, indicating that chia seeds in *D. melanogaster* promote starvation resistance. Additionally, compared to mated males and females, unmated males and females were more resistant to hunger. Thus, we conclude that the quality and quantity of different concentration of Chia seed may cause the greater resistant to starvation.

Acknowledgement

The author extends their gratitude to the chairman, Department of studies in Zoology, University of Mysore, Manasagangotri, Mysuru and *Drosophila* stock centre, University of Mysore for providing the facilities to carry out the above work. And also extends gratitude to our beloved seniors Mr. Kiran and Miss Anusree for their guidance.

Reference

1. Aishwarya KC, Ashwini M, Shilpashree BM, Suma S, Tanmayi Kishore, Yashaswini SP, *et al.*, The Effect of the Ensure nutrition supplement on the starvation resistance in *Drosophila melanogaster*. IJARQD, 2024, 11(6)
2. Anusree KA, Asniati Jabbar, Aysha Barira HM, Harshitha L, Harshitha T, Jashwanth G, *et al.*, The Effect of Mass Gainer on the Starvation Resistance in *Drosophila melanogaster*. IJARQG, 2024, 11(6)
3. Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*,1995:373:241–244.
4. Chippindale AK, Chu TJF, Rose MR. Complex trade-offs and the evolution of starvation resistance in *Drosophila*. *Evolution*,1996:50:753–766.
5. Cristiane Freitas Rodrigues, William Salgueiro, Matheus Bianchini, Juliana Cristina Veit, Robson Luiz Puntel, Tatiana Emanuelli, Cristiane Casagrande Dernadin., Diana Silva Avila. *Salvia hispanica* L (Chia) seeds oil extracts reduce lipid accumulation and produce stress resistance in *Caenorhabditis elegans*. *Nutrition and metabolism*, 2018.
6. Harshman AG Schmid JL. Evolution of Starvation Resistance in *Drosophila melanogaster*: Aspects of Metabolism and Counter-Impact Selection. *Journal of evolution*,1998:52(6):1679–1685.
7. Hoffmann AA. Harshman LG. Desiccation starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. *Heredity*,1999:83:637–643.
8. Hoffman AA. Parsons PA. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford university press, 1991.
9. Huey RB, Suess J, Hamilton H Gilchrist GW. Starvation resistance in *Drosophila melanogaster*: testing for a possible ‘cannibalism’ bias. *Funct. Ecol*,2004:18:952–954.
10. Jang T Lee T. Exploring the nutritional basis of starvation resistance in *Drosophila melanogaster*. *Funct. Ecol*,2014:28:1144–1155.
11. Kiran K Krishna MS. The effect of the Jeeni millet traditional mix on the starvation resistance in *Drosophila melanogaster*,2023:10(8):115–126.
12. Matzkin LM, Watts TD, Markow TA. Evolution of stress resistance in *Drosophila*: interspecific variation in tolerance to desiccation and starvation. *Functional Ecology*,2009:23:521–527.
13. McCue MD. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comparative Biochemistry and Physiology Part A*,2010:156:1–18.
14. Rion S. Kawecki TJ. Evolutionary biology of starvation resistance: what have we learned from *Drosophila*. *Journal of Evolutionary Biology*,2007:20:1655–1664.
15. Rush B, Sandver S, Bruer J, Roche R, Wells M Giebultowicz J. Mating increases starvation resistance and decreases oxidative stress resistance in *Drosophila melanogaster* females. *Aging Cell*,2007:6:723–726
16. Service PM. The effect of mating status on lifespan, egg laying, and starvation resistance in *Drosophila melanogaster* in relation to selection on longevity. *J. Insect Physiol*,1989:35:447–452
17. Shreeraksha, Shreejani HK Krishna MS. Spirulina Supplement Increases Starvation Resistance in *Drosophila melanogaster*,2023:14(08):4046–4050.
18. Simpson SJ, Sibly RM, Lee K, Raubenheimer D. Optimal foraging with multiple nutrient requirements. *Animal Behav.* 68: 1299–1311. Sisodia, S., Singh, B.N. 2012. Experimental Evidence for Nutrition Regulated Stress Resistance in *Drosophila ananassae*. *PLoS ONE*,2004:7(10):46131.
19. Sithembiso Sebastian Mamba, Mysore Siddaiah Krishna. Creatine monohydrate supplementation decreases starvation resistance of *D. melanogaster*. *Int J Recent Sci Res*,14(08):4022– 4026.
20. Vermeulen CJ, Van De Zande L, Bijlsma R. Developmental and age-specific effects of selection on divergent virgin life span on fat content and starvation resistance in *Drosophila melanogaster*. *J. Insect Physiol*,2006:52:910–919.